

8378163



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UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

May 23, 2023

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OF:**

APPLICATION NUMBER: 16/613,203
FILING DATE: *November 13, 2019*
PATENT NUMBER: 11446386
ISSUE DATE: *September 20, 2022*



Certified by

Kathi

Performing the Functions and Duties of the
Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

DocCode – SEQ.TXT

SCORE Placeholder Sheet for IFW Content

Application Number: 16613203

Document Date: 11/13/2019

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PTO-1390 (01-18)

Approved for use through 8/31/2019. OMB 0651-0021

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A SUBMISSION UNDER 35 U.S.C. 371		Attorney Docket No. 098065-0249
		U.S. Application No. (if known, see 37 CFR 1.5) Not Yet Assigned
International Application No. PCT/JP2018/018572	International Filing Date May 14, 2018	Priority Date Claimed May 15, 2017
Title of Invention ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE		
First Named Inventor Atsuko SAITO		
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.</p> <ol style="list-style-type: none"> 1. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). NOTE: The express request under 35 U.S.C. 371(f) will not be effective unless the requirements under 35 U.S.C. 371(c)(1), (2), and (4) for payment of the basic national fee, copy of the International Application and English translation thereof (if required), and the oath or declaration of the inventor(s) have been received. 2. ☒ A copy of the International Application (35 U.S.C. 371(c)(2)) is attached hereto (not required if the International Application was previously communicated by the International Bureau or was filed in the United States Receiving Office (RO/US)). 3. An English language translation of the International Application (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> a. ☒ is attached hereto. b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4). 4. An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) <ol style="list-style-type: none"> a. ☐ is attached. b. ☐ was previously filed in the international phase under PCT Rule 4.17(iv). <p>Items 5 to 8 below concern amendments made in the international phase.</p> <p><u>PCT Article 19 and 34 amendments</u></p> <ol style="list-style-type: none"> 5. ☐ Amendments to the claims under PCT Article 19 are attached (not required if communicated by the International Bureau) (35 U.S.C. 371(c)(3)). 6. ☐ English translation of the PCT Article 19 amendment is attached (35 U.S.C. 371(c)(3)). 7. ☐ English translation of annexes (Article 19 and/or 34 amendments only) of the International Preliminary Examination Report is attached (35 U.S.C. 371(c)(5)). <p><u>Cancellation of amendments made in the international phase</u></p> <ol style="list-style-type: none"> 8a. ☐ Do not enter the amendment made in the international phase under PCT Article 19. 8b. ☐ Do not enter the amendment made in the international phase under PCT Article 34. <p>NOTE: A proper amendment made in English under Article 19 or 34 will be entered in the U.S. national phase application absent a clear instruction from applicant not to enter the amendment(s).</p> <p>The following items 9 to 17 concern a document(s) or information included.</p> <ol style="list-style-type: none"> 9. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 10. ☒ A preliminary amendment. 11. ☒ An Application Data Sheet under 37 CFR 1.76. 12. ☐ A substitute specification. NOTE: A substitute specification cannot include claims. See 37 CFR 1.125(b). 13. ☐ A power of attorney and/or change of address letter. 14. ☒ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.3 and 37 CFR 1.821-1.825 (not required if sequence listing in text format was indicated on the PCT Request as part of the International Application and the sequence listing was published as part of the international application). 15. ☐ Assignment papers (cover sheet and document(s)). Name of Assignee: _____ 16. ☐ 37 CFR 3.73(c) Statement (when there is an Assignee). _____ 		

This collection of information is required by 37 CFR 1.414 and 1.491-1.492. The information is required to obtain or retain a benefit by the public, which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 15 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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U.S. APPLN. No. (if known – see 37 CFR 1.5)		INTERNATIONAL APPLICATION No.		ATTORNEY DOCKET No.	
Not Yet Assigned		PCT/JP2018/018572		098065-0249	
17. ☐ Other items or information:					
The following fees have been submitted.					CALCULATIONS
18. ☒ Basic national fee (37 CFR 1.492(a))					\$300
19. ☒ Examination fee (37 CFR 1.492(c))					\$
• If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4).....					\$0
• All other situations					\$760
20. ☒ Search fee (37 CFR 1.492(b))					\$
• If the written opinion prepared by ISA/US or the international preliminary examination report prepared by IPEA/US indicates all claims satisfy provisions of PCT Article 33(1)-(4).....					\$0
• Search fee (37 CFR 1.445(a)(2)) has been paid on the international application to the USPTO as an International Searching Authority					\$140
• International Search Report prepared by an ISA other than the US and provided to the Office or previously communicated to the US by the IB.....					\$520
• All other situations					\$660
TOTAL OF 18, 19, and 20 =					\$1580.00
☒ Additional fee for specification and drawings filed in paper over 100 sheets (excluding sequence listing in compliance with 37 CFR 1.821(c) or (e) in an electronic medium or computer program listing in an electronic medium) (37 CFR 1.492(j)).					800.00
Fee for each additional 50 sheets of paper or fraction thereof					\$400
Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof (round up to a whole number)	RATE		
179 - 100 =	79 / 50 =	2	x \$400		\$
Surcharge for furnishing any of the search fee, examination fee, or the oath or declaration after the date of commencement of the national stage (37 CFR 1.492(h)).....					\$140
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	20 - 20 =	0	x \$100		\$ 0.00
Independent claims	3 - 3 =	0	x \$460		\$ 0.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$820		\$
Fee for submission of Sequence Listing text file of 300 MB to 800 MB (37 CFR 1.21(o)(1)).....					\$1,000
Fee for submission of Sequence Listing text file of more than 800 MB (37 CFR 1.21(o)(2)).....					\$10,000
Processing fee for furnishing the English translation later than 30 months from the earliest claimed priority date (37 CFR 1.492(i)).....					\$140 +
TOTAL OF ABOVE CALCULATIONS =					\$ 2520.00
☐ Applicant asserts small entity status. See 37 CFR 1.27. Fees above are reduced by ½.					
☐ Applicant certifies micro entity status. See 37 CFR 1.29. Fees above are reduced by ¾. Applicant must attach form PTO/SB/15A or B or equivalent.					
TOTAL NATIONAL FEE =					\$ 2520.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31).....					\$50.00 per property +
TOTAL FEES ENCLOSED =					\$ 2520.00

PTO-1390 (01-18)

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- a. ☐ A check in the amount of \$_____ to cover the above fees is enclosed.
- b. ☐ Please charge my Deposit Account No. _____ in the amount of \$_____ to cover the above fees.
- c. ☒ The Director is hereby authorized to charge additional fees which may be required, or credit any overpayment, to Deposit Account No. 19-0741 _____ as follows:
- i. ☐ any required fee.
- ii. ☒ any required fee except for excess claims fees required under 37 CFR 1.492(d) and (e) and multiple dependent claim fee required under 37 CFR 1.492(f).
- d. ☒ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038. The PTO-2038 should only be mailed or faxed to the USPTO. However, when paying the basic national fee, the PTO-2038 may NOT be faxed to the USPTO.
- ADVISORY:** If filing by EFS-Web, do NOT attach the PTO-2038 form as a PDF along with your EFS-Web submission. Please be advised that this is **not** recommended and by doing so your **credit card information may be displayed via PAIR**. To protect your information, it is recommended to pay fees online by using the electronic payment method.

NOTE: Where an appropriate time limit under 37 CFR 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the International Application to pending status.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

- ☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013, and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE 1: By providing this statement under 37 CFR 1.55 or 1.78, **this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.**

NOTE 2: A U.S. national stage application may not claim priority to the international application of which it is the national phase. The filing date of a U.S. national stage application is the international filing date. See 35 U.S.C. 363.

Correspondence Address

- ☒ The address associated with Customer Number: 22428 OR ☐ Correspondence address below

Name					
Address					
City		State		Zip Code	
Country				Telephone	
Email					

Signature	/Michael D. Kaminski/	Date	2019-11-13
Name (Print/Type)	Michael D. Kaminski	Registration No. (Attorney/Agent)	32,904

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal				
Application Number:				
Filing Date:				
Title of Invention:		ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE		
First Named Inventor/Applicant Name:		Atsuko SAITO		
Filer:		Michael D. Kaminski/Clarissa Wilky		
Attorney Docket Number:		098065-0249		
Filed as Large Entity				
Filing Fees for U.S. National Stage under 35 USC 371				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
NATIONAL STAGE FEE	1631	1	300	300
NATL STAGE SEARCH FEE - REPORT PROVIDED	1642	1	520	520
NATIONAL STAGE EXAM - ALL OTHER CASES	1633	1	760	760
Pages:				
NATL STAGE APPL SZ FEE PER 50 PGS >100	1681	2	400	800
Claims:				
Miscellaneous-Filing:				
OATH/DECL > 30 MOS FROM 371 COMMENCEMENT	1617	1	140	140

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				2520

Electronic Acknowledgement Receipt	
EFS ID:	37736292
Application Number:	16613203
International Application Number:	PCT/JP2018/018572
Confirmation Number:	6337
Title of Invention:	ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE
First Named Inventor/Applicant Name:	Atsuko SAITO
Customer Number:	22428
Filer:	Michael D. Kaminski/Clarissa Wilky
Filer Authorized By:	Michael D. Kaminski
Attorney Docket Number:	098065-0249
Receipt Date:	13-NOV-2019
Filing Date:	
Time Stamp:	12:43:53
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	\$2520
RAM confirmation Number	E2019ACC46160865
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Application Data Sheet	098065-0249_ADS_2019-11-13.pdf	1256010	no	9
			882efd0e0d665a1252cac7dfa103d6d175b026aa		
Warnings:					
Information:					
2	Documents submitted with 371 Applications	098065-0249_PCT_Specification.pdf	13274807	no	183
			2206a238024e8785fb52f187a7235798b3140d01		
Warnings:					
Information:					
3		098065-0249_EnglishSpecification.pdf	8501578	yes	202
			d57a8aad1d53f23c02e68fedcf345875767037ce		
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Specification		1	180	
	Claims		181	201	
	Abstract		202	202	
Warnings:					
Information:					
4	Drawings-only black and white line drawings	098065-0249_EnglishDrawings.pdf	4155153	no	29
			800c3fe2e7aa68454a019301eee5acc65d9e1d40		
Warnings:					
Information:					
5		098065-0249_Preliminary-Amendment_2019-11-13.pdf	146813	yes	6
			2769b21dab419a14fd1d32064eda3f5f1baa5267		

	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Preliminary Amendment		1	1	
	Specification		2	5	
	Applicant Arguments/Remarks Made in an Amendment		6	6	
Warnings:					
Information:					
6	Sequence Listing (Text File)	098065-0249_SL.TXT	109133	no	-
Warnings:					
Information:					
7	Documents submitted with 371 Applications	098065-0249_IB_304.pdf	93095	no	1
			a3c7b2af95da000ff4ad32ebcc0078258f525db		
Warnings:					
Information:					
8	Transmittal Letter	098065-0249_IDS-Transmittal_2019-11-13.pdf	125947	no	2
			05489d41d3f79b64e34d4c332ec501fb4af5b760		
Warnings:					
Information:					
9	Information Disclosure Statement (IDS) Form (SB08)	098065-0249_IDS-SB08_2019-11-13.pdf	138841	no	2
			e1ee3e9bee356b1affdf3751f2134cc0ea12abc		
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
10	Foreign Reference	WO2014057687.pdf	15097047	no	331
			bc7aa74aa4518dfc87a9d0daed8bea8d0f2a8b68		
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Information:					

11	Foreign Reference	WO2016024195.pdf	13951492	no	259
			a2d74dbfe9964fa51637e0cfd9fbb3998c588081		
Warnings:					
Information:					
12	Other Reference-Patent/App/Search documents	PCTJP2018018572_ISR_2018-08-07.pdf	388919	no	4
			6563774ae9f77b1b104062f324093960eae258		
Warnings:					
Information:					
13	Other Reference-Patent/App/Search documents	PCTJP2018018572_WrittenOpinion_2018-08-07.pdf	389116	no	4
			87b759f7458d5dc16cb62e666bc1a9cd93426e1f		
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Information:					
14	Non Patent Literature	SHIMOYAMA_Isolation_and_Sequence.pdf	1968743	no	7
			70d1cb6bccd9b94ff9a64602ebc472b28b741f18		
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Information:					
15	Non Patent Literature	INOUE_Cadherin-6_Expression.pdf	3774790	no	12
			270f54f373bb0bf88aba2c323c1977199dce2a45		
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Information:					
16	Non Patent Literature	OSTERHOUT_Cadherin-6_Mediations.pdf	2743751	no	8
			df00dc2891f24d5612921c69683a3932d90f5b47		
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Information:					
17	Non Patent Literature	CHO_DifferentialExpression.pdf	4248569	no	10
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Warnings:					
Information:					

18	Non Patent Literature	MAH_KidneyDevelopment.pdf	5106104	no	16
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Warnings:					
Information:					
19	Non Patent Literature	PAUL_Cadherin-6_a_CellAdhesion.pdf	3334510	no	9
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20	Non Patent Literature	SHIMAZUI_The_Level.pdf	1677255	no	6
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Information:					
21	Non Patent Literature	KOEDEL_OvarianCarcinomaSubtypes.pdf	3766894	no	12
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Information:					
22	Non Patent Literature	GUGNONI_Cadherin-6_promotes_EMT.pdf	3712586	no	11
			aa2175d55a718dedc2ee852d09326a87f73e3e77		
Warnings:					
Information:					
23	Non Patent Literature	POLAKIS_Antibody.pdf	4885453	no	17
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Warnings:					
Information:					
24	Non Patent Literature	PETERS_Antibody-DrugConjugates.pdf	5705047	no	20
			5d69f34a3eb18b1ab4be9786cada887de24f4603		
Warnings:					
Information:					

25	Non Patent Literature	GOEPPERT_Cadherin-6_is_a.pdf	3523504	no	12
			33f2d99abed902406bdb074e4e708dc086eb287d		
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Information:					
26	Non Patent Literature	YOKOI_A_NovelTargetGene.pdf	2548688	no	10
			62f28dc6fca8b6c2776e17362cd42bfe5675454		
Warnings:					
Information:					
27	Transmittal of New Application	098065-0249_371-Transmittal_2019-11-13.pdf	257673	no	4
			3b925bb49697aa4d5a0a04957a686bb106a1b4a1		
Warnings:					
Information:					
28	Fee Worksheet (SB06)	fee-info.pdf	38705	no	2
			33567194d968f9f43dd9c110b1791836f18934a2		
Warnings:					
Information:					
Total Files Size (in bytes):			104920223		
<p>This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.</p> <p>New Applications Under 35 U.S.C. 111 If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p>National Stage of an International Application under 35 U.S.C. 371 If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p>New International Application Filed with the USPTO as a Receiving Office If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p>					

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	098065-0249
		Application Number	
Title of Invention	ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE		
<p>The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.</p> <p>This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.</p>			

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor	1				Remove	
Legal Name						
Prefix	Given Name	Middle Name	Family Name	Suffix		
	Atsuko		SAITO			
Residence Information (Select One) ☐ US Residency ☒ Non US Residency ☐ Active US Military Service						
City	Tokyo		Country of Residence ⁱ	JP		
Mailing Address of Inventor:						
Address 1	c/o Daiichi Sankyo Company, Limited					
Address 2	3-5-1, Nihonbashi Honcho, Chuo-ku					
City	Tokyo		State/Province			
Postal Code	103-8426		Country ⁱ	JP		
Inventor	2				Remove	
Legal Name						
Prefix	Given Name	Middle Name	Family Name	Suffix		
	Tsuyoshi		HIRATA			
Residence Information (Select One) ☐ US Residency ☒ Non US Residency ☐ Active US Military Service						
City	Tokyo		Country of Residence ⁱ	JP		
Mailing Address of Inventor:						
Address 1	c/o DAIICHI SANKYO RD NOVARE CO., LTD.					
Address 2	1-16-13, Kitakasai, Edogawa-ku					
City	Tokyo		State/Province			
Postal Code	134-8630		Country ⁱ	JP		
Inventor	3				Remove	
Legal Name						

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	098065-0249
		Application Number	
Title of Invention	ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE		

Prefix	Given Name	Middle Name	Family Name	Suffix
	Kensuke		NAKAMURA	
Residence Information (Select One) US Residency ☒ Non US Residency Active US Military Service				
City	Tokyo		Country of Residence ⁱ	
Mailing Address of Inventor:				
Address 1	c/o Daiichi Sankyo Company, Limited			
Address 2	3-5-1, Nihonbashi Honcho, Chuo-ku			
City	Tokyo	State/Province		
Postal Code	103-8426	Country ⁱ	JP	
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button. Add				

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).	
☐ An Address is being provided for the correspondence Information of this application.	
Customer Number	22428
Email Address	IPDocketing@foley.com Add Email Remove Email

Application Information:

Title of the Invention	ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE		
Attorney Docket Number	098065-0249	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	29	Suggested Figure for Publication (if any)	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	098065-0249
		Application Number	
Title of Invention	ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE		

Publication Information:

☐	Request Early Publication (Fee required at time of Request 37 CFR 1.219)
☐	Request Not to Publish. I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.											
<table border="1"> <tr> <td>Please Select One:</td> <td>☒ Customer Number</td> <td>☐ US Patent Practitioner</td> <td>☐ Limited Recognition (37 CFR 11.9)</td> </tr> <tr> <td>Customer Number</td> <td colspan="3">22428</td> </tr> </table>				Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)	Customer Number	22428		
Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)								
Customer Number	22428										

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78. When referring to the current application, please leave the "Application Number" field blank.			
Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	a 371 of international	PCT/JP2018/018572	2018-05-14
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.			Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)¹ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	098065-0249
		Application Number	
Title of Invention	ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE		

			Remove
Application Number	Country ⁱ	Filing Date (YYYY-MM-DD)	Access Code ^j (if applicable)
2017-096749	JP	2017-05-15	9E70
Additional Foreign Priority Data may be generated within this form by selecting the Add button.			Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013. NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	098065-0249
		Application Number	
Title of Invention	ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE		

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	098065-0249
		Application Number	
Title of Invention	ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE		

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.			
Applicant	1		<input type="button" value="Remove"/>
<p>If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.</p>			
		<input type="button" value="Clear"/>	
☐ Assignee	Legal Representative under 35 U.S.C. 117		Joint Inventor
Person to whom the inventor is obligated to assign.		Person who shows sufficient proprietary interest	
If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:			
<div style="border: 1px solid black; height: 20px; width: 100%;"></div>			
Name of the Deceased or Legally Incapacitated Inventor: <div style="border: 1px solid black; width: 400px; height: 20px;"></div>			
If the Applicant is an Organization check here. ☒			
Organization Name	DAIICHI SANKYO COMPANY, LIMITED		
Mailing Address Information For Applicant:			
Address 1	3-5-1, Nihonbashi Honcho, Chuo-ku		
Address 2			
City	Tokyo	State/Province	
Country	JP	Postal Code	103-8426
Phone Number		Fax Number	
Email Address			
Additional Applicant Data may be generated within this form by selecting the Add button. <input type="button" value="Add"/>			

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	098065-0249
		Application Number	
Title of Invention	ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE		

Assignee	1		
Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.			
			Remove
If the Assignee or Non-Applicant Assignee is an Organization check here. ☒			
Organization Name	DAIICHI SANKYO COMPANY, LIMITED		
Mailing Address Information For Assignee including Non-Applicant Assignee:			
Address 1	3-5-1, Nihonbashi Honcho, Chuo-ku		
Address 2			
City	Tokyo	State/Province	
Country i	JP	Postal Code	103-8426
Phone Number		Fax Number	
Email Address			
Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.			Add

Signature: [Remove](#)

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).

This Application Data Sheet must be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, all joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of all joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Michael D. Kaminski/		Date (YYYY-MM-DD)	2019-11-13	
First Name	Michael D.	Last Name	KAMINSKI	Registration Number	32,904
Additional Signature may be generated within this form by selecting the Add button.				Add	

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	098065-0249
		Application Number	
Title of Invention	ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE		

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

(12) 特許協力条約に基づいて公開された国際出願

(19) 世界知的所有権機関
国際事務局(43) 国際公開日
2018年11月22日(22.11.2018)

(10) 国際公開番号

WO 2018/212136 A1

(51) 国際特許分類:

C12N 15/13 (2006.01) A61P 43/00 (2006.01)
 A61K 9/00 (2006.01) C07K 16/28 (2006.01)
 A61K 31/4745 (2006.01) C12N 1/15 (2006.01)
 A61K 39/395 (2006.01) C12N 1/19 (2006.01)
 A61K 47/68 (2017.01) C12N 1/21 (2006.01)
 A61P 13/12 (2006.01) C12N 5/10 (2006.01)
 A61P 15/08 (2006.01) C12P 21/08 (2006.01)
 A61P 35/00 (2006.01)

(21) 国際出願番号: PCT/JP2018/018572

(22) 国際出願日: 2018年5月14日(14.05.2018)

(25) 国際出願の言語: 日本語

(26) 国際公開の言語: 日本語

(30) 優先権データ:
特願 2017-096749 2017年5月15日(15.05.2017) JP

(71) 出願人: 第一三共株式会社(DAICHI SANKYO COMPANY, LIMITED) [JP/JP]; 〒1038426 東京都中央区日本橋本町三丁目5番1号 Tokyo (JP).

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(74) 代理人: 石橋 公樹, 外(ISHIBASHI Koki et al.); 〒1400005 東京都品川区広町一丁目2番58号 第一三共株式会社内 Tokyo (JP).

(81) 指定国(表示のない限り、全ての種類の国内保護が可能): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) 指定国(表示のない限り、全ての種類の広域保護が可能): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), ユーラシア (AM, AZ, BY, KG, KZ, RU, TJ, TM), ヨーロッパ (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

添付公開書類:

- 一 国際調査報告(条約第21条(3))
- 一 明細書の別個の部分として表した配列リスト(規則5.2(a))

(54) Title: ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE

(54) 発明の名称: 抗CDH6抗体及び抗CDH6抗体-薬物コンジュゲート

(57) Abstract: The present invention addresses the problem of providing: an antibody having an internalization activity of binding to CDH6; an antibody-drug conjugate consisting of the aforesaid antibody and a drug having an antitumor activity; a medicine using the antibody-drug conjugate, said medicine having a therapeutic effect on tumors; a method for treating tumors using the antibody, the antibody-drug conjugate or the medicine; etc. Provided are: an anti-CDH6 antibody having an internalization activity; an antibody-drug conjugate consisting of the aforesaid antibody and a drug having an antitumor activity; and a medicine and a method for treating tumors using the same.

(57) 要約: 本発明の課題は、CDH6に結合する内在化活性を有する抗体、該抗体と抗腫瘍活性を有する薬物との抗体-薬物コンジュゲート、該抗体-薬物コンジュゲートを用いた腫瘍に対する治療効果を有する医薬品、及び、該抗体、該抗体-薬物コンジュゲート又は該医薬品を用いた腫瘍の治療方法等を提供することである。本発明により、内在化活性を有する抗CDH6抗体、該抗体と抗腫瘍活性を有する薬物との抗体-薬物コンジュゲート、これらを用いる医薬品および腫瘍の治療方法が提供される。

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PCT/JP2018/018572

明 細 書

発明の名称：

抗CDH6抗体及び抗CDH6抗体－薬物コンジュゲート

技術分野

[0001] 本発明は、CDH6に結合し、内在化作用を有する抗CDH6抗体、該抗CDH6抗体の製造方法、ならびに該抗体を含む抗体－薬物コンジュゲート、該抗体－薬物コンジュゲートを含む抗腫瘍剤等に関する。

背景技術

[0002] カドヘリン (C a d h e r i n) は、細胞膜表面に存在する糖タンパク質で、カルシウムイオン依存的にN末端側の細胞外ドメイン同士が結合することで、細胞間接着分子として、また細胞間相互作用を担うシグナル分子として機能する。カドヘリンスーパーファミリーの内、クラシックカドヘリンに分類される分子群は、5個の細胞外ドメイン (e x t r a c e l l u l a r d o m a i n、ECドメイン) と1個の膜貫通領域、及び細胞内ドメインから構成される1回膜貫通タンパク質である。クラシックカドヘリンはアミノ酸配列の相同性から、EーカドヘリンやNーカドヘリンに代表されるタイプIファミリーと、タイプIIファミリーに分類される。

[0003] カドヘリン6 (C a d h e r i n - 6、CDH6) は、タイプIIカドヘリンファミリーに分類される、790アミノ酸からなる1回膜貫通タンパク質であり、N末端側を細胞外、C末端側を細胞内に持つ。ヒトCDH6遺伝子は1995年に初めてクローニングされ (非特許文献1)、NM____004932、NP____004923 (NCBI) 等のアクセッション番号により参照可能である。

[0004] CDH6は発生期において脳や腎臓で特異的に発現しており、中枢神経系の回路形成 (非特許文献2、非特許文献3) や腎臓のネフロン発生時 (非特許文献4、非特許文献5) に重要な役割を担うことが報告されている。成人の正常組織では、CDH6の発現は腎尿管や胆管上皮細胞などに限局され

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ている。

[0005] 一方で、成人のいくつかの癌種において、腫瘍部で特異的にCDH6の発現が亢進することが知られており、ヒト腎細胞癌、特に腎淡明細胞癌では、CDH6発現と予後不良との相関や、腫瘍マーカーとしての利用可能性が報告されている（非特許文献6、非特許文献7）。ヒト卵巣癌でもCDH6の高発現が報告されている（非特許文献8）、ヒト甲状腺癌の上皮間葉変換と転移にCDH6が関与するとの報告もある（非特許文献9）。また、CDH6は、ヒト胆管癌およびヒト小細胞肺癌でも発現していることが報告されている（非特許文献12、13）。

[0006] がんは死亡原因の上位を占め、その罹患数は人口の高齢化と共に増加することが予想されているが、未だ治療ニーズは十分に満たされていない。従来化学療法剤は、その選択性の低さから腫瘍細胞だけでなく正常細胞に対しても傷害性を持つことによる副作用や、十分な薬剤量を投与できないことで薬剤の効果を十分に得ることが出来ないことが問題となっている。このため近年では、がん細胞に特徴的な変異や高発現を示す分子、細胞のがん化に関与する特定の分子を標的としたより選択性の高い分子標的薬や抗体医薬の開発が行われている。

[0007] 抗体は血中安定性が高く、標的抗原に特異的に結合することから副作用の軽減が期待されており、がん細胞表面に高発現している分子に対する抗体医薬が多数開発されている。抗体の抗原特異的な結合能を利用した技術の一つとして、抗体-薬物コンジュゲート（Antibody-Drug Conjugate；ADC）が挙げられる。ADCは、がん細胞表面に発現している抗原に結合し、その結合によって抗原を細胞内に内在化できる抗体に、細胞傷害活性を有する薬物を結合させたものである。ADCは、がん細胞に効率的に薬物を送達できることによって、がん細胞内に薬物を蓄積させ、がん細胞を死滅させることが期待できる（非特許文献10、特許文献1及び2）。ADCとして例えば、抗CD30モノクローナル抗体にモノメチルアウリスチンEを結合させたアドセトリス（商標）（ブレンツキシマブ ペド

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チン）がホジキンリンパ腫と未分化大細胞リンパ腫の治療薬として認可されている。また、抗HER2モノクローナル抗体にエムタンシンを結合させたカドサイラ（商標）（トラスツズマブ エムタンシン）がHER2陽性の進行、再発乳癌の治療に用いられている。

【0008】 抗腫瘍薬としてのADCに適した標的抗原の特徴としては、がん細胞表面で特異的に高発現し、正常細胞では低発現又は発現していないこと、細胞内に内在化できること、抗原が細胞表面から分泌されないことなどが挙げられる。また、ADCに適した抗体の重要な特徴としては、標的となる抗原に特異的に結合することに加えて、高い内在化能を有することが挙げられる。抗体の内在化能は標的抗原と抗体の両方の性質に依存するものであり、標的の分子構造から内在化に適した抗原結合部位を推定したり、抗体の結合強度や物性等から容易に内在化能の高い抗体を推測したりすることは困難である。このため、標的抗原に対して高い内在化能を有する抗体を取得することは、有効性の高いADCを開発する上で重要な課題となっている（非特許文献1）。

【0009】 CDH6を標的としたADCとしては、CDH6のECドメイン5（EC5）に特異的に結合する抗CDH6抗体にDM4を結合させたADCが知られている（特許文献3）。

先行技術文献

特許文献

- 【0010】 特許文献1：WO2014/057687
特許文献2：US2016/0297890
特許文献3：WO2016/024195

非特許文献

- 【0011】 非特許文献1：Shimoyama Y, et al., Cancer Research, 2206-2211, 55, May 15, 1995
非特許文献2：Inoue T, et al., Developmental Biology, 183-194, 1997

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非特許文献3: Osterhout J A, et al., Neuron, 632-639, 71, Aug 25, 2011

非特許文献4: Cho E A, et al., Development, 803-812, 125, 1998

非特許文献5: Mah S P, et al., Developmental Biology, 38-53, 223, 2000

非特許文献6: Paul R, et al., Cancer Research, 2741-2748, July 1, 57, 1997

非特許文献7: Shimazui T, et al., Cancer, 963-968, 101 (5), Sep. 1, 2004

非特許文献8: Koebel M, et al., PLoS Medicine, 1749-1760, 5 (12), e232, Dec. 2008

非特許文献9: Gugnoni M, et al., Oncogene, 667-677, 36, 2017

非特許文献10: Polakis P., Pharmacological Reviews, 3-19, 68, 2016

非特許文献11: Peters C, et al., Bioscience Reports, 1-20, 35, 2015

非特許文献12: Goepfert B, et al., Epigenetics, 780-790, 11 (11), 2016

非特許文献13: Yokoi S, et al., American Journal of Pathology, 207-216, 161, 1, 2002

発明の概要

発明が解決しようとする課題

[0012] 本発明の課題は、CDH6に対して特異的に結合する高い内在化活性を有する抗体、該抗体を含む高い抗腫瘍活性を有する抗体-薬物コンジュゲート、該抗体-薬物コンジュゲートを用いた腫瘍に対する治療効果を有する医薬

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品、及び、当該抗体、抗体－薬物コンジュゲート又は医薬品を用いた腫瘍の治療方法等を提供することである。

課題を解決するための手段

[0013] 本発明者らは、上記課題を達成するために鋭意検討したところ、驚くべきことにCDH6の細胞外ドメイン3（本明細書中、EC3とも称する）に特異的に結合する抗体が、CDH6を発現する細胞において極めて高い内在化活性を有しADC抗体として有用であることを見出した。更に、当該抗CDH6抗体に、細胞内で毒性を発揮する薬剤を特定の構造のリンカーを介して結合させた抗CDH6抗体－薬物コンジュゲートは、従来のCDH6薬物複合体よりも強い抗腫瘍活性を示すことを見出した。

[0014] 本願発明は以下の発明を包含する；

[1] 配列番号4に記載のアミノ酸配列に特異的に結合し、細胞内に取り込まれる内在化能を有する、抗体又は当該抗体の機能性断片；

[2] 配列番号4に記載のアミノ酸配列への結合に対して、以下の（1）～（5）；

（1）配列番号53の21～233番目に記載のアミノ酸配列からなる軽鎖及び配列番号56の20～471番目に記載のアミノ酸配列からなる重鎖を有する抗体、

（2）配列番号61の21～233番目に記載のアミノ酸配列からなる軽鎖及び配列番号69の20～471番目に記載のアミノ酸配列からなる重鎖を有する抗体、

（3）配列番号61の21～233番目に記載のアミノ酸配列からなる軽鎖及び配列番号73の20～471番目に記載のアミノ酸配列からなる重鎖を有する抗体、

（4）配列番号65の21～233番目に記載のアミノ酸配列からなる軽鎖及び配列番号73の20～471番目に記載のアミノ酸配列からなる重鎖を有する抗体、及び

（5）配列番号61の21～233番目に記載のアミノ酸配列からなる軽鎖

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及び配列番号 77 の 20～471 番目に記載のアミノ酸配列からなる重鎖を有する抗体、

からなる群から選択される抗体のうち少なくともいずれか 1 つと競合阻害活性を有する [1] に記載の抗体又は当該抗体の機能性断片；

[3] 以下の (1)～(4)：

(1) 配列番号 12 に記載のアミノ酸配列からなる CDR L 1、配列番号 13 に記載のアミノ酸配列からなる CDR L 2 及び配列番号 14 に記載のアミノ酸配列からなる CDR L 3、

(2) 配列番号 22 に記載のアミノ酸配列からなる CDR L 1、配列番号 23 に記載のアミノ酸配列からなる CDR L 2 及び配列番号 24 に記載のアミノ酸配列からなる CDR L 3、

(3) 配列番号 32 に記載のアミノ酸配列からなる CDR L 1、配列番号 33 に記載のアミノ酸配列からなる CDR L 2 及び配列番号 34 に記載のアミノ酸配列からなる CDR L 3、及び

(4) 配列番号 42 に記載のアミノ酸配列からなる CDR L 1、配列番号 43 に記載のアミノ酸配列からなる CDR L 2 及び配列番号 44 に記載のアミノ酸配列からなる CDR L 3、

からなる群から選択されるいずれか 1 つに記載の CDR L 1、CDR L 2 及び CDR L 3、並びに、

以下の (5)～(9)：

(5) 配列番号 17 に記載のアミノ酸配列からなる CDR H 1、配列番号 18 に記載のアミノ酸配列からなる CDR H 2 及び配列番号 19 に記載のアミノ酸配列からなる CDR H 3、

(6) 配列番号 27 に記載のアミノ酸配列からなる CDR H 1、配列番号 28 に記載のアミノ酸配列からなる CDR H 2 及び配列番号 29 に記載のアミノ酸配列からなる CDR H 3、

(7) 配列番号 37 に記載のアミノ酸配列からなる CDR H 1、配列番号 38 に記載のアミノ酸配列からなる CDR H 2 及び配列番号 39 に記載のアミ

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ノ酸配列からなるCDRH3、

(8) 配列番号47に記載のアミノ酸配列からなるCDRH1、配列番号48に記載のアミノ酸配列からなるCDRH2及び配列番号49に記載のアミノ酸配列からなるCDRH3、及び

(9) 配列番号17に記載のアミノ酸配列からなるCDRH1、配列番号60に記載のアミノ酸配列からなるCDRH2及び配列番号19に記載のアミノ酸配列からなるCDRH3、

からなる群から選択されるいずれか1つに記載のCDRH1、CDRH2及びCDRH3を含む、

[1] 又は [2] のいずれか1項に記載の抗体又は当該抗体の機能性断片；

[4] 以下の(1)～(5)：

(1) 配列番号12に記載のアミノ酸配列からなるCDRL1、配列番号13に記載のアミノ酸配列からなるCDRL2及び配列番号14に記載のアミノ酸配列からなるCDRL3、及び、配列番号17に記載のアミノ酸配列からなるCDRH1、配列番号18に記載のアミノ酸配列からなるCDRH2及び配列番号19に記載のアミノ酸配列からなるCDRH3、

(2) 配列番号22に記載のアミノ酸配列からなるCDRL1、配列番号23に記載のアミノ酸配列からなるCDRL2及び配列番号24に記載のアミノ酸配列からなるCDRL3、及び、配列番号27に記載のアミノ酸配列からなるCDRH1、配列番号28に記載のアミノ酸配列からなるCDRH2及び配列番号29に記載のアミノ酸配列からなるCDRH3、

(3) 配列番号32に記載のアミノ酸配列からなるCDRL1、配列番号33に記載のアミノ酸配列からなるCDRL2及び配列番号34に記載のアミノ酸配列からなるCDRL3、及び、配列番号37に記載のアミノ酸配列からなるCDRH1、配列番号38に記載のアミノ酸配列からなるCDRH2及び配列番号39に記載のアミノ酸配列からなるCDRH3、

(4) 配列番号42に記載のアミノ酸配列からなるCDRL1、配列番号43に記載のアミノ酸配列からなるCDRL2及び配列番号44に記載のアミ

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ノ酸配列からなるCDRL3、及び、配列番号47に記載のアミノ酸配列からなるCDRH1、配列番号48に記載のアミノ酸配列からなるCDRH2及び配列番号49に記載のアミノ酸配列からなるCDRH3、及び

(5) 配列番号12に記載のアミノ酸配列からなるCDRL1、配列番号13に記載のアミノ酸配列からなるCDRL2及び配列番号14に記載のアミノ酸配列からなるCDRL3、及び、配列番号17に記載のアミノ酸配列からなるCDRH1、配列番号60に記載のアミノ酸配列からなるCDRH2及び配列番号19に記載のアミノ酸配列からなるCDRH3、

からなる群から選択されるCDRL1、CDRL2及びCDRL3、並びに、CDRH1、CDRH2及びCDRH3を含む、

[1]～[3]のいずれか1項に記載の抗体又は当該抗体の機能性断片；

[5] ヒト化されている[1]～[4]のいずれか1項に記載の抗体又は当該抗体の機能性断片；

[6] 以下の(1)～(4)：

(1) 配列番号63に記載のアミノ酸配列からなる軽鎖可変領域、

(2) 配列番号67に記載のアミノ酸配列からなる軽鎖可変領域、

(3) (1)～(2)のアミノ酸配列において各CDR配列以外のフレームワーク領域の配列に対して少なくとも95%以上の配列同一性を有するアミノ酸配列、及び

(4) (1)～(3)のアミノ酸配列において各CDR配列以外のフレームワーク領域の配列において1又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列

からなる群から選択されるいずれか1つに記載の軽鎖可変領域、並びに、

以下の(5)～(9)：

(5) 配列番号71に記載のアミノ酸配列からなる重鎖可変領域、

(6) 配列番号75に記載のアミノ酸配列からなる重鎖可変領域、

(7) 配列番号79に記載のアミノ酸配列からなる重鎖可変領域、

(8) (5)～(7)のアミノ酸配列において各CDR配列以外のフレーム

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ワーク領域の配列に対して少なくとも95%以上の配列相同性を有するアミノ酸配列、及び

(9) (5)～(8)のアミノ酸配列において各CDR配列以外のフレームワーク領域の配列において1又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列

からなる群から選択されるいずれか1つに記載の重鎖可変領域、
を有する[1]～[5]のいずれか1項に記載の抗体又は当該抗体の機能性断片；

[7]以下の(1)～(4)：

(1) 配列番号63に記載のアミノ酸配列からなる軽鎖可変領域及び配列番号71に記載のアミノ酸配列からなる重鎖可変領域、

(2) 配列番号63に記載のアミノ酸配列からなる軽鎖可変領域及び配列番号75に記載のアミノ酸配列からなる重鎖可変領域、

(3) 配列番号67に記載のアミノ酸配列からなる軽鎖可変領域及び配列番号75に記載のアミノ酸配列からなる重鎖可変領域、又は

(4) 配列番号63に記載のアミノ酸配列からなる軽鎖可変領域及び配列番号79に記載のアミノ酸配列からなる重鎖可変領域、

のいずれかの軽鎖可変領域及び重鎖可変領域、

を含む[1]～[6]のいずれか1項に記載の抗体又は当該抗体の機能性断片；

[8]以下の(1)～(4)：

(1) 配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号69の20～471番目のアミノ酸配列からなる重鎖、

(2) 配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号73の20～471番目のアミノ酸配列からなる重鎖、

(3) 配列番号65の21～233番目のアミノ酸配列からなる軽鎖及び配列番号73の20～471番目のアミノ酸配列からなる重鎖、又は

(4) 配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配

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列番号 77 の 20～471 番目のアミノ酸配列からなる重鎖、
のいずれかを有する [1] ～ [7] のいずれか 1 項に記載の抗体又は当該抗体の機能性断片；

[9] 配列番号 61 の 21～233 番目のアミノ酸配列からなる軽鎖及び配列番号 69 の 20～471 番目のアミノ酸配列からなる重鎖を有する [8] に記載の抗体又は当該抗体の機能性断片；

[10] 配列番号 61 の 21～233 番目のアミノ酸配列からなる軽鎖及び配列番号 73 の 20～471 番目のアミノ酸配列からなる重鎖を有する [8] に記載の抗体又は当該抗体の機能性断片；

[11] 配列番号 65 の 21～233 番目のアミノ酸配列からなる軽鎖及び配列番号 73 の 20～471 番目のアミノ酸配列からなる重鎖を有する [8] に記載の抗体又は当該抗体の機能性断片；

[12] 配列番号 61 の 21～233 番目のアミノ酸配列からなる軽鎖及び配列番号 77 の 20～471 番目のアミノ酸配列からなる重鎖を有する [8] に記載の抗体又は当該抗体の機能性断片；

[13] 機能性断片が Fab、F(ab')₂、Fab' 及び Fv からなる群から選択される [1] ～ [12] のいずれか 1 項に記載の抗体の機能性断片；

[14] [1] ～ [13] のいずれか 1 項に記載の抗体又は当該抗体の機能性断片をコードするポリヌクレオチド；

[15] 以下の (1) ～ (5)：

(1) 配列番号 12 に記載のアミノ酸配列からなる CDR L1、配列番号 13 に記載のアミノ酸配列からなる CDR L2 及び配列番号 14 に記載のアミノ酸配列からなる CDR L3 を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号 17 に記載のアミノ酸配列からなる CDR H1、配列番号 18 に記載のアミノ酸配列からなる CDR H2 及び配列番号 19 に記載のアミノ酸配列からなる CDR H3 を含む重鎖可変領域をコードするポリヌクレオチド、

(2) 配列番号 22 に記載のアミノ酸配列からなる C D R L 1、配列番号 23 に記載のアミノ酸配列からなる C D R L 2 及び配列番号 24 に記載のアミノ酸配列からなる C D R L 3 を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号 27 に記載のアミノ酸配列からなる C D R H 1、配列番号 28 に記載のアミノ酸配列からなる C D R H 2 及び配列番号 29 に記載のアミノ酸配列からなる C D R H 3 を含む重鎖可変領域をコードするポリヌクレオチド、

(3) 配列番号 32 に記載のアミノ酸配列からなる C D R L 1、配列番号 33 に記載のアミノ酸配列からなる C D R L 2 及び配列番号 34 に記載のアミノ酸配列からなる C D R L 3 を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号 37 に記載のアミノ酸配列からなる C D R H 1、配列番号 38 に記載のアミノ酸配列からなる C D R H 2 及び配列番号 39 に記載のアミノ酸配列からなる C D R H 3 を含む重鎖可変領域をコードするポリヌクレオチド、

(4) 配列番号 42 に記載のアミノ酸配列からなる C D R L 1、配列番号 43 に記載のアミノ酸配列からなる C D R L 2 及び配列番号 44 に記載のアミノ酸配列からなる C D R L 3 を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号 47 に記載のアミノ酸配列からなる C D R H 1、配列番号 48 に記載のアミノ酸配列からなる C D R H 2 及び配列番号 49 に記載のアミノ酸配列からなる C D R H 3 を含む重鎖可変領域をコードするポリヌクレオチド、並びに

(5) 配列番号 12 に記載のアミノ酸配列からなる C D R L 1、配列番号 13 に記載のアミノ酸配列からなる C D R L 2 及び配列番号 14 に記載のアミノ酸配列からなる C D R L 3 を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号 17 に記載のアミノ酸配列からなる C D R H 1、配列番号 60 に記載のアミノ酸配列からなる C D R H 2 及び配列番号 19 に記載のアミノ酸配列からなる C D R H 3 を含む重鎖可変領域をコードするポリヌクレオチド、

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からなる群から選択されるいずれか1つに記載のポリヌクレオチドを含む〔14〕に記載のポリヌクレオチド；

〔16〕配列番号61の21～233番目のアミノ酸配列からなる軽鎖をコードするポリヌクレオチド及び配列番号69の20～471番目のアミノ酸配列からなる重鎖をコードするポリヌクレオチドを含む〔14〕又は〔15〕に記載のポリヌクレオチド；

〔17〕配列番号61の21～233番目のアミノ酸配列からなる軽鎖をコードするポリヌクレオチド及び配列番号73の20～471番目のアミノ酸配列からなる重鎖をコードするポリヌクレオチドを含む〔14〕又は〔15〕に記載のポリヌクレオチド；

〔18〕配列番号65の21～233番目のアミノ酸配列からなる軽鎖をコードするポリヌクレオチド及び配列番号73の20～471番目のアミノ酸配列からなる重鎖をコードするポリヌクレオチドを含む〔14〕又は〔15〕に記載のポリヌクレオチド；

〔19〕配列番号61の21～233番目のアミノ酸配列からなる軽鎖をコードするポリヌクレオチド及び配列番号77の20～471番目のアミノ酸配列からなる重鎖をコードするポリヌクレオチドを含む〔14〕又は〔15〕に記載のポリヌクレオチド；

〔20〕〔14〕～〔19〕のいずれか1項に記載のポリヌクレオチドを含む発現ベクター；

〔21〕〔20〕に記載の発現ベクターにより形質転換された宿主細胞；

〔22〕宿主細胞が真核細胞である〔21〕に記載の宿主細胞；

〔23〕〔21〕又は〔22〕に記載の宿主細胞を培養する工程、及び当該工程で得られた培養物から目的の抗体又は当該抗体の機能性断片を採取する工程を含むことを特徴とする当該抗体又は当該抗体の機能性断片の製造方法；

〔24〕重鎖又は軽鎖が、N-結合への糖鎖付加、O-結合への糖鎖付加、N末のプロセッシング、C末のプロセッシング、脱アミド化、アスパラギン

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酸の異性化、メチオニンの酸化、N末にメチオニン残基の付加、プロリン残基のアミド化、N末グルタミンもしくはN末グルタミン酸のピログルタミン酸化、及びカルボキシル末端における1つ又は2つのアミノ酸欠失からなる群より選択される1又は2以上の修飾をうけた、[1]～[13]のいずれか1項に記載の抗体又は当該抗体の機能性断片；

[25] 重鎖のカルボキシル末端において1つ又は2つのアミノ酸が欠失している[24]に記載の抗体；

[26] 2本の重鎖の双方でカルボキシル末端において1つのアミノ酸が欠失している[25]に記載の抗体；

[27] 重鎖のカルボキシル末端のプロリン残基が更にアミド化されている[24]～[26]のいずれか1項に記載の抗体；

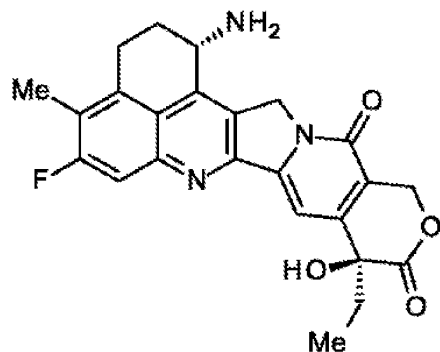
[28] 抗体依存性細胞傷害活性を増強させるために糖鎖修飾が調節されている[1]～[13]及び[24]～[27]からなる群から選択されるいずれか1項に記載の抗体又は当該抗体の機能性断片；

[29] [1]～[13]及び[24]～[28]からなる群から選択されるいずれか1項に記載の抗体又は当該抗体の機能性断片に薬物が結合している抗体－薬物コンジュゲート；

[30] 薬物が抗腫瘍性化合物である、[29]に記載の抗体－薬物コンジュゲート；

[31] 抗腫瘍性化合物が次式：

[0015] [化1]



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[0016] で示される抗腫瘍性化合物である [30] に記載の抗体-薬物コンジュゲート；

[32] 抗体と薬物が、次式 (a) ~ (f) ；

(a) - (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -、

(b) - (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -、

(c) - (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂-O-CH₂-C(=O) -、

(d) - (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂-O-CH₂-C(=O) -、

(e) - (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -、及び

(f) - (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -、
 からなる群から選択されるいずれかの構造のリンカーを介して結合している、
 [29] ~ [31] のいずれか1項に記載の抗体-薬物コンジュゲート。

(ここで、抗体は、- (Succinimid-3-yl-N) の末端において結合する。抗腫瘍性化合物は、1位のアミノ基の窒素原子を結合部位として、(a)、(b)、(e) 又は (f) の -CH₂CH₂CH₂-C(=O) - 部分、(c) の CH₂-O-CH₂-C(=O) - 部分又は (d) の CH₂CH₂-O-CH₂-C(=O) - 部分のカルボニル基に結合する。上記式中 GGFG は、グリシン-グリシン-フェニルアラニン-グリシンからなるペプチド結合でつながっているアミノ酸配列を示す。

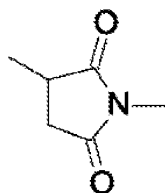
- (Succinimid-3-yl-N) - は次式：

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[0017] [化2]



[0018] で示される構造であり、このものの3位で抗体と結合し、1位の窒素原子上でこれを含むリンカー構造内のメチレン基と結合する。) ;

[33] リンカーが以下の (c)、(d) 及び (e) からなる群から選択されるいずれかの式で示される [29] ~ [32] のいずれか1項に記載の抗体-薬物コンジュゲート :

(c) - (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂-O-CH₂-C(=O)-

(d) - (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂-O-CH₂-C(=O)-

(e) - (Succinimid-3-yl-N) -CH₂CH₂-C(=O)-NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)- ;

[34] リンカーが以下の (c) 又は (e) の式で示される [29] ~ [33] のいずれか1項に記載の抗体-薬物コンジュゲート :

(c) - (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂-O-CH₂-C(=O)-

(e) - (Succinimid-3-yl-N) -CH₂CH₂-C(=O)-NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)- ;

[35]

以下の式 :

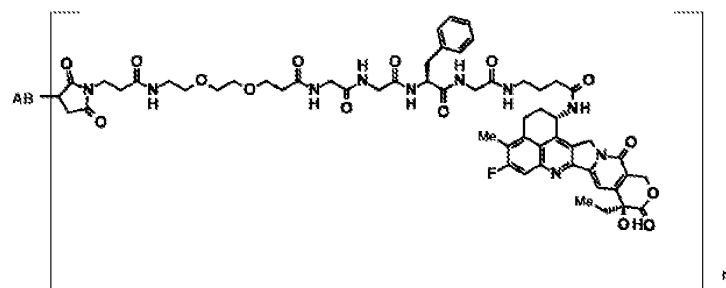
[0019]

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[化3]

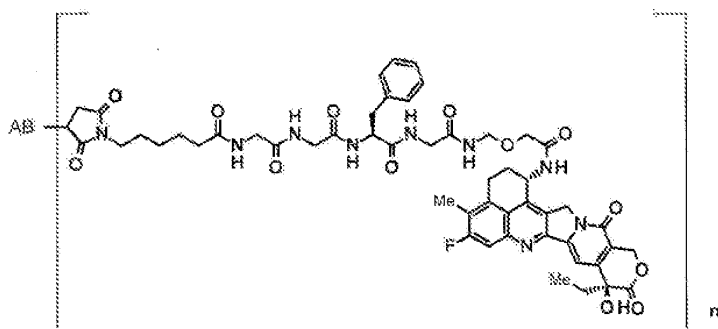


[0020] で示される構造を有する、[29]～[34]のいずれか1項に記載の抗体－薬物コンジュゲート：

ここで、ABは抗体又は該抗体の機能性断片を示す。nは抗体と結合している薬物－リンカー構造の1抗体あたりの平均結合数を示す。抗体とリンカーは抗体由来のスルフヒドリル基を介して結合している；

[36]以下の式：

[0021] [化4]



[0022] で示される構造を有する、[29]～[34]のいずれか1項に記載の抗体－薬物コンジュゲート：

ここで、ABは抗体又は該抗体の機能性断片を示す。nは抗体と結合している薬物－リンカー構造の1抗体あたりの平均結合数を示す。抗体とリンカーは抗体由来のスルフヒドリル基を介して結合している；

[37]抗体が以下の(1)～(4)：

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(1) 配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号69の20～471番目のアミノ酸配列からなる重鎖、

(2) 配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号73の20～471番目のアミノ酸配列からなる重鎖、

(3) 配列番号65の21～233番目のアミノ酸配列からなる軽鎖及び配列番号73の20～471番目のアミノ酸配列からなる重鎖、又は

(4) 配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号77の20～471番目のアミノ酸配列からなる重鎖、

からなる群から選択されるいずれか1つに記載の軽鎖及び重鎖を含む抗体又は当該抗体の機能性断片である、[29]～[36]のいずれか1項に記載の抗体-薬物コンジュゲート；

[38] 抗体が、配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号69の20～471番目のアミノ酸配列からなる重鎖を含む抗体又は当該抗体の機能性断片である、[37]に記載の抗体-薬物コンジュゲート；

[39] 抗体が、配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号77の20～471番目のアミノ酸配列からなる重鎖を含む抗体又は当該抗体の機能性断片である、[37]に記載の抗体-薬物コンジュゲート；

[40] 重鎖又は軽鎖が、N-結合への糖鎖付加、O-結合への糖鎖付加、N末のプロセッシング、C末のプロセッシング、脱アミド化、アスパラギン酸の異性化、メチオニンの酸化、N末にメチオニン残基の付加、プロリン残基のアミド化、N末グルタミンもしくはN末グルタミン酸のピログルタミン酸化及びカルボキシル末端における1つ又は2つのアミノ酸欠失からなる群から選択される1又は2以上の修飾をうけた、[29]～[39]のいずれか1項に記載の抗体-薬物コンジュゲート；

[41] 選択された1種の薬物-リンカー構造の1抗体あたりの平均結合数が1～10個の範囲である[29]～[40]のいずれか1項に記載の抗体

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ー薬物コンジュゲート；

〔42〕選択された1種の薬物ーリンカー構造の1抗体あたりの平均結合数が2～8個の範囲である〔41〕に記載の抗体ー薬物コンジュゲート；

〔43〕選択された1種の薬物ーリンカー構造の1抗体あたりの平均結合数が5～8個の範囲である〔42〕に記載の抗体ー薬物コンジュゲート；

〔44〕選択された1種の薬物ーリンカー構造の1抗体あたりの平均結合数が7～8個である〔43〕に記載の抗体ー薬物コンジュゲート；

〔45〕〔29〕～〔44〕のいずれか1項に記載の抗体ー薬物コンジュゲート、その塩、又はそれらの水和物を含むことを特徴とする医薬組成物；

〔46〕抗腫瘍薬であることを特徴とする、〔45〕に記載の医薬組成物；

〔47〕腫瘍がCDH6を発現する腫瘍であることを特徴とする、〔46〕に記載の医薬組成物；

〔48〕腫瘍が、腎細胞癌、腎淡明細胞癌、乳頭状腎細胞癌、卵巢癌、卵巢漿液性腺癌、甲状腺癌、胆管癌、肺癌、小細胞肺癌、神経膠芽腫、中皮腫、子宮癌、脾臓癌、ウィルムス腫瘍又は神経芽腫であることを特徴とする、〔46〕又は〔47〕に記載の医薬組成物；

〔49〕〔29〕～〔44〕のいずれか1項に記載の抗体ー薬物コンジュゲート、その塩、又はそれらの水和物から選択されるいずれかを個体に投与することを特徴とする腫瘍の治療方法；

〔50〕腫瘍がCDH6が発現している腫瘍であることを特徴とする、〔49〕に記載の治療方法；

〔51〕腫瘍が、腎細胞癌、腎淡明細胞癌、乳頭状腎細胞癌、卵巢癌、卵巢漿液性腺癌、甲状腺癌、胆管癌、肺癌、小細胞肺癌、神経膠芽腫、中皮腫、子宮癌、脾臓癌、ウィルムス腫瘍又は神経芽腫であることを特徴とする、〔49〕又は〔50〕に記載の治療方法；

〔52〕〔29〕～〔44〕のいずれか1項に記載の抗体ー薬物コンジュゲート、その塩、又はそれらの水和物から選択される少なくとも一つを含む医薬組成物及び少なくとも一つの抗腫瘍薬を、同時に、別々に又は連続して個

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体に投与することを特徴とする腫瘍の治療方法；

〔５３〕〔１〕～〔１３〕及び〔２４〕～〔２８〕からなる群から選択されるいずれか１項に記載の抗体又は当該抗体の機能性断片或いは〔２３〕に記載の製造方法で得られる抗体又は当該抗体の機能性断片と、薬物－リンカー中間化合物を反応させる工程を含むことを特徴とする、抗体－薬物コンジュゲートの製造方法；又は

〔５４〕〔２１〕又は〔２２〕に記載の宿主細胞を培養する工程、当該工程で得られた培養物から目的の抗体又は当該抗体の機能性断片を採取する工程、及び当該工程で得られた抗体又は当該抗体の機能性断片と薬物－リンカー中間化合物を反応させる工程を含むことを特徴とする、抗体－薬物コンジュゲートの製造方法。

発明の効果

〔００２３〕 本発明の抗ＣＤＨ６抗体は、ＣＤＨ６のＥＣドメイン３（ＥＣ３）を特異的に認識し、高い内在化活性を有することを特徴とする。本発明の抗ＣＤＨ６抗体に、細胞内で毒性を発揮する薬剤を特定の構造のリンカーを介して結合させた抗ＣＤＨ６抗体－薬物コンジュゲートは、ＣＤＨ６を発現するがん細胞を有する患者に投与することによって、優れた抗腫瘍効果及び安全性を達成することが期待できる。即ち、本発明の抗ＣＤＨ６抗体－薬物コンジュゲートは、抗腫瘍剤として有用である。

図面の簡単な説明

〔００２４〕〔図１〕図１は、ラット抗ＣＤＨ６モノクローナル抗体４種（クローン番号は、ｒＧ０１９、ｒＧ０５５、ｒＧ０５６及びｒＧ０６１）又はラットＩｇＧコントロールの、コントロール細胞又はｈＣＤＨ６導入２９３Ｔ細胞に対する結合を調べたフローサイトメトリーの結果を示す。横軸は抗体結合量を表すＦＩＴＣの蛍光強度、縦軸は細胞数を示す。

〔図２-１〕図２－１は、ラット抗ＣＤＨ６モノクローナル抗体４種（ｒＧ０１９、ｒＧ０５５、ｒＧ０５６及びｒＧ０６１）又は陰性コントロール抗体Ｒａｔ ｌ ｇ Ｇ ２ ｂ の、コントロール細胞又は全長ｈＣＤＨ６導入２９３細胞に対

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する結合性を示す。横軸は抗体結合量を表すF I T Cの蛍光強度、縦軸は細胞数を示す。

[図2-2]図2-2は、ラット抗CDH6モノクローナル抗体4種（rG019、rG055、rG056及びrG061）又はラットIgGコントロールの、コントロール細胞又はEC1欠失hCDH6導入293細胞に対する結合性を示す。横軸は抗体結合量を表すF I T Cの蛍光強度、縦軸は細胞数を示す。

[図2-3]図2-3は、ラット抗CDH6モノクローナル抗体4種（rG019、rG055、rG056及びrG061）又はラットIgGコントロールの、コントロール細胞又はEC2欠失hCDH6導入293細胞に対する結合性を示す。横軸は抗体結合量を表すF I T Cの蛍光強度、縦軸は細胞数を示す。

[図2-4]図2-4は、ラット抗CDH6モノクローナル抗体4種（rG019、rG055、rG056及びrG061）又はラットIgGコントロールの、コントロール細胞又はEC3欠失hCDH6導入293細胞に対する結合性を示す。横軸は抗体結合量を表すF I T Cの蛍光強度、縦軸は細胞数を示す。

[図2-5]図2-5は、ラット抗CDH6モノクローナル抗体4種（rG019、rG055、rG056及びrG061）又はラットIgGコントロールの、コントロール細胞又はEC4欠失hCDH6導入293細胞に対する結合性を示す。横軸は抗体結合量を表すF I T Cの蛍光強度、縦軸は細胞数を示す。

[図2-6]図2-6は、ラット抗CDH6モノクローナル抗体4種（rG019、rG055、rG056及びrG061）又はラットIgGコントロールの、コントロール細胞又はEC5欠失hCDH6導入293細胞に対する結合性を示す。横軸は抗体結合量を表すF I T Cの蛍光強度、縦軸は細胞数を示す。

[図3]図3は、4種類のヒト腫瘍細胞株（ヒト卵巣腫瘍細胞株NIH:OVC

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AR-3, PA-1, ES-2及びヒト腎細胞腫瘍細胞株786-O)の細胞膜表面でのCDH6の発現を評価したフローサイトメトリーの結果を示す。横軸は抗体結合量を表すFITCの蛍光強度、縦軸は細胞数を示す。

[図4]図4は、4種類のラット抗CDH6抗体(rG019、rG055、rG056及びrG061)又はラットIgGコントロールの内在化活性を、タンパク質合成を阻害する毒素(サボリン)を結合させた抗ラットIgG試薬Rat-ZAP又は陰性コントロールとして毒素を結合していないGoat Anti-Rat IgG, Fc(gamma) Fragment Specificを用いて、NIH:OVCAR-3細胞及び786-O細胞において評価したグラフを示す。グラフの縦軸は、ATP活性(RLU)を示す。各グラフの下に、Rat-ZAPの代わりに陰性コントロールを添加したウェルの生存細胞数を100%とした相対生存率として算出された細胞生存率(%)を示す。

[図5]図5は、ヒトキメラ抗CDH6抗体chG019のヒトCDH6及びサルCDH6に対する結合を示す。横軸は抗体濃度を示し、縦軸は結合量をMeanFluorescent Intensity(平均蛍光強度)で示す。

[図6-1]図6-1及び図6-2は、ヒト化hG019抗体4種(H01L02、H02L02、H02L03及びH04L02)又は陰性コントロール抗体ヒトIgG1の、ヒトCDH6、サルCDH6、マウスCDH6、ラットCDH6に対する結合性を示す。横軸は抗体濃度を示し、縦軸は結合量をMeanFluorescent Intensity(平均蛍光強度)で示す。

[図6-2]図6-1及び図6-2は、ヒト化hG019抗体4種(H01L02、H02L02、H02L03及びH04L02)又は陰性コントロール抗体ヒトIgG1の、ヒトCDH6、サルCDH6、マウスCDH6、ラットCDH6に対する結合性を示す。横軸は抗体濃度を示し、縦軸は結合量をMeanFluorescent Intensity(平均蛍光強度)で示す。

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す。

[図7-1]図7-1は、ヒト化hG019抗体4種（H01L02、H02L02、H02L03及びH04L02）、抗CDH6抗体NOV0712又は陰性コントロール抗体hIgG1の、コントロール細胞又は全長hCDH6導入293 α 細胞に対する結合性を示す。横軸は抗体結合量を表すAPCの蛍光強度、縦軸は細胞数を示す。

[図7-2]図7-2は、ヒト化hG019抗体4種（H01L02、H02L02、H02L03及びH04L02）、抗CDH6抗体NOV0712又は陰性コントロール抗体hIgG1の、コントロール細胞又はEC1欠失hCDH6導入293 α 細胞に対する結合性を示す。横軸は抗体結合量を表すAPCの蛍光強度、縦軸は細胞数を示す。

[図7-3]図7-3は、ヒト化hG019抗体4種（H01L02、H02L02、H02L03及びH04L02）、抗CDH6抗体NOV0712又は陰性コントロール抗体hIgG1の、コントロール細胞又はEC2欠失hCDH6導入293 α 細胞に対する結合性を示す。横軸は抗体結合量を表すAPCの蛍光強度、縦軸は細胞数を示す。

[図7-4]図7-4は、ヒト化hG019抗体4種（H01L02、H02L02、H02L03及びH04L02）、抗CDH6抗体NOV0712又は陰性コントロール抗体hIgG1の、コントロール細胞又はEC3欠失hCDH6導入293 α 細胞に対する結合性を示す。横軸は抗体結合量を表すAPCの蛍光強度、縦軸は細胞数を示す。

[図7-5]図7-5は、ヒト化hG019抗体4種（H01L02、H02L02、H02L03及びH04L02）、抗CDH6抗体NOV0712又は陰性コントロールのhIgG1の、コントロール細胞又はEC4欠失hCDH6導入293 α 細胞に対する結合性を示す。横軸は抗体結合量を表すAPCの蛍光強度、縦軸は細胞数を示す。

[図7-6]図7-6は、ヒト化hG019抗体4種（H01L02、H02L02、H02L03及びH04L02）、抗CDH6抗体NOV0712又は

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陰性コントロールのh I g G 1の、コントロール細胞又はE C 5欠失h C D H 6導入293 α 細胞に対する結合性を示す。横軸は抗体結合量を表すA P Cの蛍光強度、縦軸は細胞数を示す。

[図8]図8は、786-O/hCDH6安定発現細胞株及び親細胞株786-OにおけるヒトCDH6の発現を調べたフローサイトメトリーの結果を示す。横軸は抗体結合量を表すA l e x a F l u o r 647の蛍光強度、縦軸は細胞数を示す。

[図9]図9は、(a)ラベル化NOV0712又は(b)ラベル化H01L02を用いた、ラベル化していないヒト化hG019抗体4種(H01L02、H02L02、H02L03及びH04L02)、抗CDH6抗体NOV0712又は陰性コントロールのh I g G 1との結合競合アッセイを示す。横軸はラベル化していない抗体の添加時の終濃度を示し、縦軸は結合量をM e a n F l u o r e s c e n t I n t e n s i t y (平均蛍光強度)で示す。

[図10-1]図10-1は、ヒト化hG019抗体4種(H01L02、H02L02、H02L03及びH04L02)、抗CDH6抗体NOV0712及び陰性コントロール抗体の内在化活性を、タンパク質合成を阻害する毒素(サボリン)を結合させた抗ヒトI g G試薬Hum-ZAP又は陰性コントロールとして毒素を結合していないF (a b ') 2 F r a g m e n t G o a t A n t i - h u m a n I g G , F c (g a m m a) F r a g m e n t S p e c i f i cを用いて、NIH:OVCAR-3細胞において評価したグラフを示す。グラフの縦軸は、ATP活性(RLU)を示す。各グラフの下に、Hum-ZAPの代わりに陰性コントロールを添加したウェルの生存細胞数を100%とした相対生存率として算出された細胞生存率(%)を示す。

[図10-2]図10-2は、ヒト化hG019抗体4種(H01L02、H02L02、H02L03及びH04L02)、抗CDH6抗体NOV0712及び陰性コントロール抗体の内在化活性を、タンパク質合成を阻害する毒素

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(サボリン)を結合させた抗ヒトIgG試薬Hum-ZAP又は陰性コントロールとして毒素を結合していないF(ab')₂ Fragment Goat Anti-human IgG, Fc(gamma) Fragment Specificを用いて、786-O細胞において評価したグラフを示す。グラフの縦軸は、ATP活性(RLU)を示す。各グラフの下に、Hum-ZAPの代わりに陰性コントロールを添加したウェルの生存細胞数を100%とした相対生存率として算出された細胞生存率(%)を示す。

[図10-3]図10-3は、ヒト化hG019抗体4種(H01L02、H02L02、H02L03及びH04L02)、抗CDH6抗体NOV0712及び陰性コントロール抗体の内在化活性を、タンパク質合成を阻害する毒素(サボリン)を結合させた抗ヒトIgG試薬Hum-ZAP又は陰性コントロールとして毒素を結合していないF(ab')₂ Fragment Goat Anti-human IgG, Fc(gamma) Fragment Specificを用いて、PA-1細胞において評価したグラフを示す。グラフの縦軸は、ATP活性(RLU)を示す。各グラフの下に、Hum-ZAPの代わりに陰性コントロールを添加したウェルの生存細胞数を100%とした相対生存率として算出された細胞生存率(%)を示す。

[図11]図11は、ヒト化hG019-薬物コンジュゲート4種(H01L02-DXd、H02L02-DXd、H02L03-DXd及びH04L02-DXd)、又はNOV0712-DM4のPA-1細胞に対する*in vitro*細胞増殖抑制活性評価を示す。横軸は抗体-薬物コンジュゲートの濃度を示し、縦軸は細胞生存率(%)を示す。

[図12]図12は、ヒト化hG019-薬物コンジュゲート4種(H01L02-DXd、H02L02-DXd、H02L03-DXd及びH04L02-DXd)、又はNOV0712-DM4の*in vivo*抗腫瘍効果を示す。CDH6陽性ヒト腎細胞腫瘍細胞株786-Oを免疫不全マウスに移植した動物モデルを用いて評価した。横軸は日数、縦軸は腫瘍体積、誤差範囲は標準誤差(SE)値を示す。

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[図13]図13は、ヒト化hG019-薬物コンジュゲートH01L02-DXd又はNOV0712-DM4又はNOV0712-DXdの*in vivo*抗腫瘍効果を示す。CDH6陽性ヒト卵巣腫瘍細胞株PA-1を免疫不全マウスに移植した動物モデルを用いて評価した。横軸は日数、縦軸は腫瘍体積、誤差範囲はSE値を示す。

[図14]図14は、ヒト化hG019-薬物コンジュゲートH01L02-DXd又はNOV0712-DM4の*in vivo*抗腫瘍効果を示す。CDH6陽性ヒト卵巣腫瘍細胞株NIH:OVCAR-3を免疫不全マウスに移植した動物モデルを用いて評価した。横軸は日数、縦軸は腫瘍体積、誤差範囲はSE値を示す。

[図15]図15は、ヒト化hG019-薬物コンジュゲートH01L02-DXd又はNOV0712-DM4の*in vivo*抗腫瘍効果を示す。CDH6陽性ヒト腎細胞腫瘍細胞株786-Oを免疫不全マウスに移植した動物モデルを用いて評価した。横軸は日数、縦軸は腫瘍体積、誤差範囲はSE値を示す。

[図16]図16は、ヒト化hG019-薬物コンジュゲートH01L02-DXd又はNOV0712-DM4の*in vivo*抗腫瘍効果を示す。CDH6陰性ヒト卵巣腫瘍細胞株ES-2を免疫不全マウスに移植した動物モデルを用いて評価した。横軸は日数、縦軸は腫瘍体積、誤差範囲はSE値を示す。

発明を実施するための形態

[0025] 以下、本発明を実施するための好適な形態について図面を参照しながら説明する。なお、以下に説明する実施形態は、本発明の代表的な実施形態の一例を示したものであり、これにより本発明の範囲が狭く解釈されることはない。

[0026] 本明細書中、「がん」と「腫瘍」は同じ意味に用いられる。

[0027] 本明細書中、「遺伝子」という語には、DNAのみならずそのmRNA、cDNA及びそのcRNAも含まれる。

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- [0028] 本明細書中、「ポリヌクレオチド」又は「ヌクレオチド」は、核酸と同じ意味で用いており、DNA、RNA、プローブ、オリゴヌクレオチド、及びプライマーも含まれる。本明細書中、「ポリヌクレオチド」と「ヌクレオチド」は、特に指定されない限り、交換可能に使用され得る。
- [0029] 本明細書中、「ポリペプチド」と「タンパク質」は交換可能に使用され得る。
- [0030] 本明細書中、「細胞」には、動物個体内の細胞、培養細胞も含む。
- [0031] 本明細書中、「CDH6」は、CDH6タンパク質と同じ意味で使用され得る。本明細書中、ヒトCDH6を「hCDH6」と記載する場合がある。
- [0032] 本明細書中、「細胞傷害活性」とは、何らかの形で、細胞に病理的な変化を引き起こすことをいい、直接的な外傷にとどまらず、DNAの切断や塩基の二量体の形成、染色体の切断、細胞分裂装置の損傷、各種酵素活性の低下などあらゆる細胞の構造や機能上の損傷を引き起こすことをいう。
- [0033] 本明細書中、「細胞内で毒性を発揮する」とは、何らかの形で、細胞内で毒性を示すことをいい、直接的な外傷にとどまらず、DNAの切断や塩基の二量体の形成、染色体の切断、細胞分裂装置の損傷、各種酵素活性の低下、細胞増殖因子の作用の抑制などあらゆる細胞の構造や機能、代謝に影響をもたらすことをいう。
- [0034] 本明細書中、「抗体の機能性断片」とは、「抗体の抗原結合断片」とも呼ばれ、抗原との結合活性を有する抗体の部分断片を意味しており、Fab、F(ab')₂、Fv、scFv、diabody、線状抗体及び抗体断片より形成された多特異性抗体等を含む。また、F(ab')₂を還元条件下で処理した抗体の可変領域の一価の断片であるFab'も抗体の抗原結合断片に含まれる。但し、抗原との結合能を有している限りこれらの分子に限定されない。また、これらの抗原結合断片には、抗体蛋白質の全長分子を適当な酵素で処理したもののみならず、遺伝子工学的に改変された抗体遺伝子を用いて適当な宿主細胞において産生された蛋白質も含まれる。
- [0035] 本明細書中、「エピトープ」とは、特定の抗CDH6抗体の結合するCD

H6の部分ペプチド又は部分立体構造を意味する。前記のCDH6の部分ペプチドであるエピトープは免疫アッセイ法等当業者にはよく知られている方法によって、決定することができる。まず、抗原の様々な部分構造を作製する。部分構造の作製にあたっては、公知のオリゴヌクレオチド合成技術を用いることができる。例えば、CDH6のC末端又はN末端から適当な長さで順次短くした一連のポリペプチドを当業者に周知の遺伝子組み換え技術を用いて作製した後、それらに対する抗体の反応性を検討し、大まかな認識部位を決定した後に、更に短いペプチドを合成してそれらのペプチドとの反応性を検討することによって、エピトープを決定することができる。また、複数の細胞外ドメインからなる膜タンパク質に結合する抗体が、複数のドメインからなる立体構造をエピトープとしている場合は、特定の細胞外ドメインのアミノ酸配列を改変することによって、立体構造を改変することによってどのドメインと結合するかを決定することができる。特定の抗体の結合する抗原の部分立体構造であるエピトープは、X線構造解析によって抗体と隣接する抗原のアミノ酸残基を特定することによっても決定することができる。

[0036] 本明細書中、「同一のエピトープに結合する」とは、共通のエピトープに結合する抗体を意味している。第一の抗体の結合する部分ペプチド又は部分立体構造に第二の抗体が結合すれば、第一の抗体と第二の抗体が同一のエピトープに結合すると判定することができる。あるいは、第一の抗体の抗原に対する結合に第二の抗体が競合する（即ち、第二の抗体が第一の抗体と抗原の結合を妨げる）ことを確認することによって、具体的なエピトープの配列又は構造が決定されていなくても、第一の抗体と第二の抗体の同一のエピトープに結合すると判定することができる。本明細書中、「同一のエピトープに結合する」とは、第一の抗体と第二の抗体が、当該判定方法のいずれか一方又は両方により、共通のエピトープに結合すると判定された場合をいう。第一の抗体と第二の抗体が同一のエピトープに結合し、かつ第一の抗体が抗腫瘍活性や内在化活性等の特殊な効果を有する場合、第二の抗体も同様の活性を有することが期待できる。

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[0037] 本明細書中、「CDR」とは、相補性決定領域（CDR ; Complementarity Determining Region）を意味する。抗体分子の重鎖及び軽鎖にはそれぞれ3箇所のCDRがあることが知られている。CDRは、超可変領域（hypervariable region）とも呼ばれ、抗体の重鎖及び軽鎖の可変領域内にあって、一次構造の変異性が特に高い部位であり、重鎖及び軽鎖のポリペプチド鎖の一次構造上において、それぞれ3ヶ所に分離している。本明細書中においては、抗体のCDRについて、重鎖のCDRを重鎖アミノ酸配列のアミノ末端側からCDRH1、CDRH2、CDRH3と表記し、軽鎖のCDRを軽鎖アミノ酸配列のアミノ末端側からCDRL1、CDRL2、CDRL3と表記する。これらの部位は立体構造の上で相互に近接し、結合する抗原に対する特異性を決定している。

[0038] 本明細書中、「ストリンジェントな条件下でハイブリダイズする」とは、市販のハイブリダイゼーション溶液ExpressHyb Hybridization Solution（クロンテック社）中、68℃でハイブリダイズすること、又はDNAを固定したフィルターを用いて0.7～1.0MのNaCl存在下68℃でハイブリダイゼーションを行った後、0.1～2倍濃度のSSC溶液（1倍濃度SSCとは150mM NaCl、15mMクエン酸ナトリウムからなる）を用い、68℃で洗浄することによって同定することができる条件又はそれと同等の条件でハイブリダイズすることをいう。

[0039] 本明細書中、「1～数個」とは、1～10個、1～9個、1～8個、1～7個、1～6個、1～5個、1～4個、1～3個又は1～2個を意味する。

[0040] 1. CDH6

カドヘリンは、細胞膜表面に存在する糖タンパク質で、カルシウムイオン依存的にN末端側の細胞外ドメイン同士が結合することで、細胞間接着分子として、また細胞間相互作用を担うシグナル分子として機能する。カドヘリンスーパーファミリーの内、クラシックカドヘリンに分類される分子群は、

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細胞外に5個の細胞外ドメイン（ECドメイン）と1個の膜貫通領域、及び細胞内ドメインから構成される1回膜貫通タンパク質である。

[0041] CDH6（Cadherin-6）は、タイプIIカドヘリンファミリーに分類される、790アミノ酸からなる1回膜貫通タンパク質であり、N末端側を細胞外、C末端側を細胞内に持つ。ヒトCDH6遺伝子は1995年に初めてクローニングされ（非特許文献1）、NM_004932、NP_004923（NCBI）等のアクセッション番号により参照可能である。

[0042] 本発明で用いるCDH6タンパク質は、ヒト、非ヒト哺乳動物（ラット、マウス、サル等）のCDH6発現細胞から直接精製して使用するか、あるいは当該細胞の細胞膜画分を調製して使用することができ、また、CDH6を*in vitro*にて合成する、あるいは遺伝子操作によって宿主細胞に産生させることによって得ることができる。遺伝子操作では、具体的には、CDH6 cDNAを発現可能なベクターに組み込んだ後、転写と翻訳に必要な酵素、基質及びエネルギー物質を含む溶液中で合成する、あるいは他の原核生物、又は真核生物の宿主細胞を形質転換させることによってCDH6を発現させることによって、該タンパク質を得ることが出来る。また、前記の遺伝子操作によるCDH6発現細胞、あるいはCDH6を発現している細胞株をCDH6タンパク質として使用することも可能である。また、CDH6のcDNAを組み込んだ発現ベクターを直接被免疫動物に投与し被免疫動物の体内でCDH6を発現させることもできる。

[0043] また、上記CDH6のアミノ酸配列において、1又は数個のアミノ酸が置換、欠失及び／又は付加されたアミノ酸配列からなり、当該タンパク質と同等の生物活性を有するタンパク質もCDH6に含まれる。

[0044] ヒトCDH6タンパク質は、配列番号1に記載のアミノ酸配列を有する。ヒトCDH6タンパク質の細胞外領域は、配列番号1に記載のアミノ酸配列の54～159番目のアミノ酸配列を有する細胞外ドメイン1（本明細書中、EC1とも称する）、配列番号1に記載のアミノ酸配列の160～268番目のアミノ酸配列を有する細胞外ドメイン2（本明細書中、EC2とも称

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する。）、配列番号1に記載のアミノ酸配列の269～383番目のアミノ酸配列を有する細胞外ドメイン3（本明細書中、EC3とも称する。）、配列番号1に記載のアミノ酸配列の384～486番目のアミノ酸配列を有する細胞外ドメイン4（本明細書中、EC4とも称する。）、及び配列番号1に記載のアミノ酸配列の487～608番目のアミノ酸配列を有する細胞外ドメイン5（本明細書中、EC5とも称する。）から構成される。EC1～EC5のアミノ酸配列を、それぞれ、配列番号2～6とする（表1）。

[0045] 2. 抗CDH6抗体の製造

本発明の抗CDH6抗体の一例として、配列番号4に示すアミノ酸配列を含むアミノ酸配列を認識し、かつ内在化活性を有する抗CDH6抗体を挙げることができる。本発明の抗CDH6抗体の一例として、配列番号4に示すアミノ酸配列を含むアミノ酸配列を特異的に認識し、かつ内在化活性を有する抗CDH6抗体を挙げることができる。本発明の抗CDH6抗体の一例として、配列番号4に示すアミノ酸配列からなるアミノ酸配列を認識し、かつ内在化活性を有する抗CDH6抗体を挙げることができる。本発明の抗CDH6抗体の一例として、配列番号4に示すアミノ酸配列からなるアミノ酸配列を特異的に認識し、かつ内在化活性を有する抗CDH6抗体を挙げることができる。抗体が「配列番号4に示すアミノ酸配列を含むアミノ酸配列を特異的に認識」する又は「EC3ドメインを特異的に認識」するとは、抗体が、CDH6のEC3ドメインをCDH6の他の細胞外ドメインと比較して強く認識する又は強く結合することをいう。

[0046] 本発明の抗CDH6抗体は、いずれの種に由来してもよいが、好ましくは、ヒト、サル、ラット、マウス又はウサギを例示できる。ヒト以外の種に由来する場合は、周知の技術を用いて、キメラ化又はヒト化することが好ましい。本発明の抗体は、ポリクローナル抗体であっても、モノクローナル抗体であってもよいが、モノクローナル抗体が好ましい。

[0047] 本発明の抗CDH6抗体は腫瘍細胞を標的にできる抗体であり、すなわち腫瘍細胞を認識できる特性、腫瘍細胞に結合できる特性、及び／又は腫瘍細胞

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胞内に取り込まれて内在化する特性等を備えている。したがって、本発明の抗CDH6抗体と抗腫瘍活性を有する化合物を、リンカーを介して結合させて抗体-薬物コンジュゲートとすることができる。

[0048] 抗体の腫瘍細胞への結合性は、フローサイトメトリーを用いて確認できる。腫瘍細胞内への抗体の取り込みは、(1) 治療抗体に結合する二次抗体（蛍光標識）を用いて細胞内に取り込まれた抗体を蛍光顕微鏡で可視化するアッセイ（Cell Death and Differentiation, 2008, 15, 751-761）、(2) 治療抗体に結合する二次抗体（蛍光標識）を用いて細胞内に取り込まれた蛍光量を測定するアッセイ（Molecular Biology of the Cell Vol. 15, 5268-5282, December 2004）又は(3) 治療抗体に結合するイムノトキシンを用いて、細胞内に取り込まれると毒素が放出されて細胞増殖が抑制されるというMab-ZAPアッセイ（Bio Techniques 28:162-165, January 2000）を用いて確認できる。イムノトキシンとしては、ジフテリヤ毒素の触媒領域とプロテインGとのリコンビナント複合タンパク質タンパク質も使用可能である。

[0049] 本明細書で言う「高い内在化能」とは、当該抗体とサボリン標識抗ラットIgG抗体を投与したCDH6発現細胞の生存率（抗体未添加時の細胞生存率を100%とした相対率で表したもの）が、好ましくは70%以下、より好ましくは60%以下であることを意味する。

[0050] 抗体-薬物コンジュゲートは抗腫瘍効果を発揮する化合物を結合させてあるので、抗体自体が抗腫瘍効果を有することは、好ましいが、必須ではない。抗腫瘍性化合物の細胞傷害性を腫瘍細胞において特異的及び／又は選択的に発揮させる目的からは、抗体が内在化して腫瘍細胞内に移行する性質のあることが重要であり、好ましい。

[0051] 抗CDH6抗体は、この分野で通常実施される方法を用いて、抗原となるポリペプチドを動物に免疫し、生体内に産生される抗体を採取、精製するこ

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とによって得ることができるが、好ましくは、立体構造を保持したCDH6を抗原として用いることが好ましい。そのような方法として、DNA免疫法を挙げることができる。

[0052] 抗原の由来はヒトに限定されず、マウス、ラット等のヒト以外の動物に由来する抗原を動物に免疫することもできる。この場合には、取得された異種抗原に結合する抗体とヒト抗原との交差性を試験することによって、ヒトの疾患に適用可能な抗体を選別できる。

[0053] また、公知の方法（例えば、Kohler and Milstein, Nature (1975) 256, 495-497、Kennet, R. ed., Monoclonal Antibodies, 365-367, Plenum Press, N. Y. (1980)）に従って、抗原に対する抗体を産生する抗体産生細胞とミエローマ細胞とを融合させることによってハイブリドーマを樹立し、モノクローナル抗体を得ることもできる。

[0054] 以下、具体的にCDH6に対する抗体の取得方法を説明する。

[0055] (1) 抗原の調製

抗原は抗原タンパク質をコードする遺伝子を遺伝子操作によって宿主細胞に産生させることによって得ることができる。具体的には、抗原遺伝子を発現可能なベクターを作製し、これを宿主細胞に導入して該遺伝子を発現させ、発現した抗原を精製すればよい。上記の遺伝子操作による抗原発現細胞、あるいは抗原を発現している細胞株を動物に免疫する方法を用いることによって抗体を取得できる。

[0056] また、抗原タンパク質を用いずに、抗原タンパク質のcDNAを発現ベクターに組み込んで被免疫動物に投与し、被免疫動物の体内で抗原タンパク質を発現させ、抗原タンパク質に対する抗体を産生させることによって抗体を取得することができる。

[0057] (2) 抗CDH6モノクローナル抗体の製造

本発明で使用される抗CDH6抗体は、特に制限はないが、例えば、本願の配列表で示されたアミノ酸配列で特定される抗体を好適に使用することが

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できる。本発明において使用される抗CDH6抗体としては、以下の特性を有するものが望ましい。

(1) 以下の特性を有することを特徴とする抗体；

(a) CDH6に特異的に結合する

(b) CDH6と結合することによってCDH6発現細胞に内在化する活性を有する

(2) CDH6がヒトCDH6である上記(1)に記載の抗体又は当該抗体

。

(3) ヒトCDH6のEC3を特異的に認識し、かつ内在化活性を有する

本発明のCDH6に対する抗体の取得方法は、抗CDH6抗体を取得できる限りにおいて、特に制限されないが、高次構造を保持したCDH6を抗原として用いることが好ましい。

[0058] 好ましい抗体の取得方法の一例としてDNA免疫法を挙げることができる。DNA免疫法とは、抗原発現プラスミドをマウスやラットなどの動物個体に遺伝子導入し、抗原を個体内で発現させることによって、抗原に対する免疫を誘導する手法である。遺伝子導入の手法には、直接プラスミドを筋肉に注射する方法や、リポソームやポリエチレンジアミンなどの導入試薬を静脈注射する方法、ウイルスベクターを用いる手法、プラスミドを付着させた金粒子をGene Gunにより打ち込む手法、急速に大量のプラスミド溶液を静脈注射するHydrodynamic法などが存在する。発現プラスミドの筋注による遺伝子導入法に関して、発現量を向上させる手法として、プラスミドを筋注後、同部位にエレクトロポレーションを加える*in vivo electroporation*という技術が知られている(Aihara H, Miyazaki J. Nat Biotechnol. 1998 Sep; 16(9): 867-70又はMir LM, Bureau MF, Gehl J, Rangara R, Rouy D, Caillaud JM, Delaere P, Branellec D, Schwartz B, Scherman D. Proc Natl Acad Sci U

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SA, 1999 Apr 13; 96 (8): 4262-7.)。本手法はプラスミドの筋注前にヒアルロニダーゼで筋肉を処理することにより、さらに発現量が向上する (McMahon JM1, Signori E, Wells KE, Fazio VM, Wells DJ. Gene Ther. 2001 Aug; 8 (16): 1264-70)。また、ハイブリドーマの作製は公知の方法によって行うことができ、例えば、Hybrimmune Hybridoma Production System (Cyt o Pulse Sciences社) を用いて行うこともできる。

[0059] 具体的なモノクローナル抗体取得の例としては、以下を挙げることができる。

(a) CDH6のcDNAを発現ベクター (例えば、pcDNA3.1:Thermo Fisher Scientific) に組み込み、エレクトロポレーションや遺伝子銃等の方法によって、そのベクターを直接被免疫動物 (例えば、ラットやマウス) に投与することによって、動物体内においてCDH6を発現させることによって、免疫反応を誘起させることができる。エレクトロポレーション等によるベクターの投与は抗体価を上げるために必要であれば、1回でも複数回でもよく、好ましくは複数回である；

(b) 免疫反応を誘起された上述の動物より、抗体産生細胞を含む組織 (例えばリンパ節) を採取する；

(c) 骨髓腫細胞 (以下「ミエローマ」という) (例えば、マウスミエローマSP2/O-ag14細胞) の調製；

(d) 抗体産生細胞とミエローマとの細胞融合；

(e) 目的とする抗体を産生するハイブリドーマ群の選別；

(f) 単一細胞クローンへの分割 (クローニング) ；

(g) 場合によっては、モノクローナル抗体を大量に製造するためのハイブリドーマの培養、又はハイブリドーマを移植した動物の飼育；及び／又は

(h) このようにして製造されたモノクローナル抗体の生理活性 (内在化活性)、及びその結合特異性の検討、あるいは標識試薬としての特性の検定。

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- [0060] ここで用いられる抗体価の測定法としては、例えば、フローサイトメトリー又はCell-ELISA法を挙げることができるがこれらの方法に制限されない。
- [0061] このようにして樹立されたハイブリドーマ株の例としては、抗CDH6抗体産生ハイブリドーマrG019、rG055、rG056及びrG061を挙げることができる。なお、本明細書中においては、抗CDH6抗体産生ハイブリドーマrG019が産生する抗体を、「rG019抗体」又は単に「rG019」と記載し、ハイブリドーマrG055が産生する抗体を、「rG055抗体」又は単に「rG055」と記載し、ハイブリドーマrG056が産生する抗体を、「rG056抗体」又は単に「rG056」と記載し、ハイブリドーマrG061が産生する抗体を、「rG061抗体」又は単に「rG061」とする。
- [0062] rG019抗体の軽鎖可変領域は、配列番号10に示されるアミノ酸配列からなる。該rG019抗体の軽鎖可変領域のアミノ酸配列は、配列番号11に示されるヌクレオチド配列でコードされる。rG019抗体の軽鎖可変領域は、配列番号12に示されるアミノ酸配列からなるCDRL1、配列番号13に示されるアミノ酸配列からなるCDRL2及び配列番号14に示されるアミノ酸配列からなるCDRL3を有する。rG019抗体の重鎖可変領域は、配列番号15に示されるアミノ酸配列からなる。該rG019抗体の重鎖可変領域のアミノ酸配列は、配列番号16に示されるヌクレオチド配列でコードされる。rG019抗体の重鎖可変領域は、配列番号17に示されるアミノ酸配列からなるCDRH1、配列番号18に示されるアミノ酸配列からなるCDRH2及び配列番号19に示されるアミノ酸配列からなるCDRH3を有する。rG019抗体の配列を表1に示す。
- [0063] rG055抗体の軽鎖可変領域は、配列番号20に示されるアミノ酸配列からなる。該rG055抗体の軽鎖可変領域のアミノ酸配列は、配列番号21に示されるヌクレオチド配列でコードされる。rG055抗体の軽鎖可変領域は、配列番号22に示されるアミノ酸配列からなるCDRL1、配列番

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号23に示されるアミノ酸配列からなるCDRL2及び配列番号24に示されるアミノ酸配列からなるCDRL3を有する。rG055抗体の重鎖可変領域は、配列番号25に示されるアミノ酸配列からなる。該rG055抗体の重鎖可変領域のアミノ酸配列は、配列番号26に示されるヌクレオチド配列でコードされる。rG055抗体の重鎖可変領域は、配列番号27に示されるアミノ酸配列からなるCDRH1、配列番号28に示されるアミノ酸配列からなるCDRH2及び配列番号29に示されるアミノ酸配列からなるCDRH3を有する。rG055抗体の配列を表1に示す。

[0064] rG056抗体の軽鎖可変領域は、配列番号30に示されるアミノ酸配列からなる。該rG056抗体の軽鎖可変領域のアミノ酸配列は、配列番号31に示されるヌクレオチド配列でコードされる。rG056抗体の軽鎖可変領域は、配列番号32に示されるアミノ酸配列からなるCDRL1、配列番号33に示されるアミノ酸配列からなるCDRL2及び配列番号34に示されるアミノ酸配列からなるCDRL3を有する。rG056抗体の重鎖可変領域は、配列番号35に示されるアミノ酸配列からなる。該rG056抗体の重鎖可変領域のアミノ酸配列は、配列番号36に示されるヌクレオチド配列でコードされる。rG056抗体の重鎖可変領域は、配列番号37に示されるアミノ酸配列からなるCDRH1、配列番号38に示されるアミノ酸配列からなるCDRH2及び配列番号39に示されるアミノ酸配列からなるCDRH3を有する。rG056抗体の配列を表1に示す。

[0065] rG061抗体の軽鎖可変領域は、配列番号40に示されるアミノ酸配列からなる。該rG061抗体の軽鎖可変領域のアミノ酸配列は、配列番号41に示されるヌクレオチド配列でコードされる。rG061抗体の軽鎖可変領域は、配列番号42に示されるアミノ酸配列からなるCDRL1、配列番号43に示されるアミノ酸配列からなるCDRL2及び配列番号44に示されるアミノ酸配列からなるCDRL3を有する。rG061抗体の重鎖可変領域は、配列番号45に示されるアミノ酸配列からなる。該rG061抗体の重鎖可変領域のアミノ酸配列は、配列番号46に示されるヌクレオチド配

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列でコードされる。rG061抗体の重鎖可変領域は、配列番号47に示されるアミノ酸配列からなるCDRH1、配列番号48に示されるアミノ酸配列からなるCDRH2及び配列番号49に示されるアミノ酸配列からなるCDRH3を有する。rG061抗体の配列を表1に示す。

[0066] さらに、「2. 抗CDH6抗体の製造」(a)乃至(h)の工程を再度実施して別途に独立してモノクローナル抗体を取得した場合や他の方法によって別途にモノクローナル抗体を取得した場合においても、rG019抗体、rG055抗体、rG056抗体又はrG061抗体と同等の内在化活性を有する抗体を取得することが可能である。このような抗体の一例として、rG019抗体、rG055抗体、rG056抗体又はrG061抗体と同一のエピトープに結合する抗体を挙げることができる。新たに作製されたモノクローナル抗体が、rG019抗体、rG055抗体、rG056抗体又はrG061抗体の結合する部分ペプチド又は部分立体構造に結合すれば、該モノクローナル抗体がrG019抗体、rG055抗体、rG056抗体又はrG061抗体と同一のエピトープに結合すると判定することができる。また、rG019抗体、rG055抗体、rG056抗体又はrG061抗体のCDH6に対する結合に対して該モノクローナル抗体が競合する（即ち、該モノクローナル抗体が、rG019抗体、rG055抗体、rG056抗体又はrG061抗体とCDH6の結合を妨げる）ことを確認することによって、具体的なエピトープの配列又は構造が決定されていなくても、該モノクローナル抗体が抗CDH6抗体と同一のエピトープに結合すると判定することができる。エピトープが同一であることが確認された場合、該モノクローナル抗体がrG019抗体、rG055抗体、rG056抗体又はrG061抗体と同等の抗原結合能、生物活性及び／又は内在化活性を有していることが強く期待される。

[0067] (3) その他の抗体

本発明の抗体には、上記CDH6に対するモノクローナル抗体に加え、ヒトに対する異種抗原性を低下させること等を目的として人為的に改変した遺

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伝子組換え型抗体、例えば、キメラ（Chimeric）抗体、ヒト化（Humanized）抗体又はヒト抗体等も含まれる。これらの抗体は、既知の方法を用いて製造することができる。

【0068】 キメラ抗体としては、抗体の可変領域と定常領域が互いに異種である抗体、例えばマウス又はラット由来抗体の可変領域をヒト由来の定常領域に接合したキメラ抗体を挙げることができる（Proc. Natl. Acad. Sci. U. S. A., 81, 6851-6855, (1984) 参照）。

【0069】 ラット抗ヒトCDH6抗体由来のキメラ抗体の例としては、例えば、本明細書に記載のラット抗ヒトCDH6抗体（例えば、rG019抗体、rG055抗体、rG056抗体又はrG061抗体）の各軽鎖可変領域とヒト由来の定常領域を含む軽鎖、及び、各重鎖可変領域とヒト由来の定常領域を含む重鎖からなる抗体が挙げられる。

【0070】 ラット抗ヒトCDH6抗体由来のキメラ抗体の別の例としては、例えば、本明細書に記載のラット抗ヒトCDH6抗体（例えば、rG019抗体、rG055抗体、rG056抗体又はrG061抗体）の各軽鎖可変領域中の1～数残基、1～3残基、1～2残基、好ましくは1残基のアミノ酸を別のアミノ酸残基に置換した軽鎖可変領域を含む軽鎖、及び、各重鎖可変領域中の1～数残基、1～3残基、1～2残基、好ましくは1残基のアミノ酸を別のアミノ酸残基に置換した重鎖可変領域を含む重鎖からなる抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有してよい。

【0071】 ラット抗ヒトCDH6抗体由来のキメラ抗体の別の例としては、例えば、本明細書に記載のラット抗ヒトCDH6抗体（例えば、rG019抗体、rG055抗体、rG056抗体又はrG061抗体）の各軽鎖可変領域中のいずれか1～3個のCDR中の1～2残基、好ましくは1残基のアミノ酸を別のアミノ酸残基に置換した軽鎖可変領域を含む軽鎖、及び、各重鎖可変領域中のいずれか1～3個のCDR中の1～2残基、好ましくは1残基のアミノ酸を別のアミノ酸残基に置換した重鎖可変領域を含む重鎖からなる抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有してよい。

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- [0072] r G O 1 9 抗体由来のキメラ抗体としては、例えば、配列番号 1 0 に示されるアミノ酸配列からなる軽鎖可変領域を含む軽鎖、及び、配列番号 1 5 に示されるアミノ酸配列からなる重鎖可変領域を含む重鎖からなる抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有してよい。
- [0073] r G O 1 9 抗体由来のキメラ抗体の別の例としては、例えば、配列番号 1 0 に示されるアミノ酸配列からなる軽鎖可変領域中の 1 ～数残基、1 ～3 残基、1 ～2 残基、好ましくは 1 残基のアミノ酸を別のアミノ酸残基に置換した軽鎖可変領域を含む軽鎖、及び、配列番号 1 5 に示されるアミノ酸配列からなる重鎖可変領域中の 1 ～数残基、1 ～3 残基、1 ～2 残基、好ましくは 1 残基のアミノ酸を別のアミノ酸残基に置換した重鎖可変領域を含む重鎖からなる抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有してよい。
- [0074] r G O 1 9 抗体由来のキメラ抗体の別の例としては、例えば、配列番号 1 0 に示されるアミノ酸配列からなる軽鎖可変領域中のいずれか 1 ～3 個の C D R 中の 1 又は 2 残基（好ましくは 1 残基）のアミノ酸を別のアミノ酸残基に置換した軽鎖可変領域を含む軽鎖、及び、配列番号 1 5 に示されるアミノ酸配列からなる重鎖可変領域中のいずれか 1 ～3 個の C D R 中の 1 又は 2 残基（好ましくは 1 残基）のアミノ酸を別のアミノ酸残基に置換した重鎖可変領域を含む重鎖からなる抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有してよい。
- [0075] r G O 1 9 抗体由来のキメラ抗体の別の例としては、例えば、配列番号 1 0 に示されるアミノ酸配列からなる軽鎖可変領域を含む軽鎖、及び、配列番号 5 8 に示されるアミノ酸配列からなる重鎖可変領域を含む重鎖からなる抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有してよい。配列番号 5 8 に示されるアミノ酸配列は、配列番号 1 5 に示されるアミノ酸配列中の C D R H 2 中のシステイン残基をプロリン残基に置換した配列である。
- [0076] r G O 1 9 抗体由来のキメラ抗体の具体例としては、配列番号 5 3 に示さ

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れる軽鎖全長アミノ酸配列からなる軽鎖、及び、配列番号56に示される重鎖全長アミノ酸配列からなる重鎖からなる抗体が挙げられ、当該キメラ抗ヒトCDH6抗体を本明細書中、「キメラG019抗体」、「chG019抗体」又は「chG019」と称する。chG019抗体の軽鎖全長アミノ酸配列は配列番号54に示されるヌクレオチド配列でコードされ、chG019抗体の重鎖全長アミノ酸配列は配列番号57に示されるヌクレオチド配列でコードされる。

[0077] chG019抗体の軽鎖可変領域アミノ酸配列は、rG019抗体の軽鎖可変領域アミノ酸配列と同一であり、配列番号10に示されるアミノ酸配列からなる。chG019抗体の軽鎖は、配列番号12に示されるアミノ酸配列からなるCDRL1、配列番号13に示されるアミノ酸配列からなるCDRL2及び配列番号14に示されるアミノ酸配列からなるCDRL3を有し、それぞれ、rG019の軽鎖のCDRL1、CDRL2及びCDRL3と同一である。chG019抗体の軽鎖可変領域アミノ酸は、配列番号55に示されるヌクレオチド配列でコードされる。

[0078] chG019抗体の重鎖可変領域アミノ酸配列は、配列番号58に示されるアミノ酸配列からなる。chG019抗体の重鎖は、配列番号17に示されるアミノ酸配列からなるCDRH1、配列番号60に示されるアミノ酸配列からなるCDRH2及び配列番号19に示されるアミノ酸配列からなるCDRH3を有する。配列番号58に示されるアミノ酸配列は、配列番号15に示されるアミノ酸配列中のCDRH2中のシステイン残基をプロリン残基に置換した配列である。配列番号60に示されるアミノ酸配列からなるCDRH2は、配列番号18に示されるrG019 CDRH2中のシステイン残基をプロリン残基に置換した配列である。chG019抗体の重鎖可変領域アミノ酸配列は、配列番号59に示されるヌクレオチド配列でコードされる。

[0079] chG019抗体の配列を表1に示す。

[0080] ラット抗ヒトCDH6抗体rG055抗体由来のキメラ抗体としては、例

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例えば、配列番号20に示されるアミノ酸配列からなる軽鎖可変領域を含む軽鎖、及び、配列番号25に示されるアミノ酸配列からなる重鎖可変領域を含む重鎖からなるキメラ抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有していてもよい。

【0081】 ラット抗ヒトCDH6抗体rG056抗体由来のキメラ抗体としては、例えば、配列番号30に示されるアミノ酸配列からなる軽鎖可変領域を含む軽鎖、及び、配列番号35に示されるアミノ酸配列からなる重鎖可変領域を含む重鎖からなるキメラ抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有していてもよい。

【0082】 ラット抗ヒトCDH6抗体rG061抗体由来のキメラ抗体としては、例えば、配列番号40に示されるアミノ酸配列からなる軽鎖可変領域を含む軽鎖、及び、配列番号45に示されるアミノ酸配列からなる重鎖可変領域を含む重鎖からなるキメラ抗体が挙げられ、当該抗体は任意のヒト由来の定常領域を有していてもよい。

【0083】 ヒト化抗体としては、相補性決定領域（CDR；Complementarity Determining Region）のみをヒト由来の抗体に組み込んだ抗体（Nature（1986）321，p．522－525参照）、CDR移植法によって、CDRの配列に加え一部のフレームワークのアミノ酸残基もヒト抗体に移植した抗体（国際公開第90／07861号）、更に、抗原に対する結合能を維持しつつ、一部のCDRのアミノ酸配列を改変した抗体を挙げることができる。

【0084】 本明細書において、rG019抗体、rG055抗体、rG056抗体、rG061抗体又はchG019抗体由来のヒト化抗体とは、rG019抗体、rG055抗体、rG056抗体、rG061抗体又はchG019抗体のいずれかについて、各々に固有の6つのCDR配列を全て保持し、かつ、内在化活性を有するヒト化抗体であればよく、特定のヒト化抗体に限定されない。当該ヒト化抗体は、内在化活性を有する限り、更に一部のCDRの一部のアミノ酸配列が改変されていてもよい。

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[0085] c h G O 1 9 抗体のヒト化抗体の実例としては、（１）配列番号 6 3 又は 6 7 に記載のアミノ酸配列、（２）上記（１）のアミノ酸配列に対して少なくとも 9 5 % 以上の同一性を有するアミノ酸配列（好ましくは、各 C D R 配列以外のフレームワーク領域の配列に対して少なくとも 9 5 % 以上の配列同一性を有するアミノ酸配列）、及び（３）上記（１）のアミノ酸配列において 1 又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列からなる群から選択されるいずれか 1 つに記載の軽鎖可変領域を含む軽鎖、並びに（４）配列番号 7 1、7 5 又は 7 9 に記載のアミノ酸配列、（５）上記（４）のアミノ酸配列に対して少なくとも 9 5 % 以上の同一性を有するアミノ酸配列（好ましくは、各 C D R 配列以外のフレームワーク領域の配列に対して少なくとも 9 5 % 以上の配列同一性を有するアミノ酸配列）、及び（６）上記（４）のアミノ酸配列において 1 又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列からなる群から選択されるいずれか 1 つに記載の重鎖可変領域を含む重鎖の任意の組合せを挙げることができる。

[0086] また、重鎖又は軽鎖の一方をヒト化し、他方をラット抗体やキメラ抗体の軽鎖又は重鎖とした抗体も用いることができる。そのような抗体の例としては、（１）配列番号 6 3 又は 6 7 に記載のアミノ酸配列、（２）上記（１）のアミノ酸配列に対して少なくとも 9 5 % 以上の同一性を有するアミノ酸配列（好ましくは、各 C D R 配列以外のフレームワーク領域の配列に対して少なくとも 9 5 % 以上の配列同一性を有するアミノ酸配列）、及び（３）上記（１）のアミノ酸配列において 1 又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列からなる群から選択されるいずれか 1 つに記載の軽鎖可変領域を含む軽鎖、並びに（４）配列番号 1 5、2 5、3 5、4 5 又は 5 8 に記載のアミノ酸配列、（５）上記（４）のアミノ酸配列に対して少なくとも 9 5 % 以上の同一性を有するアミノ酸配列（好ましくは、各 C D R 配列以外のフレームワーク領域の配列に対して少なくとも 9 5 % 以上の配列同一性を有するアミノ酸配列）、及び（６）上記（４）のアミノ酸配列において 1 又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列からなる群か

ら選択されるいずれか1つに記載の重鎖可変領域を含む重鎖の任意の組合せを挙げることができる。他の例としては、(1) 配列番号10、20、30又は40に記載のアミノ酸配列、(2) 上記(1)のアミノ酸配列に対して少なくとも95%以上の同一性を有するアミノ酸配列(好ましくは、各CDR配列以外のフレームワーク領域の配列に対して少なくとも95%以上の配列同一性を有するアミノ酸配列)、及び(3) 上記(1)のアミノ酸配列において1又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列からなる群から選択されるいずれか1つに記載の軽鎖可変領域を含む軽鎖、並びに(4) 配列番号71、75又は79に記載のアミノ酸配列、(5) 上記(4)のアミノ酸配列に対して少なくとも95%以上の同一性を有するアミノ酸配列(好ましくは、各CDR配列以外のフレームワーク領域の配列に対して少なくとも95%以上の配列同一性を有するアミノ酸配列)、及び(6) 上記(4)のアミノ酸配列において1又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列からなる群から選択されるいずれか1つに記載の重鎖可変領域を含む重鎖の任意の組合せを挙げることができる。

[0087] また、本明細書中におけるアミノ酸の置換としては保存的アミノ酸置換が好ましい。保存的アミノ酸置換とは、アミノ酸側鎖に関連のあるアミノ酸グループ内で生じる置換である。好適なアミノ酸グループは、以下のとおりである：酸性グループ＝アスパギン酸、グルタミン酸；塩基性グループ＝リシン、アルギニン、ヒスチジン；非極性グループ＝アラニン、バリン、ロイシン、イソロイシン、プロリン、フェニルアラニン、メチオニン、トリプトファン；及び非常電極性ファミリー＝グリシン、アスパラギン、グルタミン、システイン、セリン、スレオニン、チロシン。他の好適なアミノ酸グループは次のとおりである：脂肪族ヒドロキシグループ＝セリン及びスレオニン；アミド含有グループ＝アスパラギン及びグルタミン；脂肪族グループ＝アラニン、バリン、ロイシン及びイソロイシン；並びに芳香族グループ＝フェニルアラニン、トリプトファン及びチロシン。かかるアミノ酸置換は元のアミノ酸配列を有する物質の特性を低下させない範囲で行うのが好ましい。

[0088] 上記軽鎖及び重鎖の好適な組合せの抗体としては、配列番号63に示される軽鎖可変領域アミノ酸配列（本明細書中、hL02軽鎖可変領域アミノ酸配列とも称する）を有する軽鎖又は配列番号67に示される軽鎖可変領域アミノ酸配列（本明細書中、hL03軽鎖可変領域アミノ酸配列とも称する）を有する軽鎖、及び、配列番号71に示される重鎖可変領域アミノ酸配列（本明細書中、hH01重鎖可変領域アミノ酸配列とも称する）を有する重鎖、配列番号75に示される重鎖可変領域アミノ酸配列（本明細書中、hH02重鎖可変領域アミノ酸配列とも称する）を有する重鎖又は配列番号79に示される重鎖可変領域アミノ酸配列（本明細書中、hH04重鎖可変領域アミノ酸配列とも称する）を有する重鎖からなる抗体が挙げられる。好ましい例としては、配列番号63に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号71に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体；配列番号63に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号75に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体；配列番号63に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号79に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体；配列番号67に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号71に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体；配列番号67に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号75に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体；又は、配列番号67に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号79に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体が挙げられる。より好ましい例としては、配列番号63に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号71に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体；配列番号63に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号75に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体；配列番号63に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号79に示される重鎖可変領域ア

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ミノ酸配列を有する重鎖からなる抗体；又は、配列番号 67 に示される軽鎖可変領域アミノ酸配列を有する軽鎖及び配列番号 75 に示される重鎖可変領域アミノ酸配列を有する重鎖からなる抗体が挙げられる。

[0089] 上記軽鎖及び重鎖の好適な組合せの抗体の別の例としては、配列番号 61 に示される軽鎖全長アミノ酸配列（本明細書中、hL02 軽鎖全長アミノ酸配列とも称する）の 21～233 番目のアミノ酸配列からなる軽鎖又は配列番号 65 に示される軽鎖全長アミノ酸配列（本明細書中、hL03 軽鎖全長アミノ酸配列とも称する）の 21～233 番目のアミノ酸配列からなる軽鎖、及び、配列番号 69 に示される重鎖全長アミノ酸配列（本明細書中、hH01 重鎖全長アミノ酸配列とも称する）の 20～471 番目のアミノ酸配列からなる重鎖、配列番号 73 に示される重鎖全長アミノ酸配列（本明細書中、hH02 重鎖全長アミノ酸配列とも称する）の 20～471 番目のアミノ酸配列からなる重鎖又は配列番号 77 に示される重鎖全長アミノ酸配列（本明細書中、hH04 重鎖全長アミノ酸配列とも称する）の 20～471 番目のアミノ酸配列からなる重鎖からなる抗体が挙げられる。好ましい例としては、配列番号 61 に示される軽鎖全長アミノ酸配列の 21～233 番目のアミノ酸配列からなる軽鎖及び配列番号 69 に示される重鎖全長アミノ酸配列の 20～471 番目のアミノ酸配列からなる重鎖からなる抗体；配列番号 61 に示される軽鎖全長アミノ酸配列の 21～233 番目のアミノ酸配列からなる軽鎖及び配列番号 73 に示される重鎖全長アミノ酸配列の 20～471 番目のアミノ酸配列からなる重鎖からなる抗体；配列番号 61 に示される軽鎖全長アミノ酸配列の 21～233 番目のアミノ酸配列からなる軽鎖及び配列番号 77 に示される重鎖全長アミノ酸配列の 20～471 番目のアミノ酸配列からなる重鎖からなる抗体；配列番号 65 に示される軽鎖全長アミノ酸配列の 21～233 番目のアミノ酸配列からなる軽鎖及び配列番号 69 に示される重鎖全長アミノ酸配列の 20～471 番目のアミノ酸配列からなる重鎖からなる抗体；配列番号 65 に示される軽鎖全長アミノ酸配列の 21～233 番目のアミノ酸配列からなる軽鎖及び配列番号 73 に示される重鎖全長

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アミノ酸配列の20～471番目のアミノ酸配列からなる重鎖からなる抗体；又は、配列番号65に示される軽鎖全長アミノ酸配列の21～233番目のアミノ酸配列からなる軽鎖及び配列番号77に示される重鎖全長アミノ酸配列の20～471番目のアミノ酸配列からなる重鎖からなる抗体が挙げられる。より好ましい例としては、配列番号61に示される軽鎖全長アミノ酸配列の21～233番目のアミノ酸配列からなる軽鎖及び配列番号69に示される重鎖全長アミノ酸配列の20～471番目のアミノ酸配列からなる重鎖からなる抗体（本明細書中、「H01L02抗体」又は「H01L02」とも称する）；配列番号61に示される軽鎖全長アミノ酸配列の21～233番目のアミノ酸配列からなる軽鎖及び配列番号73に示される重鎖全長アミノ酸配列の20～471番目のアミノ酸配列からなる重鎖からなる抗体（本明細書中、「H02L02抗体」又は「H02L02」とも称する）；配列番号61に示される軽鎖全長アミノ酸配列の21～233番目のアミノ酸配列からなる軽鎖及び配列番号77に示される重鎖全長アミノ酸配列の20～471番目のアミノ酸配列からなる重鎖からなる抗体（本明細書中、「H04L02抗体」又は「H04L02」とも称する）；又は、配列番号65に示される軽鎖全長アミノ酸配列の21～233番目のアミノ酸配列からなる軽鎖及び配列番号73に示される重鎖全長アミノ酸配列の20～471番目のアミノ酸配列からなる重鎖からなる抗体（本明細書中、「H02L03抗体」又は「H02L03」とも称する）が挙げられる。H01L02抗体、H02L02抗体、H02L03抗体又はH04L02抗体の配列を表1に示す。

[0090] 上記の重鎖アミノ酸配列及び軽鎖アミノ酸配列と高い同一性を示す配列を組み合わせることによって、上記の各抗体と同等の生物活性を有する抗体を選択することが可能である。このような同一性は、一般的には80%以上の同一性であり、好ましくは90%以上の同一性であり、より好ましくは95%以上の同一性であり、最も好ましくは99%以上の同一性である。また、重鎖又は軽鎖のアミノ酸配列に1乃至数個のアミノ酸残基が置換、欠失又は

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付加されたアミノ酸配列を組み合わせることによっても、上記の各抗体と同等の生物活性を有する抗体を選択することが可能である。

[0091] 二種類のアミノ酸配列間の同一性は、Clustal W version 2 (Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ and Higgins DG (2007), 「Clustal W and Clustal X version 2.0」, Bioinformatics, 23 (21) : 2947-2948) のデフォルトパラメーターを使用して配列を整列させることによって決定することができる。

[0092] なお、配列番号61に示されるhL02軽鎖全長アミノ酸配列中、1～20番目のアミノ酸残基からなるアミノ酸配列はシグナル配列であり、21～128番目のアミノ酸残基からなるアミノ酸配列は可変領域であり、129～233番目のアミノ酸残基からなるアミノ酸配列は定常領域である。配列番号62に示されるhL02軽鎖全長ヌクレオチド配列中、1～60番目のヌクレオチドからなるヌクレオチド配列はシグナル配列をコードし、61～384番目のヌクレオチドからなるヌクレオチド配列は可変領域をコードし、385～699番目のヌクレオチドからなるヌクレオチド配列は定常領域をコードしている。

[0093] 配列番号65に示されるhL03軽鎖全長アミノ酸配列中、1～20番目のアミノ酸残基からなるアミノ酸配列はシグナル配列であり、21～128番目のアミノ酸残基からなるアミノ酸配列は可変領域であり、129～233番目のアミノ酸残基からなるアミノ酸配列は定常領域である。配列番号66に示されるhL03軽鎖全長ヌクレオチド配列中、1～60番目のヌクレオチドからなるヌクレオチド配列はシグナル配列をコードし、61～384番目のヌクレオチドからなるヌクレオチド配列は可変領域をコードし、38

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5～699番目のヌクレオチドからなるヌクレオチド配列は定常領域をコードしている。

【0094】 配列番号69に示されるhH01重鎖全長アミノ酸配列中、1～19番目のアミノ酸残基からなるアミノ酸配列はシグナル配列であり、20～141番目のアミノ酸残基からなるアミノ酸配列は可変領域であり、142～471番目のアミノ酸残基からなるアミノ酸配列は定常領域である。配列番号70に示されるhH01重鎖全長ヌクレオチド配列中、1～57番目のヌクレオチドからなるヌクレオチド配列はシグナル配列をコードし、58～423番目のヌクレオチドからなるヌクレオチド配列は可変領域をコードし、424～1413番目のヌクレオチドからなるヌクレオチド配列は定常領域をコードしている。

【0095】 配列番号73に示されるhH02重鎖全長アミノ酸配列中、1～19番目のアミノ酸残基からなるアミノ酸配列はシグナル配列であり、20～141番目のアミノ酸残基からなるアミノ酸配列は可変領域であり、142～471番目のアミノ酸残基からなるアミノ酸配列は定常領域である。配列番号74に示されるhH02重鎖全長ヌクレオチド配列中、1～57番目のヌクレオチドからなるヌクレオチド配列はシグナル配列をコードし、58～423番目のヌクレオチドからなるヌクレオチド配列は可変領域をコードし、424～1413番目のヌクレオチドからなるヌクレオチド配列は定常領域をコードしている。

【0096】 配列番号77に示されるhH04重鎖全長アミノ酸配列中、1～19番目のアミノ酸残基からなるアミノ酸配列はシグナル配列であり、20～141番目のアミノ酸残基からなるアミノ酸配列は可変領域であり、142～471番目のアミノ酸残基からなるアミノ酸配列は定常領域である。配列番号78に示されるhL04重鎖全長ヌクレオチド配列で、1～57番目のヌクレオチドからなるヌクレオチド配列はシグナル配列であり、58～423番目のヌクレオチドからなるヌクレオチド配列は可変領域をコードしており、424～1413番目のヌクレオチドからなるヌクレオチド配列は定常領域を

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コードしている。

[0097] [表1-1]

配列 番号		配列
1	ヒトCDH6 ORFのアミノ酸 配列	MRTYRYFLLLFVVGQPYPTLSTPLSKRTSGFPKAKRAELSGNSKNEINRSKRSWNN NGFFLLEEYTGSDYGYVGLHSDQDRGDGSLKYILSGDGAGDLFIINENTGDIGATK RLDREKPYVYILRAGAINRRTGRPEPESEFIIKIHQINDNEPIFTKEVYTATVPEM SDVGTFFVGVATDADDPYGNsAKVYYSILOGGPYFSVESETGIKTALLNMOREN REQYGVVIGAKDMGGGNGGLSGTTTNNITLTDVNDNPPRFPQSTYQFKTPESSPPGT PIGRJKASDADVGENAEIEYSITDGEGLDMFDVITQETQEGITVKKLLDFEKKKV YTLKVEASNPVVEPRFLYLGPFKDSATVRIVVEDVDEPPVFSKLAYILQIREDAQIN TTIGSVTAGDPDAARNPVKYSVORHTMDRIFNIDSGNGSIFTSKLLDRETLWHNI TVIATEINNPKQSSRVPLYIKVLDVNDNAPEFAEFYETFVCEKAKADQLIQTLHAYD KDDPYSGHGFSLAPEAASGNSFTIQDNKONTAGILTRKNGYNRHEMSTYLLPVV SNDYPVGSSTGTIVRVACADHGNMGSCHAELIHP TGLSTGALVAIILLCIVILL VTVVLFAALRRORKKEPLISKEDIRDNIVSYNDEGGGEEDTQAFDIGTLRNPEAIE DNKLRRDIVPEALFLPRRTPTARDNTDVRDFINGRLKENDTDTAPPYDSLATYAYE GTGSVADSLSSLESVTTDAGGQDYDLSDWGPRFKKLADMYGQVDSOKOS
2	ヒトCDH6 EC1	SWNNNGFFLLEEYTGSDYGYVGLHSDQDRGDGSLKYILSGDGAGDLFIINENTGDIGATKRLDREKPYVYILRAGAINRRTGRPEPESEFIIKIHQINDNEPIF
3	ヒトCDH6 EC2	TKEVYTATVPEMSDVGTFFVGVATDADDPYGNsAKVYYSILOGGPYFSVESETGIKTALLNMORENREQYGVVIGAKDMGGGNGGLSGTTTNNITLTDVNDNPPRF
4	ヒトCDH6 EC3	POSTYQFKTPESSPPGTPIGRJKASDADVGENAEIEYSITDGEGLDMFDVITQETQEGITVKKLLDFEKKKVYTLKVEASNPVVEPRFLYLGPFKDSATVRIVVEDVDEPPVF
5	ヒトCDH6 EC4	SKLAYILQIREDAQINTTIGSVTAGDPDAARNPVKYSVORHTMDRIFNIDSGNGSIFTSKLLDRETLWHNITVIATEINNPKQSSRVPLYIKVLDVNDNAP
6	ヒトCDH6 EC5	EFAEFYETFVCEKAKADQLIQTLHAYDKDDPYSGHGFSLAPEAASGNSFTIQDNKONTAGILTRKNGYNRHEMSTYLLPVVISNDYPVGSSTGTIVRVACADHGNMGSCHAELIHP
7	マウスCDH6 ORFのアミノ酸 配列	MRTYRYFLLLFVVGQPYPTFSNPLSKRTSGFPKAKRAELSANSRNELSRSKRSWNN NGFFLLEEYTGSDYGYVGLHSDQDRGDGSLKYILSGDGAGDLFIINENTGDIGATK RLDREKPYVYILRAGAVNRRTGRPEPESEFIIKIHQINDNEPIFTKDYVYATVPEM ADVGTFFVGVATDADDPYGNsAKVYYSILOGGPYFSVESETGIKTALLNMOREN REQYGVVIGAKDMGGGNGGLSGTTTNNITLTDVNDNPPRFPQSTYQFKTPESSPPGT PIGRJKASDADVGENAEIEYSITDGEGLDMFDVITQETQEGITVKKLLDFEKKKV YTLKVEASNPVVEPRFLYLGPFKDSATVRIVVDDVDEPPVFSKLAYILQIREDAQIN TTIGSVAAAGDPDAARNPVKYSVORHTMDRIFNIDSGNGSIFTSKLLDRETLWHNI TVIATEINNPKQSSRVPLYIKVLDVNDNAPEFAEFYETFVCEKAKADQLIQTLRAYD KDDPYSGHGFSLAPEAASSNFTIQDNKONTAGILTRKNGYNRHEMSTYLLPVV SNDYPVGSSTGTIVRVACADHGNMGSCHAELIHP TGLSTGALVAIILLCIVILL VTVVLFAALRRORKKEPLISKEDIRDNIVSYNDEGGGEEDTQAFDIGTLRNPEAME DGKSRDIVPEALFLPRRTPTARDNTDVRDFINGRLKENDTDTAPPYDSLATYAYE GTGSVADSLSSLESVTTDAGGQDYDLSDWGPRFKKLADMYGQVDSOKOS

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[表1-2]

8	ラットCDH6 ORFのアミノ酸 配列	<p> MRTYRYFLLLFWVGGPYPTFSNPLSKRTSGFPKRRALELSANSRNELSRCKRSWNY NQFFLLEEYTGSDYGYVGKLSHQDQDGDGSLKYJLSGDGAGDLFIINENTGDIQATK RLDRECKPVYILRAGAINRRTGRPEPESEFIKIHQINDNEPIFTKDYVYATVPDM ADVGTFFVQVATDADDPYGNKAKVYYSILQGGPYFSVESETGIKTALLNMDREN REQYQVVIQAKDMGGGSGGLSGTTTNNITLTDVNDNPPRFPQSTYQFKTPESSPPGT PIGRJKASDADVGENAEIYSITDGEGLDMFDVITDQETQEGIIIVKKLLDFEKKRV YTLKVEASNPHEPRFLYLGPFKDSATVRIIVDDVDEPPVFSKPAYILQIREDAGIN TTIGSVAAQDPAARNPVKYSVDRTMDRIFNIDSGNGSIFTSKLLDRETLNHN TVIATEINNPKQSSRPVLYIKVLVDNNAPEFAEFYETFYCEKAKADGLIQTLRAVD KDDPYSGHQFSFLAPEAASGSNFTIQDNKONTAGILTRKNGYNRHNSTYLLPVVI SDNDYPVQSSSTGTVTVRVCACDHGNNMOSCHAEALIHPTGLSTGALVAIILCIVILL VTVVLFAALRRQRKKEPLIISKEDIRDNIVSYNDEGGGEEDTQAFDITGLRNPKNR QGSRRDMVPEALFLPRRTPTARDNTDVRDFISGRLRKMHTDPTAPPYDSLATYAYEG TGSVADSLSSLESVTTDGGQDYGYLSDWGPRFKKLADMYGGVDSKDS </p>
9	カニウイザル CDH6 ORFのア ミノ酸配列	<p> MRTYRYFLLLFWVGGPYPTLSTPLSKRTSGFPKRRALELSGNSKNELNRSKRSWNY NQFFLLEEYTGSDYGYVGKLSHQDQDGDGSLKYJLSGDGAGDLFIINENTGDIQATK RLDRECKPVYILRAGAINRRTGRPEPESEFIKIHQINDNEPIFTKEVYATVPDM SDVGTFFVQVATDADDPYGNKAKVYYSILQGGPYFSVESETGIKTALLNMDREN REQYQVVIQAKDMGGGSGGLSGTTTNNITLTDVNDNPPRFPQSTYQFKTPESSPPGT PIGRJKASDADVGENAEIYSITDGEGLDMFDVITDQETQEGIIIVKKLLDFEKKRV YTLKVEASNPHEPRFLYLGPFKDSATVRIIVDDVDEPPVFSKLAYILQIREDAGIN TTIGSVTAQDPAARNPVKYSVDRTMDRIFNIDSGNGSIFTSKLLDRETLNHN TVIATEINNPKQSSRPVLYIKVLVDNNAPEFAEFYETFYCEKAKADGLIQTLRAVD KDDPYSGHQFSFLAPEAASGSNFTIQDNKONTAGILTRKNGYNRHNSTYLLPVVI SDNDYPVQSSSTGTVTVRVCACDHGNNMOSCHAEALIHPTGLSTGALVAIILCIVILL VTVVLFAALRRQRKKEPLIISKEDIRDNIVSYNDEGGGEEDTQAFDITGLRNPKEIE DNKLRRDIPVPEALFLPRRTPTARDNTDVRDFINORLKENDTDPTAPPYDSLATYAYE GTGSVADSLSSLESVTTDGGQDYGYLSDWGPRFKKLADMYGGVDSKDS </p>
10	rG019軽鎖可変 領域アミノ酸 配列	<p> DIQMTQSPSLLSASVGDRTVLNCKASQNIYKNLAWYQQKLGEGPKLLYYDANTLQTG IPSRFSGSGSGSDFTLTITSSLQPEDVATYFCQQYYSYSGWAFGGVTNLELKRA </p>
11	rG019軽鎖可変 領域ヌクレオ チド配列	<p> GAGATCGAGATGACCCAGTCTCCTTCAGTCCTGCTGCATCTGTGGGAGACAGAGTG ACTCTCAACTGCAAGGAAGTCAGAAATTTATAGAACTTAGCGTGGTATCAGCAA AAGCTTGAGAGAGGTGCGAACTCCTGATTATGATGCAAGACGTTGCAACGGGC ATGCCATCAAGGTTTCAAGTGGCAGTGGATGCTGGTTCAGATTTCACACTCAGCATGAGC AGCCTGCAGCCTGAAGATGTTGCCACATATTTCTGCCAGCAGTACTATAGCGGCTGG CGCTTCGGTGGAGTGAAGCACTGGAATTGAACGGGCT </p>
12	rG019 CDRL1	KASQNIYKNLA
13	rG019 CDRL2	DANTLOT
14	rG019 CDRL3	QQYYSQWA

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[表1-3]

15	rGG19重鎖可変領域アミノ酸配列	QYQLQQSGAELVKPGSSVKISCKASGYTFTRNFMHWIKQDPGNGLWIGWIYCGDGE TEYNQKFNKATLTADRSSSTAYNELSRLTSEDSAVYFCARGVYGGFAGGYFQFQG GVMVTYSS
16	rGG19重鎖可変領域ヌクレオチド配列	CAGGTACAGCTGCAGCAATCTGGGGCTGAACGGTGAAGCCTGGGCTCCTCAGTGA ATTTCTGCAAGGCTTCTGGCTACAGCTTCACGAGAACTTTATGCACTGGATAAA CAGCAGGCTGGAAAAGGGCTTGAATGGATTGGGTGSAATTTATGTGAGATGGTGA AGAGAGTACAATCAAAAGTTCAATGGGAAGGCAACACTCAGTGGGAGAGATGCTCC AGCACAGCCTATATGGAGCTCAGCAGACTGACATCTGAGGACTCTGCAGTCTATTT TGTGCAAGAGGGGTTTACGGAGGGTTTCCCGGGGCTAGTTTGAATTTCTGGGGCAA GGAATCATGGTCACAGTCTCTCA
17	rGG19 CDRH1	GYTFTRNFMH
18	rGG19 CDRH2	WIYCGDGEYE
19	rGG19 CDRH3	GVYGGFAGGYFDF
20	rGG55軽鎖可変領域アミノ酸配列	DYQMTHTPSYLAASPGEVSISCKTSKNISNYLVWYGGKPGAEYKLLIYSSSTLQSG TPSRFSGSGSDTDFLTIRSLPEDFGLYFCGGVYKPFYFGSGTKLEIKRA
21	rGG55軽鎖可変領域ヌクレオチド配列	GATGTCCAGATGACCCACTCTCGGTCTTATCTTGTCTGCTCTCCTGGAGAAAGTGT TCCATCAGTTGCAAGACAAGTAAGAACATTAGTAATTTATGCTCTGGTATCAACAG AAACCTGGGGAAGCATATAAGCTTCTTATCTATTCTGGGTCAACTTTGCAATCTGGA ACTCCATCAAGTTTCAAGTGGCAGTGGATCTGGTACAGATTCACTCTGAGTATCAGA AGCCTGGAGCCTGAAGATTTGGACTCTATTTCTGTCAACAGTATTATGAAAACCA TTCACGTTGGGTCAGGGACGAAGTTGGAATAAAAGGGCT
22	rGG55 CDRL1	KTSKNISNYLV
23	rGG55 CDRL2	SGSTLQS
24	rGG55 CDRL3	QGYYEKPFY
25	rGG55重鎖可変領域アミノ酸配列	EVQLQESGPGLVRPSQSLSLSCSVTDYSITSNYHWIRFPGNKMEHWGIIYSGYT SYNPSLQSRISITRDTSKNQFFLQNSVTAEDTATYYCARSINHGYSYVVDAWPGP ASVTYSS
26	rGG55重鎖可変領域ヌクレオチド配列	GAGGTGCAACTTCAGGAGTCAGGAGCTGGCCTTGTGAGACCTCAGATCAGTCTCC CTCTCTGTTCTGTCACTGATTACTCCATCACTAGTAATTACTGGGGCTGGATCCGG AGGTTCCAGGAAATAAATGGAGTGGATGGGATAGATAACCTATAGTGGTTACACT AGGTACAACCATCTCTCAAAGTGAATCTCCATTACTAGAGACACATCGAAGAAT CAGTTCTTCTGCACTTGAACCTCTGTAACCTGCTGAGGACACAGGCATATTACTGT GCAAGATCGAATTAACACGGAGGATATAGTTATGTTGTGGATGCTGGGTCGGGA GTTCACTCACTGTCTCTCA
27	rGG55 CDRH1	DYSITSNYWG
28	rGG55 CDRH2	YITYSGYTS
29	rGG55 CDRH3	SINHGYSYVVDA

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[表1-4]

30	rG056 軽鎖可変領域アミノ酸配列	DVQMTQSPSSLAASPGESVSI SGRATKSI G1YLA WYQQKPKRTFKLLI YSGSTLQSGTPSRFSGSGSGTDFTLTISRLEPEDFGLYFCQQFYENPFTFGSGTKLEIRRA
31	rG056 軽鎖可変領域ヌクレオチド配列	GATGTCCAGATGACCCAGTCTCCGTCTTCTCTTGCTGCGTCTCCTGGAGAAAGTGTTCATCAGTTGCAGGGCAAGTAAGAGCATTGGTATTTATTAGCCTGGTATCAACAGAAACCTGGGAAACATTTAAGCTTCTTATCTACTCTGGTCAACTTTGCAATCTGGAATCGATCAAGGTCAGTGGCAGTGGGTCTGGTACAGATTTCACTCTCACCATCAGAGCCTGGAGCCTGAAGATTTTGGACTCTATTTCTGTCAACAGTTTTATGAAAACCAATCACGTTCCGGCTCAGGGACGAAGTTGAAAATAAGACGGGCT
32	rG056 CDR1	RATKSI G1YLA
33	rG056 CDR2	SGSTLQSG
34	rG056 CDR3	QQFYENPFT
35	rG056 重鎖可変領域アミノ酸配列	EVQLQESGPGLVKPSQSLSLTCSVTDSYITTFYWGNI RKPFGNKMENMGYMSYRGGTSYNPSLKSRI SIFRDTSKNGFFLQLSVTTEDTATYYCARCPNYGGHSLVFDYWGSGVMYTVSS
36	rG056 重鎖可変領域ヌクレオチド配列	GAGGTGCAGCTTCAGGAGTCAGGACCTGGCCTTGTAACCCCTCAGAGTCACTCTCCCTCACCTGTTCTGTCACTGATTACTCCATCACTACTTATTTCTGGGCTGGATCCGGAAGTTCACAGGAAATAAAATGGAGTGGATGGGATACATGAGCTACCGTGGTGGCACTTCCTACAAACCATCTCTCAAGAGATCGAATCTCCATTACTAGAGAGAGATCGAAGAATCAGTTCTTCTGCGAGTTGAACCTGTAACTACTGAGGACACAGCCACATATTACTGTGCAAGATGGGCTAACTACGGAGGGCATTCCTTGTTTTGATTACTGGGGCAAGGAGTCATGGTCACAGTGTCTGA
37	rG056 CDRH1	DYSITTFYWG
38	rG056 CDRH2	YMSYRGGTS
39	rG056 CDRH3	CPNYGGHSLVFDY
40	rG061 軽鎖可変領域アミノ酸配列	DVQMTQSPSYLAASPGESVSI SCKATKSI SNYLA WYQQKPGEAYKVL IYSGSTLQSGTPSRFSGSGSGTDFTLTISRLEPEDFGLYSCQGYEKLPTFGSGTKLEIKRA
41	rG061 軽鎖可変領域ヌクレオチド配列	GATGTCCAGATGACCCAGTCTCCGTCTTATCTTGCTGCGTCTCCTGGAGAAAGTGTTCATCAGTTGCAAGGCAAGTAAGAGCATTAGTAATTATTAGCCTGGTATCAACAGAAACCTGGGAAAGCATATAAGGTTCTTATCTATTCTGGTCAACTTTGCAATCTGGAATCGATCAAGGTCAGTGGCAGTGGATCTGGTACAGATTTCACTCTCACCATCAGAGCCTGGAGCCTGAAGATTTTGGAGTGTATTCTGTCAACAGTATTATGAAAACCGCTCACGTTCCGGTTCTGGGACCAAGCTGGAGATCAAAACGGGCT
42	rG061 CDR1	KATKSI SNYLA
43	rG061 CDR2	SGSTLQSG
44	rG061 CDR3	QQYIEKPLT

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[表1-5]

45	rG06f 重鎖可変領域アミノ酸配列	EVQLQESGPGLVKPSQSLSLTCSVTGYSITITYWGNIIRKFPGNKMEHNGYISYSGRISYNPSLKSIRMSITRDASKNGFFLQLNSVITDDTATYYCARSPINHGGYNYFDWFPGTMTVTVSS
46	rG06f 重鎖可変領域ヌクレオチド配列	GAGGTGCAGCTTCAGGAGTCAGGACCTGGCCTTGTGAAACCCACAGTCACCTCTCCCTCACCTGTTCTGTCACTGTTACTCCATCACTACTTATTACTGGGGTGGATCCGGAAATTCCAGGAAATAAAATGGAGTGGATGGGTACATAAGCTAGAGTGGTCCCACTAGTTATAACCGATGCTCAAAAGTCGAATGTGCATTACTAGAGACGCATCGAAGAATCAGTTCTTCTACAGTTGAACCTGTGTAACCTAGTACGACACAGCCACATATTAGTGTGCAAGATCCCAATTAAACGAGGAGGACTGGTACTTTGAGTTCTGGGGCCAGGAACCATGGTCACCGTGCTCA
47	rG06f CORH1	GYSITITYWG
48	rG06f CORH2	YISYSGRIS
49	rG06f CORH3	SPINHGGYNYFD
50	ヒト軽鎖シグナル配列及びヒト κ 鎖定常領域をコードするDNA配列を含むDNA断片	gcctccggactctagagccaccatggctgctgcagagccaggtgttcattctccctgctgctgtggatctccggccgtacggcgatctgtgatgattaaacgtacgggtggcggccccctccgtgttcattcttcccccctccgacgacgactgaagtccggcaccgcctccgtgtgtgctgtgtaataacttctacccagagagggccaaggtgcagtggaaggtggacaaagccctgcagtcgggaactccagggaggggtgaccgagcaggacagcaaggacgacctacagcctgagcagcaccctgaccctgagcaaaagccgactacgagaaacaaaggtgtacggctggcaggtgacccaccagggcctgagctcccccgtaccaaaggttcaacagggggagtgtagggcccggttaaacgggggaggtg

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[表1-6]

51	ヒト重鎖シグナル配列及びヒトIgG1定常領域をコードするDNA配列を含むDNA断片	gccctccggaactctagagccaccATGAACACCTGTGGTTCTTCTCCTGCTGGTGGC AGCTCCAGATGGGTGGTGAAGCAGGTGCAATTGTGCAGGCGGTAGCTCAGCCTCC AGCAAGGGGCGAAGCGTCTTCCCCCTGGCAGGCTCCTCCAAGAGCACCTCTGGG99C ACAGCGCCCTGGGGTGGCTGGTCAAGGACTACTTCCCCGAAGCGGTGACCGTGAGC TGGAACTCAGGCGCCCTGACCAGCGCGTGCACACCTTCCCCGCTGTCTGCAGTCC TCAGGAGTCTACTTCCCTCAGCAGCGTGGTGAAGGTGGCTTCCAGCAGCTTGGGAGC CAGACCTACATCTGCAAGGTGAATCAAGCCAGCAACACAGGTGACAGAGA GTTGAGCCCAATCTTGTACAAAACCTACACATGCCACCGTGGCCAGCACCTGAA CTGCTGGGGGACCTCAGTCTTCTCTTCCGCGGCAAAACCAAGGACACCTCATG ATCTCCGGGACCCCTGAGGTGACATGCGTGGTGGAGCTGAGCCAGGAGACCCCT GAGGTCAAGTTCAAGTGTGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAG CCGGGGAGGAGCAGTACAACAGCACGTACGGGGTGGTCAAGCTCTCACCCTGCTG CACCAGGACTGGCTGAATGGCAAGGAGTCAAGTGAAGGTCTCCAACAAAGCCCTC CCAGCCCCCATCGAGAAAGCATCTCCAAGCCAAAGGCCAGGGGGGAGACGACAG GTGTACACCGTGGCCCCATCCCGGGAGGAGATGACCAAGAACAGGTGACGCTGAGC TGGCTGGTCAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGC CAGCCGAGAACACTACAGAGCACCCCTCCGCTGCTGGAGTGGGAGGCTCCTTC TTCTCTACAGCAGCTACCGTGGACAGAGCAGGTGGCAGCAGGGCAACGCTTTC TCATGGTCCGTGATGCATGAGGCTCTGCGACAGCACTACACCAGAGAGGCTCTCC CTGTCTCCCGCAATgagatactggcccggtttaaagggggaggcta
52	chG019軽鎖をコードするDNA配列を含むDNA断片	ccagcctccggaactctagagccaccATGGTGGTGCAGACCCAGGTGTTGATCAGCCT GCTGCTGTGGATCAGGCGCGCTACGCGGACATCCAGATGAGCGAGAGCCCTAGCCT GCTGAGGGGAGGGTGGGCGATAGAGTGAGGCTGAAGTGCAGGCCAGCCAGAACAT CTACAAGAACCTGGCTGGTATCAGCAGAAAGCTGGGCGAGGGCCCCAAGCTGCTGAT CTACGACGCCAACACCGCTGCGAGCCGCGCATCCCCAGCAGATTCTGCGAGCGGAG CGGCTCGGACTTCACCTGACAAATCAGCAGCTGCAGCCCGAGGAGCTGGCCACCTA CTTTTGGCAGCAGTACTACAGCGGCTGGGGCTTGGGCGCGGTGACCAAGCTGGAAGT GAAGAGAGCGGTGGGGGTGGCTGGTGTTCATCTTCCAGCTAGGAGCAGCAGCT GAAGTCGGGCACAGCTCTGTGCTGTGCTGCTGAACAATTCTACCCCGCGGAGGC CAAGGTGCACTGGAAGGTGACAAATGCCCTGCACTGTGCAACAGCCAGGAAAGGCT GACCGAGCAGGACAGCAAGGAGTCCACCTACAGCCTGAGCAGGAGGCTGACCTGAG CAAGGCCGAUTACGAGAAGCACAAGGTGTACGCTTGGAGTGAACCAAGGAGGCT GTCTAGCCCGGTGACCAAGAGCTTCAACCGGGGAGTGTTgagtttaagggggagg ggttaact
53	chG019軽鎖全長アミノ酸配列	MYLQTVFISLLWISGAYGDIQNTQSPSLLSASVGDRTVLNCKASQNIYKNLANYQ QKLGEQKLLIYDANTLGTGIPSRFSGSGSGSDFTLTISLQPEQVATYFCQYYSG WAFGGVTNLELKRAVAAPSVFIFPPSDEOLKSGTASVVCLLNNFYFREAKVQWKVDN ALQSGNSQESVTEQDSKDSYLSSTLTLSKADYKHKVYAGEVTHQSLSSPVTKSF NRGEC

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[表1-7]

54	ch9019経鎖全長ヌクレオチド配列	ATGGTGCTGCAGACCCAGGTGTTTCATGAGCCTGCTGCTGATCAGCGGGCGCTAG GGCGACATCCAGATGAGCCAGAGCCCTAGCCTGCTGAGCGCCAGCGTGGGCGATAGA GTGACCCCTGAACTGCAAGGCCAGCCAGAACATCTACAAGAAGGTGGCCTGGTATCAG CAGAAGGTGGGCGAGGGGCCCAAGCTGCTGATCTACGACGCCAACACCTGCAGAGC GGCATCCCCAGCAGATTTTCTGGCAGGCGCAGGGCTCCGACTTCAGCTGACAAATC AGCAGCCTGCAGCGGAGGAGCTGGCCACCTACTTTTGGCAGGAGTACTACAGCGGC TGGGCGCTTCGGCGGGGTGACCAACCTGGAAGTGAAGAGAGCCGTGGCCGCTCCCTCG GTGTTTCATCTTCCACCTAGCGACGAGCAGCTGAAGTCCGGCACAGCTCTGTGCTG TGCCTGCTGAACAAGTTCTAGCCCGCGAGGCCAAGGTGCAATGGAAGGTGACAAAT GGCTTGAGTCTGGCAACAGCCAGGAAGGGTGAAGCAGCAGGACAGCAAGGACTCG AGGTACAGCCTGAGCAGCAGCTGACGCTGAGCAAGGCCACTACGAGAAGCACAAG GTGTACGCTGCGAAGTGACCCACCGGGCTGTCTAGCGCGGTGACCAAGAGCTTC AAGCGGGCGAGTGT
10	ch9019経鎖可変領域アミノ酸配列	DIQNTQSPSLLSASVGDRTVLNCKASQNIYKNLAWYQKLGEGPKLLIYDANTLOTG JPSRFSGSGSGDFTLTISSLQPEDVATYFCGGYYSGNAFAGVYNLELKRA
55	ch9019経鎖可変領域ヌクレオチド配列	GACATCCAGATGACCGAGAGCCCTAGCCTGCTGAGCGCCAGCGTGGGCGATAGAGTG ACCTGGAAGTGCAAGGCCAGCCAGAACATCTACAAGAAGCTGGCCTGGTATCAGCAG AAGGTGGGCGAGGGGCCCAAGCTGCTGATCTAGGAGGCCAACACCTGCAGAGCGGC ATCCCCAGCAGATTTTCTGGCAGCGCAGCGGCTCCGACTTCAGCTGACAAATCAGC AGCCTGAGCGCGAGGAGCTGGCCACCTACTTTTGGCAGCAGTACTACAGCGGCTGG GGCTTGGGCGGGGTGACCAACCTGGAAGTGAAGAGAGGC
12	ch9019 CDRL1	KASQNIYKNLA
13	ch9019 CDRL2	DANTLOT
14	ch9019 CDRL3	GGYYSGNA
56	ch9019重鎖全長アミノ酸配列	MKHLWFFLLIWAAPRWVLSQVQLQQSGAELVPGSSVNTISCKASGYTFTRNFMHNIK GGPQNGLEWIGWIIYPGDGETEYNGKFNKGATLTADRSSSTAYNELSRLTSEDSAVYF CARSVYGGFAGGYFDWGGGVMTVSSASTKGPSVFPPLAPSSKSTSGGTAALGGLVK DYFPEPVTVSNNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNNVH KPSNTKVDKRVPEKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFMWYVDGVEVHNAIKPREEDYNSTYRVVSVLYVLDHQLNSKE YKCKVSNKALPAPIEKTIISKAKGQPREPGVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEKESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEAL HNHYTQKSLSLSPGK

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[表1-8]

57	dhG019重鎖全長ヌクレオチド配列	ATGAAACACCTGTGGTTCTTCTCTCTGCTGGTGGCAGCTCCAGATGGGTGCTGAGC CAGGTCAGCTGCAGCAGCTCTGGCGCCGAGCTCGTGAAGCCTGGCAGCAGCGTGAAG ATCAGCTGCAAGGGGAGGGGTACACCTTCACCCGGAAGTTGATGCACTGGATCAAG CAGCAGCCCGGCAACGGCCTGGAATGGATCGGCTGGATCTATCCGGCGACGGCGAG ACAGAGTACAACCAAGATTCAACGGCAAGGCCACCTGAGCGCGGACAGAGGAGC TCCACCGCTAGATGGAAGTGAAGCGGCTGACCAGCGAGGAGAGCGCGTGAAGTTT TGGCGCAGAGGGCGTGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTGGGGCCAG GGGCTGATGGTACCGTCAGCTCAGCTCCACCAAGGGCCCAAGCGCTCTGGCGGTG GCACCTCTCTCAAGAGAGCTCTGGCGGCACAGCGCGCTGGGCTGGCTGGTCAAG GACTAGTTCCCGGAACCGGTGACCGTGAGCTGGAAGCTAGGCGCGCTGACGAGCGGG GTGACAGCTTCCCGCTGTCTGCGAGTCTCAGGACTCTACTCCCTCAGGAGGCTG GTGACCGTGGCTCCAGCAGCTTGGGCAACCCAGACCTAGATCTGCAACGTGAATCAC AAGCCAGCAACACCAAGGTGGACAAGAGATTGAGGCCAAATCTTGTGACAAAAT CAGACATGCCACCTGCCAGCAGCTGAAGCTCTGGGGGACCTCAGTCTTCTG TTCCCGCCAAAGCCAGGAGAGCTCAGATCTCCCGGAGCGCTGAGGTCACATGC GTGGTGGTGGAGGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGAC GGGCTGGAGGTGCATAATGCCAAGACAAGCGCGGGAGGAGCAGTACAACAGCAAG TAGCGGGTGGTCAAGCTCTGACCGTCTGACCGAGGAGTGGCTGAATGGCAAGGAG TACAAGTGCAGGGTGTGCAACAAGCCCTCCAGCGCCGATGAGAGAAACCATCTCC AAAGCCAAAGGCCAGCCCGGGAACCAAGGTGTACACCTTGCCCCCATCCGGGAG GAGATGACCAAGAACCAAGGTGAGCTGACCTGCGCTGGTCAAGGGTTCTATCCAGC GACATCGCGGTGGAGTGGGAGAGCAATGGCCAGCCGAGAGCAACTACAAGACCAAC CCTCCCGTGGTGGACTCCGACGGCTCGTTCTTCTCTACAGCAAGCTCAGCGTGGAG AAGAGCAGGTGGCAGCAGGGCAACGTGTTCTCATGCTCCGTGATGCTAGGCTGTG CACAACCACTAGACCCAGAGAGCCTCTCCCTGTCTCCGGGAAA
58	dhG019重鎖可変領域アミノ酸配列	GVQLQQSGAELVKPGSSVKISCKASGYTFTRNFMHWIKQDPSNGLENIGWLYPGDGE TEYNQKFNKGKATLTADRSSSTAYMELSRLTSEDSAVYFCARGVYGGFAGSYFDWGG GVNVTYSS
59	dhG019重鎖可変領域ヌクレオチド配列	CAGGTGCAGCTGCAGCAGCTCTGGCGCGAGCTCGTGAAGCCTGGCAGCAGCGTGAAG ATCAGCTGCAAGGCCAGCGGCTACACCTTCACCCGGAAGTTGATGCACTGGATCAAG CAGCAGCCCGGCAACGGCCTGGAATGGATCGGCTGGATCTATCCGGCGACGGCGAG ACAGAGTACAACCAAGTTCAACGGCAAGGCCACCTGAGCGCGGACAGAGGAGC TGCAGCGCTTACATGGAAGTGAAGCGGCTGAGCAGCGAGGACAGCGCGTGTAGTTT TGGCGCAGAGGGGTGTAGGGCGGCTTCGCTGCCGCTAGTTGATTTTGGGGCCAG GGCGTGATGGTACCGCTCAGCTCA
17	dhG019 CDRH1	GYTFTRNFMH
60	dhG019 CDRH2	WLYPGDGETE
19	dhG019 CDRH3	GVYGGFAGGYDF

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61	hL02経線全長 アミノ酸配列	MVLQTQVFISSLNWSGAYGDIQMTQSPSSLSASVGRVITITCKASQNIYKNLAWYQKPKGAPKLLIYDANTLQTGVPSRFSGSGSGDSFTLTLSLQPEDFATFYCQQQYYSWAFGGTGKVEIKRTVAAPSVEIFPPDSDEQLKSGTASVCLNNFYPREAKYQWVKDALQSGNSGSESVTEQDSKDSITYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
62	hL02経線全長 ヌクレオチド 配列	ATGGTGTCTGCAGACCCAGGTGTTTCATCTCCCTGCTGCTGGATCTCCGGCCGGTACGGCAGCATCCAGATGACCCAGAGGCCCTAGCAGCCTGAGCGCCAGCGTGGGGCAGACAGTGTGACCATCAGATGCAGGGCCAGCCAGAACATCTACAAGAACCTGGCCTGGTATCAGCAGAAAGCCGGCAGAGGCCGCCAACCTGCCTGATCTAGSAGGCCAACACCCCTGCAGACCGGGCTGCCCAGCAGATTTTCTGGCAGCGGACAGGGCTCGGACTTCAGCCTGACAAATAGCAGCCTCGACCGCCAGGAGCTTCGCCACCTACTTTTGGCAGCAGTACTACAGCGCGCTGGGGCTTCGGCCAGGGCACCAAGGTGGAATCAAGGCTAGGGTGGCCGCCCCCTCGGTGTTCACTTCCTCCCTCCGAGGAGCAGCTGAAGTCCGGCACCGCTTCGGTGGTGTGCTGCTGAATAACTTCTACGCCAGAGAGGCCAAGGTGCAGTGGAAAGTGGACAAACGCCCTGCAGTCGCGGAACCTCCACAGGAGAGCGGTGAGGAGCAGGACAGGACAGGACAGGACCTTGCAGCTGACAGCAGCACTCCAGCAACCTGAGCAAAAGCGACTACGAGAAAGCACAAGGTGTATACGCTGCGAGGTGACCGACGAGAGGCTGAGCTCCCGCTGACGAAAGAGTTC AACAGGGGGGAGTGT
63	hL02経線可変 領域アミノ酸 配列	DIQMTQSPSSLSASVGRVITITCKASQNIYKNLAWYQKPKGAPKLLIYDANTLQTGVPSRFSGSGSGDSFTLTLSLQPEDFATFYCQQYYSWAFGGTGKVEIKRT
64	hL02経線可変 領域ヌクレオ チド配列	GACATCCACATGACCCAGAGGCCCTAGCAGCCTGAGCGGCCAGCGTGGGCGACAGAGTACCATACATGCAAGGCCAGGCGAGAACATCTACAAGAACCTGGCCTGGTATGAGCAGACGCGCCGGCAGGGGCCCAAGCTGCTGATCTAGCAGGCCAACACCCCTGCAGACCGCGGTGCCACGAGCAGATTCTTGGCAGCGGACAGCGCTCGGACTCACCTGCACCAATCAGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTTTTCCAGCAGTACTACAGGGGCTGGCCTTCGGCCAGGGCACCAAGGTGGAATCAAGCGTACG
65	hL03経線全長 アミノ酸配列	MVLQTQVFISSLNWSGAYGDIQMTQSPSSLSASVGRVITITCKASQNIYKNLAWYQKLGEGPKLLIYDANTLQTGVPSRFSGSGSGDSFTLTLSLQPEDFATFYCQQYYSWAFGGTGKVEIKRTVAAPSVEIFPPDSDEQLKSGTASVCLNNFYPREAKYQWVKDALQSGNSGSESVTEQDSKDSITYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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【表1-10】

66	hL03経鎖全長 ヌクレオチド 配列	ATGGTGCTGCAGACCGAGGTGTTTCATCTCCCTGCTGCTGTGATGTCGGGCGGTAC GGCGACATCCASATGACCCAGAGCCCTAGCAGGCTGAGCGCCAGCGTGGGCGACAGA GTGAGGATCAGATGCAAGGCCAGCCAGAACATCTACAAGAACCTGGCTTGTATCAG CAGAAAGCTGGGCGAGGGGGCAAGCTGCTGATCTACGACGCCAAGACCTGCAGAGCC GGGCTGCCAGCAGATTTTCTGGCAGGGGCTGGGCGACGACTTACCCCTGACAATC AGCAGGCTGCAGCCGAGGAGTTTGGCCACCTACTACTGCCAGCAGTACTACAGGGGC TGGGCTTTTGGCCAGGGAGCAAGGTGGAAATCAAGCTACGGTGGCGGCCCTCC GTGTTTCATCTTCCGCCCCCTCCGACGAGCAGGTGAAGTCCGGCACCCTCCGTGGTG TGGCTGCTGAATAACTTCTACCCAGAGAGGGGCAAGGTGCAGTGGAAAGTGGAGAAC GCCCTGCAGTCCGGGAAGTCCCAAGAGAGCGGTGACCGAGCAGGACAGCAAGGACAGC ACCTACAGGCTGAGCAGCAGCCCTGACCGTGAGCAAGGCCGACTACGAGAAGCACAAG GTGTAGGCTTGGAGGTGACCCACAGGGGCTGAGCTCCCGGTCACCAAGAGGTTG AACAGGGGGAGTGT
67	hL03経鎖可変 領域アミノ酸 配列	DIGMTSPSSLSASVGRVTITCKASGNLYKNLAWYGGKLGEGPKLLIYDANTLQTS VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQDYYSQWAFGGGKVEIKRT
68	hL03経鎖可変 領域ヌクレオ チド配列	GACATCCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCCAGGCTGGGCGACAGAGTG AGCATCAGATGCAAGGCCAGCCAGAGATCTACAAGAACCTGGCTTGTATCAGAG AAGCTGGGCGAGGGGCCCAAGCTGCTGATCTACGACGCCAAGCCTGCAGAGCGGC GTGCCAGCAGATTTTCTGGCAGCGGCTCCGGCACCAGCTTACCCCTGACAATCAGC AGGCTGAGGCGGAGGACTTCGCCACCTACTAGTGGCAGCAGTACTACAGCGGCTGG GCTTTGGCAGGGGAGCAAGGTGGAAATCAAGCGTACG
69	hH01重鎖全長 アミノ酸配列	MKHLNFFLLLVAAPRWYLSEVQLVQSGAEVKKPGASVKVSCKASGYTFIRNFMHVR QAPGGGLFWMGWLYPGDGE TEYAQKFGGRVTITADTSTSTAYMELSSLRSEDYVYY CARGVYGGFAGSYFDWGGGTLVTYSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVYSWNNGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICRWNH KPSNTKVDKRVKPKCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEYKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHGDWLNKGE YKGVSNKALPAPIEKTISKAKGQPREPDVYTLPPSREEMTKNGVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRNKKGNVFGCSVMHEAL NNHYTKSLSLSPGK

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[表1-11]

70	hH01重鎖全長 ヌクレオチド 配列	ATGAAAGAGCTGTGGTTCTTCTCTCTGCTGGTGGGAGCTCCGAGATGGGTGCTGAGG GAAGTGCAGCTGGTGCAGTCTGGGCGCGAAGTGAAGAAACCAAGGCGCCAGCGTGAAG GTGTCTGCAAGGCCAGCGGCTACACCTTTACCGGAACTTCATGCACTGGGTGGCG CAGGCTGCAGGCCAGGGAAGTGAATGGATGGGTGGATCTATCCCGGCACGGCGAG ACAGAGTACGCCAGAAATTCAGGGCAGAGTGACCATCACCGCCGACACCAAGCACC TCCACCGCTACATGGAAGTGAGCAGCCTGCGGAGGAGGACACCGCGGTGTACTAT TGTGGCAGAGGCGGTACGGCGGCTTCGCTGGGGGCTACTTGGATTTTGGGGCCAG GGCACCTCTGTACCGCTCAGCTGAGCTCCACCAAGGGCCCAAGGCTTTGGCCCTG GCACCTCTCTCAAGAGCAGCTCTGGCGGCACAGCGCGCTGGGCTGCCTGGTCAAG GACTACTTGGCGAAGCGGTGACCGTGAGCTGGAAGTCAAGGCGCCTGACCAAGCGG GTGCACACCTTCCCGCTGTCTGCACTCTCAGGACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCAGCAGCTTGGGACCCAGAGCTACATCTGCAAGCTGAATCAC AAGCGGAGCAACACCAAGGTGGACAAGAGAGTTGAGGCGAAATCTTGTGACAAAAC CACACATGCCCAACCTGCCAGCAGCTGAAGTCTGGGGGACCGCTGAGTCTTCTC TTCCCCCAAAACCAAGGAGACCGTCAATGATCTCCCGAACCTGAGGTACATGC GTGGTGGTGGAGTGAAGCAGAGACCGTGAAGTGAAGTTCAACTGGTACGTGGAG GCGGTGGAGGTGCATAATGCCAAGACAAAGCGCGGGAGGAGCAGTACAAAGCAGG TACCGGGTGGTCAAGCTCTCAGCGTCTGCAAGAGGAGTGGTGAATGGCAAGGAG TACAAGTGCAAGGTCTCCAAACAAGCGCTCCAGGCGCATGGAGAAAACCATCTCC AAAGCCAAAGGCCAGCCCGGGAGCAGAGGTGTACACCTGCCCGCATCCGCGGAG GAGATGACCAAGAACCAGGTGAGCGTGAACCTGCTGGTCAAGGGTTGTATCCGAGC GACATCGCGGTGGAGTGGGAGAGCAATGCCAGCGCGAGAACCACTACAAGACCC CTTCCCGTGTGGACTCCGAGCGCTCTTCTTCTCTACAGCAAGCTACCGTGGAC AAGAGCAGGTGGCAGCAGGGCAAGCTTCTCATGCTCCGTGATGATGAGGCTCTG CACAAGCACTACACCGAGAGAGCCTCTCCTGTCTCCCGGCAAA
71	hH01重鎖可変 領域アミノ酸 配列	EVQLVQSGAEVKKPGASVKYSCKASGYTFTRNFMHWVRGAPGGLENNMGNIPQDGE TEYAGKFGGRVITADTSTSTAYMELSSLRSEDATVYYCARGVYGGFAGGYFDWGG GTLVTVSS
72	hH01重鎖可変 領域ヌクレオ チド配列	GAAGTGCAGCTGGTGCAGTCTGGCGCGAAGTGAAGAAACCAAGGCGCCAGCGTGAAG GTGTCTGCAAGGCCAGCGGCTACACCTTTACCGGAACTTCATGCACTGGGTGGCG CAGGCTCCAGGCCAGGGAAGTGAATGGATGGGTGGATCTATCCCGGCACGGCGAG ACAGAGTACGCCAGAAATTCAGGGCAGAGTGACCATCACCGCCGACACCAAGCACC TCCACCGCTACATGGAAGTGAGCAGCCTGCGGAGGAGGACACCGCGGTGTACTAT TGTGGCAGAGGCGGTACGGCGGCTTCGCTGGGGGCTACTTGGATTTTGGGGCCAG GGCACCGCTGTGACCGTCAAGTCA

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[表1-12]

73	hH02重鎖全長 アミノ酸配列	NKHLWFFLLI.VAAPRRVLSEVQLVQSGAEVKKPGASVKVSCKASGYTFRNFMHWVR QAPGGLENMGWIYPGDGETEYNQKFQGRVTITADRSTSTAYMELSSLRSEDTAVYF CARGVYGGFAGGYDFWGGGLVTVSSASTKGPSVFPLAPSSKISGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYIGNVNH KPSNTKVDKRVKPKSCDKHTHTCPCPAPELLGGPSVFLPPPKBDTLMISRTPEVTC VVVDVSHEDPEVKFMWYVDGVEVHNAKTKPREEGYNSTYRVVSVLTVLRHQLNGKE YKCKVSNKALPAPIEKTIISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVENESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRNQQGNVFSQSVNHREAL HNHYTQKSLSLSPGK
74	hH02重鎖全長 ヌクレオチド 配列	ATGAACACCTGTGGTCTTCTCCTCCTGCTGGTGGCAGCTCCAGATGGGTGCTGAGC GAAGTGCAGCTGGTGCAGTCTGGCGCGAAGTGAAGAAACAGGCGCCAGCGTGAAG GTGTCTGCAAGGCCAGCGGTACACSTTTACCGGAACCTCATGCACTGGGTGGGC CAGGCTCCAGGCCAGGAGTGAATGGATGGGCTGGATCTATCCGCGGACAGGCGAG ACAGAGTACAACAGAAATTCCAGGGCAGAGTACCATCAGCGCGACAGAAAGCACC AGCAGCGCTACATGGAACTGAGCAGCGTGGGAGCGAGGATACCGCCGTGTAGTTCC TGTGCCAGAGGCGTGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTGGGGCCAG GGCAGCCTCGTGACCGTGAGCTCAGCCTCCACCAAGGGGCCAAGCGTCTTCCCGCTG GCAGCCTCCTCCAGAGCACCTCTGGCGGACAGCGCGCTGGGCTGCTGGTGAAG GACTACTTCCCGAAGCGGTGACCGTGAAGTGGAACTCAGGCGCGCTGACCGAGCGC GTGCACACCTTCCCGGCTGTCTCTGAGTCTCAGGACTGTAGTGCCTCAGCAGCGTG GTGACCGTGCCTCCAGCAGCTTGGGCGGACAGCCTACATCTGCAAGTGAATCAG AAGCCAGCAACACCAAGGTGGACAAGAGTGTAGCCCAATCTTGTGACAAAACCT CACACATGCCACCGTGGCGAGCACCTGAACTCTGGGGGAGCGCTCAGTCTTCTCTC TTGGGGCCAAACCCCAAGGACACCTGATGATCTCCGGGACCCCTGAGTGAGATGG GTGGTGGTGGAGTGAAGCAGAAAGACCTGAGGTCAAGTTCAAGTGGTACGTGGAC GGCGTGGAGGTGCATAATGCAAGACAAAGCCCGGGAGGAGAGTACAACAGCACG TACCGGGTGGTCAAGCTCTCACCGCTGTCACCGAGGACTGGCTGAATGGCAAGGAG TACAAGTGCAAGGTCTCCAAAGCCCTCCAGCGCCCATCGAGAAAACCATCTCC AAAGCCAAAGGCGAGCGCGGGGAACACAGGTGTACACCTGCGCGCATCCCGGAG GAGATGACCAAGAACAGGTCAAGCTGAGCTGGCTGGTGGTCAAGGGCTCTATCCGAGC GACATGCGCGTGGAGTGGGAGAGCAATGGCGAGCGGAGAACAACTACAAGAGCAGC CCTCCCGTGTGAGTGGAGCGGCTCCTTCTCTCTAGAGCAAGTCAAGCGTGGAC AAGAGCAGGTGGCAGCGGCAACGCTCTTCTCATGCTCGTGAATGCATGAGGCTCTG CACAAGCACTACACCCAGAAGAGCCTGTGGGTGTCTCCCGGCAAA
75	hH02重鎖可変 領域アミノ酸 配列	EVGLVQSGAEVKKPGASVKVSCKASGYTFRNFMHWVRQAPGGLENMGWIYPGDGE TEYNQKFQGRVTITADRSTSTAYMELSSLRSEDTAVYFCARGVYGGFAGGYDFWGG GLTVTVSS

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[表1-13]

76	HH02重鎖可変領域ヌクレオチド配列	GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCAGGGGCCAGCGTGAAG GTGTCTGCAAGGCCAGCGGCTACACCTTTACCGGAACCTTCATGCAGTGGGTGCGC CAGGGTCCAGGCCAGGGACTGGAAATGGATGGGCTGGATCTATCCCGCGACGGCGAG ACAGAGTACAACCAGAAATTGCAGGGCAGAGTGACCATCACGGCGACAGAAGCACCC AGCACCGGCTACATGGAAGTGAGGAGCGCTGGGAGCGAGGATAGCGCGGTGTAAGTTC TGTGGCAGAGGCGGTGACGGCGGCTTCGCTGGCGGCTACTTCGATTTTGGGGGAG GGCACCGCTCGTGACCGTGAGGTGA
77	HH04重鎖全長アミノ酸配列	MKHLWFFLLLVAAAPRWVLGGVQLVGSAGVKKPGASVKVSCASGYTFTRNFMHWIR QAPGGGLEWNGWYYPGDGEIYAGKFGGRVLTADRSTSTAYMELSSLRSEDTAVYY CARGVYGGFAGGYDFWGGGTLVTSSASTKGPSVFPLAPSSKSTSGGTAALGGLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNH KPSNTKVDKRYEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVDVSHEDPEVKFNWYVDGVEVHNARTKPREEDYNSTYRVSVLTIVLHGDWLNKGE YKCKVSNKALPAPIEKTIISKAKGQPREPOVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWGGSNVFSCSVMEAL HHHTQKSLSLSPGK
78	HH04重鎖全長ヌクレオチド配列	ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCGAGATGGGTGCTGAGC CAGGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCAGGGGCCAGCGTGAAG GTGTCTGCAAGGCCAGCGGCTACACCTTTACCGGAACCTTCATGCAGTGGATCCGG CAGGCCCTGGACAGGGCGTGGAAATGGATGGGCTGGATCTATCCCGCGACGGCGAG ACAGAGTACGCCAGAAATTCCAGGGCAGAGTGACCGTGAACGCCGACAGAAGCACCC AGCAGGCGCTACATGGAAGTGAGCAGCGCTGGGAGCGAGGACACCGCGGTGTACTAT TGTGCCAGAGCGGTGTACGGCGGCTTCGCTGGCGGCTACTTGGATTTTGGGGCCAG GGCACCGCTCGTGACCGTCAAGTCAAGCTCGAGCAAGGGCCCAAGCGTCTCCCGCTG GCACGGTCTCCAAAGAGCACCTCTGGCGGACAGCGCGCGCTGGGCTGCTGGTCAAG GACTACTTCCCCGAACCGGTGACCGTGAGCTGGAACCTCAGGGGCCGTGACCAAGCGGC GTGCACACCTTCCCGGCTGCTCCTGAGTCTCAGGACTCTACTCCCTCAGCAGCGTG GTGAGCGTGGCTCCAGCAGCTTGGGACGGAGAGCTAGATCTGCAACGTGAATGAC AAGCCAGCAACACCAAGGTGGAGAGAGAGTTGAGCCCAATGTTGTGACAAAACCT CACACATGGCCACCGTGGCCAGCACCTGAAGTCTGGGGGACCGCTCAGTCTTCTCT TTGGCCCAAAACCAAGGACACCTCATGATCTCCCGGACCGCTGAGGTGACATGG GTGGTGGTGGAGTGAAGCAGGAGACCGTGAAGTCAAGTTCAACTGGTACGTGGAC GGCGTGGAGGTGCATAATGCCAAGACAAAGCCCGGGAGGAGCAGTACAACAGCACG TACCGGGTGGTCAAGCTCCTACCGTCTGACCGAGGACTGGCTGAATGGCAAGGAG TACAAGTGAAGGTCTGCAACAAAGCCCTCCAGCCCCATCGAGAAAAGCATCTCC AAAGCCAAAGGCCAGCGCGGGGAACACAGGTGTACACCTGGCGGATCCCGGGAG GAGATGACCAAGAACCGGTGACCGTGAAGTGGCTGGTCAAGGCTTCTATCCAGC GACATGCGCGTGGAGTGGGAGAGCAATGGCCAGCCGAGAACAACTACAAGACCAAC CCTCCCGTGTGGAGTGGAGCGGCTCTCTCTCTCTACAGCAAGCTACCGTGGAC AAGAGCAGGTGGCAGCAGGCAACGCTCTCTCATGCTGGTGAATGATGAGGCTGTG CACACGACTACACCCAGAGAGGCTCTCGCTGTCTCCCGGCAAA

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[表1-14]

79	hh04重鎖可変領域アミノ酸配列	OVGLVQSGAEVKKPGASVKVSKASGYTFRNFMHWIRGAPGGGLEWNGWYYPGDGE TEYAKKFGGRVTLADHSTSTAYMELSSLRSEDTAVYYCARGVYGGFAGGYDFWGG GTLVTIVSS
80	hh04重鎖可変領域ヌクレオチド配列	CAGGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACAGGCCAGCGTGAAG GTGTCTTGCAGGCCAGCGGCTACACCTTTACCCGGAACITCATGCACTGGATCCGG CAGGCCCTGGACAGGGCTGGAATGGATGGGCTGATCTATCCCGCGACGGCGAG ACAGAGTACGCCAGAAATTCAGGGGAGAGTGACCTGACCGCCGACAGAAAGCAGC AGCACCCTACATGGAAGTGAAGCAGCTGGGAGCGAGAGACCGCCGTGTACTAT TGTGCCAGAGCGGTGTACGGCGGCTTGGCTGGCGGCTACTTCGATTTTGGGGCCAG GSCACCTCGTGACCGTCAGCTCA
81	NOV0712軽鎖全長アミノ酸配列	MVLGTQVFIQLLWISGAYGDTQMTQSPSSLSASVGGRRVITTCRASQSISSYLNWYQ QKPGKAPKLLIYAVSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQSGTF PPITFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVYCLLNRFYPREAKVQHWY DNALQSGNSGESVTEQDSKDSYSLSSLTILSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC
82	NOV0712軽鎖全長ヌクレオチド配列	ATGGTGCTGCAGACCGAGGTGTTTCATCTCCCTGCTGCTGTGAATCTCGGCGGTAC GGCGACATCCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGCGACAGA GTGACCATCACCTGTAGAGCCAGCCAGCATCAGCAGCTACCTGAAGTGGTATCAG CAGAAGCCCGGCAAGGGCCCAAACTGCTGATCTACGGCGGTGTCCACACTGCAGAGC GGCGTGCCCGAGCAGATTTCTGGCAGCGGCTCGGGCAGGAGTTACCGCTGACAATC AGCAGCCTGCAGCCCGAGGACTTCGGCAGCTACTACTGTACAGCAGTCCGGCAGCTT CCCCCACCACATTTGGCGAGGCAACAGGTGGAAATCAAGGGTAGGGTGGCGGCC CCCTCCGTGTTTCATCTTCCGCCCTCCGACGAGCAGCTGAAGTGGCGACCGCTCC GTGGTGCCCTGCTGAATAACTTCTACGGCAGAGAGGCCAAGGTGCAGTGGAAAGGTG GACAACGCCCTGCAGTCCGGGAACCTCCAGGAGAGCGTGACCGAGGAGGACAGCAAG GACAGCACCTACAGCGTGAAGCAGACCTGACCGCTGAGGAAAAGCGGACTACGAGAAG CAGAAGGTGTACGCTGCGAGGTGACGAGCAGGAGGCTGAGCTCCCCGTGAGCAAG AGCTTCAACAGGGGGAGTGT
83	NOV0712重鎖全長アミノ酸配列	NKRLWFFLLIIVAPRWVLSQVQLLESQGGGLVQPGGSLRLSCAASGFTFSHGMHWVR QAPGKGLEKWSVJSGSGSNTGYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYY CARGNGSYAFDSWGGTLVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP EPVTVSNNGALTSQVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKREPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSKEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCK VSNKALPAPIETISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV ENESNGUPENNYKTTIPVLDSDGSFFLYSKLTVDKSRWGGGNVFSCSVNHREALHNNY TGKSLSLSPGK

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[表1-15]

84	NOV0712重鎖全長ヌクレオチド配列	ATGAAACACCTGTGGTTCTTCTCTCTGCTGGTGGCAGCTCCAGATGGGTGCTGAGC CAGGTGCAGCTGTGGAATCTGGCGGAGGACTGGTGCAGGCTGGCGGCTCTCTGAGA CTGTCTTGTGCCCGCAGCGGCTTCAGCTTCAGCAGCCACGGAATGCACTGGGTGGC CAGGCCCTGGAAAGGGACTGGAATGGGTGTCCGTGATCAGCGGCAAGGCTGCAAT ACCGGCTACGGCGATAGCGTGAAGGGCCGGTTCAACATCAGCCGGGACACAGCAAG AACACCCCTGTACCTGCAGATGAACAGGCTGGGGGGGAGGACACCGCGGTGATAT TGTCCAGACAGTGGGGGAGCTACGGCTTCGATTCTTGGGGCCAGGGACCCCTGGT ACCGTGAGGTGAGGCTCCACCAAGGGCCCAAGGGTCTTCCCGCTGGACCCCTCCTCC AAGAGCAGCTCTGGGGGACAGCGCGCTGGGCTGCCTGGTCAAGGACTACTTCCCG GAACCCGTGACCGTGAGCTGGAACCTCAGCGCCCTGACCAAGCGGCTGCACACCTTC CCCGCTGTCTGCAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC TCCAGCAGCTTGGGCACCCAGAGCTACATCTGCAACGTGAATCACAAGCCAGCAAC ACCAAGGTGGACAAGAGAGTTGAGCGCAATCTTGTGACAAACTGAGAGATGGCCA CCCTGCCAGCAGCTGAACCTCTGGGGGACCCCTCAGTCTTCTCTTCCCGCCAAA CCCAAGGAGACCCCTCATGATCTCCGGACCCCTGAGGTACATGCGTGGTGGGAC GTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGAGGGGTGGAGGTG CATAATGCCAAGACAAGCCCGGGAGGAGCAGTACAACAGCACSTACCGGTGGTC AGCGTCTCAGCGTCTGCAACGAGCTGGCTGAATGGCAAGGAGTACAAGTGAAG GTCTCCAAACAAGCCCTCCAGCGCCCATCGAGAAAACCATCTCCAAAGCCAAAGGC CAGCCCGGGGAACGAGGTGTACACCTGCCCGCATCCGGGAGGAGATGACCAAG AACCAGGTGAGCGTGACCTGCCTGGTCAAAGGGTTCTATCCCAAGCGACATCGCCGTG GAGTGGGAGAGCAATGGCCAGCCGGAACAACCTACAAGACCCCTCCCGTGGT GACTCCGACGGCTCTTCTCTCTACAGCAAGCTCAACGTGGACAAGAGCAGGTGG CAGCAGGGCAACGTCTTCTCATGCTCCGTGATGATGAGGCTCTGCACACCACTAC ACCCAGAGAGCCCTCTCCGTGTCTCCGGGAAA
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[0112] (本明細書中、表1-1～表1-15を表1と総称する場合がある。)

本発明の抗体としては、さらに、CDH6に結合する、ヒト抗体を挙げることができる。抗CDH6ヒト抗体とは、ヒト染色体由来の抗体の遺伝子配列のみを有するヒト抗体を意味する。抗CDH6ヒト抗体は、ヒト抗体の重鎖と軽鎖の遺伝子を含むヒト染色体断片を有するヒト抗体産生マウスを用いた方法(Tomizuka, K. et al., Nature Genetics (1997) 16, p. 133-143; Kuroiwa, Y. et al., Nucl. Acids Res. (1998) 26, p. 3447-3448; Yoshida, H. et al., Animal Cell Technology: Basic and Applied Aspects vol. 10, p. 69-73 (Kitagawa, Y., Matsuda, T. and Iijima, S. eds.), Kluwer Academic Publishers, 1999.; Tomizuka, K. et al., Proc. Natl. Acad. Sci. USA (2000) 97, p. 722-727等を参照。)によって取得することができる。

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- [0113] このようなヒト抗体産生マウスは、具体的には、内在性免疫グロブリン重鎖及び軽鎖の遺伝子座が破壊され、代わりに酵母人工染色体（*Yeast artificial chromosome*, YAC）ベクター等を介してヒト免疫グロブリン重鎖及び軽鎖の遺伝子座が導入された遺伝子組み換え動物として、ノックアウト動物及びトランスジェニック動物の作製及びこれらの動物同士を掛け合わせることによって作り出すことができる。
- [0114] また、遺伝子組換え技術によって、そのようなヒト抗体の重鎖及び軽鎖の各々をコードするcDNA、好ましくは該cDNAを含むベクターによって真核細胞を形質転換し、遺伝子組換えヒトモノクローナル抗体を産生する形質転換細胞を培養することによって、この抗体を培養上清中から得ることもできる。
- [0115] ここで、宿主としては例えば真核細胞、好ましくはCHO細胞、リンパ球やミエローマ等の哺乳動物細胞を用いることができる。
- [0116] また、ヒト抗体ライブラリーより選別したファージディスプレイ由来のヒト抗体を取得する方法（*Wormstone, I. M. et. al, Investigative Ophthalmology & Visual Science*, (2002) 43 (7), p. 2301–2308; *Carmen, S. et. al., Briefings in Functional Genomics and Proteomics* (2002), 1 (2), p. 189–203; *Siriwardena, D. et. al., Ophthalmology* (2002) 109 (3), p. 427–431等参照。）も知られている。
- [0117] 例えば、ヒト抗体の可変領域を一本鎖抗体（*scFv*）としてファージ表面に発現させて、抗原に結合するファージを選択するファージディスプレイ法（*Nature Biotechnology* (2005), 23, (9), p. 1105–1116）を用いることができる。
- [0118] 抗原に結合することで選択されたファージの遺伝子を解析することによって、抗原に結合するヒト抗体の可変領域をコードするDNA配列を決定する

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ことができる。

- [0119] 抗原に結合する s c F v の DNA 配列が明らかになれば、当該配列を有する発現ベクターを作製し、適当な宿主に導入して発現させることによってヒト抗体を取得することができる（国際公開第 92/01047 号、同 92/20791 号、同 93/06213 号、同 93/11236 号、同 93/19172 号、同 95/01438 号、同 95/15388 号、Annu. R. ev. Immunol (1994) 12, p. 433-455、Nature Biotechnology (2005) 23 (9), p. 1105-1116）。
- [0120] 新たに作製されたヒト抗体が、本明細書に記載のラット抗ヒト CDH6 抗体、キメラ抗ヒト CDH6 抗体又はヒト化抗ヒト CDH6 抗体（例えば、rG019 抗体、rG055 抗体、rG056 抗体、rG061 抗体、chG019 抗体、H01L02 抗体、H02L02 抗体、H02L03 抗体又は H04L02 抗体）のいずれか 1 つが結合する部分ペプチド又は部分立体構造に結合すれば、該ヒト抗体が当該ラット抗ヒト CDH6 抗体、キメラ抗ヒト CDH6 抗体又はヒト化抗ヒト CDH6 抗体と同一のエピトープに結合すると判定することができる。あるいは、本明細書に記載のラット抗ヒト CDH6 抗体、キメラ抗ヒト CDH6 抗体又はヒト化抗ヒト CDH6 抗体（例えば、rG019 抗体、rG055 抗体、rG056 抗体、rG061 抗体、chG019 抗体、H01L02 抗体、H02L02 抗体、H02L03 抗体又は H04L02 抗体）の CDH6 に対する結合に対して該ヒト抗体が競合する（例えば、該ヒト抗体が、rG019 抗体、rG055 抗体、rG056 抗体、rG061 抗体、chG019 抗体、H01L02 抗体、H02L02 抗体、H02L03 抗体又は H04L02 抗体と CDH6、好ましくは、CDH6 の EC3 への結合を妨げる）ことを確認することによって、具体的なエピトープの配列又は構造が決定されていなくても、該ヒト抗体が本明細書に記載のラット抗ヒト CDH6 抗体、キメラ抗ヒト CDH6 抗体又はヒト化抗ヒト CDH6 抗体と同一のエピトープに結合すると判定することが

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できる。本明細書において、当該判定方法の少なくともいずれか一方の方法により「同一のエピトープに結合する」と判定された場合、新たに作製されたヒト抗体が、本明細書に記載のラット抗ヒトCDH6抗体、キメラ抗ヒトCDH6抗体又はヒト化抗ヒトCDH6抗体と「同一のエピトープに結合する」といえる。エピトープが同一であることが確認された場合、該ヒト抗体がラット抗ヒトCDH6抗体、キメラ抗ヒトCDH6抗体又はヒト化抗ヒトCDH6抗体（例えば、rG019抗体、rG055抗体、rG056抗体、rG061抗体、chG019抗体、H01L02抗体、H02L02抗体、H02L03抗体又はH04L02抗体）と同等の生物活性を有していることが期待される。

[0121] 以上の方法によって得られたキメラ抗体、ヒト化抗体、又はヒト抗体は、公知の方法等によって抗原に対する結合性を評価し、好適な抗体を選抜することができる。

[0122] 抗体の性質を比較する際の別の指標の一例としては、抗体の安定性を挙げることができる。示差走査カロリーメトリー（DSC）は、蛋白の相対的構造安定性のよい指標となる熱変性中点（ T_m ）を素早く、また正確に測定することができる装置である。DSCを用いて T_m 値を測定し、その値を比較することによって、熱安定性の違いを比較することができる。抗体の保存安定性は、抗体の熱安定性とある程度の相関を示すことが知られており（Lor i Burton, et. al., *Pharmaceutical Development and Technology* (2007) 12, p. 265-273）、熱安定性を指標に、好適な抗体を選抜することができる。抗体を選抜するための他の指標としては、適切な宿主細胞における収量が高いこと、及び水溶液中での凝集性が低いことを挙げることができる。例えば収量の最も高い抗体が最も高い熱安定性を示すとは限らないので、以上に述べた指標に基づいて総合的に判断して、ヒトへの投与に最も適した抗体を選抜する必要がある。

[0123] 本発明の抗体には抗体の修飾体も含まれる。当該修飾体とは、本発明の抗

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体に化学的又は生物学的な修飾が施されてなるものを意味する。化学的な修飾体には、アミノ酸骨格への化学部分の結合、N-結合又はO-結合炭水化物鎖の化学修飾体等が含まれる。生物学的な修飾体には、翻訳後修飾（例えば、N-結合又はO-結合への糖鎖付加、N末又はC末のプロセッシング、脱アミド化、アスパラギン酸の異性化、メチオニンの酸化、又はN末グルタミンもしくはN末グルタミン酸のピログルタミン酸化）されたもの、原核生物宿主細胞を用いて発現させることによってN末にメチオニン残基が付加したもの等が含まれる。また、本発明の抗体又は抗原の検出又は単離を可能にするために標識されたもの、例えば、酵素標識体、蛍光標識体、アフィニティ標識体もかかる修飾物の意味に含まれる。このような本発明の抗体の修飾物は、抗体の安定性及び血中滞留性の改善、抗原性の低減、抗体又は抗原の検出又は単離等に有用である。

[0124] また、本発明の抗体に結合している糖鎖修飾を調節すること（グリコシル化、脱フコース化等）によって、抗体依存性細胞傷害活性を増強することが可能である。抗体の糖鎖修飾の調節技術としては、国際公開第1999/54342号、同2000/61739号、同2002/31140号等が知られているが、これらに限定されるものではない。本発明の抗体には当該糖鎖修飾を調節された抗体も含まれる。

[0125] 抗体遺伝子を一旦単離した後、適当な宿主に導入して抗体を作製する場合には、適当な宿主と発現ベクターの組み合わせを使用することができる。抗体遺伝子の具体例としては、本明細書に記載された抗体の重鎖配列をコードする遺伝子、及び軽鎖配列をコードする遺伝子を組み合わせたものを挙げることができる。宿主細胞を形質転換する際には、重鎖配列遺伝子と軽鎖配列遺伝子は、同一の発現ベクターに挿入されていることが可能であり、又別々の発現ベクターに挿入されていることも可能である。

[0126] 真核細胞を宿主として使用する場合、動物細胞、植物細胞、真核微生物を用いることができる。特に動物細胞としては、哺乳類細胞、例えば、サルの細胞であるCOS細胞（Gluzman, Y. Cell (1981) 23,

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p. 175-182、ATCC CRL-1650)、マウス線維芽細胞NIH3T3(ATCC No. CRL-1658)やチャイニーズ・ハムスター卵巣細胞(CHO細胞、ATCC CCL-61)のジヒドロ葉酸還元酵素欠損株(Urlaub, G. and Chasin, L. A. Proc. Natl. Acad. Sci. U. S. A. (1980) 77, p. 4126-4220)、FreeStyle 293F細胞(Invitrogen社)を挙げることができる。

[0127] 原核細胞を使用する場合は、例えば、大腸菌、枯草菌を挙げることができる。

[0128] これらの細胞に目的とする抗体遺伝子を形質転換によって導入し、形質転換された細胞を*in vitro*で培養することによって抗体が得られる。当該培養においては抗体の配列によって収量が異なる場合があり、同等な結合活性を持つ抗体の中から収量を指標に医薬としての生産が容易なものを選別することが可能である。よって、本発明の抗体には、上記形質転換された宿主細胞を培養する工程、及び当該工程で得られた培養物から目的の抗体又は当該抗体の機能性断片を採取する工程を含むことを特徴とする当該抗体の製造方法によって得られる抗体も含まれる。

[0129] なお、哺乳類培養細胞で生産される抗体の重鎖のカルボキシル末端のリシン残基が欠失することが知られており(Journal of Chromatography A, 705:129-134(1995))、また、同じく重鎖カルボキシル末端のグリシン、リシンの2アミノ酸残基が欠失し、新たにカルボキシル末端に位置するプロリン残基がアミド化されることが知られている(Analytical Biochemistry, 360:75-83(2007))。しかし、これらの重鎖配列の欠失及び修飾は、抗体の抗原結合能及びエフェクター機能(補体の活性化や抗体依存性細胞傷害作用等)には影響を及ぼさない。従って、本発明に係る抗体には、当該修飾を受けた抗体及び当該抗体の機能性断片も含まれ、重鎖カルボキシル末端において1又は2つのアミノ酸が欠失した欠失体、及びアミド化された当

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該欠失体（例えば、カルボキシル末端部位のプロリン残基がアミド化された重鎖）等も包含される。但し、抗原結合能及びエフェクター機能が保たれている限り、本発明に係る抗体の重鎖のカルボキシル末端の欠失体は上記の種類に限定されない。本発明に係る抗体を構成する2本の重鎖は、完全長及び上記の欠失体からなる群から選択される重鎖のいずれか一種であってもよいし、いずれか二種を組み合わせたものであってもよい。各欠失体の量比は本発明に係る抗体を産生する哺乳類培養細胞の種類及び培養条件に影響を受け得るが、本発明に係る抗体の主成分としては2本の重鎖の双方でカルボキシル末端の1つのアミノ酸残基が欠失している場合を挙げることができる。

【0130】 本発明の抗体のアイソタイプとしては、例えばIgG（IgG1、IgG2、IgG3、IgG4）等を挙げることができるが、好ましくはIgG1又はIgG4を挙げることができる。

【0131】 抗体の生物活性としては、一般的には抗原結合活性、抗原と結合することによって該抗原を発現する細胞に内在化する活性、抗原の活性を中和する活性、抗原の活性を増強する活性、抗体依存性細胞傷害（ADCC）活性、補体依存性細胞傷害（CDC）活性及び抗体依存性細胞媒介食作用（ADCP）を挙げることができるが、本発明に係る抗体が有する機能は、CDH6に対する結合活性であり、好ましくはCDH6と結合することによってCDH6発現細胞に内在化する活性である。さらに、本発明の抗体は、細胞内在化活性に加えて、ADCC活性、CDC活性及び／又はADCP活性を併せ持っていることも良い。

【0132】 得られた抗体は、均一にまで精製することができる。抗体の分離、精製は通常のタンパク質で使用されている分離、精製方法を使用すればよい。例えばカラムクロマトグラフィー、フィルター濾過、限外濾過、塩析、透析、調製用ポリアクリルアミドゲル電気泳動、等電点電気泳動等を適宜選択、組み合わせれば、抗体を分離、精製することができる（Strategies for Protein Purification and Characterization: A Laboratory Course Ma

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nual, Daniel R. Marshak et al., eds., Cold Spring Harbor Laboratory Press (1996); Antibodies: A Laboratory Manual, Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988)) が、これらに限定されるものではない。

[0133] クロマトグラフィーとしては、アフィニティークロマトグラフィー、イオン交換クロマトグラフィー、疎水性クロマトグラフィー、ゲル濾過クロマトグラフィー、逆相クロマトグラフィー、吸着クロマトグラフィー等を挙げることができる。

[0134] これらのクロマトグラフィーは、HPLCやFPLC等の液体クロマトグラフィーを用いて行うことができる。

[0135] アフィニティークロマトグラフィーに用いるカラムとしては、プロテインAカラム、プロテインGカラムを挙げることができる。例えばプロテインAカラムを用いたカラムとして、Hyper D, POROS, Sepharose F, F, (ファルマシア)等を挙げることができる。

[0136] また抗原を固定化した担体を用いて、抗原への結合性を利用して抗体を精製することも可能である。

[0137] 3. 抗CDH6抗体-薬物コンジュゲート

(1) 薬物

上記「2. 抗CDH6抗体の製造」にて取得された抗CDH6抗体はリンカー構造部分を介して薬物を結合させることによって、抗CDH6抗体-薬物コンジュゲートとすることができる。薬物としては、リンカー構造に結合できる置換基、部分構造を有するものであれば特に制限はない。抗CDH6抗体-薬物コンジュゲートは結合する薬物に応じて種々の用途に用いることができる。そのような薬物の例としては、抗腫瘍活性を有する物質、血液疾患に対する効果を有する物質、自己免疫疾患に対する効果を有する物質、抗炎症物質、抗菌物質、抗真菌物質、抗寄生虫物質、抗ウイルス物質、抗麻酔

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物質等を挙げることができる。

[0138] (1) - 1 抗腫瘍化合物

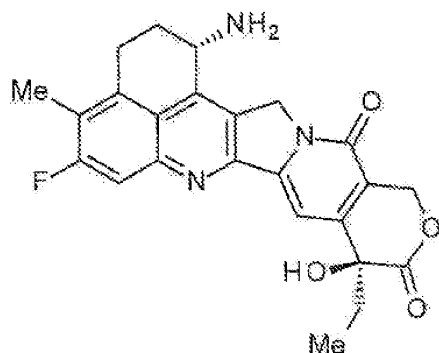
本発明の抗CDH6抗体-薬物コンジュゲートに結合される化合物として抗腫瘍性化合物を用いる例について以下に述べる。抗腫瘍性化合物としては、抗腫瘍効果を有する化合物であって、リンカー構造に結合できる置換基、部分構造を有するものであれば特に制限はない。抗腫瘍性化合物は、リンカーの一部又は全部が腫瘍細胞内で切断されて抗腫瘍性化合物部分が遊離されて抗腫瘍効果が発現される。リンカーが薬物との結合部分で切断されれば抗腫瘍性化合物が本来の構造で遊離され、その本来の抗腫瘍効果が発揮される。

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[0139] 上記「2. 抗CDH6抗体の製造」にて取得された抗CDH6抗体はリンカー構造部分を介して抗腫瘍性化合物を結合させることによって、抗CDH6抗体-薬物コンジュゲートとすることができる。

[0140] 本発明で使用する抗腫瘍性化合物の一つの例として、カンプトテシン誘導体であるエキサテカン（(1S, 9S)-1-アミノ-9-エチル-5-フルオロ-2, 3-ジヒドロ-9-ヒドロキシ-4-メチル-1H, 12H-ベンゾ[d,e]ピラノ[3', 4': 6, 7]インドリジノ[1, 2-b]キノリン-10, 13(9H, 15H)-ジオン；次式：）

[0141] [化5]



[0142] を好適に使用することができる。同化合物は、例えば、米国特許公開公報U

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S 2 0 1 6 / 0 2 9 7 8 9 0 号に記載の方法やその他の公知の方法で容易に取得でき、1位のアミノ基をリンカー構造への結合部位として好適に使用することができる。また、エキサテカンハリンカーの一部が結合した状態で腫瘍細胞内で遊離される場合もあるが、このような状態でも優れた抗腫瘍効果が発揮される化合物である。

[0143] エキサテカンはカンプトテシン構造を有するので、酸性水性媒体中（例えばpH3程度）ではラクトン環が形成された構造（閉環体）に平衡が偏り、一方、塩基性水性媒体中（例えばpH10程度）ではラクトン環が開環した構造（開環体）に平衡が偏ることが知られている。このような閉環構造及び開環構造に対応するエキサテカン残基を導入した薬物コンジュゲートであっても同等の抗腫瘍効果が期待され、いずれのものも本発明の範囲に包含されることはいうまでもない。

[0144] 他の抗腫瘍性化合物として例えば、文献（*Pharmacological Reviews*, 68, p3-19, 2016）記載の抗腫瘍化合物を挙げることができ、その一例として、ドキソルビシン（Doxorubicin）、カルケマイシン（Calicheamicin）、ドラスタチン（Dorastatin）10、モノメチルアウリスタチンE（MMAE）、モノメチルアウリスタチンF（MMAF）等のアウリスタチン類（Auristatins）、DM1、DM4等のメイタンシノイド類（Maytansinoids）、ピロロベンゾジアゼピン（Pyrrolodiazepine）二量体のSG2000（SJG-136）、カンプトテシンの誘導体であるSN-38、CC-1065等のデュオカルマイシン類（Duocarmycins）、アマニチン（Amanitin）、ダウノルビシン、マイトマイシンC、ブレオマイシン、シクロシチジン、ビンクリスチン、ビンブラスチン、メトトレキサート、白金系抗腫瘍剤（シスプラチン若しくはその誘導体）、タキソール若しくはその誘導体等を挙げることができる。

[0145] 抗体-薬物コンジュゲートにおいて、抗体1分子への薬物の結合数は、そ

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の有効性、安全性に影響する重要因子である。抗体－薬物コンジュゲートの製造は、薬物の結合数が一定の数となるよう、反応させる原料・試薬の使用量等の反応条件を規定して実施されるが、低分子化合物の化学反応とは異なり、異なる数の薬物が結合した混合物として得られるのが通常である。抗体 1 分子への薬物の結合数は平均値、すなわち、平均薬物結合数として特定され、表記される。本発明でも原則として断りのない限り、すなわち、異なる薬物結合数をもつ抗体－薬物コンジュゲート混合物に含まれる特定の薬物結合数をもつ抗体－薬物コンジュゲートを示す場合を除き、薬物の結合数は平均値を意味する。抗体分子へのエキサテカンの結合数はコントロール可能であり、1 抗体あたりの薬物平均結合数として、1～10 個程度のエキサテカン結合させることができるが、好ましくは 2～8 個、3～8 個、4～8 個、5～8 個、6～8 個、7～8 個であり、より好ましくは 5～8 個であり、更に好ましくは 7～8 個であり、更により好ましくは 8 個である。なお、当業者であれば本願の実施例の記載から抗体に必要な数の薬物を結合させる反応を設計することができ、エキサテカンの結合数をコントロールした抗体－薬物コンジュゲートを取得することができる。

[0146] (2) リンカー構造

本発明の抗 CDH6 抗体－薬物コンジュゲートにおいて薬物を抗 CDH6 抗体に結合させるリンカー構造について述べる。

[0147] 本願の抗体－薬物コンジュゲートにおいて、抗 CDH6 抗体と薬物とを結合するリンカー構造は、抗体－薬物コンジュゲートとして用いることができる限りにおいて特に制限されず、使用目的に応じて適宜選択して用いることもできる。リンカー構造の一例としては、公知文献 (Pharmacol Rev 68:3-19, January 2016, Protein Cell DOI 10.1007/s13238-016-0323-0 等) に記載のリンカーを挙げることができ、更に具体的な例としては、VC (バリン－シトルリン: valine-citrulline)、MC (マレインアミドカプロイル: maleimidocaproyl)、SMCC (

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スクシニミジル 4- (N-マレイミドメチル) シクロヘキサノ-1-カルボキシレート: succinimidyl 4- (N-maleimido methyl) cyclohexane-1-carboxylate)、SPP (N-スクシニミジル 4- (2-ピリジルジチオ) ペンタン酸: N-succinimidyl 4- (2-pyridyldithio) pentanoate、SS (ジスルフィド: disulfide)、SPDB (N-スクシニミジル 4- (2-ピリジルジチオ) ブチレート) N-succinimidyl 4- (2-pyridyldithio) butyrate、SS/ヒドラゾン、ヒドラゾン、カルボネートを挙げることができる。

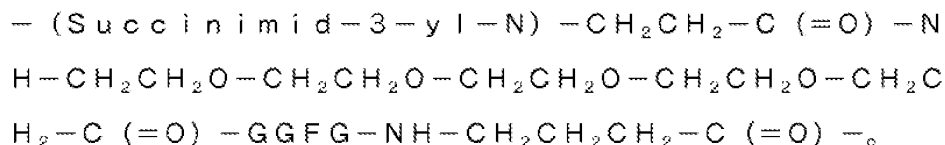
[0148] 他の一例としては、例えば、米国特許公開公報US2016/0297890に記載のリンカー構造（一例として、段落[0260]～[0289]に記載のもの）を挙げることができ、以下の構造のものを好適に使用することができる。なお以下に示す構造の左端が抗体との結合部位であり、右端が薬物との結合部位である。また、下記のリンカー構造中のGGFGは、グリシン-グリシン-フェニルアラニン-グリシン (GGFG) からなるペプチド結合でつながっているアミノ酸配列を示す。

— (Succinimid-3-yl-N) —CH₂CH₂—C(=O)—GGFG—NH—CH₂CH₂CH₂—C(=O)—、
 — (Succinimid-3-yl-N) —CH₂CH₂CH₂CH₂CH₂—C(=O)—GGFG—NH—CH₂CH₂CH₂—C(=O)—、
 — (Succinimid-3-yl-N) —CH₂CH₂CH₂CH₂CH₂—C(=O)—GGFG—NH—CH₂—O—CH₂—C(=O)—、
 — (Succinimid-3-yl-N) —CH₂CH₂CH₂CH₂CH₂—C(=O)—GGFG—NH—CH₂CH₂—O—CH₂—C(=O)—、
 — (Succinimid-3-yl-N) —CH₂CH₂—C(=O)—NH—CH₂CH₂O—CH₂CH₂O—CH₂CH₂—C(=O)—GGFG—NH—CH₂CH₂CH₂—C(=O)—、

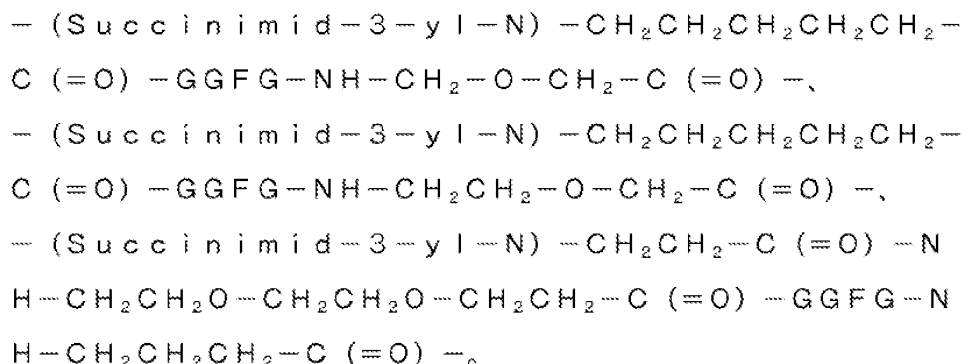
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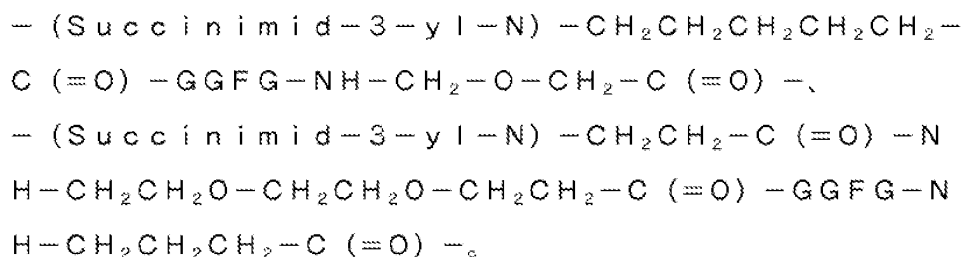
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[0149] より好ましくは、次のものを挙げることができる。



さらにより好ましくは、



を挙げることができる。

[0150] 抗体は $-(\text{Succinimid-3-yl-N})$ の末端、(例えば、『 $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$ 』において、 $(-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-)$ が結合するのは反対側の末端(左末端))で結合し、抗腫瘍性化合物は $-(\text{Succinimid-3-yl-N})$ とは反対側の末端(上記例では右末端の、 $\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$ のカルボニル基で結合する。『 $-(\text{Succinimid-3-yl-N})-$ 』は、次式：

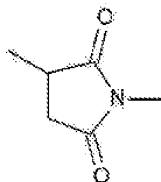
[0151]

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[化6]



[0152] で示される構造を有する。この部分構造における3位が抗CDH6抗体への結合部位である。この3位での該抗体との結合は、チオエーテルを形成して結合することが特徴である。この構造部分の1位の窒素原子は、この構造が含まれるリンカー内に存在するメチレンの炭素原子と結合する。

[0153] 薬物をエキサテカンとする本発明の抗体-薬物コンジュゲートにおいては、下記の構造の薬物-リンカー構造部分を抗体に結合させたものが好ましい。これらの薬物-リンカー構造部分は、1抗体あたりの平均結合数として、1から10を結合させればよいが、好ましくは2から8であり、より好ましくは5から8であり、更に好ましくは7から8であり、更により好ましくは8である。

—(Succinimid-3-yl-N)—CH₂CH₂—C(=O)—G
GGFG—NH—CH₂CH₂CH₂—C(=O)—(NH—DX)、

—(Succinimid-3-yl-N)—CH₂CH₂CH₂CH₂CH₂—
C(=O)—GGFG—NH—CH₂CH₂CH₂—C(=O)—(NH—DX)
)、

—(Succinimid-3-yl-N)—CH₂CH₂CH₂CH₂CH₂—
C(=O)—GGFG—NH—CH₂—O—CH₂—C(=O)—(NH—D
X)、

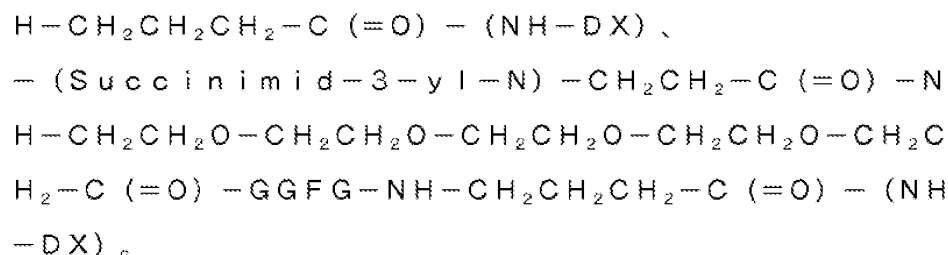
—(Succinimid-3-yl-N)—CH₂CH₂CH₂CH₂CH₂—
C(=O)—GGFG—NH—CH₂CH₂—O—CH₂—C(=O)—(NH
—DX)、

—(Succinimid-3-yl-N)—CH₂CH₂—C(=O)—N
H—CH₂CH₂O—CH₂CH₂O—CH₂CH₂—C(=O)—GGFG—N

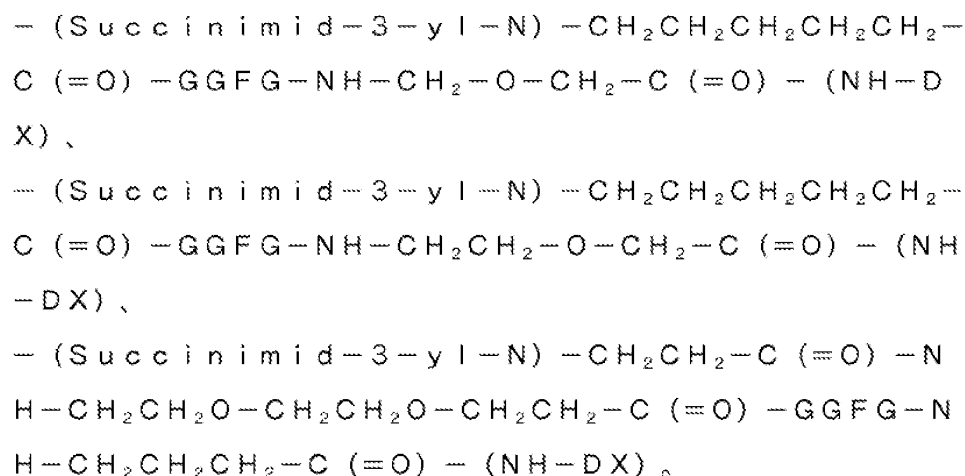
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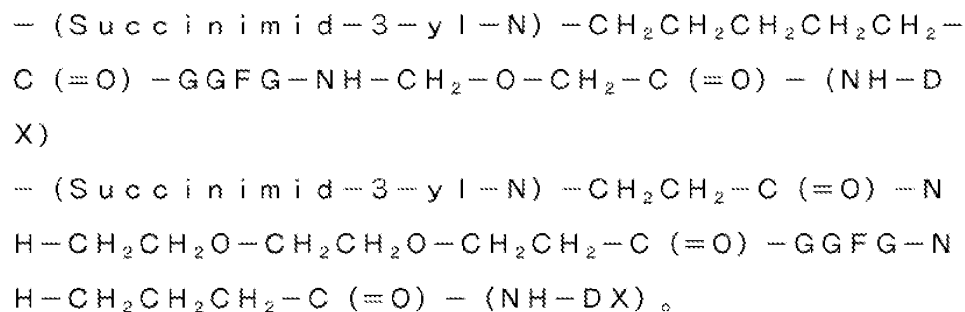
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[0154] より、好ましくは、次のものである。



[0155] さらに、より好ましくは、次のものを挙げることができる。

[0156] また、 $-(\text{NH}-\text{DX})$ は次式：

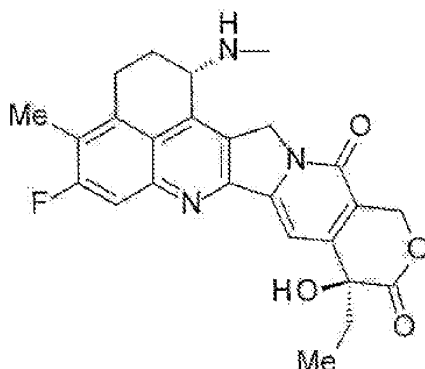
[0157]

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[化7]



[0158] で示される構造であり、エキサテカンの1位のアミノ基の水素原子1個が除かれて生成する基を示す。

[0159] (3) 抗体-薬物コンジュゲートの製造方法

本発明の抗体-薬物コンジュゲートに用いることができる抗体は、上記「2. 抗CDH6抗体の製造」の項及び実施例に記載の内在化活性を有する抗CDH6抗体及び該抗体の機能性断片であれば特に制限がない。

[0160] 次に、本発明の抗体-薬物コンジュゲートの代表的な製造方法について説明する。なお、以下において、化合物を示すために、各反応式中に示される化合物の番号を用いる。すなわち、『式(1)の化合物』、『化合物(1)』等と称する。また、これ以外の番号の化合物についても同様に記載する。

[0161] (3)-1 製造方法1

下記式(1)で示される抗体-薬物コンジュゲートのうち、チオエーテルを介して抗CDH6抗体とリンカー構造が結合しているものは抗CDH6抗体を還元してジスルフィド結合をスルヒドリル基に変換した抗体に対して、既知の方法によって入手しうる化合物(2)（例えば、US2016/297890号公開特許公報に記載の方法（例えば段落[0336]～[0374]に記載の方法）で入手可能）を反応させることによって製造することができる。例えば下記の方法によって製造することができる。

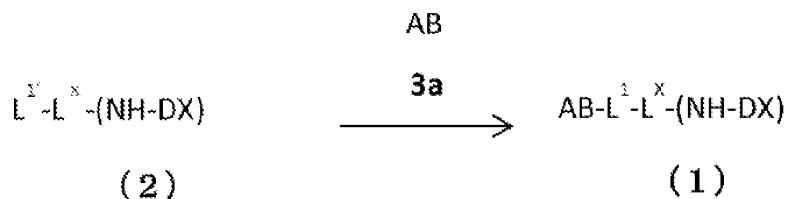
[0162]

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[数1]



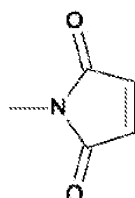
[0163] [式中、ABは、スルフヒドリル基を有する抗体を示す。

ここで、L¹は、

- (Succinimid-3-yl-N) - の構造で示される。

L¹ は次式で示される、マレイミジル基を示す。]

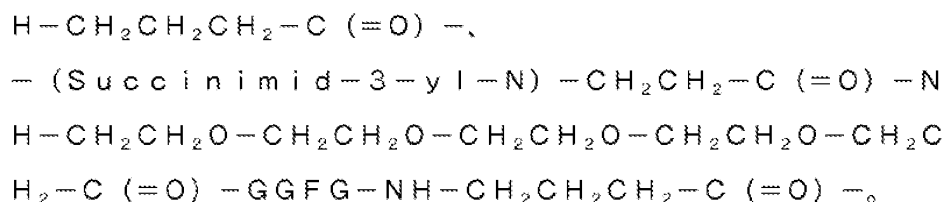
[0164] [化8]

[0165] -L¹-L^Xは、以下の式で示されるいずれかの構造を有する。- (Succinimid-3-yl-N) -CH₂CH₂-C(=O)-GGGFG-NH-CH₂CH₂CH₂-C(=O)-、- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-、- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂-O-CH₂-C(=O)-、- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂-O-CH₂-C(=O)-、- (Succinimid-3-yl-N) -CH₂CH₂-C(=O)-NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O)-GGFG-N

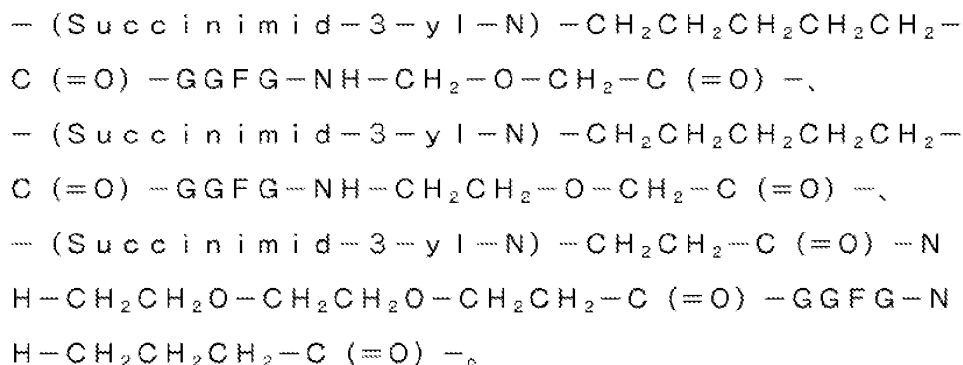
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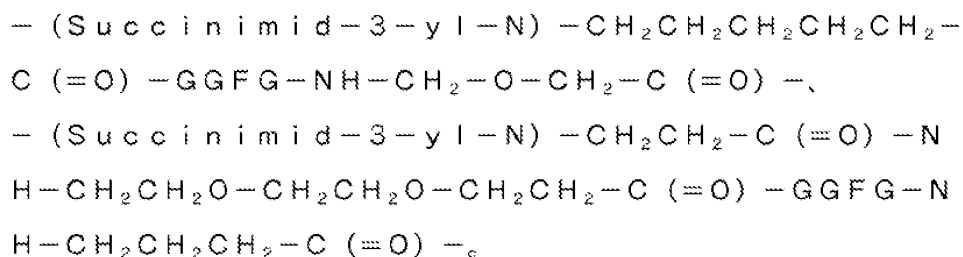
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[0166] これらのうちでより好ましくは、次のものである。



[0167] さらに、好ましくは、次のものを挙げることができる。



[0168] また、上記の反応式において抗体-薬物コンジュゲート(1)では、薬物からリンカー末端までの構造部分1個が1個の抗体に対して結合した構造として記載されているが、これは説明のための便宜的な記載であって、実際には当該構造部分が1個の抗体分子に対して複数個が結合している場合が多い。この状況は以下の製造方法の説明においても同様である。

[0169] すなわち、既知の方法によって入手しうる化合物(2)(例えば、US 2016/297890号公開特許公報に記載の方法(例えば段落[0336]～[0374]に記載の方法)で入手可能)で入手可能)と、スルフヒドリル基を有する抗体(3a)を反応させることによって、抗体-薬物コンジュゲート(1)を製造することができる。

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[0170] スルフヒドリル基を有する抗体（3a）は、当業者周知の方法で得ることができる（Hermanson, G. T., *Bioconjugate Techniques*, pp. 56-136, pp. 456-493, Academic Press (1996)）。例えば、Traut's 試薬を抗体のアミノ基に作用させる；N-サクシンイミジルS-アセチルチオアルカノエート類を抗体のアミノ基に作用させた後、ヒドロキシルアミンを作用させる；N-サクシンイミジル3-（ピリジルジチオ）プロピオネートを作用させた後、還元剤を作用させる；ジチオトレイトール、2-メルカプトエタノール、トリス（2-カルボキシエチル）ホスフィン塩酸塩（TCEP）等の還元剤を抗体に作用させて抗体内鎖間部のジスルフィド結合を還元しスルフヒドリル基を生成させる；等の方法を挙げることができるがこれらに限定されることはない。

[0171] 具体的には、還元剤としてTCEPを、抗体内鎖間部ジスルフィド一個当たりに対して0.3乃至3モル当量用い、キレート剤を含む緩衝液中で、抗体と反応させることで、抗体内鎖間部ジスルフィドが部分的もしくは完全に還元された抗体を得ることができる。キレート剤としては、例えばエチレンジアミン四酢酸（EDTA）やジエチレントリアミン5酢酸（DTPA）等を挙げることができる。これ等を1mM乃至20mMの濃度で用いればよい。緩衝液としては、リン酸ナトリウムやホウ酸ナトリウム、酢酸ナトリウム溶液等を用いることができる。具体的な例において、抗体は4℃乃至37℃にて1乃至4時間TCEPと反応させることで部分的もしくは完全に還元されたスルフヒドリル基を有する抗体（3a）を得ることが出来る。

[0172] なお、ここでスルフヒドリル基を薬物-リンカー部分に付加させる反応を実施させることでチオエーテル結合によって薬物-リンカー部分を結合させることができる。

[0173] 次に、スルフヒドリル基を有する抗体（3a）一個あたり、2乃至20モル当量の化合物（2）を使用して、抗体1個当たり2個乃至8個の薬物が結合した抗体-薬物コンジュゲート（1）を製造することができる。具体的に

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は、スルフヒドリル基を有する抗体（3 a）を含む緩衝液に、化合物（2）を溶解させた溶液を加えて反応させればよい。ここで、緩衝液としては、酢酸ナトリウム溶液、リン酸ナトリウムやホウ酸ナトリウム、等を用いればよい。反応時のpHは5乃至9であり、より好適にはpH7付近で反応させればよい。化合物（2）を溶解させる溶媒としては、ジメチルスルホキシド（DMSO）、ジメチルホルムアミド（DMF）、ジメチルアセトアミド（DMA）、N-メチル-2-ピリドン（NMP）等の有機溶媒を用いることができる。化合物（2）を溶解させた有機溶媒溶液を、スルフヒドリル基を有する抗体（3 a）を含む緩衝液に1乃至20% v/vを加えて反応させればよい。反応温度は、0乃至37℃、より好適には10乃至25℃であり、反応時間は、0.5乃至2時間である。反応は、未反応の化合物（2）の反応性をチオール含有試薬によって失活させることによって終了できる。チオール含有試薬は例えば、システイン又はN-アセチル-L-システイン（NAC）である。より具体的には、NACを、用いた化合物（2）に対して、1乃至2モル当量加え、室温で10乃至30分インキュベートすることにより反応を終了できる。

[0174] （4）抗体-薬物コンジュゲートの同定

製造した抗体-薬物コンジュゲート（1）は、以下の共通操作によって濃縮、バッファー交換、精製、抗体濃度及び抗体-分子あたりの薬物平均結合数の測定を行い、抗体-薬物コンジュゲート（1）の同定を行うことができる。

[0175] （4）-1 共通操作A：抗体もしくは抗体-薬物コンジュゲート水溶液の濃縮

Amicon Ultra（50,000 MWCO, Millipore Corporation）の容器内に抗体もしくは抗体-薬物コンジュゲート溶液を入れ、遠心機（Allegra X-15R, Beckman Coulter, Inc.）を用いた遠心操作（2000G乃至3800Gにて5乃至20分間遠心）にて、抗体もしくは抗体-薬物コンジュゲート

溶液を濃縮した。

[0176] (4) - 2 共通操作B：抗体の濃度測定

UV測定器 (Nanodrop 1000, Thermo Fisher Scientific Inc.) を用いて、メーカー規定の方法に従い、抗体濃度の測定を行った。その際に、抗体ごとに異なる280nm吸光係数 ($1.3 \text{ mL mg}^{-1} \text{ cm}^{-1}$ 乃至 $1.8 \text{ mL mg}^{-1} \text{ cm}^{-1}$) を用いた。

[0177] (4) - 3 共通操作C：抗体のバッファー交換

Sephadex G-25担体を使用したNAP-25カラム (Cat. No. 17-0852-02, GE Healthcare Japan Corporation) を、メーカー規定の方法に従い、塩化ナトリウム (50mM) 及びEDTA (2mM) を含むリン酸緩衝液 (50mM, pH 6.0) (本明細書でPBS 6.0/EDTAと称する。) にて平衡化させた。このNAP-25カラム一本につき、抗体水溶液2.5mLをのせたのち、PBS 6.0/EDTA 3.5mLで溶出させた画分 (3.5mL) を分取した。この画分を共通操作Aによって濃縮し、共通操作Bを用いて抗体濃度の測定を行ったのちに、PBS 6.0/EDTAを用いて20mg/mLに抗体濃度を調整した。

[0178] (4) - 4 共通操作D：抗体-薬物コンジュゲートの精製

市販のSorbitol (5%) を含む酢酸緩衝液 (10mM, pH 5.5; 本明細書でABSと称する。) のいずれかの緩衝液でNAP-25カラムを平衡化させた。このNAP-25カラムに、抗体-薬物コンジュゲート反応水溶液 (約2.5mL) をのせ、メーカー規定の量の緩衝液で溶出させることで、抗体画分を分取した。この分取画分を再びNAP-25カラムにのせ緩衝液で溶出させるゲルろ過精製操作を計2乃至3回繰り返すことで、未結合の薬物リンカーや低分子化合物 (トリス (2-カルボキシエチル) ホスフィン塩酸塩 (TCEP), N-アセチル-L-システイン (NAC), ジメチルスルホキシド) を除いた抗体-薬物コンジュゲートを得た。

[0179] (4) - 5 共通操作E：抗体-薬物コンジュゲートにおける抗体濃度及

び抗体一分子あたりの薬物平均結合数の測定

抗体－薬物コンジュゲートにおける結合薬物濃度は、抗体－薬物コンジュゲート水溶液の280 nm及び370 nmの二波長におけるUV吸光度を測定したのちに下記の計算を行うことで、算出することができる。

[0180] ある波長における全吸光度は系内に存在する全ての吸収化学種の吸光度の和に等しい〔吸光度の加成性〕ことから、抗体と薬物のコンジュゲーション前後において、抗体及び薬物のモル吸光係数に変化がないと仮定すると、抗体－薬物コンジュゲートにおける抗体濃度及び薬物濃度は、下記の関係式で示される。

$$A_{280} = A_{D, 280} + A_{A, 280} = \varepsilon_{D, 280} C_D + \varepsilon_{A, 280} C_A \quad \text{式 (1)}$$

$$A_{370} = A_{D, 370} + A_{A, 370} = \varepsilon_{D, 370} C_D + \varepsilon_{A, 370} C_A \quad \text{式 (2)}$$

ここで、 A_{280} は280 nmにおける抗体－薬物コンジュゲート水溶液の吸光度を示し、 A_{370} は370 nmにおける抗体－薬物コンジュゲート水溶液の吸光度を示し、 $A_{A, 280}$ は280 nmにおける抗体の吸光度を示し、 $A_{A, 370}$ は370 nmにおける抗体の吸光度を示し、 $A_{D, 280}$ は280 nmにおけるコンジュゲート前駆体の吸光度を示し、 $A_{D, 370}$ は370 nmにおけるコンジュゲート前駆体の吸光度を示し、 $\varepsilon_{A, 280}$ は280 nmにおける抗体のモル吸光係数を示し、 $\varepsilon_{A, 370}$ は370 nmにおける抗体のモル吸光係数を示し、 $\varepsilon_{D, 280}$ は280 nmにおけるコンジュゲート前駆体のモル吸光係数を示し、 $\varepsilon_{D, 370}$ は370 nmにおけるコンジュゲート前駆体のモル吸光係数を示し、 C_A は抗体－薬物コンジュゲートにおける抗体濃度を示し、 C_D は抗体－薬物コンジュゲートにおける薬物濃度を示す。

[0181] ここで、 $\varepsilon_{A, 280}$ 、 $\varepsilon_{A, 370}$ 、 $\varepsilon_{D, 280}$ 、 $\varepsilon_{D, 370}$ は、事前に用意した値（計算推定値もしくは化合物のUV測定から得られた実測値）が用いられる。例えば、 $\varepsilon_{A, 280}$ は、抗体のアミノ酸配列から、既知の計算方法（Protein Science, 1995, vol. 4, 2411–2423）によって推定することが出来る。 $\varepsilon_{A, 370}$ は、通常、ゼロである。 $\varepsilon_{D, 280}$ 及び $\varepsilon_{D, 370}$ は、用いるコンジュゲート前駆体をあるモル濃度に溶解させた溶液の吸

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光度を測定することで、ランベルト・ベールの法則（吸光度＝モル濃度×モル吸光係数×セル光路長）によって、得ることができる。抗体－薬物コンジュゲート水溶液の A_{280} 及び A_{370} を測定し、これらの値を式（１）及び（２）に代入して連立方程式を解くことによって、 C_A 及び C_D を求めることができる。さらに C_D を C_A で除することで１抗体あたりの薬物平均結合数が求められることができる。

[0182] （４）－６ 共通操作F：抗体－薬物コンジュゲートにおける抗体一分子あたりの薬物平均結合数の測定（２）

抗体－薬物コンジュゲートにおける抗体一分子あたりの薬物平均結合数は、前述の「（４）－５ 共通操作E」に加え、以下の方法を用いる高速液体クロマトグラフィー（HPLC）分析によっても求めることができる。以下に、抗体と薬物リンカーがジスルフィド結合している場合のHPLCによる薬物平均結合数の測定方法を記載する。当業者は、この方法を参照して、抗体と薬物リンカーとの結合様式に依存して適宜、HPLCにより薬物平均結合数を測定し得る。

[0183] F－１．HPLC分析用サンプルの調製（抗体－薬物コンジュゲートの還元）

抗体－薬物コンジュゲート溶液（約 1 mg/mL 、 $60\text{ }\mu\text{L}$ ）をジチオトレイトール（DTT）水溶液（ 100 mM 、 $15\text{ }\mu\text{L}$ ）と混合する。混合物を 37°C で 30 分インキュベートすることで、抗体－薬物コンジュゲートの軽鎖及び重鎖間のジスルフィド結合を切断したサンプルを、HPLC分析に用いる。

[0184] F－２．HPLC分析

HPLC分析を、下記の測定条件にて行う。

[0185] HPLCシステム：Agilent 1290 HPLCシステム（Agilent Technologies）

検出器：紫外吸光度計（測定波長： 280 nm ）

カラム：ACQUITY UPLC BEH Phenyl（ 2.1×5

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0 mm、1.7 μ m、130 Å; Waters, P/N 186002884)

カラム温度: 80°C

移動相 A: 0.10% トリフルオロ酢酸 (TFA)、15% 2-プロパノールを含む水溶液

移動相 B: 0.075% TFA、15% 2-プロパノールを含むアセトニトリル溶液

グラジエントプログラム: 14%–36% (0分–15分)、36%–80% (15分–17分)、80%–14% (17分–17.01分)、14% (17.01分–25分)

サンプル注入量: 10 μ L

F-3. データ解析

F-3-1 薬物の結合していない抗体の軽鎖 (L0) 及び重鎖 (H0) に対して、薬物の結合した軽鎖 (薬物が i 個結合した軽鎖: L_i) 及び重鎖 (薬物が i 個結合した重鎖: H_i) は、結合した薬物の数に比例して疎水性が増し保持時間が大きくなることから、例えば、L0、L1、H0、H1、H2、H3の順に溶出される。L0及びH0との保持時間比較により検出ピークをL0、L1、H0、H1、H2、H3のいずれかに割り当てることができる。薬物結合数は、当業者によって定義され得るが、好ましくは、L0、L1、H0、H1、H2、H3である。

[0186] F-3-2 薬物リンカーにUV吸収があるため、薬物リンカーの結合数に応じて、軽鎖、重鎖及び薬物リンカーのモル吸光係数を用いて下式に従ってピーク面積値の補正を行う。

[0187] [数2]

$$\text{薬物が } i \text{ 個結合した軽鎖ピーク面積補正值}(A_{Li}) = \text{ピーク面積} \\ \times \frac{\text{軽鎖のモル吸光係数}}{\text{軽鎖のモル吸光係数} + \text{結合薬物数}(i) \times \text{薬物リンカーのモル吸光係数}}$$

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[0188] [数3]

$$\begin{aligned} \text{薬物が } i \text{ 個結合した重鎖ピーク面積補正值 } (A_{HI}) &= \text{ピーク面積} \\ &\times \frac{\text{重鎖のモル吸光係数}}{\text{重鎖のモル吸光係数} + \text{結合薬物数 } (i) \times \text{薬物リンカーのモル吸光係数}} \end{aligned}$$

[0189] ここで、各抗体における軽鎖及び重鎖のモル吸光係数（280nm）は、既知の計算方法（Protein Science, 1995, vol. 4, 2411-2423）によって、各抗体の軽鎖及び重鎖のアミノ酸配列から推定される値を用いることができる。H01L02の場合、そのアミノ酸配列に従って、軽鎖のモル吸光係数として31710を、重鎖のモル吸光係数として79990を推定値として用いた。また、薬物リンカーのモル吸光係数（280nm）は、各薬物リンカーをメルカプトエタノール又はN-アセチルシステインで反応させ、マレイミド基をサクシニイミドチオエーテルに変換した化合物の実測のモル吸光係数（280nm）を用いた。吸光度を測定する波長は、当業者によって適宜設定され得るが、好ましくは、抗体のピークが測定され得る波長であり、より好ましくは280nmである。

[0190] F-3-3 ピーク面積補正值合計に対する各鎖ピーク面積比（%）を下式に従って計算する。

[0191] [数4]

$$\begin{aligned} \text{薬物が } i \text{ 個結合した軽鎖ピーク面積比} &= \frac{A_{LI}}{A_{LO} + A_{LI}} \times 100 \\ \text{薬物が } i \text{ 個結合した重鎖ピーク面積比} &= \frac{A_{HI}}{A_{HO} + A_{HI} + A_{H2} + A_{H3}} \times 100 \\ A_{LI}, \quad A_{HI} : L_i, \quad H_i &\text{ 各々のピーク面積補正值} \end{aligned}$$

[0192] F-3-4 抗体-薬物コンジュゲートにおける抗体一分子あたりの薬物平均結合数を、下式に従って計算する。

[0193] 薬物平均結合数 = (L₀ピーク面積比 × 0 + L₁ピーク面積比 × 1 + H₀ピー

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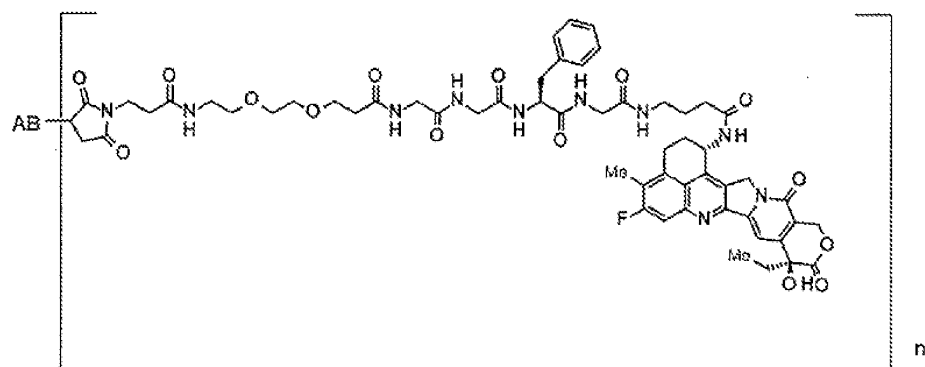
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ク面積比×0+H₁ピーク面積比×1+H₂ピーク面積比×2+H₃ピーク面積比×3)／100×2

なお、抗体－薬物コンジュゲートの量を確保するために、同様な条件で作製して得られた平均薬物数が同程度の複数の抗体－薬物コンジュゲート（例えば±1程度）を混合して新たなロットにすることができる。その場合、平均薬物数は混合前の平均薬物数の間に収まる。

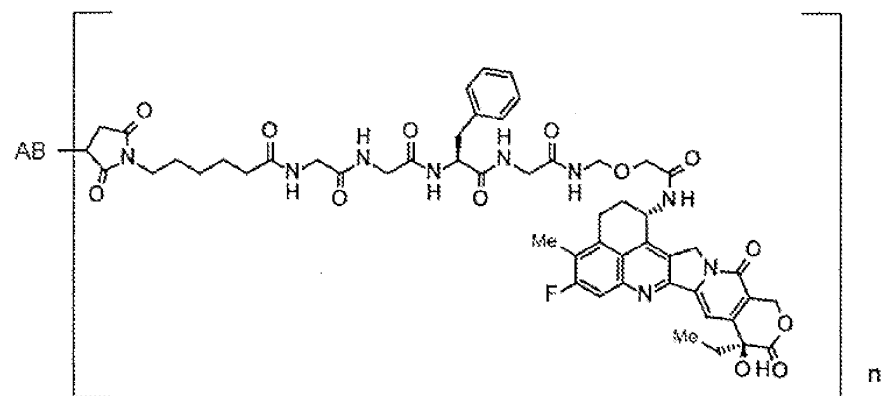
[0194] 本発明の抗体－薬物コンジュゲートの一つの具体例としては次式：

[0195] [化9]



[0196] 又は次式：

[0197] [化10]



[0198] で示される構造を有するものを挙げることができる。

[0199] ここで、ABは本明細書で開示される抗CDH6抗体を示し、抗体由来の

スルフヒドリル基を介して結合リンカーと結合している。ここで、 n はいわゆるDAR (Drug-to-Antibody Ratio) と同義であり、1抗体あたりの薬物抗体比を示す。すなわち、抗体1分子への薬物の結合数を示すが、これは平均値、すなわち、平均薬物結合数として特定され、表記される数値である。本発明の[化9]及び[化10]で示される抗体-薬物コンジュゲートの場合、共通操作Fによる測定で、 n は、2から8であればよく、好ましくは5から8であり、更に好ましくは7から8であり、更に好ましくは8である。

[0200] 本発明の抗体薬物-コンジュゲートの一例として、上記式[化9]又は[化10]に示される構造において、ABで示される抗体が、以下の(a)乃至(g)からなる群から選択されるいずれか1項に記載の重鎖及び軽鎖の抗体又はその機能性断片を含む、抗体-薬物コンジュゲート又はその薬理学的許容される塩を挙げることができる：

(a) 配列番号61に示される軽鎖全長アミノ酸配列の21～233番目のアミノ酸配列からなる軽鎖及び配列番号69に示される重鎖全長アミノ酸配列の20～471番目のアミノ酸配列からなる重鎖からなる抗体；

(b) 配列番号61に示される軽鎖全長アミノ酸配列の21～233番目のアミノ酸配列からなる軽鎖及び配列番号73に示される重鎖全長アミノ酸配列の20～471番目のアミノ酸配列からなる重鎖からなる抗体；

(c) 配列番号61に示される軽鎖全長アミノ酸配列の21～233番目のアミノ酸配列からなる軽鎖及び配列番号77に示される重鎖全長アミノ酸配列の20～471番目のアミノ酸配列からなる重鎖からなる抗体；

(d) 配列番号65に示される軽鎖全長アミノ酸配列の21～233番目のアミノ酸配列からなる軽鎖及び配列番号69に示される重鎖全長アミノ酸配列の20～471番目のアミノ酸配列からなる重鎖からなる抗体；

(e) 配列番号65に示される軽鎖全長アミノ酸配列の21～233番目のアミノ酸配列からなる軽鎖及び配列番号73に示される重鎖全長アミノ酸配列の20～471番目のアミノ酸配列からなる重鎖からなる抗体；

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(f) 配列番号 65 に示される軽鎖全長アミノ酸配列の 21～233 番目のアミノ酸配列からなる軽鎖及び配列番号 77 に示される重鎖全長アミノ酸配列の 20～471 番目のアミノ酸配列からなる重鎖からなる抗体；又は

(g) 重鎖又は軽鎖が N-結合への糖鎖付加、O-結合への糖鎖付加、N 末のプロセッシング、C 末のプロセッシング、脱アミド化、アスパラギン酸の異性化、メチオニンの酸化、N 末にメチオニン残基の付加、プロリン残基のアミド化、N 末グルタミンや N 末グルタミン酸のピログルタミン酸化などに代表される翻訳後修飾、及びカルボキシル末端における 1 つ又は 2 つのアミノ酸欠失からなる群より選択される 1 又は 2 以上の修飾を含む、(a)～(f) からなる群から選択されるいずれか 1 つに記載の抗体。

[0201] 4. 医薬

上記、「2. 抗 CDH6 抗体の製造」の項及び実施例に記載された本発明の抗 CDH6 抗体及び当該抗体の機能性断片は、腫瘍細胞表面の CDH6 に結合し、内在化活性を有することから、単独であるいは他の薬剤と組み合わせて、医薬として、腎細胞腫瘍及び卵巣腫瘍等のがん、例えば、腎細胞癌、腎臓明細胞癌、乳頭状腎細胞癌、卵巣癌、卵巣漿液性腺癌、甲状腺癌、胆管癌、肺癌（例えば、小細胞肺癌又は非小細胞肺癌）、神経膠芽腫、中皮腫、子宮癌、膵臓癌、ウィルムス腫瘍又は神経芽腫の治療剤として用いることができる。

[0202] また、CDH6 を発現している細胞の検出に用いることができる。

[0203] 更に、本発明の抗 CDH6 抗体及び当該抗体の機能性断片は、内在化活性を有することから、抗体-薬物コンジュゲートに用いる抗体として供することができる。

[0204] 上記「3. 抗 CDH6 抗体-薬物コンジュゲート」の項及び実施例に記載の本発明の抗 CDH6 抗体-薬物コンジュゲートのうちで薬物として細胞傷害活性等の抗腫瘍活性を有する薬物が用いられているものは、内在化活性を有する抗 CDH6 抗体及び／又は当該抗体の機能性断片と細胞傷害活性等の抗腫瘍活性を有する薬物のコンジュゲートであり、CDH6 を発現している

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がん細胞に対して抗腫瘍活性を示すことから、医薬として、特にがんに対する治療剤及び／又は予防剤として使用することができる。

[0205] 本発明の抗CDH6抗体-薬物コンジュゲートは、大気中に放置したり、又は再結晶や精製操作をしたりすることにより、水分を吸収し、あるいは吸着水が付着するなどして、水和物になる場合があり、そのような水を含む化合物又は薬理学的に許容され得る塩も本発明に包含される。

[0206] 本発明の抗CDH6抗体-薬物コンジュゲートが、アミノ基などの塩基性基を有する場合、所望により薬理学的に許容され得る酸付加塩を形成することができる。そのような酸付加塩としては、例えばフッ化水素酸塩、塩酸塩、臭化水素酸塩、ヨウ化水素酸塩などのハロゲン化水素酸塩；硝酸塩、過塩素酸塩、硫酸塩、燐酸塩などの無機酸塩；メタンスルホン酸塩、トリフルオロメタンスルホン酸塩、エタンスルホン酸塩などの低級アルカンスルホン酸塩；ベンゼンスルホン酸塩、p-トルエンスルホン酸塩などのアリールスルホン酸塩；蟻酸塩、酢酸塩、トリフルオロ酢酸塩、リンゴ酸塩、フマル酸塩、コハク酸塩、クエン酸塩、酒石酸塩、シュウ酸塩、マレイン酸塩などの有機酸塩；又はオルニチン酸塩、グルタミン酸塩、アスパラギン酸塩などのアミノ酸塩などを挙げることができる。

[0207] 本発明の抗CDH6抗体-薬物コンジュゲートが、カルボキシ基などの酸性基を有する場合、所望により薬理学的に許容され得る塩基付加塩を形成することができる。そのような塩基付加塩としては、例えばナトリウム塩、カリウム塩、リチウム塩などのアルカリ金属塩；カルシウム塩、マグネシウム塩などのアルカリ土類金属塩；アンモニウム塩などの無機塩；ジベンジルアミン塩、モルホリン塩、フェニルグリシンアルキルエステル塩、エチレンジアミン塩、N-メチルグルカミン塩、ジエチルアミン塩、トリエチルアミン塩、シクロヘキシルアミン塩、ジシクロヘキシルアミン塩、N, N'-ジベンジルエチレンジアミン塩、ジエタノールアミン塩、N-ベンジル-N-(2-フェニルエトキシ)アミン塩、ピペラジン塩、テトラメチルアンモニウム塩、トリス(ヒドロキシメチル)アミノメタン塩などの有機アミン塩など

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を挙げることができる。

[0208] 本発明はまた、抗体－薬物コンジュゲートを構成する原子の1以上が、その原子の同位体で置換された抗CDH6抗体－薬物コンジュゲートを包含し得る。同位体には放射性同位体及び安定同位体の2種類が存在し、同位体の例としては、例えば、水素の同位体（ 2H 及び 3H ）、炭素の同位体（ 11C 、 13C 及び 14C ）、窒素の同位体（ 13N 及び 15N ）、酸素の同位体（ 15O 、 17O 及び 18O ）、フッ素の同位体（ 18F ）などを挙げることができる。同位体で標識された抗体－薬物コンジュゲートを含む組成物は、例えば、治療剤、予防剤、研究試薬、アッセイ試薬、診断剤、インビボ画像診断剤などとして有用である。同位体で標識された抗体－薬物コンジュゲート、及び、同位体で標識された抗体－薬物コンジュゲートの任意の割合の混合物もすべて本発明に包含される。同位体で標識された抗体－薬物コンジュゲートは、当該分野で公知の方法により、例えば、後述する本発明の製造方法における原料の代わりに同位体で標識された原料を用いることにより、製造することができる。

[0209] *in vitro*での殺細胞活性は例えば、細胞の増殖抑制活性で測定することができる。例えば、CDH6を過剰発現している癌細胞株を培養し、培養系に種々の濃度で抗CDH6抗体－薬物コンジュゲートを添加し、フォーカス活性、コロニー形成及びスフェロイド増殖に対する抑制活性を測定することができる。ここで、例えば、腎細胞腫瘍及び卵巣腫瘍由来がん細胞株等を用いることで、腎細胞腫瘍及び卵巣腫瘍に対する細胞増殖抑制活性を調べることができる。

[0210] *in vivo*での実験動物を用いたがんに対する治療効果は、例えば、CDH6を高発現している腫瘍細胞株を移植したヌードマウスに抗CDH6抗体－薬物コンジュゲートを投与し、がん細胞の変化を測定することができる。ここで、例えば、腎細胞癌、腎淡明細胞癌、乳頭状腎細胞癌、卵巣癌、卵巣漿液性腺癌又は甲状腺癌由来の細胞を免疫不全マウスに移植した動物モデルを用いることで、腎細胞癌、腎淡明細胞癌、乳頭状腎細胞癌、卵巣癌、

卵巣漿液性腺癌又は甲状腺癌に対する治療効果を測定することができる。

[0211] 本発明の抗CDH6抗体-薬物コンジュゲートが適用されるがんの種類としては、治療対象となるがん細胞においてCDH6を発現しているがんであれば特に制限されないが、例えば、腎細胞癌（例えば、腎淡明細胞癌又は乳頭状腎細胞癌）、卵巣癌、卵巣漿液性腺癌、甲状腺癌、胆管癌、肺癌（例えば、小細胞肺癌又は非小細胞肺癌）、神経膠芽腫、中皮腫、子宮癌、膵臓癌、ウィルムス腫瘍又は神経芽腫を挙げることができるが、CDH6を発現している限りこれらに制限されない。より好ましいがんの例としては、腎細胞癌（例えば、腎淡明細胞癌、乳頭状腎細胞癌）又は卵巣癌を挙げることができる。

[0212] 本発明の抗CDH6抗体-薬物コンジュゲートは、哺乳動物に対して好適に投与することができるが、より好ましくはヒトである。

[0213] 本発明の抗CDH6抗体-薬物コンジュゲートが含まれる医薬組成物において使用される物質としては、投与量や投与濃度において、この分野において通常使用される製剤添加物その他から適宜選択して適用することができる。

[0214] 本発明の抗CDH6抗体-薬物コンジュゲートは、1種以上の薬学的に適合性の成分を含む薬学的組成物として投与され得る。例えば、上記薬学的組成物は、代表的には、1種以上の薬学的キャリア（例えば、滅菌した液体（例えば、水及び油（石油、動物、植物、又は合成起源の油（例えば、ラッカセイ油、大豆油、鉱油、ごま油など）を含む））を含む。水は、上記薬学的組成物が静脈内投与される場合に、より代表的なキャリアである。食塩水溶液、ならびにデキストロース水溶液及びグリセロール水溶液もまた、液体キャリアとして、特に、注射用溶液のために使用され得る。適切な薬学的賦形剤は、当該分野で公知である。上記組成物はまた、所望であれば、微量の湿潤剤もしくは乳化剤、又はpH緩衝化剤を含み得る。適切な薬学的キャリアの例は、E. W. Martinによる「Remington's Pharmaceutical Sciences」に記載される。その処方、投

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与の態様に対応する。

【0215】 種々の送達システムが公知であり、本発明の抗CDH6抗体-薬物コンジュゲートを投与するために使用され得る。導入方法としては、皮内、筋肉内、腹腔内、静脈内、及び皮下の経路が挙げられるが、これらに限定されない。投与は、例えば、注入又はボラス注射によるものであり得る。特定の好ましい実施形態において、上記抗体-薬物コンジュゲートの投与は、注入によるものである。非経口的投与は、好ましい投与経路である。

【0216】 代表的実施形態において、上記薬学的組成物は、ヒトへの静脈内投与に適合した薬学的組成物として、常習的手順に従って処方される。代表的には、静脈内投与のための組成物は、滅菌の等張性の水性緩衝液中の溶液である。必要である場合、上記医薬はまた、可溶化剤及び注射部位での疼痛を和らげるための局所麻酔剤（例えば、リグノカイン）を含み得る。一般に、上記成分は、（例えば、活性剤の量を示すアンプル又はサシェなどに密封してシールされた容器中の乾燥凍結乾燥粉末又は無水の濃縮物として、別個に、又は単位剤形中で一緒に混合して、のいずれかで供給される。上記医薬が注入によって投与される予定である場合、それは、例えば、滅菌の製薬グレードの水又は食塩水を含む注入ボトルで投薬され得る。上記医薬が注射によって投与される場合、注射用滅菌水又は食塩水のアンプルは、例えば、上記成分が投与前に混合され得るように、提供され得る。

【0217】 本発明の医薬組成物には本願の抗CDH6抗体-薬物コンジュゲートのみを含む医薬組成物であってもよいし、抗CDH6抗体-薬物コンジュゲート及び少なくとも一つのこれ以外のがん治療剤を含む医薬組成物であってもよい。本発明の抗CDH6抗体-薬物コンジュゲートは、他のがん治療剤と共に投与することもでき、これによって抗がん効果を増強させることができる。このような目的で使用される他の抗がん剤は、抗体-薬物コンジュゲートと同時に、別々に、あるいは連続して個体に投与されてもよいし、それぞれの投与間隔を変えて投与してもよい。このようながん治療剤として、Imatinib、Sunitinib、Regorafenibなどを含むチロ

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シンキナーゼ阻害剤、Palbociclibなどを含むCDK4／6阻害剤、TAS-116などを含むHSP90阻害剤、MEK162などを含むMEK阻害剤、ニボルマブ、ペンブロリスマブ、イピリムマブなどを含む免疫チェックポイント阻害剤等を挙げることができるが、抗腫瘍活性を有する薬剤であれば限定されることはない。

【0218】 このような医薬組成物は、選択された組成と必要な純度を持つ製剤として、凍結乾燥製剤あるいは液状製剤として製剤化すればよい。凍結乾燥製剤として製剤化する際には、この分野において使用される適当な製剤添加物が含まれる製剤であってもよい。また液剤においても同様にして、この分野において使用される各種の製剤添加物を含む液状製剤として製剤化することができる。

【0219】 医薬組成物の組成及び濃度は投与方法によっても変化するが、本発明の医薬組成物に含まれる抗CDH6抗体-薬物コンジュゲートは、抗体-薬物コンジュゲートの抗原に対する親和性、すなわち、抗原に対する解離定数（ K_d 値）の点において、親和性が高い（ K_d 値が低い）ほど、少量の投与量であっても薬効を発揮させることができる。したがって、抗体-薬物コンジュゲートの投与量の決定に当たっては、抗体-薬物コンジュゲートと抗原との親和性の状況に基づいて投与量を設定することもできる。本発明の抗体-薬物コンジュゲートをヒトに対して投与する際には、例えば、約0.001～100mg/kgを1回あるいは1～180日間に1回の間隔で複数回投与すればよい。好適には、0.1～50mg/kgを、さらに好適には、1～50mg/kg、1～30mg/kg、1～20mg/kg、1～15mg/kg、2～50mg/kg、2～30mg/kg、2～20mg/kg又は2～15mg/kgを1～4週間に1回、好ましくは2～3週間に1回の間隔で複数回投与すればよい。

実施例

【0220】 以下に示す実施例によって本発明を具体的に説明するが、本発明はこれらに限定されるものではない。また、これらはいかなる意味においても限定的

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に解釈されるものではない。なお、下記実施例において遺伝子操作に関する各操作は特に明示がない限り、「モレキュラークローニング (Molecular Cloning)」(Sambrook, J., Fritsch, E. F. 及び Maniatis, T. 著, Cold Spring Harbor Laboratory Pressより1989年発刊) に記載の方法及びその他の当業者が使用する実験書に記載の方法により行うか、又は、市販の試薬やキットを用いる場合には市販品の指示書に従って行った。また、本明細書において、特に記載のない試薬、溶媒及び出発材料は、市販の供給源から容易に入手可能である。

[0221] [実施例1：内在化活性を有するラット抗ヒトCDH6抗体の取得]

1) -1 ヒト、マウス、ラットおよびカニクイザルCDH6発現ベクターの構築

ヒトCDH6タンパク質 (NP_004923) をコードするcDNA発現ベクター (OriGene社、RC217889) を用いて、当業者に公知な方法に従い哺乳動物発現用ベクターに組み込むことによってヒトCDH6発現ベクターpCDNA3.1-hCDH6が作製された。ヒトCDH6 ORF (Open Reading Frame) のアミノ酸配列を配列番号1に示す。

[0222] マウスCDH6タンパク質 (NP_031692) をコードするcDNA発現ベクター (OriGene社、MC221619) を用いて、当業者に公知な方法に従い哺乳動物発現用ベクターに組み込むことによってマウスCDH6発現ベクターpCDNA3.1-mCDH6、及びp3xFLAG-CMV-9-mCDH6が作製された。マウスCDH6 ORFのアミノ酸配列を配列番号7に示す。

[0223] ラットCDH6タンパク質 (NP_037059) をコードするcDNA発現ベクター (OriGene社、RN211850) の各cDNA部分を用いて、当業者に公知な方法に従い哺乳動物発現用ベクターに組み込むことによってヒトCDH6発現ベクターpCDNA3.1-rCDH6、及びp

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3xFLAG-CMV-9-rCDH6が作製された。ラットCDH6 ORFのアミノ酸配列を配列番号8に示す。

[0224] カニクイザルCDH6タンパク質をコードするcDNAは、カニクイザル腎臓のtotal RNAより合成したcDNAを鋳型にprimer 1 (5'-CACCATGAGAACTTACCGCTACTTCTTGTGCTC-3') (配列番号85) 及びprimer 2 (5'-TTAGGAGTCTTTGTCACTGTCCACTCCTCC-3') (配列番号86) を用いてクローニングを行った。得られた配列が、カニクイザルCDH6 (NCBI, XP_005556691.1) の細胞外領域と一致することを確認した。また、EMBLで登録されたカニクイザルCDH6 (EHH54180.1) と全長配列が一致することを確認した。当業者に公知な方法に従い哺乳動物発現用ベクターに組み込むことによってカニクイザルCDH6発現ベクターpcDNA3.1-cynoCDH6が作製された。カニクイザルCDH6 ORFのアミノ酸配列を配列番号9に示す。

[0225] 作製したプラスミドDNAの大量調製は、EndoFree Plasmid Giga Kit (QIAGEN) を用いて行った。

[0226] 1) -2 免疫

免疫にはWKY/Izmラットの雌 (日本エスエルシー社) を使用した。まずラット両足下腿部をHyaluronidase (SIGMA-ALDRICH社) にて前処理後、同部位に実施例1) -1で作製したヒトCDH6発現ベクターpcDNA3.1-hCDH6を筋肉内注射した。続けて、ECM830 (BTX社) を使用し、2ニードル電極を用いて、同部位にインビボエレクトロポレーションを実施した。約二週間に一度、同様のインビボエレクトロポレーションを繰り返した後、ラットのリンパ節又は脾臓を採取しハイブリドーマ作製に用いた。

[0227] 1) -3 ハイブリドーマ作製

リンパ節細胞あるいは脾臓細胞とマウスミエローマSP2/O-ag14細胞 (ATCC, No. CRL-1581) とをLF301 Cell

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Fusion Unit (BEX社)を用いて電気細胞融合した後、ClonaCell-HY Selection Medium D (Stem Cell Technologies社)に懸濁し、希釈して37℃、5% CO₂の条件下で培養した。出現した各々のハイブリドーマコロニーは、モノクローンとして回収され、ClonaCell-HY Selection Medium E (Stem Cell Technologies社)に懸濁して37℃、5% CO₂の条件下で培養した。適度に細胞が増殖した後、各々のハイブリドーマ細胞の凍結ストックを作製すると共に、得られたハイブリドーマ培養上清を抗ヒトCDH6抗体産生ハイブリドーマのスクリーニングに用いた。

[0228] 1) -4 Cell-ELISA法による抗体産生ハイブリドーマのスクリーニング

1) -4-1 Cell-ELISA用抗原遺伝子発現細胞の調製

293 α 細胞(インテグリン α v及びインテグリン β 3を発現するHEK 293由来の安定発現細胞株)を10% FBS含有DMEM培地中5 $\times$ 10⁵細胞/mLになるよう調製した。Lipofectamine 2000 (Thermo Fisher Scientific社)を用いた形質移入手順に従って、この293 α 細胞に対して、pcDNA3.1-hCDH6もしくはpcDNA3.1-cynoCDH6または、陰性コントロールとしてpcDNA3.1のDNAを導入し、96-well plate (Corning社)に100 μ Lずつ分注後、10% FBS含有DMEM培地中で37℃、5% CO₂の条件下で24から27時間培養した。得られた形質移入細胞を接着状態のまま、Cell-ELISAに使用した。

[0229] 1) -4-2 Cell-ELISA

実施例1) -4-1で調製した発現ベクター導入293 α 細胞の培養上清を除去後、pcDNA3.1-hCDH6もしくはpcDNA3.1-cynoCDH6またはpcDNA3.1導入293 α 細胞の各々に対しハイブリドーマ培養上清を添加し、4℃で1時間静置した。well中の細胞を5

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% FBS含有PBS (+) で1回洗浄後、5% FBS含有PBS (+) で500倍に希釈したAnti-Rat IgG-Peroxidase antibody produced in rabbit (SIGMA社) を加えて、4℃で1時間静置した。well中の細胞を5% FBS含有PBS (+) で3回洗浄した後、OPD発色液 (OPD溶解液 (0.05 M クエン酸3ナトリウム、0.1 M リン酸水素2ナトリウム・12水 pH4.5) にo-フェニレンジアミン二塩酸塩 (和光純薬社)、H₂O₂をそれぞれ0.4 mg/mL、0.6% (v/v) になるように溶解) を100 μ L/wellで添加した。時々攪拌しながら発色反応を行い、1 M HClを100 μ L/wellで添加して発色反応を停止させた後、プレートリーダー (ENVISION: PerkinElmer社) で490 nmの吸光度を測定した。コントロールのpcDNA3.1導入293 α 細胞と比較し、pcDNA3.1-hCDH6ならびにpcDNA3.1-cynoCDH6発現ベクター導入293 α 細胞の方でより高い吸光度を示す培養上清を産生するハイブリドーマをヒト、かつ、カニクイサルCDH6に結合する抗体産生ハイブリドーマとして選択した。

[0230] 1) -5 フローサイトメトリーによるカニクイサルCDH6への選択的結合抗体のスクリーニング

1) -5-1 フローサイトメトリー解析用抗原遺伝子発現細胞の調製

293T細胞を5 $\times$ 10⁴細胞/cm²になるよう225 cm²フラスコ (住友ベークライト社) に播種し、10% FBS含有DMEM培地中で37℃、5% CO₂の条件下で一晩培養した。この293T細胞に、pcDNA3.1-cynoCDH6または、陰性コントロールとしてpcDNA3.1をLipofectamine 2000を用いて導入し、37℃、5% CO₂の条件下でさらに一晩培養した。各ベクターを導入した293T細胞をTrypLE Express (Thermo Fisher Scientific社) で処理し、10% FBS含有DMEMで細胞を洗浄した後、5% FBS含有PBSに懸濁した。得られた細胞懸濁液をフローサイ

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トメトリー解析に使用した。

[0231] 1) -5-2 フローサイトメトリー解析

実施例1)-4のCell-ELISAで選択されたヒト、かつ、カニクイサルCDH6に結合する抗体産生ハイブリドーマが産生する抗体のカニクイサルCDH6に対する結合特異性をフローサイトメトリー法によりさらに確認した。実施例1)-5-1で調製した一過性発現293T細胞の懸濁液を遠心し、上清を除去した後、各々に対しハイブリドーマ培養上清を加えて懸濁し、4℃で1時間静置した。5% FBS含有PBSで2回洗浄した後、5% FBS含有PBSで500倍に希釈したAnti-Rat IgG FITC conjugate (SIGMA社)を加えて懸濁し、4℃で1時間静置した。5% FBS含有PBSで2回洗浄した後、2 μg/mL 7-aminoactinomycin D (Molecular Probes社)を含む5% FBS含有PBSに再懸濁し、フローサイトメーター (FC500: Beckman Coulter社)で検出した。データ解析はFlowJo (TreeStar社)で行った。7-aminoactinomycin D陽性の死細胞をゲートで除外した後、生細胞のFITC蛍光強度のヒストグラムを作成した。コントロールであるpcDNA3.1導入293T細胞の蛍光強度ヒストグラムに対しpcDNA3.1-cynoCDH6導入293T細胞のヒストグラムが強蛍光強度側にシフトしている抗体を産生するハイブリドーマを細胞膜表面上に発現するカニクイサルCDH6に特異的に結合する抗体産生ハイブリドーマとして選択した。

[0232] 1) -6 ラットモノクローナル抗体のアイソタイプの決定

実施例1)-5で選択したラット抗CDH6抗体産生ハイブリドーマの中から、ヒトならびにサルCDH6に強く特異的に結合することが示唆されたクローンrG019、rG055、rG056、rG061を選抜し、各抗体のアイソタイプを同定した。抗体の重鎖のサブクラス、軽鎖のタイプは、RAT MONOCLONAL ANTIBODY ISOTYPING TEST KIT (DSファーマバイオメディカル社)により決定された。

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。その結果、rG019、rG055、rG056、rG061の4クローンともサブクラスは、IgG2b、タイプは κ 鎖であることが確認された。

[0233] 1) -7 ラット抗CDH6抗体の調製

1) -7-1 培養上清作製

ラット抗ヒトCDH6モノクローナル抗体は、ハイブリドーマ培養上清から精製した。まず、ラット抗CDH6モノクローナル抗体産生ハイブリドーマをClonaCell-HY Selection Medium E (StemCell Technologies社)で充分量まで増殖させた後、Ultra Low IgG FBS (Thermo Fisher Scientific社)を20%添加したHybridoma SFM (Thermo Fisher Scientific社)に培地交換し、4-5日間培養した。本培養上清を回収し、0.8 μ mのフィルターに通した後、さらに0.2 μ mのフィルターに通して不溶物を除去した。

[0234] 1) -7-2 ラット抗CDH6抗体の精製

実施例1) -7-1で作製したハイブリドーマの培養上清から抗体(ラット抗CDH6抗体(rG019, rG055, rG056, rG061))をProtein Gアフィニティークロマトグラフィーで精製した。Protein Gカラム(GE Healthcare Bioscience社)に抗体を吸着させ、PBSでカラムを洗浄後に0.1M グリシン/塩酸水溶液(pH2.7)で溶出した。溶出液に1M Tris-HCl(pH9.0)を加えてpH7.0~7.5に調整した後に、Centrifugal UF Filter Device VIVASPIN20(分画分子量UF30K, Sartorius社)にてHBSor(25mM ヒスチジン/5% ソルビトール、pH6.0)へのバッファー置換を行うとともに抗体の濃縮を行い、抗体濃度を1mg/mLに調製した。最後にMinisart-Plus filter(Sartorius社)でろ過し、精製サンプルとした。

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[実施例2：ラット抗CDH6抗体の*in vitro*評価]

2) -1 フローサイトメトリーによるラット抗CDH6抗体の結合能評価

実施例1) -7で作製したラット抗CDH6抗体のヒトCDH6結合性をフローサイトメトリー法により評価した。実施例1) -1で作製したpcDNA3、1-hCDH6を293T細胞(ATCC)にLipofectamine 2000(Thermo Fisher Scientific)を用いて一過性に導入し、37℃、5% CO₂の条件下で一晩培養した後、細胞懸濁液を調製した。遺伝子導入した293T細胞懸濁液を遠心し、上清を除去した後、実施例1) -7で調製したラット抗CDH6モノクローナル抗体4種(クローン番号は、rG019、rG055、rG056及びrG061)又はラットIgGコントロール(R&D Systems)を終濃度10ng/mL加えて懸濁し、4℃で1時間静置した。5% FBS含有PBSで2回洗浄した後、5% FBS含有PBSで50倍に希釈したAnti-Rat IgG(whole molecule)-FITC antibody produced in rabbit(SIGMA)を加えて懸濁し、4℃で1時間静置した。5% FBS含有PBSで2回洗浄した後、フローサイトメーター(FC500:Beckman Coulter)で検出を行った。データ解析はFlowJo(TreeStar)で行った。その結果を図1に示す。図1のヒストグラムにおいて、横軸は抗体結合量を表すFITCの蛍光強度、縦軸は細胞数を示す。網掛けで示すヒストグラムはhCDH6を導入していない陰性コントロール293T細胞を用いた場合を示し、白抜き実線で示すヒストグラムは、hCDH6導入293T細胞を用いた場合を示す。細胞表面のhCDH6に抗体が結合したことによって蛍光強度が増強したことを示す。ラットIgGコントロールはいずれの細胞にも結合しない。この結果、作製したラット抗CDH6モノクローナル抗体4種はpcDNA3、1-hCDH6導入293T細胞に結合することを確認した。

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[0236] 2) - 2 フローサイトメトリーによるラット抗CDH6抗体のCDH6結合部位の解析

2) - 2 - 1 ヒトCDH6各ドメイン欠失体発現ベクターの構築

ヒトCDH6の細胞外全長には5つの細胞外ドメイン、EC1（配列番号2）、EC2（配列番号3）、EC3（配列番号4）、EC4（配列番号5）、EC5（配列番号6）が存在する。ヒトCDH6全長から、5つのECドメインを各一箇所ずつ欠失発現した遺伝子をGeneArt社で合成し、当業者に公知な方法に従い哺乳動物発現用ベクターp3xFLAG-CMV-9ベクター（SIGMA-ALDRICH社）へ組み込み、EC1からEC5をそれぞれ欠失した、各ドメイン欠失体発現ベクターを作製した。

[0237] 2) - 2 - 2 フローサイトメトリーによるドメイン欠失体を用いたラット抗CDH6抗体のエピトープ解析

各ECドメイン欠失ベクターを導入した293 α 細胞株を用いたフローサイトメトリー解析により、ラット抗ヒトCDH6抗体の結合エピトープを同定した。インテグリン α vおよびインテグリン β 3発現ベクターをHEK293細胞内に安定形質移入した細胞株293 α 細胞株に対して、実施例2) - 2 - 1で作成した各ドメイン欠失体発現ベクター、及び全長ヒトCDH6を発現するpcDNA3.1-hCDH6をLipofectamine 2000 (Thermo Fisher Scientific)を用いて一過性に導入し、37℃、5% CO₂の条件下で一晩培養した後、細胞懸濁液を調製した。導入293 α 細胞懸濁液を遠心し、上清を除去した後、実施例1) - 7で調製したラット抗CDH6モノクローナル抗体4種（クローン番号は、rG019、rG055、rG056及びrG061）又はラットIgGコントロール（R&D Systems）を終濃度20nMで加えて懸濁し、4℃で1時間静置した。5% FBS含有PBSで2回洗浄した後、5% FBS含有PBSで50倍に希釈したAnti-Rat IgG (whole molecule) - FITC antibody produced in rabbit (SIGMA)を加えて懸濁し、4℃で1時

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間静置した。5% FBS含有PBSで2回洗浄した後、フローサイトメーター（CantoII: BD Biosciences）で検出を行った。データ解析はFlowJo（TreeStar）で行った。その結果を図2-1～図2-6に示す。図2-1～図2-6のヒストグラムにおいて、横軸は抗体結合量を表すFITCの蛍光強度、縦軸は細胞数を示す。網掛けで示すヒストグラムは遺伝子を導入していない陰性コントロール293 α 細胞を用いた場合を示し、白抜き実線で示すヒストグラムは、全長hCDH6又は各ECドメイン欠失293細胞を用いた場合を示す。細胞表面の全長hCDH6又は各ECドメイン欠失体に抗体が結合した場合は、蛍光強度が増強する。ラットIgGコントロールはいずれの導入細胞にも結合しない。作製したラット抗CDH6モノクローナル抗体4種は、全長hCDH6、EC1欠失体、EC2欠失体、EC4欠失体、及びEC5欠失体には結合するが、EC3欠失体には結合しない。この結果から、ラット抗CDH6モノクローナル抗体4種はhCDH6のEC3をエピトープとして特異的に結合することが示された。

[0238] 2) - 3 ラット抗CDH6抗体の内在化活性

2) - 3 - 1 ヒト腫瘍細胞株におけるCDH6の発現確認

取得抗体の評価に用いるCDH6陽性ヒト腫瘍細胞株を選抜するため、公知データベースからCDH6発現情報を検索し、フローサイトメトリー法により細胞膜表面でのCDH6の発現を評価した。ヒト卵巣腫瘍細胞株NIH: OVCAR-3, PA-1, ES-2及びヒト腎細胞腫瘍細胞株786-O（全てATCCから入手）を37℃、5% CO₂の条件下で培養した後、細胞懸濁液を調製した。細胞を遠心し、上清を除去した後、市販抗ヒトCDH6抗体（MABU2715, R&D Systems）又は陰性コントロールとしてマウスIgG1（BD Pharmingen）を終濃度50 μ g/mL加えて懸濁し、4℃で1時間静置した。5% FBS含有PBSで2回洗浄した後、5% FBS含有PBSで50倍に希釈したF(ab')₂ Fragment of FITC-conjugated Goat

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Anti-mouse immunoglobulins (Dako) を加えて懸濁し、4℃で1時間静置した。5% FBS含有PBSで2回洗浄した後、フローサイトメーター (Canto II: BD Biosciences) で検出を行った。データ解析はFlowJo (TreeStar) で行った。その結果を図3に示す。図3のヒストグラムにおいて、横軸は抗体結合量を表すFITCの蛍光強度、縦軸は細胞数を示す。網掛けで示すヒストグラムは陰性コントロールmIgG1で染色した場合を示し、白抜き実線で示すヒストグラムは抗ヒトCDH6抗体で染色した場合を示す。細胞表面のhCDH6に抗体が結合したことによって蛍光強度が増強したことを示す。mIgG1コントロールはいずれの細胞にも結合しない。この結果、NIH: OVCAR-3, PA-1 及び786-O細胞株は、内在的にCDH6を細胞表面上に発現することが示された。一方、ES-2細胞株はCDH6を全く発現しないことが示された。

[0239] 2) -3-2 ラット抗CDH6抗体の内在化活性評価

ラット抗CDH6抗体の内在化活性は、タンパク質合成を阻害する毒素(サボリン)を結合させた抗ラットIgG試薬Rat-ZAP (ADVANCED TARGETING SYSTEMS)を用いて評価した。すなわち、ヒトCDH6陽性卵巣腫瘍細胞株NIH: OVCAR-3 (ATCC)を 4×10^3 cells/wellで96wellプレートに播種して37℃、5% CO₂の条件下で一晩培養した。ヒトCDH6陽性腎細胞腫瘍細胞株786-O (ATCC)は 1×10^3 cells/wellで96wellプレートに播種して一晩培養した。翌日、ラット抗CDH6抗体(終濃度: 1 nM)又は、陰性コントロール抗体としてラットIgG2b抗体(R&D Systems)を添加した。さらに、Rat-ZAP(終濃度: 0.5 nM)または、陰性コントロールとして毒素を結合していないGoat Anti-Rat IgG, Fc(gamma) Fragment Specific (JACKSON IMMUNORESEARCH)(終濃度: 0.5 nM)を添加し、3日間37℃、5%CO₂条件下で培養した。生

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存細胞数は、CellTiter-Glo™ Luminescent Cell Viability Assay (Promega) によるATP 活性 (RLU) の定量で測定した。この評価では、ラット抗CDH6抗体の 内在化活性に依存してRat-ZAPが細胞内に取り込まれ、タンパク合成 を阻害するサボリンが細胞内に放出されることで、細胞増殖が抑制される。 抗CDH6抗体添加による細胞増殖抑制作用は、Rat-ZAPの代わりに 陰性コントロールを添加したウェルの生存細胞数を100%とした相対生存 率で表記した。図4にグラフおよび細胞生存率の表を示す。この結果、ラッ ト抗CDH6抗体はCDH6に結合して内在化を引き起こすことが示された。

[0240]

〔実施例3：ラット抗CDH6抗体の可変領域をコードするcDNAのヌ クレオチド配列の決定〕

3) -1 rG019重鎖可変領域及び軽鎖可変領域の遺伝子断片の増幅 および配列決定

3) -1-1 G019からのtotal RNAの調製

rG019の可変領域を含むcDNAを増幅するためG019よりTRIzol Reagent (Ambion社) を用いてtotal RNAを 調製した。

[0241] 3) -1-2 5' -RACE PCRによるrG019の重鎖可変領域 を含むcDNAの増幅とヌクレオチド配列の決定

重鎖可変領域を含むcDNAの増幅は、実施例3) -1-1で調製したt otal RNAの約1μgとSMARTer RACE cDNA Am plification Kit (Clontech社) を用いて実施した。rG019の重鎖遺伝子の可変領域のcDNAをPCRで増幅するための プライマーとして、UPM (Universal Primer A M ix: SMARTer RACE cDNA Amplification Kitに付属)、及び公知のラット重鎖の定常領域の配列から設計したブ

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ライマーを用いた。

[0242] 5' - RACE PCRで増幅した重鎖の可変領域を含むcDNAをプラスミドにクローニングし、次に重鎖の可変領域のcDNAのヌクレオチド配列のシーケンス解析を実施した。

[0243] 決定されたrG019の重鎖の可変領域をコードするcDNAのヌクレオチド配列を配列番号16に示し、アミノ酸配列を配列番号15に示した。

[0244] 3) - 1 - 3 5' - RACE PCRによるrG019の軽鎖可変領域を含むcDNAの増幅とヌクレオチド配列の決定

実施例3) - 1 - 2と同様の方法で実施した。ただし、rG019の軽鎖遺伝子の可変領域のcDNAをPCRで増幅するためのプライマーとして、UPM (Universal Primer A Mix: SMARTer RACE cDNA Amplification Kitに付属)、及び公知のラット軽鎖の定常領域の配列から設計したプライマーを用いた。

[0245] 決定されたrG019の軽鎖の可変領域をコードするcDNAのヌクレオチド配列を配列番号11に示し、アミノ酸配列を配列番号10に示した。

[0246] 3) - 2 rG055重鎖可変領域及び軽鎖可変領域の遺伝子断片の増幅および配列決定

実施例3) - 1と同様の方法で配列を決定した。

[0247] 決定されたrG055の重鎖の可変領域をコードするcDNAのヌクレオチド配列を配列番号26に示し、アミノ酸配列を配列番号25に示した。軽鎖の可変領域をコードするcDNAのヌクレオチド配列を配列番号21に示し、アミノ酸配列を配列番号20に示した。

[0248] 3) - 3 rG056重鎖可変領域及び軽鎖可変領域の遺伝子断片の増幅および配列決定

実施例3) - 1と同様の方法で配列を決定した。

[0249] 決定されたrG056の重鎖の可変領域をコードするcDNAのヌクレオチド配列を配列番号36に示し、アミノ酸配列を配列番号35に示した。軽鎖の可変領域をコードするcDNAのヌクレオチド配列を配列番号31に示

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し、アミノ酸配列を配列番号30に示した。

[0250] 3) - 4 rG061 重鎖可変領域及び軽鎖可変領域の遺伝子断片の増幅
および配列決定

実施例3) - 1と同様の方法で配列を決定した。

[0251] 決定されたrG061の重鎖の可変領域をコードするcDNAのヌクレオ
チド配列を配列番号46に示し、アミノ酸配列を配列番号45に示した。軽
鎖の可変領域をコードするcDNAのヌクレオチド配列を配列番号41に示
し、アミノ酸配列を配列番号40に示した。

[0252]

[実施例4：ヒトキメラ化抗CDH6抗体chG019の作製]

4) - 1 ヒトキメラ化抗CDH6抗体chG019の発現ベクターの構
築

4) - 1 - 1 キメラ及びヒト化軽鎖発現ベクターpCMA-LKの構築
プラスミドpcDNA3.3-TOPO/LacZ (Invitrogen社)を制限酵素XbaI及びPmeIで消化して得られる約5.4kbの
フラグメントと、配列番号50に示すヒト軽鎖シグナル配列及びヒトκ鎖定
常領域をコードするDNA配列を含むDNA断片をIn-Fusion Advantage PCRクローニングキット (Clontech社)を用
いて結合して、pcDNA3.3/LKを作製した。

[0253] pcDNA3.3/LKからネオマイシン発現ユニットを除去すること
によりpCMA-LKを構築した。

[0254] 4) - 1 - 2 キメラ及びヒト化IgG1タイプ重鎖発現ベクターpCM
A-G1の構築

pCMA-LKをXbaI及びPmeIで消化して軽鎖シグナル配列及び
ヒトκ鎖定常領域を取り除いたDNA断片と、配列番号51で示されるヒト
重鎖シグナル配列及びヒトIgG1定常領域をコードするDNA配列を含む
DNA断片をIn-Fusion Advantage PCRクローニング
キット (Clontech社)を用いて結合して、pCMA-G1を構築

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した。

[0255] 4) - 1 - 3 chG019重鎖発現ベクターの構築

配列番号57に示すchG019重鎖のヌクレオチド配列のヌクレオチド番号36乃至440に示されるDNA断片を合成した(GENEART社)。In-Fusion HD PCRクローニングキット(Clontech社)を用いて、pCMA-G1を制限酵素BspIで切断した箇所に合成したDNA断片を挿入することによりchG019重鎖発現ベクターを構築した。なお、chG019重鎖は予期せぬジスルフィド結合を防ぐため、CDR中のシステインをプロリンに置換した配列を用いた。

[0256] 4) - 1 - 4 chG019軽鎖発現ベクターの構築

配列番号52に示すchG019軽鎖をコードするDNA配列を含むDNA断片を合成した(GENEART社)。In-Fusion HD PCRクローニングキット(Clontech社)を用いて、合成したDNA断片とpCMA-LKをXbaI及びPmeIで消化して軽鎖シグナル配列及びヒトκ鎖定常領域を取り除いたDNA断片を結合することにより、chG019軽鎖発現ベクターを構築した。

[0257] 4) - 2 ヒトキメラ化抗CDH6抗体chG019の生産及び精製

4) - 2 - 1 chG019の生産

FreeStyle 293F細胞(Invitrogen社)はマニュアルに従い、継代、培養をおこなった。対数増殖期の 1.2×10^9 個のFreeStyle 293F細胞(Invitrogen社)を3L Fernbach Erlenmeyer Flask(CORNING社)に播種し、FreeStyle293 expression medium(Invitrogen社)で希釈して 2.0×10^6 細胞/mlに調製した。40mlのOpti-Pro SFM培地(Invitrogen社)に0.24mgの重鎖発現ベクターと0.36mgの軽鎖発現ベクターと1.8mgのPolyethyleneimine(Polyscience #24765)を加えて穏やかに攪拌し、さらに5分間放置した後にFree

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Style 293F細胞に添加した。37℃、8%CO₂インキュベーターで4時間、90rpmで振とう培養後に600mlのEX-CELL VPRO培地 (SAFC Biosciences社)、18mlのGlutaMAX 1 (GIBCO社)、及び30mlのYeastolate Ultrafiltrate (GIBCO社)を添加し、37℃、8%CO₂インキュベーターで7日間、90rpmで振とう培養して得られた培養上清をDisposable Capsule Filter (Advantec #CCS-045-E1H)でろ過した。

[0258] 4) -2-2 chG019の精製

実施例4) -2-1で得られた培養上清をrProtein Aアフィニティークロマトグラフィーの1段階工程で精製した。培養上清をPBSで平衡化したMabSelect SuReが充填されたカラム (GE Healthcare Bioscience社製) にアプライしたのちに、カラム容量の2倍以上のPBSでカラムを洗浄した。次に2Mアルギニン塩酸塩溶液 (pH4.0) で溶出し、抗体の含まれる画分を集めた。その画分を透析 (Thermo Scientific社、Slide-A-Lyzer Dialysis Cassette) によりHBSor (25mM ヒスチジン/5% ソルビトール、pH6.0) へのバッファー置換を行った。Centrifugal UF Filter Device VIVASPIN20 (分画分子量UF10K, Sartorius社) で抗体を濃縮し、IgG濃度を5mg/ml以上に調製した。最後にMinisart-Plus filter (Sartorius社) でろ過し、精製サンプルとした。

[0259] 4) -3 ヒトキメラ化抗CDH6抗体chG019の結合性評価

4) -2で精製したヒトキメラ化抗CDH6抗体chG019のCDH6結合性をフローサイトメトリー法により確認した。実施例1) -1で作製したpcDNA3.1-hCDH6、又はpcDNA3.1-cynoCDH6、又はpcDNA3.1をそれぞれ293α細胞にLipofectam

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ine 2000を用いて一過性に導入し、37℃、5% CO₂の条件下で一晩培養した後、細胞懸濁液を調製した。これらの細胞懸濁液にchG019を加えて4℃で1時間静置した後、5% FBS含有PBSで2回洗浄し、5% FBS含有PBSで500倍に希釈したPE標識F(ab')₂ Fragment 抗ヒトIgG、Fcγ抗体 (JACKSON IMMUNORESEARCH) を加えて懸濁し、4℃で1時間静置した。5% FBS含有PBSで2回洗浄した後、5% FBS含有PBSに再懸濁し、フローサイトメーター (CantoII: BD Biosciences) で検出を行った。データ解析はFlowJo (TreeStar) で行った。図5に示すとおり、chG019は陰性コントロールであるpcDNA3.1導入293T細胞には結合せず、pcDNA3.1-hCDH6及びpcDNA3.1-cynoCDH6導入293T細胞に抗体濃度依存的に結合した。図5において横軸は抗体濃度を示し、縦軸は結合量をMean Fluorescent Intensity (平均蛍光強度) で示す。この結果から、chG019が、ヒトおよびカニクイザルCDH6に対して特異的に結合し、結合活性はほぼ同等であることがわかる。

[0260]

[実施例5：ヒト化抗CDH6抗体の作製]

5) -1 抗CDH6抗体のヒト化体デザイン

5) -1-1 chG019の可変領域の分子モデリング

chG019の可変領域の分子モデリングは、ホモロジーモデリングとして公知の方法 (Methods in Enzymology, 203, 121-153, (1991)) を利用した。chG019の重鎖と軽鎖の可変領域に対して高い配列同一性を有するProtein Data Bank (Nuc. Acid Res. 35, D301-D303 (2007)) に登録されている構造 (PDB ID: 2I9L) を鋳型に、市販のタンパク質立体構造解析プログラムBioLuminate (Schrodinger社製) を用いて行った。

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[0261] 5) -1-2 ヒト化hG019に対するアミノ酸配列の設計

chG019は、CDRグラフティング (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)) によりヒト化した。KABAT et al. (Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service National Institutes of Health, Bethesda, MD, (1991)) において既定されるヒトのgamma鎖サブグループ1およびkappa鎖サブグループ1のコンセンサス配列が、chG019のフレームワーク領域に対して高い同一性を有することから、それぞれ、重鎖と軽鎖のアクセプターとして選択された。アクセプター上に移入すべきドナー残基は、Queen et al. (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)) によって与えられる基準などを参考に三次元モデルを分析することで選択された。

[0262] 5) -2 chG019重鎖のヒト化

設計された3種の重鎖をhH01、hH02及びhH04と命名した。hH01の重鎖全長アミノ酸配列を、配列番号69に記載する。配列番号69のアミノ酸配列をコードするヌクレオチド配列を、配列番号70に記載する。hH02の重鎖全長アミノ酸配列を、配列番号73に記載する。配列番号73のアミノ酸配列をコードするヌクレオチド配列を、配列番号74に記載する。hH04の重鎖全長アミノ酸配列を、配列番号77に記載する。配列番号77のアミノ酸配列をコードするヌクレオチド配列を、配列番号78に記載する。

[0263] 5) -3 chG019軽鎖のヒト化

設計された2種の軽鎖をhL02およびhL03と命名した。hL02の軽鎖全長アミノ酸配列を、配列番号61に記載する。配列番号61のアミノ酸配列をコードするヌクレオチド配列を、配列番号62に記載する。hL03の軽鎖全長アミノ酸配列を、配列番号65に記載する。配列番号65のア

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ミノ酸配列をコードするヌクレオチド配列は、配列番号66に記載する。

- [0264] 5) - 4 重鎖及び軽鎖の組み合わせによるヒト化hG019の設計
- hH01及びhL02からなる抗体を「H01L02抗体」又は「H01L02」と称する。hH02及びhL02からなる抗体を「H02L02抗体」又は「H02L02」と称する。hH02及びhL03からなる抗体を「H02L03抗体」又は「H02L03」と称する。hH04及びhL02からなる抗体を「H04L02抗体」又は「H04L02」と称する。

- [0265] 5) - 5 ヒト化抗CDH6抗体の発現
- 5) - 5 - 1 ヒト化hG019の重鎖発現ベクターの構築
- 5) - 5 - 1 - 1 ヒト化hG019-H01タイプ重鎖発現ベクターの構築

配列番号70に示すヒト化hG019-H01タイプ重鎖のヌクレオチド配列のヌクレオチド番号36乃至440に示されるDNA断片を合成した(GENEART社)。実施例4) - 1 - 3と同様の方法でヒト化hG019-H01タイプ重鎖発現ベクターを構築した。

- [0266] 5) - 5 - 1 - 2 ヒト化hG019-H02タイプ重鎖発現ベクターの構築

配列番号74に示すヒト化hG019-H02タイプ重鎖のヌクレオチド配列のヌクレオチド番号36乃至440に示されるDNA断片を合成した(GENEART社)。実施例4) - 1 - 3と同様の方法でヒト化hG019-H02タイプ重鎖発現ベクターを構築した。

- [0267] 5) - 5 - 1 - 3 ヒト化hG019-H04タイプ重鎖発現ベクターの構築

配列番号78に示すヒト化hG019-H04タイプ重鎖のヌクレオチド配列のヌクレオチド番号36乃至440に示されるDNA断片を合成した(GENEART社)。実施例4) - 1 - 3と同様の方法でヒト化hG019-H04タイプ重鎖発現ベクターを構築した。

- [0268] 5) - 5 - 2 ヒト化hG019の軽鎖発現ベクターの構築

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5) - 5 - 2 - 1 ヒト化hG019-L02タイプ軽鎖発現ベクターの構築

配列番号62に示すヒト化hG019-L02タイプ軽鎖のヌクレオチド配列のヌクレオチド番号37乃至399に示されるヒト化hG019-L02タイプ軽鎖の可変領域をコードするDNA配列を含むDNA断片を合成した(GENEART社)。In-Fusion HD PCRクローニングキット(Clontech社)を用いて、pCMA-LKを制限酵素BsiWIで切断した箇所に合成したDNA断片を挿入することによりヒト化hG019-L02タイプ軽鎖発現ベクターを構築した。

[0269] 5) - 5 - 2 - 2 ヒト化hG019-L03タイプ軽鎖発現ベクターの構築

配列番号66に示すヒト化hG019-L03タイプ軽鎖のヌクレオチド配列のヌクレオチド番号37乃至399に示されるヒト化hG019-L03タイプ軽鎖の可変領域をコードするDNA配列を含むDNA断片を合成した(GENEART社)。実施例5) - 5 - 2 - 1と同様の方法でヒト化hG019-L03タイプ軽鎖発現ベクターを構築した。

[0270] 5) - 5 - 3 ヒト化hG019の調製

5) - 5 - 3 - 1 H01L02、H02L02、H02L03、H04L02の生産

実施例4) - 2 - 1と同様の方法で生産した。実施例5) - 4に示した重鎖と軽鎖の組み合わせにより、H01L02、H02L02、H02L03、H04L02を生産した。

[0271] 5) - 5 - 3 - 2 H01L02、H02L02、H02L03、H04L02の2step精製

実施例5) - 5 - 3 - 1で得られた培養上清をrProtein Aアフィニティークロマトグラフィーとセラミックハイドロキシアパタイトの2段階工程で精製した。培養上清をPBSで平衡化したMabSelect Sureが充填されたカラム(GE Healthcare Biosciences)

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ce社製) にアブライした後に、カラム容量の2倍以上のPBSでカラムを洗浄した。次に2Mアルギニン塩酸塩溶液(pH4.0)で抗体を溶出した。抗体の含まれる画分を透析(Thermo Scientific社、Slide-A-Lyzer Dialysis Cassette)によりPBSへのバッファー置換を行い、5mMリン酸ナトリウム/50mM MES/pH7.0のバッファーで5倍希釈した後に、5mM NaPi/50mM MES/30mM NaCl/pH7.0のバッファーで平衡化したセラミックハイドロキシアパタイトカラム(日本バイオラッド、Bio-Scale CHT Type-1 Hydroxyapatite Column) にアブライした。塩化ナトリウムによる直線的濃度勾配溶出を実施し、抗体の含まれる画分を集めた。その画分を透析(Thermo Scientific社、Slide-A-Lyzer Dialysis Cassette)によりHBSor(25mM ヒスチジン/5% ソルビトール、pH6.0)へのバッファー置換を行った。Centrifugal UF Filter Device VIVASPIN20(分画分子量UF10K, Sartorius社)にて抗体を濃縮し、IgG濃度を20mg/mlに調製した。最後にMinisart-Plus filter(Sartorius社)でろ過し、精製サンプルとした。

[0272]

[参考例1：抗CDH6抗体NOV0712の作製]

実施例で用いた抗CDH6抗体NOV0712は、国際公開第2016/024195号に記載のNOV0712の軽鎖全長、及び重鎖全長のアミノ酸配列(それぞれ、国際公開第2016/024195の配列番号235及び配列番号234)を参照して作製した。

[0273] 参考例1) - 1 抗CDH6抗体NOV0712

参考例1) - 1 - 1 抗CDH6抗体NOV0712の重鎖発現ベクターの構築

配列番号84に示すNOV0712の重鎖のヌクレオチド配列のヌクレオ

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チド番号36乃至428に示されるNOV0712の重鎖の可変領域を含むDNA断片を合成した（GENEART社）。実施例4）－1－3と同様の方法でNOV0712の重鎖発現ベクターを構築した。当該NOV0712の重鎖発現ベクターにより発現するNOV0712の重鎖のアミノ酸配列を、配列番号83に示す。配列番号83に示されるアミノ酸配列中、1～19番目のアミノ酸残基からなるアミノ酸配列はシグナル配列である。

[0274] 参考例1）－1－2 抗CDH6抗体NOV0712の軽鎖発現ベクターの構築

配列番号82に示すNOV0712の軽鎖のヌクレオチド配列のヌクレオチド番号37乃至405に示されるNOV0712の軽鎖の可変領域をコードするDNA配列を含むDNA断片を合成した（GENEART社）。実施例5）－5－2－1と同様の方法でNOV0712の軽鎖発現ベクターを構築した。当該NOV0712の軽鎖発現ベクターにより発現するNOV0712の軽鎖のアミノ酸配列を、配列番号81に示す。配列番号81に示されるアミノ酸配列中、1～20番目のアミノ酸残基からなるアミノ酸配列はシグナル配列である。

[0275] 参考例1）－2 抗CDH6抗体NOV0712の調製

参考例1）－2－1 抗CDH6抗体NOV0712の生産

実施例4）－2－1と同様の方法でNOV0712を生産した。

[0276] 参考例1）－2－2 抗CDH6抗体NOV0712の1step精製

参考例1）－2－1で得られた培養上清から実施例4）－2－2と同様の方法で抗CDH6抗体NOV0712を精製した（抗体濃度HBSorで5mg/l）。

[0277]

[実施例6：ヒト化hG019およびNOV0712のin vitro 評価]

6）－1 ヒト化hG019の結合性評価

6）－1－1 ヒト化hG019のヒトCDH6抗原結合能

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抗体と抗原 (Recombinant Human CDH6 Fc His chimera, R&D Systems) との解離定数測定は、Biacore T200 (GEヘルスケアバイオサイエンス) を使用し、固定化した抗His抗体に抗原をリガンドとして捕捉 (キャプチャー) し、抗体をアナライトとして測定するキャプチャー法にて行った。抗ヒスチジン抗体 (His capture kit, GEヘルスケアバイオサイエンス) は、センサーチップCM5 (GEヘルスケアバイオサイエンス) へ、アミンカップリング法にて約1000RU共有結合させた。リファレンスセルにも同様に固定化した。ランニングバッファーとして1mM CaCl_2 を添加したHBS-P+ (10mM HEPES pH7.4, 0.15M NaCl, 0.05% Surfactant P20) を用いた。抗ヒスチジン抗体を固定化したチップ上に、抗原を60秒間添加した後、抗体の希釈系列溶液 (0.391-100nM) を流速30 μl /分で300秒間添加し、引き続き600秒間の解離相をモニターした。再生溶液として、5M MgCl_2 を添加したGlycine溶液pH1.5を流速10 μl /分で30秒間、2回添加した。データの解析は、分析ソフトウェア (BIAevaluation software, version 4.1) のSteady State Affinityモデルを用いて、解離定数 (KD) を算出した。結果を表2に示す。

[0278] [表2]

	抗体	KD (M)
1	H02L02	1.5E-09
2	H02L02	1.1E-09
3	H02L03	1.4E-09
4	H04L02	1.1E-09

[0279] 6) -1-2 ヒト、サル、マウス、ラットCDH6に対する結合性

実施例1) -1で作製したp cDNA3、1-hCDH6、p cDNA3、1-cynoCDH6、p3xFLAG-CMV-9-mCDH6、p3xFLAG-CMV-9-rCDH6を、293 α 細胞にLipofectamine 2000を用いて一過性に導入し、37℃、5% CO₂の条件下で一晩培養した後、細胞懸濁液を調製した。陰性コントロールとして、遺伝子導入していない293 α 細胞を用いた。上記にて作製した293 α 細胞懸濁液を遠心し、上清を除去した後、実施例5) -5-3で調製したヒト化hG019抗体4種（クローン番号は、H01L02、H02L02、H02L03およびH04L02）、又は、ヒトIgG1コントロール（Calbiochem）を加えて懸濁し、4℃で1時間静置した。5% FBS含有PBSで2回洗浄した後、5% FBS含有PBSで500倍に希釈したanti-human Fcg PE goat F(ab')（Jackson laboratory）を加えて懸濁し、4℃で1時間静置した。5% FBS含有PBSで2回洗浄した後、フローサイトメーター（Canto II: BD Biosciences）で検出を行った。データ解析はFlowJo (TreeStar)で行った。図6-1及び図6-2において横軸は抗体濃度を示し、縦軸は結合量をMean Fluorescent Intensity（平均蛍光強度）で示す。図6-1及び図6-2に示すとおり、陰性コントロールであるヒトIgG1コントロールは、いずれのCDH6遺伝子導入細胞に対しても結合しない。ヒト化hG019抗体4種（クローン番号は、H01L02、H02L02、H02L03およびH04L02）はヒト及びカニクイザルCDH6に結合するが、マウス及びラットCDH6には結合しない。いずれの抗体も、陰性コントロールである空ベクターp cDNA3、1導入細胞には結合しない。一方、NOV0712抗体はヒト、カニクイザル、マウス、及びラットCDH6全てに結合活性を示すことが国際公開第2016/024195号に示されている。この結果、本明細書で取得したヒト化hG019抗体4種は、NOV0712抗体と異なる結合性質を示す抗CDH6抗体であることが示された。

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[0280] 6) - 2 ヒト化hG019およびNOV0712のCDH6結合部位の解析

6) - 2 - 1 ドメイン欠失体を用いたエピトープ解析

実施例2) - 2 - 1で作成した各ドメイン欠失体発現ベクター、及び全長hCDH6を発現するpcDNA3.1-hCDH6をLipofectamine 2000 (Thermo Fisher Scientific)を用いて一過性に導入し、37℃、5% CO₂の条件下で一晩培養した後、細胞懸濁液を調製した。遺伝子導入した293α細胞懸濁液を遠心し、上清を除去した後、実施例5) - 5 - 3で調製したヒト化hG019抗体4種(クローン番号は、H01L02、H02L02、H02L03およびH04L02)、又は、参考例1で調製した抗CDH6抗体NOV0712、又は、陰性コントロールとしてヒトIgG1 (Calbiochem)を加えて懸濁し、4℃で1時間静置した。5% FBS含有PBSで2回洗浄した後、5% FBS含有PBSで500倍に希釈したAPC-anti-human IgG goat F(ab')₂ (Jackson laboratory)を加えて懸濁し、4℃で1時間静置した。5% FBS含有PBSで2回洗浄した後、フローサイトメーター(CantoII: BD Biosciences)で検出を行った。データ解析はFlowJo (TreeStar)で行った。その結果を図7-1～図7-6に示す。図7-1～図7-6のヒストグラムにおいて、横軸は抗体結合量を表すAPCの蛍光強度、縦軸は細胞数を示す。網掛けで示すヒストグラムは遺伝子を導入していない陰性コントロール293α細胞を用いた場合を示し、白抜き実線で示すヒストグラムは、全長hCDH6又は各ECドメイン欠失293α細胞を用いた場合を示す。細胞表面の全長hCDH6又は各ECドメイン欠失体に抗体が結合した場合は、蛍光強度が増強する。ヒトIgG1コントロールはいずれの導入細胞にも結合しない。ヒト化hG019抗体4種(クローン番号は、H01L02、H02L02、H02L03およびH04L02)は、全長hCDH6、EC1欠失体、EC2欠失体、EC4欠失体、及びEC5

欠失体には結合するが、EC3欠失体には結合しない。すなわち、ヒト化hG019抗体4種はhCDH6のEC3をエピトープとして特異的に結合することが示された。一方、抗CDH6抗体NOV0712は、全長hCDH6、EC1欠失体、EC2欠失体、EC3欠失体、及びEC4欠失体には結合するが、EC5欠失体には結合しない。すなわち、抗CDH6抗体NOV0712抗体はhCDH6のEC5をエピトープとして特異的に結合することが示され、国際公開第2016/024195号に記載されているNOV0712のエピトープ情報と一致する。この結果から、NOV0712と本明細書で取得したヒト化hG019抗体4種は、異なる性質を示す抗CDH6抗体であることが示された。

[0281] 6) -2-2 抗体の結合競合アッセイ

6) -2-2-1 786-O/hCDH6安定発現細胞株の作製

786-O/hCDH6安定発現細胞株は、786-O細胞(ATCC)にヒトCDH6全長発現用組換えレトロウイルスを感染させることにより作製した。ヒトCDH6発現レトロウイルスベクター(pQCXIN-hCDH6)は、ヒトCDH6タンパク質(NP_004923)をコードするcDNA発現ベクター(OriGene社RC217889)を用いて、当業者に公知な方法に従いレトロウイルスベクターpQCXIN(CLONTECH)に組み込むことにより作製された。FuGene HD(Promega)を用いてレトロウイルスパッケージング細胞RetroPack P T67(CLONTECH)にpQCXIN-hCDH6を一過性に導入し、48時間後に組換えレトロウイルスを含む培養上清を回収し、786-O細胞培養系に添加することで同細胞に感染させた。感染3日後から、G418(Gibco)を終濃度50mg/mLで添加した培地で37℃、5%CO₂の条件下で培養を行い感染細胞の薬剤選抜を行うことで、ヒトCDH6を安定的に発現する細胞株786-O/hCDH6を樹立した。実施例2)-3-1と同様にフローサイトメトリーにて安定発現株でのヒトCDH6の高発現を確認した(図8)。検出用抗体には5% FBS含有PBSで5

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00倍に希釈したGoat anti-Mouse IgG1 Secondary Antibody Alexa Fluor 647 (Thermo Fisher Scientific)を使用した。その結果を図8に示す。図8のヒストグラムにおいて、横軸は抗体結合量を表すAlexa Fluor 647の蛍光強度、縦軸は細胞数を示す。網掛けで示すヒストグラムは陰性コントロールmIgG1で染色した場合を示し、白抜き実線で示すヒストグラムは抗ヒトCDH6抗体で染色した場合を示す。細胞表面のhCDH6に抗体が結合したことによって蛍光強度が増強したことを示す。mIgG1コントロールはいずれの細胞にも結合しない。この結果、786-O/hCDH6安定発現細胞株では親株786-O細胞と比較して、ヒトCDH6を高発現することが示された。

【0282】 6) -2-2-2 ラベル化H01L02及びラベル化NOV0712を用いた結合競合アッセイ

Alexa Fluor 488 Monoclonal Antibody Labeling Kit (Thermo Fisher)を用いて、ラベル化H01L02及びラベル化NOV0712を作製した。7) -2-2-1で作製した786-O/hCDH6安定発現細胞株の細胞懸濁液を遠心し、上清を除去した後、ラベル化NOV0712又はラベル化H01L02を終濃度5 nMで添加し、さらに実施例5) -5-3で調製したヒト化hG019抗体4種（クローン番号は、H01L02、H02L02、H02L03およびH04L02）、又は、参考例1で調製した抗CDH6抗体NOV0712、又は、陰性コントロールとしてヒトIgG1 (Calbiochem)を図9の横軸で示す終濃度で添加して懸濁し、4℃で1時間静置した。5% FBS含有PBSで2回洗浄した後、フローサイトメーター (Cantoll: BD Biosciences) で検出を行った。データ解析はFlowJo (TreeStar)で行った。その結果を図9に示す。横軸はラベル化していない抗体の添加時の終濃度を示し、縦軸は結合量をMean Fluorescent Intensity (平均蛍光強度)

で示す。ラベル化NOV0712を添加した細胞にラベル化していないNOV0712を添加した場合は、同じエピトープを持ち結合が競合するため、添加濃度依存的にラベル化していない抗体に置き換わりラベル化抗体の結合量が減少する。一方、ラベル化NOV0712を添加した細胞にヒト化hG019抗体4種、又は陰性コントロールとしてヒトIgG1を添加しても、ラベル化抗体の結合量に変化は無いことから、これらの抗体はエピトープが異なり結合が競合しないことが示される。同様に、ラベル化H01L02を添加した細胞にラベル化していないヒト化hG019抗体4種を添加した場合は、同じエピトープを持ち結合が競合するため、添加濃度依存的にラベル化していない抗体に置き換わりラベル化抗体の結合量が減少する。一方、ラベル化H01L02を添加した細胞にNOV0712、又は陰性コントロールとしてヒトIgG1を添加しても、ラベル化抗体の結合量に変化は無いことから、これらの抗体はエピトープが異なり結合が競合しないことが示される。

[0283] 6) -3 ヒト化hG019およびNOV0712の内在化活性評価

ヒト化hG019およびNOV0712の内在化活性は、タンパク質合成を阻害する毒素（サポリン）を結合させた抗ヒトIgG試薬Hum-ZAP（ADVANCED TARGETING SYSTEMS）を用いて評価した。すなわち、ヒトCDH6陽性卵巣腫瘍細胞株NIH: OVCAR-3（ATCC）を 4×10^3 cells/wellで96wellプレートに播種して37℃、5% CO₂の条件下で一晩培養した。ヒトCDH6陽性腎細胞腫瘍細胞株786-O（ATCC）は 1×10^3 cells/wellで96wellプレートに播種して一晩培養した。ヒトCDH6陽性卵巣腫瘍細胞株PA-1（ATCC）を 1×10^3 cells/wellで96wellプレートに播種して37℃、5% CO₂の条件下で一晩培養した。翌日、抗CDH6抗体（終濃度：1 nM）、又は、陰性コントロール抗体としてヒトIgG1抗体（Calbiochem）を添加した。さらにHum-ZAP（終濃度：0.5 nM）または、陰性コントロールとして毒素を

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結合していないF(ab')₂ Fragment Goat Anti-human IgG, Fc(gamma) Fragment Specific (JACKSON IMMUNORESEARCH) (終濃度: 0.5 nM) を添加し、3日間37℃、5%CO₂条件下で培養した。生存細胞数は、CellTiter-Glo™ Luminescent Cell Viability AssayによるATP活性(RLU)の定量で測定した。この評価では、ヒト化抗CDH6抗体の内在化活性に依存してHum-ZAPが細胞内に取り込まれ、タンパク合成を阻害するサボリンが細胞内に放出されることで、細胞増殖が抑制される。抗CDH6抗体添加による細胞増殖抑制作用は、Hum-ZAPの代わりに陰性コントロールを添加したウェルの生存細胞数を100%とした相対生存率で表記した。図10-1～図10-3にグラフおよび細胞生存率の表を示す。本実験において内在化活性が強い抗体は低い細胞生存率を示すと考えられる。この結果、NOV0712はヒト化hG019抗体4種は3種の細胞株いずれにおいても細胞生存率から予測される内在化率は約50～75%であり、非常に高い内在化活性を示し、NOV0712と比較して、さらに高い内在活性を示す。ADCの薬効メカニズムから、高い内在化活性を有する抗体は、ADC化抗体をして、より適していると考えられる。

[0284]

[実施例7: ヒト化hG019-薬物コンジュゲートの作製]

7)-1 抗体-薬物コンジュゲートの作製 H01L02-DXd

工程1: 抗体-薬物コンジュゲート(1)

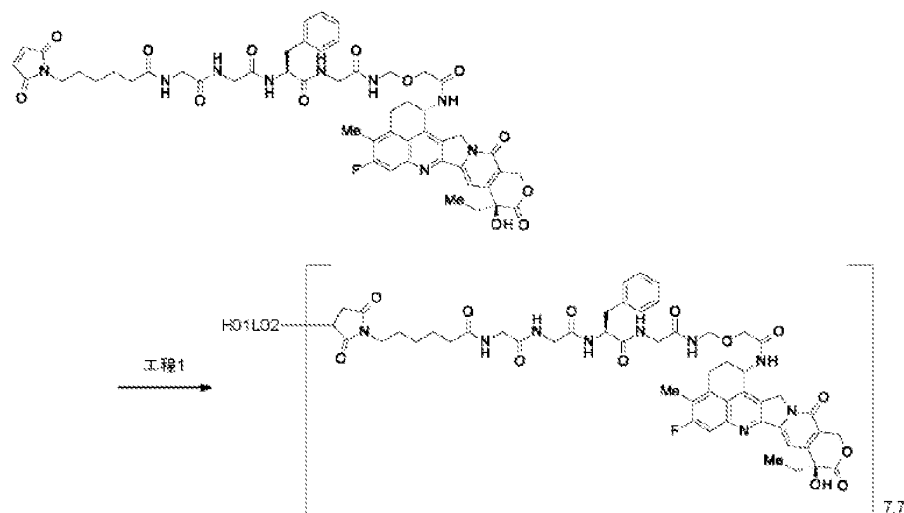
[0285]

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[化11]



【0286】 抗体の還元：実施例5にて作製したH01L02を、製造方法1に記載した共通操作B（280nm吸光係数として $1.53\text{ mL mg}^{-1}\text{ cm}^{-1}$ を使用）及びCを用いて、PBS6.0/EDTAにて9.85mg/mLに調製した。本溶液（5.7mL）に10mM TCEP（東京化成工業株式会社）水溶液（0.231mL；抗体一分子に対して6.0当量）及び1Mリン酸水素二カリウム水溶液（Nacalai Tesque, Inc.；0.0855mL）を加えた。本溶液のpHが 7.0 ± 0.1 内であることを確認した後、 37°C で2時間インキュベートすることによって、抗体内鎖間部のジスルフィド結合を還元した。

【0287】 抗体と薬物リンカーのコンジュゲーション：上記溶液を 15°C で10分間インキュベートした。次いでN-[6-(2,5-ジオキソ-2,5-ジヒドロ-1H-ピロール-1-イル)ヘキサノイル]グリシルグリシル-L-フェニルアラニル-N-[(2-{[(1S,9S)-9-エチル-5-フルオロ-9-ヒドロキシ-4-メチル-10,13-ジオキソ-2,3,9,10,13,15-ヘキサヒドロ-1H,12H-ベンゾ[d,e]ピラノ[3',4':6,7]インドリジノ[1,2-b]キノリン-1-イル]

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アミノ}-2-オキソエトキシ)メチル]グリシンアミドの10mM ジメチルスルホキシド溶液(0.386mL;抗体一分子に対して10当量)を加え、15℃で1時間インキュベートし、薬物リンカーを抗体へ結合させた。次に、100mM NAC(Sigma-Aldrich Co. LLC)水溶液(0.0347mL;抗体一分子に対して9当量)を加え、さらに室温にて20分間攪拌し、薬物リンカーの反応を停止させた。

[0288] 精製:上記溶液を、製造方法1に記載した共通操作Dによる精製を行い、標記抗体-薬物コンジュゲート「H01L02-ADC」を含有する溶液を19mL得た。

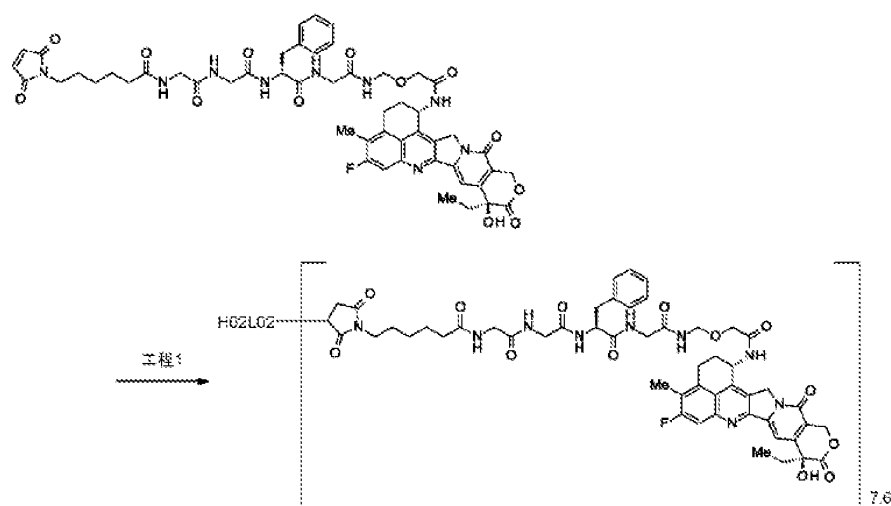
[0289] 特性評価:製造方法1に記載した共通操作E($\varepsilon_{D,280}=5440$ 、 $\varepsilon_{D,370}=21240$ を使用)を使用して、下記の特性値を得た。

抗体濃度:2.26mg/mL, 抗体収量:42.9mg(76%), 共通操作Eにて測定された抗体一分子あたりの薬物平均結合数(n):5.9; 共通操作Fにて測定された抗体一分子あたりの薬物平均結合数(n):7.7。

[0290] 7)-2 抗体-薬物コンジュゲートの作製 H02L02-DXd

工程1:抗体-薬物コンジュゲート(2)

[0291] [化12]



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[0292] 抗体の還元：実施例5にて作製したH02L02を、製造方法1に記載した共通操作B（280nm吸光係数として $1.51\text{ mL mg}^{-1}\text{ cm}^{-1}$ を使用）及びCを用いて、PBS6.0/EDTAにて9.95mg/mLに調製した。本溶液（5.7mL）に10mM TCEP（東京化成工業株式会社）水溶液（0.234mL；抗体一分子に対して6.0当量）及び1Mリン酸水素二カリウム水溶液（Nacalai Tesque, Inc.；0.0855mL）を加えた。本溶液のpHが 7.0 ± 0.1 内であることを確認した後に、37℃で2時間インキュベートすることによって、抗体内鎖間部のジスルフィド結合を還元した。

[0293] 抗体と薬物リンカーのコンジュゲーション：上記溶液を15℃で10分間インキュベートした。次いでN-[6-(2,5-ジオキソ-2,5-ジヒドロ-1H-ピロール-1-イル)ヘキサノイル]グリシルグリシル- α -フェニルアラニル-N-[(2-{[(1S,9S)-9-エチル-5-フルオロ-9-ヒドロキシ-4-メチル-10,13-ジオキソ-2,3,9,10,13,15-ヘキサヒドロ-1H,12H-ベンゾ[de]ピラノ[3',4':6,7]インドリジノ[1,2-b]キノリン-1-イル]アミノ}-2-オキソエトキシ)メチル]グリシンアミドの10mMジメチルスルホキシド溶液（0.389mL；抗体一分子に対して10当量）を加え、15℃で1時間インキュベートし、薬物リンカーを抗体へ結合させた。次に、100mM NAC（Sigma-Aldrich Co, LLC）水溶液（0.0350mL；抗体一分子に対して9当量）を加え、さらに室温にて20分間攪拌し、薬物リンカーの反応を停止させた。

[0294] 精製：上記溶液を、製造方法1に記載した共通操作Dによる精製を行い、標記抗体-薬物コンジュゲート「H02L02-ADC」を含有する溶液を19mL得た。

[0295] 特性評価：製造方法1に記載した共通操作E（ $\epsilon_{D,280}=5440$ 、 $\epsilon_{D,370}=21240$ を使用）を使用して、下記の特性値を得た。

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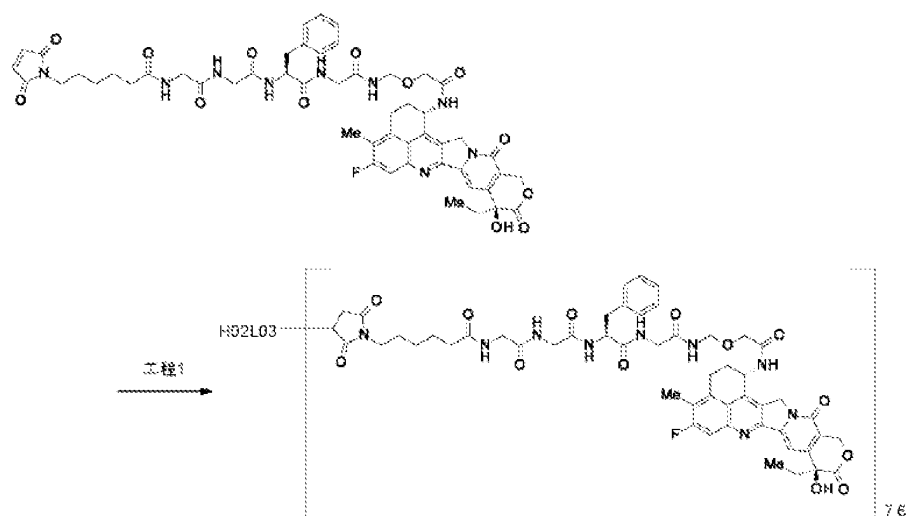
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抗体濃度：2.61 mg/mL、抗体収量：49.6 mg（87%）、共通操作Eにて測定された抗体一分子あたりの薬物平均結合数（n）：5.9；共通操作Fにて測定された抗体一分子あたりの薬物平均結合数（n）：7.6。

[0296] 7) -3 抗体-薬物コンジュゲートの作製 H02L03-DXd

工程1：抗体-薬物コンジュゲート（3）

[0297] [化13]



[0298] 抗体の還元：実施例5にて作製したH02L03を、製造方法1に記載した共通操作B（280nm吸光係数として1.53 mL mg⁻¹ cm⁻¹を使用）及びCを用いて、PBS 6.0/EDTAにて9.86 mg/mLに調製した。本溶液（5.7 mL）に10 mM TCEP（東京化成工業株式会社）水溶液（0.270 mL；抗体一分子に対して7.0当量）及び1 Mリン酸水素二カリウム水溶液（Nacalai Tesque, Inc.；0.0855 mL）を加えた。本溶液のpHが7.0±0.1内であることを確認した後に、37℃で2時間インキュベートすることによって、抗体内鎖間のジスルフィド結合を還元した。

[0299] 抗体と薬物リンカーのコンジュゲーション：上記溶液を15℃で10分間

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インキュベートした。次いでN-[6-(2,5-ジオキソ-2,5-ジヒドロ-1H-ピロール-1-イル)ヘキサノイル]グリシルグリシル-L-フェニルアラニル-N-[(2-{[(1S,9S)-9-エチル-5-フルオロ-9-ヒドロキシ-4-メチル-10,13-ジオキソ-2,3,9,10,13,15-ヘキサヒドロ-1H,12H-ベンゾ[de]ピラノ[3',4':6,7]インドリジノ[1,2-b]キノリン-1-イル]アミノ}-2-オキソエトキシ)メチル]グリシンアミドの10mMジメチルスルホキシド溶液(0.386mL;抗体一分子に対して10当量)を加え、15℃で1時間インキュベートし、薬物リンカーを抗体へ結合させた。次に、100mM NAC(Sigma-Aldrich Co. LLC)水溶液(0.0347mL;抗体一分子に対して9当量)を加え、さらに室温にて20分間攪拌し、薬物リンカーの反応を停止させた。

[0300] 精製：上記溶液を、製造方法1に記載した共通操作Dによる精製を行い、標記抗体-薬物コンジュゲート「H01L02-ADC」を含有する溶液を19mL得た。

[0301] 特性評価：製造方法1に記載した共通操作E($\epsilon_{D,280}=5440$ 、 $\epsilon_{D,370}=21240$ を使用)を使用して、下記の特性値を得た。

抗体濃度：2.71mg/mL、抗体収量：51.4mg(91%)、共通操作Eにて測定された抗体一分子あたりの薬物平均結合数(n)：5.7；共通操作Fにて測定された抗体一分子あたりの薬物平均結合数(n)：7.6。

[0302] 7)-4 抗体-薬物コンジュゲートの作製 H04L02-DXd
工程1：抗体-薬物コンジュゲート(4)

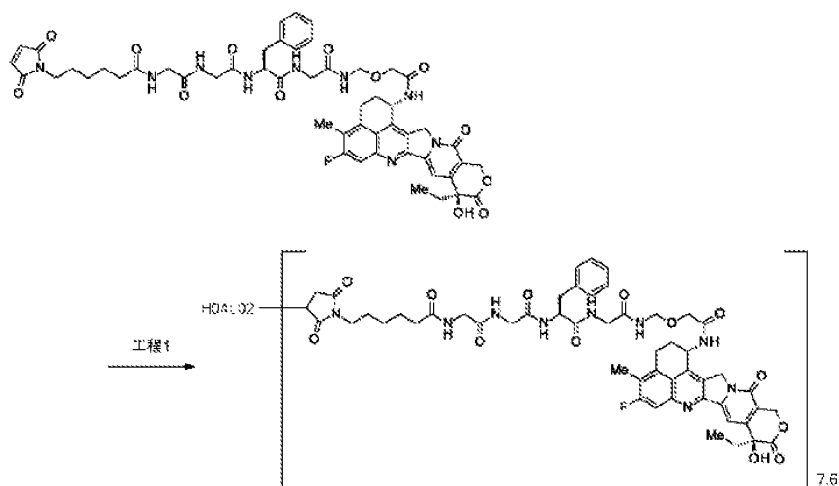
[0303]

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[化14]



[0304] 抗体の還元：実施例5にて作製したH04L02を、製造方法1に記載した共通操作B（280nm吸光係数として $1.53\text{ mL mg}^{-1}\text{ cm}^{-1}$ を使用）及びCを用いて、PBS6.0/EDTAにて9.86mg/mLに調製した。本溶液（5.7mL）に10mM TCEP（東京化成工業株式会社）水溶液（0.232mL；抗体一分子に対して6.0当量）及び1Mリン酸水素二カリウム水溶液（Nacalai Tesque, Inc.；0.0855mL）を加えた。本溶液のpHが 7.0 ± 0.1 内であることを確認した後に、37℃で2時間インキュベートすることによって、抗体内鎖間部のジスルフィド結合を還元した。

[0305] 抗体と薬物リンカーのコンジュゲーション：上記溶液を15℃で10分間インキュベートした。次いでN-[6-(2,5-ジオキソ-2,5-ジヒドロ-1H-ピロール-1-イル)ヘキサノイル]グリシルグリシル-L-フェニルアラニル-N-[(2-{[(1S,9S)-9-エチル-5-フルオロ-9-ヒドロキシ-4-メチル-10,13-ジオキソ-2,3,9,10,13,15-ヘキサヒドロ-1H,12H-ベンゾ[de]ピラノ[3',4':6,7]インドリジノ[1,2-b]キノリン-1-イル]

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アミノ}-2-オキソエトキシ)メチル]グリシンアミドの10mM ジメチルスルホキシド溶液(0.386mL;抗体一分子に対して10当量)を加え、15℃で1時間インキュベートし、薬物リンカーを抗体へ結合させた。次に、100mM NAC(Sigma-Aldrich Co. LLC)水溶液(0.0347mL;抗体一分子に対して9当量)を加え、さらに室温にて20分間攪拌し、薬物リンカーの反応を停止させた。

[0306] 精製:上記溶液を、製造方法1に記載した共通操作Dによる精製を行い、標記抗体-薬物コンジュゲート「H04L02-ADC」を含有する溶液を19mL得た。

[0307] 特性評価:製造方法1に記載した共通操作E($\epsilon_{D,280}=5440$ 、 $\epsilon_{D,370}=21240$ を使用)を使用して、下記の特性値を得た。

抗体濃度:2.56mg/mL, 抗体収量:48.7mg(87%), 共通操作Eにて測定された抗体一分子あたりの薬物平均結合数(n):5.8; 共通操作Fにて測定された抗体一分子あたりの薬物平均結合数(n):7.6。

[0308]

[参考例2:NOV0712-薬物コンジュゲートの作製]

参考例2)-1 抗体-薬物コンジュゲートの作製 NOV0712-DM4

抗体-薬物コンジュゲート(5)

抗体と薬物リンカーのコンジュゲーション:参考例1にて作製したNOV0712を、製造方法1に記載した共通操作B(280nm吸光係数として1.51mLmg⁻¹cm⁻¹を使用)及びCを用いて、20mM HEPES8.1(LIFE TECHNOLOGIES社製 HEPES, 1M Buffer Solution(20mL)を1M水酸化ナトリウムでpH8.1とした後、蒸留水にて1Lとした)にて9.7mg/mLに調製し、20℃で10分間インキュベートした。次いでWO2016/024195号公開特許公報に記載の10mM 1-(2,5-ジオキソピロリジン-1

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－イルオキシ）－１－オキソ－４－（ピリジン－２－イルジスルファニル）
 ブタン－２－スルホン酸のDMA溶液（０．３６６ｍＬ；抗体一分子に対
 して５．２当量）、１０ｍＭ　Ｎ２－デアセチル－デアセチル－Ｎ２－（４
 －メチル－４－メルカプト－１－オキソペンチル）－メイタンシン（DM4
 ）のDMA溶液（０．３６６ｍＬ；抗体一分子に対して６．８当量）、及び
 ０．２４３ｍＬのDMAを加え、２０℃で１６時間インキュベートし、薬物
 リンカーを抗体へ結合させた。次に、１Ｍ　酢酸水溶液を加えｐＨ５．０と
 し、さらに室温にて２０分間攪拌し、薬物リンカーの反応を停止させた。

【0309】　精製：上記溶液を、製造方法１に記載した共通操作Dによる精製を行い、
 標記抗体－薬物コンジュゲート「NOV0712－DM4」を含有する溶液
 を２８ｍＬ得た。

【0310】　特性評価：製造方法１に記載した共通操作E（ $\epsilon_{A, 280} = 200500$ 、 $\epsilon_{A, 252} = 76295$ 、 $\epsilon_{D, 280} = 43170$ 、及び $\epsilon_{D, 252} = 23224$ を使
 用）を使用して、下記の特性値を得た。

抗体濃度：２．５８ｍｇ／ｍＬ、抗体収量：７２．２ｍｇ（９３％）、共通
 操作Eにて測定された抗体一分子あたりの薬物平均結合数（ n ）：３．０。

【0311】　参考例２）－２　抗体－薬物コンジュゲートの作製　NOV0712－D
 X d

工程１：抗体－薬物コンジュゲート（６）

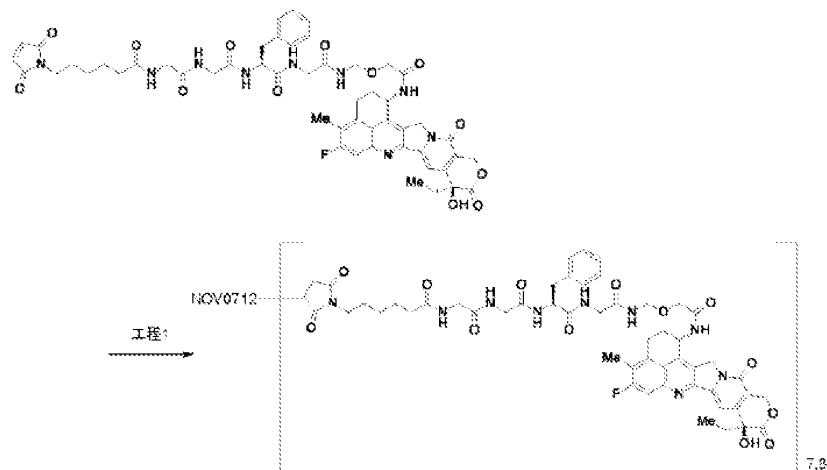
【0312】

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[化15]



[0313] 抗体の還元：参考例1にて作製したNOV0712を、製造方法1に記載した共通操作B（280nm吸光係数として1.51mLmg⁻¹cm⁻¹を使用）及びCを用いて、PBS6.0/EDTAにて9.26mg/mLに調製した。本溶液（6.6mL）に10mM TCEP（東京化成工業株式会社）水溶液（0.254mL；抗体一分子に対して6.0当量）及び1Mリン酸水素二カリウム水溶液（Nacalai Tesque, Inc.；0.0990mL）を加えた。本溶液のpHが7.0±0.1内であることを確認した後に、37℃で2時間インキュベートすることによって、抗体内鎖間部のジスルフィド結合を還元した。

[0314] 抗体と薬物リンカーのコンジュゲーション：上記溶液を15℃で10分間インキュベートした。次いでN-[6-(2,5-ジオキソ-2,5-ジヒドロ-1H-ピロール-1-イル)ヘキサノイル]グリシルグリシル-L-フェニルアラニル-N-[(2-{[(1S,9S)-9-エチル-5-フルオロ-9-ヒドロキシ-4-メチル-10,13-ジオキソ-2,3,9,10,13,15-ヘキサヒドロ-1H,12H-ベンゾ[de]ピラノ[3',4':6,7]インドリジノ[1,2-b]キノリン-1-イル]アミノ}-2-オキソエトキシ)メチル]グリシンアミドの10mMジメ

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チルスルホキンド溶液（0.381 mL；抗体一分子に対して9当量）を加え、15℃で1時間インキュベートし、薬物リンカーを抗体へ結合させた。次に、100 mM NAC（Sigma-Aldrich Co. LLC）水溶液（0.0381 mL；抗体一分子に対して9当量）を加え、さらに室温にて20分間攪拌し、薬物リンカーの反応を停止させた。

[0315] 精製：上記溶液を、製造方法1に記載した共通操作Dによる精製を行い、標記抗体-薬物コンジュゲート「NOV0712-ADC」を含有する溶液を23.5 mL得た。

[0316] 特性評価：製造方法1に記載した共通操作E（ $\epsilon_{D, 280} = 5440$ 、 $\epsilon_{D, 370} = 21240$ を使用）を使用して、下記の特性値を得た。
抗体濃度：2.26 mg/mL、抗体収量：56.4 mg（92%）、共通操作Eにて測定された抗体一分子あたりの薬物平均結合数（n）：6.4；共通操作Fにて測定された抗体一分子あたりの薬物平均結合数（n）：7.8。

[0317]

[参考例3：H01L02-DM4の作製]

参考例3) - 1 抗体-薬物コンジュゲートの作製 H01L02-DM4

抗体-薬物コンジュゲート（7）

抗体と薬物リンカーのコンジュゲーション：実施例5にて作製したH01L02を、製造方法1に記載した共通操作B（280 nm吸光係数として1.53 mL mg⁻¹ cm⁻¹を使用）及びCを用いて、20 mM HEPES 8.1（LIFE TECHNOLOGIES社製 HEPES, 1M Buffer Solution（20 mL）を1M水酸化ナトリウムでpH 8.1とした後、蒸留水にて1 Lとした）にて9.8 mg/mLに調製し、20℃で10分間インキュベートした。次いでWO2016/024195号公開特許公報に記載の10 mM 1-（2,5-ジオキソピロリジン-1-イルオキシ）-1-オキソ-4-（ピリジン-2-イルジスルファニル）

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ブタン-2-スルホン酸のDMA溶液（0.062 mL；抗体一分子に対して11.5当量）、10 mM N2-デアセチル-N2-（4-メチル-4-メルカプト-1-オキソペンチル）-メイトンシン（DM4）のDMA溶液（0.082 mL；抗体一分子に対して15.1当量）を加え、20℃で18時間インキュベートし、薬物リンカーを抗体へ結合させた。次に、1 M 酢酸水溶液を加えpH 5.0とし、さらに室温にて20分間攪拌し、薬物リンカーの反応を停止させた。

[0318] 精製：上記溶液を、製造方法1に記載した共通操作Dによる精製を行い、標記抗体-薬物コンジュゲート「H01L02-DM4」を含有する溶液を3.5 mL得た。

[0319] 特性評価：製造方法1に記載した共通操作E（εA, 280=223400、εA, 252=85646、εD, 280=4317、及びεD, 252=23224を使用）を使用して、下記の特性値を得た。

抗体濃度：1.97 mg/mL、抗体収量：6.90 mg（88%）、共通操作Eにて測定された抗体一分子あたりの薬物平均結合数（n）：3.6。

[0320]

〔実施例8：抗体-薬物コンジュゲートの*in vitro*活性評価〕

8)-1 CDH6陽性ヒト腫瘍細胞株に対する抗体-薬物コンジュゲートの*in vitro*細胞増殖抑制活性評価

CDH6陽性ヒト卵巣腫瘍細胞株PA-1を10% FBS含有MEM培地中 2×10^3 細胞/100 μL/wellになるよう96ウェルプレートに播種し、37℃、5% CO₂の条件下で一晩培養した。翌日、実施例7で作製したヒト化hG019-薬物コンジュゲート4種（クローン名は、H01L02-DXd、H02L02-DXd、H02L03-DXd及びH04L02-DXd）、又は参考例2で作製したNOV0712-薬物コンジュゲート（NOV0712-DM4）を終濃度0.0001（nM）から100（nM）となるように添加した。4日間培養後に生存細胞数をCellTiter-Glo™ Luminescent Cell Viabili

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ty Assay (Promega) によるATPの定量で測定した。図11に抗体-薬物コンジュゲートを各々添加した際の濃度依存的な細胞増殖抑制活性を示す。この結果、ヒト化hG019-薬物コンジュゲート4種は、NOV0712-薬物コンジュゲートと比較して、より低い添加濃度から腫瘍細胞の増殖抑制活性を示し、抗腫瘍活性が高いことが示される。

[0321]

[実施例9：抗体-薬物コンジュゲートのin vivo抗腫瘍効果]

抗体-薬物コンジュゲートの抗腫瘍効果は、CDH6陽性ヒト腫瘍細胞株の細胞を免疫不全マウスに移植した動物モデルを用いて評価した。4-5週齢のBALB/cヌードマウス(CAnN、Cg-Foxn1[nu]/Cr1Cr1j[Foxn1nu/Foxn1nu]、日本チャールス・リバー)及び、SCIDマウス(CB17/1cr-Prkdc[scid]/Cr1Cr1j、日本チャールス・リバー)を実験使用前にSPF条件化で3日間以上馴化した。マウスには滅菌した固形飼料(FR-2, Funabashi Farms Co., Ltd)を給餌し、滅菌した水道水(5-15ppm次亜塩素酸ナトリウム溶液を添加して調製)を与えた。移植した腫瘍の長径及び短径を電子式デジタルノギス(CD-15CX, Mitutoyo Corp.)で1週間に2回測定し、以下に示す計算式により腫瘍体積を算出した。

腫瘍体積(mm³) = 1/2 × 長径(mm) × [短径(mm)]²

抗体-薬物コンジュゲートは全てABS緩衝液(10mM-Acetate Buffer, 5% Sorbitol, pH5.5)(NACALAI)で希釈し、各実施例に示す用量にて尾静脈内投与した。また、コントロール群(Vehicle群)としてABS緩衝液を同様に投与した。1群あたり6匹のマウスを実験に用いた。

[0322] 9) - 1 抗腫瘍効果(1)

実施例2) - 3 - 1にてCDH6発現を確認した、CDH6陽性ヒト腎細胞腫瘍細胞株786-O(ATCC)をマトリゲル(コーニング社)に懸濁

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し、 5×10^5 cells を雄 SCID マウスの右側腹部に皮下移植し (Day 0)、Day 18 に無作為に群分けを実施した。群分け実施日に、実施例 7 で作製した抗体-薬物コンジュゲート 4 種 (クローン名は、H01L02-DXd、H02L02-DXd、H02L03-DXd 及び H04L02-DXd)、又は参考例 2 で作製した NOV0712-DM4 を 3 mg/kg の用量で尾静脈内投与した。結果を図 12 に示す。横軸は日数、縦軸は腫瘍体積、誤差範囲は SE 値を示す。

[0323] NOV0712-DM4 は本腫瘍モデルにおいて顕著な抗腫瘍効果を示さなかった。実施例 7 で作成した抗体-薬物コンジュゲート 4 種はいずれも投与後腫瘍体積が減少し、顕著な腫瘍の退縮が認められ、投与後 24 日の間、腫瘍退縮効果が持続した (図 12)。

[0324] 9) - 2 抗腫瘍効果 (2)

実施例 2) - 3 - 1 にて CDH6 発現を確認した、CDH6 陽性ヒト卵巣腫瘍細胞株 PA-1 (ATCC) をマトリゲル (コーニング社) に懸濁し、 5×10^5 cells を雌ヌードマウスの右側腹部に皮下移植し (Day 0)、Day 11 に無作為に群分けを実施した。群分け実施日に、実施例 7 で作製した抗体-薬物コンジュゲート H01L02-DXd 又は参考例 2 で作製した NOV0712-DM4 もしくは NOV0712-DXd を、1 及び 3 mg/kg の用量で尾静脈内投与した。結果を図 13 に示す。横軸は日数、縦軸は腫瘍体積、誤差範囲は SE 値を示す。

[0325] NOV0712-DM4 は本腫瘍モデルにおいて 1 及び 3 mg/kg いずれの用量でも抗腫瘍効果を示さなかった。一方、H01L02-DXd は 1 及び 3 mg/kg いずれの用量でも投与後腫瘍体積が著しく減少し、腫瘍を縮退させる効果を発揮した (図 13)。また、本明細書で取得した H01L02 抗体もしくは NOV0712 抗体に同じ薬物 DXd をコンジュゲートした検体の薬効を比較したところ、H01L02-DXd は、1 及び 3 mg/kg いずれの用量でも NOV0712-DXd に比べて強い抗腫瘍効果を発揮した。すなわち、本発明の H01L02 抗体は、NOV0712 抗体に比

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べて、抗腫瘍剤としての抗体-薬物コンジュゲートの抗体としてより優れた抗体であることが示された（図13）。

[0326] 9) - 3 抗腫瘍効果 (3)

実施例2) - 3 - 1にてCDH6発現を確認した、CDH6陽性ヒト卵巣腫瘍細胞株NIH:OVCAR-3 (ATCC)をマトリゲル（コーニング社）に懸濁し、 1×10^7 cellsを雌ヌードマウスの右側腹部に皮下移植し（Day 0）、Day 22に無作為に群分けを実施した。群分け実施日に、実施例7で作製した抗体-薬物コンジュゲートH01L02-DXd又は参考例2で作製したNOV0712-DM4を、1及び3 mg/kgの用量で尾静脈内投与した。結果を図14に示す。横軸は日数、縦軸は腫瘍体積、誤差範囲はSE値を示す。

[0327] NOV0712-DM4は、1 mg/kgでは全く抗腫瘍効果を示さず、3 mg/kgでは抗腫瘍効果を示すものの、投与2週間後から腫瘍の再増殖が認められた。一方、H01L02-DXdは1及び3 mg/kgいずれの用量でも、投与後腫瘍体積の増加を著しく抑制し、特に3 mg/kgでは投与後31日間の長期にわたって腫瘍増殖抑制効果が持続した。（図14）。

[0328] また、PA-1細胞を用いて同様に、参考例2で作製したNOV0712-DM4もしくは参考例3で作成したH01L02-DM4の腫瘍増殖抑制効果を評価した。H01L02-DM4はNOV0712-DM4よりも腫瘍体積を縮退させ、本発明のH01L02抗体は、NOV0712抗体に比べて、抗腫瘍剤としての抗体-薬物コンジュゲートの抗体としてより優れた抗体であった。

[0329] 9) - 4 抗腫瘍効果 (4)

実施例2) - 3 - 1にてCDH6発現を確認した、CDH6陽性ヒト腎細胞腫瘍細胞株786-O (ATCC)をマトリゲル（コーニング社）に懸濁し、 5×10^6 cellsを雄SCIDマウスの右側腹部に皮下移植し（Day 0）、Day 20に無作為に群分けを実施した。群分け実施日に、実施例7で作製した抗体-薬物コンジュゲートH01L02-DXd又は参考例2

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で作製したNOVO712-DM4を、1及び3mg/kgの用量で尾静脈内投与した。結果を図15に示す。横軸は日数、縦軸は腫瘍体積、誤差範囲はSE値を示す。

[0330] NOVO712-DM4は本腫瘍モデルにおいて1及び3mg/kgいずれの用量でも顕著な抗腫瘍効果を示さなかった。一方、H01L02-DXdは1及び3mg/kgいずれの用量でも投与後腫瘍体積が減少し、特に3mg/kgでは顕著な腫瘍の退縮が認められ、投与後20日の間、腫瘍退縮効果が持続した（図15）。

[0331] 9) - 5 抗腫瘍効果 (5)

実施例2) - 3 - 1にてCDH6を発現しないことを確認した、CDH6陰性ヒト卵巣腫瘍細胞株ES-2 (ATCC)を生理食塩水に懸濁し、 1×10^6 cellsを雌ヌードマウスの右側腹部に皮下移植し (Day 0)、Day 7に無作為に群分けを実施した。群分け実施日に、実施例7で作成した抗体-薬物コンジュゲートH01L02-DXd又は参考例2で作製したNOVO712-DM4を、1及び3mg/kgの用量で尾静脈内投与した。結果を図16に示す。横軸は日数、縦軸は腫瘍体積、誤差範囲はSE値を示す。

[0332] CDH6を発現しない本腫瘍モデルでは、H01L02-DXd及びNOVO712-DM4はいずれの用量でも抗腫瘍効果を全く示さなかった。この結果から、実施例9) - 1、9) - 2、9) - 3、9) - 4で示されたCDH6陽性腫瘍モデルでの抗体-薬物コンジュゲートの抗腫瘍効果は、腫瘍細胞のCDH6発現に依存して起こる効果であり、CDH6陽性の腫瘍に特異的に抗腫瘍効果を示し、CDH6陰性の正常組織には細胞障害を起こさない選択的で安全な抗腫瘍薬剤と考えられる（図16）。

産業上の利用可能性

[0333] 本発明により、内在化活性を有する抗CDH6抗体及び該抗体を含む抗体-薬物コンジュゲートが提供された。該抗体-薬物コンジュゲートは癌に対する治療薬等として用いることができる。

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請求の範囲

- 【請求項1】 配列番号4に記載のアミノ酸配列に特異的に結合し、細胞内に取り込まれる内在化能を有する、抗体又は当該抗体の機能性断片。
- 【請求項2】 配列番号4に記載のアミノ酸配列への結合に対して、以下の（1）～（5）：
- （1）配列番号53の21～233番目に記載のアミノ酸配列からなる軽鎖及び配列番号56の20～471番目に記載のアミノ酸配列からなる重鎖を有する抗体、
 - （2）配列番号61の21～233番目に記載のアミノ酸配列からなる軽鎖及び配列番号69の20～471番目に記載のアミノ酸配列からなる重鎖を有する抗体、
 - （3）配列番号61の21～233番目に記載のアミノ酸配列からなる軽鎖及び配列番号73の20～471番目に記載のアミノ酸配列からなる重鎖を有する抗体、
 - （4）配列番号65の21～233番目に記載のアミノ酸配列からなる軽鎖及び配列番号73の20～471番目に記載のアミノ酸配列からなる重鎖を有する抗体、及び
 - （5）配列番号61の21～233番目に記載のアミノ酸配列からなる軽鎖及び配列番号77の20～471番目に記載のアミノ酸配列からなる重鎖を有する抗体、
- からなる群から選択される抗体のうち少なくともいずれか1つと競合阻害活性を有する請求項1に記載の抗体又は当該抗体の機能性断片。
- 【請求項3】 以下の（1）～（4）：
- （1）配列番号12に記載のアミノ酸配列からなるCDRL1、配列番号13に記載のアミノ酸配列からなるCDRL2及び配列番号14に記載のアミノ酸配列からなるCDRL3、
 - （2）配列番号22に記載のアミノ酸配列からなるCDRL1、配列番号23に記載のアミノ酸配列からなるCDRL2及び配列番号24

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に記載のアミノ酸配列からなるCDRL3、

(3) 配列番号32に記載のアミノ酸配列からなるCDRL1、配列番号33に記載のアミノ酸配列からなるCDRL2及び配列番号34に記載のアミノ酸配列からなるCDRL3、及び

(4) 配列番号42に記載のアミノ酸配列からなるCDRL1、配列番号43に記載のアミノ酸配列からなるCDRL2及び配列番号44に記載のアミノ酸配列からなるCDRL3、

からなる群から選択されるいずれか1つに記載のCDRL1、CDRL2及びCDRL3、並びに、

以下の(5)～(9)：

(5) 配列番号17に記載のアミノ酸配列からなるCDRH1、配列番号18に記載のアミノ酸配列からなるCDRH2及び配列番号19に記載のアミノ酸配列からなるCDRH3、

(6) 配列番号27に記載のアミノ酸配列からなるCDRH1、配列番号28に記載のアミノ酸配列からなるCDRH2及び配列番号29に記載のアミノ酸配列からなるCDRH3、

(7) 配列番号37に記載のアミノ酸配列からなるCDRH1、配列番号38に記載のアミノ酸配列からなるCDRH2及び配列番号39に記載のアミノ酸配列からなるCDRH3、

(8) 配列番号47に記載のアミノ酸配列からなるCDRH1、配列番号48に記載のアミノ酸配列からなるCDRH2及び配列番号49に記載のアミノ酸配列からなるCDRH3、及び

(9) 配列番号17に記載のアミノ酸配列からなるCDRH1、配列番号60に記載のアミノ酸配列からなるCDRH2及び配列番号19に記載のアミノ酸配列からなるCDRH3、

からなる群から選択されるいずれか1つに記載のCDRH1、CDRH2及びCDRH3を含む、

請求項1又2のいずれか1項に記載の抗体又は当該抗体の機能性断片

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[請求項4]

以下の（１）～（５）：

（１）配列番号１２に記載のアミノ酸配列からなるCDRL１、配列番号１３に記載のアミノ酸配列からなるCDRL２及び配列番号１４に記載のアミノ酸配列からなるCDRL３、及び、配列番号１７に記載のアミノ酸配列からなるCDRH１、配列番号１８に記載のアミノ酸配列からなるCDRH２及び配列番号１９に記載のアミノ酸配列からなるCDRH３、

（２）配列番号２２に記載のアミノ酸配列からなるCDRL１、配列番号２３に記載のアミノ酸配列からなるCDRL２及び配列番号２４に記載のアミノ酸配列からなるCDRL３、及び、配列番号２７に記載のアミノ酸配列からなるCDRH１、配列番号２８に記載のアミノ酸配列からなるCDRH２及び配列番号２９に記載のアミノ酸配列からなるCDRH３、

（３）配列番号３２に記載のアミノ酸配列からなるCDRL１、配列番号３３に記載のアミノ酸配列からなるCDRL２及び配列番号３４に記載のアミノ酸配列からなるCDRL３、及び、配列番号３７に記載のアミノ酸配列からなるCDRH１、配列番号３８に記載のアミノ酸配列からなるCDRH２及び配列番号３９に記載のアミノ酸配列からなるCDRH３、

（４）配列番号４２に記載のアミノ酸配列からなるCDRL１、配列番号４３に記載のアミノ酸配列からなるCDRL２及び配列番号４４に記載のアミノ酸配列からなるCDRL３、及び、配列番号４７に記載のアミノ酸配列からなるCDRH１、配列番号４８に記載のアミノ酸配列からなるCDRH２及び配列番号４９に記載のアミノ酸配列からなるCDRH３、及び

（５）配列番号１２に記載のアミノ酸配列からなるCDRL１、配列番号１３に記載のアミノ酸配列からなるCDRL２及び配列番号１４

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に記載のアミノ酸配列からなるCDRL3、及び、配列番号17に記載のアミノ酸配列からなるCDRH1、配列番号60に記載のアミノ酸配列からなるCDRH2及び配列番号19に記載のアミノ酸配列からなるCDRH3、

からなる群から選択されるCDRL1、CDRL2及びCDRL3、並びに、CDRH1、CDRH2及びCDRH3を含む、

請求項1～3のいずれか1項に記載の抗体又は当該抗体の機能性断片。

〔請求項5〕 ヒト化されている請求項1～4のいずれか1項に記載の抗体又は当該抗体の機能性断片。

〔請求項6〕 以下の(1)～(4)：

(1) 配列番号63に記載のアミノ酸配列からなる軽鎖可変領域、
(2) 配列番号67に記載のアミノ酸配列からなる軽鎖可変領域、
(3) (1)～(2)のアミノ酸配列において各CDR配列以外のフレームワーク領域の配列に対して少なくとも95%以上の配列同一性を有するアミノ酸配列、及び

(4) (1)～(3)のアミノ酸配列において各CDR配列以外のフレームワーク領域の配列において1又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列
からなる群から選択されるいずれか1つに記載の軽鎖可変領域、並びに、

以下の(5)～(9)：

(5) 配列番号71に記載のアミノ酸配列からなる重鎖可変領域、
(6) 配列番号75に記載のアミノ酸配列からなる重鎖可変領域、
(7) 配列番号79に記載のアミノ酸配列からなる重鎖可変領域、
(8) (5)～(7)のアミノ酸配列において各CDR配列以外のフレームワーク領域の配列に対して少なくとも95%以上の配列相同性を有するアミノ酸配列、及び

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(9) (5)～(8)のアミノ酸配列において各CDR配列以外のフレームワーク領域の配列において1又は数個のアミノ酸が欠失、置換又は付加されたアミノ酸配列からなる群から選択されるいずれか1つに記載の重鎖可変領域、を有する請求項1～5のいずれか1項に記載の抗体又は当該抗体の機能性断片。

[請求項7]

以下の(1)～(4)：

(1) 配列番号63に記載のアミノ酸配列からなる軽鎖可変領域及び配列番号71に記載のアミノ酸配列からなる重鎖可変領域、

(2) 配列番号63に記載のアミノ酸配列からなる軽鎖可変領域及び配列番号75に記載のアミノ酸配列からなる重鎖可変領域、

(3) 配列番号67に記載のアミノ酸配列からなる軽鎖可変領域及び配列番号75に記載のアミノ酸配列からなる重鎖可変領域、又は

(4) 配列番号63に記載のアミノ酸配列からなる軽鎖可変領域及び配列番号79に記載のアミノ酸配列からなる重鎖可変領域、

のいずれかの軽鎖可変領域及び重鎖可変領域、

を含む請求項1～6のいずれか1項に記載の抗体又は当該抗体の機能性断片。

[請求項8]

以下の(1)～(4)：

(1) 配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号69の20～471番目のアミノ酸配列からなる重鎖、

(2) 配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号73の20～471番目のアミノ酸配列からなる重鎖、

(3) 配列番号65の21～233番目のアミノ酸配列からなる軽鎖及び配列番号73の20～471番目のアミノ酸配列からなる重鎖、

又は

(4) 配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号77の20～471番目のアミノ酸配列からなる重鎖、

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- のいずれかを有する請求項1～7のいずれか1項に記載の抗体又は当該抗体の機能性断片。
- 【請求項9】 配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号69の20～471番目のアミノ酸配列からなる重鎖を有する請求項8に記載の抗体又は当該抗体の機能性断片。
- 【請求項10】 配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号73の20～471番目のアミノ酸配列からなる重鎖を有する請求項8に記載の抗体又は当該抗体の機能性断片。
- 【請求項11】 配列番号65の21～233番目のアミノ酸配列からなる軽鎖及び配列番号73の20～471番目のアミノ酸配列からなる重鎖を有する請求項8に記載の抗体又は当該抗体の機能性断片。
- 【請求項12】 配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号77の20～471番目のアミノ酸配列からなる重鎖を有する請求項8に記載の抗体又は当該抗体の機能性断片。
- 【請求項13】 機能性断片がFab、F(ab')₂、Fab'及びFvからなる群から選択される請求項1～12のいずれか1項に記載の抗体の機能性断片。
- 【請求項14】 請求項1～13のいずれか1項に記載の抗体又は当該抗体の機能性断片をコードするポリヌクレオチド。
- 【請求項15】 以下の(1)～(5)：
- (1) 配列番号12に記載のアミノ酸配列からなるCDRL1、配列番号13に記載のアミノ酸配列からなるCDRL2及び配列番号14に記載のアミノ酸配列からなるCDRL3を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号17に記載のアミノ酸配列からなるCDRH1、配列番号18に記載のアミノ酸配列からなるCDRH2及び配列番号19に記載のアミノ酸配列からなるCDRH3を含む重鎖可変領域をコードするポリヌクレオチド、
- (2) 配列番号22に記載のアミノ酸配列からなるCDRL1、配列

番号23に記載のアミノ酸配列からなるCDRL2及び配列番号24に記載のアミノ酸配列からなるCDRL3を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号27に記載のアミノ酸配列からなるCDRH1、配列番号28に記載のアミノ酸配列からなるCDRH2及び配列番号29に記載のアミノ酸配列からなるCDRH3を含む重鎖可変領域をコードするポリヌクレオチド、

(3) 配列番号32に記載のアミノ酸配列からなるCDRL1、配列番号33に記載のアミノ酸配列からなるCDRL2及び配列番号34に記載のアミノ酸配列からなるCDRL3を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号37に記載のアミノ酸配列からなるCDRH1、配列番号38に記載のアミノ酸配列からなるCDRH2及び配列番号39に記載のアミノ酸配列からなるCDRH3を含む重鎖可変領域をコードするポリヌクレオチド、

(4) 配列番号42に記載のアミノ酸配列からなるCDRL1、配列番号43に記載のアミノ酸配列からなるCDRL2及び配列番号44に記載のアミノ酸配列からなるCDRL3を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号47に記載のアミノ酸配列からなるCDRH1、配列番号48に記載のアミノ酸配列からなるCDRH2及び配列番号49に記載のアミノ酸配列からなるCDRH3を含む重鎖可変領域をコードするポリヌクレオチド、並びに

(5) 配列番号12に記載のアミノ酸配列からなるCDRL1、配列番号13に記載のアミノ酸配列からなるCDRL2及び配列番号14に記載のアミノ酸配列からなるCDRL3を含む軽鎖可変領域をコードするポリヌクレオチド、及び、配列番号17に記載のアミノ酸配列からなるCDRH1、配列番号60に記載のアミノ酸配列からなるCDRH2及び配列番号19に記載のアミノ酸配列からなるCDRH3を含む重鎖可変領域をコードするポリヌクレオチド、
からなる群から選択されるいずれか1つに記載のポリヌクレオチドを

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- 含む請求項14に記載のポリヌクレオチド。
- 〔請求項16〕 配列番号61の21～233番目のアミノ酸配列からなる軽鎖をコードするポリヌクレオチド及び配列番号69の20～471番目のアミノ酸配列からなる重鎖をコードするポリヌクレオチドを含む請求項14又は15に記載のポリヌクレオチド。
- 〔請求項17〕 配列番号61の21～233番目のアミノ酸配列からなる軽鎖をコードするポリヌクレオチド及び配列番号73の20～471番目のアミノ酸配列からなる重鎖をコードするポリヌクレオチドを含む請求項14又は15に記載のポリヌクレオチド。
- 〔請求項18〕 配列番号65の21～233番目のアミノ酸配列からなる軽鎖をコードするポリヌクレオチド及び配列番号73の20～471番目のアミノ酸配列からなる重鎖をコードするポリヌクレオチドを含む請求項14又は15に記載のポリヌクレオチド。
- 〔請求項19〕 配列番号61の21～233番目のアミノ酸配列からなる軽鎖をコードするポリヌクレオチド及び配列番号77の20～471番目のアミノ酸配列からなる重鎖をコードするポリヌクレオチドを含む請求項14又は15に記載のポリヌクレオチド。
- 〔請求項20〕 請求項14～19のいずれか1項に記載のポリヌクレオチドを含有する発現ベクター。
- 〔請求項21〕 請求項20に記載の発現ベクターにより形質転換された宿主細胞。
- 〔請求項22〕 宿主細胞が真核細胞である請求項21に記載の宿主細胞。
- 〔請求項23〕 請求項21又は22に記載の宿主細胞を培養する工程、及び当該工程で得られた培養物から目的の抗体又は当該抗体の機能性断片を採取する工程を含むことを特徴とする当該抗体又は当該抗体の機能性断片の製造方法。
- 〔請求項24〕 重鎖又は軽鎖が、N-結合への糖鎖付加、O-結合への糖鎖付加、N末のプロセッシング、C末のプロセッシング、脱アミド化、アスパラギン酸の異性化、メチオニンの酸化、N末にメチオニン残基の付加、

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プロリン残基のアミド化、N末グルタミンもしくはN末グルタミン酸のピログルタミン酸化、及びカルボキシル末端における1つ又は2つのアミノ酸欠失からなる群より選択される1又は2以上の修飾をうけた、請求項1～13のいずれか1項に記載の抗体又は当該抗体の機能性断片。

〔請求項25〕 重鎖のカルボキシル末端において1つ又は2つのアミノ酸が欠失している請求項24に記載の抗体。

〔請求項26〕 2本の重鎖の双方でカルボキシル末端において1つのアミノ酸が欠失している請求項25に記載の抗体。

〔請求項27〕 重鎖のカルボキシル末端のプロリン残基が更にアミド化されている請求項24～26のいずれか1項に記載の抗体。

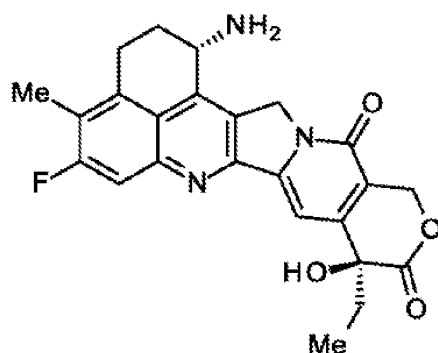
〔請求項28〕 抗体依存性細胞傷害活性を増強させるために糖鎖修飾が調節されている請求項1～13及び請求項24～27からなる群から選択されるいずれか1項に記載の抗体又は当該抗体の機能性断片。

〔請求項29〕 請求項1～13及び請求項24～28からなる群から選択されるいずれか1項に記載の抗体又は当該抗体の機能性断片に薬物が結合している抗体－薬物コンジュゲート。

〔請求項30〕 薬物が抗腫瘍性化合物である、請求項29に記載の抗体－薬物コンジュゲート。

〔請求項31〕 抗腫瘍性化合物が次式：

〔化1〕



で示される抗腫瘍性化合物である請求項30に記載の抗体-薬物コンジュゲート。

【請求項32】 抗体と薬物が、次式 (a) ~ (f) :

(a) - (Succinimid-3-yl-N) -CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-、

(b) - (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-、

(c) - (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂-O-CH₂-C(=O)-、

(d) - (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂-O-CH₂-C(=O)-、

(e) - (Succinimid-3-yl-N) -CH₂CH₂-C(=O)-NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-、及び

(f) - (Succinimid-3-yl-N) -CH₂CH₂-C(=O)-NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-、

からなる群から選択されるいずれかの構造のリンカーを介して結合している、請求項29~31のいずれか1項に記載の抗体-薬物コンジュゲート。

(ここで、抗体は、- (Succinimid-3-yl-N) の末端において結合する。抗腫瘍性化合物は、1位のアミノ基の窒素原子を結合部位として、(a)、(b)、(e)又は(f)の-CH₂CH₂CH₂CH₂-C(=O)-部分、(c)のCH₂-O-CH₂-C(=

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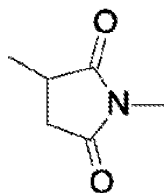
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O) 一部分又は (d) の $\text{CH}_2\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})$ 一部分のカルボニル基に結合する。上記式中GGFGは、グリシン-グリシン-フェニルアラニン-グリシンからなるペプチド結合でつながっているアミノ酸配列を示す。

—(Succinimid-3-yl-N)—は次式：

[化2]



で示される構造であり、このものの3位で抗体と結合し、1位の窒素原子上でこれを含むリンカー構造内のメチレン基と結合する。)

[請求項33] リンカーが以下の (c)、(d) 及び (e) からなる群から選択されるいずれかの式で示される請求項29～32のいずれか1項に記載の抗体-薬物コンジュゲート：

(c) —(Succinimid-3-yl-N)— $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$ 、

(d) —(Succinimid-3-yl-N)— $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$ 、

(e) —(Succinimid-3-yl-N)— $\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$ 。

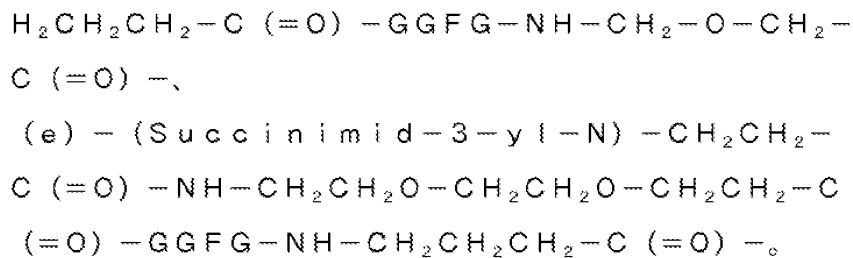
[請求項34] リンカーが以下の (c) 又は (e) の式で示される請求項29～33のいずれか1項に記載の抗体-薬物コンジュゲート：

(c) —(Succinimid-3-yl-N)— $\text{CH}_2\text{CH}_2\text{C}$

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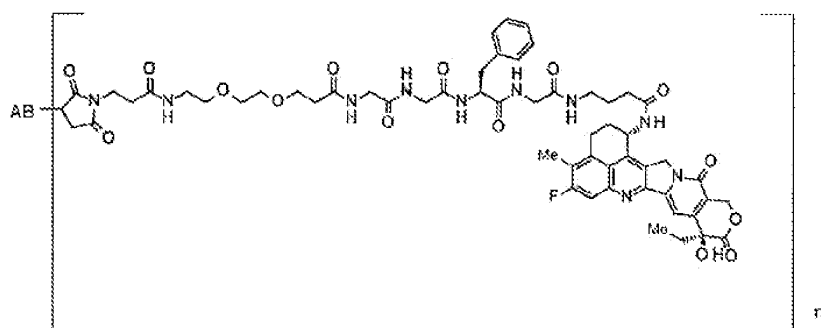
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【請求項35】 以下の式：

【化3】

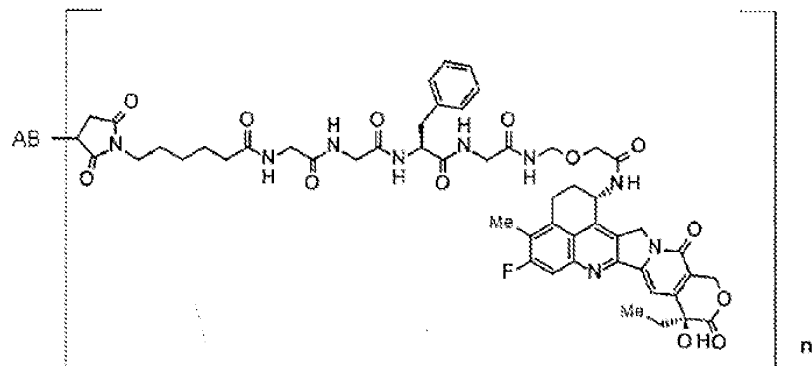


で示される構造を有する、請求項29～34のいずれか1項に記載の抗体-薬物コンジュゲート：

ここで、ABは抗体又は該抗体の機能性断片を示す。nは抗体と結合している薬物-リンカー構造の1抗体あたりの平均結合数を示す。抗体とリンカーは抗体由来のスルフヒドリル基を介して結合している。

【請求項36】 以下の式：

【化4】



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で示される構造を有する、請求項29～34のいずれか1項に記載の抗体-薬物コンジュゲート；

ここで、ABは抗体又は該抗体の機能性断片を示す。nは抗体と結合している薬物-リンカー構造の1抗体あたりの平均結合数を示す。抗体とリンカーは抗体由来のスルフヒドリル基を介して結合している。

〔請求項37〕 抗体が以下の(1)～(4)；

(1) 配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号69の20～471番目のアミノ酸配列からなる重鎖、

(2) 配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号73の20～471番目のアミノ酸配列からなる重鎖、

(3) 配列番号65の21～233番目のアミノ酸配列からなる軽鎖及び配列番号73の20～471番目のアミノ酸配列からなる重鎖、又は

(4) 配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号77の20～471番目のアミノ酸配列からなる重鎖、からなる群から選択されるいずれか1つに記載の軽鎖及び重鎖を含む抗体又は当該抗体の機能性断片である、請求項29～36のいずれか1項に記載の抗体-薬物コンジュゲート。

〔請求項38〕 抗体が、配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号69の20～471番目のアミノ酸配列からなる重鎖を含む抗体又は当該抗体の機能性断片である、請求項37に記載の抗体-薬物コンジュゲート。

〔請求項39〕 抗体が、配列番号61の21～233番目のアミノ酸配列からなる軽鎖及び配列番号77の20～471番目のアミノ酸配列からなる重鎖を含む抗体又は当該抗体の機能性断片である、請求項37に記載の抗体-薬物コンジュゲート。

〔請求項40〕 重鎖又は軽鎖が、N-結合への糖鎖付加、O-結合への糖鎖付加、N末のプロセッシング、C末のプロセッシング、脱アミド化、アスパラ

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ギン酸の異性化、メチオニンの酸化、N末にメチオニン残基の付加、プロリン残基のアミド化、N末グルタミンもしくはN末グルタミン酸のピログルタミン酸化及びカルボキシル末端における1つ又は2つのアミノ酸欠失からなる群から選択される1又は2以上の修飾をうけた、請求項29～39のいずれか1項に記載の抗体－薬物コンジュゲート。

【請求項41】 選択された1種の薬物－リンカー構造の1抗体あたりの平均結合数が1～10個の範囲である請求項29～40のいずれか1項に記載の抗体－薬物コンジュゲート。

【請求項42】 選択された1種の薬物－リンカー構造の1抗体あたりの平均結合数が2～8個の範囲である請求項41に記載の抗体－薬物コンジュゲート。

【請求項43】 選択された1種の薬物－リンカー構造の1抗体あたりの平均結合数が5～8個の範囲である請求項42に記載の抗体－薬物コンジュゲート。

【請求項44】 選択された1種の薬物－リンカー構造の1抗体あたりの平均結合数が7～8個である請求項43に記載の抗体－薬物コンジュゲート。

【請求項45】 請求項29～44のいずれか1項に記載の抗体－薬物コンジュゲート、その塩、又はそれらの水和物を含むことを特徴とする医薬組成物。

【請求項46】 抗腫瘍薬であることを特徴とする、請求項45に記載の医薬組成物。

【請求項47】 腫瘍がCDH6を発現する腫瘍であることを特徴とする、請求項46に記載の医薬組成物。

【請求項48】 腫瘍が、腎細胞癌、腎臓明細胞癌、乳頭状腎細胞癌、卵巣癌、卵巣漿液性腺癌、甲状腺癌、胆管癌、肺癌、小細胞肺癌、神経膠芽腫、中皮腫、子宮癌、膵臓癌、ウィルムス腫瘍又は神経芽腫であることを特徴とする、請求項46又は47に記載の医薬組成物。

【請求項49】 請求項29～44のいずれか1項に記載の抗体－薬物コンジュゲート、その塩、又はそれらの水和物から選択されるいずれかを個体に投与

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することを特徴とする腫瘍の治療方法。

【請求項50】 腫瘍がCDH6が発現している腫瘍であることを特徴とする、請求項49に記載の治療方法。

【請求項51】 腫瘍が、腎細胞癌、腎淡明細胞癌、乳頭状腎細胞癌、卵巢癌、卵巢漿液性腺癌、甲状腺癌、胆管癌、肺癌、小細胞肺癌、神経膠芽腫、中皮腫、子宮癌、膵臓癌、ウィルムス腫瘍又は神経芽腫であることを特徴とする、請求項49又は50に記載の治療方法。

【請求項52】 請求項29～44のいずれか1項に記載の抗体－薬物コンジュゲート、その塩、又はそれらの水和物から選択される少なくとも一つを含む医薬組成物及び少なくとも一つの抗腫瘍薬を、同時に、別々に又は連続して個体に投与することを特徴とする腫瘍の治療方法。

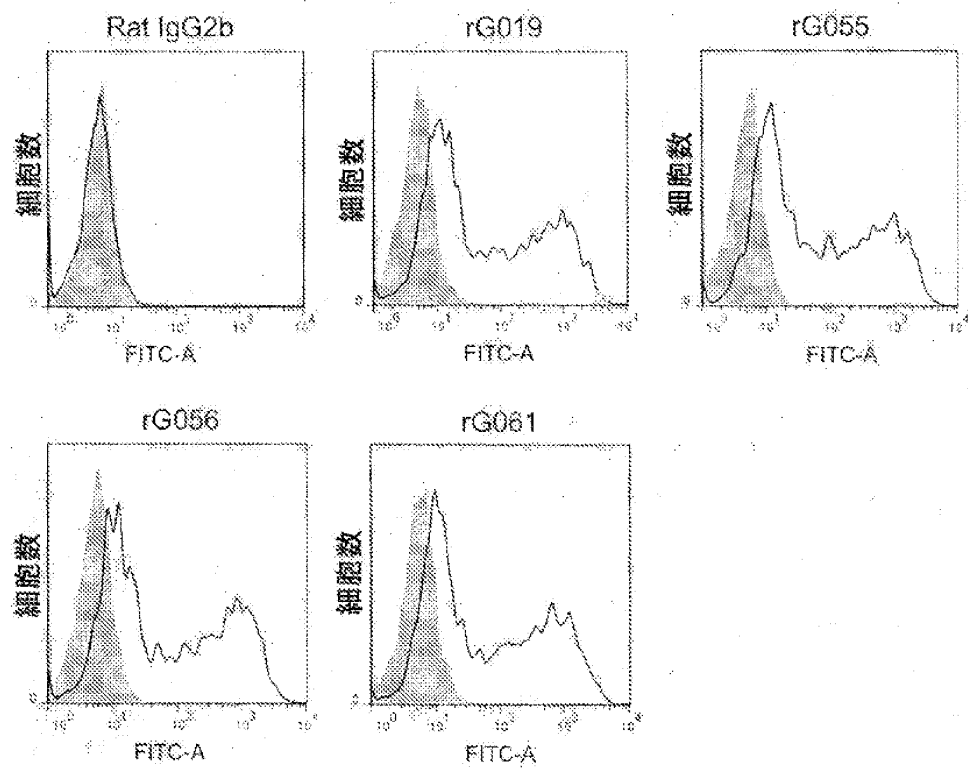
【請求項53】 請求項1～13及び請求項24～28からなる群から選択されるいずれか1項に記載の抗体又は当該抗体の機能性断片或いは請求項23に記載の製造方法で得られる抗体又は当該抗体の機能性断片と、薬物－リンカー中間化合物を反応させる工程を含むことを特徴とする、抗体－薬物コンジュゲートの製造方法。

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[図1]

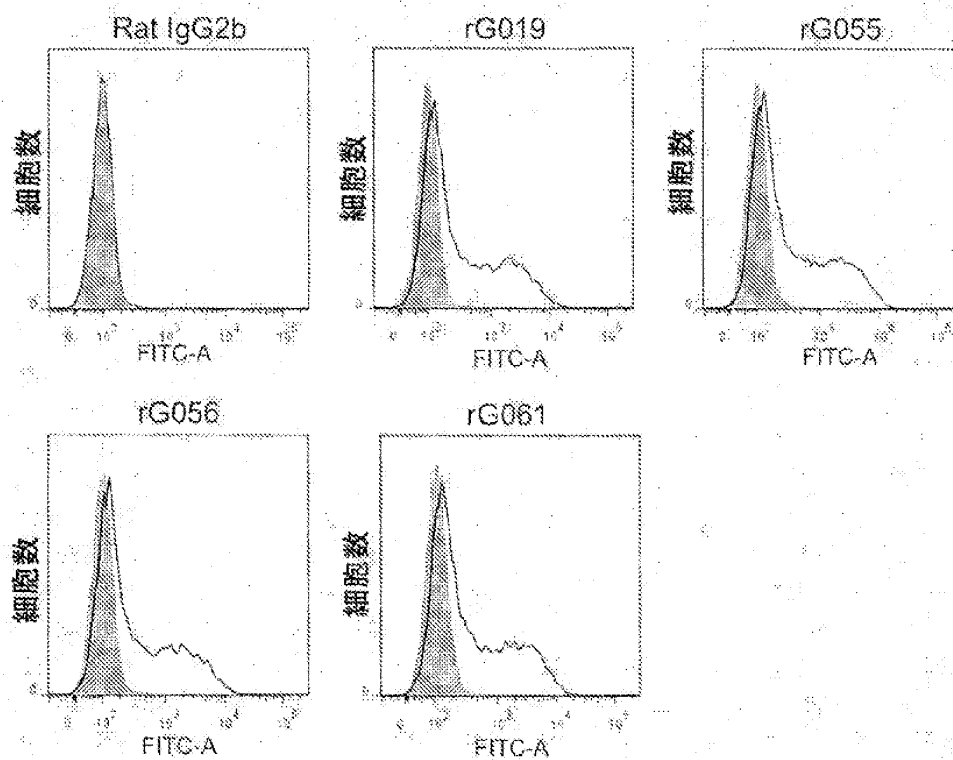


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[図2-1]

(1) コントロール細胞又は全長hCDH6導入細胞



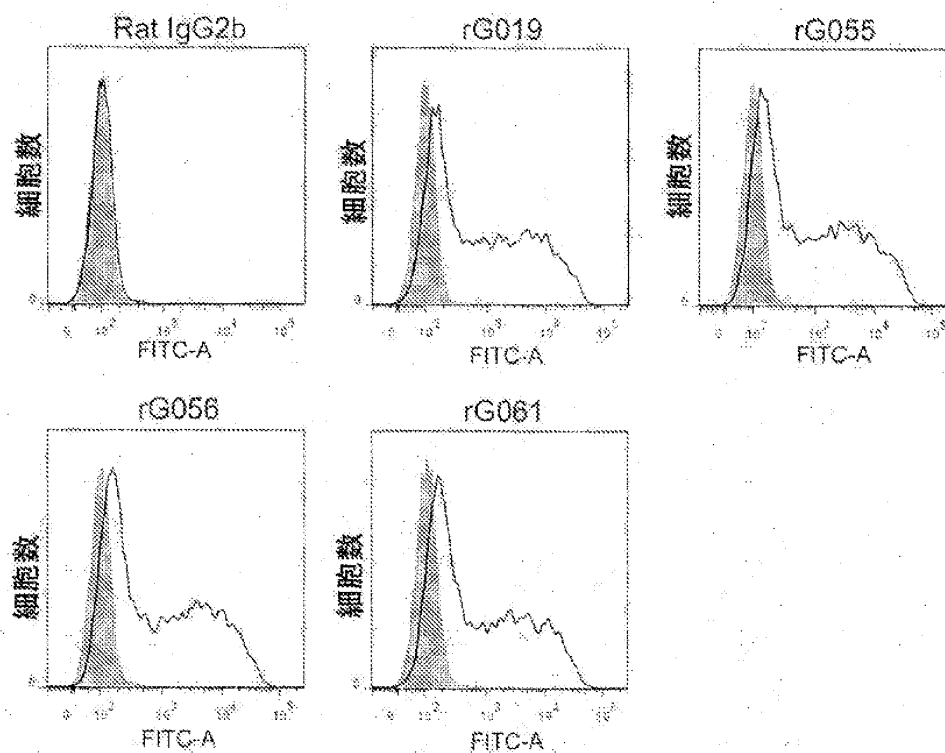
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[図2-2]

(2) コントロール細胞又はEC1欠失hCDH6導入細胞



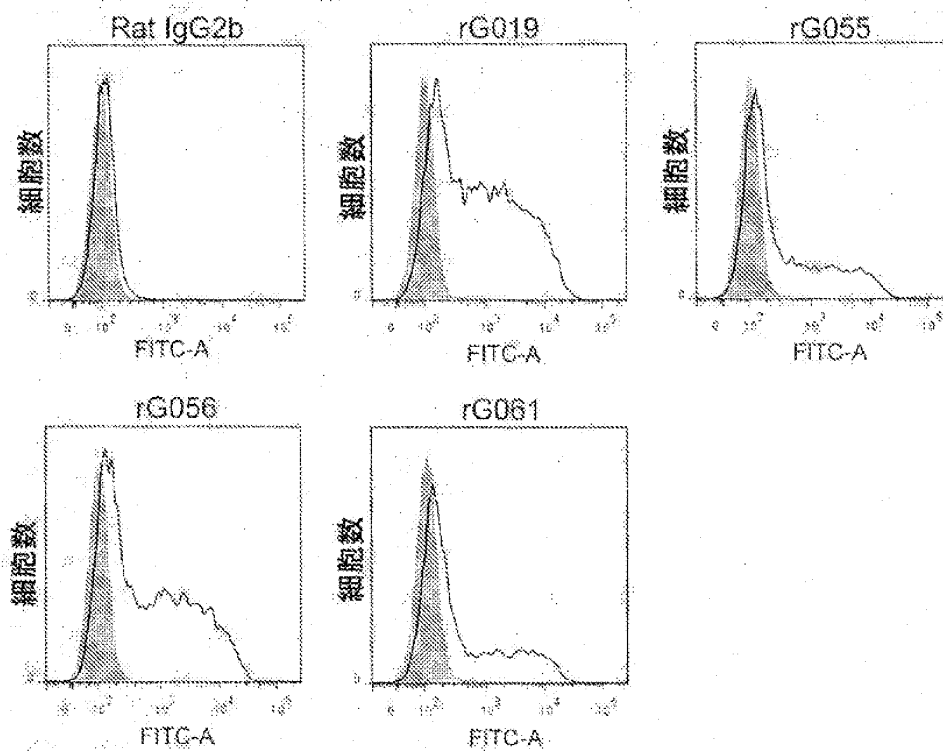
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[図2-3]

(3) コントロール細胞又はEC2欠失hCDH6導入細胞



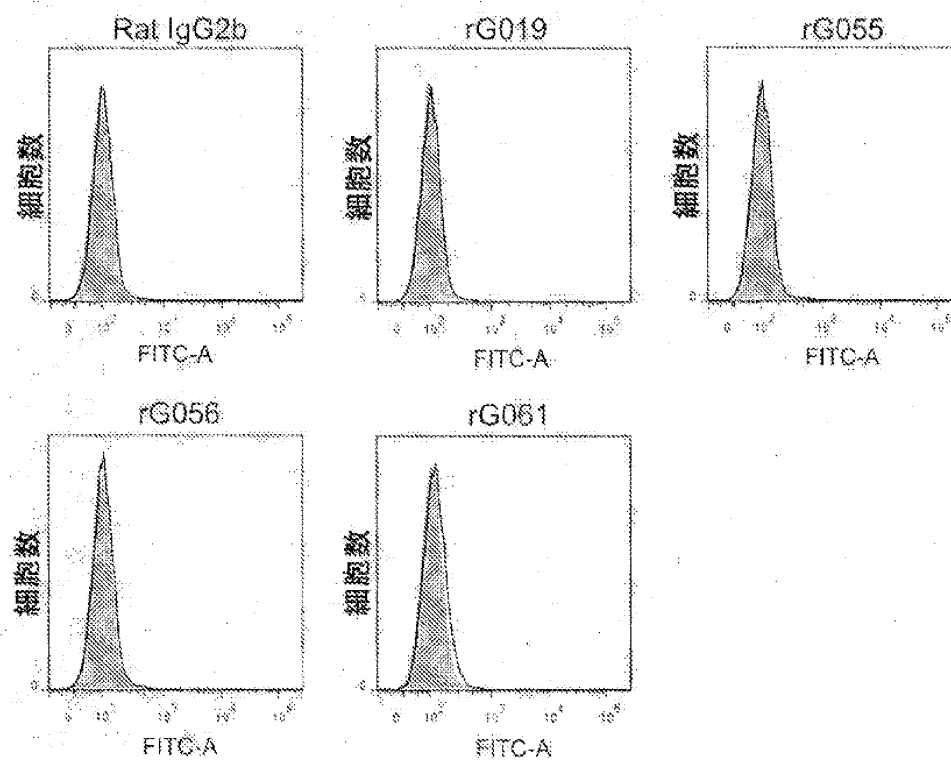
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[図2-4]

(4) コントロール細胞又はEC3欠失hCDH6導入細胞



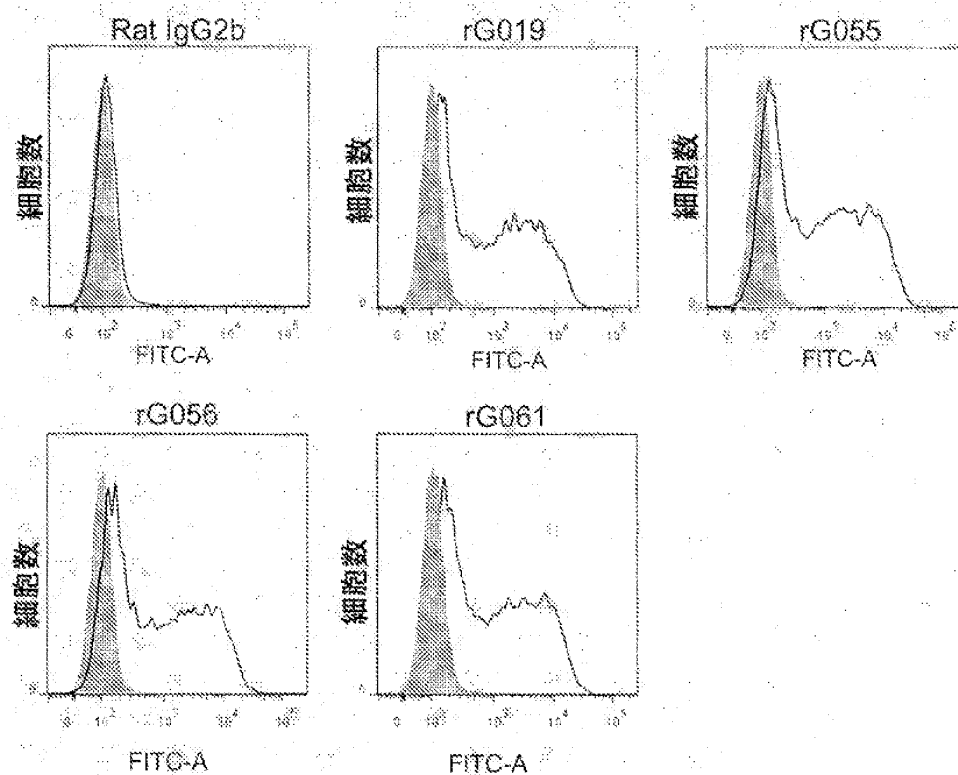
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[図2-5]

(5) コントロール細胞又はEC4欠失hCDH6導入細胞



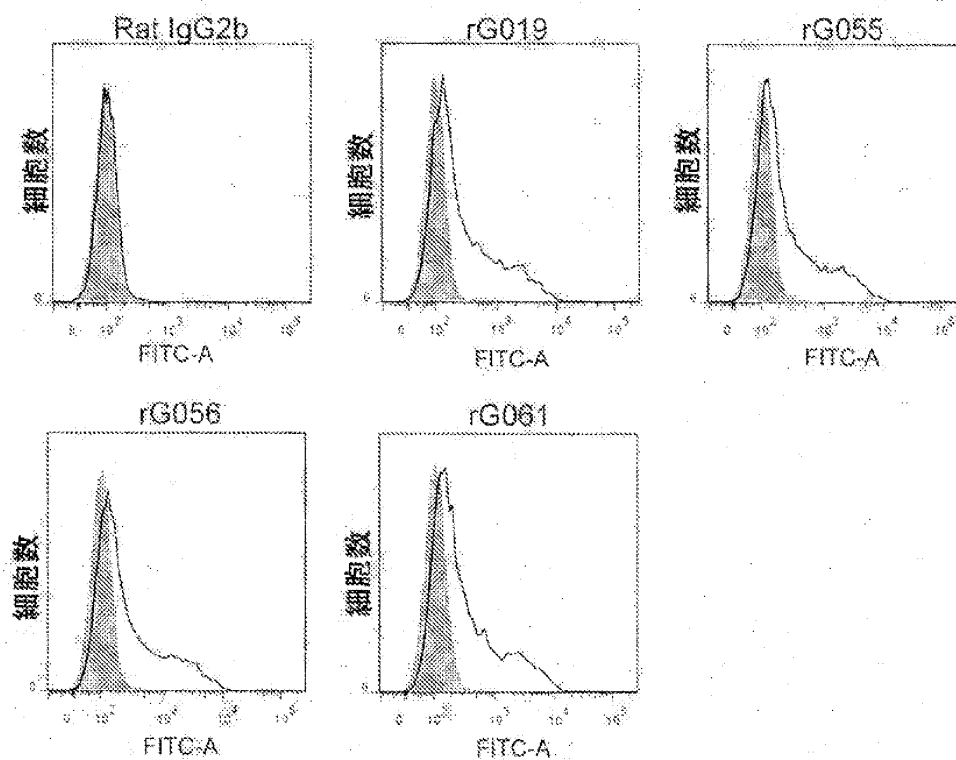
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[図2-6]

(6) コントロール細胞又はEC5欠失hCDH6導入細胞

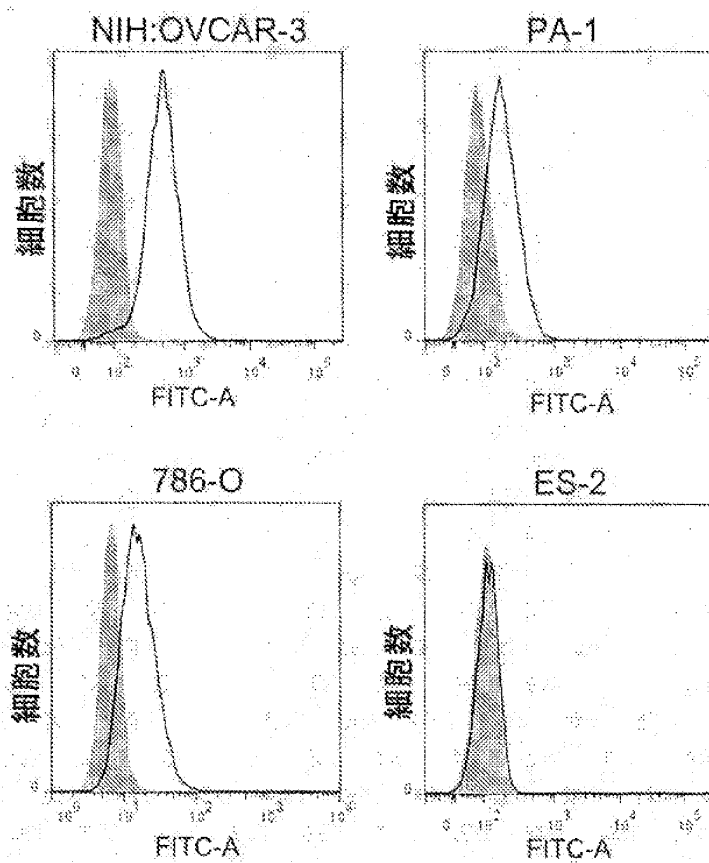


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[図3]

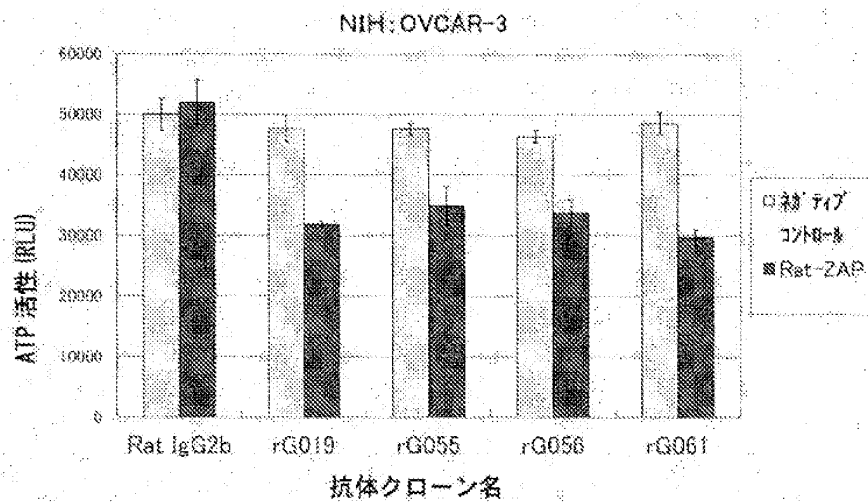


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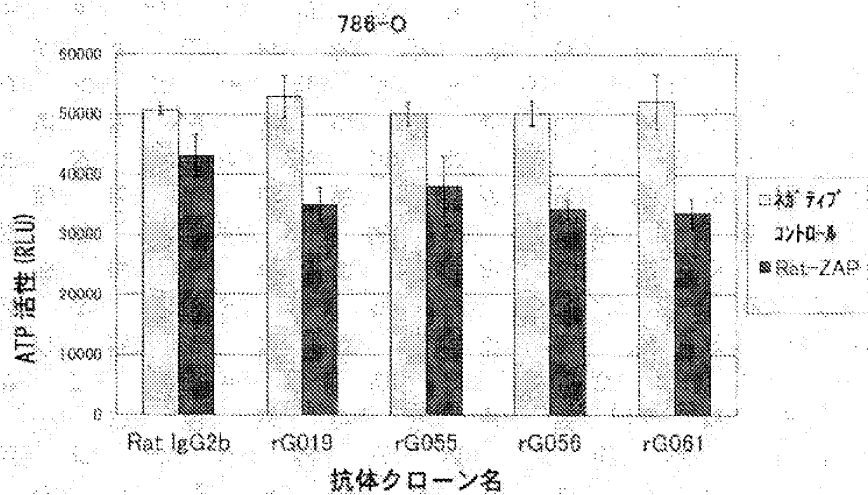
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[図4]



Rat-ZAPを用いた NIH: OVCAR-3 細胞生存率 (%)

Rat IgG2b	rG019	rG055	rG056	rG061
104.1	66.8	73.3	72.8	61.2



Rat-ZAPを用いた 786-O 細胞生存率 (%)

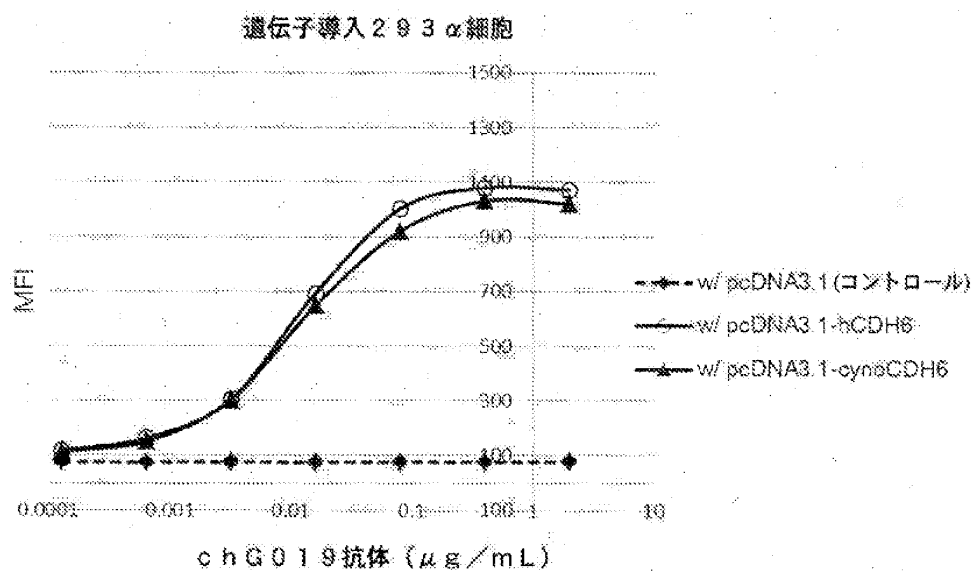
Rat IgG2b	rG019	rG055	rG056	rG061
85.2	66.1	75.7	68.1	64.2

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[図5]

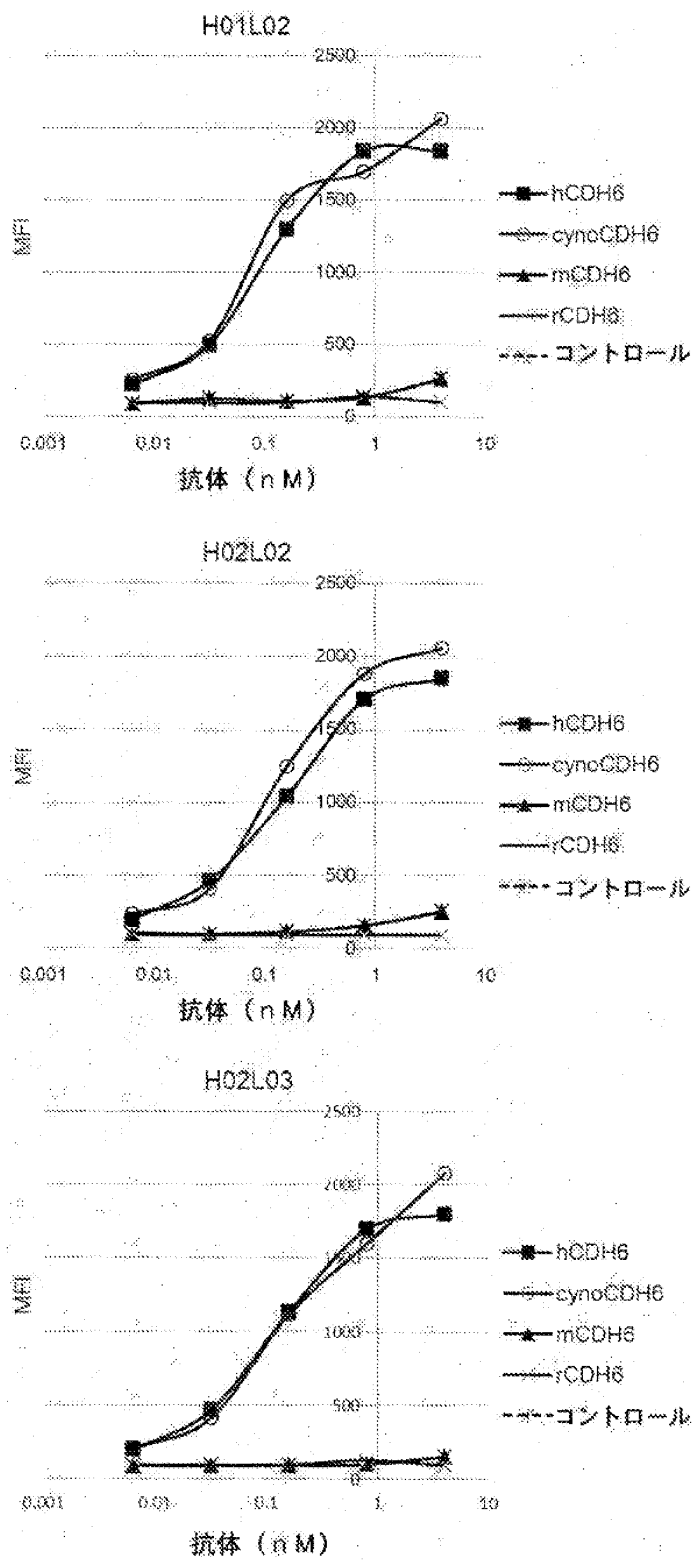


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[図6-1]

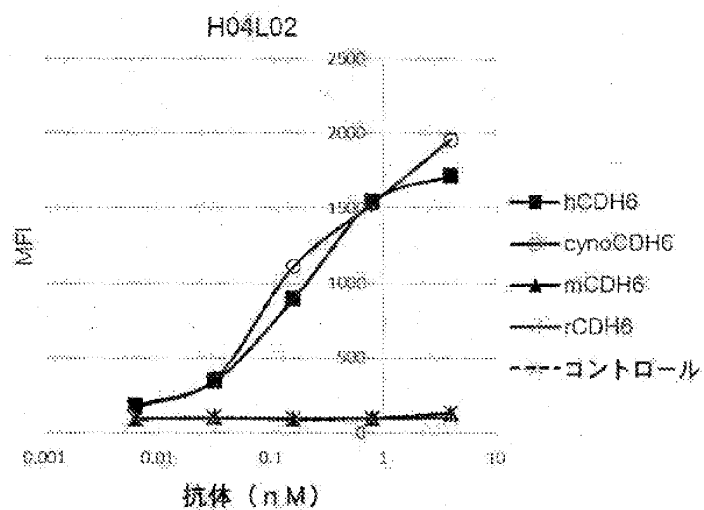
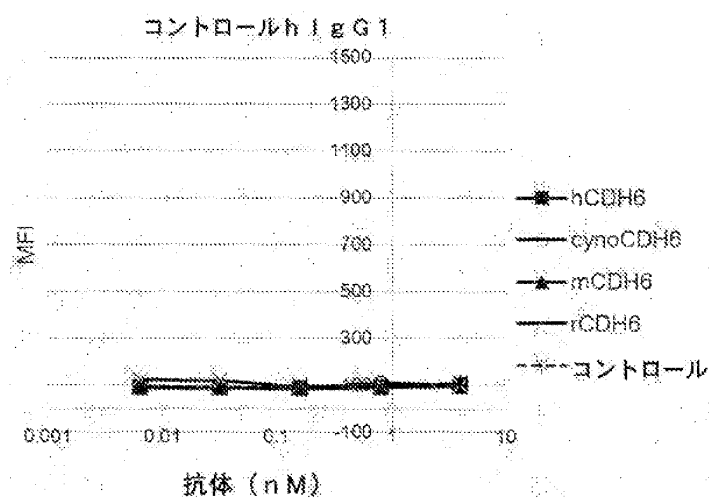


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[図6-2]

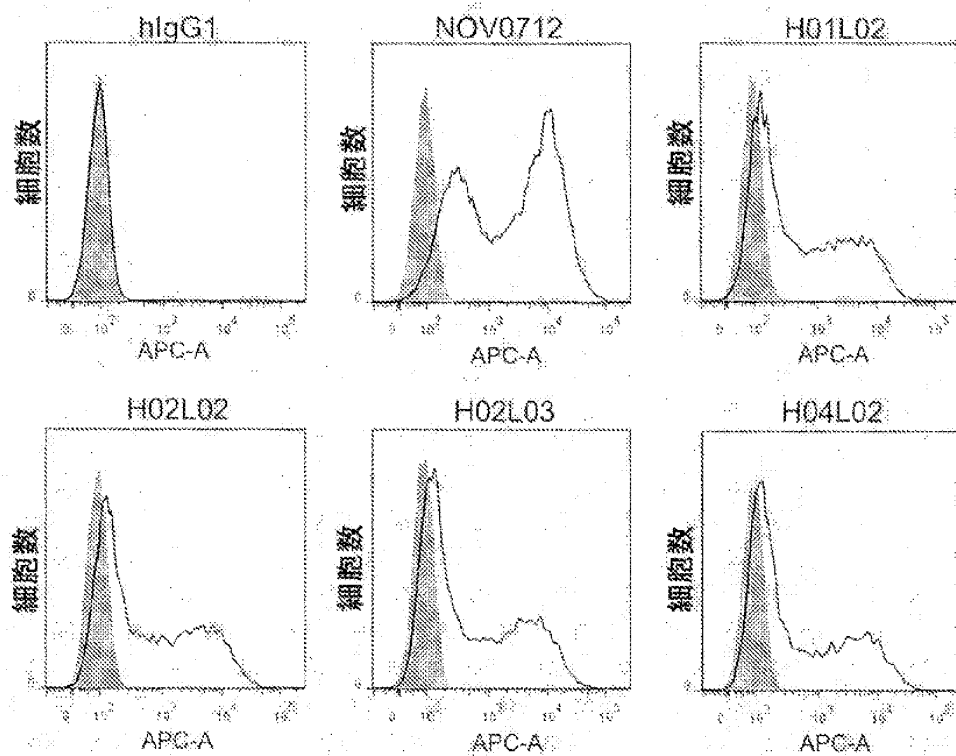


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[図7-1]

(1) コントロール細胞又は全長hCDH6導入細胞



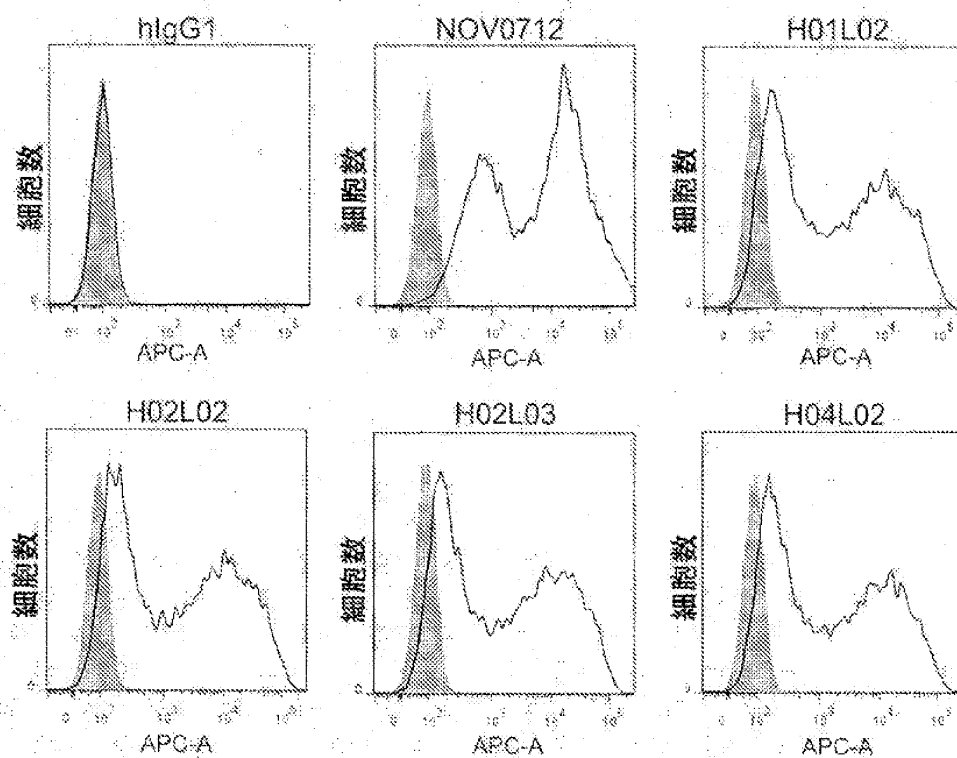
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[図7-2]

(2) コントロール細胞又はEC1欠失hCDH6導入細胞

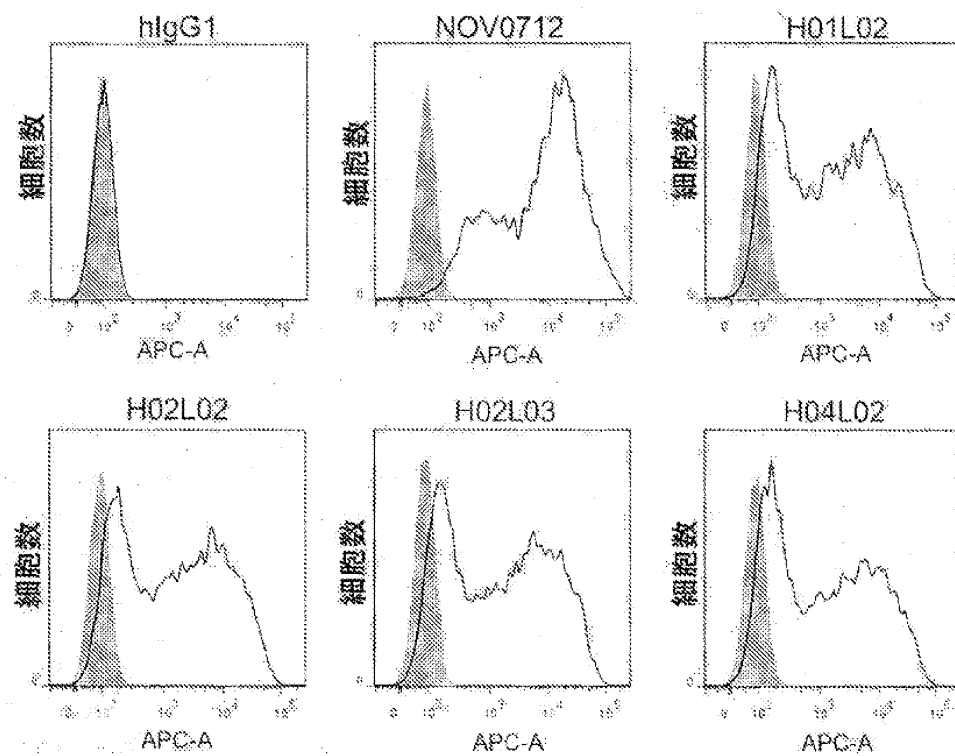


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[図7-3]

(3) コントロール細胞又はEC2欠失hCDH6導入細胞

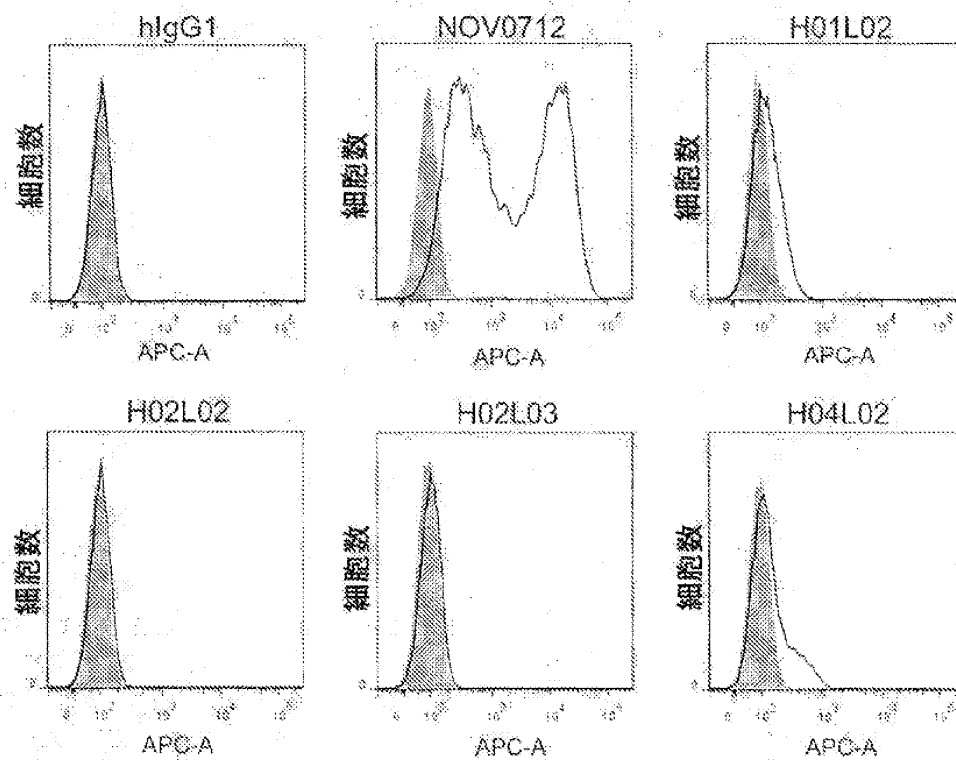


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[図7-4]

(4) コントロール細胞又はEC3欠失hCDH6導入細胞



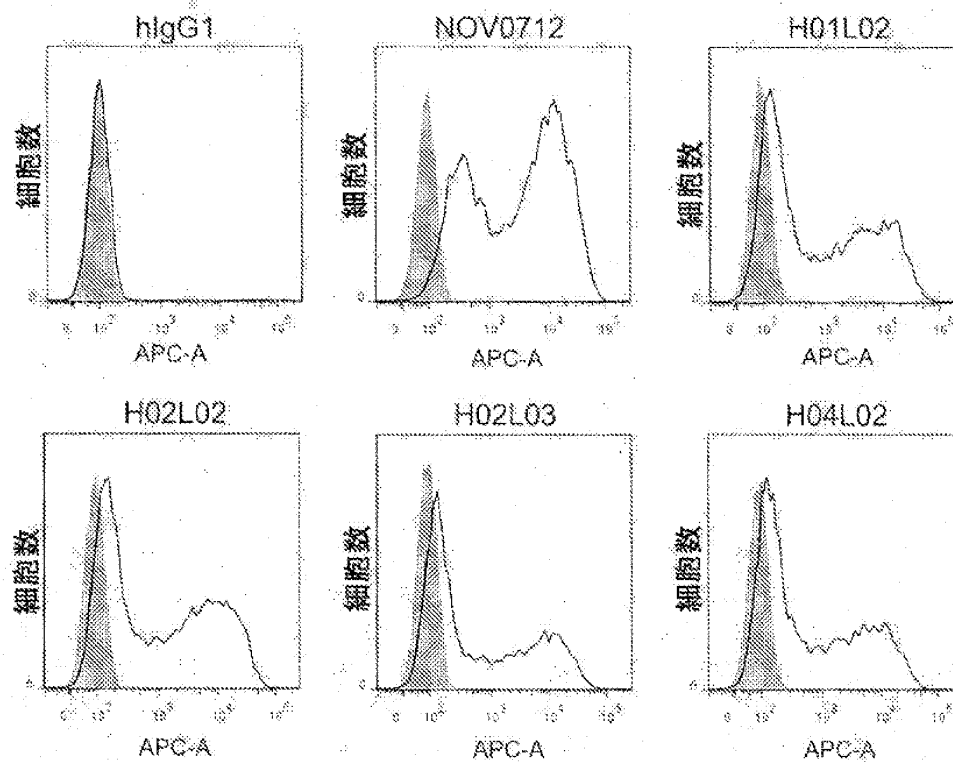
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[図7-5]

(5) コントロール細胞又はEC4欠失hCDH6導入細胞

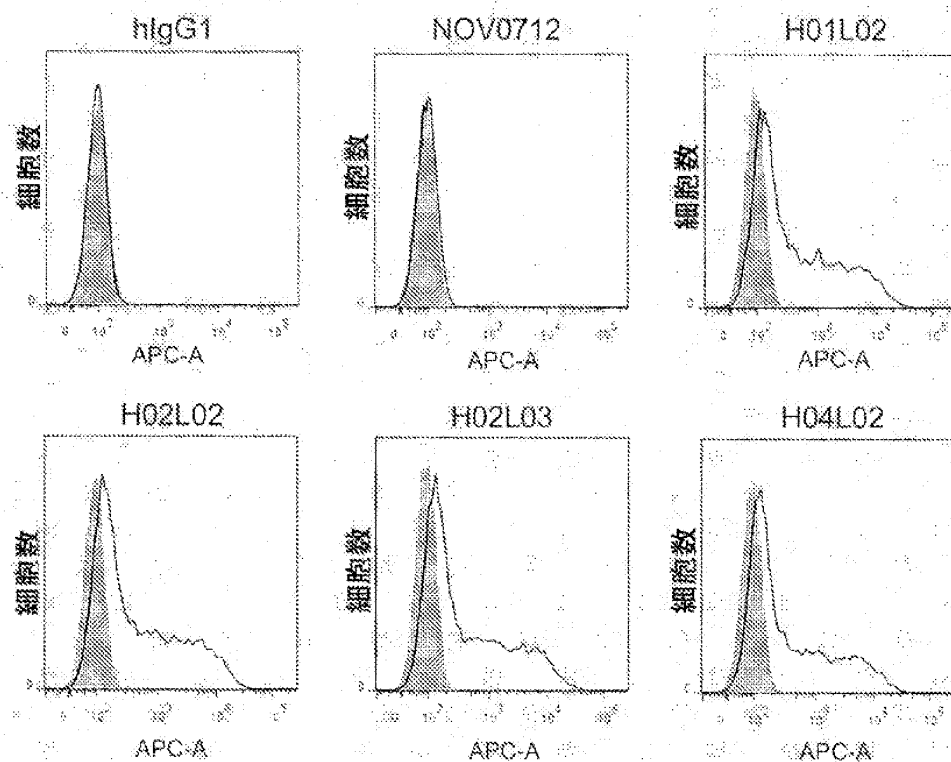


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[図7-6]

(6) コントロール細胞又はEC5欠失hCDH6導入細胞



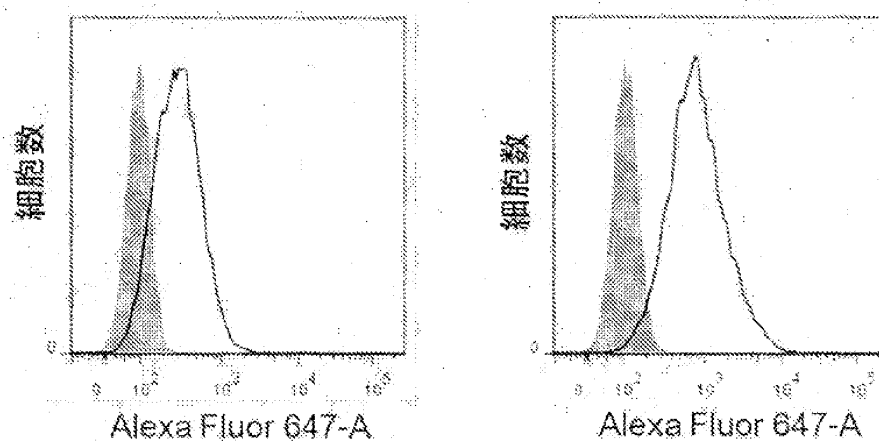
[図8]

786-O

親細胞株

786-O / hCDH6

安定発現細胞株

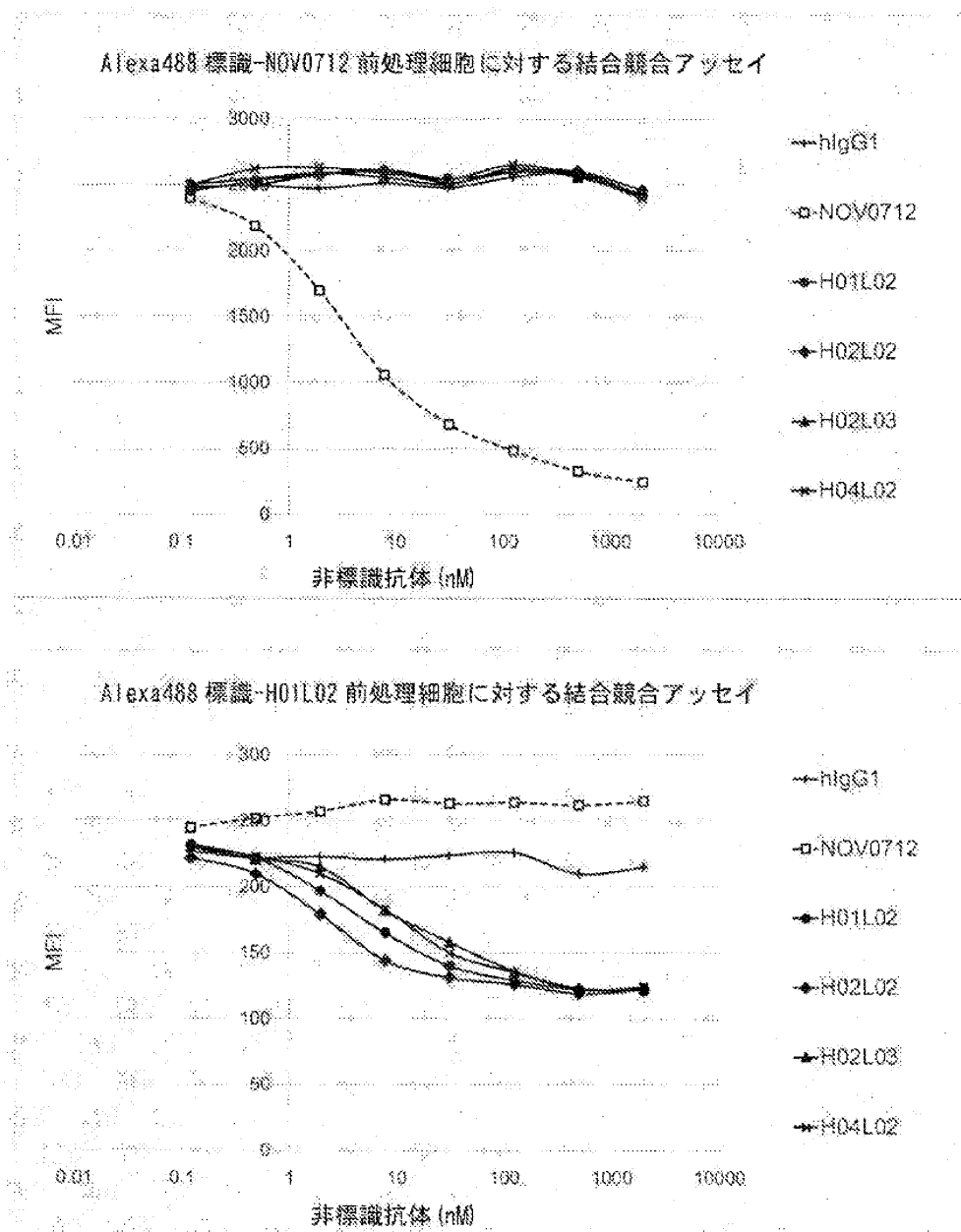


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[図9]

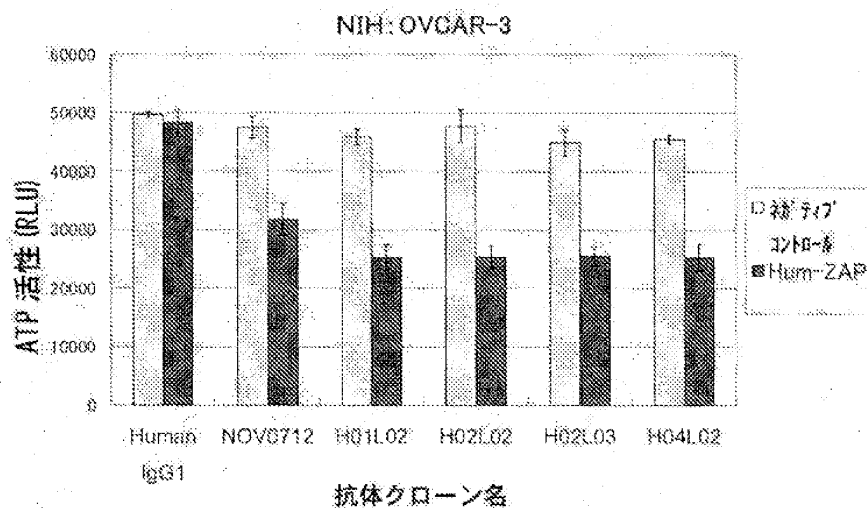


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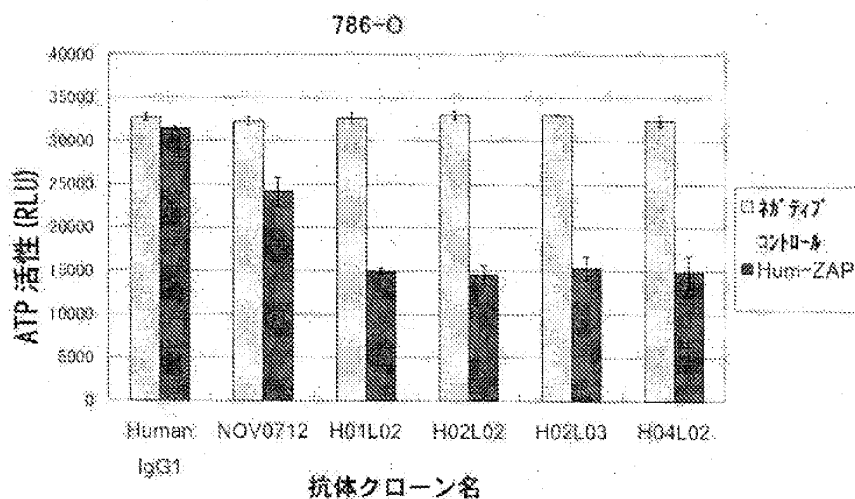
[図10-1]



Hum-ZAPを用いたNIH: OVCAR-3細胞生存率 (%)

Human IgG1	NOV0712	H01L02	H02L02	H02L03	H04L02
97.3	67.0	55.1	53.1	50.7	55.6

[図10-2]



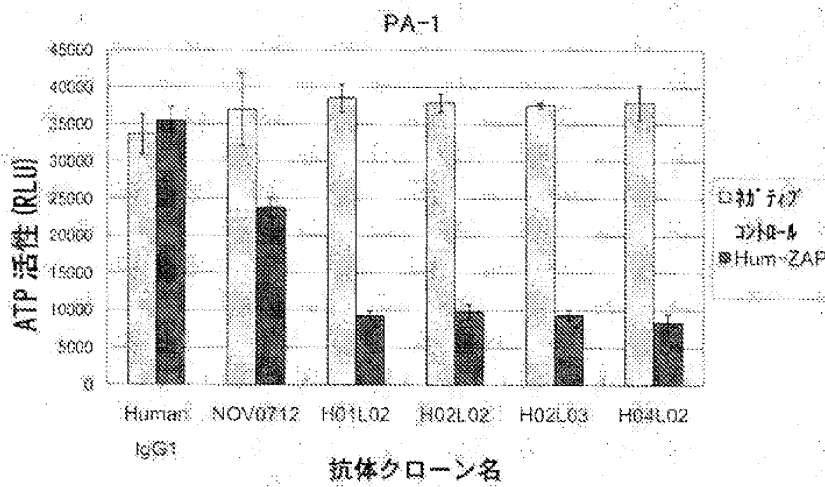
Hum-ZAPを用いた786-O細胞生存率 (%)

Human IgG1	NOV0712	H01L02	H02L02	H02L03	H04L02
95.9	74.8	46.0	44.1	46.5	45.9

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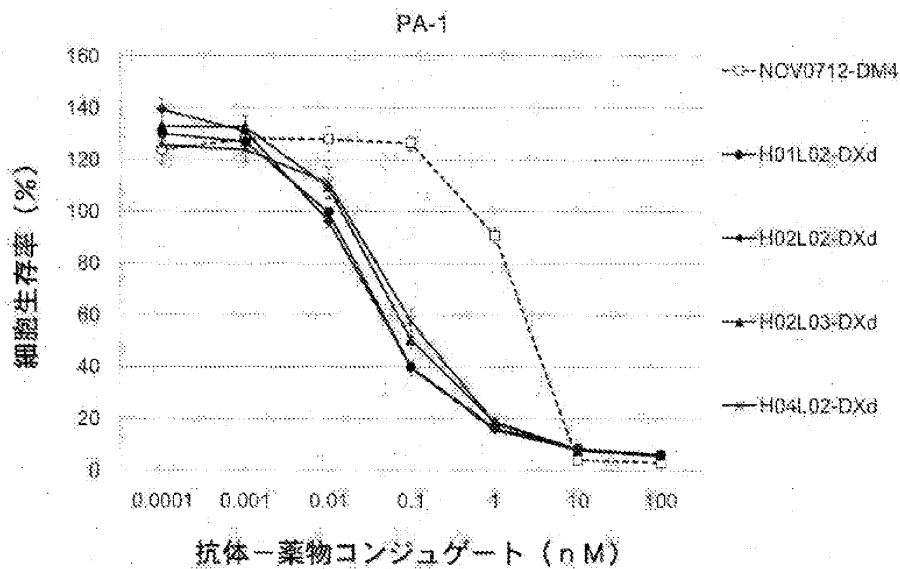
[図10-3]



Hum-ZAPを用いたPA-1細胞生存率 (%)

Human IgG1	NOV0712	H01L02	H02L02	H02L03	H04L02
105.6	64.5	24.3	25.8	25.1	22.2

[図11]

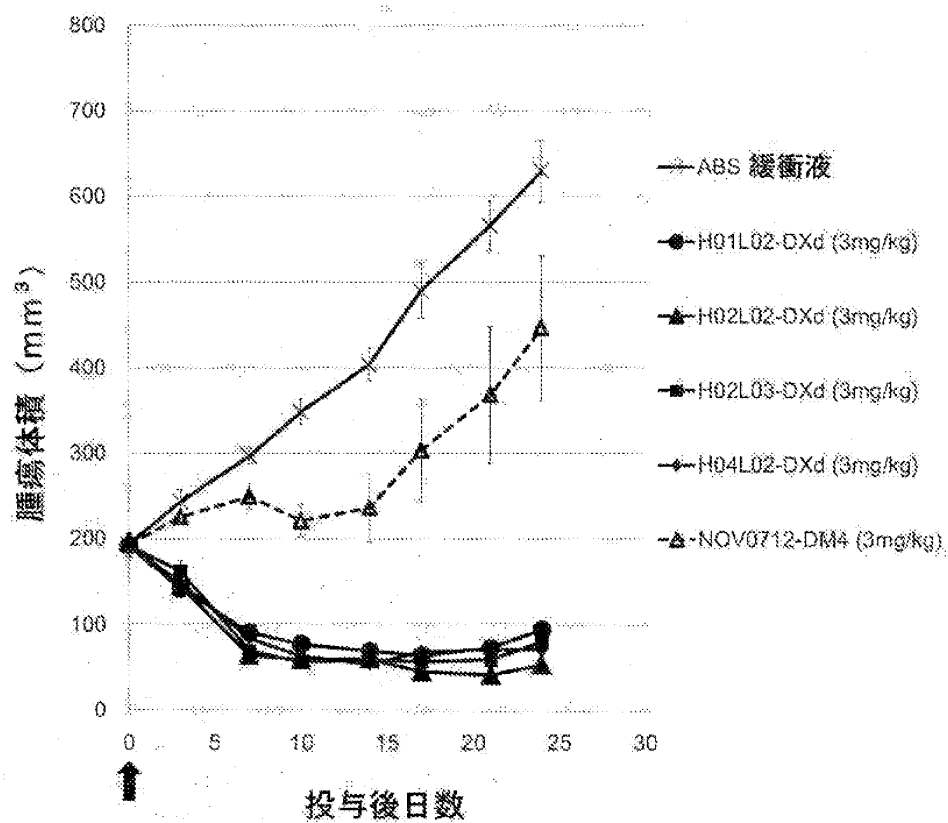


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[図12]

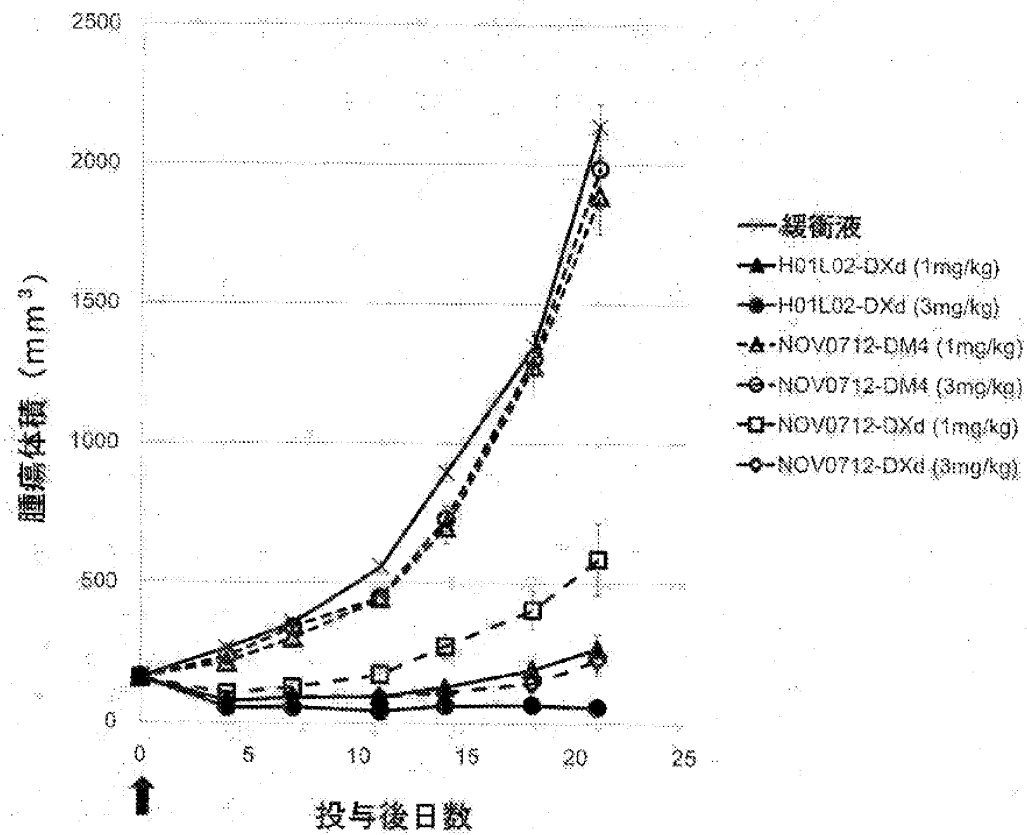


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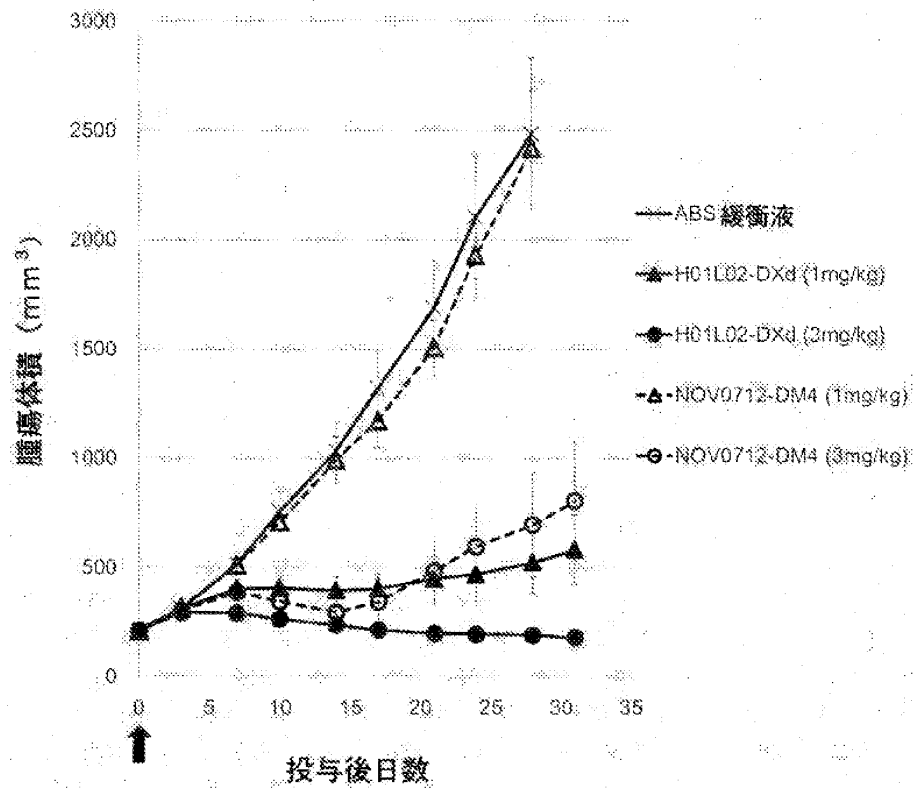
[図13]



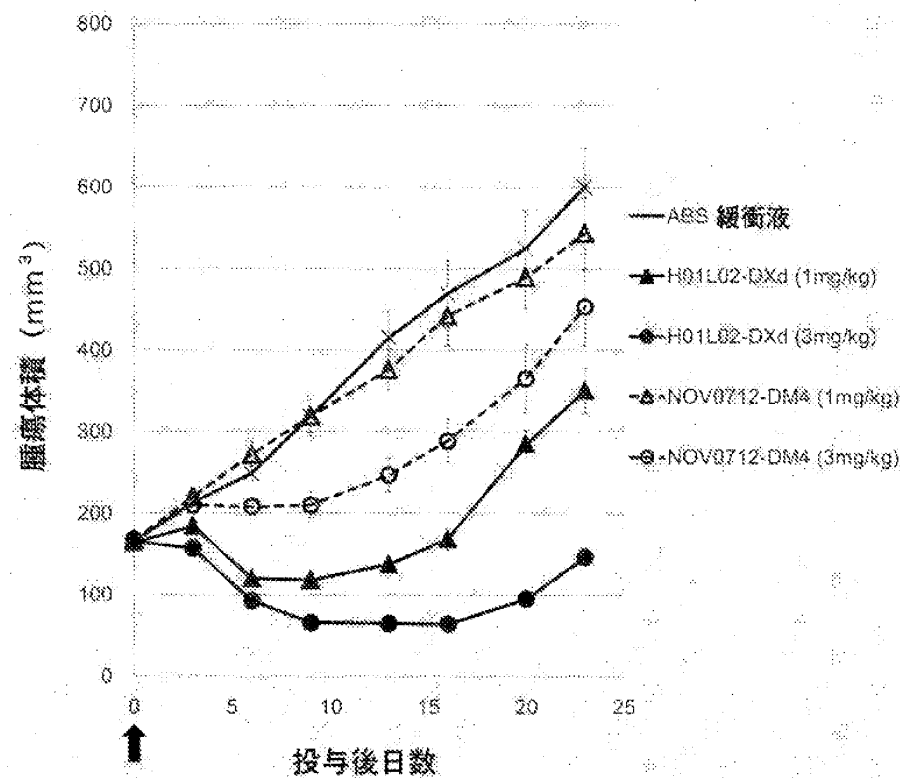
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[図14]



[図15]

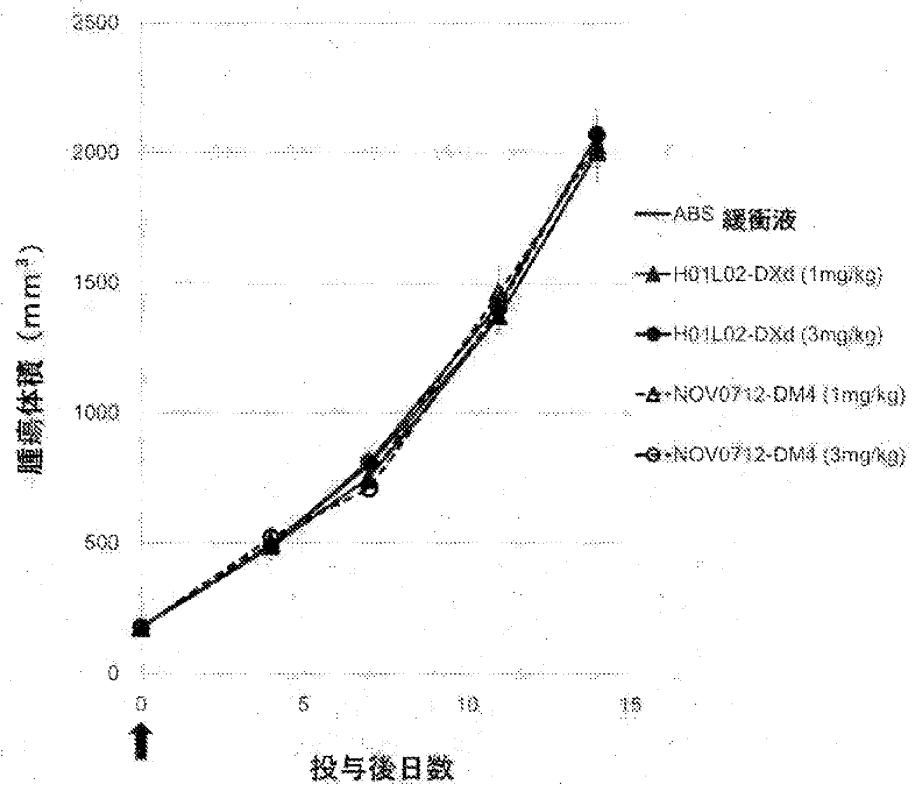


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[図16]



INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2018/018572

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. See extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl. C12N15/13, A61K9/00, A61K31/4748, A61K39/395, A61K47/68,
A61P13/12, A61P15/08, A61P35/00, A61P43/00, C07K16/28,
C12N1/15, C12N1/19, C12N1/21, C12N5/10, C12P21/08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2018
Registered utility model specifications of Japan 1996-2018
Published registered utility model applications of Japan 1994-2018

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

JSTPlus/JMEDPlus/JST7580 (JDreamIII), CAPLUS/MEDLINE/EMBASE/Biosis (STN),
WPIDS/WPIX (STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2016/024195 A1 (NOVARTIS AG) 18 February 2016, claims 1-61, examples, in particular, example 7, fig. 4, etc. & US 2016/0046711 A1, claims 1-61, examples, in particular, example 7, fig. 4, etc.	1-53
A	WO 2014/057697 A1 (DAIICHI SANKYO CO., LTD.) 17 April 2014, claims 1-49, examples, etc. & US 2015/0297748 A1, claims 1-49, examples, etc.	1-53

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

30 July 2018 (30.07.2018)

Date of mailing of the international search report

07 August 2018 (07.08.2018)

Name and mailing address of the ISA/
Japan Patent Office
3-4-3, Kasumigaseki, Chiyoda-ku,
Tokyo 100-8915, Japan

Authorized officer

Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2018/018572

CLASSIFICATION OF SUBJECT MATTER

C12N15/13(2006.01)i, A61K9/00(2006.01)i, A61K31/4745(2006.01)i,
A61K39/395(2006.01)i, A61K47/68(2017.01)i, A61P13/12(2006.01)i,
A61P15/08(2006.01)i, A61P35/00(2006.01)i, A61P43/00(2006.01)i,
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A. 発明の属する分野の分類 (国際特許分類 (IPC)) Int.Cl. 特別ページ参照			
B. 調査を行った分野 調査を行った最小限資料 (国際特許分類 (IPC)) Int.Cl. C12N15/13, A61K9/00, A61K31/4745, A61K39/395, A61K47/68, A61P13/12, A61P15/08, A61P35/00, A61P43/00, C07K16/28, C12N1/15, C12N1/19, C12N1/21, C12N5/19, C12P21/08 最小限資料以外の資料で調査を行った分野に含まれるもの 日本国実用新案公報 1922-1996年 日本国公開実用新案公報 1971-2018年 日本国実用新案登録公報 1996-2018年 日本国登録実用新案公報 1994-2018年 国際調査で利用した電子データベース (データベースの名称、調査に使用した用語) JSTPlus/JMEDPlus/JST7580 (JDreamIID, Cplus/MEDLINE/EMBASE/BIOSIS (STN), WPIIOS/WPIIX (STN))			
C. 関連すると認められる文献			
引用文献の カテゴリー*	引用文献名 及び一部の箇所が関連するときは、その関連する箇所の表示	関連する 請求項の番号	
A	WO 2016/024195 A1 (NOVARTIS AG) 2016.02.18, 請求項 1-61, 実施例特に実施例 7, 図 4 等 & US 2016/0046711 A1, 請求項 1-61, 実施例特に実施例 7, 図 4 等	1-53	
A	WO 2014/057687 A1 (第一三共株式会社) 2014.04.17, 請求項 1-49, 実施例等 & US 2015/0297748 A1, 請求項 1-49, 実施例等	1-53	
☐ C欄の続きにも文献が列挙されている。 ☐ パテントファミリーに関する別紙を参照。			
* 引用文献のカテゴリー 「A」 特に関連のある文献ではなく、一般的技術水準を示すもの 「E」 国際出願日前の出願または特許であるが、国際出願日以後に公表されたもの 「L」 優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献 (理由を付す) 「O」 口頭による開示、使用、展示等に言及する文献 「P」 国際出願日前で、かつ優先権の主張の基礎となる出願日の後に公表された文献 「T」 国際出願日又は優先日後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの 「X」 特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの 「Y」 特に関連のある文献であって、当該文献と他の 1 以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの 「&」 同一パテントファミリー文献			
国際調査を完了した日 30.07.2018		国際調査報告の発注日 07.08.2018	
国際調査機関の名称及びあて先 日本国特許庁 (ISA/J P) 郵便番号 100-8915 東京都千代田区霞が関三丁目 4 番 3 号		特許庁審査官 (権限のある職員) 田ノ上 拓自 電話番号 03-3581-1101 内線 3448	4B 6115

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発明の属する分野の分類

C12N15/13(2006.01)i, A61K9/00(2006.01)i, A61K31/4745(2006.01)i,
A61K39/395(2006.01)i, A61K47/68(2017.01)i, A61P13/12(2006.01)i, A61P15/08(2006.01)i,
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Description

Title of Invention:

ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG CONJUGATE

Technical Field

[0001] The present invention relates to an anti-CDH6 antibody binding to CDH6 and having an internalization effect, a method for producing the anti-CDH6 antibody, an antibody-drug conjugate comprising the antibody, an antitumor agent comprising the antibody-drug conjugate, and the like.

Background Art

[0002] Cadherins are glycoproteins present on the surface of cell membranes and function as cell-cell adhesion molecules through the calcium ion-dependent binding of their N-terminal extracellular domains, or as signal molecules responsible for cell-cell interaction. Classic cadherins are in the cadherin superfamily and are single-pass transmembrane proteins composed of five extracellular domains (EC domains), one transmembrane region, and an intracellular domain. The classic cadherins are classified into the type I family typified by E-cadherin and N-cadherin, and the type II family according to the homologies of their amino acid sequences.

[0003] Cadherin-6 (CDH6) is a single-pass transmembrane protein composed of 790 amino acids, which is classified into the type II cadherin family, and this protein has N-terminal extracellular and C-terminal intracellular domains. The human CDH6 gene was cloned for the first time in 1995 (Non Patent

Literature 1), and its sequence can be referred to under, for example, accession Nos. NM_004932 and NP_004923 (NCBI).

[0004] CDH6 is specifically expressed in the brain or the kidney at the stage of development and has been reported to play an important role in the circuit formation of the central nervous system (Non Patent Literature 2 and 3) and nephron development in the kidney (Non Patent Literature 4 and 5). The expression of CDH6 in the normal tissues of adult humans is localized to the tubules of the kidney, bile duct epithelial cells, and the like.

[0005] Meanwhile, it is known that CDH6 is specifically overexpressed at tumor sites in some types of human adult cancers. The correlation of CDH6 expression with poor prognosis and its applicability as a tumor marker has been reported with respect to human renal cell carcinoma, particularly, renal clear cell carcinoma (Non Patent Literature 6 and 7). The high expression of CDH6 has also been reported with respect to human ovarian cancer (Non Patent Literature 8). It has also been reported that CDH6 is involved in the epithelial-mesenchymal transition of human thyroid cancer (Non Patent Literature 9). Furthermore, it has been reported that CDH6 is also expressed in human bile duct cancer and human small-cell lung cancer (Non Patent Literature 12 and 13).

[0006] Cancers rank high in causes of death. Although the number of cancer patients is expected to increase with aging of the population, treatment needs have not yet been sufficiently satisfied. The problems of conventional chemotherapeutics are that: due to their low selectivity,

these chemotherapeutics are toxic not only to tumor cells but also to normal cells and thereby have adverse reactions; and the chemotherapeutics cannot be administered in sufficient amounts and thus cannot produce their effects sufficiently. Hence, in recent years, more highly selective molecular target drugs or antibody drugs have been developed, which target molecules that exhibit mutations or a high expression characteristic in cancer cells, or specific molecules involved in malignant transformation of cells.

[0007] Antibodies are highly stable in blood, and specifically bind to their target antigens. For these reasons, a reduction in adverse reaction is expected, and a large number of antibody drugs have been developed for molecules highly expressed on the surface of cancer cells. One of the techniques relying on the antigen-specific binding ability of antibodies is to use an antibody-drug conjugate (ADC). ADC is a conjugate in which an antibody that binds to an antigen expressed on the surface of cancer cells and can internalize the antigen into the cell through the binding is conjugated to a drug having cytotoxic activity. ADC can efficiently deliver the drug to cancer cells, and can thereby be expected to kill the cancer cells by accumulating the drug in the cancer cells (Non Patent Literature 10 and Patent Literature 1 and 2).

With regard to ADC, for example, Adcetris(TM) (brentuximab vedotin) comprising an anti-CD30 monoclonal antibody conjugated to monomethyl auristatin E has been approved as a therapeutic drug for Hodgkin's lymphoma and anaplastic large cell lymphoma. Also, Kadcyla(TM) (trastuzumab emtansine) comprising an anti-HER2 monoclonal antibody conjugated to

emtansine is used in the treatment of HER2-positive progressive or recurrent breast cancer.

[0008] The features of a target antigen suitable for ADC as an antitumor drug are that: the antigen is specifically highly expressed on the surface of cancer cells but has low expression or is not expressed in normal cells; the antigen can be internalized into cells; the antigen is not secreted from the cell surface; etc. The internalization ability of the antibody depends on the properties of both the target antigen and the antibody. It is difficult to predict an antigen-binding site suitable for internalization from the molecular structure of a target or to predict an antibody having high internalization ability from binding strength, physical properties, and the like of the antibody. Hence, an important challenge in developing ADC having high efficacy is obtaining an antibody having high internalization ability against the target antigen (Non Patent Literature 11).

[0009] ADC comprising DM4 conjugated to an anti-CDH6 antibody specifically binding to EC domain 5 (EC5) of CDH6 are known as ADC targeting CDH6 (Patent Literature 3).

Citation List

Patent Literature

[0010]

Patent Literature 1: WO2014/057687

Patent Literature 2: US2016/0297890

Patent Literature 3: WO2016/024195

Non Patent Literature

[0011]

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Non Patent Literature 1: Shimoyama Y, et al., Cancer Research, 2206-2211, 55, May 15, 1995

Non Patent Literature 2: Inoue T, et al., Developmental Biology, 183-194, 1997

Non Patent Literature 3: Osterhout J A, et al., Neuron, 632-639, 71, Aug 25, 2011

Non Patent Literature 4: Cho E A, et al., Development, 803-812, 125, 1998

Non Patent Literature 5: Mah S P, et al., Developmental Biology, 38-53, 223, 2000

Non Patent Literature 6: Paul R, et al., Cancer Research, 2741-2748, July 1, 57, 1997

Non Patent Literature 7: Shimazui T, et al., Cancer, 963-968, 101(5), Sep.1, 2004

Non Patent Literature 8: Koebel M, et al., PLoS Medicine, 1749-1760, 5(12), e232, Dec.2008

Non Patent Literature 9: Gugnoni M, et al., Oncogene, 667-677, 36, 2017

Non Patent Literature 10: Polakis P., Pharmacological Reviews, 3-19, 68, 2016

Non Patent Literature 11: Peters C, et al., Bioscience Reports, 1-20, 35, 2015

Non Patent Literature 12: Goeppert B, et al., Epigenetics, 780-790, 11(11), 2016

Non Patent Literature 13: Yokoi S, et al., American Journal of Pathology, 207-216, 161, 1, 2002

Summary of Invention

Technical Problem

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[0012] It is an object of the present invention to provide an antibody specifically binding to CDH6 and having high internalization activity, an antibody-drug conjugate comprising the antibody and having high antitumor activity, a pharmaceutical product comprising the antibody-drug conjugate and having therapeutic effects on a tumor, a method for treating a tumor using the antibody, the antibody-drug conjugate or the pharmaceutical product, and the like.

Solution to Problem

[0013] The present inventors have conducted intensive studies directed towards achieving the above-described object, and found that, surprisingly, an antibody specifically binding to extracellular domain 3 (in the present description, also referred to as EC3) of CDH6 has exceedingly high internalization activity against cells expressing CDH6 and is useful as an antibody for ADC. The inventors have further found that an anti-CDH6 antibody-drug conjugate comprising the aforementioned anti-CDH6 antibody conjugated to a drug exerting toxicity in cells via a linker having a specific structure exhibits stronger antitumor activity than that of conventional CDH6-drug conjugates.

[0014] The present invention includes the following aspects of the invention:

[1] an antibody specifically binding to the amino acid sequence shown in SEQ ID NO: 4 and having internalization ability that permits cellular uptake, or a functional fragment of the antibody;

[2] the antibody or the functional fragment of the antibody according to [1], which has competitive inhibitory activity, for binding to the amino acid sequence shown in SEQ ID NO: 4, against at least any one antibody selected from the group consisting of the following antibodies (1) to (5):

(1) an antibody having a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 53 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 56,

(2) an antibody having a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 69,

(3) an antibody having a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73,

(4) an antibody having a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 65 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73, and

(5) an antibody having a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 77;

[3] the antibody or the functional fragment of the antibody according to [1] or [2], which comprises CDRL1, CDRL2 and CDRL3 in any one combination selected from the group consisting of the following combinations (1) to (4):

- (1) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 12, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 13, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 14,
- (2) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 22, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 23, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 24,
- (3) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 32, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 33, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 34, and
- (4) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 42, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 43, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 44, and
- CDRH1, CDRH2 and CDRH3 in any one combination selected from the group consisting of the following combinations (5) to (9):
- (5) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 18, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 19,
- (6) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 27, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 28, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 29,
- (7) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 37, CDRH2 consisting of the amino acid sequence shown

in SEQ ID NO: 38, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 39,

(8) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 47, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 48, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 49, and

(9) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 60, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 19;

[4] the antibody or the functional fragment of the antibody according to any one of [1] to [3], which comprises CDRL1, CDRL2 and CDRL3, and CDRH1, CDRH2 and CDRH3 in any combination selected from the group consisting of the following combinations (1) to (5):

(1) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 12, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 13, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 14, and CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 18, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 19,

(2) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 22, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 23, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 24, and CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 27, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 28, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 29,

(3) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 32, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 33, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 34, and CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 37, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 38, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 39,

(4) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 42, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 43, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 44, and CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 47, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 48, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 49, and

(5) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 12, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 13, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 14, and CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 60, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 19;

[5] the antibody or the functional fragment of the antibody according to any one of [1] to [4], which is humanized;

[6] the antibody or the functional fragment of the antibody according to any one of [1] to [5], which has any one light chain variable region selected from the group consisting of the following variable regions (1) to (4):

- (1) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 63,
- (2) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 67,
- (3) an amino acid sequence having a sequence identity of at least 95% or more to the sequence of a framework region other than at each CDR sequence in the amino acid sequences of (1) and (2), and
- (4) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the sequence of a framework region other than at each CDR sequence in the amino acid sequences of (1) to (3), and
any one heavy chain variable region selected from the group consisting of the following variable regions (5) to (9):
 - (5) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 71,
 - (6) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 75,
 - (7) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 79,
 - (8) an amino acid sequence having a sequence homology of at least 95% or more to the sequence of a framework region other than at each CDR sequence in the amino acid sequences of (5) to (7), and
 - (9) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the sequence of a framework region other than at each CDR sequence in the amino acid sequences of (5) to (8);

[7] the antibody or the functional fragment of the antibody according to any one of [1] to [6], which comprises a light chain variable region and a heavy chain variable region in any of the following combinations (1) to (4):

(1) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 63 and a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 71,

(2) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 63 and a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 75,

(3) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 67 and a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 75, and

(4) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 63 and a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 79;

[8] the antibody or the functional fragment of the antibody according to any one of [1] to [7], which has any of the following combinations (1) to (4):

(1) a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 69,

(2) a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain

consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73,

(3) a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 65 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73, and

(4) a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 77;

[9] the antibody or the functional fragment of the antibody according to [8], which has a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 69;

[10] the antibody or the functional fragment of the antibody according to [8], which has a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73;

[11] the antibody or the functional fragment of the antibody according to [8], which has a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 65 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73;

[12] the antibody or the functional fragment of the antibody according to [8], which has a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61

and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 77;

[13] the functional fragment of the antibody according to any one of [1] to [12], wherein the functional fragment is selected from the group consisting of Fab, F(ab')₂, Fab' and Fv;

[14] a polynucleotide encoding the antibody or the functional fragment of the antibody according to any one of [1] to [13];

[15] the polynucleotide according to [14], which comprises polynucleotides in any one combination selected from the group consisting of the following combinations (1) to (5):

(1) a polynucleotide encoding a light chain variable region comprising CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 12, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 13 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 14, and a polynucleotide encoding a heavy chain variable region comprising CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 18 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 19,

(2) a polynucleotide encoding a light chain variable region comprising CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 22, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 23 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 24, and a polynucleotide encoding a heavy chain variable region comprising CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 27, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 28

and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 29,

(3) a polynucleotide encoding a light chain variable region comprising CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 32, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 33 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 34, and a polynucleotide encoding a heavy chain variable region comprising CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 37, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 38 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 39,

(4) a polynucleotide encoding a light chain variable region comprising CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 42, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 43 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 44, and a polynucleotide encoding a heavy chain variable region comprising CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 47, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 48 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 49, and

(5) a polynucleotide encoding a light chain variable region comprising CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 12, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 13 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 14, and a polynucleotide encoding a heavy chain variable region comprising CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 17, CDRH2

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consisting of the amino acid sequence shown in SEQ ID NO: 60 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 19;

[16] the polynucleotide according to [14] or [15], which comprises a polynucleotide encoding a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a polynucleotide encoding a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 69;

[17] the polynucleotide according to [14] or [15], which comprises a polynucleotide encoding a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a polynucleotide encoding a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73;

[18] the polynucleotide according to [14] or [15], which comprises a polynucleotide encoding a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 65 and a polynucleotide encoding a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73;

[19] the polynucleotide according to [14] or [15], which comprises a polynucleotide encoding a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a polynucleotide encoding a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 77;

[20] an expression vector comprising the polynucleotide according to any one of [14] to [19];

[21] host cells transformed with the expression vector according to [20];

[22] the host cells according to [21], wherein the host cells are eukaryotic cells;

[23] a method for producing an antibody of interest or a functional fragment of the antibody, which comprises the step of culturing the host cells according to [21] or [22], and the step of collecting an antibody of interest or a functional fragment of the antibody from the culture obtained by the aforementioned step;

[24] the antibody or the functional fragment of the antibody according to any one of [1] to [13], wherein the heavy chain or the light chain has undergone one or two or more modifications selected from the group consisting of N-linked glycosylation, O-linked glycosylation, N-terminal processing, C-terminal processing, deamidation, isomerization of aspartic acid, oxidation of methionine, addition of a methionine residue to the N-terminus, amidation of a proline residue, conversion of N-terminal glutamine or N-terminal glutamic acid to pyroglutamic acid, and a deletion of one or two amino acids from the carboxyl terminus;

[25] the antibody according to [24], wherein one or two amino acids are deleted from the carboxyl terminus of a heavy chain thereof;

[26] the antibody according to [25], wherein one amino acid is deleted from each of the carboxyl termini of both of the heavy chains thereof;

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[27] the antibody according to any one of [24] to [26], wherein a proline residue at the carboxyl terminus of a heavy chain thereof is further amidated;

[28] the antibody or the functional fragment of the antibody according to any one of [1] to [13] and [24] to [27], wherein sugar chain modification is regulated in order to enhance antibody-dependent cellular cytotoxic activity;

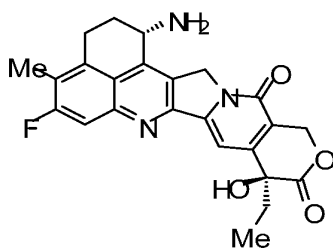
[29] an antibody-drug conjugate comprising the antibody or the functional fragment of the antibody according to any one of [1] to [13] and [24] to [28] conjugated to a drug;

[30] the antibody-drug conjugate according to [29], wherein the drug is an antitumor compound;

[31] the antibody-drug conjugate according to [30], wherein the antitumor compound is an antitumor compound represented by the following formula:

[0015]

[Formula 1]



[0016]

[32] the antibody-drug conjugate according to any one of [29] to [31], wherein the antibody is conjugated to the drug via a linker having any structure selected from the group consisting of the following formulas (a) to (f):

(a) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$,

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(b) - (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-,

(c) - (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂-O-CH₂-C(=O)-,

(d) - (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂-O-CH₂-C(=O)-,

(e) - (Succinimid-3-yl-N) -CH₂CH₂-C(=O)-NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-, and

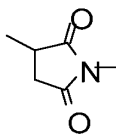
(f) - (Succinimid-3-yl-N) -CH₂CH₂-C(=O)-NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-,

wherein the antibody is connected to the terminus of -

(Succinimid-3-yl-N), the antitumor compound is connected to the carbonyl group of the -CH₂CH₂CH₂-C(=O)- moiety of (a), (b), (e) or (f), the CH₂-O-CH₂-C(=O)- moiety of (c) or the CH₂CH₂-O-CH₂-C(=O)- moiety of (d) with the nitrogen atom of the amino group at position 1 as a connecting position, GGFG represents an amino acid sequence consisting of glycine-glycine-phenylalanine-glycine linked through peptide bonds, and -(Succinimid-3-yl-N)- has a structure represented by the following formula:

[0017]

[Formula 2]



[0018]

which is connected to the antibody at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1;

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[33] the antibody-drug conjugate according to any one of [29] to [32], wherein the linker is represented by any formula selected from the group consisting of the following formulas

(c), (d) and (e):

(c) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$,

(d) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$, and

(e) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$;

[34] the antibody-drug conjugate according to any one of [29] to [33], wherein the linker is represented by the following formula (c) or (e):

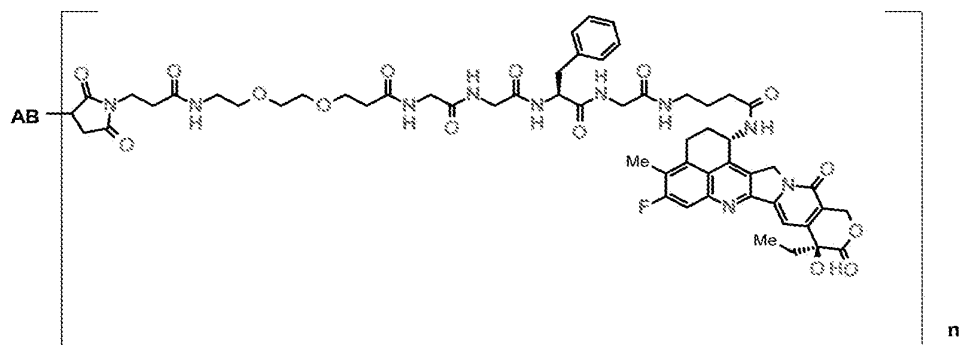
(c) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$, and

(e) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$;

[35] the antibody-drug conjugate according to any one of [29] to [34], which has a structure represented by the following formula:

[0019]

[Formula 3]



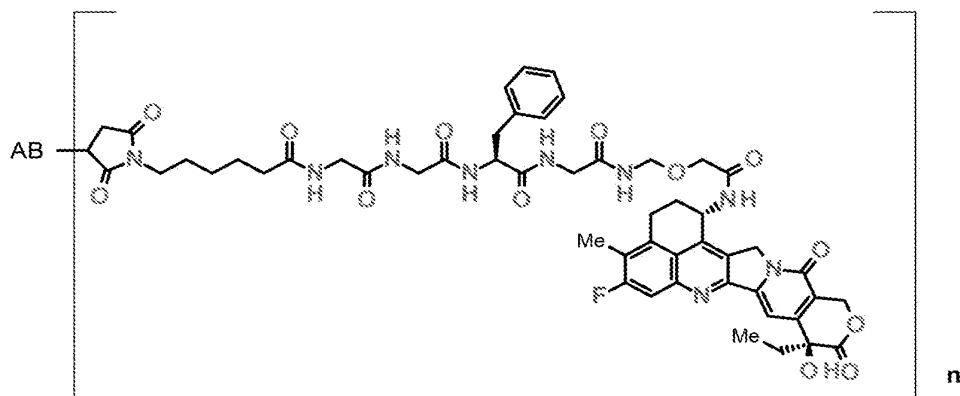
[0020]

wherein AB represents the antibody or the functional fragment of the antibody, n represents the average number of units of the drug-linker structure conjugated to the antibody per antibody, and the antibody is connected to the linker via a sulfhydryl group derived from the antibody;

[36] the antibody-drug conjugate according to any one of [29] to [34], which has a structure represented by the following formula:

[0021]

[Formula 4]



[0022]

wherein AB represents the antibody or the functional fragment of the antibody, n represents the average number of units of the drug-linker structure conjugated to the antibody per antibody, and the antibody is connected to the linker via a sulfhydryl group derived from the antibody;

[37] the antibody-drug conjugate according to any one of [29] to [36], wherein the antibody is an antibody comprising a light chain and a heavy chain in any one combination selected from the group consisting of the following combinations (1) to (4), or a functional fragment of the antibody:

(1) a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 69,

(2) a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73,

(3) a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 65 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73, and

(4) a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 77;

[38] the antibody-drug conjugate according to [37], wherein the antibody is an antibody comprising a light chain

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consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 69, or a functional fragment of the antibody;

[39] the antibody-drug conjugate according to [37], wherein the antibody is an antibody comprising a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 77, or a functional fragment of the antibody;

[40] the antibody-drug conjugate according to any one of [29] to [39], wherein the heavy chain or the light chain has undergone one or two or more modifications selected from the group consisting of N-linked glycosylation, O-linked glycosylation, N-terminal processing, C-terminal processing, deamidation, isomerization of aspartic acid, oxidation of methionine, addition of a methionine residue to the N-terminus, amidation of a proline residue, conversion of N-terminal glutamine or N-terminal glutamic acid to pyroglutamic acid, and a deletion of one or two amino acids from the carboxyl terminus;

[41] the antibody-drug conjugate according to any one of [29] to [40], wherein the average number of units of the selected drug-linker structure conjugated per antibody is in the range of from 1 to 10;

[42] the antibody-drug conjugate according to [41], wherein the average number of units of the selected drug-linker structure conjugated per antibody is in the range of from 2 to 8;

[43] the antibody-drug conjugate according to [42], wherein the average number of units of the selected drug-linker structure conjugated per antibody is in the range of from 5 to 8;

[44] the antibody-drug conjugate according to [43], wherein the average number of units of the selected drug-linker structure conjugated per antibody is in the range of from 7 to 8;

[45] a pharmaceutical composition comprising the antibody-drug conjugate according to any one of [29] to [44], a salt thereof, or a hydrate of the conjugate or the salt;

[46] the pharmaceutical composition according to [45], which is an antitumor drug;

[47] the pharmaceutical composition according to [46], wherein the tumor is a tumor expressing CDH6;

[48] the pharmaceutical composition according to [46] or [47], wherein the tumor is renal cell carcinoma, renal clear cell carcinoma, papillary renal cell carcinoma, ovarian cancer, ovarian serous adenocarcinoma, thyroid cancer, bile duct cancer, lung cancer, small-cell lung cancer, glioblastoma, mesothelioma, uterine cancer, pancreatic cancer, Wilms' tumor or neuroblastoma;

[49] a method for treating a tumor, which comprises administering any component selected from the antibody-drug conjugate according to any one of [29] to [44], a salt thereof, and a hydrate of the conjugate or the salt to an individual;

[50] the treatment method according to [49], wherein the tumor is a tumor expressing CDH6;

[51] the treatment method according to [49] or [50], wherein the tumor is renal cell carcinoma, renal clear cell carcinoma, papillary renal cell carcinoma, ovarian cancer, ovarian serous adenocarcinoma, thyroid cancer, bile duct cancer, lung cancer, small-cell lung cancer, glioblastoma, mesothelioma, uterine cancer, pancreatic cancer, Wilms' tumor or neuroblastoma;

[52] a method for treating a tumor, which comprises administering a pharmaceutical composition comprising at least one component selected from the antibody-drug conjugate according to any one of [29] to [44], a salt thereof, and a hydrate of the conjugate or the salt, and at least one antitumor drug to an individual, simultaneously, separately, or continuously;

[53] a method for producing an antibody-drug conjugate, which comprises the step of reacting the antibody or the functional fragment of the antibody according to any one of [1] to [13] and [24] to [28], or an antibody or a functional fragment of the antibody obtained by the production method according to [23] with a drug-linker intermediate compound; and

[54] a method for producing an antibody-drug conjugate, which comprises the step of culturing the host cells according to [21] or [22], the step of collecting an antibody of interest or a functional fragment of the antibody from the culture obtained by the aforementioned step, and the step of reacting the antibody or the functional fragment of the antibody obtained by the aforementioned step with a drug-linker intermediate compound.

Advantageous Effects of Invention

[0023] Features of the anti-CDH6 antibody of the present invention are to specifically recognize EC domain 3 (EC3) of CDH6 and to have high internalization activity. An anti-CDH6 antibody-drug conjugate comprising the anti-CDH6 antibody of the present invention conjugated to a drug exerting toxicity in cells via a linker having a specific structure can be expected to achieve an excellent antitumor effect and safety by administration to patients having cancer cells expressing CDH6. Specifically, the anti-CDH6 antibody-drug conjugate of the present invention is useful as an antitumor agent.

Brief Description of Drawings

[0024]

[Figure 1] Figure 1 shows flow cytometry results of examining the binding of four rat anti-CDH6 monoclonal antibodies (clone Nos. rG019, rG055, rG056 and rG061) or rat IgG control to control cells or hCDH6-transfected 293T cells. The abscissa depicts FITC fluorescence intensity indicating the amount of the antibody bound, and the ordinate depicts cell count.

[Figure 2-1] Figure 2-1 shows the binding activity of four rat anti-CDH6 monoclonal antibodies (rG019, rG055, rG056 and rG061) or negative control antibody Rat IgG2b against control cells or full-length hCDH6-transfected 293 cells. The abscissa depicts FITC fluorescence intensity indicating the amount of the antibody bound, and the ordinate depicts cell count.

[Figure 2-2] Figure 2-2 shows the binding activity of four rat anti-CDH6 monoclonal antibodies (rG019, rG055, rG056 and rG061) or rat IgG control against control cells or EC1-deleted

hCDH6-transfected 293 cells. The abscissa depicts FITC fluorescence intensity indicating the amount of the antibody bound, and the ordinate depicts cell count.

[Figure 2-3] Figure 2-3 shows the binding activity of four rat anti-CDH6 monoclonal antibodies (rG019, rG055, rG056 and rG061) or rat IgG control against control cells or EC2-deleted hCDH6-transfected 293 cells. The abscissa depicts FITC fluorescence intensity indicating the amount of the antibody bound, and the ordinate depicts cell count.

[Figure 2-4] Figure 2-4 shows the binding activity of four rat anti-CDH6 monoclonal antibodies (rG019, rG055, rG056 and rG061) or rat IgG control against control cells or EC3-deleted hCDH6-transfected 293 cells. The abscissa depicts FITC fluorescence intensity indicating the amount of the antibody bound, and the ordinate depicts cell count.

[Figure 2-5] Figure 2-5 shows the binding activity of four rat anti-CDH6 monoclonal antibodies (rG019, rG055, rG056 and rG061) or rat IgG control against control cells or EC4-deleted hCDH6-transfected 293 cells. The abscissa depicts FITC fluorescence intensity indicating the amount of the antibody bound, and the ordinate depicts cell count.

[Figure 2-6] Figure 2-6 shows the binding activity of four rat anti-CDH6 monoclonal antibodies (rG019, rG055, rG056 and rG061) or rat IgG control against control cells or EC5-deleted hCDH6-transfected 293 cells. The abscissa depicts FITC fluorescence intensity indicating the amount of the antibody bound, and the ordinate depicts cell count.

[Figure 3] Figure 3 shows flow cytometry results of evaluating the expression of CDH6 on the cell membrane surface of 4 types

of human tumor cell lines (human ovarian tumor cell lines NIH:OVCAR-3, PA-1, and ES-2 and human renal cell tumor cell line 786-O). The abscissa depicts FITC fluorescence intensity indicating the amount of the antibody bound, and the ordinate depicts cell count.

[Figure 4] Figure 4 shows a graph on which the internalization activity of 4 types of rat anti-CDH6 antibodies (rG019, rG055, rG056 and rG061) or rat IgG control was evaluated in NIH:OVCAR-3 cells and 786-O cells using anti-rat IgG reagent Rat-ZAP conjugated with a toxin (saporin) inhibiting protein synthesis, or Goat Anti-Rat IgG, Fc (gamma) Fragment Specific unconjugated with the toxin as a negative control. The ordinate of the graph depicts ATP activity (RLU). A cell survival rate (%), calculated as a relative survival rate when the number of live cells in a well supplemented with the negative control instead of Rat-ZAP was defined as 100%, is shown below each graph.

[Figure 5] Figure 5 shows the binding of human chimeric anti-CDH6 antibody chG019 to human CDH6 and monkey CDH6. The abscissa depicts antibody concentration, and the ordinate depicts the amount of antibody bound based on mean fluorescence intensity.

[Figure 6-1] Figures 6-1 and 6-2 each show the binding activity of four humanized hG019 antibodies (H01L02, H02L02, H02L03 and H04L02) or a negative control antibody human IgG1 against human CDH6, monkey CDH6, mouse CDH6, and rat CDH6. The abscissa depicts antibody concentration, and the ordinate depicts the amount of the antibody bound based on mean fluorescence intensity.

[Figure 6-2] Figures 6-1 and 6-2 each show the binding activity of four humanized hG019 antibodies (H01L02, H02L02, H02L03 and H04L02) or negative control antibody human IgG1 against human CDH6, monkey CDH6, mouse CDH6, and rat CDH6. The abscissa depicts antibody concentration, and the ordinate depicts the amount of the antibody bound based on mean fluorescence intensity.

[Figure 7-1] Figure 7-1 shows the binding activity of four humanized hG019 antibodies (H01L02, H02L02, H02L03 and H04L02), anti-CDH6 antibody NOV0712 or negative control antibody hIgG1 against control cells or full-length hCDH6-transfected 293 α cells. The abscissa depicts APC fluorescence intensity indicating the amount of the antibody bound. The ordinate depicts cell count.

[Figure 7-2] Figure 7-2 shows the binding activity of four humanized hG019 antibodies (H01L02, H02L02, H02L03 and H04L02), anti-CDH6 antibody NOV0712 or negative control antibody hIgG1 against control cells or EC1-deleted hCDH6-transfected 293 α cells. The abscissa depicts APC fluorescence intensity indicating the amount of the antibody bound. The ordinate depicts cell count.

[Figure 7-3] Figure 7-3 shows the binding activity of four humanized hG019 antibodies (H01L02, H02L02, H02L03 and H04L02), anti-CDH6 antibody NOV0712 or negative control antibody hIgG1 against control cells or EC2-deleted hCDH6-transfected 293 α cells. The abscissa depicts APC fluorescence intensity indicating the amount of the antibody bound. The ordinate depicts cell count.

[Figure 7-4] Figure 7-4 shows the binding activity of four humanized hG019 antibodies (H01L02, H02L02, H02L03 and H04L02), anti-CDH6 antibody NOV0712 or negative control antibody hIgG1 against control cells or EC3-deleted hCDH6-transfected 293 α cells. The abscissa depicts APC fluorescence intensity indicating the amount of the antibody bound. The ordinate depicts cell count.

[Figure 7-5] Figure 7-5 shows the binding activity of four humanized hG019 antibodies (H01L02, H02L02, H02L03 and H04L02), anti-CDH6 antibody NOV0712 or negative control hIgG1 against control cells or EC4-deleted hCDH6-transfected 293 α cells. The abscissa depicts APC fluorescence intensity indicating the amount of the antibody bound. The ordinate depicts cell count.

[Figure 7-6] Figure 7-6 shows the binding activity of four humanized hG019 antibodies (H01L02, H02L02, H02L03 and H04L02), anti-CDH6 antibody NOV0712 or negative control hIgG1 against control cells or EC5-deleted hCDH6-transfected 293 α cells. The abscissa depicts APC fluorescence intensity indicating the amount of the antibody bound. The ordinate depicts cell count.

[Figure 8] Figure 8 shows flow cytometry results of examining the expression of human CDH6 in 786-O/hCDH6 stably expressing cell line and its parent cell line 786-O. The abscissa depicts Alexa Fluor 647 fluorescence intensity indicating the amount of the antibody bound, and the ordinate depicts a cell count.

[Figure 9] Figure 9 shows the binding competition assay of four unlabeled humanized hG019 antibodies (H01L02, H02L02, H02L03 and H04L02), anti-CDH6 antibody NOV0712 or negative control hIgG1 using (a) labeled NOV0712 or (b) labeled H01L02.

The abscissa depicts the final concentration of the added unlabeled antibody, and the ordinate depicts the amount of the antibody bound based on mean fluorescence intensity.

[Figure 10-1] Figure 10-1 shows a graph on which the internalization activity of four humanized hG019 antibodies (H01L02, H02L02, H02L03 and H04L02), anti-CDH6 antibody NOV0712 and a negative control antibody was evaluated in NIH:OVCAR-3 cells using anti-human IgG reagent Hum-ZAP conjugated with a toxin (saporin) inhibiting protein synthesis, or F(ab')₂ Fragment Goat Anti-human IgG, Fc (gamma) Fragment Specific unconjugated with the toxin as a negative control. The ordinate of the graph depicts ATP activity (RLU). A cell survival rate (%), calculated as a relative survival rate when the number of live cells in a well supplemented with the negative control instead of Hum-ZAP was defined as 100%, is shown below each graph.

[Figure 10-2] Figure 10-2 shows a graph on which the internalization activity of four humanized hG019 antibodies (H01L02, H02L02, H02L03 and H04L02), anti-CDH6 antibody NOV0712 and a negative control antibody was evaluated in 786-O cells using anti-human IgG reagent Hum-ZAP conjugated with a toxin (saporin) inhibiting protein synthesis, or F(ab')₂ Fragment Goat Anti-human IgG, Fc (gamma) Fragment Specific unconjugated with the toxin as a negative control. The ordinate of the graph depicts ATP activity (RLU). A cell survival rate (%), calculated as a relative survival rate when the number of live cells in a well supplemented with the negative control instead of Hum-ZAP was defined as 100%, is shown below each graph.

[Figure 10-3] Figure 10-3 shows a graph on which the internalization activity of four humanized hG019 antibodies (H01L02, H02L02, H02L03 and H04L02), anti-CDH6 antibody NOV0712 and a negative control antibody was evaluated in PA-1 cells using anti-human IgG reagent Hum-ZAP conjugated with a toxin (saporin) inhibiting protein synthesis, or F(ab')₂ Fragment Goat Anti-human IgG, Fc (gamma) Fragment Specific unconjugated with the toxin as a negative control. The ordinate of the graph depicts ATP activity (RLU). A cell survival rate (%), calculated as a relative survival rate when the number of live cells in a well supplemented with the negative control instead of Hum-ZAP was defined as 100%, is shown below each graph.

[Figure 11] Figure 11 shows results of evaluating the *in vitro* cell growth inhibition activity of four humanized hG019-drug conjugates (H01L02-DXd, H02L02-DXd, H02L03-DXd and H04L02-DXd) or NOV0712-DM4 against PA-1 cells. The abscissa depicts an antibody-drug conjugate concentration, and the ordinate depicts cell survival rate (%).

[Figure 12] Figure 12 shows the *in vivo* antitumor effects of four humanized hG019-drug conjugates (H01L02-DXd, H02L02-DXd, H02L03-DXd and H04L02-DXd) or NOV0712-DM4. The evaluation was conducted using animal models in which CDH6-positive human renal cell tumor cell line 786-O was inoculated into immunodeficient mice. The abscissa depicts the number of days, and the ordinate depicts tumor volume. The error range depicts a standard error (SE) value.

[Figure 13] Figure 13 shows the *in vivo* antitumor effects of the humanized hG019-drug conjugate H01L02-DXd or NOV0712-DM4

or NOV0712-DXd. The evaluation was conducted using animal models in which CDH6-positive human ovarian tumor cell line PA-1 was inoculated into immunodeficient mice. The abscissa depicts the number of days, and the ordinate depicts tumor volume. The error range depicts a SE value.

[Figure 14] Figure 14 shows the *in vivo* antitumor effects of the humanized hG019-drug conjugate H01L02-DXd or NOV0712-DM4. The evaluation was conducted using animal models in which CDH6-positive human ovarian tumor cell line NIH:OVCAR-3 was inoculated into immunodeficient mice. The abscissa depicts the number of days, and the ordinate depicts tumor volume. The error range depicts a SE value.

[Figure 15] Figure 15 shows the *in vivo* antitumor effects of the humanized hG019-drug conjugate H01L02-DXd or NOV0712-DM4. The evaluation was conducted using animal models in which CDH6-positive human renal cell tumor cell line 786-O was inoculated into immunodeficient mice. The abscissa depicts the number of days, and the ordinate depicts tumor volume. The error range depicts a SE value.

[Figure 16] Figure 16 shows the *in vivo* antitumor effects of the humanized hG019-drug conjugate H01L02-DXd or NOV0712-DM4. The evaluation was conducted using animal models in which CDH6-negative human ovarian tumor cell line ES-2 was inoculated into immunodeficient mice. The abscissa depicts the number of days, and the ordinate depicts tumor volume. The error range depicts a SE value.

Description of Embodiments

[0025] Hereinafter, the preferred embodiments for carrying out the present invention will be described with reference to the drawings. It is to be noted that the embodiments described below merely illustrate the representative embodiments of the present invention, and the scope of the present invention shall not be narrowly interpreted due to these examples.

[0026] In the present description, the term "cancer" is used to have the same meaning as that of the term "tumor".

[0027] In the present description, the term "gene" is used to include not only DNA but also its mRNA and cDNA, and cRNA thereof.

[0028] In the present description, the term "polynucleotide" or "nucleotide" is used to have the same meaning as that of a nucleic acid, and also includes DNA, RNA, a probe, an oligonucleotide, and a primer. In the present description, the terms "polynucleotide" and "nucleotide" can be used interchangeably with each other unless otherwise specified.

[0029] In the present description, the terms "polypeptide" and "protein" can be used interchangeably with each other.

[0030] In the present description, the term "cell" includes cells in an individual animal, and cultured cells.

[0031] In the present description, the term "CDH6" can be used to have the same meaning as that of the CDH6 protein. In the present description, human CDH6 is also referred to as "hCDH6".

[0032] In the present description, the term "cytotoxic activity" is used to mean that a pathologic change is caused to cells in any given way. The term not only means a direct trauma, but also means all types of structural or functional damage caused to cells, such as DNA cleavage, formation of a

base dimer, chromosomal cleavage, damage to cell mitotic apparatus, and a reduction in the activities of various types of enzymes.

[0033] In the present description, the phrase "exerting toxicity in cells" is used to mean that toxicity is exhibited in cells in any given way. The term not only means a direct trauma, but also means all types of structural, functional, or metabolic influences caused to cells, such as DNA cleavage, formation of a base dimer, chromosomal cleavage, damage to cell mitotic apparatus, a reduction in the activities of various types of enzymes, and suppression of effects of cell growth factors.

[0034] In the present description, the term "functional fragment of an antibody", also called "antigen-binding fragment of an antibody", is used to mean a partial fragment of the antibody having binding activity against an antigen, and includes Fab, F(ab')₂, scFv, a diabody, a linear antibody and a multispecific antibody formed from antibody fragments, and the like. Fab', which is a monovalent fragment of antibody variable regions obtained by treating F(ab')₂ under reducing conditions, is also included in the antigen-binding fragment of an antibody. However, the antigen-binding fragment of an antibody is not limited to these molecules, as long as the antigen-binding fragment has antigen-binding ability. These antigen-binding fragments include not only those obtained by treating a full-length molecule of an antibody protein with an appropriate enzyme, but proteins produced in appropriate host cells using a genetically engineered antibody gene.

[0035] In the present description, the term "epitope" is used to mean the partial peptide or partial three-dimensional structure of CDH6, to which a specific anti-CDH6 antibody binds. Such an epitope, which is the above-described partial peptide of CDH6, can be determined by a method well known to a person skilled in the art, such as an immunoassay. First, various partial structures of an antigen are produced. As regards production of such partial structures, a known oligopeptide synthesis technique can be applied. For example, a series of polypeptides, in which CDH6 has been successively truncated at an appropriate length from the C-terminus or N-terminus thereof, are produced by a genetic recombination technique well known to a person skilled in the art. Thereafter, the reactivity of an antibody to such polypeptides is studied, and recognition sites are roughly determined. Thereafter, further shorter peptides are synthesized, and the reactivity thereof to these peptides can then be studied, so as to determine an epitope. When an antibody binding to a membrane protein having a plurality of extracellular domains is directed to a three-dimensional structure composed of a plurality of domains as an epitope, the domain to which the antibody binds can be determined by modifying the amino acid sequence of a specific extracellular domain, and thereby modifying the three-dimensional structure. The epitope, which is a partial three-dimensional structure of an antigen that binds to a specific antibody, can also be determined by specifying the amino acid residues of an antigen adjacent to the antibody by X-ray structural analysis.

[0036] In the present description, the phrase "antibodies binding to the same epitope" is used to mean antibodies that bind to a common epitope. If a second antibody binds to a partial peptide or a partial three-dimensional structure to which a first antibody binds, it can be determined that the first antibody and the second antibody bind to the same epitope. Alternatively, by confirming that a second antibody competes with a first antibody for the binding of the first antibody to an antigen (i.e., a second antibody interferes with the binding of a first antibody to an antigen), it can be determined that the first antibody and the second antibody bind to the same epitope, even if the specific sequence or structure of the epitope has not been determined. In the present description, the phrase "binding to the same epitope" refers to the case where it is determined that the first antibody and the second antibody bind to a common epitope by any one or both of these determination methods. When a first antibody and a second antibody bind to the same epitope and further, the first antibody has special effects such as antitumor activity or internalization activity, the second antibody can be expected to have the same activity as that of the first antibody.

[0037] In the present description, the term "CDR" is used to mean a complementarity determining region. It is known that the heavy chain and light chain of an antibody molecule each have three CDRs. Such a CDR is also referred to as a hypervariable region, and is located in the variable regions of the heavy chain and light chain of an antibody. These regions have a particularly highly variable primary structure

and are separated into three sites on the primary structure of the polypeptide chain in each of the heavy chain and light chain. In the present description, with regard to the CDR of an antibody, the CDRs of a heavy chain are referred to as CDRH1, CDRH2 and CDRH3, respectively, from the amino-terminal side of the amino acid sequence of the heavy chain, whereas the CDRs of a light chain are referred to as CDRL1, CDRL2 and CDRL3, respectively, from the amino-terminal side of the amino acid sequence of the light chain. These sites are located close to one another on the three-dimensional structure, and determine the specificity of the antibody to an antigen to which the antibody binds.

[0038] In the present invention, the phrase "hybridizing under stringent conditions" is used to mean that hybridization is carried out in the commercially available hybridization solution ExpressHyb Hybridization Solution (manufactured by Clontech Laboratories, Inc.) at 68°C, or that hybridization is carried out under conditions in which hybridization is carried out using a DNA-immobilized filter in the presence of 0.7 to 1.0 M NaCl at 68°C, and the resultant is then washed at 68°C with a 0.1- to 2-fold concentration of SSC solution (wherein 1-fold concentration of SSC consists of 150 mM NaCl and 15 mM sodium citrate) for identification, or conditions equivalent thereto.

[0039] In the present description, the term "one to several" is used to mean 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 or 2.

[0040] 1. CDH6

Cadherins are glycoproteins present on the surface of cell membranes and function as cell-cell adhesion molecules through the calcium ion-dependent binding of their N-terminal extracellular domains, or as signal molecules responsible for cell-cell interaction. Classic cadherins are in the cadherin superfamily and are single-pass transmembrane proteins composed of five extracellular domains (EC domains), one transmembrane region, and an intracellular domain.

[0041] CDH6 (cadherin-6) is a single-pass transmembrane protein composed of 790 amino acids, which is classified into the type II cadherin family, and this protein has N-terminal extracellular and C-terminal intracellular domains. The human CDH6 gene was cloned for the first time in 1995 (Non Patent Literature 1), and its sequence can be referred to under, for example, accession Nos. NM_004932 and NP_004923 (NCBI).

[0042] The CDH6 protein used in the present invention can be directly purified from the CDH6-expressing cells of a human or a non-human mammal (e.g., a rat, a mouse or a monkey) and can then be used, or a cell membrane fraction of the aforementioned cells can be prepared and can be used as the CDH6 protein. Alternatively, CDH6 can also be obtained by synthesizing it *in vitro*, or by allowing host cells to produce CDH6 by genetic manipulation. According to such genetic manipulation, the CDH6 protein can be obtained, specifically, by incorporating CDH6 cDNA into a vector capable of expressing the CDH6 cDNA, and then synthesizing CDH6 in a solution containing enzymes, substrate and energetic materials necessary for transcription and translation, or by transforming the host cells of other prokaryotes or eukaryotes,

so as to allow them to express CDH6. Also, CDH6-expressing cells based on the above-described genetic manipulation, or a cell line expressing CDH6 may be used to present the CDH6 protein. Alternatively, the expression vector into which CDH6 cDNA has been incorporated can be directly administered to an animal to be immunized, and CDH6 can be expressed in the body of the animal thus immunized.

[0043] Moreover, a protein which consists of an amino acid sequence comprising a substitution, deletion and/or addition of one or several amino acids in the above-described amino acid sequence of CDH6, and has a biological activity equivalent to that of the CDH6 protein, is also included within the term "CDH6".

[0044] The human CDH6 protein has the amino acid sequence shown in SEQ ID NO: 1. The extracellular region of the human CDH6 protein is composed of extracellular domain 1 (in the present description, also referred to as EC1) having the amino acid sequence at positions 54 to 159 in the amino acid sequence shown in SEQ ID NO: 1, extracellular domain 2 (in the present description, also referred to as EC2) having the amino acid sequence at positions 160 to 268 in the amino acid sequence shown in SEQ ID NO: 1, extracellular domain 3 (in the present description, also referred to as EC3) having the amino acid sequence at positions 269 to 383 in the amino acid sequence shown in SEQ ID NO: 1, extracellular domain 4 (in the present description, also referred to as EC4) having the amino acid sequence at positions 384 to 486 in the amino acid sequence shown in SEQ ID NO: 1, and extracellular domain 5 (in the present description, also referred to as EC5) having the amino

acid sequence at positions 487 to 608 in the amino acid sequence shown in SEQ ID NO: 1. The amino acid sequences of EC1 to EC5 are shown in SEQ ID NOs: 2 to 6, respectively (Table 1).

[0045] 2. Production of anti-CDH6 antibody

One example of the anti-CDH6 antibody of the present invention can include an anti-CDH6 antibody which recognizes an amino acid sequence comprising the amino acid sequence shown in SEQ ID NO: 4, and has internalization activity. One example of the anti-CDH6 antibody of the present invention can include an anti-CDH6 antibody which specifically recognizes an amino acid sequence comprising the amino acid sequence shown in SEQ ID NO: 4, and has internalization activity. One example of the anti-CDH6 antibody of the present invention can include an anti-CDH6 antibody which recognizes an amino acid sequence consisting of the amino acid sequence shown in SEQ ID NO: 4, and has internalization activity. One example of the anti-CDH6 antibody of the present invention can include an anti-CDH6 antibody which specifically recognizes an amino acid sequence consisting of the amino acid sequence shown in SEQ ID NO: 4, and has internalization activity. The phrase "specifically recognize an amino acid sequence comprising the amino acid sequence shown in SEQ ID NO: 4" or "specifically recognize an EC3 domain" as applied to an antibody is used to mean that the antibody strongly recognizes or strongly binds to the EC3 domain of CDH6 compared with the other extracellular domains of CDH6.

[0046] The anti-CDH6 antibody of the present invention may be derived from any species. Preferred examples of the species

can include humans, monkeys, rats, mice and rabbits. When the anti-CDH6 antibody of the present invention is derived from a species other than humans, it is preferred to chimerize or humanize the anti-CDH6 antibody by a well-known technique. The antibody of the present invention may be a polyclonal antibody or may be a monoclonal antibody, and a monoclonal antibody is preferred.

[0047] The anti-CDH6 antibody of the present invention is an antibody that can target tumor cells. Specifically, the anti-CDH6 antibody of the present invention possesses the property of being able to recognize tumor cells, the property of being able to bind to tumor cells, and/or the property of being internalized into tumor cells by cellular uptake, and the like. Accordingly, the anti-CDH6 antibody of the present invention can be conjugated to a compound having antitumor activity via a linker to prepare an antibody-drug conjugate.

[0048] The binding activity of an antibody against tumor cells can be confirmed by flow cytometry. The uptake of an antibody into tumor cells can be confirmed by (1) an assay of visualizing a cellularly taken-up antibody under a fluorescent microscope using a secondary antibody (fluorescently labeled) binding to the antibody (Cell Death and Differentiation, 2008, 15, 751-761), (2) an assay of measuring the amount of cellularly taken-up fluorescence using a secondary antibody (fluorescently labeled) binding to the antibody (Molecular Biology of the Cell Vol. 15, 5268-5282, December 2004) or (3) a Mab-ZAP assay using an immunotoxin binding to the antibody, wherein the toxin is released upon cellular uptake, so as to suppress cell growth (Bio Techniques 28: 162-165, January

2000). A recombinant conjugated protein of a catalytic region of diphtheria toxin and protein G may be used as the immunotoxin.

[0049] In the present description, the term "high internalization ability" is used to mean that the survival rate (which is indicated by a ratio relative to a cell survival rate without antibody addition defined as 100%) of CDH6-expressing cells to which the aforementioned antibody and a saporin-labeled anti-rat IgG antibody have been administered is preferably 70% or less, and more preferably 60% or less.

[0050] The antitumor antibody-drug conjugate of the present invention comprises a conjugated compound exerting an antitumor effect. Therefore, it is preferred, but not essential, that the antibody itself should have an antitumor effect. For the purpose of specifically and/or selectively exerting the cytotoxicity of the antitumor compound in tumor cells, it is important and preferred that the antibody should have a property of being internalized and transferred into tumor cells.

[0051] The anti-CDH6 antibody can be obtained by immunizing an animal with a polypeptide serving as an antigen by a method usually performed in this field, and then collecting and purifying an antibody produced in a living body thereof. It is preferred to use CDH6 retaining a three-dimensional structure as an antigen. Examples of such a method can include a DNA immunization method.

[0052] The origin of the antigen is not limited to a human, and thus, an animal can also be immunized with an antigen derived from a non-human animal such as a mouse or a rat. In this

case, an antibody applicable to the disease of a human can be selected by examining the cross-reactivity of the obtained antibody binding to the heterologous antigen with the human antigen.

[0053] Furthermore, antibody-producing cells that produce an antibody against the antigen can be fused with myeloma cells according to a known method (e.g., Kohler and Milstein, Nature (1975) 256, 495-497; and Kennet, R. ed., Monoclonal Antibodies, 365-367, Plenum Press, N. Y. (1980)) to establish hybridomas, so as to obtain a monoclonal antibody.

[0054] Hereinafter, the method for obtaining an antibody against CDH6 will be specifically described.

[0055] (1) Preparation of antigen

The antigen can be obtained by allowing host cells to produce a gene encoding the antigen protein according to genetic manipulation. Specifically, a vector capable of expressing the antigen gene is produced, and the vector is then introduced into host cells, so that the gene is expressed therein, and thereafter, the expressed antigen may be purified. The antibody can also be obtained by a method of immunizing an animal with the antigen-expressing cells based on the above-described genetic manipulation, or a cell line expressing the antigen.

[0056] Alternatively, the antibody can also be obtained, without the use of the antigen protein, by incorporating cDNA of the antigen protein into an expression vector, then administering the expression vector to an animal to be immunized, and expressing the antigen protein in the body of

the animal thus immunized, so that an antibody against the antigen protein is produced therein.

[0057] (2) Production of anti-CDH6 monoclonal antibody

The anti-CDH6 antibody used in the present invention is not particularly limited. For example, an antibody specified by an amino acid sequence shown in the sequence listing of the present application can be suitably used. The anti-CDH6 antibody used in the present invention is desirably an antibody having the following properties:

- (1) an antibody having the following properties:
 - (a) specifically binding to CDH6, and
 - (b) having the activity of being internalized into CDH6-expressing cells by binding to CDH6;
- (2) the antibody according to the above (1) or the aforementioned antibody, wherein the CDH6 is human CDH6; or
- (3) specifically recognizing EC3 of human CDH6, and having internalization activity.

The method for obtaining the antibody against CDH6 of the present invention is not particularly limited as long as an anti-CDH6 antibody can be obtained. It is preferred to use CDH6 retaining its conformation as an antigen.

[0058] One preferred example of the method for obtaining the antibody can include a DNA immunization method. The DNA immunization method is an approach which involves transfecting an animal (e.g., mouse or rat) individual with an antigen expression plasmid, and then expressing the antigen in the individual to induce immunity against the antigen. The transfection approach includes a method of directly injecting the plasmid to the muscle, a method of injecting a

transfection reagent such as a liposome or polyethylenimine to the vein, an approach using a viral vector, an approach of injecting gold particles attached with the plasmid using a gene gun, a hydrodynamic method of rapidly injecting a plasmid solution in a large amount to the vein, and the like. With regard to the transfection method of injecting the expression plasmid to the muscle, a technique called *in vivo* electroporation, which involves applying electroporation to the intramuscular injection site of the plasmid, is known as an approach for improving expression levels (Aihara H, Miyazaki J. Nat Biotechnol. 1998 Sep; 16 (9): 867-70 or Mir LM, Bureau MF, Gehl J, Rangara R, Rouy D, Caillaud JM, Delaere P, Branellec D, Schwartz B, Scherman D. Proc Natl Acad Sci U S A. 1999 Apr 13; 96 (8): 4262-7). This approach further improves the expression level by treating the muscle with hyaluronidase before the intramuscular injection of the plasmid (McMahon JM1, Signori E, Wells KE, Fazio VM, Wells DJ., Gene Ther. 2001 Aug; 8 (16): 1264-70). Furthermore, the hybridoma production can be performed by a known method, and can also be performed using, for example, a Hybrimmune Hybridoma Production System (Cyto Pulse Sciences, Inc.).

[0059] Specific examples of obtaining a monoclonal antibody can include the following procedures:

(a) immune response can be induced by incorporating CDH6 cDNA into an expression vector (e.g., pCDNA3.1; Thermo Fisher Scientific Inc.), and directly administering the vector to an animal (e.g., a rat or a mouse) to be immunized by a method such as electroporation or a gene gun, so as to express CDH6 in the body of the animal. The administration of the vector

by electroporation or the like may be performed one or more times, preferably a plurality of times, if necessary for enhancing antibody titer;

(b) collection of tissue (e.g., a lymph node) containing antibody-producing cells from the aforementioned animal in which the immune response has been induced;

(c) preparation of myeloma cells (hereinafter, referred to as "myelomas") (e.g., mouse myeloma SP2/0-ag14 cells);

(d) cell fusion between the antibody-producing cells and the myelomas;

(e) selection of a hybridoma group producing an antibody of interest;

(f) division into single cell clones (cloning);

(g) optionally, the culture of hybridomas for the mass production of monoclonal antibodies, or the breeding of animals into which the hybridomas are inoculated; and/or

(h) study of the physiological activity (internalization activity) and binding specificity of the monoclonal antibody thus produced, or examination of the properties of the antibody as a labeling reagent.

[0060] Examples of the method for measuring the antibody titer used herein can include, but are not limited to, flow cytometry and Cell-ELISA.

[0061] Examples of the hybridoma strain thus established can include anti-CDH6 antibody-producing hybridomas rG019, rG055, rG056 and rG061. It is to be noted that, in the present description, an antibody produced by the anti-CDH6 antibody-producing hybridoma rG019 is referred to as a "rG019 antibody" or simply "rG019", an antibody produced by the hybridoma rG055

is referred to as a "rG055 antibody" or simply "rG055", an antibody produced by the hybridoma rG056 is referred to as a "rG056 antibody" or simply "rG056", and an antibody produced by the hybridoma rG061 is referred to as a "rG061 antibody" or simply "rG061".

[0062] The light chain variable region of the rG019 antibody consists of the amino acid sequence shown in SEQ ID NO: 10. The amino acid sequence of the light chain variable region of the rG019 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 11. The light chain variable region of the rG019 antibody has CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 12, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 13, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 14. The heavy chain variable region of the rG019 antibody consists of the amino acid sequence shown in SEQ ID NO: 15. The amino acid sequence of the heavy chain variable region of the rG019 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 16. The heavy chain variable region of the rG019 antibody has CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 18, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 19. The sequence of the rG019 antibody is shown in Table 1.

[0063] The light chain variable region of the rG055 antibody consists of the amino acid sequence shown in SEQ ID NO: 20. The amino acid sequence of the light chain variable region of the rG055 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 21. The light chain variable region of the

rG055 antibody has CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 22, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 23, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 24. The heavy chain variable region of the rG055 antibody consists of the amino acid sequence shown in SEQ ID NO: 25. The amino acid sequence of the heavy chain variable region of the rG055 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 26. The heavy chain variable region of the rG055 antibody has CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 27, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 28, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 29. The sequence of the rG055 antibody is shown in Table 1.

[0064] The light chain variable region of the rG056 antibody consists of the amino acid sequence shown in SEQ ID NO: 30. The amino acid sequence of the light chain variable region of the rG056 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 31. The light chain variable region of the rG056 antibody has CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 32, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 33, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 34. The heavy chain variable region of the rG056 antibody consists of the amino acid sequence shown in SEQ ID NO: 35. The amino acid sequence of the heavy chain variable region of the rG056 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 36. The heavy chain variable region of the rG056 antibody has CDRH1 consisting of the amino acid sequence shown in SEQ ID

NO: 37, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 38, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 39. The sequence of the rG056 antibody is shown in Table 1.

[0065] The light chain variable region of the rG061 antibody consists of the amino acid sequence shown in SEQ ID NO: 40. The amino acid sequence of the light chain variable region of the rG061 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 41. The light chain variable region of the rG061 antibody has CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 42, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 43, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 44. The heavy chain variable region of the rG061 antibody consists of the amino acid sequence shown in SEQ ID NO: 45. The amino acid sequence of the heavy chain variable region of the rG061 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 46. The heavy chain variable region of the rG061 antibody has CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 47, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 48, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 49. The sequence of the rG061 antibody is shown in Table 1.

[0066] Furthermore, in the case where the steps (a) to (h) in the above "2. Production of anti-CDH6 antibody" are carried out again to obtain independently a monoclonal antibody separately and also in the case where a monoclonal antibody is obtained separately by other methods, an antibody having internalization activity equivalent to that of the rG019

antibody, the rG055 antibody, the rG056 antibody or the rG061 antibody can be obtained. One example of such an antibody can include an antibody binding to the same epitope to which the rG019 antibody, the rG055 antibody, the rG056 antibody or the rG061 antibody binds. If a newly prepared monoclonal antibody binds to a partial peptide or a partial three-dimensional structure to which the rG019 antibody, the rG055 antibody, the rG056 antibody or the rG061 antibody binds, it can be determined that the monoclonal antibody binds to the same epitope to which the rG019 antibody, the rG055 antibody, the rG056 antibody or the rG061 antibody binds. Moreover, by confirming that the monoclonal antibody competes with the rG019 antibody, the rG055 antibody, the rG056 antibody or the rG061 antibody in the binding of the antibody to CDH6 (i.e., the monoclonal antibody interferes with the binding of the rG019 antibody, the rG055 antibody, the rG056 antibody or the rG061 antibody to CDH6), it can be determined that the monoclonal antibody binds to the same epitope to which the anti-CDH6 antibody binds, even if the specific sequence or structure of the epitope has not been determined. When it is confirmed that the monoclonal antibody binds to the same epitope to which the rG019 antibody, the rG055 antibody, the rG056 antibody or the rG061 antibody binds, then it is strongly expected that the monoclonal antibody should have antigen-binding ability, biological activity and/or internalization activity equivalent to that of the rG019 antibody, the rG055 antibody, the rG056 antibody or the rG061 antibody.

[0067] (3) Other antibodies

The antibody of the present invention also includes genetically recombinant antibodies that have been artificially modified for the purpose of reducing heterogenetic antigenicity to humans, such as a chimeric antibody, a humanized antibody and a human antibody, as well as the above-described monoclonal antibody against CDH6. These antibodies can be produced by known methods.

[0068] Example of the chimeric antibody can include antibodies in which a variable region and a constant region are heterologous to each other, such as a chimeric antibody formed by conjugating the variable region of a mouse- or rat-derived antibody to a human-derived constant region (see Proc. Natl. Acad. Sci. U.S.A., 81, 6851-6855, (1984)).

[0069] Examples of the chimeric antibody derived from the rat anti-human CDH6 antibody include an antibody consisting of a light chain comprising the light chain variable region of each rat anti-human CDH6 antibody described in the present description (e.g., the rG019 antibody, the rG055 antibody, the rG056 antibody or the rG061 antibody) and a human-derived constant region, and a heavy chain comprising the heavy chain variable region thereof and a human-derived constant region.

[0070] Other examples of the chimeric antibody derived from the rat anti-human CDH6 antibody include an antibody consisting of a light chain comprising a light chain variable region having a substitution of one to several residues, 1 to 3 residues, 1 or 2 residues, preferably 1 residue, of amino acids in the light chain variable region of each rat anti-human CDH6 antibody described in the present description (e.g., the rG019 antibody, the rG055 antibody, the rG056 antibody or the rG061

antibody) with other amino acid residues, and a heavy chain comprising a heavy chain variable region having a substitution of one to several residues, 1 to 3 residues, 1 or 2 residues, preferably 1 residue, of amino acids in the heavy chain variable region thereof with other amino acid residues. This antibody may have any given human-derived constant region.

[0071] Other examples of the chimeric antibody derived from the rat anti-human CDH6 antibody include an antibody consisting of a light chain comprising a light chain variable region having a substitution of 1 or 2 residues, preferably 1 residue, of amino acids in any 1 to 3 CDRs in the light chain variable region of each rat anti-human CDH6 antibody described in the present description (e.g., the rG019 antibody, the rG055 antibody, the rG056 antibody or the rG061 antibody) with other amino acid residues, and a heavy chain comprising a heavy chain variable region having a substitution of 1 or 2 residues, preferably 1 residue, of amino acids in any 1 to 3 CDRs in the heavy chain variable region thereof with other amino acid residues. This antibody may have any given human-derived constant region.

[0072] Examples of the chimeric antibody derived from the rG019 antibody include an antibody consisting of a light chain comprising a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 10, and a heavy chain comprising a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 15. This antibody may have any given human-derived constant region.

[0073] Other examples of the chimeric antibody derived from the rG019 antibody include an antibody consisting of a light chain

comprising a light chain variable region having a substitution of one to several residues, 1 to 3 residues, 1 or 2 residues, preferably 1 residue, of amino acids in the light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 10 with other amino acid residues, and a heavy chain comprising a heavy chain variable region having a substitution of one to several residues, 1 to 3 residues, 1 or 2 residues, preferably 1 residue, of amino acids in the heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 15 with other amino acid residues. This antibody may have any given human-derived constant region.

[0074] Other examples of the chimeric antibody derived from the rG019 antibody include an antibody consisting of a light chain comprising a light chain variable region having a substitution of 1 or 2 residues (preferably 1 residue) of amino acids in any 1 to 3 CDRs in the light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 10 with other amino acid residues, and a heavy chain comprising a heavy chain variable region having a substitution of 1 or 2 residues (preferably 1 residue) of amino acids in any 1 to 3 CDRs in the heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 15 with other amino acid residues. This antibody may have any given human-derived constant region.

[0075] Other examples of the chimeric antibody derived from the rG019 antibody include an antibody consisting of a light chain comprising a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 10, and a heavy chain comprising a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 58. This antibody may

have any given human-derived constant region. The amino acid sequence shown in SEQ ID NO: 58 is a sequence with a cysteine residue substituted with a proline residue in CDRH2 in the amino acid sequence shown in SEQ ID NO: 15.

[0076] Specific examples of the chimeric antibody derived from the rG019 antibody include an antibody consisting of a light chain consisting of the light chain full-length amino acid sequence shown in SEQ ID NO: 53, and a heavy chain consisting of the heavy chain full-length amino acid sequence shown in SEQ ID NO: 56. In the present description, this chimeric anti-human CDH6 antibody is referred to as a "chimeric G019 antibody", a "chG019 antibody" or "chG019". The light chain full-length amino acid sequence of the chG019 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 54, and the heavy chain full-length amino acid sequence of the chG019 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 57.

[0077] The amino acid sequence of the light chain variable region of the chG019 antibody is identical to the amino acid sequence of the light chain variable region of the rG019 antibody, and consists of the amino acid sequence shown in SEQ ID NO: 10. The light chain of the chG019 antibody has CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 12, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 13, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 14, which are identical to the light chain CDRL1, CDRL2 and CDRL3, respectively, of rG019. The amino acid of the light chain variable region of the chG019 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 55.

[0078] The amino acid sequence of the heavy chain variable region of the chG019 antibody consists of the amino acid sequence shown in SEQ ID NO: 58. The heavy chain of the chG019 antibody has CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 60, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 19. The amino acid sequence shown in SEQ ID NO: 58 is a sequence with a cysteine residue substituted with a proline residue in CDRH2 in the amino acid sequence shown in SEQ ID NO: 15. The CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 60 is a sequence with a cysteine residue substituted with a proline residue in the rG019 CDRH2 shown in SEQ ID NO: 18. The amino acid sequence of the heavy chain variable region of the chG019 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 59.

[0079] The sequence of the chG019 antibody is shown in Table 1.

[0080] Examples of the chimeric antibody derived from the rat anti-human CDH6 antibody rG055 antibody include a chimeric antibody consisting of a light chain comprising a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 20, and a heavy chain comprising a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 25. This antibody may have any given human-derived constant region.

[0081] Examples of the chimeric antibody derived from the rat anti-human CDH6 antibody rG056 antibody include a chimeric antibody consisting of a light chain comprising a light chain variable region consisting of the amino acid sequence shown in

SEQ ID NO: 30, and a heavy chain comprising a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 35. This antibody may have any given human-derived constant region.

[0082] Examples of the chimeric antibody derived from the rat anti-human CDH6 antibody rG061 antibody include a chimeric antibody consisting of a light chain comprising a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 40, and a heavy chain comprising a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 45. This antibody may have any given human-derived constant region.

[0083] Examples of the humanized antibody can include an antibody formed by incorporating only complementarity determining regions (CDRs) into a human-derived antibody (see Nature (1986) 321, p. 522-525), an antibody formed by incorporating the amino acid residues from some frameworks, as well as CDR sequences, into a human antibody according to a CDR grafting method (International Publication No. WO90/07861), and an antibody formed by modifying the amino acid sequences of some CDRs while maintaining antigen-binding ability.

[0084] In the present description, the humanized antibody derived from the rG019 antibody, the rG055 antibody, the rG056 antibody, the rG061 antibody or the chG019 antibody is not limited to a specific humanized antibody as long as the humanized antibody retains all 6 CDR sequences unique to the rG019 antibody, the rG055 antibody, the rG056 antibody, the rG061 antibody or the chG019 antibody and has internalization activity. The amino acid sequences of some CDRs of this

humanized antibody may be further modified as long as it has internalization activity.

[0085] Concrete examples of the humanized antibody of the chG019 antibody can include any given combination of: a light chain comprising a light chain variable region consisting of any one amino acid sequence selected from the group consisting of (1) the amino acid sequence shown in SEQ ID NO: 63 or 67, (2) an amino acid sequence having an identity of at least 95% or more (preferably an amino acid sequence having a sequence identity of at least 95% or more to the sequence of a framework region other than at each CDR sequence) to the above-described amino acid sequence (1), and (3) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the above-described amino acid sequence (1); and a heavy chain comprising a heavy chain variable region consisting of any one amino acid sequence selected from the group consisting of (4) the amino acid sequence shown in SEQ ID NO: 71, 75 or 79, (5) an amino acid sequence having an identity of at least 95% or more (preferably an amino acid sequence having a sequence identity of at least 95% or more to the sequence of a framework region other than at each CDR sequence) to the above-described amino acid sequence (4), and (6) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the above-described amino acid sequence (4).

[0086] Alternatively, an antibody having a humanized heavy chain or light chain and the other chain derived from a rat antibody or a chimeric antibody can also be used. Examples of such an antibody can include any given combination of: a light

chain comprising a light chain variable region consisting of any one amino acid sequence selected from the group consisting of (1) the amino acid sequence shown in SEQ ID NO: 63 or 67, (2) an amino acid sequence having an identity of at least 95% or more (preferably an amino acid sequence having a sequence identity of at least 95% or more to the sequence of a framework region other than at each CDR sequence) to the above-described amino acid sequence (1), and (3) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the above-described amino acid sequence (1); and a heavy chain comprising a heavy chain variable region consisting of any one amino acid sequence selected from the group consisting of (4) the amino acid sequence shown in SEQ ID NO: 15, 25, 35, 45 or 58, (5) an amino acid sequence having an identity of at least 95% or more (preferably an amino acid sequence having a sequence identity of at least 95% or more to the sequence of a framework region other than at each CDR sequence) to the above-described amino acid sequence (4), and (6) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the above-described amino acid sequence (4). Other examples of such an antibody can include any given combination of: a light chain comprising a light chain variable region consisting of any one amino acid sequence selected from the group consisting of (1) the amino acid sequence shown in SEQ ID NO: 10, 20, 30 or 40, (2) an amino acid sequence having an identity of at least 95% or more (preferably an amino acid sequence having a sequence identity of at least 95% or more to the sequence of a framework region other than at each CDR

sequence) to the above-described amino acid sequence (1), and (3) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the above-described amino acid sequence (1); and a heavy chain comprising a heavy chain variable region consisting of any one amino acid sequence selected from the group consisting of (4) the amino acid sequence shown in SEQ ID NO: 71, 75 or 79, (5) an amino acid sequence having an identity of at least 95% or more (preferably an amino acid sequence having a sequence identity of at least 95% or more to the sequence of a framework region other than at each CDR sequence) to the above-described amino acid sequence (4), and (6) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the above-described amino acid sequence (4).

[0087] The amino acid substitution in the present description is preferably a conservative amino acid substitution. The conservative amino acid substitution is a substitution occurring within an amino acid group associated with certain amino acid side chains. Preferred amino acid groups are the following: acidic group = aspartic acid and glutamic acid; basic group = lysine, arginine, and histidine; non-polar group = alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, and tryptophan; and uncharged polar family = glycine, asparagine, glutamine, cysteine, serine, threonine, and tyrosine. Other preferred amino acid groups are the following: aliphatic hydroxy group = serine and threonine; amide-containing group = asparagine and glutamine; aliphatic group = alanine, valine, leucine and isoleucine; and aromatic

group = phenylalanine, tryptophan and tyrosine. Such amino acid substitution is preferably carried out without impairing the properties of a substance having the original amino acid sequence.

[0088] Examples of the antibody having a preferred combination of the above-described light chains and heavy chains include an antibody consisting of a light chain having the light chain variable region amino acid sequence shown in SEQ ID NO: 63 (in the present description, also referred to as a hL02 light chain variable region amino acid sequence) or a light chain having the light chain variable region amino acid sequence shown in SEQ ID NO: 67 (in the present description, also referred to as a hL03 light chain variable region amino acid sequence), and a heavy chain having the heavy chain variable region amino acid sequence shown in SEQ ID NO: 71 (in the present description, also referred to as a hH01 heavy chain variable region amino acid sequence), a heavy chain having the heavy chain variable region amino acid sequence shown in SEQ ID NO: 75 (in the present description, also referred to as a hH02 heavy chain variable region amino acid sequence) or a heavy chain having the heavy chain variable region amino acid sequence shown in SEQ ID NO: 79 (in the present description, also referred to as a hH04 heavy chain variable region amino acid sequence). Preferred examples thereof include: an antibody consisting of a light chain having the light chain variable region amino acid sequence shown in SEQ ID NO: 63 and a heavy chain having the heavy chain variable region amino acid sequence shown in SEQ ID NO: 71; an antibody consisting of a light chain having the light chain variable region amino

acid sequence shown in SEQ ID NO: 63 and a heavy chain having the heavy chain variable region amino acid sequence shown in SEQ ID NO: 75; an antibody consisting of a light chain having the light chain variable region amino acid sequence shown in SEQ ID NO: 63 and a heavy chain having the heavy chain variable region amino acid sequence shown in SEQ ID NO: 79; an antibody consisting of a light chain having the light chain variable region amino acid sequence shown in SEQ ID NO: 67 and a heavy chain having the heavy chain variable region amino acid sequence shown in SEQ ID NO: 71; an antibody consisting of a light chain having the light chain variable region amino acid sequence shown in SEQ ID NO: 67 and a heavy chain having the heavy chain variable region amino acid sequence shown in SEQ ID NO: 75; and an antibody consisting of a light chain having the light chain variable region amino acid sequence shown in SEQ ID NO: 67 and a heavy chain having the heavy chain variable region amino acid sequence shown in SEQ ID NO: 79. More preferred examples thereof include: an antibody consisting of a light chain having the light chain variable region amino acid sequence shown in SEQ ID NO: 63 and a heavy chain having the heavy chain variable region amino acid sequence shown in SEQ ID NO: 71; an antibody consisting of a light chain having the light chain variable region amino acid sequence shown in SEQ ID NO: 63 and a heavy chain having the heavy chain variable region amino acid sequence shown in SEQ ID NO: 75; an antibody consisting of a light chain having the light chain variable region amino acid sequence shown in SEQ ID NO: 63 and a heavy chain having the heavy chain variable region amino acid sequence shown in SEQ ID NO: 79; and an

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antibody consisting of a light chain having the light chain variable region amino acid sequence shown in SEQ ID NO: 67 and a heavy chain having the heavy chain variable region amino acid sequence shown in SEQ ID NO: 75.

[0089] Other examples of the antibody having a preferred combination of the above-described light chains and heavy chains include an antibody consisting of a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 61 (in the present description, also referred to as the hL02 light chain full-length amino acid sequence) or a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 65 (in the present description, also referred to as the hL03 light chain full-length amino acid sequence), and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 69 (in the present description, also referred to as the hH01 heavy chain full-length amino acid sequence), a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 73 (in the present description, also referred to as the hH02 heavy chain full-length amino acid sequence) or a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 77 (in the present description, also referred to as the hH04 heavy chain full-length amino acid sequence). Preferred examples thereof include: an antibody consisting of a light

chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 69; an antibody consisting of a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 73; an antibody consisting of a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 77; an antibody consisting of a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 65 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 69; an antibody consisting of a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 65 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 73; and an antibody consisting of a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length

amino acid sequence shown in SEQ ID NO: 65 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 77. More preferred examples thereof include: an antibody consisting of a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 69 (in the present description, also referred to as the "H01L02 antibody" or "H01L02"); an antibody consisting of a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 73 (in the present description, also referred to as the "H02L02 antibody" or "H02L02"); an antibody consisting of a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 77 (in the present description, also referred to as the "H04L02 antibody" or "H04L02"); and an antibody consisting of a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 65 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length

amino acid sequence shown in SEQ ID NO: 73 (in the present description, also referred to as the "H02L03 antibody" or "H02L03"). The sequences of the H01L02 antibody, the H02L02 antibody, the H02L03 antibody or the H04L02 antibody are shown in Table 1.

[0090] By combining together sequences showing a high identity to the above-described heavy chain amino acid sequences and light chain amino acid sequences, it is possible to select an antibody having a biological activity equivalent to that of each of the above-described antibodies. Such an identity is an identity of generally 80% or more, preferably 90% or more, more preferably 95% or more, and most preferably 99% or more. Moreover, also by combining amino acid sequences of a heavy chain and a light chain comprising a substitution, deletion or addition of one or several amino acid residues thereof with respect to the amino acid sequence of a heavy chain or a light chain, it is possible to select an antibody having a biological activity equivalent to that of each of the above-described antibodies.

[0091] The identity between two types of amino acid sequences can be determined by aligning the sequences using the default parameters of Clustal W version 2 (Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ and Higgins DG (2007), "Clustal W and Clustal X version 2.0", Bioinformatics. 23 (21): 2947-2948).

[0092] It is to be noted that, in the hL02 light chain full-length amino acid sequence shown in SEQ ID NO: 61, the amino acid sequence consisting of the amino acid residues at

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positions 1 to 20 is the signal sequence, the amino acid sequence consisting of the amino acid residues at positions 21 to 128 is the variable region, and the amino acid sequence consisting of the amino acid residues at positions 129 to 233 is the constant region. In the hL02 light chain full-length nucleotide sequence shown in SEQ ID NO: 62, the nucleotide sequence consisting of the nucleotides at positions 1 to 60 encodes the signal sequence, the nucleotide sequence consisting of the nucleotides at positions 61 to 384 encodes the variable region, and the nucleotide sequence consisting of the nucleotides at positions 385 to 699 encodes the constant region.

[0093] In the hL03 light chain full-length amino acid sequence shown in SEQ ID NO: 65, the amino acid sequence consisting of the amino acid residues at positions 1 to 20 is the signal sequence, the amino acid sequence consisting of the amino acid residues at positions 21 to 128 is the variable region, and the amino acid sequence consisting of the amino acid residues at positions 129 to 233 is the constant region. In the hL03 light chain full-length nucleotide sequence shown in SEQ ID NO: 66, the nucleotide sequence consisting of the nucleotides at positions 1 to 60 encodes the signal sequence, the nucleotide sequence consisting of the nucleotides at positions 61 to 384 encodes the variable region, and the nucleotide sequence consisting of the nucleotides at positions 385 to 699 encodes the constant region.

[0094] In the hH01 heavy chain full-length amino acid sequence shown in SEQ ID NO: 69, the amino acid sequence consisting of the amino acid residues at positions 1 to 19 is the signal

sequence, the amino acid sequence consisting of the amino acid residues at positions 20 to 141 is the variable region, and the amino acid sequence consisting of the amino acid residues at positions 142 to 471 is the constant region. In the hH01 heavy chain full-length nucleotide sequence shown in SEQ ID NO: 70, the nucleotide sequence consisting of the nucleotides at positions 1 to 57 encodes the signal sequence, the nucleotide sequence consisting of the nucleotides at positions 58 to 423 encodes the variable region, and the nucleotide sequence consisting of the nucleotides at positions 424 to 1413 encodes the constant region.

[0095] In the hH02 heavy chain full-length amino acid sequence shown in SEQ ID NO: 73, the amino acid sequence consisting of the amino acid residues at positions 1 to 19 is the signal sequence, the amino acid sequence consisting of the amino acid residues at positions 20 to 141 is the variable region, and the amino acid sequence consisting of the amino acid residues at positions 142 to 471 is the constant region. In the hH02 heavy chain full-length nucleotide sequence shown in SEQ ID NO: 74, the nucleotide sequence consisting of the nucleotides at positions 1 to 57 encodes the signal sequence, the nucleotide sequence consisting of the nucleotides at positions 58 to 423 encodes the variable region, and the nucleotide sequence consisting of the nucleotides at positions 424 to 1413 encodes the constant region.

[0096] In the hH04 heavy chain full-length amino acid sequence shown in SEQ ID NO: 77, the amino acid sequence consisting of the amino acid residues at positions 1 to 19 is the signal sequence, the amino acid sequence consisting of the amino acid

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residues at positions 20 to 141 is the variable region, and the amino acid sequence consisting of the amino acid residues at positions 142 to 471 is the constant region. In the hH04 heavy chain full-length nucleotide sequence shown in SEQ ID NO: 78, the nucleotide sequence consisting of the nucleotides at positions 1 to 57 encodes the signal sequence, the nucleotide sequence consisting of the nucleotides at positions 58 to 423 encodes the variable region, and the nucleotide sequence consisting of the nucleotides at positions 424 to 1413 encodes the constant region.

[0097]

[Table 1-1]

SEQ ID NO		Sequence
1	Amino acid sequence of human CDH6 ORF	MRTYRYFLLLFVVGQPYPTLSTPLSKRTSGFPAKKRALELSGNSKNELNRSKRSMW NQFFLLEEYTGSDYQYVGKLHSDQDRGDGSLKYILSGDGAGDLFIINENTGDIQATK RLDREKPYVYILRAQAINRRTGRPVEPESEFIKIHNDINNEPIFTKEVYTATVP SDVGTFFVQVTATDADDPYGNNAKVYVYILQGQPYFSVESETGIKTALLNMDREN REQYQVVIQAKDMGGQMGGLSGTTVNITLTDVNDNPPRFQSTYQFKTPESSPPGT PIGRIKASDADVGNAEIEYSITDGEGLDMFDVITDQETQEGIIITVKKLLDFEKKKV YTLKVEASNPYVEPRFLYLGPFKDSATVRIVVEDVDEPPVFSKLAYILQIREDAQIN TTIGSVTAQDPDAARNPVKYSVDRHTMDRIFNIDSGNGSIFTSKLLDRETLWHNI TVIATEINNPKQSSRVPLYIKVLDVNDNAPEFAEFYETFVCEKAKADQLIQTLHAVD KDDPYSGHQFSFLAPEAASGSNFTIQDNKDNNTAGILTRKNGYNRHEMSTYLLPVVI SDNDYPVQSSTGTVTVRVCACDHGNNMQSCHAEALIHPTGLSTGALVAIILLCIVILL VTVVLFALRRQRKKEPLISKEDIRDNIVSYNDEGGGEEDTQAFDIGTLRNP EAE DNKLRRDIVPEALFLPRRTPTARDNTDVRDFINQRLKENDTDPAPPYDSLATYAYE GTGSVADSLSSLESVTTDADQDYDYLSDWGPFRFKKLADMYGGVDSKDS
2	Human CDH6 EC1	SWMWVQFFLLEEYTGSDYQYVGKLHSDQDRGDGSLKYILSGDGAGDLFIINENTGDI QATKRLDREKPYVYILRAQAINRRTGRPVEPESEFIKIHNDINNEPIF
3	Human CDH6 EC2	TKEVYTATVPMSDVGTFFVQVTATDADDPYGNNAKVYVYILQGQPYFSVESETGI IKTALLNMDRENREQYQVVIQAKDMGGQMGGLSGTTVNITLTDVNDNPPRF
4	Human CDH6 EC3	QSTYQFKTPESSPPGTPIGRIKASDADVGNAEIEYSITDGEGLDMFDVITDQETQ EGIIITVKKLLDFEKKKVYTLKVEASNPYVEPRFLYLGPFKDSATVRIVVEDVDEPPV F
5	Human CDH6 EC4	SKLAYILQIREDAQINTTIGSVTAQDPDAARNPVKYSVDRHTMDRIFNIDSGNGSI FTSKLLDRETLWHNITVIATEINNPKQSSRVPLYIKVLDVNDNAP
6	Human CDH6 EC5	EFAEFYETFVCEKAKADQLIQTLHAVDKDDPYSGHQFSFLAPEAASGSNFTIQDNK DNNTAGILTRKNGYNRHEMSTYLLPVVISDNDYPVQSSTGTVTVRVCACDHGNNMQSC HAEALIH
7	Amino acid sequence of mouse CDH6 ORF	MRTYRYFLLLFVVGQPYPTFSNPLSKRTSGFPAKKRALELSANSRNELSRSKRSMW NQFFLLEEYTGSDYQYVGKLHSDQDRGDGSLKYILSGDGAGDLFIINENTGDIQATK RLDREKPYVYILRAQAVNRRTPVEPESEFIKIHNDINNEPIFTKDVYTATVP ADVGTFFVQVTATDADDPYGNNAKVYVYILQGQPYFSVESETGIKTALLNMDREN REQYQVVIQAKDMGGQMGGLSGTTVNITLTDVNDNPPRFQSTYQFKTPESSPPGT PIGRIKASDADVGNAEIEYSITDGEHEMFVITDQETQEGIIITVKKLLDFEKKKV YTLKVEASNPYVEPRFLYLGPFKDSATVRIVDDVDEPPVFSKLAYILQIREDAQIN TTIGSVAAQDPDAARNPVKYSVDRHTMDRIFNIDSGNGSIFTSKLLDRETLWHNI TVIATEINNPKQSSRVPLYIKVLDVNDNAPEFAEFYETFVCEKAKADQLIQTLRAVD KDDPYSGHQFSFLAPEAASSNFTIQDNKDNNTAGILTRKNGYNRHEMSTYLLPVVI SDNDYPVQSSTGTVTVRVCACDHGNNMQSCHAEALIHPTGLSTGALVAIILLCIVILL VTVVLFALRRQRKKEPLISKEDIRDNIVSYNDEGGGEEDTQAFDIGTLRNP EAE DSKSRRDIVPEALFLPRRTPTARDNTDVRDFINQRLKENDTDPAPPYDSLATYAYE GTGSVADSLSSLESVTTDGQDYDYLSDWGPFRFKKLADMYGGMDSKDS

[0098]

[Table 1-2]

8	Amino acid sequence of rat CDH6 ORF	MRTYRYFLLLFVVGQPYPTFSNPLSKRTSGFPAKKRALELSANSRNELSRSKRSMW NQFFLLEEYTGSDYQYVGKLHSDQDRGDGSLKYILSGDGAGDLFIINENTGDIQATK RLDREEKPYYILRAQAINRRRTGRPVEPESEFIKIHDIINDNEPIFTKDYYTATVPEM ADVGTFFVQVTATDADDPYGNNAKVVYSILQGQPYFSVESETGIKKTALLNMDREN REQYQVVIQAKDMGGMGGLSGTTVNITLTDVNDNPPRFPQSTYQFKTPESPPGT PIGRKASDADVGNAEIEYSITDGEHDMFDVITDQETQEGITVKKLLDFEKKRV YTLKVEASNPHIEPRFLYLGPFKDSATVRIVDDVDEPPVFSKPAYILQIREDAQIN TTIGSVAQDPDAARNPVKYSVDRHTMDRIFNIDSGNGSIFTSKLLDRETLLWHNI TVIATEINNPKQSSRVPLYIKVLDVNDNAPEFAEFYETFVCEKAKADQLIQLTHAVD KDDPYSGHQFSFSLAPEAASGSNFTIQDNKONTAGILTRKNGYNRHEMSTYLLPVVI SDNDYPVQSSTGTVTVRVCADHHGNMQSCHAEALIHPTGLSTGALVAILLCIVILL VTVVLFALRRQRKKEPLISKEDI RDNIVSYNDEGGGEEDTQAFDIGTLRNPKPWR QQSRRDMVPEALFLPRRTPTARDNTDVRDFISQRLRKMNTDPTAPPYDSLATYAYEG TGSVADSLSSLESVTTDGDQDYGYLSDWGPFRFKKLADMYGGMDSKDS
9	Amino acid sequence of cynomolgus monkey CDH6 ORF	MRTYRYFLLLFVVGQPYPTLSTPLSKRTSGFPAKKRALELSGNSKNELNRSKRSMW NQFFLLEEYTGSDYQYVGKLHSDQDRGDGSLKYILSGDGAGDLFIINENTGDIQATK RLDREEKPYYILRAQAINRRRTGRPVEPESEFIKIHDIINDNEPIFTKEVYTATVPEM SDVGTFFVQVTATDADDPYGNNAKVVYSILQGQPYFSVESETGIKKTALLNMDREN REQYQVVIQAKDMGGMGGLSGTTVNITLTDVNDNPPRFPQSTYQFKTPESPPGT PIGRKASDADVGNAEIEYSITDGEGLDMFDVITDQETQEGITVKKLLDFEKKKV YTLKVEASNPHVEPRFLYLGPFKDSATVRIVVEDVDEPPVFSKLAYILQIREDAQIN TTIGSVTAQDPDAARNPVKYSVDRHTMDRIFNIDSGNGSIFTSKLLDRETLLWHNI TVIATEINNPKQSSRVPLYIKVLDVNDNAPEFAEFYETFVCEKAKADQLIQLTHAVD KDDPYSGHQFSFSLAPEAASGSNFTIQDNKONTAGILTRKNGYNRHEMSTYLLPVVI SDNDYPVQSSTGTVTVRVCADHHGNMQSCHAEALIHPTGLSTGALVAILLCIVILL VTVVLFALRRQRKKEPLISKEDI RDNIVSYNDEGGGEEDTQAFDIGTLRNPKEIE DNKLRRDIVPEALFLPRRTPTARDNTDVRDFINQRLKENDTPTAPPYDSLATYAYE GTGSVADSLSSLESVTTDGDQDYGYLSDWGPFRFKKLADMYGGVDSKDS
10	rG019 light chain variable region amino acid sequence	DIQMTQSPSLLSASVGDRVTLNCKASQNIYKNLAWYQQKLGEQKLLIYDANTLTGT IPSRFSGSGSGSDFTLTISLQPEDVATYFCQQYYSGWAFGGVTNLELKRA
11	rG019 light chain variable region nucleotide sequence	GACATCCAGATGACCCAGTCTCCTTCACTCCTGTCTGCATCTGTGGGAGACAGAGTC ACTCTCAACTGCAAAGCAAGTCAGAATATTTATAAGAACTTAGCCTGGTATCAGCAA AAGCTTGGAGAAGGTCCAAACTCCTGATTTATGATGCAAACTTTGCAACGGGC ATCCCATCAAGGTTCAAGTGGCAGTGGATCTGGTTCAGATTTCACTCACCATCAGC AGCCTGCAGCTGAAGATGTTGCCACATATTCTGCCAGCAGTACTATAGCGGGTGG GCGTTCGGTGGAGTCACCAACCTGGAATTGAAACGGGCT
12	rG019 CDRL1	KASQNIYKNLA
13	rG019 CDRL2	DANTLQT
14	rG019 CDRL3	QQYYSGWA

[0099]

[Table 1-3]

15	rG019 heavy chain variable region amino acid sequence	QVQLQQSGAELVKPGSSVKISCKASGYTFRNFMHWIKQQPGNGLEWIGWIYCGDGE TEYNQKFNGKATLTADRSSSTAYMELSRLTSEDSAVYFCARGVYGGFAGGYDFWGG GVMVTVSS
16	rG019 heavy chain variable region nucleotide sequence	CAGGTACAGCTGCAGCAATCTGGGGCTGAACTGGTGAAGCCTGGGTCTCAGTGA ATTCCTGCAAGGCTTCTGGCTACACCTTCACCAGGAACCTTATGCACTGGATAAA CAGCAGCCTGGAAATGGCCTTGAGTGGATTGGGTGGATTTATTGTGGAGATGGTGAG ACAGAGTACAATCAAAAGTTCAATGGGAAGGCAACACTCACTGCGGACAGATCCTCC AGCACAGCCTATATGGAGCTCAGCAGACTGACATCTGAGGACTCTGCAGTCTATTTG TGTGCAAGAGGGGTTTACGGAGGGTTTGCCGGGGGCTACTTTGATTCTGGGGCCAA GGAGTCATGGTCACAGTCTCCTCA
17	rG019 CDRH1	GYTFTRNFMH
18	rG019 CDRH2	WIYCGDGETE
19	rG019 CDRH3	GVYGGFAGGYDF
20	rG055 light chain variable region amino acid sequence	DVQMTSPSYLAASPGESVSI SCKTSKNISNYLVWYQKPGEAYKLLIYSGSTLQSG TPSRFSGSGSDFTLTIRSLPEPDFGLYFCQYYEKPFTFGSGTKLEIKRA
21	rG055 light chain variable region nucleotide sequence	GATGTCCAGATGACCCACTCTCCGTCTTATCTTGCTGCGTCTCCTGGAGAAAGTGT TCCATCAGTTGCAAGACAAGTAAGAACATTAGTAATTATTAGTCTGGTATCAACAG AAACCTGGGGAAGCATATAAGCTTCTTATCTATTCTGGGTCACTTTGCAATCTGGA ACTCCATCAAGGTTCAAGTGGCAGTGGATCTGGTACAGATTTCCTCTCACTATCAGA AGCCTGGAGCCTGAAGATTTTGGACTCTATTCTGTCAACAGTATTATGAAAAACCA TTCACGTTCCGGTCAAGGACGAAGTTGGAATAAAACGGGCT
22	rG055 CDRL1	KTSKNISNYLV
23	rG055 CDRL2	SGSTLQS
24	rG055 CDRL3	QYYEKPFT
25	rG055 heavy chain variable region amino acid sequence	EVQLQESGPGLVRPQSLSLSCSVTDYSITSNYWGWIIRFPGNKMEWMGYITYSGYT SYNPSLQSRISITRDTSKNQFFLQLNSVTAEDTATYYCARSINHGGSYVVDWGP ASVTVSS
26	rG055 heavy chain variable region nucleotide sequence	GAGGTGCAACTTCAGGAGTCAGGACCTGGCCTTGAGAGCCCTCACAGTCACTCTCC CTCTCCTGTTCTGCTCACTGATTACTCCATCACTAGTAATTACTGGGGCTGGATCCGG AGGTTCCAGGAAATAAAATGGAGTGGATGGGATACATAACCTATAGTGGTTACACT AGCTACAACCCATCTCTCCAAAGTCGAATCTCCATTACTAGAGACACATCGAAGAAT CAGTTCTTCCTGCAGTTGAACTCTGTAAGTCTGAGGACACAGCCACATATTACTGT GCAAGATCGATTAAACACGGAGGATATAGTTATGTTGTGGATGCCTGGGGTCCGGGA GCTTCAGTCACTGTCTCCTCA
27	rG055 CDRH1	DYSITSNYWG
28	rG055 CDRH2	YITYSGYTS
29	rG055 CDRH3	SINHGGSYVVD

[0100]

[Table 1-4]

30	rG056 light chain variable region amino acid sequence	DVQMTQSPSSLAASPGESVSI SCRATKSI GTIYLA WYQQKPGKTFKLLI YSGSTLQSGTPSRFSGSGSDFTLTIRSLPEDFGLYFCQQFYENPFTFGSGTKLEIRRA
31	rG056 light chain variable region nucleotide sequence	GATGTCCAGATGACCCAGTCTCCGTCTTCTCTTGCTGCGTCTCCTGGAGAAAGTGTTTCCATCAGTTGCAGGGCAACTAAGAGCATTGGTATTTATTAGCCTGGTATCAACAGAAACCTGGGAAACATTTAAGCTTCTTATCTACTCTGGGTCAACTTTGCAATCTGGAACCTCATCAAGGTTCAAGTGGCAGTGGGTCTGGTACAGATTTCACTCTCACCATCAGAAGCCTGGAGCCTGAAGATTTTGGACTCTATTCTGTCAACAGTTTATGAAAACCCA TTCACGTTCCGGCTCAGGGACGAAGTTGAAAATAAGACGGGCT
32	rG056 CDR1	RATKSI GTIYLA
33	rG056 CDR2	SGSTLQSG
34	rG056 CDR3	QQFYENPFT
35	rG056 heavy chain variable region amino acid sequence	EVQLQESGPGLVKPSQSLSLTCSVTDY SITT YFWG WIRKFPGNKMEWMGYMSYRGGTSYNPSLKSRI S ITRDTSKNQFFLQLNSVTTEDTATYYCARCPNYGGHSLVFDYWGQGVMVTVSS
36	rG056 heavy chain variable region nucleotide sequence	GAGGTGCAGCTTCAGGAGTCAGGACCTGGCCTTGTAACCCCTCACAGTCAGTCTCCCTCACCTGTTCTGTCACTGATTACTCCATCACTACTTATTTCTGGGGCTGGATCCGGAAGTTCCAGGAAATAAAATGGAGTGGATGGGATACATGAGCTACCGTGGTGGCACTTCCTACAACCCATCTCTCAAGAGTCGAATCTCCATTACTAGAGACACATCGAAGAATCAGTTCTTCTGCAGTTGAAGTCTGTAAGTACTGAGGACACAGCCACATATTACTGTGCAAGATGCCCTAACTACGGAGGGCATTCCCTTGTTTTTGATTACTGGGGCCAAGGAGTCATGGTCACAGTGCCTCA
37	rG056 CDRH1	DYSITTYFWG
38	rG056 CDRH2	YMSYRGGTS
39	rG056 CDRH3	CPNYGGHSLVFDY
40	rG061 light chain variable region amino acid sequence	DVQMTQSPSYLAASPGESVSI SCKATKSI SNYLA WYQQKPGEAYKVL IYSGSTLQSGTPSRFSGSGSDFTLTIRSLPEDFGLYSCQYYEKPLTFGSGTKLEIKRA
41	rG061 light chain variable region nucleotide sequence	GATGTCCAGATGACCCAGTCTCCGTCTTATCTTGCTGCGTCTCCTGGAGAAAGTGTTTCCATCAGTTGCAAGGCAACTAAGAGCATTAGTAATTATTAGCCTGGTATCAACAGAAACCTGGGGAAGCATATAAGGTTCTTATCTATTCTGGGTCAACTTTGCAATCTGGAACCTCATCAAGGTTCAAGTGGCAGTGGATCTGGTACAGATTTCACTCTCACCATCAGAAGCCTGGAGCCTGAAGATTTTGGACTCTATTCTGTCAACAGTATTATGAAAACCGCTCACGTTCCGTTCTGGGACCAAGCTGGAGATCAAACGGGCT
42	rG061 CDR1	KATKSI SNYLA
43	rG061 CDR2	SGSTLQSG
44	rG061 CDR3	QQYYEKPLT

[0101]

[Table 1-5]

45	rG061 heavy chain variable region amino acid sequence	EVQLQESGPGLVKPSQSLSLTCSVTGYSITTTYWGWIRKFPGNKMEWMGYISYSGRTSYNPSLKSRRMSITRDASKNQFFLQLNSVTDDTATYYCARSPINHGGYWYDFWGPGLTMVTVSS
46	rG061 heavy chain variable region nucleotide sequence	GAGGTGCAGCTTCAGGAGTCAGGACCTGGCCTTGTAACCCCTCACAGTCACTCTCCCTCACCTGTTCTGTCACTGGTTACTCCATCACTACTTATTACTGGGGCTGGATCCGGAAAGTCCCAGGAAATAAAATGGAGTGGATGGGGTACATAAGCTACAGTGGTCGCACTAGTTATAACCCATCTCTCAAAGTGAATGTCCATTACTAGAGACGCATCGAAGAATCAGTTCTTCTACAGTTGAACCTCTGTAAGTACTGACGACACAGCCACATATTACTGTGCAAGATCCCCAATTAACACGGAGGGTACTGGTACTTTGACTTCTGGGGCCAGGAACCATGGTCACCGTGTCTCA
47	rG061 CDRH1	GYSITTYWG
48	rG061 CDRH2	YISYSGRTS
49	rG061 CDRH3	SPINHGGYWYDF
50	DNA fragment comprising DNA sequence encoding human light chain signal sequence and human κ chain constant region	gcctccggactctagagccaccATGGTGCTGCAGACCCAGGTGTTTCATCTCCCTGCTGCTGTGGATCTCCGGCGGTACGGCGATATCGTGATGATTAAACGTACGGTGGCCGC CCCCTCCGTGTTTCATCTTCCCCCCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTC CGTGGTGTGCTGCTGAATAACTTCTACCCAGAGAGGCCAAGGTGCAGTGAAGGTGGACAACGCCCTGCAGTCCGGGAACCTCCAGGAGAGCGTGACCGAGCAGGACAGCAA GGACAGCACCTACAGCCTGAGCAGCACCTGACCCTGAGCAAAGCCGACTACGAGAA GCACAAGGTGTACGCCCTGCGAGGTGACCCACAGGGCCTGAGCTCCCCCGTCACGAA GAGCTTCAACAGGGGGGAGTGTTtagggggccgtttaaacggggaggcta

[0102]

[Table 1-6]

51	DNA fragment comprising DNA sequence encoding human heavy chain signal sequence and human IgG1 constant region	gcctccggactctagagccaccATGAAACACCTGTGGTTCTTCCTCTGCTGGTGGG AGCTCCCAGATGGGTGCTGAGCCAGGTGCAATTGTGCAGGCGGTTAGCTCAGCCTCC ACCAAGGGCCCAAGCGTCTTCCCCCTGGCACCTCCTCCAAGAGCACCTCTGGCGGC ACAGCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGTGACCGTGAGC TGGAACTCAGGCGCCCTGACCAGCGGCTGCACACCTTCCCCGCTGTCTGCAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACC CAGACCTACATCTGCAACGTGAATCACAAGCCAGCAACACCAAGGTGGACAAGAGA GTTGAGCCCAATCTTGTGACAAAACCTCACACATGCCACCCCTGCCAGCACCTGAA CTCTGGGGGGACCCCTCAGTCTTCTCTTCCCCCAAACCAAGGACACCCCTCATG ATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCT GAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAG CCCCAGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAAGCGTCTCACCCTCCTG CACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGAAGGTCTCCAACAAAGCCCTC CCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGCCAGCCCCGGGAACCCAG GTGTACACCTGCCCCATCCCGGAGGAGATGACCAAGAACCAGGTGAGCCTGACC TGGCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGC CAGCCCGAGAACTACAAGACCACCTCCCGTGGTGGTGGTGGTGGTGGTGGTGGTGGT TTCTCTACAGCAAGCTCACCCTGGACAAGAGCAGGTGGCAGCAGGGCAACGCTTCT TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACCCAGAAGAGCCTCTCC CTGTCTCCCGGCAAAAgagatatcgggccggtttaaacggggaggcta
52	DNA fragment comprising DNA sequence encoding chG019 light chain	ccagcctccggactctagagccaccATGGTGTGCAGACCCAGGTGTTTCATCAGCCT GCTGCTGTGGATCAGCGGCGCCTACGGCGACATCCAGATGACCCAGAGCCCTAGCCT GCTGAGCGCCAGCGTGGGCGATAGAGTGACCCCTGAAGTGAAGGCCAGCCAGAACAT CTACAAGAACCTGGCTGGTATCAGCAGAAGCTGGGCGAGGGCCCAAGCTGCTGAT CTAGGACGCCAACACCTGCAGACCGGATCCCGAGCAGATTTCTGGCAGCGGCAG CGGCTCCGACTTCACCTGACAATCAGCAGCCTGCAGCCGAGGACGTGGCCACCTA CTTTTCGAGCAGTACTACAGCGGCTGGGCTTCGGCGGCGTGACCAACCTGGAAGT GAAGAGAGCCGTGGCGCTCCCTCCGTGTTTCATCTTCCACCTAGCGACGAGCAGCT GAAGTCCGGCACAGCCTCTGTGTGTGCTGTGAACAACCTTACCCCGCGGAGGC CAAGGTGCAGTGAAGGTGGACAATGCCCTGCAGTCTGGCAACAGCCAGGAAGCGT GACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCTGACCCTGAG CAAGGCCGACTACGAGAAGCACAAGGTGTACGCTGCGAAGTGACCCACAGGGCCT GTCTAGCCCGTGACCAAGAGCTTCAACCGGGGCGAGTGTtaggtttaaacggggga ggctaact
53	chG019 light chain full-length amino acid sequence	MVLQTQVFISLLWISGAYGDIQMTQSPSLLSASVGDRTLNCKASQNIYKNLAWYQ QKLGEQPKLLIYDANTLQTGIPSRFSGSGSDFTLTISLQPEDVATYFCQQYYSG WAFGGVTNLELKRAVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWVDN ALQSGNSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC

[0103]

[Table 1-7]

54	chG019 light chain full-length nucleotide sequence	ATGGTGCTGCAGACCCAGGTGTTTCATCAGCCTGCTGCTGTGGATCAGCGGGCGCTAC GGCGACATCCAGATGACCCAGAGCCCTAGCCTGCTGAGCGCCAGCGTGGGCGATAGA GTGACCCTGAACTGCAAGGCCAGCCAGAACATCTACAAGAACCTGGCCTGGTATCAG CAGAAGCTGGGCGAGGGCCCCAAGCTGCTGATCTACGACGCCAACACCCTGCAGACC GGCATCCCCAGCAGATTTTCTGGCAGCGGCAGCGGCTCCGACTTCACCCTGACAATC AGCAGCCTGCAGCCCGAGGACGTGGCCACCTACTTTGCCAGCAGTACTACAGCGGC TGGGCTTCGGCGGCGTGACCAACCTGGAAGTGAAGAGAGCCGTGGCGGCTCCCTCC GTGTTTCATCTTCCACCTAGCGACGAGCAGCTGAAGTCCGGCACAGCCTCTGTCGTG TGCCTGCTGAACAATTCTACCCCGCGAGGCCAAGGTGCAGTGAAGGTGGACAAT GCCCTGCAGTCTGGCAACAGCCAGGAAGCGTGACCGAGCAGGACAGCAAGGACTCC ACCTACAGCCTGAGCAGCACCTGACCTGAGCAAGGCCGACTACGAGAAGCACAAG GTGTACGCTGCGAAGTGACCCACCAGGGCTGTCTAGCCCGTGACCAAGAGCTTC AACCGGGGCGAGTGT
10	chG019 light chain variable region amino acid sequence	DIQMTQSPSLLSASVGDRTVLNCKASQNIYKNLAWYQQKLGEGPKLLIYDANTLQTG IPSRFSGSGSGDFTLTISSLQPEDVATYFCQQYYSGWAFGGVTNLELKRA
55	chG019 light chain variable region nucleotide sequence	GACATCCAGATGACCCAGAGCCCTAGCCTGCTGAGCGCCAGCGTGGGCGATAGAGTG ACCCTGAACTGCAAGGCCAGCCAGAACATCTACAAGAACCTGGCCTGGTATCAGCAG AAGCTGGGCGAGGGCCCCAAGCTGCTGATCTACGACGCCAACACCCTGCAGACCGGC ATCCCAGCAGATTTTCTGGCAGCGGCAGCGGCTCCGACTTCACCCTGACAATCAGC AGCCTGCAGCCCGAGGACGTGGCCACCTACTTTGCCAGCAGTACTACAGCGGCTGG GCCTTCGGCGGCGTGACCAACCTGGAAGTGAAGAGAGCC
12	chG019 CDRL1	KASQNIYKNLA
13	chG019 CDRL2	DANTLQT
14	chG019 CDRL3	QQYYSGWA
56	chG019 heavy chain full-length amino acid sequence	NKHLWFFLLLVAAAPRWVLSQVQLQQSGAELVKPGSSVKISCKASGYTFTRNFMHWIK QQPGNGLEWIGWIYPGDGETEYNQKFNKGATLTADRSSSTAYMELSRLTSEDSAVYF CARGVYGGFAGGYDFWGGVMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHLEA HNHYTQKSLSLSPGK

[0104]

[Table 1-8]

57	chG019 heavy chain full-length nucleotide sequence	ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGC CAGGTGCAGCTGCAGCAGTCTGGCGCCGAGCTCGTGAAGCCTGGCAGCAGCGTGAAG ATCAGCTGCAAGGCCAGCGGCTACACCTTCACCCGGAACCTCATGCACTGGATCAAG CAGCAGCCCGGCAACGGCTGGAATGGATCGGCTGGATCTATCCCGGCGACGGCGAG ACAGAGTACAACCAGAAGTTCAACGGCAAGGCCACCCTGACCGCCGACAGAAGCAGC TCCACCGCTACATGGAAGTGAAGCGGCTGACCAGCGAGGACAGCGCCGTGACTTT TGGCCAGAGGCGGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTGGGGCCAG GGGCTGATGGTCACCGTCAGCTCAGCCTCCACCAAGGGCCCAAGCGTCTTCCCCGTG GCACCTCCTCCAAGAGCAGCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCGAACCCTGACCGTGAAGTGAAGTCAAGCGCCCTGACCAGCGGC GTGCACAGCTTCCCCGCTGTCTGCAGTCTCAGGACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCAC AAGCCAGCAACACCAAGGTGGACAAGAGATTGAGCCCAAATCTTGTGACAAAAGT CACACATGCCACCTGCCCAGCACCTGAAGTCTGGGGGACCCCTCAGTCTTCTC TTCCCCCAAAACCCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTACATGC GTGGTGGTGGAGTGAAGCCAGAACCCCTGAGGTCAAGTTCAACTGGTACGTGGAC GGGCTGGAGGTGCATAATGCCAAGACAAGCCCCGGGAGGAGCAGTACAACAGCAG TACCGGGTGGTCAGCGTCTCACCCTCTGCACAGGACTGGCTGAATGGCAAGGAG TACAAGTGCAAGGTCTCCAACAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCC AAAGCCAAAGGCCAGCCCCGGGAACCAAGGTGTACACCTGCCCCATCCCGGGAG GAGATGACCAAGAACCAGGTGAGCTGACCTGCCTGGTCAAAGCTTCTATCCAGC GACATCGCGTGGAGTGGGAGAGCAATGGCAGCCGAGAACAACTACAAGACCACC CCTCCCGTGGTGGACTCCGACGGCTCTTCTTCTCTACAGCAAGCTCACCCTGGAC AAGAGCAGGTGGCAGCAGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACCAGAAGAGCTCTCCCTGTCTCCCGGCAAA
58	chG019 heavy chain variable region amino acid sequence	QVQLQQSGAELVKPGSSVKISCKASGYTFRNFMHWIKQDPGNGLIEWIGWIYPGDGE TEYNQKFNGKATLTADRSSSTAYMELSRLTSEDSAVYFCARGVYGGFAGGYDFWQG GVMVTVSS
59	chG019 heavy chain variable region nucleotide sequence	CAGGTGCAGCTGCAGCAGTCTGGCGCCGAGCTCGTGAAGCCTGGCAGCAGCGTGAAG ATCAGCTGCAAGGCCAGCGGCTACACCTTCACCCGGAACCTCATGCACTGGATCAAG CAGCAGCCCGGCAACGGCTGGAATGGATCGGCTGGATCTATCCCGGCGACGGCGAG ACAGAGTACAACCAGAAGTTCAACGGCAAGGCCACCCTGACCGCCGACAGAAGCAGC TCCACCGCTACATGGAAGTGAAGCGGCTGACCAGCGAGGACAGCGCCGTGACTTT TGGCCAGAGGCGGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTGGGGCCAG GGGCTGATGGTCACCGTCAGCTCA
17	chG019 CDRH1	GYTFTRNFMH
60	chG019 CDRH2	WIYPGDGETE
19	chG019 CDRH3	GVYGGFAGGYDF

[0105]

[Table 1-9]

61	hL02 light chain full-length amino acid sequence	MVLQTQVFISLLWISGAYGDIQMTQSPSSLSASVGDRVTITCKASQNIYKNLAWYQ QKPGKAPKLLIYDANTLQTGVPSRFSGSGSDFTLTISLQPEDFATYFCQQYYSG WAFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC
62	hL02 light chain full-length nucleotide sequence	ATGGTGCTGCAGACCCAGGTGTTTCATCTCCCTGCTGCTGGATCTCCGGCGCGTAC GGCGACATCCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGCGACAGA GTGACCATCACATGCAAGGCCAGCCAGAACATCTACAAGAACCTGGCCTGGTATCAG CAGAAGCCCGGCAAGGCCCAAGCTGCTGATCTACGACGCCAACCCCTGCAGACC GGCGTGCCCGAGCAGATTTTCTGGCAGCGGCAGCGGCTCCGACTTCACCCTGACAATC AGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTTTTGCAGCAGTACTACAGCGGC TGGGCCTTCGGCCAGGGCACCAAGGTGGAATCAAGCGTACGGTGGCCGCCCCCTCC GTGTTTCATCTTCCCCCCTCCGACGAGCAGCTGAAGTCCGGCACCCTCCGTGGTG TGCTGCTGAATAACTTCTACCCAGAGAGGCCAAGGTGCAAGTGAAGGTGGACAAC GCCCTGCAGTCCGGGAACCTCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACAGC ACCTACAGCCTGAGCAGCACCTGACCCCTGAGCAAAAGCCGACTACGAGAAGCACAAAG GTGTACGCCTGCGAGGTGACCCACCAGGGCCTGAGCTCCCCCGTCACCAAGAGCTTC AACAGGGGGGAGTGT
63	hL02 light chain variable region amino acid sequence	DIQMTQSPSSLSASVGDRVTITCKASQNIYKNLAWYQKPGKAPKLLIYDANTLQTG VPSRFSGSGSDFTLTISLQPEDFATYFCQQYYSGWAFGGGKVEIKRT
64	hL02 light chain variable region nucleotide sequence	GACATCCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGCGACAGAGTG ACCATCACATGCAAGGCCAGCCAGAACATCTACAAGAACCTGGCCTGGTATCAGCAG AAGCCCGGCAAGGCCCAAGCTGCTGATCTACGACGCCAACCCCTGCAGACCGGC GTGCCAGCAGATTTTCTGGCAGCGGCAGCGGCTCCGACTTCACCCCTGACAATCAGC AGCCTGCAGCCGAGGACTTCGCCACCTACTTTTGCAGCAGTACTACAGCGGCTGG GCCTTCGGCCAGGGCACCAAGGTGGAATCAAGCGTACG
65	hL03 light chain full-length amino acid sequence	MVLQTQVFISLLWISGAYGDIQMTQSPSSLSASVGDRVTITCKASQNIYKNLAWYQ QKLGEKPKLLIYDANTLQTGVPSRFSGSGSDFTLTISLQPEDFATYFCQQYYSG WAFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC

[0106]

[Table 1-10]

66	hL03 light chain full-length nucleotide sequence	ATGGTGCTGCAGACCCAGGTGTTTCATCTCCCTGCTGCTGTGGATCTCCGGCGGTAC GGCGACATCCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGCGACAGA GTGACCATCACATGCAAGGCCAGCCAGAACATCTACAAGAACCTGGCCTGGTATCAG CAGAAGCTGGGCGAGGGCCCCAAGCTGCTGATCTACGACGCCAACCCCTGCAGACC GGCGTGCCAGCAGATTTTCTGGCAGCGGCTCCGGCACCGACTTCACCTGACAATC AGCAGCCTGCAGCCGAGGACTTCGCCACCTACTACTGCCAGCAGTACTACAGCGGC TGGGCCTTTGGCCAGGGCACCAAGGTGGAATCAAGCGTACGGTGGCCGCCCCCTCC GTGTTTCATCTTCCCCCCTCCGACGAGCAGCTGAAGTCCGGCACCGCTCCGTGGTG TGCCTGCTGAATAACTTCTACCCAGAGAGGCCAAGGTGCAAGTGAAGGTGGACAAC GCCCTGCAGTCCGGGAACCTCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACAGC ACCTACAGCCTGAGCAGCACCTGACCTGAGCAAGCCGACTACGAGAAGCACAAG GTGTACGCTGCGAGGTGACCCACCAGGGCCTGAGCTCCCCGTCACCAAGAGCTTC AACAGGGGGGAGTGT
67	hL03 light chain variable region amino acid sequence	DIQMTQSPSSLSASVGDRVTITCKASQNIYKNLAWYQQKLGEGPKLLIYDANTLQTG VPSRFSGSGSGTDFLTITSSLPEDFATYYCQQYYSGWAFGQGKVEIKRT
68	hL03 light chain variable region nucleotide sequence	GACATCCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGGCGACAGAGTG ACCATCACATGCAAGGCCAGCCAGAACATCTACAAGAACCTGGCCTGGTATCAGCAG AAGCTGGGCGAGGGCCCCAAGCTGCTGATCTACGACGCCAACCCCTGCAGACCGGC GTGCCAGCAGATTTTCTGGCAGCGGCTCCGGCACCGACTTCACCTGACAATCAGC AGCCTGCAGCCGAGGACTTCGCCACCTACTACTGCCAGCAGTACTACAGCGGCTGG GCCTTTGGCCAGGGCACCAAGGTGGAATCAAGCGTACG
69	hH01 heavy chain full-length amino acid sequence	MKHLWFFLLLVAAPRWVLSEVQLVQSGAEVKKPGASVKVSCKASGYTFTRNFMHWVR QAPGGGLEWMGWIPGDGETEYAKFGGRVTITADTSTSTAYMELSSLRSEDTAVYY CARGVYGGFAGGYDFWGGGTLVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTIISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DI AVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEAL HNHYTQKSLSLSPGK

[0107]

[Table 1-11]

70	hH01 heavy chain full-length nucleotide sequence	ATGAAACACCTGTGGTTCTTCCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGC GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCAGGCGCCAGCGTGAAG GTGTCTTGAAGGCCAGCGGCTACACCTTTACCCGGAACCTTCATGCACTGGGTGCGC CAGGCTCCAGGCCAGGGACTGGAATGGATGGGCTGGATCTATCCCGGCGACGGCGAG ACAGAGTACGCCCAGAAATTCAGGGCAGAGTGACCATCACCGCCGACACCGAGCACC TCCACCGCCTACATGGAACAGCAGCCTGCGGAGCGAGGACACCGCCGTGTACTAT TGTGCCAGAGGCGTGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTGGGGCCAG GGCACCCCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCAAGCGTCTTCCCCCTG GCACCTCCTCCAAGAGCACCTCTGGCGGCACAGCGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCGAACCCTGACCGTGAGCTGGAACAGGCGCCCTGACCGAGCGGC GTGCACACCTTCCCCGCTGTCTGCAGTCTCAGGACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCAC AAGCCACAGCAACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAAC CACACATGCCACCCCTGCCAGCACCTGAACCTCTGGGGGACCCTCAGTCTTCTC TTCCCCCAAAACCCAAAGGACACCTCATGATCTCCCGGACCCCTGAGGTACATGC GTGGTGGTGGAGCTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGAC GGCGTGGAGGTGCATAATGCCAAGACAAGCCCGGGAGGAGCAGTACAACAGCAGC TACCGGGTGGTCAGCGTCTCACCCTCTGCACAGGACTGGCTGAATGGCAAGGAG TACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCC AAAGCCAAAGGCCAGCCCCGGGAACACAGGTGTACACCCTGCCCCCATCCCGGAG GAGATGACCAAGAACCAGGTGAGCTGACCTGCCTGGTCAAAGGCTTCTATCCAGC GACATCGCCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACATAAGACCACC CCTCCCGTGTGGAGTCCGACGGCTCCTTCTTCTCTACAGCAAGCTACCGTGGAC AAGAGCAGGTGGCAGCAGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACTACACCCAGAAGAGCTCTCCCTGTCTCCGGGCAAA
71	hH01 heavy chain variable region amino acid sequence	EVQLVQSGAEVKKPGASVKVSKASGYTFTRNFMHWVRQAPGGGLEWMGWIYPGDGE TEYAKFKGGRVTITADTSTSTAYMELSSLRSEDTAVYYCARGVYGGFAGGYDFWQG GTLVTVSS
72	hH01 heavy chain variable region nucleotide sequence	GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCAGGCGCCAGCGTGAAG GTGTCTTGAAGGCCAGCGGCTACACCTTTACCCGGAACCTTCATGCACTGGGTGCGC CAGGCTCCAGGCCAGGGACTGGAATGGATGGGCTGGATCTATCCCGGCGACGGCGAG ACAGAGTACGCCCAGAAATTCAGGGCAGAGTGACCATCACCGCCGACACCGAGCACC TCCACCGCCTACATGGAACAGCAGCCTGCGGAGCGAGGACACCGCCGTGTACTAT TGTGCCAGAGGCGTGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTGGGGCCAG GGCACCCCTCGTGACCGTCAGCTCA

[0108]

[Table 1-12]

73	hH02 heavy chain full-length amino acid sequence	MKHLWFFLLVAAPRWLSEVQLVQSGAEVKKPGASVKVSKASGYTFRNFMHWVR QAPGGGLEWMGWLYPGDGETEYNQKFQGRVTITADRSTSTAYMELSSLRSEDVAVYF CARGVYGGFAGGYDFWGGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSKDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSYMHEAL HNHYTQKSLSLSPGK
74	hH02 heavy chain full-length nucleotide sequence	ATGAAACACCTGTGGTTCTTCTCCTGCTGGTGGCAGCTCCCAGATGGGTGCTGAGC GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCAGCGCCAGCGTGAAG GTGTCTGCAAGGCCAGCGGCTACACCTTTACCCGGAAGTTCATGCACTGGGTGGCG CAGGCTCCAGGCCAGGACTGGAATGGATGGGCTGGATCTATCCCGGCGACGGCGAG ACAGAGTACAACAGAAATTCCAGGGCAGAGTGACCATCACCGCCGACAGAAAGCACC AGCACCGCTACATGGAAGTGAAGCAGCTGCGGAGCGAGGATACCGCGTGTACTTC TGTGCCAGAGGCGGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTGGGGCCAG GGCACCCCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCAAGCGTCTCCCCCTG GCACCCCTCCTCCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCGAACCCTGACCGTGAGCTGGAAGTCAAGCGCCCTGACCAAGCGG GTGCACACCTTCCCGCTGTCTGCAAGTCTCAGGACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCAC AAGCCACGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAAC CACACATGCCACCCCTGCCAGCACCTGAATCCTGGGGGACCCCTCAGTCTTCTC TTCCCCCAAAACCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTACATGC GTGGTGGTGGAGCTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGAC GGCGTGGAGGTGCATAATGCCAAGACAAGCCCGGGAGGAGCAGTACAACAGCAG TACCGGGTGGTCAGCGTCTCACCCTCTGCACAGGACTGGCTGAATGGCAAGGAG TACAAGTGCAAGGTCTCCAACAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCC AAAGCCAAAGGCCAGCCCCGGGAACCAAGGTGTACACCTGCCCCATCCCGGGAG GAGATGACCAAGAACCAGGTGAGCTGACCTGCCTGGTCAAAGGCTTCTATCCAGC GACATCGCGTGGAGTGGGAGAGCAATGGCCAGCCCGAGAACAACTACAAGACCACC CCTCCCGTGTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCCTGGAC AAGAGCAGGTGGCAGCAGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAAACCTACACCAAGAAGAGCTCTCCCTGTCTCCCGGCAAA
75	hH02 heavy chain variable region amino acid sequence	EVQLVQSGAEVKKPGASVKVSKASGYTFRNFMHWVRQAPGGGLEWMGWLYPGDGE TEYNQKFQGRVTITADRSTSTAYMELSSLRSEDVAVYFCARGVYGGFAGGYDFWGG GTLVTVSS

[0109]

[Table 1-13]

76	hH02 heavy chain variable region nucleotide sequence	GAAGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCAGGCGCCAGCGTGAAG GTGTCTGCAAGGCCAGCGGCTACACCTTTACCCGGAACCTCATGCACTGGGTGCCG CAGGCTCCAGGCCAGGGACTGGAATGGATGGGCTGGATCTATCCCGGCGACGGCGAG ACAGAGTACAACCAGAAATTCCAGGGCAGAGTGACCATCACCGCCGACAGAAGCACC AGCACCGCTACATGGAACGTAGCAGCCTGCGGAGCGAGGATACCGCGGTGACTTC TGTGCCAGAGGCGTGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTGGGGCCAG GGCACCTCGTGACCGTCAGCTCA
77	hH04 heavy chain full-length amino acid sequence	MKHLWFFLLLVAAPRWLSQVQLVQSGAEVKKPGASVKVSCKASGYTFTRNFMHWIR QAPGGGLEWMGWLYPGDGETEYAKFKGRVLTADRSTSTAYMELSSLRSEDATVYY CARGVYGGFAGGYDFWGGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVKDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTIISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEAL HNHYTQKSLSLSPGK
78	hH04 heavy chain full-length nucleotide sequence	ATGAAACACCTGTGGTTCCTCCTCCTGCTGGTGGCAGCTCCAGATGGGTGCTGAGC CAGGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCAGGCGCCAGCGTGAAG GTGTCTGCAAGGCCAGCGGCTACACCTTTACCCGGAACCTCATGCACTGGATCCGG CAGGCCCCCTGGACAGGGCCTGGAATGGATGGGCTGGATCTATCCCGGCGACGGCGAG ACAGAGTACGCCAGAAATTCCAGGGCAGAGTGACCATCACCGCCGACAGAAGCACC AGCACCGCTACATGGAACGTAGCAGCCTGCGGAGCGAGGACACCGCGGTGACTAT TGTGCCAGAGGCGTGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTGGGGCCAG GGCACCTCGTGACCGTCAGCTCAGCCTCCACCAAGGGCCCAAGCGTCTTCCCGCTG GCACCTCTCTCAAGAGCACCTCTGGCGGCACAGCCGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCGGAACCCGTGACCGTGAGCTGGAACCTCAGGCGCCCTGACCAGCGGC GTGCACACCTTCCCCGCTGTCTCAGTCTCAGGACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCAC AAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAATCTTGTGACAAAAC CACACATGCCACCTGCCAGCACCTGAACCTCTGGGGGAGCCCTCAGTCTTCTCTC TTCCCCCAAAACCCAGGACACCTCATGATCTCCCGGACCCCTGAGGTACATGC GTGGTGGTGGAGCTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGAC GGCGTGGAGGTGCATAATGCCAAGACAAAGCCCCGGGAGGAGCAGTACAACAGCAG TACCGGGTGGTCAGCGTCTCACCCTGCTGCACAGGACTGGCTGAATGGCAAGGAG TACAAGTGAAGGTCTCCAACAAAGCCCTCCAGCCCCATCGAGAAAACCATCTCC AAAGCCAAAGGCCAGCCCCGGGAACACAGGTGTACACCTGCCCCCATCCGGGGAG GAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAGC GACATCGCCGTGGAGTGGGAGAGCAATGCCAGCCGAGAACAACTACAAGACCACC CCTCCCGTGTGAGTCCGACGGCTCTTCTCTCTACAGCAAGCTACCGTGGAC AAGAGCAGGTGGCAGCAGGGCAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACCAGAAGAGCTCTCCCTGTCTCCCGGCAA

[0110]

[Table 1-14]

79	hH04 heavy chain variable region amino acid sequence	QVQLVQSGAEVKKPGASVKVSKASGYTFRNFMHWIRQAPGGGLEWMGWYIPGDGE TEYAQKFQGRVTL TADRSTSTAYMELSSLRSED TAVYYCARGVYGGFAGGYDFWQG GTLVTVSS
80	hH04 heavy chain variable region nucleotide sequence	CAGGTGCAGCTGGTGCAGTCTGGCGCCGAAGTGAAGAAACCAGGCGCCAGCGTGAAG GTGTCTGCAAGGCCAGCGGCTACACCTTTACCCGGAAC TTCATGCACTGGATCCGG CAGGCCCTGGACAGGCCCTGGAATGGATGGGCTGGATCTATCCCGGCGACGGCGAG ACAGAGTACGCCAGAAATTCAGGGCAGAGTGACCCTGACCGCCGACAGAAGCACC AGCACCGCTACATGGAAC TGAGCAGCCTGCGGAGCGAGGACACCGCGTGTACTAT TGTGCCAGAGGCGGTACGGCGGCTTCGCTGGCGGCTACTTCGATTTTGGGGCCAG GGCACCTCGTGACCGTCAGCTCA
81	NOV0712 light chain full-length amino acid sequence	MVLQTQVFISLLLWISGAYGDIQMTQSPSSLSASVGDRTITCRASQSISSYLNWYQ QKPGKAPKLLIYAVSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSGTF PPTTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC
82	NOV0712 light chain full-length nucleotide sequence	ATGGTGCTGCAGACCCAGGTGTTTCATCTCCCTGCTGCTGTGGATCTCCGGCGGTAC GGCGACATCCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGGCGACAGA GTGACCATCACCTGTAGAGCCAGCCAGAGCATCAGCAGTACCTGAAC TGGTATCAG CAGAAGCCCGGCAAGGCCCCAAACTGCTGATCTACGCCGTGTCCACACTGCAGAGC GGCGTGCCAGCAGATTTTCTGGCAGCGGCTCCGGCACC GACTTCACCTGACAA TC AGCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGTCAGCAGTCCGGCACCTTC CCCCCACCACATTTGGCCAGGGCACCAAGGTGGAATCAAGCGTACGGTGGCCGCC CCCTCCGTGTTTCATCTCCCGCCCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCC GTGGTGTGCTGCTGAATAACTTCTACCCAGAGAGGCCAAGGTGCAGTGAAGGTG GACAAAGCCCTGCAGTCCGGGAAC TCCAGGAGAGCGTGACCGAGCAGGACAGCAAG GACAGCACCTACAGCCTGAGCAGCACCTGACCTGAGCAAAGCCGACTACGAGAAG CACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGCCTGAGCTCCCCGTCACCAAG AGCTTCAACAGGGGGGAGTGT
83	NOV0712 heavy chain full-length amino acid sequence	MKHLWFFLLLVAAPRWVLSQVQLLESGGGLVQPGGSLRLSCAASGFTFSSGHMHWVR QAPGKGLEWVSVISGSGSNTGYADSVKGRFTISRDN SKNTLYLQMNSLR AEDTAVYY CARQWGSYAFDSWGGGT LVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN TKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTIKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHY TQKSLSLSPGK

[0111]

[Table 1-15]

84	NOV0712 heavy chain full-length nucleotide sequence	ATGAAACACCTGTGGTTCTTCTCCTGCTGGTGGCAGCTCCAGATGGGTGCTGAGC CAGGTGCAGCTGCTGGAATCTGGCGGAGGACTGGTGACGCTGGCGGCTCTCTGAGA CTGTCTTTGTGCCGCCAGCGGCTTCACCTTCAGCAGCCACGGAATGCACTGGGTGCGC CAGGCCCTGGAAGGGACTGGAATGGGTGTCGGTGATCAGCGCAGCGGCTCCAAT ACCGGCTACGCCGATAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAG AACACCCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGACTAT TGTGCCAGACAGTGGGGCAGCTACGCCCTTCGATTCTTGGGGCCAGGGCACCGTCGTG ACCGTCAGCTCAGCCTCCACCAAGGGCCCAAGCGTCTCCCCCTGGCACCGCTCCTCC AAGAGCACTCTGGCGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCC GAACCCGTGACCGTGAGCTGGAAGTCAAGGCCCTGACCAAGCGCGTGCACACCTTC CCCGCTGTCTGCAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAGTGAATCACAAGCCAGCAAC ACCAAGGTGGACAAGAGAGTTGAGCCCAATCTTGTGACAAAATCAGACATGCCCA CCCTGCCAGCACCTGAAGTCTCTGGGGGACCCTCAGTCTTCTCTTCCCCCAAAA CCCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGAC GTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTG CATAATGCCAAGACAAGCCCCGGGAGGAGCAGTACAACAGCAGTACCGGGTGGTC AGCGTCTCACCCTCTGCACAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG GTCTCCAACAAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAGCCAAAGGC CAGCCCCGGGAACCAAGGTGTACACCTGCCCCATCCGGGAGGAGATGACCAAG AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTG GAGTGGGAGAGCAATGGCCAGCCGAGAACAACTACAAGACCACCCCTCCCGTGCTG GACTCCGACGGCTCTTCTCTCTACAGCAAGCTACCGTGGACAAGAGCAGGTGG CAGCAGGGCAACGCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTAC ACCCAGAAGAGCCTCTCCCTGTCTCCCGGCAAA
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[0112] In the present description, Tables 1-1 to 1-15 are also collectively referred to as Table 1.

Further examples of the antibody of the present invention can include a human antibody binding to CDH6. The anti-CDH6 human antibody means a human antibody having only the gene sequence of an antibody derived from human chromosomes. The anti-CDH6 human antibody can be obtained by a method using a human antibody-producing mouse having a human chromosomal fragment comprising the heavy chain and light chain genes of a human antibody (see Tomizuka, K. et al., *Nature Genetics* (1997) 16, p. 133-143; Kuroiwa, Y. et al., *Nucl. Acids Res.* (1998) 26, p. 3447-3448; Yoshida, H. et al., *Animal Cell Technology: Basic and Applied Aspects* vol. 10, p. 69-73 (Kitagawa, Y., Matsuda, T. and Iijima, S. eds.), Kluwer

Academic Publishers, 1999; Tomizuka, K. et al., Proc. Natl. Acad. Sci. USA (2000) 97, p. 722-727; etc.).

[0113] Such a human antibody-producing mouse can be specifically produced by using a genetically modified animal, the gene loci of endogenous immunoglobulin heavy chain and light chain of which have been disrupted and instead the gene loci of human immunoglobulin heavy chain and light chain have been then introduced using a yeast artificial chromosome (YAC) vector or the like, then producing a knock-out animal and a transgenic animal from such a genetically modified animal, and then breeding such animals with one another.

[0114] Otherwise, the anti-CDH6 human antibody can also be obtained by transforming eukaryotic cells with cDNA encoding each of the heavy chain and light chain of such a human antibody, or preferably with a vector comprising the cDNA, according to genetic recombination techniques, and then culturing the transformed cells producing a genetically modified human monoclonal antibody, so that the antibody can be obtained from the culture supernatant.

[0115] In this context, eukaryotic cells, and preferably, mammalian cells such as CHO cells, lymphocytes or myelomas can, for example, be used as a host.

[0116] Furthermore, a method of obtaining a phage display-derived human antibody that has been selected from a human antibody library (see Wormstone, I. M. et al., Investigative Ophthalmology & Visual Science. (2002) 43 (7), p. 2301-2308; Carmen, S. et al., Briefings in Functional Genomics and Proteomics (2002), 1 (2), p. 189-203; Siriwardena, D. et al., Ophthalmology (2002) 109 (3), p. 427-431; etc.) is also known.

[0117] For example, a phage display method, which comprises allowing the variable regions of a human antibody to express as a single chain antibody (scFv) on the surface of phages, and then selecting a phage binding to an antigen, can be applied (Nature Biotechnology (2005), 23, (9), p. 1105-1116).

[0118] By analyzing the phage gene that has been selected because of its binding ability to the antigen, DNA sequences encoding the variable regions of a human antibody binding to the antigen can be determined.

[0119] Once the DNA sequence of scFv binding to the antigen is determined, an expression vector having the aforementioned sequence is produced, and the produced expression vector is then introduced into an appropriate host and can be allowed to express therein, thereby obtaining a human antibody (International Publication Nos. WO92/01047, WO92/20791, WO93/06213, WO93/11236, WO93/19172, WO95/01438, and WO95/15388, Annu. Rev. Immunol (1994) 12, p. 433-455, Nature Biotechnology (2005) 23 (9), p. 1105-1116).

[0120] If a newly produced human antibody binds to a partial peptide or a partial three-dimensional structure to which any one rat anti-human CDH6 antibody, chimeric anti-human CDH6 antibody or humanized anti-human CDH6 antibody described in the present description (e.g., the rG019 antibody, the rG055 antibody, the rG056 antibody, the rG061 antibody, the chG019 antibody, the H01L02 antibody, the H02L02 antibody, the H02L03 antibody or the H04L02 antibody) binds, it can be determined that the human antibody binds to the same epitope to which the rat anti-human CDH6 antibody, the chimeric anti-human CDH6 antibody or the humanized anti-human CDH6 antibody binds.

Alternatively, by confirming that the human antibody competes with the rat anti-human CDH6 antibody, the chimeric anti-human CDH6 antibody or the humanized anti-human CDH6 antibody described in the present description (e.g., the rG019 antibody, the rG055 antibody, the rG056 antibody, the rG061 antibody, the chG019 antibody, the H01L02 antibody, the H02L02 antibody, the H02L03 antibody or the H04L02 antibody) in the binding of the antibody to CDH6 (e.g., the human antibody interferes with the binding of the rG019 antibody, the rG055 antibody, the rG056 antibody, the rG061 antibody, the chG019 antibody, the H01L02 antibody, the H02L02 antibody, the H02L03 antibody or the H04L02 antibody to CDH6, preferably EC3 of CDH6), it can be determined that the human antibody binds to the same epitope to which the rat anti-human CDH6 antibody, the chimeric anti-human CDH6 antibody or the humanized anti-human CDH6 antibody described in the present description binds, even if the specific sequence or structure of the epitope has not been determined. In the present description, when it is determined by at least one of these determination methods that the human antibody "binds to the same epitope", it is concluded that the newly prepared human antibody "binds to the same epitope" as that for the rat anti-human CDH6 antibody, the chimeric anti-human CDH6 antibody or the humanized anti-human CDH6 antibody described in the present description. When it is confirmed that the human antibody binds to the same epitope, then it is expected that the human antibody should have a biological activity equivalent to that of the rat anti-human CDH6 antibody, the chimeric anti-human CDH6 antibody or the humanized anti-human CDH6 antibody (e.g., the rG019

antibody, the rG055 antibody, the rG056 antibody, the rG061 antibody, the chG019 antibody, the H01L02 antibody, the H02L02 antibody, the H02L03 antibody or the H04L02 antibody).

[0121] The chimeric antibodies, the humanized antibodies, or the human antibodies obtained by the above-described methods are evaluated for their binding activity against the antigen according to a known method, etc., so that a preferred antibody can be selected.

[0122] One example of another indicator for comparison of the properties of antibodies can include the stability of an antibody. A differential scanning calorimeter (DSC) is an apparatus capable of promptly and exactly measuring a thermal denaturation midpoint (T_m) serving as a good indicator for the relative structural stability of a protein. By using DSC to measure T_m values and making a comparison regarding the obtained values, differences in thermal stability can be compared. It is known that the preservation stability of an antibody has a certain correlation with the thermal stability of the antibody (Lori Burton, et al., Pharmaceutical Development and Technology (2007) 12, p. 265-273), and thus, a preferred antibody can be selected using thermal stability as an indicator. Other examples of the indicator for selection of an antibody can include high yield in suitable host cells and low agglutination in an aqueous solution. For example, since an antibody with the highest yield does not always exhibit the highest thermal stability, it is necessary to select an antibody most suitable for administration to a human by comprehensively determining it based on the aforementioned indicators.

[0123] The antibody of the present invention also includes a modification of an antibody. The modification is used to mean the antibody of the present invention, which is chemically or biologically modified. Examples of such a chemical modification include the binding of a chemical moiety to an amino acid skeleton, and the chemical modification of an N-linked or O-linked carbohydrate chain. Examples of such a biological modification include antibodies which have undergone a posttranslational modification (e.g., N-linked or O-linked glycosylation, N-terminal or C-terminal processing, deamidation, isomerization of aspartic acid, oxidation of methionine, and conversion of N-terminal glutamine or N-terminal glutamic acid to pyroglutamic acid), and antibodies, to the N-terminus of which a methionine residue is added as a result of having been allowed to be expressed using prokaryote host cells. In addition, such a modification is also meant to include labeled antibodies for enabling detection or isolation of the antibody of the present invention or an antigen, for example, an enzymatically labeled antibody, a fluorescently labeled antibody, and an affinity-labeled antibody. Such a modification of the antibody of the present invention is useful for the improvement of the stability and retention in blood of an antibody; a reduction in antigenicity; detection or isolation of an antibody or an antigen; etc.

[0124] Moreover, by regulating a sugar chain modification (glycosylation, de-fucosylation, etc.) that binds to the antibody of the present invention, antibody-dependent cellular cytotoxic activity can be enhanced. As techniques of regulating the sugar chain modification of an antibody, those

described in International Publication Nos. WO1999/54342, WO2000/61739, and WO2002/31140, etc. are known, though the techniques are not limited thereto. The antibody of the present invention also includes antibodies in respect of which the aforementioned sugar chain modification has been regulated.

[0125] Once an antibody gene is isolated, the gene can be introduced into an appropriate host to produce an antibody, using an appropriate combination of a host and an expression vector. A specific example of the antibody gene can be a combination of a gene encoding the heavy chain sequence of the antibody described in the present description and a gene encoding the light chain sequence of the antibody described therein. Upon transformation of host cells, such a heavy chain sequence gene and a light chain sequence gene may be inserted into a single expression vector, or these genes may instead each be inserted into different expression vectors.

[0126] When eukaryotic cells are used as hosts, animal cells, plant cells or eukaryotic microorganisms can be used. In particular, examples of the animal cells can include mammalian cells such as COS cells which are monkey cells (Gluzman, Y., Cell (1981) 23, p. 175-182, ATCC CRL-1650), mouse fibroblasts NIH3T3 (ATCC No. CRL-1658), a dihydrofolate reductase-deficient cell line of Chinese hamster ovary cells (CHO cells, ATCC CCL-61) (Urlaub, G. and Chasin, L. A. Proc. Natl. Acad. Sci. U.S.A. (1980) 77, p. 4126-4220), and FreeStyle 293F cells (Invitrogen Corp.).

[0127] When prokaryotic cells are used as hosts, *Escherichia coli* or *Bacillus subtilis* can be used, for example.

[0128] An antibody gene of interest is introduced into these cells for transformation, and the transformed cells are then cultured *in vitro* to obtain an antibody. In the aforementioned culture, there are cases where yield is different depending on the sequence of the antibody, and thus, it is possible to select an antibody, which is easily produced as a medicament, from antibodies having equivalent binding activity, using the yield as an indicator. Accordingly, the antibody of the present invention also includes an antibody obtained by the above-described method for producing an antibody, which comprises a step of culturing the transformed host cells and a step of collecting an antibody of interest or a functional fragment of the antibody from the culture obtained in the aforementioned step.

[0129] It is known that the lysine residue at the carboxyl terminus of the heavy chain of an antibody produced in cultured mammalian cells is deleted (Journal of Chromatography A, 705: 129-134 (1995)), and also, it is known that the two amino acid residues at the heavy chain carboxyl terminus, glycine and lysine, are deleted, and that the proline residue newly positioned at the carboxyl terminus is amidated (Analytical Biochemistry, 360: 75-83 (2007)). However, such deletion and modification of these heavy chain sequences does not have an influence on the antigen-binding activity and effector function (activation of complement, antibody-dependent cellular cytotoxicity, etc.) of an antibody. Accordingly, the antibody according to the present invention also includes an antibody that has undergone the aforementioned modification, and a functional fragment of the

antibody, and specific examples of such an antibody include a deletion mutant comprising a deletion of 1 or 2 amino acids at the heavy chain carboxyl terminus, and a deletion mutant formed by amidating the aforementioned deletion mutant (e.g., a heavy chain in which the proline residue at the carboxyl-terminal site is amidated). However, deletion mutants involving a deletion at the carboxyl terminus of the heavy chain of the antibody according to the present invention are not limited to the above-described deletion mutants, as long as they retain antigen-binding activity and effector function. Two heavy chains constituting the antibody according to the present invention may be any one type of heavy chain selected from the group consisting of a full-length antibody and the above-described deletion mutants, or may be a combination of any two types selected from the aforementioned group. The ratio of individual deletion mutants can be influenced by the types of cultured mammalian cells that produce the antibody according to the present invention, and the culture conditions. Examples of the main ingredient of the antibody according to the present invention can include antibodies where one amino acid residue is deleted at each of the carboxyl termini of the two heavy chains.

[0130] Examples of the isotype of the antibody of the present invention can include IgG (IgG1, IgG2, IgG3, and IgG4). Among others, IgG1 and IgG4 are preferable.

[0131] Examples of the biological activity of an antibody can generally include antigen-binding activity, activity of being internalized into cells expressing an antigen by binding to the antigen, activity of neutralizing the activity of an

antigen, activity of enhancing the activity of an antigen, antibody-dependent cellular cytotoxic (ADCC) activity, complement-dependent cytotoxic (CDC) activity, and antibody-dependent cellular phagocytosis (ADCP). The function of the antibody according to the present invention is binding activity against CDH6 and is preferably the activity of being internalized into CDH6-expressing cells by binding to CDH6. Moreover, the antibody of the present invention may have ADCC activity, CDC activity and/or ADCP activity, as well as cellular internalization activity.

[0132] The obtained antibody can be purified to a homogenous state. For separation and purification of the antibody, separation and purification methods used for ordinary proteins may be used. For example, column chromatography, filtration, ultrafiltration, salting-out, dialysis, preparative polyacrylamide gel electrophoresis, and isoelectric focusing are appropriately selected and combined with one another, so that the antibody can be separated and purified (Strategies for Protein Purification and Characterization: A Laboratory Course Manual, Daniel R. Marshak et al. eds., Cold Spring Harbor Laboratory Press (1996); and Antibodies: A Laboratory Manual. Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988)), though examples of the separation and purification methods are not limited thereto.

[0133] Examples of the chromatography can include affinity chromatography, ion exchange chromatography, hydrophobic chromatography, gel filtration chromatography, reverse phase chromatography, and absorption chromatography.

[0134] These chromatographic techniques can be carried out using liquid chromatography such as HPLC or FPLC.

[0135] Examples of the column used in the affinity chromatography can include a Protein A column and a Protein G column. Examples of the column involving the use of Protein A can include Hyper D, POROS, and Sepharose F. F. (Pharmacia).

[0136] Also, using an antigen-immobilized carrier, the antibody can be purified by utilizing the binding activity of the antibody to the antigen.

[0137] 3. Anti-CDH6 antibody-drug conjugate

(1) Drug

The anti-CDH6 antibody obtained in the above "2. Production of anti-CDH6 antibody" can be conjugated to a drug via a linker structure moiety to prepare an anti-CDH6 antibody-drug conjugate. The drug is not particularly limited as long as it has a substituent or a partial structure that can be connected to a linker structure. The anti-CDH6 antibody-drug conjugate can be used for various purposes according to the conjugated drug. Examples of such a drug can include substances having antitumor activity, substances effective for blood diseases, substances effective for autoimmune diseases, anti-inflammatory substances, antimicrobial substances, antifungal substances, antiparasitic substances, antiviral substances, and anti-anesthetic substances.

[0138] (1)-1 Antitumor compound

An example using an antitumor compound as a compound to be conjugated in the anti-CDH6 antibody-drug conjugate of the present invention will be described below. The antitumor

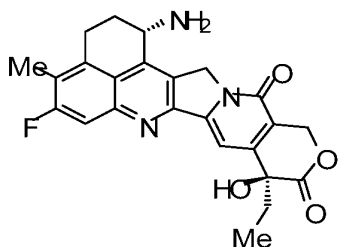
compound is not particularly limited as long as the compound has an antitumor effect and has a substituent or a partial structure that can be connected to a linker structure. Upon cleavage of a part or the whole of the linker in tumor cells, the antitumor compound moiety is released so that the antitumor compound exhibits an antitumor effect. As the linker is cleaved at a connecting position with the drug, the antitumor compound is released in its original structure to exert its original antitumor effect.

[0139] The anti-CDH6 antibody obtained in the above "2. Production of anti-CDH6 antibody" can be conjugated to the antitumor compound via a linker structure moiety to prepare an anti-CDH6 antibody-drug conjugate.

[0140] As one example of the antitumor compound used in the present invention, exatecan, a camptothecin derivative ((1S,9S)-1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinoline-10,13(9H,15H)-dione represented by the following formula) can preferably be used.

[0141]

[Formula 5]



[0142] The compound can be easily obtained by, for example, a method described in U.S. Patent Publication No. US2016/0297890 or other known methods, and the amino group at position 1 can

be preferably used as a connecting position to the linker structure. Further, exatecan may be released in tumor cells while a part of the linker is still attached thereto. However, the compound exerts an excellent antitumor effect even in such a state.

[0143] Since exatecan has a camptothecin structure, it is known that the equilibrium shifts to a structure with a formed lactone ring (closed ring) in an acidic aqueous medium (e.g., of the order of pH 3) whereas the equilibrium shifts to a structure with an opened lactone ring (open ring) in a basic aqueous medium (e.g., of the order of pH 10). A drug conjugate into which exatecan residues corresponding to such a closed ring structure and an open ring structure have been introduced is also expected to have an equivalent antitumor effect, and it is needless to say that any of such drug conjugate is included within the scope of the present invention.

[0144] Other examples of the antitumor compound can include antitumor compounds described in the literature (Pharmacological Reviews, 68, p. 3-19, 2016). Specific examples thereof can include doxorubicin, calicheamicin, dolastatin 10, auristatins such as monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF), maytansinoids such as DM1 and DM4, a pyrrolobenzodiazepine dimer SG2000 (SJG-136), a camptothecin derivative SN-38, duocarmycins such as CC-1065, amanitin, daunorubicin, mitomycin C, bleomycin, cyclocytidine, vincristine, vinblastine, methotrexate, platinum-based antitumor agents (cisplatin and derivatives thereof), and Taxol and derivatives thereof.

[0145] In the antibody-drug conjugate, the number of conjugated drug molecules per antibody molecule is a key factor having an influence on the efficacy and safety thereof. The production of the antibody-drug conjugate is carried out by specifying reaction conditions such as the amounts of starting materials and reagents used for reaction, so as to attain a constant number of conjugated drug molecules. Unlike the chemical reaction of a low-molecular-weight compound, a mixture containing different numbers of conjugated drug molecules is usually obtained. The number of conjugated drug molecules per antibody molecule is defined and indicated as an average value, i.e., the average number of conjugated drug molecules. Unless otherwise specified, i.e., except in the case of representing an antibody-drug conjugate having a specific number of conjugated drug molecules that is included in an antibody-drug conjugate mixture having different numbers of conjugated drug molecules, the number of conjugated drug molecules according to the present invention also means an average value as a rule. The number of exatecan molecules conjugated to an antibody molecule is controllable, and as an average number of conjugated drug molecules per antibody, approximately 1 to 10 exatecan molecules can be conjugated. The number of exatecan molecules is preferably 2 to 8, 3 to 8, 4 to 8, 5 to 8, 6 to 8, or 7 to 8, more preferably 5 to 8, further preferably 7 to 8, still further preferably 8. It is to be noted that a person skilled in the art can design a reaction for conjugating a required number of drug molecules to an antibody molecule based on the description of Examples of the present

application, and can obtain an antibody-drug conjugate with a controlled number of conjugated exatecan molecules.

[0146] (2) Linker structure

The linker structure which conjugates the drug to the anti-CDH6 antibody in the anti-CDH6 antibody-drug conjugate of the present invention will be described.

[0147] In the antibody-drug conjugate of the present application, the linker structure which conjugates the anti-CDH6 antibody to the drug is not particularly limited as long as the resulting antibody-drug conjugate can be used. The linker structure may be appropriately selected and used according to the purpose of use. One example of the linker structure can include a linker described in known literature (Pharmacol Rev 68: 3-19, January 2016, Protein Cell DOI 10.1007/s13238-016-0323-0, etc.). Further specific examples thereof can include VC (valine-citrulline), MC (maleimidocaproyl), SMCC (succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate), SPP (N-succinimidyl 4-(2-pyridyldithio)pentanoate, SS (disulfide), SPDB (N-succinimidyl 4-(2-pyridyldithio)butyrate, SS/hydrazone, hydrazone and carbonate.

[0148] Another example can include a linker structure described in U.S. Patent Publication No. US2016/0297890 (as one example, those described in paragraphs [0260] to [0289] thereof). Any linker structure given below can preferably be used. It is to be noted that the left terminus of the structure is a connecting position to the antibody, and the right terminus thereof is a connecting position to the drug. Furthermore, GGFG in the linker structures given below represents an amino

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acid sequence consisting of glycine-glycine-phenylalanine-glycine (GGFG) linked through peptide bonds.

- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -,
- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -,
- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂-O-CH₂-C(=O) -,
- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂-O-CH₂-C(=O) -,
- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -, and
- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -.

[0149] More preferred are the following:

- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂-O-CH₂-C(=O) -,
- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂-O-CH₂-C(=O) -, and
- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -.

Still more preferred are the following:

- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂-O-CH₂-C(=O) -, and
- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -.

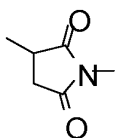
[0150] The antibody is connected to the terminus of -(Succinimid-3-yl-N) (e.g., a terminus opposite (left terminus) to the terminus to which -CH₂CH₂CH₂CH₂CH₂- is connected in "-(Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-

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NH-CH₂-O-CH₂-C(=O)-"), and the antitumor compound is connected to a terminus (the carbonyl group of CH₂-O-CH₂-C(=O)- at the right terminus in the above-described example) opposite to the terminus to which the antibody is connected to -(Succinimid-3-yl-N). "-(Succinimid-3-yl-N)-" has a structure represented by the following formula:

[0151]

[Formula 6]



[0152] Position 3 of this partial structure is the connecting position to the anti-CDH6 antibody. This connection to the antibody at position 3 is characterized by forming a thioether bond. The nitrogen atom at position 1 of this structure moiety is connected to the carbon atom of methylene which is present within the linker including the structure.

[0153] In the antibody-drug conjugate of the present invention having exatecan as the drug, a drug-linker structure moiety having any structure given below is preferred for conjugation to the antibody. For these drug-linker structure moieties, the average number conjugated per antibody may be 1 to 10 and is preferably 2 to 8, more preferably 5 to 8, further preferably 7 to 8, and still further preferably 8.

-(Succinimid-3-yl-N)-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-(NH-DX),

-(Succinimid-3-yl-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-(NH-DX),

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- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂-O-CH₂-
C(=O) - (NH-DX) ,
- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂-O-CH₂-
C(=O) - (NH-DX) ,
- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-
C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) - (NH-DX) , and
- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂O-
CH₂CH₂O-CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) - (NH-DX) .

[0154] More preferred are the following:

- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂-O-CH₂-
C(=O) - (NH-DX) ,
- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂-O-CH₂-
C(=O) - (NH-DX) , and
- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-
C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) - (NH-DX) .

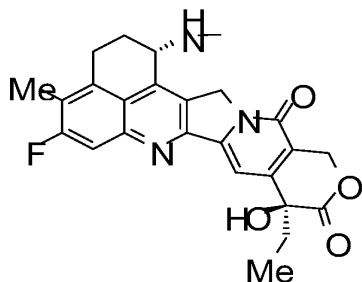
[0155] Still more preferred are the following:

- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂-O-CH₂-
C(=O) - (NH-DX) , and
- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-
C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) - (NH-DX) .

[0156] - (NH-DX) has a structure represented by the following
formula:

[0157]

[Formula 7]



[0158] and it represents a group that is derived by removing one hydrogen atom from the amino group at position 1 of exatecan.

[0159] (3) Method for producing antibody-drug conjugate

The antibody that can be used in the antibody-drug conjugate of the present invention is not particularly limited as long as it is an anti-CDH6 antibody having internalization activity or a functional fragment of the antibody, as described in the above section "2. Production of anti-CDH6 antibody" and the Examples.

[0160] Next, a typical method for producing the antibody-drug conjugate of the present invention will be described. It is to be noted that, in the description below, "compound No." shown in each reaction scheme is used to represent a compound. Specifically, each compound is referred to as a "compound of formula (1)", "compound (1)", or the like. The same holds true for the other compound Nos.

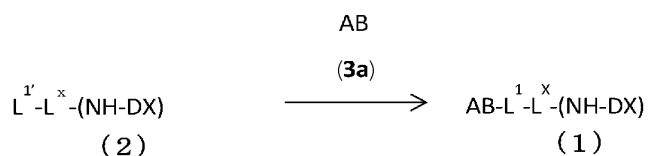
[0161] (3)-1 Production method 1

The antibody-drug conjugate represented by formula (1) given below in which the anti-CDH6 antibody is connected to the linker structure via a thioether can be produced by reacting an antibody having a sulfhydryl group converted from

a disulfide bond by the reduction of the anti-CDH6 antibody, with the compound (2), the compound (2) being obtainable by a known method (e.g., obtainable by a method described in the patent publication literature US2016/297890 (e.g., a method described in the paragraphs [0336] to [0374])). This antibody-drug conjugate can be produced by the following method, for example.

[0162]

[Expression 1]



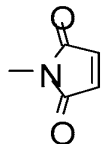
[0163]

wherein AB represents an antibody with a sulfhydryl group, wherein

L^I has a structure represented by -(Succinimid-3-yl-N)-, and L^{I'} represents a maleimidyl group represented by the following formula.

[0164]

[Formula 8]



[0165] -L^I-L^x has a structure represented by any of the following formulas:

-(Succinimid-3-yl-N)-CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-,
 -(Succinimid-3-yl-N)-CH₂CH₂CH₂CH₂CH₂-C(=O)-GGFG-NH-CH₂CH₂CH₂-C(=O)-,

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- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂-O-CH₂-
C(=O) -,
- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂-O-CH₂-
C(=O) -,
- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-
C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -, and
- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂O-
CH₂CH₂O-CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -.

[0166] Among them, more preferred are the following:

- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂-O-CH₂-
C(=O) -,
- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂CH₂-O-CH₂-
C(=O) -, and
- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-
C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -.

[0167] Further preferred are the following:

- (Succinimid-3-yl-N) -CH₂CH₂CH₂CH₂CH₂-C(=O) -GGFG-NH-CH₂-O-CH₂-
C(=O) -, and
- (Succinimid-3-yl-N) -CH₂CH₂-C(=O) -NH-CH₂CH₂O-CH₂CH₂O-CH₂CH₂-
C(=O) -GGFG-NH-CH₂CH₂CH₂-C(=O) -.

[0168] In the above-described reaction scheme, the antibody-drug conjugate (1) can be understood as having a structure in which one structure moiety from the drug to the linker terminus is connected to one antibody. However, this description is given for the sake of convenience, and there are actually many cases in which a plurality of the aforementioned structure moieties is connected to one antibody molecule. The same holds true for the explanation of the production method described below.

[0169] Specifically, the antibody-drug conjugate (1) can be produced by reacting the compound (2) obtainable by a known method (e.g., obtainable by a method described in the patent publication literature US2016/297890 (e.g., obtainable by a method described in the paragraphs [0336] to [0374])), with the antibody (3a) having a sulfhydryl group.

[0170] The antibody (3a) having a sulfhydryl group can be obtained by a method well known to a person skilled in the art (Hermanson, G.T, Bioconjugate Techniques, pp. 56-136, pp. 456-493, Academic Press (1996)). Examples of the method can include, but are not limited to: Traut's reagent being reacted with the amino group of the antibody; N-succinimidyl S-acetylthioalkanoates being reacted with the amino group of the antibody followed by reaction with hydroxylamine; N-succinimidyl 3-(pyridyldithio)propionate being reacted with the antibody, followed by reaction with a reducing agent; the antibody being reacted with a reducing agent such as dithiothreitol, 2-mercaptoethanol, or tris(2-carboxyethyl)phosphine hydrochloride (TCEP) to reduce the interchain disulfide bond in the antibody, so as to form a sulfhydryl group.

[0171] Specifically, an antibody with interchain disulfide bonds partially or completely reduced can be obtained by using 0.3 to 3 molar equivalents of TCEP as a reducing agent per interchain disulfide bond in the antibody, and reacting the reducing agent with the antibody in a buffer solution containing a chelating agent. Examples of the chelating agent can include ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA). The chelating

agent can be used at a concentration of 1 mM to 20 mM. A solution of sodium phosphate, sodium borate, sodium acetate, or the like can be used as the buffer solution. As a specific example, the antibody (3a) having partially or completely reduced sulfhydryl groups can be obtained by reacting the antibody with TCEP at 4°C to 37°C for 1 to 4 hours.

[0172] It is to be noted that by carrying out an addition reaction of a sulfhydryl group to a drug-linker moiety, the drug-linker moiety can be conjugated by a thioether bond.

[0173] Then, using 2 to 20 molar equivalents of the compound (2) per antibody (3a) having a sulfhydryl group, the antibody-drug conjugate (1) in which 2 to 8 drug molecules are conjugated per antibody can be produced. Specifically, a solution containing the compound (2) dissolved therein may be added to a buffer solution containing the antibody (3a) having a sulfhydryl group for the reaction. In this context, a sodium acetate solution, sodium phosphate, sodium borate, or the like can be used as the buffer solution. pH for the reaction is 5 to 9, and more preferably, the reaction may be performed near pH 7. An organic solvent such as dimethyl sulfoxide (DMSO), dimethylformamide (DMF), dimethylacetamide (DMA), or N-methyl-2-pyrrolidone (NMP) can be used as a solvent for dissolving the compound (2). The reaction may be performed by adding the solution containing the compound (2) dissolved in the organic solvent at 1 to 20% v/v to a buffer solution containing the antibody (3a) having a sulfhydryl group. The reaction temperature is 0 to 37°C, more preferably 10 to 25°C, and the reaction time is 0.5 to 2 hours. The reaction can be terminated by deactivating the reactivity of

unreacted compound (2) with a thiol-containing reagent. The thiol-containing reagent is, for example, cysteine or N-acetyl-L-cysteine (NAC). More specifically, the reaction can be terminated by adding 1 to 2 molar equivalents of NAC to the compound (2) used, and incubating the obtained mixture at room temperature for 10 to 30 minutes.

[0174] (4) Identification of antibody-drug conjugate

The produced antibody-drug conjugate (1) can be subjected to concentration, buffer exchange, purification, and measurement of antibody concentration and the average number of conjugated drug molecules per antibody molecule according to common procedures described below, to identify the antibody-drug conjugate (1).

[0175] (4)-1 Common procedure A: Concentration of aqueous solution of antibody or antibody-drug conjugate

To an Amicon Ultra (50,000 MWCO, Millipore Corporation) container, a solution of an antibody or an antibody-drug conjugate was added, and the solution of the antibody or the antibody-drug conjugate was concentrated by centrifugation (centrifugation at 2000 G to 3800 G for 5 to 20 minutes) using a centrifuge (Allegra X-15R, Beckman Coulter, Inc.)

[0176] (4)-2 Common procedure B: Measurement of antibody concentration

Using a UV detector (Nanodrop 1000, Thermo Fisher Scientific Inc.), measurement of the antibody concentration was carried out according to the method defined by the manufacturer. In this respect, 280 nm absorption coefficient differing among antibodies ($1.3 \text{ mLmg}^{-1}\text{cm}^{-1}$ to $1.8 \text{ mLmg}^{-1}\text{cm}^{-1}$) was used.

[0177] (4)-3 Common procedure C: Buffer exchange for antibody

A NAP-25 column (Cat. No. 17-0852-02, GE Healthcare Japan Corporation) using Sephadex G-25 carrier was equilibrated with a phosphate buffer (50 mM, pH 6.0) (referred to as PBS6.0/EDTA in the present description) containing sodium chloride (50 mM) and EDTA (2 mM) according to the method defined by the manufacturer. An aqueous solution of the antibody was applied in an amount of 2.5 mL per NAP-25 column, and thereafter, a fraction (3.5 mL) eluted with 3.5 mL of PBS6.0/EDTA was collected. This fraction was concentrated by common procedure A. After measurement of the concentration of the antibody using common procedure B, the antibody concentration was adjusted to 20 mg/mL using PBS6.0/EDTA.

[0178] (4)-4 Common procedure D: Purification of antibody-drug conjugate

A NAP-25 column was equilibrated with any commercially available buffer solution such as an acetate buffer containing sorbitol (5%) (10 mM, pH 5.5; referred to as ABS in the present description). An aqueous reaction solution of the antibody-drug conjugate (approximately 2.5 mL) was applied to the NAP-25 column, and thereafter, elution was carried out with the buffer solution in an amount defined by the manufacturer, so as to collect an antibody fraction. A gel filtration purification process, in which the collected fraction was applied again to the NAP-25 column, and elution was carried out with the buffer solution, was repeated a total of 2 or 3 times to obtain the antibody-drug conjugate excluding non-conjugated drug linker and low-molecular-weight

compounds (tris(2-carboxyethyl)phosphine hydrochloride (TCEP), N-acetyl-L-cysteine (NAC), and dimethyl sulfoxide).

[0179] (4)-5 Common procedure E: Measurement of antibody concentration in antibody-drug conjugate and average number of conjugated drug molecules per antibody molecule

The conjugated drug concentration in the antibody-drug conjugate can be calculated by measuring UV absorbance of an aqueous solution of the antibody-drug conjugate at two wavelengths of 280 nm and 370 nm, and thereafter performing the calculation shown below.

[0180] The total absorbance at any given wavelength is equal to the sum of the absorbance of all light-absorbing chemical species that are present in a system [additivity of absorbance]. Therefore, based on the hypothesis that the molar absorption coefficients of the antibody and the drug do not vary between before and after conjugation between the antibody and the drug, the antibody concentration and the drug concentration in the antibody-drug conjugate are represented by the following equations.

$$A_{280} = A_{D,280} + A_{A,280} = \epsilon_{D,280}C_D + \epsilon_{A,280}C_A \text{ Equation (1)}$$

$$A_{370} = A_{D,370} + A_{A,370} = \epsilon_{D,370}C_D + \epsilon_{A,370}C_A \text{ Equation (2)}$$

In this context, A_{280} represents the absorbance of an aqueous solution of the antibody-drug conjugate at 280 nm, A_{370} represents the absorbance of an aqueous solution of the antibody-drug conjugate at 370 nm, $A_{A,280}$ represents the absorbance of the antibody at 280 nm, $A_{A,370}$ represents the absorbance of the antibody at 370 nm, $A_{D,280}$ represents the absorbance of a conjugate precursor at 280 nm, $A_{D,370}$ represents the absorbance of a conjugate precursor at 370 nm, $\epsilon_{A,280}$

represents the molar absorption coefficient of the antibody at 280 nm, $\epsilon_{A,370}$ represents the molar absorption coefficient of the antibody at 370 nm, $\epsilon_{D,280}$ represents the molar absorption coefficient of a conjugate precursor at 280 nm, $\epsilon_{D,370}$ represents the molar absorption coefficient of a conjugate precursor at 370 nm, C_A represents the antibody concentration in the antibody-drug conjugate, and C_D represent the drug concentration in the antibody-drug conjugate.

[0181] In this context, with regard to $\epsilon_{A,280}$, $\epsilon_{A,370}$, $\epsilon_{D,280}$, and $\epsilon_{D,370}$, preliminarily prepared values (estimated values based on calculation or measurement values obtained by UV measurement of the compound) are used. For example, $\epsilon_{A,280}$ can be estimated from the amino acid sequence of the antibody by a known calculation method (Protein Science, 1995, vol. 4, 2411-2423). $\epsilon_{A,370}$ is generally zero. $\epsilon_{D,280}$ and $\epsilon_{D,370}$ can be obtained according to Lambert-Beer's law (Absorbance = Molar concentration $\times$ Molar absorption coefficient $\times$ Cell path length) by measuring the absorbance of a solution in which the conjugate precursor used is dissolved at a certain molar concentration. C_A and C_D can be determined by measuring A_{280} and A_{370} of an aqueous solution of the antibody-drug conjugate, and then solving the simultaneous equations (1) and (2) by substitution of these values. Further, by dividing C_D by C_A , the average number of conjugated drug molecules per antibody can be determined.

[0182] (4)-6 Common procedure F: Measurement of average number of conjugated drug molecules per antibody molecule in antibody-drug conjugate - (2)

The average number of conjugated drug molecules per antibody molecule in the antibody-drug conjugate can also be determined by high-performance liquid chromatography (HPLC) analysis using the following method, in addition to the aforementioned "(4)-5 Common procedure E". Hereinafter, the method for measuring the average number of conjugated drug molecules by HPLC when the antibody is conjugated to the drug linker by a disulfide bond will be described. A person skilled in the art is capable of appropriately measuring the average number of conjugated drug molecules by HPLC, depending on the connecting manner between the antibody and the drug linker, with reference to this method.

[0183] F-1. Preparation of sample for HPLC analysis (Reduction of antibody-drug conjugate)

An antibody-drug conjugate solution (approximately 1 mg/mL, 60 μ L) is mixed with an aqueous solution of dithiothreitol (DTT) (100 mM, 15 μ L). By incubating the mixture at 37°C for 30 minutes, the disulfide bond between the light chain and heavy chain of the antibody-drug conjugate is cleaved. The resulting sample is used in HPLC analysis.

[0184] F-2. HPLC analysis

The HPLC analysis is carried out under the following measurement conditions.

[0185] HPLC system: Agilent 1290 HPLC system (Agilent Technologies, Inc.)

Detector: Ultraviolet absorption spectrometer
(measurement wavelength: 280 nm)

Column: ACQUITY UPLC BEH Phenyl (2.1 $\times$ 50 mm, 1.7 μ m, 130 angstroms; Waters Corp., P/N 186002884)

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Column temperature: 80°C

Mobile phase A: Aqueous solution containing 0.10% trifluoroacetic acid (TFA) and 15% 2-propanol

Mobile phase B: Acetonitrile solution containing 0.075% TFA and 15% 2-propanol

Gradient program: 14%-36% (0 min-15 min), 36%-80% (15 min-17 min), 80%-14% (17 min-17.01 min.), and 14% (17.01 min-25 min)

Sample injection: 10 µL

F-3. Data analysis

F-3-1. Compared with non-conjugated antibody light (L0) and heavy (H0) chains, a light chain bound to drug molecule(s) (light chain bound to i drug molecule(s): L_i) and a heavy chain bound to drug molecule(s) (heavy chain bound to i drug molecule(s): H_i) exhibit higher hydrophobicity in proportion to the number of conjugated drug molecules and thus have a larger retention time. These chains are therefore eluted in the order of, for example, L0 and L1 or H0, H1, H2, and H3. Detection peaks can be assigned to any of L0, L1, H0, H1, H2, and H3 by the comparison of retention times with L0 and H0. The number of conjugated drug molecules can be defined by a person skilled in the art, but is preferably L0, L1, H0, H1, H2, and H3.

[0186] F-3-2. Since the drug linker has UV absorption, peak area values are corrected in response to the number of conjugated drug linker molecules according to the following expression using the molar absorption coefficients of the light chain or heavy chain and the drug linker.

[0187]

[Expression 2]

Corrected value of peak area of light chain bound to i drug molecule(s) (A_{Li}) = Peak area

$$\times \frac{\text{Molar absorption coefficient of light chain}}{\text{Molar absorption coefficient of light chain} + \text{The number of conjugated drug molecules (i)} \times \text{Molar absorption coefficient of drug linker}}$$

[0188]

[Expression 3]

Corrected value of peak area of heavy chain bound to i drug molecule(s) (A_{Hi}) = Peak area

$$\times \frac{\text{Molar absorption coefficient of heavy chain}}{\text{Molar absorption coefficient of heavy chain} + \text{The number of conjugated drug molecules (i)} \times \text{Molar absorption coefficient of drug linker}}$$

[0189] In this context, a value estimated from the amino acid sequence of the light chain or heavy chain of each antibody by a known calculation method (Protein Science, 1995, vol. 4, 2411-2423) can be used as the molar absorption coefficient (280 nm) of the light chain or heavy chain of the antibody. In the case of H01L02, a molar absorption coefficient of 31710 and a molar absorption coefficient of 79990 were used as estimated values for the light chain and heavy chain, respectively, according to the amino acid sequence of the antibody. The actually measured molar absorption coefficient (280 nm) of a compound in which the maleimide group has been converted to succinimide thioether by the reaction of each drug linker with mercaptoethanol or N-acetylcysteine was used as the molar absorption coefficient (280 nm) of the drug linker. The wavelength for absorbance measurement can be appropriately set by a person skilled in the art, but is preferably a wavelength at which the peak of the antibody can be measured, and more preferably 280 nm.

[0190] F-3-3. The peak area ratio (%) of each chain is calculated for the total of the corrected values of peak areas according to the following expression.

[0191]

[Expression 4]

$$\text{Peak area ratio of light chain bound to } i \text{ drug molecule(s)} = \frac{A_{Li}}{A_{L0} + A_{L1}} \times 100$$

$$\text{Peak area ratio of heavy chain bound to } i \text{ drug molecule(s)} = \frac{A_{Hi}}{A_{H0} + A_{H1} + A_{H2} + A_{H3}} \times 100$$

A_{Li} and A_{Hi} : Corrected values of peak areas of L_i and H_i , respectively

[0192] F-3-4. The average number of conjugated drug molecules per antibody molecule in the antibody-drug conjugate is calculated according to the following expression.

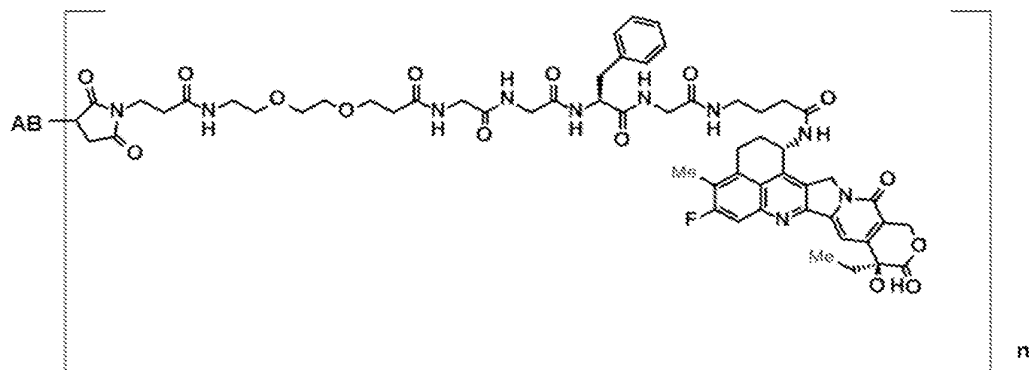
[0193] Average number of conjugated drug molecules = (L_0 peak area ratio $\times 0$ + L_1 peak area ratio $\times 1$ + H_0 peak area ratio $\times 0$ + H_1 peak area ratio $\times 1$ + H_2 peak area ratio $\times 2$ + H_3 peak area ratio $\times 3$) / 100×2

It is to be noted that, in order to secure the amount of the antibody-drug conjugate, a plurality of antibody-drug conjugates having almost the same average number of conjugated drug molecules (e.g., on the order of ± 1), which have been produced under similar conditions, can be mixed to prepare a new lot. In this case, the average number of drug molecules of the new lot falls between the average numbers of drug molecules before the mixing.

[0194] One specific example of the antibody-drug conjugate of the present invention can include an antibody-drug conjugate having a structure represented by the following formula:

[0195]

[Formula 9]

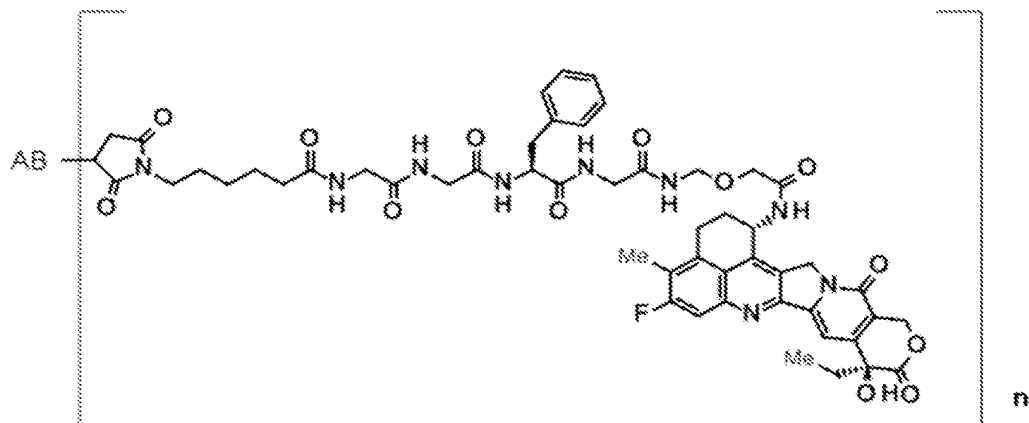


[0196]

or the following formula:

[0197]

[Formula 10]



[0198]

[0199] In this context, AB represents the anti-CDH6 antibody disclosed in the present description, and the antibody is conjugated to the drug linker via a sulfhydryl group stemming from the antibody. In this context, n has the same meaning as that of the so-called DAR (drug-to-antibody Ratio), and represents a drug-to-antibody ratio per antibody. Specifically, n represents the number of conjugated drug molecules per antibody molecule, which is a numeric value

defined and indicated as an average value, i.e., the average number of conjugated drug molecules. In the case of the antibody-drug conjugate represented by [Formula 9] or [Formula 10] of the present invention, n can be 2 to 8 and is preferably 5 to 8, more preferably 7 to 8, and still more preferably 8, in measurement by common procedure F.

[0200] One example of the antibody-drug conjugate of the present invention can include an antibody-drug conjugate having a structure represented by the above-described formula [Formula 9] or [Formula 10] wherein the antibody represented by AB comprises any one antibody selected from the group consisting of the following antibodies (a) to (g), or a functional fragment of the antibody, or a pharmacologically acceptable salt of the antibody-drug conjugate:

(a) an antibody consisting of a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 69;

(b) an antibody consisting of a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 73;

(c) an antibody consisting of a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 61 and a

heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 77;

(d) an antibody consisting of a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 65 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 69;

(e) an antibody consisting of a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 65 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 73;

(f) an antibody consisting of a light chain consisting of the amino acid sequence at positions 21 to 233 in the light chain full-length amino acid sequence shown in SEQ ID NO: 65 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in the heavy chain full-length amino acid sequence shown in SEQ ID NO: 77; and

(g) any one antibody selected from the group consisting of the antibodies (a) to (f), wherein the heavy chain or the light chain comprises one or two or more modifications selected from the group consisting of posttranslational modifications typified by N-linked glycosylation, O-linked glycosylation, N-terminal processing, C-terminal processing, deamidation, isomerization of aspartic acid, oxidation of methionine, addition of a methionine residue to the N-terminus, amidation

of a proline residue, and conversion of N-terminal glutamine or N-terminal glutamic acid to pyroglutamic acid, and a deletion of one or two amino acids at the carboxyl terminus

[0201] 4. Medicament

Since the anti-CDH6 antibody of the present invention or the functional fragment of the antibody described in the above section "2. Production of anti-CDH6 antibody" and the Examples binds to CDH6 on the surface of tumor cells and has internalization activity, it can be used as a medicament, and in particular, as a therapeutic agent for cancer such as renal cell tumor or ovarian tumor, for example, renal cell carcinoma, renal clear cell carcinoma, papillary renal cell carcinoma, ovarian cancer, ovarian serous adenocarcinoma, thyroid cancer, bile duct cancer, lung cancer (e.g., small-cell lung cancer or non-small cell lung cancer), glioblastoma, mesothelioma, uterine cancer, pancreatic cancer, Wilms' tumor or neuroblastoma, either alone or in combination with an additional drug.

[0202] Furthermore, the anti-CDH6 antibody of the present invention or the functional fragment of the antibody can be used in the detection of cells expressing CDH6.

[0203] Moreover, since the anti-CDH6 antibody of the present invention or the functional fragment of the antibody has internalization activity, it can be applied as the antibody in an antibody-drug conjugate.

[0204] When a drug having antitumor activity such as cytotoxic activity is used as the drug, the anti-CDH6 antibody-drug conjugate of the present invention described in the above section "3. Anti-CDH6 antibody-drug conjugate" and the

Examples is a conjugate of the anti-CDH6 antibody and/or the functional fragment of the antibody having internalization activity, and the drug having antitumor activity such as cytotoxic activity. Since this anti-CDH6 antibody-drug conjugate exhibits antitumor activity against cancer cells expressing CDH6, it can be used as a medicament, and in particular, as a therapeutic agent and/or a prophylactic agent for cancer.

[0205] The anti-CDH6 antibody-drug conjugate of the present invention may absorb moisture or have adsorption water, for example, to turn into a hydrate when it is left in air or subjected to recrystallization or purification procedures. Such a compound or a pharmacologically acceptable salt containing water is also included in the present invention.

[0206] When the anti-CDH6 antibody-drug conjugate of the present invention has a basic group such as an amino group, it can form a pharmacologically acceptable acid-addition salt, if desired. Examples of such an acid-addition salt can include: hydrohalides such as hydrofluoride, hydrochloride, hydrobromide, and hydroiodide; inorganic acid salts such as nitrate, perchlorate, sulfate, and phosphate; lower alkanesulfonates such as methanesulfonate, trifluoromethanesulfonate, and ethanesulfonate; arylsulfonates such as benzenesulfonate and p-toluenesulfonate; organic acid salts such as formate, acetate, trifluoroacetate, malate, fumarate, succinate, citrate, tartrate, oxalate, and maleate; and amino acid salts such as ornithine salt, glutamate, and aspartate.

[0207] When the anti-CDH6 antibody-drug conjugate of the present invention has an acidic group such as a carboxy group, it can form a pharmacologically acceptable base-addition salt, if desired. Examples of such a base-addition salt can include: alkali metal salts such as a sodium salt, a potassium salt, and lithium salt; alkaline earth metal salts such as a calcium salt and a magnesium salt; inorganic salts such as an ammonium salt; and organic amine salts such as a dibenzylamine salt, a morpholine salt, a phenylglycine alkyl ester salt, an ethylenediamine salt, an N-methylglucamine salt, a diethylamine salt, a triethylamine salt, a cyclohexylamine salt, a dicyclohexylamine salt, an N,N'-dibenzylethylenediamine salt, a diethanolamine salt, an N-benzyl-N-(2-phenylethoxy)amine salt, a piperazine salt, tetramethylammonium salt, and a tris(hydroxymethyl)aminomethane salt.

[0208] The present invention can also include an anti-CDH6 antibody-drug conjugate in which one or more atoms constituting the antibody-drug conjugate are replaced with isotopes of the atoms. There exist two types of isotopes: radioisotopes and stable isotopes. Examples of the isotope can include isotopes of hydrogen (^2H and ^3H), isotopes of carbon (^{11}C , ^{13}C and ^{14}C), isotopes of nitrogen (^{13}N and ^{15}N), isotopes of oxygen (^{15}O , ^{17}O and ^{18}O), and isotopes of fluorine (^{18}F). A composition comprising the antibody-drug conjugate labeled with such an isotope is useful as, for example, a therapeutic agent, a prophylactic agent, a research reagent, an assay reagent, a diagnostic agent, and an *in vivo* diagnostic imaging agent. Each and every antibody-drug

conjugate labeled with an isotope, and mixtures of antibody-drug conjugates labeled with an isotope at any given ratio are included in the present invention. The antibody-drug conjugate labeled with an isotope can be produced, for example, by using a starting material labeled with an isotope, instead of a starting material for the production method of the present invention mentioned later, according to a method known in the art.

[0209] *In vitro* cytotoxicity can be measured based on the activity of suppressing the proliferative responses of cells, for example. For example, a cancer cell line overexpressing CDH6 is cultured, and the anti-CDH6 antibody-drug conjugate is added at different concentrations to the culture system. Thereafter, its suppressive activity against focus formation, colony formation and spheroid growth can be measured. In this context, for example, by using a renal cell tumor- or ovarian tumor-derived cancer cell line, cell growth inhibition activity against renal cell tumor or ovarian tumor can be examined.

[0210] *In vivo* therapeutic effects on cancer in an experimental animal can be measured, for example, by administering the anti-CDH6 antibody-drug conjugate to a nude mouse into which a tumor cell line highly expressing CDH6 has been inoculated, and then measuring a change in the cancer cells. In this context, for example, by using an animal model derived from an immunodeficient mouse by the inoculation of renal cell carcinoma-, renal clear cell carcinoma-, papillary renal cell carcinoma-, ovarian cancer-, ovarian serous adenocarcinoma- or thyroid cancer-derived cells, therapeutic effects on renal

cell carcinoma, renal clear cell carcinoma, papillary renal cell carcinoma, ovarian cancer, ovarian serous adenocarcinoma or thyroid cancer can be measured.

[0211] The type of cancer to which the anti-CDH6 antibody-drug conjugate of the present invention is applied is not particularly limited as long as the cancer expresses CDH6 in cancer cells to be treated. Examples thereof can include renal cell carcinoma (e.g., renal clear cell carcinoma or papillary renal cell carcinoma), ovarian cancer, ovarian serous adenocarcinoma, thyroid cancer, bile duct cancer, lung cancer (e.g., small-cell lung cancer or non-small cell lung cancer), glioblastoma, mesothelioma, uterine cancer, pancreatic cancer, Wilms' tumor and neuroblastoma, though the cancer is not limited thereto as long as the cancer expresses CDH6. More preferred examples of the cancer can include renal cell carcinoma (e.g., renal clear cell carcinoma and papillary renal cell carcinoma) and ovarian cancer.

[0212] The anti-CDH6 antibody-drug conjugate of the present invention can preferably be administered to a mammal, and more preferably to a human.

[0213] A substance used in a pharmaceutical composition comprising the anti-CDH6 antibody-drug conjugate of the present invention can be appropriately selected from pharmaceutical additives and others usually used in this field, in terms of the applied dose or the applied concentration, and then used.

[0214] The anti-CDH6 antibody-drug conjugate of the present invention can be administered as a pharmaceutical composition comprising one or more pharmaceutically compatible components.

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For example, the pharmaceutical composition typically comprises one or more pharmaceutical carriers (e.g., sterilized liquids (e.g., water and oil (including petroleum oil and oil of animal origin, plant origin, or synthetic origin (e.g., peanut oil, soybean oil, mineral oil, and sesame oil))). Water is a more typical carrier when the pharmaceutical composition is intravenously administered. An aqueous saline solution, an aqueous dextrose solution, and an aqueous glycerol solution can also be used as a liquid carrier, in particular, for an injection solution. Suitable pharmaceutical vehicles are known in the art. If desired, the composition may also comprise a trace amount of a moisturizing agent, an emulsifying agent, or a pH buffering agent.

Examples of suitable pharmaceutical carriers are disclosed in "Remington's Pharmaceutical Sciences" by E. W. Martin. The prescription corresponds to an administration mode.

[0215] Various delivery systems are known, and they can be used for administering the anti-CDH6 antibody-drug conjugate of the present invention. Examples of the administration route can include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, and subcutaneous routes. The administration can be made by injection or bolus injection, for example. According to a specific preferred embodiment, the administration of the above-described antibody-drug conjugate is performed by injection. Parenteral administration is a preferred administration route.

[0216] According to a representative embodiment, the pharmaceutical composition is prescribed, as a pharmaceutical composition suitable for intravenous administration to a human,

according to conventional procedures. The composition for intravenous administration is typically a solution in a sterile and isotonic aqueous buffer solution. If necessary, the medicament may also contain a solubilizing agent and a local anesthetic to alleviate pain at an injection area (e.g., lignocaine). In general, the above-described ingredients are provided, either separately or together in a mixture in unit dosage form, as a freeze-dried powder or an anhydrous concentrate contained in a container which is obtained by sealing in, for example, an ampoule or a sachet indicating the amount of the active agent. When the medicament is to be administered by injection, it may be administered using, for example, an injection bottle containing water or saline of sterile pharmaceutical grade. When the medicament is to be administered by injection, an ampoule of sterile water or saline for injection may be provided such that the above-described ingredients are admixed with one another before administration.

[0217] The pharmaceutical composition of the present invention may be a pharmaceutical composition comprising only the anti-CDH6 antibody-drug conjugate of the present application, or may be a pharmaceutical composition comprising the anti-CDH6 antibody-drug conjugate and at least one other therapeutic agent for cancer. The anti-CDH6 antibody-drug conjugate of the present invention can also be administered together with an additional therapeutic agent for cancer, and can thereby enhance an anticancer effect. The additional anticancer agent used for such a purpose may be administered to an individual, simultaneously, separately, or continuously, together with the

antibody-drug conjugate. Otherwise, the additional anticancer agent and the anti-CDH6 antibody-drug conjugate may each be administered to the subject at different administration intervals. Examples of such a therapeutic agent for cancer can include tyrosine kinase inhibitors including imatinib, sunitinib, and regorafenib, CDK4/6 inhibitors including palbociclib, HSP90 inhibitors including TAS-116, MEK inhibitors including MEK162, and immune checkpoint inhibitors including nivolumab, pembrolizumab, and ipilimumab, though the therapeutic agent for cancer is not limited thereto as long as the drug has antitumor activity.

[0218] Such a pharmaceutical composition can be prepared as a formulation having a selected composition and a necessary purity in the form of a freeze-dried formulation or a liquid formulation. The pharmaceutical composition prepared as a freeze-dried formulation may be a formulation containing an appropriate pharmaceutical additive used in this field. Likewise, the liquid formulation can be prepared such that the liquid formulation contains various pharmaceutical additives used in this field.

[0219] The composition and concentration of the pharmaceutical composition also vary depending on the administration method. With regard to the affinity of the anti-CDH6 antibody-drug conjugate comprised in the pharmaceutical composition of the present invention for the antigen, i.e., the dissociation constant (K_d value) of the anti-CDH6 antibody-drug conjugate to the antigen, as the affinity increases (i.e., the K_d value is low), the pharmaceutical composition can exert medicinal effects, even if the applied dose thereof is decreased.

Accordingly, the applied dose of the antibody-drug conjugate can also be determined by setting the applied dose based on the status of the affinity of the antibody-drug conjugate for the antigen. When the antibody-drug conjugate of the present invention is administered to a human, it may be administered at a dose of, for example, from approximately 0.001 to 100 mg/kg once or a plurality of times at intervals of 1 to 180 days. It can be administered preferably at a dose of from 0.1 to 50 mg/kg and more preferably 1 to 50 mg/kg, 1 to 30 mg/kg, 1 to 20 mg/kg, 1 to 15 mg/kg, 2 to 50 mg/kg, 2 to 30 mg/kg, 2 to 20 mg/kg or 2 to 15 mg/kg a plurality of times at intervals of 1 to 4 weeks, preferably 2 to 3 weeks.

Examples

[0220] Hereinafter, the present invention will be specifically described in the following examples. However, these examples are not intended to limit the scope of the present invention. Furthermore, these examples should not be construed in a limited manner by any means. It is to be noted that, in the following examples, unless otherwise specified, individual operations regarding genetic manipulation have been carried out according to the method described in "Molecular Cloning" (Sambrook, J., Fritsch, E. F. and Maniatis, T., published by Cold Spring Harbor Laboratory Press in 1989) or other methods described in experimental manuals used by persons skilled in the art, or when commercially available reagents or kits have been used, the examples have been carried out in accordance with the instructions included in the commercially available products. In the present description, reagents, solvents and

starting materials are readily available from commercially available sources, unless otherwise specified.

[0221] [Example 1: Obtaining rat anti-human CDH6 antibody having internalization activity]

1)-1 Construction of human, mouse, rat and cynomolgus monkey CDH6 expression vectors

Using a human CDH6 protein (NP_004923)-encoding cDNA expression vector (OriGene Technologies Inc., RC217889), the cDNA was incorporated into a vector for mammalian expression according to a method known to a person skilled in the art to produce human CDH6 expression vector pcDNA3.1-hCDH6. The amino acid sequence of the human CDH6 ORF (open reading frame) is shown in SEQ ID NO: 1.

[0222] Using a mouse CDH6 protein (NP_031692)-encoding cDNA expression vector (OriGene Technologies Inc., MC221619), the cDNA was incorporated into a vector for mammalian expression according to a method known to a person skilled in the art to produce mouse CDH6 expression vectors pcDNA3.1-mCDH6 and p3xFLAG-CMV-9-mCDH6. The amino acid sequence of the mouse CDH6 ORF is shown in SEQ ID NO: 7.

[0223] Using each cDNA moiety of the rat CDH6 protein (NP_037059)-encoding cDNA expression vector (OriGene Technologies Inc., RN211850), the cDNA was incorporated into a vector for mammalian expression according to a method known to a person skilled in the art to produce human CDH6 expression vectors pcDNA3.1-rCDH6 and p3xFLAG-CMV-9-rCDH6. The amino acid sequence of the rat CDH6 ORF is shown in SEQ ID NO: 8.

[0224] cDNA encoding cynomolgus monkey CDH6 protein was cloned with cDNA synthesized from total RNA of the cynomolgus monkey

kidney as a template using primer 1 (5'-CACCATGAGAACTTACCGCTACTTCTTGCTGCTC-3') (SEQ ID NO: 85) and primer 2 (5'-TTAGGAGTCTTTGTCACTGTCCACTCCTCC-3') (SEQ ID NO: 86). It was confirmed that the obtained sequence corresponded to the extracellular region of cynomolgus monkey CDH6 (NCBI, XP_005556691.1). It was also confirmed that the sequence corresponded to the full-length sequence of cynomolgus monkey CDH6 (EHH54180.1) registered in EMBL. The cDNA was incorporated into a vector for mammalian expression according to a method known to a person skilled in the art to produce cynomolgus monkey CDH6 expression vector pcDNA3.1-cynoCDH6. The amino acid sequence of the cynomolgus monkey CDH6 ORF is shown in SEQ ID NO: 9.

[0225] EndoFree Plasmid Giga Kit (Qiagen N.V.) was used for mass production of the produced plasmid DNA.

[0226] 1)-2 Immunization

For immunization, WKY/Izm female rats (Japan SLC, Inc.) were used. First, the lower limbs of each rat were pre-treated with Hyaluronidase (Sigma-Aldrich Co. LLC), and thereafter, the human CDH6 expression vector pcDNA3.1-hCDH6 produced in Example 1)- 1 was intramuscularly injected into the same sites. Subsequently, employing ECM830 (BTX), *in vivo* electroporation was carried out on the same sites using a two-needle electrode. Approximately once every two weeks, the same *in vivo* electroporation was repeated, and thereafter, lymph nodes or the spleen were collected from the rat, and then used in production of hybridomas.

[0227] 1)-3 Production of hybridomas

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The lymph node cells or the spleen cells were fused with mouse myeloma SP2/0-ag14 cells (ATCC, No. CRL-1 581) according to electrical cell fusion, using a LF301 Cell Fusion Unit (BEX Co., Ltd.), and the cells were then suspended and diluted with ClonaCell-HY Selection Medium D (StemCell Technologies Inc.), and then cultured under conditions of 37°C and 5% CO₂. Individual hybridoma colonies that appeared in the culture medium were collected as monoclonal hybridomas, then suspended in ClonaCell-HY Selection Medium E (StemCell Technologies Inc.), and then cultured under conditions of 37°C and 5% CO₂. After moderate proliferation of cells, frozen stocks of individual hybridoma cells were produced, while the obtained hybridoma culture supernatant was used to screen for anti-human CDH6 antibody-producing hybridomas.

[0228] 1)-4 Antibody-producing hybridoma screening according to Cell-ELISA method

1)-4-1 Preparation of antigen gene-expressing cells for use in Cell-ELISA

293 α cells (a stable expression cell line derived from HEK293 cells expressing integrin α v and integrin β 3) were prepared at 5 x 10⁵ cells/mL in DMEM medium supplemented with 10% FBS. In accordance with transduction procedures for using Lipofectamine 2000 (Thermo Fisher Scientific Inc.), DNA of pcDNA3.1-hCDH6 or pcDNA3.1-cynoCDH6, or pcDNA3.1 as a negative control was introduced into the 293 α cells, and the cells were dispensed in an amount of 100 μ L/well onto a 96-well plate (Corning Inc.). Thereafter, the cells were cultured under conditions of 37°C and 5% CO₂ in DMEM medium supplemented with

10% FBS for 24 to 27 hours. The obtained transfected cells were used for Cell-ELISA in an adhesive state.

[0229] 1)-4-2 Cell-ELISA

The culture supernatant of the 293 α cells transfected with the expression vector prepared in Example 1)-4-1 was removed, and the culture supernatant from each hybridoma was then added to the 293 α cells transfected with pcDNA3.1-hCDH6 or pcDNA3.1-cynoCDH6, or pcDNA3.1. The cells were left standing at 4°C for 1 hour. The cells in the wells were washed once with PBS (+) supplemented with 5% FBS, and thereafter, Anti-Rat IgG-Peroxidase antibody produced in rabbit (Sigma-Aldrich Co. LLC) that had been 500-fold diluted with PBS (+) supplemented with 5% FBS was added to the wells. The cells were left standing at 4°C for 1 hour. The cells in the wells were washed three times with PBS (+) supplemented with 5% FBS, and thereafter, OPD coloring solution (which had been prepared by dissolving o-phenylenediamine dihydrochloride (Wako Pure Chemical Industries, Ltd.) and H₂O₂ in an OPD solution (0.05 M trisodium citrate, 0.1 M disodium hydrogen phosphate 12-water; pH 4.5), so that the substances became 0.4 mg/ml and 0.6% (v/v), respectively, was added in an amount of 100 μ L/well to the wells. A coloring reaction was carried out with occasional stirring. Thereafter, 1 M HCl was added to the plate (100 μ L/well) to terminate the coloring reaction, followed by measurement of the absorbance at 490 nm using a plate reader (ENVISION: PerkinElmer, Inc.). Hybridomas that produced a culture supernatant exhibiting higher absorbance in the 293 α cells transfected with the pcDNA3.1-hCDH6 or pcDNA3.1-cynoCDH6 expression vector than that in the 293 α

cells transfected with the control pcDNA3.1 were selected as hybridomas producing antibodies binding to human CDH6 and cynomolgus monkey CDH6.

[0230] 1)-5 Selective screening for antibody binding to cynomolgus monkey CDH6 according to flow cytometry

1)-5-1 Preparation of antigen gene-expressing cells for use in flow cytometry analysis

293T cells were seeded in a 225-cm² flask (Sumitomo Bakelite Co., Ltd.) at 5×10^4 cells/cm², and the cells were then cultured overnight under conditions of 37°C and 5% CO₂ in DMEM medium supplemented with 10% FBS. pcDNA3.1-cynoCDH6 or pcDNA3.1 as a negative control was introduced into the 293T cells using Lipofectamine 2000, and the cells were further cultured overnight under conditions of 37°C and 5% CO₂. The 293T cells transfected with each vector were treated with TrypLE Express (Thermo Fisher Scientific Corp.), and the cells were washed with DMEM supplemented with 10% FBS, and then suspended in PBS supplemented with 5% FBS. The obtained cell suspension was used in flow cytometry analysis.

[0231] 1)-5-2 Flow cytometry analysis

The binding specificity to cynomolgus monkey CDH6 of an antibody produced from the human CDH6- and cynomolgus monkey CDH6-binding antibody-producing hybridomas that had been selected by Cell-ELISA in Example 1)-4 was further confirmed by flow cytometry. The suspension of the transiently expressing 293T cells prepared in Example 1)-5-1 was centrifuged, and the supernatant was then removed. Thereafter, the cells were suspended by the addition of the culture supernatant from each hybridoma. The cells were left standing

at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, and thereafter, the cells were suspended by the addition of Anti-Rat IgG FITC conjugate (Sigma-Aldrich Co. LLC) that had been 500-fold diluted with PBS supplemented with 5% FBS. The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, and then re-suspended in PBS supplemented with 5% FBS and 2 µg/ml 7-aminoactinomycin D (Molecular Probes, Inc.), followed by detection using a flow cytometer (FC500; Beckman Coulter, Inc.). The data was analyzed using FlowJo (Tree Star, Inc.). After dead cells were removed from analysis by gating out 7-aminoactinomycin D-positive cells, a histogram of the FITC fluorescence intensity of live cells was generated. Hybridomas producing antibodies specifically binding to cynomolgus monkey CDH6 expressed on the cell membrane surface were selected based on results where the histogram for the antibody shifted to the strong fluorescence intensity side in the 293T cells transfected with pcDNA3.1-cynoCDH6 compared with the 293T cells transfected with the control pcDNA3.1.

[0232] 1)-6 Determination of isotype of rat monoclonal antibody

Clones rG019, rG055, rG056, and rG061 suggested to bind specifically and strongly to human CDH6 and monkey CDH6 were selected from among the rat anti-CDH6 antibody-producing hybridomas selected in Example 1)-5, and the isotype of each antibody was identified. The heavy chain subclass and the light chain type of the antibody were determined using a RAT MONOCLONAL ANTIBODY ISOTYPING TEST KIT (DS Pharma Biomedical Co., Ltd.). As a result, it was confirmed that all of these 4

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clones rG019, rG055, rG056, and rG061 had a heavy chain of IgG2b subclass and a light chain of κ chain type.

[0233] 1)-7 Preparation of rat anti-human CDH6 antibody

1)-7-1 Production of culture supernatant

The rat anti-human CDH6 monoclonal antibodies were purified from the hybridoma culture supernatants. First, the volume of each rat anti-CDH6 monoclonal antibody-producing hybridoma was sufficiently increased with ClonaCell-HY Selection Medium E (StemCell Technologies Inc.), and thereafter, the medium was exchanged with Hybridoma SFM (Thermo Fisher Scientific Corp.) to which 20% of Ultra Low IgG FBS (Thermo Fisher Scientific Corp.) had been added. Thereafter, the hybridoma was cultured for 4 to 5 days. The resulting culture supernatant was harvested, and insoluble matter was removed therefrom by passing through a 0.8- μ m filter, and through a 0.2- μ m filter.

[0234] 1)-7-2 Purification of rat anti-CDH6 antibody

An antibody (rat anti-CDH6 antibody (rG019, rG055, rG056 or rG061)) was purified from the culture supernatant of hybridomas prepared in Example 1)-7-1 according to Protein G affinity chromatography. The antibody was adsorbed on a Protein G column (GE Healthcare Biosciences Corp.), the column was then washed with PBS, and the antibody was then eluted with a 0.1 M glycine/HCl aqueous solution (pH 2.7). 1 M Tris-HCl (pH 9.0) was added to the eluate, so that the pH was adjusted to pH 7.0 to 7.5. Thereafter, using Centrifugal UF Filter Device VIVASPIN20 (molecular weight cutoff: UF30K, Sartorius Inc.), the buffer was replaced with HBSor (25 mM histidine/5% sorbitol, pH 6.0), while the antibody was

concentrated, so that the concentration of the antibody was adjusted to 1 mg/mL. Finally, the antibody was filtrated through a Minisart-Plus filter (Sartorius Inc.) to obtain a purified sample.

[0235] [Example 2: *In vitro* evaluation of rat anti-CDH6 antibody]

2)-1 Evaluation of binding ability of rat anti-CDH6 antibody by flow cytometry

The human CDH6-binding activity of the rat anti-CDH6 antibody produced in Example 1)-7 was evaluated by flow cytometry. Using Lipofectamine 2000 (Thermo Fisher Scientific Inc.), pcDNA3.1-hCDH6 produced in Example 1)-1 was transiently introduced into 293T cells (ATCC). The cells were cultured overnight under conditions of 37°C and 5% CO₂, and thereafter, a cell suspension was prepared. The suspension of the transfected 293T cells was centrifuged, and the supernatant was then removed. Thereafter, the cells were suspended by the addition of each of the 4 rat anti-CDH6 monoclonal antibodies (clone Nos: rG019, rG055, rG056 and rG061), which had been prepared in Example 1)-7, or rat IgG control (R&D Systems, Inc.) (final concentration: 10 ng/mL). The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, and then suspended by the addition of Anti-Rat IgG (whole molecule)-FITC antibody produced in rabbit (Sigma-Aldrich Co. LLC) that had been 50-fold diluted with PBS supplemented with 5% FBS. The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, followed by detection using a flow cytometer (FC500; Beckman Coulter, Inc.). The

data was analyzed using FlowJo (Tree Star, Inc.). The results are shown in Figure 1. In the histogram of Figure 1, the abscissa depicts FITC fluorescence intensity indicating the amount of the antibody bound, and the ordinate depicts a cell count. The shaded histogram shows that negative control 293T cells untransfected with hCDH6 were used, and the open solid line histogram shows that hCDH6-transfected 293T cells were used. As seen, fluorescence intensity was enhanced by the binding of the antibody to hCDH6 on the cell surface. The rat IgG control binds to neither of the cells. As a result, it was confirmed that the 4 produced rat anti-CDH6 monoclonal antibodies bind to 293T cells transfected with pCDNA3.1-hCDH6.

[0236] 2)-2 Analysis of CDH6-binding site of rat anti-CDH6 antibody by flow cytometry

2)-2-1 Construction of expression vector for each domain deletion mutant of human CDH6

The full-length extracellular region of human CDH6 has five extracellular domains, EC1 (SEQ ID NO: 2), EC2 (SEQ ID NO: 3), EC3 (SEQ ID NO: 4), EC4 (SEQ ID NO: 5), and EC5 (SEQ ID NO: 6). A gene to be expressed such that each one of the five EC domains could be deleted from full-length human CDH6 was synthesized by GeneArt, and incorporated into p3xFLAG-CMV-9 vectors for mammalian expression (Sigma-Aldrich Co. LLC) according to a method known to a person skilled in the art in order to produce an expression vector for each domain deletion mutant lacking any one of EC1 to EC5.

[0237] 2)-2-2 Epitope analysis of rat anti-CDH6 antibody by flow cytometry using domain deletion mutant

The epitopes to which the rat anti-human CDH6 antibodies bound were identified by flow cytometry analysis using a 293 α cell line transfected with each EC domain deletion vector. Using Lipofectamine 2000 (Thermo Fisher Scientific Inc.), each domain deletion mutant expression vector produced in Example 2)-2-1, or pcDNA3.1-hCDH6 for the expression of full-length human CDH6 was transiently introduced into a 293 α cell line, which was a cell line derived from HEK293 cells by stable transfection with integrin α v and integrin β 3 expression vectors. The cells were cultured overnight under conditions of 37°C and 5% CO₂, and thereafter, a cell suspension was prepared. The suspension of the transfected 293 α cells was centrifuged, and a supernatant was then removed. Thereafter, the cells were suspended by the addition of each of the 4 rat anti-CDH6 monoclonal antibodies (clone Nos: rG019, rG055, rG056 and rG061), which had been prepared in Example 1)-7, or rat IgG control (R&D Systems, Inc.) (final concentration: 20 nM). The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, and then suspended by the addition of Anti-Rat IgG (whole molecule)-FITC antibody produced in rabbit (Sigma-Aldrich Co. LLC) that had been 50-fold diluted with PBS supplemented with 5% FBS. The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, followed by detection using a flow cytometer (Canto II; BD Biosciences). The data was analyzed using FlowJo (Tree Star, Inc.). The results are shown in Figures 2-1 to 2-6. In the histograms of Figures 2-1 to 2-6, the abscissa depicts FITC fluorescence intensity indicating the amount of the antibody

bound, and the ordinate depicts cell count. The shaded histogram shows that negative control untransfected 293 α cells were used, and the open solid line histogram shows that 293 cells expressing full-length hCDH6 or each EC domain deletion mutant were used. Fluorescence intensity is enhanced when the antibody binds to full-length hCDH6 or each EC domain deletion mutant on the surface of cells. The rat IgG control binds to none of the transfected cells. The 4 produced rat anti-CDH6 monoclonal antibodies bind to the full-length hCDH6, the EC1 deletion mutant, the EC2 deletion mutant, the EC4 deletion mutant, and the EC5 deletion mutant, but do not bind to the EC3 deletion mutant. From this result, it was demonstrated that the 4 rat anti-CDH6 monoclonal antibodies specifically bind to hCDH6 with EC3 as an epitope.

[0238] 2)-3 Internalization activity of rat anti-CDH6 antibody

2)-3-1 Confirmation of CDH6 expression in human tumor cell line

In order to select a CDH6-positive human tumor cell line for use in the evaluation of the obtained antibodies, CDH6 expression information was retrieved from a known database, and the expression of CDH6 on the cell membrane surface was evaluated by flow cytometry. Human ovarian tumor cell lines NIH:OVCA-3, PA-1 and ES-2 and human renal cell tumor cell line 786-O (all obtained from ATCC) were each cultured under conditions of 37°C and 5% CO₂, and thereafter, a cell suspension was prepared. The cells were centrifuged, and the supernatant was then removed. Thereafter, the cells were suspended by the addition of a commercially available anti-human CDH6 antibody (MABU2715, R&D Systems, Inc.) or mouse

IgG1 (BD Pharmingen) as a negative control (final concentration: 50 µg/mL). The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, and then suspended by the addition of F(ab')₂ Fragment of FITC-conjugated Goat Anti-mouse immunoglobulins (Dako) that had been 50-fold diluted with PBS supplemented with 5% FBS. The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, followed by detection using a flow cytometer (Canto II; BD Biosciences). The data was analyzed using FlowJo (Tree Star, Inc.). The results are shown in Figure 3. In the histogram of Figure 3, the abscissa depicts FITC fluorescence intensity indicating the amount of the antibody bound, and the ordinate depicts cell count. The shaded histogram shows that the negative control mIgG1 was used in staining, and the open solid line histogram shows that the anti-human CDH6 antibody was used in staining. As seen, fluorescence intensity was enhanced by the binding of the antibody to hCDH6 on the surface of cells. The mIgG1 control binds to none of the cells. As a result, it was confirmed that the NIH:OVCA-3, PA-1 and 786-O cell lines endogenously express CDH6 on the cell surface. On the other hand, it was demonstrated that the ES-2 cell line expresses no CDH6.

[0239] 2)-3-2 Evaluation of internalization activity of rat anti-CDH6 antibody

The internalization activity of the rat anti-CDH6 antibodies was evaluated using an anti-rat IgG reagent Rat-ZAP (Advanced Targeting Systems) conjugated with a toxin (saporin) inhibiting protein synthesis. Specifically, human CDH6-

positive ovarian tumor cell line NIH:OVCAR-3 (ATCC) was seeded at 4×10^3 cells/well on a 96-well plate, and then cultured overnight under conditions of 37°C and 5% CO₂. Human CDH6-positive renal cell tumor cell line 786-O (ATCC) was seeded at 1×10^3 cells/well on a 96-well plate, and then cultured overnight. On the next day, each rat anti-CDH6 antibody (final concentration: 1 nM) or rat IgG2b antibody (R&D Systems, Inc.) as a negative control antibody was added to the plate. Rat-ZAP (final concentration: 0.5 nM) or Goat Anti-Rat IgG, Fc (gamma) Fragment Specific (Jackson ImmunoResearch Laboratories, Inc.) unconjugated with the toxin (final concentration: 0.5 nM) as a negative control was further added to the plate, and the cells were cultured under conditions of 37°C and 5% CO₂ for 3 days. The number of live cells was measured by the quantification of ATP activity (RLU) using a CellTiter-Glo(TM) Luminescent Cell Viability Assay (Promega Corp.). In this evaluation, Rat-ZAP is taken up into cells in a manner dependent on the internalization activity of the rat anti-CDH6 antibody, so that saporin inhibiting protein synthesis is released into the cells, so as to suppress cell growth. A cell growth inhibition effect brought about by the addition of the anti-CDH6 antibody was indicated by a relative survival rate when the number of live cells in a well supplemented with the negative control instead of Rat-ZAP was defined as 100%. Figure 4 shows a graph and a table of the cell survival rate. As a result, it was demonstrated that the rat anti-CDH6 antibodies bind to CDH6 and cause internalization.

[0240] [Example 3: Determination of nucleotide sequence of cDNA encoding variable region of rat anti-CDH6 antibody]

3)-1 Amplification and sequencing of rG019 heavy chain variable region and light chain variable region gene fragments

3)-1-1 Preparation of total RNA from G019

In order to amplify cDNA encoding each variable region of rG019, total RNA was prepared from G019 using TRIzol Reagent (Ambion, Inc.).

[0241] 3)-1-2 Amplification of cDNA encoding rG019 heavy chain variable region by 5'-RACE PCR and determination of nucleotide sequence

cDNA encoding the heavy chain variable region was amplified using approximately 1 µg of the total RNA prepared in Example 3)-1-1 and a SMARTer RACE cDNA Amplification Kit (Clontech Laboratories, Inc.). As primers used to amplify the cDNA of the variable region of the rG019 heavy chain gene according to PCR, UPM (Universal Primer A Mix: included with SMARTer RACE cDNA Amplification Kit) and primers designed from the sequences of the constant regions of known rat heavy chains were used.

[0242] The heavy chain variable region-encoding cDNA amplified by 5'-RACE PCR was cloned into a plasmid, and thereafter, the nucleotide sequence of the cDNA of the heavy chain variable region was subjected to sequence analysis.

[0243] The determined nucleotide sequence of the cDNA encoding the heavy chain variable region of rG019 is shown in SEQ ID NO: 16, and the amino acid sequence thereof is shown in SEQ ID NO: 15.

[0244] 3)-1-3 Amplification of cDNA encoding rG019 light chain variable region by 5'-RACE PCR and determination of nucleotide sequence

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Amplification and sequencing were carried out by the same method as that applied in Example 3)-1-2. However, as primers used to amplify the cDNA of the variable region of the rG019 light chain gene according to PCR, UPM (Universal Primer A Mix: included with SMARTer RACE cDNA Amplification Kit) and primers designed from the sequences of the constant regions of known rat light chains were used.

[0245] The determined nucleotide sequence of the cDNA encoding the light chain variable region of rG019 is shown in SEQ ID NO: 11, and the amino acid sequence thereof is shown in SEQ ID NO: 10.

[0246] 3)-2 Amplification and sequencing of rG055 heavy chain variable region and light chain variable region gene fragments

The sequences were determined by the same method as that applied in Example 3)-1.

[0247] The determined nucleotide sequence of the cDNA encoding the heavy chain variable region of rG055 is shown in SEQ ID NO: 26, and the amino acid sequence thereof is shown in SEQ ID NO: 25. The nucleotide sequence of the cDNA encoding the light chain variable region of rG055 is shown in SEQ ID NO: 21, and the amino acid sequence thereof is shown in SEQ ID NO: 20.

[0248] 3)-3 Amplification and sequencing of rG056 heavy chain variable region and light chain variable region gene fragments

The sequences were determined by the same method as that applied in Example 3)-1.

[0249] The determined nucleotide sequence of the cDNA encoding the heavy chain variable region of rG056 is shown in SEQ ID NO: 36, and the amino acid sequence thereof is shown in SEQ ID NO: 35. The nucleotide sequence of the cDNA encoding the

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light chain variable region of rG056 is shown in SEQ ID NO: 31, and the amino acid sequence thereof is shown in SEQ ID NO: 30.

[0250] 3)-4 Amplification and sequencing of rG061 heavy chain variable region and light chain variable region gene fragments

The sequences were determined by the same method as that applied in Example 3)-1.

[0251] The determined nucleotide sequence of the cDNA encoding the heavy chain variable region of rG061 is shown in SEQ ID NO: 46, and the amino acid sequence thereof is shown in SEQ ID NO: 45. The nucleotide sequence of the cDNA encoding the light chain variable region of rG061 is shown in SEQ ID NO: 41, and the amino acid sequence thereof is shown in SEQ ID NO: 40.

[0252] [Example 4: Production of human chimeric anti-CDH6 antibody chG019]

4)-1 Construction of human chimeric anti-CDH6 antibody chG019 expression vector

4)-1-1 Construction of chimeric and humanized light chain expression vector pCMA-LK

An approx. 5.4-kb fragment, which had been obtained by digesting plasmid pcDNA3.3-TOPO/LacZ (Invitrogen Corp.) with the restriction enzymes XbaI and PmeI, was bound to a DNA fragment comprising a DNA sequence (SEQ ID NO: 50) encoding a human light chain signal sequence and a human κ chain constant region, using an In-Fusion Advantage PCR cloning kit (Clontech Laboratories, Inc.), to produce pcDNA3.3/LK.

[0253] A neomycin expression unit was removed from pcDNA3.3/LK to construct pCMA-LK.

[0254] 4)-1-2 Construction of chimeric and humanized IgG1 type heavy chain expression vector pCMA-G1

A DNA fragment, which had been obtained by digesting pCMA-LK with XbaI and PmeI to remove the DNA sequence encoding the light chain signal sequence and the human κ chain constant region therefrom, was bound to a DNA fragment comprising a DNA sequence (SEQ ID NO: 51) encoding a human heavy chain signal sequence and a human IgG1 constant region, using an In-Fusion Advantage PCR cloning kit (Clontech Laboratories, Inc.), to construct pCMA-G1.

[0255] 4)-1-3 Construction of chG019 heavy chain expression vector

A DNA fragment from nucleotide positions 36 to 440 in the nucleotide sequence of the chG019 heavy chain shown in SEQ ID NO: 57 was synthesized (GENEART). Using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), the synthesized DNA fragment was inserted into a site of pCMA-G1 that had been cleaved with the restriction enzyme BlnI, so as to construct a chG019 heavy chain expression vector. It is to be noted that, for the chG019 heavy chain, a CDR sequence with cysteine substituted with proline was used in order to prevent unpredictable disulfide bonds.

[0256] 4)-1-4 Construction of chG019 light chain expression vector

A DNA fragment comprising a DNA sequence (SEQ ID NO: 52) encoding the chG019 light chain was synthesized (GENEART). Using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), the synthesized DNA fragment was bound to a DNA fragment, which had been obtained by digesting pCMA-LK with XbaI and PmeI to remove the DNA sequence encoding the light chain signal sequence and the human κ chain constant region

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therefrom, so as to construct a chG019 light chain expression vector.

[0257] 4)-2 Production and purification of human chimeric anti-CDH6 antibody chG019

4)-2-1 Production of chG019

In accordance with the manual, FreeStyle 293F cells (Invitrogen Corp.) were cultured and passaged. 1.2×10^9 FreeStyle 293F cells (Invitrogen Corp.) in the logarithmic growth phase were seeded on a 3-L Fernbach Erlenmeyer Flask (Corning Inc.), then diluted with FreeStyle 293 expression medium (Invitrogen Corp.) at 2.0×10^6 cells/mL. To 40 ml of Opti-Pro SFM medium (Invitrogen Corp.), 0.24 mg of the heavy chain expression vector, 0.36 mg of the light chain expression vector and 1.8 mg of Polyethyleneimine (Polyscience #24765) were added, and the obtained mixture was gently stirred. After incubation for 5 minutes, the mixture was added to the FreeStyle 293F cells. The cells were shake-cultured at 90 rpm in an 8% CO₂ incubator at 37°C for 4 hours, and thereafter, 600 mL of EX-CELL VPRO medium (SAFC Biosciences Inc.), 18 mL of GlutaMAX I (GIBCO), and 30 mL of Yeastolate Ultrafiltrate (GIBCO) were added to the culture. The cells were further shake-cultured at 90 rpm in an 8% CO₂ incubator at 37°C for 7 days. The obtained culture supernatant was filtrated through a Disposable Capsule Filter (Advantec #CCS-045-E1H).

[0258] 4)-2-2 Purification of chG019

An antibody was purified from the culture supernatant obtained in Example 4)-2-1 by a one-step process according to rProtein A affinity chromatography. The culture supernatant was applied to a column that had been packed with

MabSelectSuRe (GE Healthcare Biosciences Corp.) equilibrated with PBS, and thereafter, the column was washed with PBS in an amount of two or more times the volume of the column.

Subsequently, the antibody was eluted with a 2 M arginine hydrochloride solution (pH 4.0), so that a fraction containing an antibody was collected. The fraction was dialyzed (Thermo Fisher Scientific Inc., Slide-A-Lyzer Dialysis Cassette), so that the buffer was replaced with HBSor (25 mM histidine/5% sorbitol, pH 6.0). Using a Centrifugal UF Filter Device VIVASPIN20 (molecular weight cutoff: UF10K, Sartorius Inc.), the antibody was concentrated, so that the concentration of IgG was adjusted to 5 mg/ml or more. Finally, the antibody was filtrated through a Minisart-Plus filter (Sartorius Inc.) to obtain a purified sample.

[0259] 4)-3 Evaluation of binding activity of human chimeric anti-CDH6 antibody chG019

The CDH6-binding activity of the human chimeric anti-CDH6 antibody chG019 purified in 4)-2 was confirmed by flow cytometry. Using Lipofectamine 2000, pcDNA3.1-hCDH6 or pcDNA3.1-cynoCDH6 produced in Example 1)-1, or pcDNA3.1 was transiently introduced into 293 α cells. The cells were cultured overnight under conditions of 37°C and 5% CO₂, and thereafter, a cell suspension was prepared. chG019 was added to the suspension of each of these cells. The cells were left standing at 4°C for 1 hour. Thereafter, the cells were washed twice with PBS supplemented with 5% FBS, and then suspended by the addition of PE-labeled F(ab')₂ Fragment anti-human IgG, Fc γ antibody (Jackson ImmunoResearch Laboratories, Inc.) that had been 500-fold diluted with PBS supplemented with 5% FBS.

The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, and then re-suspended in PBS supplemented with 5% FBS, followed by detection using a flow cytometer (Canto II; BD Biosciences). The data was analyzed using FlowJo (Tree Star, Inc.). As shown in Figure 5, chG019 did not bind to the 293T cells transfected with pcDNA3.1 as a negative control, but did bind to the 293T cells transfected with pcDNA3.1-hCDH6 or pcDNA3.1-cynoCDH6 in an antibody concentration-dependent manner. In Figure 5, the abscissa depicts antibody concentration, and the ordinate depicts the amount of the antibody bound, based on mean fluorescence intensity. It is evident from this result that chG019 specifically binds to human CDH6 and cynomolgus monkey CDH6 with almost equivalent binding activity.

[0260] [Example 5: Production of humanized anti-CDH6 antibody]

5)-1 Design of humanized form of anti-CDH6 antibody

5)-1-1 Molecular modeling of chG019 variable region

The molecular modeling of the variable regions of chG019 exploited a method known as homology modeling (Methods in Enzymology, 203, 121-153, (1991)). The commercially available protein three-dimensional structure analysis program BioLuminate (manufactured by Schrodinger, LLC) was employed using, as a template, a structure (PDB ID: 2I9L) registered in Protein Data Bank (Nuc. Acid Res. 35, D301-D303 (2007)) with a high sequence identity to the heavy chain and light chain variable regions of chG019.

[0261] 5)-1-2 Design of amino acid sequence of humanized hG019

chG019 was humanized by CDR grafting (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). The consensus sequences of

human gamma chain subgroup 1 and kappa chain subgroup 1 determined by KABAT et al. (Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service National Institutes of Health, Bethesda, MD. (1991)) had high identity to the framework regions of chG019, and based on this, they were selected as acceptors for the heavy chain and the light chain, respectively. Donor residues to be grafted onto the acceptors were selected by analyzing three-dimensional models with reference to, for example, the criteria given by Queen et al. (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)).

[0262] 5)-2 Humanization of chG019 heavy chain

Three heavy chains thus designed were named hH01, hH02 and hH04. The full-length amino acid sequence of the hH01 heavy chain is shown in SEQ ID NO: 69. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 69 is shown in SEQ ID NO: 70. The full-length amino acid sequence of the heavy chain hH02 is shown in SEQ ID NO: 73. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 73 is shown in SEQ ID NO: 74. The full-length amino acid sequence of the heavy chain hH04 is shown in SEQ ID NO: 77. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 77 is shown in SEQ ID NO: 78.

[0263] 5)-3 Humanization of chG019 light chain

Two light chains thus designed were named hL02 and hL03. The full-length amino acid sequence of the hL02 light chain is shown in SEQ ID NO: 61. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 61 is shown in SEQ ID NO: 62. The full-length amino acid sequence of the light chain hL03 is

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shown in SEQ ID NO: 65. The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 65 is shown in SEQ ID NO: 66.
[0264] 5)-4 Design of humanized hG019 by combination of heavy chain and light chain

An antibody consisting of hH01 and hL02 was named "H01L02 antibody" or "H01L02". An antibody consisting of hH02 and hL02 was named "H02L02 antibody" or "H02L02". An antibody consisting of hH02 and hL03 was named "H02L03 antibody" or "H02L03". An antibody consisting of hH04 and hL02 was named "H04L02 antibody" or "H04L02".

[0265] 5)-5 Expression of humanized anti-CDH6 antibody

5)-5-1 Construction of humanized hG019 heavy chain expression vector

5)-5-1-1 Construction of humanized hG019-H01 type heavy chain expression vector

A DNA fragment from nucleotide positions 36 to 440 in the nucleotide sequence of the humanized hG019-H01 type heavy chain shown in SEQ ID NO: 70 was synthesized (GENEART). A humanized hG019-H01 type heavy chain expression vector was constructed by the same method as that applied in Example 4)-1-3.

[0266] 5)-5-1-2 Construction of humanized hG019-H02 type heavy chain expression vector

A DNA fragment from nucleotide positions 36 to 440 in the nucleotide sequence of the humanized hG019-H02 type heavy chain shown in SEQ ID NO: 74 was synthesized (GENEART). A humanized hG019-H02 type heavy chain expression vector was constructed by the same method as that applied in Example 4)-1-3.

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[0267] 5)-5-1-3 Construction of humanized hG019-H04 type heavy chain expression vector

A DNA fragment from nucleotide positions 36 to 440 in the nucleotide sequence of the humanized hG019-H04 type heavy chain shown in SEQ ID NO: 78 was synthesized (GENEART). A humanized hG019-H04 type heavy chain expression vector was constructed by the same method as that applied in Example 4)-1-3.

[0268] 5)-5-2 Construction of humanized hG019 light chain expression vector

5)-5-2-1 Construction of humanized hG019-L02 type light chain expression vector

A DNA fragment comprising a humanized hG019-L02 type light chain variable region-encoding DNA sequence from nucleotide positions 37 to 399 in the nucleotide sequence of the humanized hG019-L02 type light chain shown in SEQ ID NO: 62 was synthesized (GENEART). Using an In-Fusion HD PCR cloning kit (Clontech Laboratories, Inc.), the synthesized DNA fragment was inserted into a site of pCMA-LK that had been cleaved with the restriction enzyme BsiWI, so as to construct a humanized hG019-L02 type light chain expression vector.

[0269] 5)-5-2-2 Construction of humanized hG019-L03 type light chain expression vector

A DNA fragment comprising a humanized hG019-L03 type light chain variable region-encoding DNA sequence from nucleotide positions 37 to 399 in the nucleotide sequence of the humanized hG019-L03 type light chain shown in SEQ ID NO: 66 was synthesized (GENEART). A humanized hG019-L03 type

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light chain expression vector was constructed by the same method as that applied in Example 5)-5-2-1.

[0270] 5)-5-3 Preparation of humanized hG019

5)-5-3-1 Production of H01L02, H02L02, H02L03 and H04L02

The antibodies were produced by the same method as that applied in Example 4)-2-1. H01L02, H02L02, H02L03 and H04L02 were produced by the combination of the heavy chain and the light chain shown in Example 5)-4.

[0271] 5)-5-3-2 Two-step purification of H01L02, H02L02, H02L03 and H04L02

The antibody was purified from the culture supernatant obtained in Example 5)-5-3-1, by a two-step process, namely, by rProtein A affinity chromatography and ceramic hydroxyapatite. The culture supernatant was applied to a column that had been packed with MabSelectSuRe (manufactured by GE Healthcare Biosciences Corp.) equilibrated with PBS, and thereafter, the column was washed with PBS in an amount of two or more times the volume of the column. Subsequently, the antibody was eluted using a 2 M arginine hydrochloride solution (pH 4.0). A fraction containing the antibody was dialyzed (Thermo Fisher Scientific Inc., Slide-A-Lyzer Dialysis Cassette), so that the buffer was replaced with PBS. The antibody solution was 5-fold diluted with a buffer of 5 mM sodium phosphate/50 mM MES/pH 7.0, and then applied to a ceramic hydroxyapatite column (Bio-Rad Laboratories, Inc., Bio-Scale CHT Type-1 Hydroxyapatite Column) that had been equilibrated with a buffer of 5 mM NaPi/50 mM MES/30 mM NaCl/pH 7.0. Elution was carried out on a linear concentration gradient of sodium chloride, so that a fraction

containing an antibody was collected. This fraction was dialyzed (Thermo Fisher Scientific Inc., Slide-A-Lyzer Dialysis Cassette), so that the buffer was replaced with HBSor (25 mM histidine/5% sorbitol, pH 6.0). The antibody was concentrated with Centrifugal UF Filter Device VIVASPIN20 (molecular weight cutoff: UF10K, Sartorius Inc.), thereby adjusting the IgG concentration to 20 mg/ml. Finally, the antibody was filtrated through a Minisart-Plus filter (Sartorius Inc.) to obtain a purified sample.

[0272] [Reference Example 1: Production of anti-CDH6 antibody NOV0712]

The anti-CDH6 antibody NOV0712 used in the Examples was produced with reference to the light chain full-length and heavy chain full-length amino acid sequences (SEQ ID NO: 235 and SEQ ID NO: 234, respectively, in International Publication No. WO 2016/024195) of NOV0712 described in International Publication No. WO 2016/024195.

[0273] Reference Example 1)-1 Anti-CDH6 antibody NOV0712

Reference Example 1)-1-1 Construction of anti-CDH6 antibody NOV0712 heavy chain expression vector

A NOV0712 heavy chain variable region-encoding DNA fragment from nucleotide positions 36 to 428 in the nucleotide sequence of the NOV0712 heavy chain shown in SEQ ID NO: 84 was synthesized (GENEART). A NOV0712 heavy chain expression vector was constructed by the same method as that applied in Example 4)-1-3. The amino acid sequence of the NOV0712 heavy chain expressed by the NOV0712 heavy chain expression vector is shown in SEQ ID NO: 83. In the amino acid sequence shown

in SEQ ID NO: 83, the amino acid sequence consisting of the amino acid residues at positions 1 to 19 is a signal sequence.

[0274] Reference Example 1)-1-2 Construction of anti-CDH6 antibody NOV0712 light chain expression vector

A DNA fragment comprising a NOV0712 light chain variable region-encoding DNA sequence from nucleotide positions 37 to 405 in the nucleotide sequence of the NOV0712 light chain shown in SEQ ID NO: 82 was synthesized (GENEART). A NOV0712 light chain expression vector was constructed by the same method as that applied in Example 5)-5-2-1. The amino acid sequence of the NOV0712 light chain expressed by the NOV0712 light chain expression vector is shown in SEQ ID NO: 81. In the amino acid sequence shown in SEQ ID NO: 81, the amino acid sequence consisting of the amino acid residues at positions 1 to 20 is a signal sequence.

[0275] Reference Example 1)-2 Preparation of anti-CDH6 antibody NOV0712

Reference Example 1)-2-1 Production of anti-CDH6 antibody NOV0712

NOV0712 was produced by the same method as that applied in Example 4)-2-1.

[0276] Reference Example 1)-2-2 One-step purification of anti-CDH6 antibody NOV0712

The anti-CDH6 antibody NOV0712 was purified from the culture supernatant obtained in Reference Example 1)-2-1 by the same method as that applied in Example 4)-2-2 (antibody concentration: 5 mg/l HBSor).

[0277] [Example 6: *In vitro* evaluation of humanized hG019 and NOV0712]

6)-1 Evaluation of binding activity of humanized hG019

6)-1-1 Human CDH6 antigen-binding ability of humanized hG019

The dissociation constant between the antibody and the antigen (Recombinant Human CDH6 Fc His chimera, R&D Systems, Inc.) was measured by using Biacore T200 (GE Healthcare Biosciences Corp.), according to a capture method, which comprises capturing the antigen as a ligand with the immobilized anti-His antibody and then measuring the dissociation constant using an antibody as an analyte. Approximately 1000 RU of the anti-histidine antibody (His capture kit, GE Healthcare Biosciences Corp.) was covalently bound to sensor chip CM5 (GE Healthcare Biosciences Corp.) by the amine coupling method. The antibody was also immobilized onto reference cells in the same manner as above. HBS-P+ (10 mM HEPES pH 7.4, 0.15 M NaCl, 0.05% Surfactant P20) supplemented with 1 mM CaCl₂ was used as a running buffer. The antigen was added onto the anti-histidine antibody-immobilized chip for 60 seconds, and a dilution series solution (0.391 to 100 nM) of the antibody was then added at a flow rate of 30 µl/min for 300 seconds. Subsequently, the dissociation phase was monitored for 600 seconds. As a regeneration solution, a glycine solution (pH 1.5) supplemented with 5 M MgCl₂ was added twice at a flow rate of 10 µl/min for 30 seconds. A Steady State Affinity model in analysis software (BIAevaluation software, version 4.1) was used in data analysis, and the dissociation constant (K_D) was calculated. The results are shown in Table 2.

[0278]

[Table 2]

	Antibody	KD (M)
1	H02L02	1.5E-09
2	H02L02	1.1E-09
3	H02L03	1.4E-09
4	H04L02	1.1E-09

[0279] 6)-1-2 Binding activity against human, monkey, mouse or rat CDH6

Using Lipofectamine 2000 (Thermo Fisher Scientific Inc.), pcDNA3.1-hCDH6, pcDNA3.1-cynoCDH6, p3xFLAG-CMV-9-mCDH6, or p3xFLAG-CMV-9-rCDH6 produced in Example 1)-1 was transiently introduced into 293 α cells. The cells were cultured overnight under conditions of 37°C and 5% CO₂, and thereafter, a cell suspension was prepared. Untransfected 293 α cells were used as a negative control. The suspension of the 293 α cells produced as described above was centrifuged, and the supernatant was then removed. Thereafter, the cells were suspended by the addition of each of the 4 humanized hG019 antibodies (clone Nos: H01L02, H02L02, H02L03 and H04L02), which had been prepared in Example 5)-5-3, or human IgG1 control (Calbiochem). The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, and then suspended by the addition of anti-human IgG, Fc(gamma) PE goat F(ab') (Jackson ImmunoResearch Laboratories, Inc.) that had been 500-fold diluted with PBS supplemented with 5% FBS. The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, followed by detection using a flow cytometer

(Canto II; BD Biosciences). The data was analyzed using FlowJo (Tree Star, Inc.). In Figures 6-1 and 6-2, the abscissa depicts antibody concentration, and the ordinate depicts the amount of the antibody bound based on mean fluorescence intensity. As shown in Figures 6-1 and 6-2, the human IgG1 control as a negative control binds to none of the CDH6-transfected cells. The 4 humanized hG019 antibodies (clone Nos: H01L02, H02L02, H02L03 and H04L02) bind to human CDH6 and cynomolgus monkey CDH6, but bind to neither mouse nor rat CDH6. None of the antibodies bind to the cells transfected with the empty vector pcDNA3.1 as a negative control. On the other hand, International Publication No. WO 2016/024195 discloses that the NOV0712 antibody exhibits binding activity against all of human CDH6, cynomolgus monkey CDH6, mouse CDH6, and rat CDH6. As a result, it was demonstrated that the 4 humanized hG019 antibodies obtained in the present description are anti-CDH6 antibodies that exhibit binding properties different from those of the NOV0712 antibody.

[0280] 6)-2 Analysis of CDH6-binding sites of humanized hG019 and NOV0712

6)-2-1 Epitope analysis using domain deletion mutant

Using Lipofectamine 2000 (Thermo Fisher Scientific Inc.), each domain deletion mutant expression vector produced in Example 2)-2-1, or pcDNA3.1-hCDH6 for the expression of full-length human CDH6 was transiently introduced into cells. The cells were cultured overnight under conditions of 37°C and 5% CO₂, and thereafter, a cell suspension was prepared. The suspension of the transfected 293α cells was centrifuged, and

the supernatant was then removed. Thereafter, the cells were suspended by the addition of each of the 4 humanized hG019 antibodies (clone Nos: H01L02, H02L02, H02L03 and H04L02), which had been prepared in Example 5)-5-3, or the anti-CDH6 antibody NOV0712, which had been prepared in Reference Example 1, or human IgG1 (Calbiochem) as a negative control. The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, and then suspended by the addition of APC-anti-human IgG goat F(ab')₂ (Jackson ImmunoResearch Laboratories, Inc.) that had been 500-fold diluted with PBS supplemented with 5% FBS. The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, followed by detection using a flow cytometer (Canto II; BD Biosciences). The data was analyzed using FlowJo (Tree Star, Inc.). The results are shown in Figures 7-1 to 7-6. In the histograms of Figures 7-1 to 7-6, the abscissa depicts APC fluorescence intensity indicating the amount of the antibody bound, and the ordinate depicts cell count. The shaded histogram shows that negative control untransfected 293 α cells were used, and the open solid line histogram shows that 293 α cells expressing full-length hCDH6 or each EC domain deletion mutant were used. Fluorescence intensity is enhanced when the antibody binds to full-length hCDH6 or each EC domain deletion mutant on cell surface. The human IgG1 control binds to none of the transfected cells. The 4 humanized hG019 antibodies (clone Nos: H01L02, H02L02, H02L03 and H04L02) bind to the full-length hCDH6, the EC1 deletion mutant, the EC2 deletion mutant, the EC4 deletion mutant, and the EC5 deletion mutant, but do

not bind to the EC3 deletion mutant. Specifically, it was demonstrated that the 4 humanized hG019 antibodies specifically bind to hCDH6 with EC3 as an epitope. On the other hand, the anti-CDH6 antibody NOV0712 binds to the full-length hCDH6, the EC1 deletion mutant, the EC2 deletion mutant, the EC3 deletion mutant, and the EC4 deletion mutant, but does not bind to the EC5 deletion mutant. Specifically, it was demonstrated that the anti-CDH6 antibody NOV0712 specifically binds to hCDH6 with EC5 as an epitope. This is consistent with epitope information on NOV0712 described in International Publication No. WO 2016/024195. From this result, it was demonstrated that the 4 humanized hG019 antibodies obtained in the present description are anti-CDH6 antibodies that exhibit properties different from those of NOV0712.

[0281] 6)-2-2 Binding competition assay of antibodies

6)-2-2-1 Production of 786-O/hCDH6 stably expressing cell line

The 786-O/hCDH6 stably expressing cell line was produced by infecting 786-O cells (ATCC) with a recombinant retrovirus for full-length human CDH6 expression. A human CDH6 expression retrovirus vector (pQCXIN-hCDH6) was produced by using a human CDH6 protein (NP_004923)-encoding cDNA expression vector (OriGene Technologies Inc., RC217889), and incorporating the cDNA into retrovirus vector pQCXIN (Clontech Laboratories, Inc.) according to a method known to a person skilled in the art. Using FuGene HD (Promega Corp.), pQCXIN-hCDH6 was transiently introduced into retrovirus packaging cells RetroPack PT67 (Clontech Laboratories, Inc.). After 48 hours, a culture supernatant containing recombinant retrovirus

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was recovered, and then added to the 786-O cell culture system, so that the cells were infected. From 3 days after the infection, the infected cells were cultured under conditions of 37°C and 5% CO₂ in a medium supplemented with G418 (Gibco) (final concentration: 50 mg/mL) and screened with the drug, so as to establish cell line 786-O/hCDH6 stably expressing human CDH6. The high expression of human CDH6 in the stably expressing line was confirmed by flow cytometry in the same manner as that applied in Example 2)-3-1 (Figure 8). Goat anti-Mouse IgG1 Secondary Antibody Alexa Fluor 647 (Thermo Fisher Scientific Inc.) that had been 500-fold diluted with PBS supplemented with 5% FBS was used as an antibody for detection. The results are shown in Figure 8. In the histogram of Figure 8, the abscissa depicts Alexa Fluor 647 fluorescence intensity indicating the amount of the antibody bound, and the ordinate depicts cell count. The shaded histogram shows that the negative control mIgG1 was used in staining, and the open solid line histogram shows that the anti-human CDH6 antibody was used in staining. As seen, fluorescence intensity was enhanced by the binding of the antibody to hCDH6 on cell surface. The mIgG1 control binds to none of the cells. As a result, it was demonstrated that the 786-O/hCDH6 stably expressing cell line more highly expresses human CDH6 than the parent line 786-O cells.

[0282] 6)-2-2-2 Binding competition assay using labeled H01L02 and labeled NOV0712

Labeled H01L02 and labeled NOV0712 were produced using an Alexa Fluor 488 Monoclonal Antibody Labeling Kit (Thermo Fisher Scientific Inc.). The cell suspension of the 786-

O/hCDH6 stably expressing cell line produced in 7)-2-2-1 was centrifuged, and the supernatant was then removed. Thereafter, the cells were suspended by the addition of labeled NOV0712 or labeled H01L02 (final concentration: 5 nM) and, further, the addition of each of the 4 humanized hG019 antibodies (clone Nos: H01L02, H02L02, H02L03 and H04L02), which had been prepared in Example 5)-5-3, or the anti-CDH6 antibody NOV0712, which had been prepared in Reference Example 1, or human IgG1 (Calbiochem) as a negative control (final concentration: as shown in the abscissa of Figure 9). The cells were left standing at 4°C for 1 hour. Thereafter, the cells were washed twice with PBS supplemented with 5% FBS, followed by detection using a flow cytometer (Canto II; BD Biosciences). The data was analyzed using FlowJo (Tree Star, Inc.). The results are shown in Figure 9. The abscissa depicts the final concentration of the added unlabeled antibody, and the ordinate depicts the amount of the antibody bound based on mean fluorescence intensity. When unlabeled NOV0712 is added to cells supplemented with labeled NOV0712, the amount of the labeled antibody bound is decreased by replacement with the unlabeled antibody in an addition concentration-dependent manner because they compete with each other for binding to the same epitope. On the other hand, even if each of the 4 humanized hG019 antibodies or human IgG1 as a negative control is added to cells supplemented with labeled NOV0712, there is no change in the amount of the labeled antibody bound, indicating that these antibodies differ in epitope and thus do not compete with each other for binding. Likewise, when each of the 4 unlabeled humanized hG019 antibodies is added to

cells supplemented with labeled H01L02, the amount of the labeled antibody bound is decreased by replacement with the unlabeled antibody in an addition concentration-dependent manner because they compete with each other for binding to the same epitope. On the other hand, even if NOV0712 or human IgG1 as a negative control is added to cells supplemented with labeled H01L02, there is no change in the amount of the labeled antibody bound, indicating that these antibodies differ in epitope and thus do not compete with each other for binding.

[0283] 6)-3 Evaluation of internalization activity of humanized hG019 and NOV0712

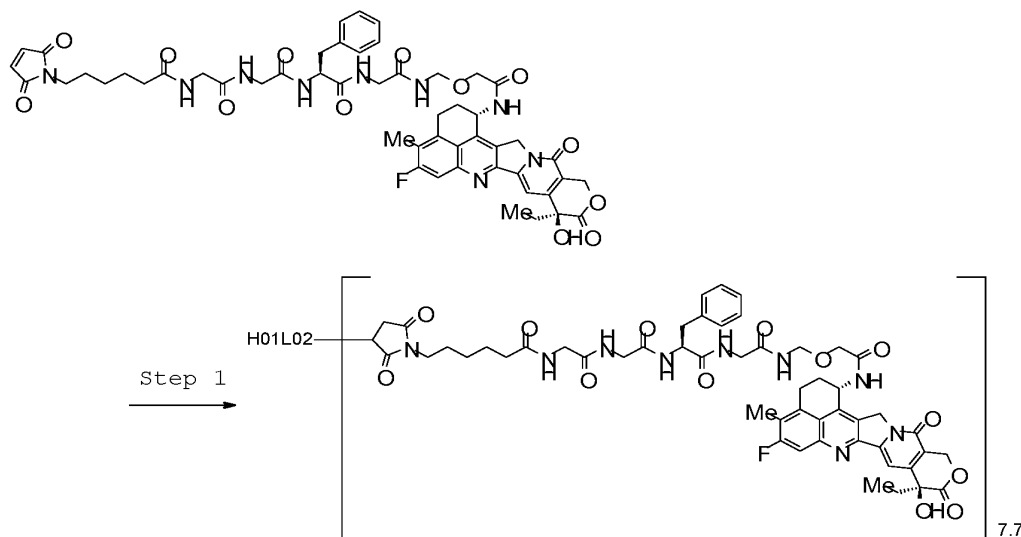
The internalization activity of humanized hG019 and NOV0712 was evaluated using an anti-human IgG reagent Hum-ZAP (Advanced Targeting Systems) conjugated with a toxin (saporin) inhibiting protein synthesis. Specifically, human CDH6-positive ovarian tumor cell line NIH:OVCAR-3 (ATCC) was seeded at 4×10^3 cells/well on a 96-well plate, and then cultured overnight under conditions of 37°C and 5% CO₂. Human CDH6-positive renal cell tumor cell line 786-O (ATCC) was seeded at 1×10^3 cells/well on a 96-well plate, and then cultured overnight. Human CDH6-positive ovarian tumor cell line PA-1 (ATCC) was seeded at 1×10^3 cells/well on a 96-well plate, and then cultured overnight under conditions of 37°C and 5% CO₂. On the next day, each anti-CDH6 antibody (final concentration: 1 nM) or human IgG1 antibody (Calbiochem) as a negative control antibody was added to the plate. Hum-ZAP (final concentration: 0.5 nM) or F(ab')₂ Fragment Goat Anti-human IgG, Fc (gamma) Fragment Specific (Jackson ImmunoResearch

Laboratories, Inc.) unconjugated with the toxin (final concentration: 0.5 nM) as a negative control was further added to the plate, and the cells were cultured under conditions of 37°C and 5% CO₂ for 3 days. The number of live cells was measured by the quantification of ATP activity (RLU) using CellTiter-Glo(TM) Luminescent Cell Viability Assay. In this evaluation, Hum-ZAP is taken up into cells in a manner dependent on the internalization activity of the humanized anti-CDH6 antibody, so that saporin, which inhibits protein synthesis, is released into the cells, so as to suppress cell growth. A cell growth inhibition effect brought about by the addition of the anti-CDH6 antibody was indicated by a relative survival rate when the number of live cells in a well supplemented with the negative control instead of Hum-ZAP was defined as 100%. Figures 10-1 to 10-3 each show a graph and a table of the cell survival rate. In this experiment, an antibody having strong internalization activity is considered to offer a low cell survival rate. As a result, the 4 humanized hG019 antibodies have an internalization rate of approximately 50 to 75% predicted from the cell survival rates for all of the 3 cell lines. Thus, the 4 humanized hG019 antibodies exhibit very high internalization activity and exhibit much higher internalization activity than that of NOV0712. From the mechanism of the medicinal effects of ADC, an antibody having higher internalization activity is considered to be more suitable as an ADC antibody.

[0284] [Example 7: Production of humanized hG019-drug conjugate]

7)-1 Production of antibody-drug conjugate H01L02-DXd

[Formula 11]



[0287] Conjugation between antibody and drug linker: The above-described solution was incubated at 15°C for 10 minutes. Subsequently, a 10 mM solution of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-(2-
{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-

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2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-2-oxoethoxy)methyl]glycinamide in dimethyl sulfoxide (0.386 mL; 10 equivalents per antibody molecule) was added thereto, and the obtained mixture was incubated at 15°C for 1 hour to conjugate the drug linker to the antibody.

Subsequently, an aqueous solution of 100 mM NAC (Sigma-Aldrich Co. LLC) (0.0347 mL; 9 equivalents per antibody molecule) was added thereto, and the obtained mixture was further stirred at room temperature for 20 minutes to terminate the reaction of the drug linker.

[0288] Purification: The above-described solution was purified by common procedure D described in production method 1 to obtain 19 mL of a solution containing the title antibody-drug conjugate "H01L02-ADC".

[0289] Characterization: Using common procedure E (using $\epsilon_{D,280} = 5440$ and $\epsilon_{D,370} = 21240$) described in production method 1, the following characteristic values were obtained.

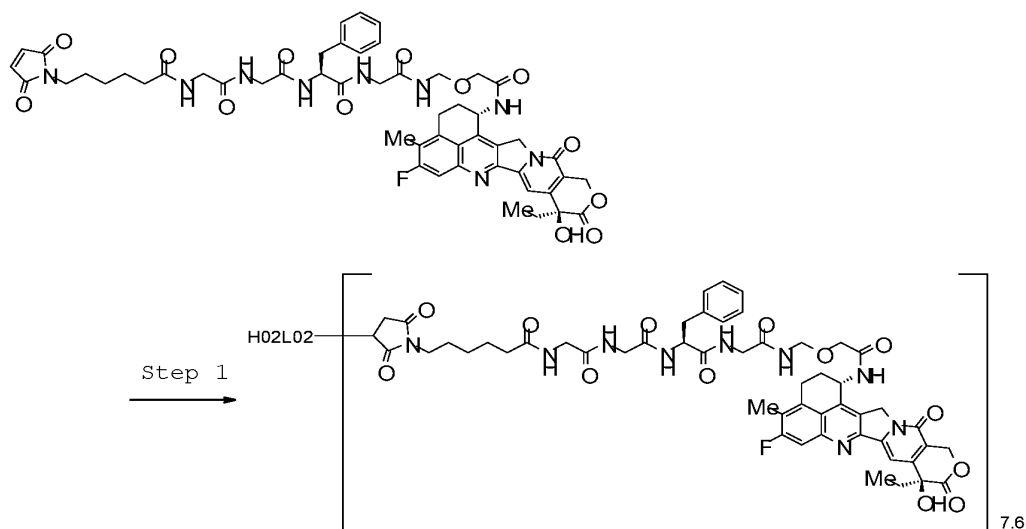
Antibody concentration: 2.26 mg/mL, antibody yield: 42.9 mg (76%), average number of conjugated drug molecules (n) per antibody molecule measured by common procedure E: 5.9, and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure F: 7.7.

[0290] 7)-2 Production of antibody-drug conjugate H02L02-DXd

Step 1: Antibody-drug conjugate (2)

[0291]

[Formula 12]



[0292] Reduction of antibody: H02L02 produced in Example 5 was adjusted to 9.95 mg/mL with PBS6.0/EDTA by using common procedures B (using $1.51 \text{ mLmg}^{-1}\text{cm}^{-1}$ as 280 nm absorption coefficient) and C described in production method 1. To this solution (5.7 mL), an aqueous solution of 10 mM TCEP (Tokyo Chemical Industry Co., Ltd.) (0.234 mL; 6.0 equivalents per antibody molecule) and a 1 M aqueous dipotassium hydrogen phosphate solution (Nacalai Tesque, Inc.; 0.0855 mL) were added. After confirming that the solution had a pH within 7.0 ± 0.1 , the interchain disulfide bond in the antibody was reduced by incubating the solution at 37°C for 2 hours.

[0293] Conjugation between antibody and drug linker: The above-described solution was incubated at 15°C for 10 minutes. Subsequently, a 10mM solution of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-

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yl]amino}-2-oxoethoxy)methyl]glycinamide in dimethyl sulfoxide (0.389 mL; 10 equivalents per antibody molecule) was added thereto, and the obtained mixture was incubated at 15°C for 1 hour to conjugate the drug linker to the antibody.

Subsequently, an aqueous solution of 100 mM NAC (Sigma-Aldrich Co. LLC) (0.0350 mL; 9 equivalents per antibody molecule) was added thereto, and the obtained mixture was further stirred at room temperature for 20 minutes to terminate the reaction of the drug linker.

[0294] Purification: The above-described solution was purified by common procedure D described in production method 1 to obtain 19 mL of a solution containing the title antibody-drug conjugate "H02L02-ADC".

[0295] Characterization: Using common procedure E (using $\epsilon_{D,280} = 5440$ and $\epsilon_{D,370} = 21240$) described in production method 1, the following characteristic values were obtained.

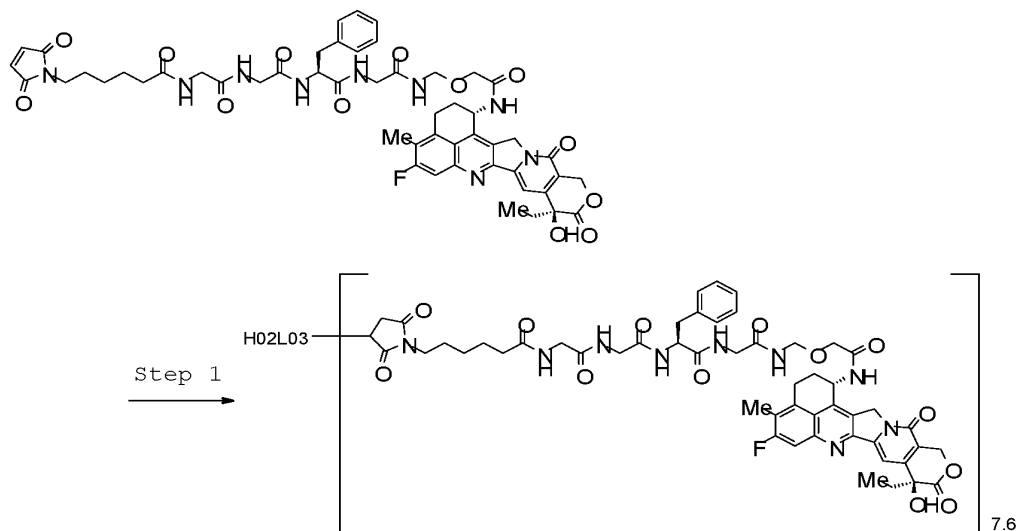
Antibody concentration: 2.61 mg/mL, antibody yield: 49.6 mg (87%), average number of conjugated drug molecules (n) per antibody molecule measured by common procedure E: 5.9, and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure F: 7.6.

[0296] 7)-3 Production of antibody-drug conjugate H02L03-DXd

Step 1: Antibody-drug conjugate (3)

[0297]

[Formula 13]



[0298] Reduction of antibody: H02L03 produced in Example 5 was adjusted to 9.86 mg/mL with PBS6.0/EDTA by using common procedures B (using $1.53 \text{ mLmg}^{-1}\text{cm}^{-1}$ as 280 nm absorption coefficient) and C described in production method 1. To this solution (5.7 mL), an aqueous solution of 10 mM TCEP (Tokyo Chemical Industry Co., Ltd.) (0.270 mL; 7.0 equivalents per antibody molecule) and a 1 M aqueous dipotassium hydrogen phosphate solution (Nacalai Tesque, Inc.; 0.0855 mL) were added. After confirming that the solution had a pH within 7.0 ± 0.1 , the interchain disulfide bond in the antibody was reduced by incubating the solution at 37°C for 2 hours.

[0299] Conjugation between antibody and drug linker: The above-described solution was incubated at 15°C for 10 minutes. Subsequently, a 10 mM solution of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-

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yl]amino}-2-oxoethoxy)methyl]glycinamide in dimethyl sulfoxide (0.386 mL; 10 equivalents per antibody molecule) was added thereto, and the obtained mixture was incubated at 15°C for 1 hour to conjugate the drug linker to the antibody.

Subsequently, an aqueous solution of 100 mM NAC (Sigma-Aldrich Co. LLC) (0.0347 mL; 9 equivalents per antibody molecule) was added thereto, and the obtained mixture was further stirred at room temperature for 20 minutes to terminate the reaction of the drug linker.

[0300] Purification: The above-described solution was purified by common procedure D described in production method 1 to obtain 19 mL of a solution containing the title antibody-drug conjugate "H01L02-ADC".

[0301] Characterization: Using common procedure E ((using $\epsilon_{D,280} = 5440$ and $\epsilon_{D,370} = 21240$) described in production method 1, the following characteristic values were obtained.

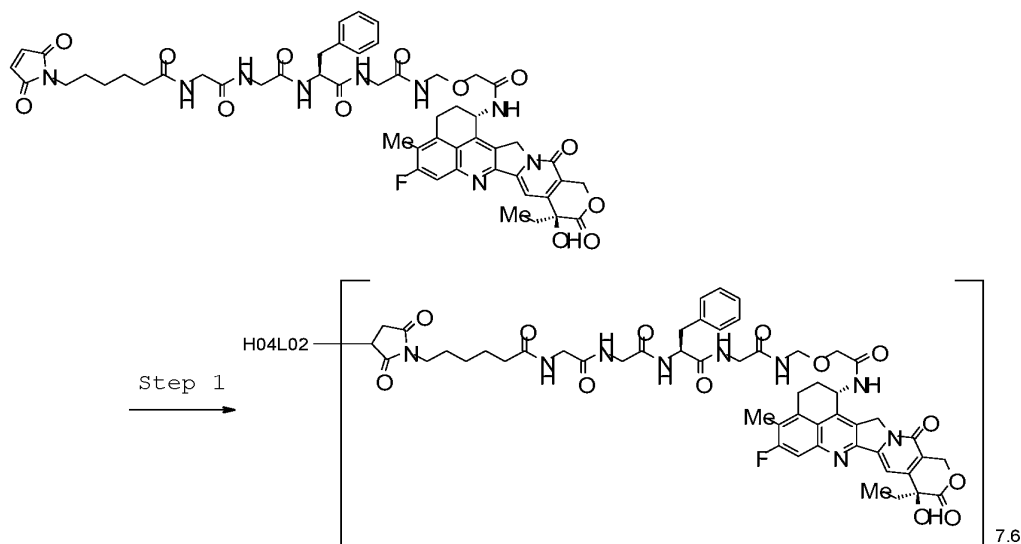
Antibody concentration: 2.71 mg/mL, antibody yield: 51.4 mg (91%), average number of conjugated drug molecules (n) per antibody molecule measured by common procedure E: 5.7, and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure F: 7.6.

[0302] 7)-4 Production of antibody-drug conjugate H04L02-DXd

Step 1: Antibody-drug conjugate (4)

[0303]

[Formula 14]



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benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-2-oxoethoxy)methyl]glycinamide in dimethyl sulfoxide (0.386 mL; 10 equivalents per antibody molecule) was added thereto, and the obtained mixture was incubated at 15°C for 1 hour to conjugate the drug linker to the antibody. Subsequently, an aqueous solution of 100 mM NAC (Sigma-Aldrich Co. LLC) (0.0347 mL; 9 equivalents per antibody molecule) was added thereto, and the obtained mixture was further stirred at room temperature for 20 minutes to terminate the reaction of the drug linker.

[0306] Purification: The above-described solution was purified by common procedure D described in production method 1 to obtain 19 mL of a solution containing the title antibody-drug conjugate "H04L02-ADC".

[0307] Characterization: Using common procedure E (using $\epsilon_{D,280} = 5440$ and $\epsilon_{D,370} = 21240$) described in production method 1, the following characteristic values were obtained.

Antibody concentration: 2.56 mg/mL, antibody yield: 48.7 mg (87%), average number of conjugated drug molecules (n) per antibody molecule measured by common procedure E: 5.8, and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure F: 7.6.

[0308] [Reference Example 2: Production of NOV0712-drug conjugate]

Reference Example 2)-1 Production of antibody-drug conjugate NOV0712-DM4

Antibody-drug conjugate(5)

Conjugation between antibody and drug linker: NOV0712 produced in Reference Example 1 was adjusted to 9.7 mg/mL with

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20 mM HEPES8.1 (HEPES, 1 M Buffer Solution (20 mL) manufactured by Life Technologies Corp. was pH-adjusted to 8.1 with 1 M sodium hydroxide, and then brought to 1 L with distilled water) by using common procedures B (using $1.51 \text{ mLmg}^{-1}\text{cm}^{-1}$ as 280 nm absorption coefficient) and C described in production method 1. The solution was incubated at 20°C for 10 minutes. Subsequently, a 10 mM solution of 1-(2,5-dioxopyrrolidin-1-yloxy)-1-oxo-4-(pyridin-2-yl)disulfanylbutane-2-sulfonic acid described in WO2016/024195 in DMA (0.366 mL; 5.2 equivalents per antibody molecule), a 10 mM solution of N2-deacetyl-deacetyl-N2-(4-methyl-4-mercapto-1-oxopentyl)-maytansine (DM4) in DMA (0.366 mL; 6.8 equivalents per antibody molecule), and 0.243 mL of DMA were added thereto, and the obtained mixture was incubated at 20°C for 16 hours to conjugate the drug linker to the antibody. Subsequently, an aqueous solution of 1 M acetic acid was added thereto to adjust the pH to 5.0, and the obtained mixture was further stirred at room temperature for 20 minutes to terminate the reaction of the drug linker.

[0309] Purification: The above-described solution was purified by common procedure D described in production method 1 to obtain 28 mL of a solution containing the title antibody-drug conjugate "NOV0712-DM4".

[0310] Characterization: Using common procedure E (using $\epsilon_{A,280} = 200500$, $\epsilon_{A,252} = 76295$, $\epsilon_{D,280} = 43170$, and $\epsilon_{D,252} = 23224$) described in production method 1, the following characteristic values were obtained.

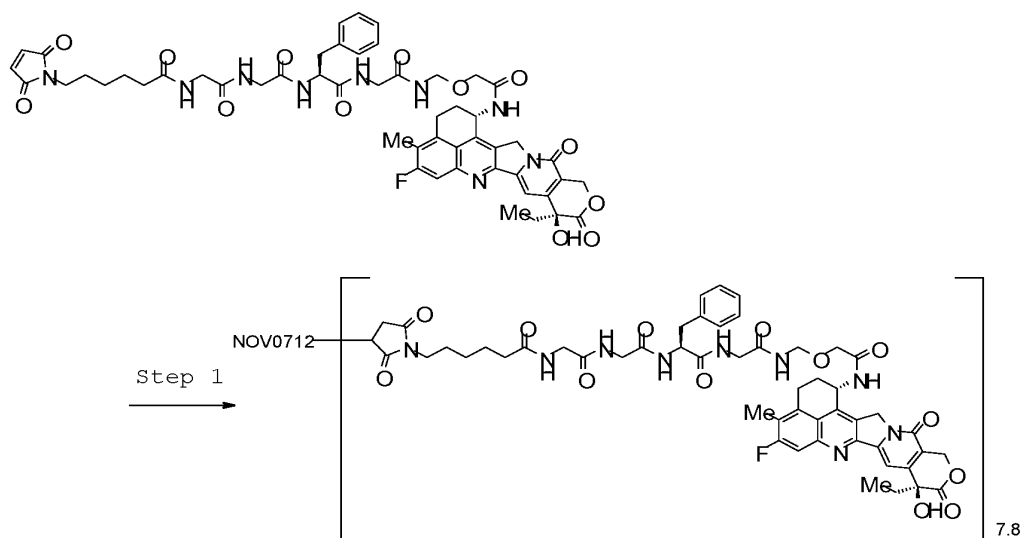
Antibody concentration: 2.58 mg/mL, antibody yield: 72.2 mg (93%), and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure E: 3.0.

[0311] Reference Example 2)-2 Production of antibody-drug conjugate NOV0712-DXd

Step 1: Antibody-drug conjugate (6)

[0312]

[Formula 15]



[0313] Reduction of antibody: NOV0712 produced in Reference Example 1 was adjusted to 9.26 mg/mL with PBS6.0/EDTA by using common procedures B (using $1.5 \text{ mLmg}^{-1}\text{cm}^{-1}$ as 280 nm absorption coefficient) and C described in production method 1. To this solution (6.6 mL), an aqueous solution of 10 mM TCEP (Tokyo Chemical Industry Co., Ltd.) (0.254 mL; 6.0 equivalents per antibody molecule) and a 1 M aqueous dipotassium hydrogen phosphate solution (Nacalai Tesque, Inc.; 0.0990 mL) were added. After confirming that the solution had a pH within 7.0 ± 0.1 , the interchain disulfide bond in the antibody was reduced by incubating the solution at 37°C for 2 hours.

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[0314] Conjugation between antibody and drug linker: The above-described solution was incubated at 15°C for 10 minutes. Subsequently, a 10 mM solution of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-{{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-2-oxoethoxy)methyl]glycinamide in dimethyl sulfoxide (0.381 mL; 9 equivalents per antibody molecule) was added thereto, and the obtained mixture was incubated at 15°C for 1 hour to conjugate the drug linker to the antibody. Subsequently, an aqueous solution of 100 mM NAC (Sigma-Aldrich Co. LLC) (0.0381 mL; 9 equivalents per antibody molecule) was added thereto, and the obtained mixture was further stirred at room temperature for 20 minutes to terminate the reaction of the drug linker.

[0315] Purification: The above-described solution was purified by common procedure D described in production method 1 to obtain 23.5 mL of a solution containing the title antibody-drug conjugate "NOV0712-ADC".

[0316] Characterization: Using common procedure E (using $\epsilon_{D,280}$ = 5440 and $\epsilon_{D,370}$ = 21240) described in production method 1, the following characteristic values were obtained.

Antibody concentration: 2.26 mg/mL, antibody yield: 56.4 mg (92%), average number of conjugated drug molecules (n) per antibody molecule measured by common procedure E: 6.4, and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure F: 7.8.

[0317] [Reference Example 3: Production of H01L02-DM4]

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Reference Example 3)-1 Production of antibody-drug
conjugate H01L02-DM4

Antibody-drug conjugate(7)

Conjugation between antibody and drug linker: H01L02
produced in Example 5 was adjusted to 9.8 mg/mL with 20 mM
HEPES8.1 (HEPES, 1 M Buffer Solution (20 mL) manufactured by
Life Technologies Corp. was pH-adjusted to 8.1 with 1 M sodium
hydroxide, and then brought to 1 L with distilled water) by
using common procedures B (using $1.53 \text{ mLmg}^{-1}\text{cm}^{-1}$ as 280 nm
absorption coefficient) and C described in production method 1.
The solution was incubated at 20°C for 10 minutes.
Subsequently, a 10 mM solution of 1-(2,5-dioxopyrrolidin-1-
yloxy)-1-oxo-4-(pyridin-2-yl)disulfanylbutane-2-sulfonic acid
described in WO2016/024195 in DMA (0.062 mL; 11.5 equivalents
per antibody molecule) and a 10 mM solution of N2-deacetyl-N2-
(4-methyl-4-mercapto-1-oxopentyl)-maytansine (DM4) in DMA
(0.082 mL; 15.1 equivalents per antibody molecule) were added
thereto, and the obtained mixture was incubated at 20°C for 18
hours to conjugate the drug linker to the antibody.
Subsequently, an aqueous solution of 1 M acetic acid was added
thereto to adjust the pH to 5.0, and the obtained mixture was
further stirred at room temperature for 20 minutes to
terminate the reaction of the drug linker.

[0318] Purification: The above-described solution was purified
by common procedure D described in production method 1 to
obtain 3.5 mL of a solution containing the title antibody-drug
conjugate "H01L02-DM4".

[0319] Characterization: Using common procedure E (using $\epsilon_{A,280}$
= 223400, $\epsilon_{A,252}$ = 85646, $\epsilon_{D,280}$ = 4317, and $\epsilon_{D,252}$ = 23224)

described in production method 1, the following characteristic values were obtained.

Antibody concentration: 1.97 mg/mL, antibody yield: 6.90 mg (88%), and average number of conjugated drug molecules (n) per antibody molecule measured by common procedure E: 3.6.
[0320] [Example 8: Evaluation of *in vitro* activity of antibody-drug conjugate]

8)-1 Evaluation of *in vitro* cell growth inhibition activity of antibody-drug conjugate against CDH6-positive human tumor cell line

CDH6-positive human ovarian tumor cell line PA-1 was seeded over a 96-well plate at 2×10^3 cells/100 μ L/well in MEM medium supplemented with 10% FBS, and the cells were then cultured overnight under conditions of 37°C and 5% CO₂. On the next day, each of the 4 humanized hG019-drug conjugates (clone names: H01L02-DXd, H02L02-DXd, H02L03-DXd and H04L02-DXd) produced in Example 7, or the NOV0712-drug conjugate (NOV0712-DM4) produced in Reference Example 2 was added to the cells such that the final concentrations were from 0.0001 (nM) to 100 (nM). After culture for 4 days, the number of live cells was measured by the quantification of ATP using CellTiter-Glo(TM) Luminescent Cell Viability Assay (Promega Corp.). Figure 11 shows concentration-dependent cell growth inhibition activity when each antibody-drug conjugate was added to the cells. From this result, it was demonstrated that the 4 humanized hG019-drug conjugates exhibit growth inhibition activity against tumor cells from a lower addition concentration than that of the NOV0712-drug conjugate, and have high antitumor activity.

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[0321] [Example 9: *In vivo* antitumor effect of antibody-drug conjugate]

The antitumor effects of the antibody-drug conjugates were evaluated using animal models derived from immunodeficient mice by the inoculation of CDH6-positive human tumor cell line cells. Four- to 5-week-old BALB/c nude mice (CAnN.Cg-Foxnl[nu]/CrlCrlj[Foxnlnu/Foxnlnu], Charles River Laboratories Japan Inc.) and SCID mice (CB17/Icr-Prkdc[scid]/CrlCrlj, Charles River Laboratories Japan Inc.) were acclimatized for 3 days or longer under SPF conditions before use in the experiment. The mice were fed with a sterilized solid diet (FR-2, Funabashi Farms Co., Ltd) and given sterilized tap water (which had been prepared by adding a 5 to 15 ppm sodium hypochlorite solution to tap water). The long diameter and short diameter of the inoculated tumor were measured twice a week using electronic digital calipers (CD-15CX, Mitutoyo Corp.), and the volume of the tumor was then calculated according to the following expression.

Tumor volume (mm³) = 1/2 × Long diameter (mm) × [Short diameter (mm)]²

Each antibody-drug conjugate was diluted with ABS buffer (10 mM acetate buffer, 5% sorbitol, pH 5.5) (Nacalai Tesque, Inc.), and the dilution was intravenously administered at a dose shown in each Example to the tail of each mouse. ABS buffer was administered in the same manner as above to a control group (vehicle group). Six mice per group were used in the experiment.

[0322] 9)-1 Antitumor effect - (1)

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The CDH6-positive human renal cell tumor cell line 786-O (ATCC), the CDH6 expression of which had been confirmed in Example 2)-3-1, was suspended in Matrigel (Corning Inc.), and the cell suspension was subcutaneously inoculated at a dose of 5×10^6 cells to the right flank region of each male SCID mouse (Day 0). On Day 18, the mice were randomly grouped. On the day of grouping, each of the 4 antibody-drug conjugates (clone names: H01L02-DXd, H02L02-DXd, H02L03-DXd and H04L02-DXd) produced in Example 7, or NOV0712-DM4 produced in Reference Example 2 was intravenously administered at a dose of 3 mg/kg to the tail of each mouse. The results are shown in Figure 12. The abscissa depicts the number of days, and the ordinate depicts tumor volume. The error range depicts a SE value. [0323] NOV0712-DM4 exhibited no significant antitumor effect in this tumor model. All the 4 antibody-drug conjugates produced in Example 7 decreased tumor volume after administration, exerted significant tumor regression, and sustained the tumor regression effect for 24 days after administration (Figure 12). [0324] 9)-2 Antitumor effect - (2)

The CDH6-positive human ovarian tumor cell line PA-1(ATCC), the CDH6 expression of which had been confirmed in Example 2)-3-1, was suspended in Matrigel (Corning Inc.), and the cell suspension was subcutaneously inoculated at a dose of 8.5×10^6 cells to the right flank region of each female nude mouse (Day 0). On Day 11, the mice were randomly grouped. On the day of grouping, the antibody-drug conjugate H01L02-DXd produced in Example 7, or NOV0712-DM4 or NOV0712-DXd produced in Reference Example 2 was intravenously administered at doses of 1 or 3 mg/kg to the tail of each mouse. The results are

shown in Figure 13. The abscissa depicts the number of days, and the ordinate depicts tumor volume. The error range depicts a SE value.

[0325] NOV0712-DM4 exhibited no antitumor effect at any of the doses of 1 and 3 mg/kg in this tumor model. On the other hand, H01L02-DXd significantly decreased tumor volume after administration at both the doses of 1 and 3 mg/kg and exerted a tumor regression effect (Figure 13). The H01L02 antibody obtained in the present description and the NOV0712 antibody were conjugated to the same drug DXd, and the medicinal effects of the resulting samples were compared. As a result, H01L02-DXd exerted a stronger antitumor effect than that of NOV0712-DXd at both the doses of 1 and 3 mg/kg. Specifically, it was demonstrated that the H01L02 antibody of the present invention is a superior antibody for antibody-drug conjugates as antitumor agents to the NOV0712 antibody (Figure 13).

[0326] 9)-3 Antitumor effect - (3)

The CDH6-positive human ovarian tumor cell line NIH:OVCA-3 (ATCC), the CDH6 expression of which had been confirmed in Example 2)-3-1, was suspended in Matrigel (Corning Inc.), and the cell suspension was subcutaneously inoculated at a dose of 1×10^7 cells to the right flank region of each female nude mouse (Day 0). On Day 22, the mice were randomly grouped. On the day of grouping, the antibody-drug conjugate H01L02-DXd produced in Example 7, or NOV0712-DM4 produced in Reference Example 2 was intravenously administered at doses of 1 or 3 mg/kg to the tail of each mouse. The results are shown in Figure 14. The abscissa depicts the

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number of days, and the ordinate depicts tumor volume. The error range depicts a SE value.

[0327] NOV0712-DM4 exhibited no antitumor effect at the dose of 1 mg/kg, and exhibited an antitumor effect at the dose of 3 mg/kg, though tumor regrowth was observed from 2 weeks after administration. On the other hand, H01L02-DXd significantly suppressed increase in tumor volume after administration at both the doses of 1 and 3 mg/kg, and sustained, particularly, at the dose of 3 mg/kg, the tumor growth inhibition effect over a long period of 31 days after administration (Figure 14).

[0328] The tumor growth inhibition effect of NOV0712-DM4 produced in Reference Example 2 or H01L02-DM4 produced in Reference Example 3 was evaluated in the same manner as above using PA-1 cells. H01L02-DM4 further decreased tumor volume than NOV0712-DM4. Thus, the H01L02 antibody of the present invention is superior as an antibody for antibody-drug conjugates acting as antitumor agents as compared with the NOV0712 antibody.

[0329] 9)-4 Antitumor effect - (4)

The CDH6-positive human renal cell tumor cell line 786-O (ATCC), the CDH6 expression of which had been confirmed in Example 2)-3-1, was suspended in Matrigel (Corning Inc.), and the cell suspension was subcutaneously inoculated at a dose of 5×10^6 cells to the right flank region of each male SCID mouse (Day 0). On Day 20, the mice were randomly grouped. On the day of grouping, the antibody-drug conjugate H01L02-DXd produced in Example 7, or NOV0712-DM4 produced in Reference Example 2 was intravenously administered at doses of 1 or 3 mg/kg to the tail of each mouse. The results are shown in

Figure 15. The abscissa depicts the number of days, and the ordinate depicts tumor volume. The error range depicts a SE value.

[0330] NOV0712-DM4 exhibited no significant antitumor effect at any of the doses of 1 and 3 mg/kg in this tumor model. On the other hand, H01L02-DXd decreased tumor volume after administration at both the doses of 1 and 3 mg/kg, and exerted, particularly, at the dose of 3 mg/kg, significant tumor regression, and sustained the tumor regression effect for 20 days after administration (Figure 15).

[0331] 9)-5 Antitumor effect - (5)

The CDH6-negative human ovarian tumor cell line ES-2 (ATCC), the absence of the CDH6 expression of which had been confirmed in Example 2)-3-1, was suspended in physiological saline, and the cell suspension was subcutaneously inoculated at a dose of 1×10^6 cells to the right flank region of each female nude mouse (Day 0). On Day 7, the mice were randomly grouped. On the day of grouping, the antibody-drug conjugate H01L02-DXd produced in Example 7, or NOV0712-DM4 produced in Reference Example 2 was intravenously administered at doses of 1 or 3 mg/kg to the tail of each mouse. The results are shown in Figure 16. The abscissa depicts the number of days, and the ordinate depicts tumor volume. The error range depicts a SE value.

[0332] In this tumor model expressing no CDH6, H01L02-DXd and NOV0712-DM4 exhibited no antitumor effect at any of the doses. From this result, the antitumor effect of the antibody-drug conjugate in the CDH6-positive tumor model demonstrated in Examples 9)-1, 9)-2, 9)-3, and 9)-4 is an effect dependent on

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CDH6 expression in the tumor cells. Thus, the antibody-drug conjugate of the present invention is considered as a selective and safe antitumor drug that specifically exhibits an antitumor effect on CDH6-positive tumor without causing cytotoxicity to CDH6-negative normal tissues (Figure 16).

Industrial Applicability

[0333] The present invention provides an anti-CDH6 antibody having internalization activity and an antibody-drug conjugate comprising the antibody. The antibody-drug conjugate can be used as a therapeutic drug for cancer, and the like.

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Claims

[Claim 1]

An antibody specifically binding to the amino acid sequence shown in SEQ ID NO: 4 and having internalization ability that permits cellular uptake, or a functional fragment of the antibody.

[Claim 2]

The antibody or the functional fragment of the antibody according to claim 1, which has competitive inhibitory activity, for binding to the amino acid sequence shown in SEQ ID NO: 4, against at least any one antibody selected from the group consisting of the following antibodies (1) to (5):

- (1) an antibody having a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 53 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 56,
- (2) an antibody having a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 69,
- (3) an antibody having a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73,
- (4) an antibody having a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 65 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73, and

(5) an antibody having a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 77.

[Claim 3]

The antibody or the functional fragment of the antibody according to claim 1 or 2, which comprises CDRL1, CDRL2 and CDRL3 in any one combination selected from the group consisting of the following combinations (1) to (4):

(1) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 12, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 13, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 14,

(2) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 22, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 23, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 24,

(3) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 32, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 33, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 34, and

(4) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 42, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 43, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 44, and

CDRH1, CDRH2 and CDRH3 in any one combination selected from the group consisting of the following combinations (5) to (9):

(5) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence shown

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in SEQ ID NO: 18, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 19,

(6) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 27, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 28, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 29,

(7) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 37, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 38, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 39,

(8) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 47, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 48, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 49, and

(9) CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 60, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 19.

[Claim 4]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 3, which comprises CDRL1, CDRL2 and CDRL3, and CDRH1, CDRH2 and CDRH3 in any combination selected from the group consisting of the following combinations (1) to (5):

(1) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 12, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 13, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 14, and CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 17, CDRH2 consisting

of the amino acid sequence shown in SEQ ID NO: 18, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 19,

(2) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 22, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 23, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 24, and CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 27, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 28, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 29,

(3) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 32, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 33, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 34, and CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 37, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 38, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 39,

(4) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 42, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 43, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 44, and CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 47, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 48, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 49, and

(5) CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 12, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 13, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 14, and CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 17, CDRH2 consisting

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of the amino acid sequence shown in SEQ ID NO: 60, and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 19.
[Claim 5]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 4, which is humanized.
[Claim 6]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 5, which has any one light chain variable region selected from the group consisting of the following variable regions (1) to (4):

- (1) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 63,
 - (2) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 67,
 - (3) an amino acid sequence having a sequence identity of at least 95% or more to the sequence of a framework region other than at each CDR sequence in the amino acid sequences of (1) and (2), and
 - (4) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the sequence of a framework region other than at each CDR sequence in the amino acid sequences of (1) to (3), and
- any one heavy chain variable region selected from the group consisting of the following variable regions (5) to (9):
- (5) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 71,
 - (6) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 75,

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(7) a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 79,

(8) an amino acid sequence having a sequence homology of at least 95% or more to the sequence of a framework region other than at each CDR sequence in the amino acid sequences of (5) to (7), and

(9) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids in the sequence of a framework region other than at each CDR sequence in the amino acid sequences of (5) to (8).

[Claim 7]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 6, which comprises a light chain variable region and a heavy chain variable region in any of the following combinations (1) to (4):

(1) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 63 and a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 71,

(2) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 63 and a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 75,

(3) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 67 and a heavy chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 75, and

(4) a light chain variable region consisting of the amino acid sequence shown in SEQ ID NO: 63 and a heavy chain variable

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region consisting of the amino acid sequence shown in SEQ ID NO: 79.

[Claim 8]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 7, which has any of the following combinations (1) to (4):

(1) a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 69,

(2) a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73,

(3) a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 65 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73, and

(4) a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 77.

[Claim 9]

The antibody or the functional fragment of the antibody according to claim 8, which has a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 69.

[Claim 10]

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The antibody or the functional fragment of the antibody according to claim 8, which has a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73.

[Claim 11]

The antibody or the functional fragment of the antibody according to claim 8, which has a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 65 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73.

[Claim 12]

The antibody or the functional fragment of the antibody according to claim 8, which has a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 77.

[Claim 13]

The functional fragment of the antibody according to any one of claims 1 to 12, wherein the functional fragment is selected from the group consisting of Fab, F(ab')₂, Fab' and Fv.

[Claim 14]

A polynucleotide encoding the antibody or the functional fragment of the antibody according to any one of claims 1 to 13.

[Claim 15]

The polynucleotide according to claim 14, which comprises polynucleotides in any one combination selected from the group consisting of the following combinations (1) to (5):

(1) a polynucleotide encoding a light chain variable region comprising CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 12, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 13 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 14, and a polynucleotide encoding a heavy chain variable region comprising CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 18 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 19,

(2) a polynucleotide encoding a light chain variable region comprising CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 22, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 23 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 24, and a polynucleotide encoding a heavy chain variable region comprising CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 27, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 28 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 29,

(3) a polynucleotide encoding a light chain variable region comprising CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 32, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 33 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 34, and a polynucleotide encoding a heavy chain variable region comprising CDRH1 consisting of

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the amino acid sequence shown in SEQ ID NO: 37, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 38 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 39,

(4) a polynucleotide encoding a light chain variable region comprising CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 42, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 43 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 44, and a polynucleotide encoding a heavy chain variable region comprising CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 47, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 48 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 49, and

(5) a polynucleotide encoding a light chain variable region comprising CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 12, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 13 and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 14, and a polynucleotide encoding a heavy chain variable region comprising CDRH1 consisting of the amino acid sequence shown in SEQ ID NO: 17, CDRH2 consisting of the amino acid sequence shown in SEQ ID NO: 60 and CDRH3 consisting of the amino acid sequence shown in SEQ ID NO: 19.

[Claim 16]

The polynucleotide according to claim 14 or 15, which comprises a polynucleotide encoding a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a polynucleotide encoding a heavy chain consisting

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of the amino acid sequence at positions 20 to 471 in SEQ ID
NO: 69.

[Claim 17]

The polynucleotide according to claim 14 or 15, which
comprises a polynucleotide encoding a light chain consisting
of the amino acid sequence at positions 21 to 233 in SEQ ID
NO: 61 and a polynucleotide encoding a heavy chain consisting
of the amino acid sequence at positions 20 to 471 in SEQ ID
NO: 73.

[Claim 18]

The polynucleotide according to claim 14 or 15, which
comprises a polynucleotide encoding a light chain consisting
of the amino acid sequence at positions 21 to 233 in SEQ ID
NO: 65 and a polynucleotide encoding a heavy chain consisting
of the amino acid sequence at positions 20 to 471 in SEQ ID
NO: 73.

[Claim 19]

The polynucleotide according to claim 14 or 15, which
comprises a polynucleotide encoding a light chain consisting
of the amino acid sequence at positions 21 to 233 in SEQ ID
NO: 61 and a polynucleotide encoding a heavy chain consisting
of the amino acid sequence at positions 20 to 471 in SEQ ID
NO: 77.

[Claim 20]

An expression vector comprising the polynucleotide
according to any one of claims 14 to 19.

[Claim 21]

Host cells transformed with the expression vector
according to claim 20.

[Claim 22]

The host cells according to claim 21, wherein the host cells are eukaryotic cells.

[Claim 23]

A method for producing an antibody of interest or a functional fragment of the antibody, which comprises the step of culturing the host cells according to claim 21 or 22, and the step of collecting an antibody of interest or a functional fragment of the antibody from the culture obtained by the aforementioned step.

[Claim 24]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 13, wherein the heavy chain or the light chain has undergone one or two or more modifications selected from the group consisting of N-linked glycosylation, O-linked glycosylation, N-terminal processing, C-terminal processing, deamidation, isomerization of aspartic acid, oxidation of methionine, addition of a methionine residue to the N-terminus, amidation of a proline residue, conversion of N-terminal glutamine or N-terminal glutamic acid to pyroglutamic acid, and a deletion of one or two amino acids from the carboxyl terminus.

[Claim 25]

The antibody according to claim 24, wherein one or two amino acids are deleted from the carboxyl terminus of a heavy chain thereof.

[Claim 26]

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The antibody according to claim 25, wherein one amino acid is deleted from each of the carboxyl termini of both of the heavy chains thereof.

[Claim 27]

The antibody according to any one of claims 24 to 26, wherein a proline residue at the carboxyl terminus of a heavy chain thereof is further amidated.

[Claim 28]

The antibody or the functional fragment of the antibody according to any one of claims 1 to 13 and 24 to 27, wherein sugar chain modification is regulated in order to enhance antibody-dependent cellular cytotoxic activity.

[Claim 29]

An antibody-drug conjugate comprising the antibody or the functional fragment of the antibody according to any one of claims 1 to 13 and 24 to 28 conjugated to a drug.

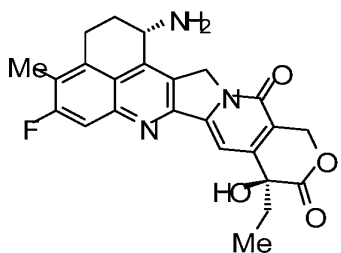
[Claim 30]

The antibody-drug conjugate according to claim 29, wherein the drug is an antitumor compound.

[Claim 31]

The antibody-drug conjugate according to claim 30, wherein the antitumor compound is an antitumor compound represented by the following formula:

[Formula 1]



[Claim 32]

The antibody-drug conjugate according to any one of claims 29 to 31, wherein the antibody is conjugated to the drug via a linker having any structure selected from the group consisting of the following formulas (a) to (f):

(a) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$,

(b) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$,

(c) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$,

(d) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$,

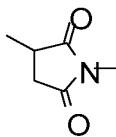
(e) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$, and

(f) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$,

wherein the antibody is connected to the terminus of -

$(\text{Succinimid-3-yl-N})$, the antitumor compound is connected to the carbonyl group of the $-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$ moiety of (a), (b), (e) or (f), the $\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$ moiety of (c) or the $\text{CH}_2\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$ moiety of (d) with the nitrogen atom of the amino group at position 1 as a connecting position, GGFG represents an amino acid sequence consisting of glycine-glycine-phenylalanine-glycine linked through peptide bonds, and $-(\text{Succinimid-3-yl-N})-$ has a structure represented by the following formula:

[Formula 2]



which is connected to the antibody at position 3 thereof and is connected to a methylene group in the linker structure containing this structure on the nitrogen atom at position 1.

[Claim 33]

The antibody-drug conjugate according to any one of claims 29 to 32, wherein the linker is represented by any formula selected from the group consisting of the following formulas (c), (d) and (e):

(c) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$,

(d) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$, and

(e) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$.

[Claim 34]

The antibody-drug conjugate according to any one of claims 29 to 33, wherein the linker is represented by the following formula (c) or (e):

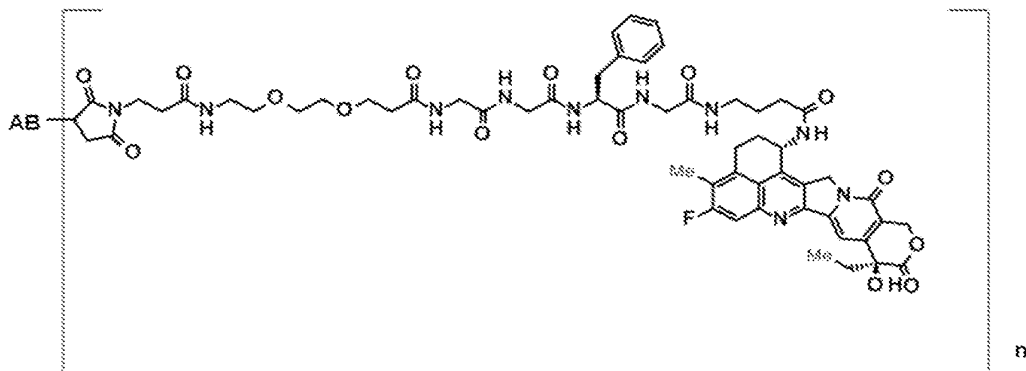
(c) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2-\text{O}-\text{CH}_2-\text{C}(=\text{O})-$, and

(e) $-(\text{Succinimid-3-yl-N})-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-\text{GGFG}-\text{NH}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{C}(=\text{O})-$.

[Claim 35]

The antibody-drug conjugate according to any one of claims 29 to 34, which has a structure represented by the following formula:

[Formula 3]

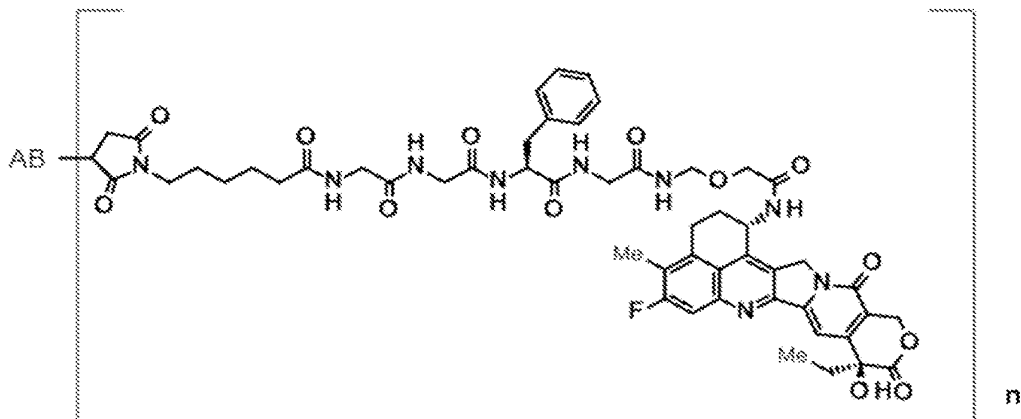


wherein AB represents the antibody or the functional fragment of the antibody, n represents the average number of units of the drug-linker structure conjugated to the antibody per antibody, and the antibody is connected to the linker via a sulfhydryl group derived from the antibody.

[Claim 36]

The antibody-drug conjugate according to any one of claims 29 to 34, which has a structure represented by the following formula:

[Formula 4]



wherein AB represents the antibody or the functional fragment of the antibody, n represents the average number of units of the drug-linker structure conjugated to the antibody per antibody, and the antibody is connected to the linker via a sulfhydryl group derived from the antibody.

[Claim 37]

The antibody-drug conjugate according to any one of claims 29 to 36, wherein the antibody is an antibody comprising a light chain and a heavy chain in any one combination selected from the group consisting of the following combinations (1) to (4), or a functional fragment of the antibody:

- (1) a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 69,
- (2) a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73,
- (3) a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 65 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 73, and
- (4) a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 77.

[Claim 38]

The antibody-drug conjugate according to claim 37, wherein the antibody is an antibody comprising a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 69, or a functional fragment of the antibody.

[Claim 39]

The antibody-drug conjugate according to claim 37, wherein the antibody is an antibody comprising a light chain consisting of the amino acid sequence at positions 21 to 233 in SEQ ID NO: 61 and a heavy chain consisting of the amino acid sequence at positions 20 to 471 in SEQ ID NO: 77, or a functional fragment of the antibody.

[Claim 40]

The antibody-drug conjugate according to any one of claims 29 to 39, wherein the heavy chain or the light chain has undergone one or two or more modifications selected from the group consisting of N-linked glycosylation, O-linked glycosylation, N-terminal processing, C-terminal processing, deamidation, isomerization of aspartic acid, oxidation of methionine, addition of a methionine residue to the N-terminus, amidation of a proline residue, conversion of N-terminal glutamine or N-terminal glutamic acid to pyroglutamic acid, and a deletion of one or two amino acids from the carboxyl terminus.

[Claim 41]

The antibody-drug conjugate according to any one of claims 29 to 40, wherein the average number of units of the

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selected drug-linker structure conjugated per antibody is in the range of from 1 to 10.

[Claim 42]

The antibody-drug conjugate according to claim 41, wherein the average number of units of the selected drug-linker structure conjugated per antibody is in the range of from 2 to 8.

[Claim 43]

The antibody-drug conjugate according to claim 42, wherein the average number of units of the selected drug-linker structure conjugated per antibody is in the range of from 5 to 8.

[Claim 44]

The antibody-drug conjugate according to claim 43, wherein the average number of units of the selected drug-linker structure conjugated per antibody is in the range of from 7 to 8.

[Claim 45]

A pharmaceutical composition comprising the antibody-drug conjugate according to any one of claims 29 to 44, a salt thereof, or a hydrate of the conjugate or the salt.

[Claim 46]

The pharmaceutical composition according to claim 45, which is an antitumor drug.

[Claim 47]

The pharmaceutical composition according to claim 46, wherein the tumor is a tumor expressing CDH6.

[Claim 48]

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The pharmaceutical composition according to claim 46 or 47, wherein the tumor is renal cell carcinoma, renal clear cell carcinoma, papillary renal cell carcinoma, ovarian cancer, ovarian serous adenocarcinoma, thyroid cancer, bile duct cancer, lung cancer, small-cell lung cancer, glioblastoma, mesothelioma, uterine cancer, pancreatic cancer, Wilms' tumor or neuroblastoma.

[Claim 49]

A method for treating a tumor, which comprises administering any component selected from the antibody-drug conjugate according to any one of claims 29 to 44, a salt thereof, and a hydrate of the conjugate or the salt to an individual.

[Claim 50]

The treatment method according to claim 49, wherein the tumor is a tumor expressing CDH6.

[Claim 51]

The treatment method according to claim 49 or 50, wherein the tumor is renal cell carcinoma, renal clear cell carcinoma, papillary renal cell carcinoma, ovarian cancer, ovarian serous adenocarcinoma, thyroid cancer, bile duct cancer, lung cancer, small-cell lung cancer, glioblastoma, mesothelioma, uterine cancer, pancreatic cancer, Wilms' tumor or neuroblastoma.

[Claim 52]

A method for treating a tumor, which comprises administering a pharmaceutical composition comprising at least one component selected from the antibody-drug conjugate according to any one of claims 29 to 44, a salt thereof, and a hydrate of the conjugate or the salt, and at least one

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antitumor drug to an individual, simultaneously, separately,
or continuously.

[Claim 53]

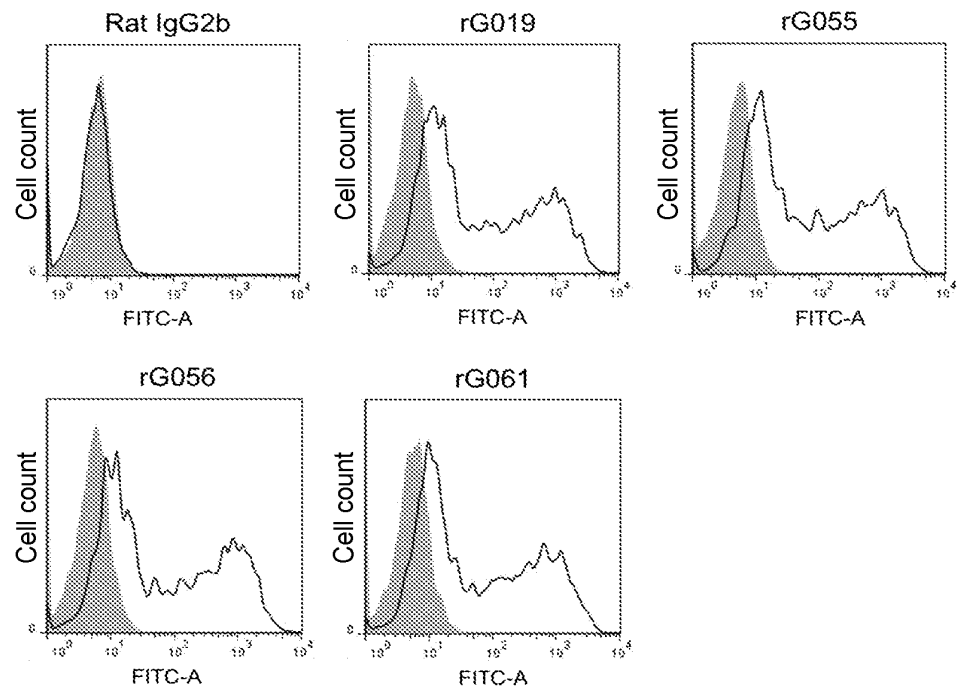
A method for producing an antibody-drug conjugate, which
comprises the step of reacting the antibody or the functional
fragment of the antibody according to any one of claims 1 to
13 and 24 to 28, or an antibody or a functional fragment of
the antibody obtained by the production method according to
claim 23 with a drug-linker intermediate compound.

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Abstract

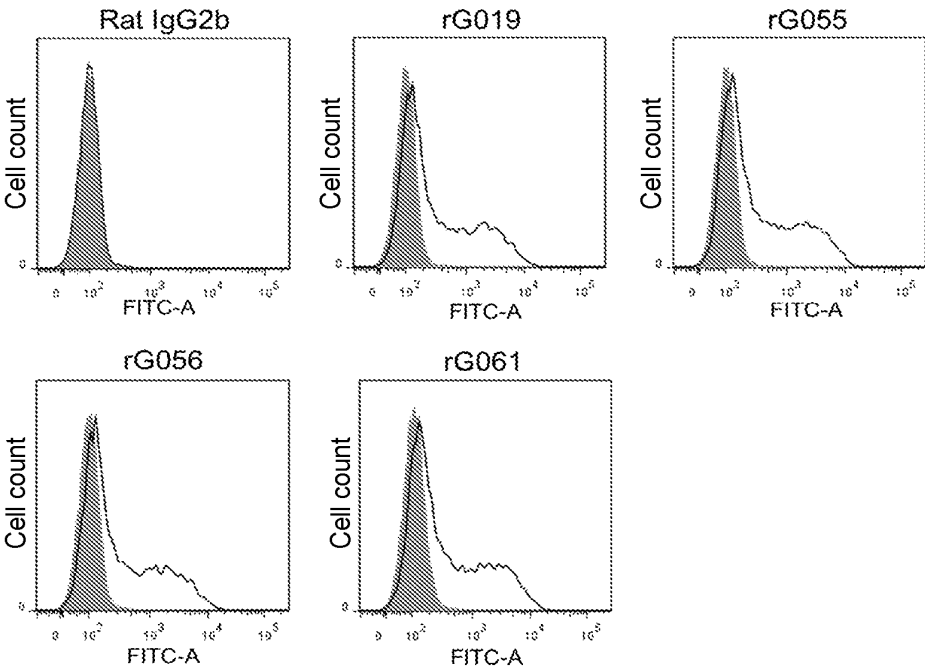
It is an object of the present invention to provide an antibody binding to CDH6 and having internalization activity, an antibody-drug conjugate of the antibody and a drug having antitumor activity, a pharmaceutical product comprising the antibody-drug conjugate and having therapeutic effects on a tumor, a method for treating a tumor using the antibody, the antibody-drug conjugate or the pharmaceutical product, and the like. The present invention provides an anti-CDH6 antibody having internalization activity, an antibody-drug conjugate of the antibody and a drug having antitumor activity, a pharmaceutical product comprising the antibody or the antibody-drug conjugate, and a method for treating a tumor.

[Figure 1]



[Figure 2-1]

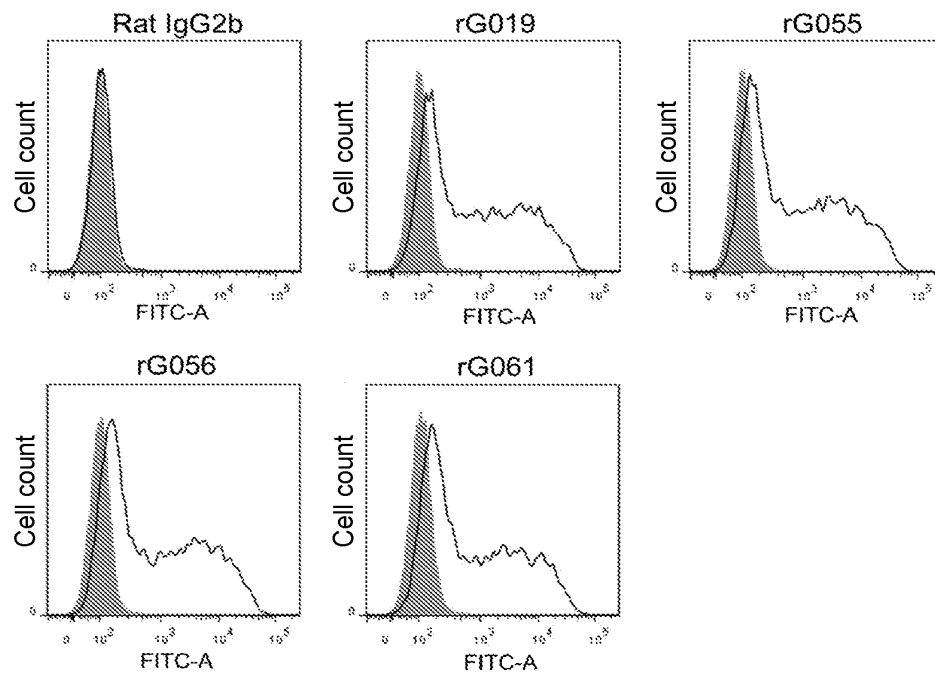
(1) Control cell or full-length hCDH6-transfected cell



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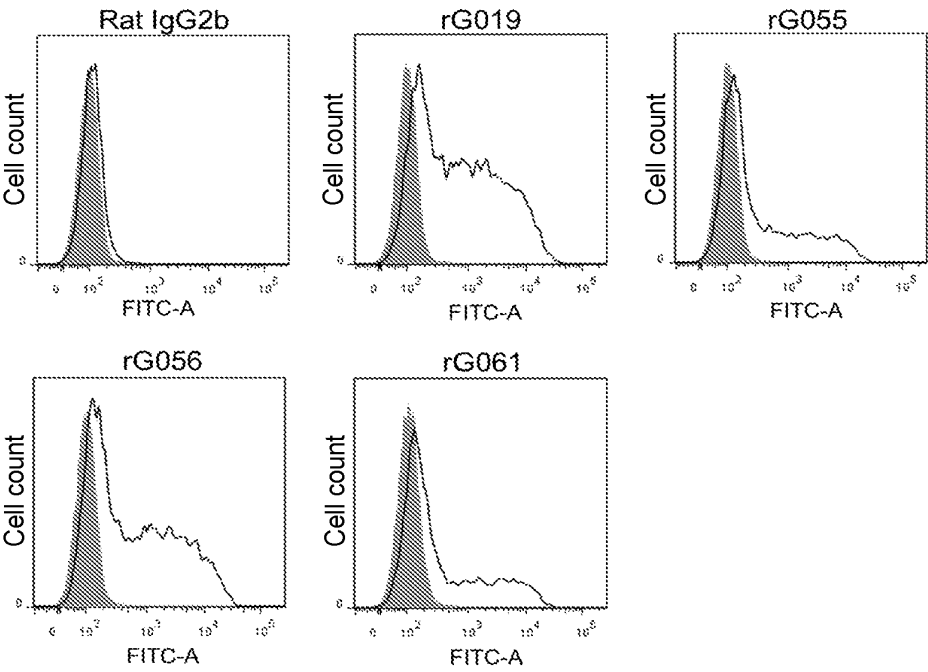
[Figure 2-2]

(2) Control cell or EC1-deleted hCDH6-transfected cell



[Figure 2-3]

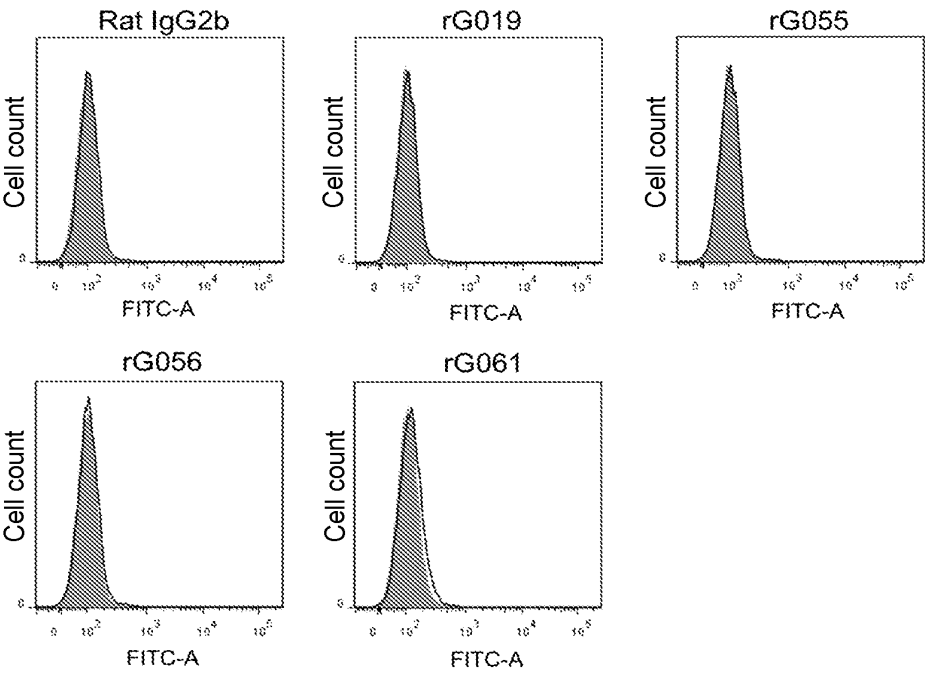
(3) Control cell or EC2-deleted hCDH6-transfected cell



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[Figure 2-4]

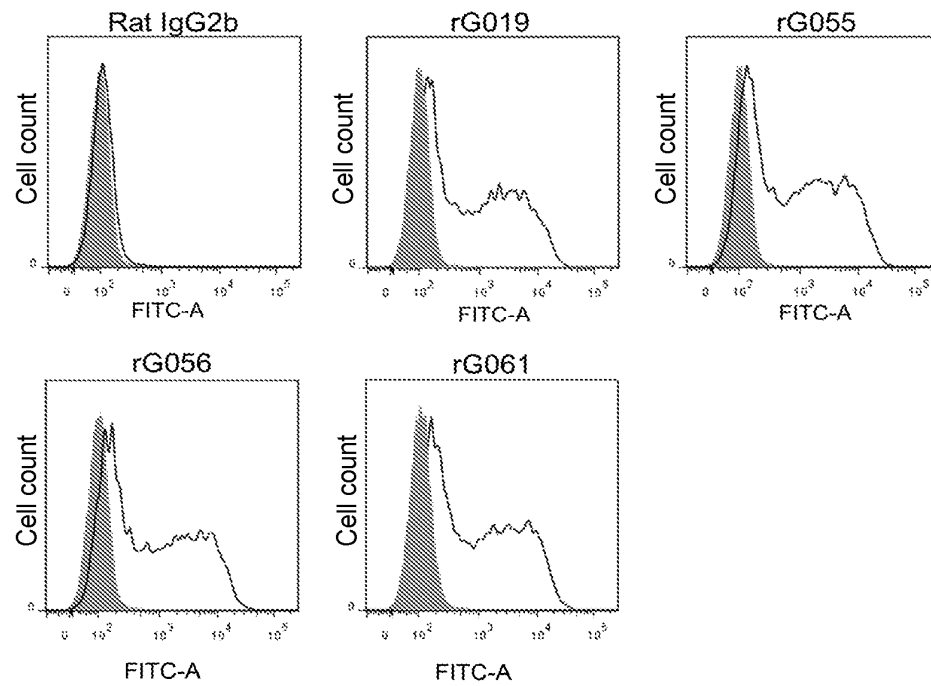
(4) Control cell or EC3-deleted hCDH6-transfected cell



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[Figure 2-5]

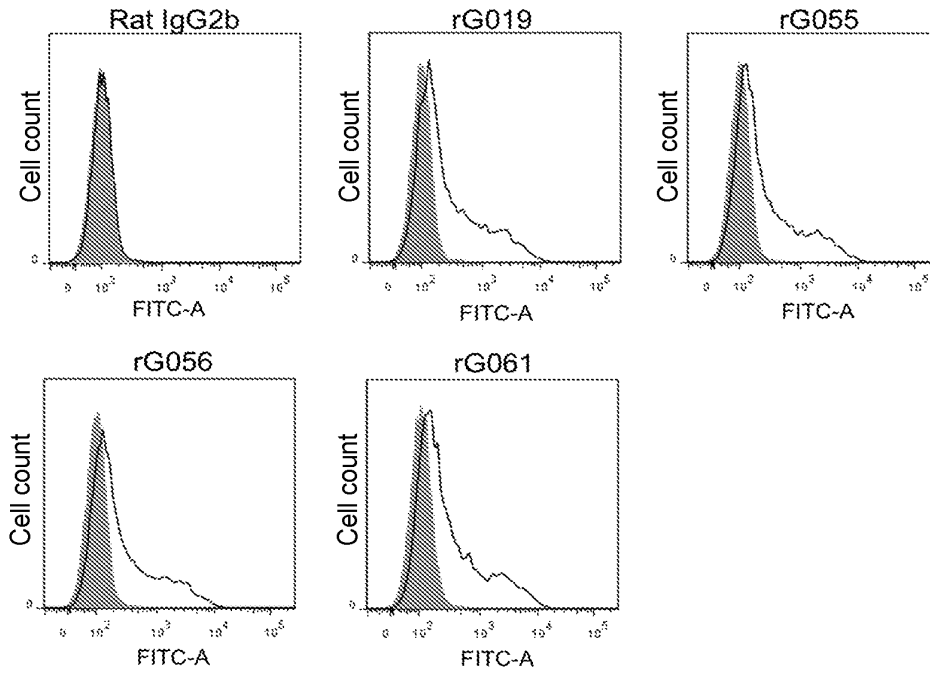
(5) Control cell or EC4-deleted hCDH6-transfected cell



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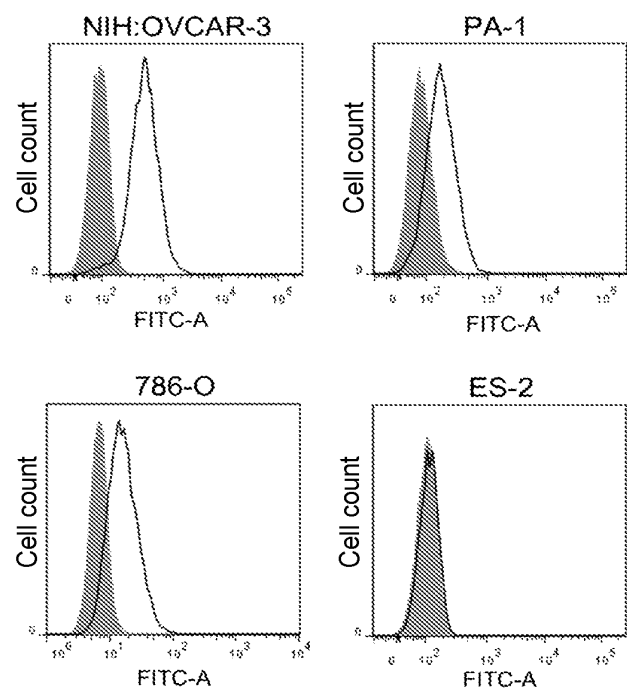
[Figure 2-6]

(6) Control cell or EC5-deleted hCDH6-transfected cell



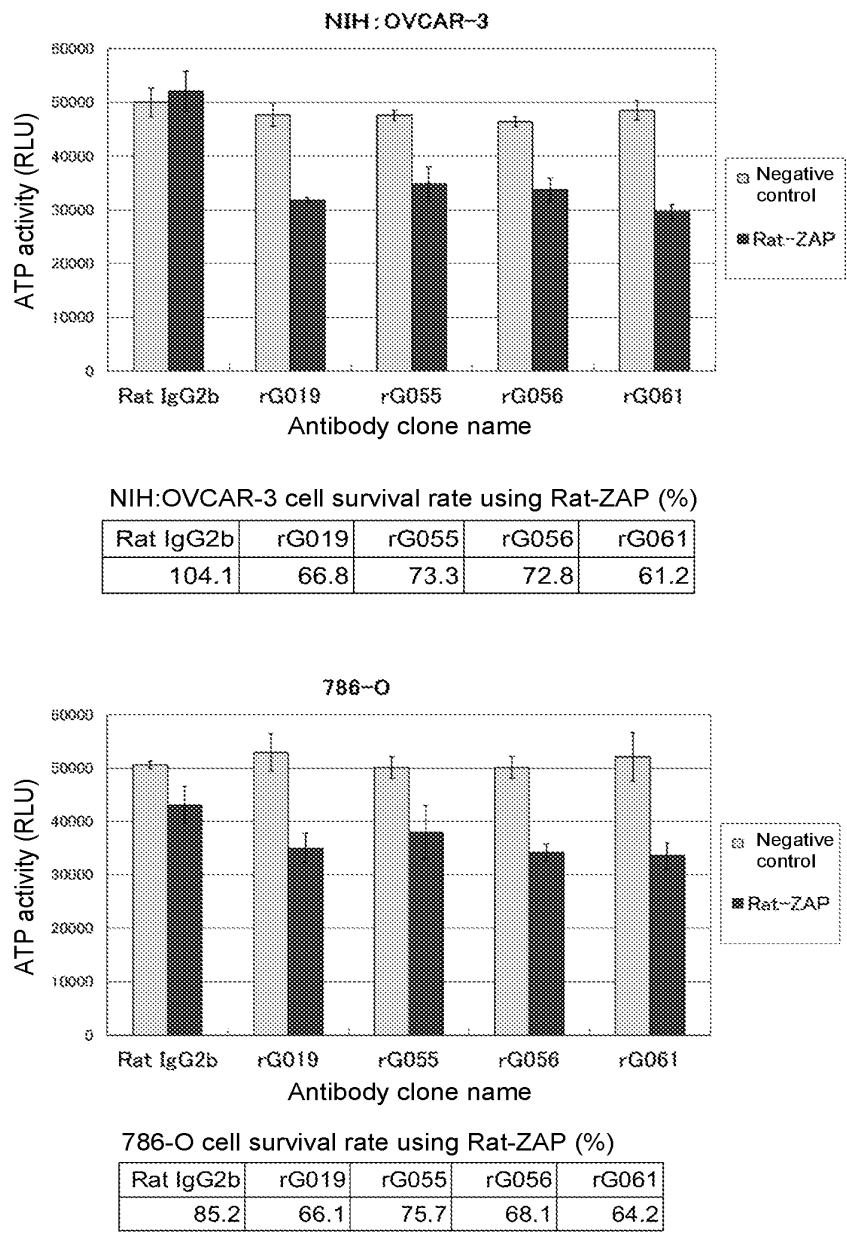
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[Figure 3]



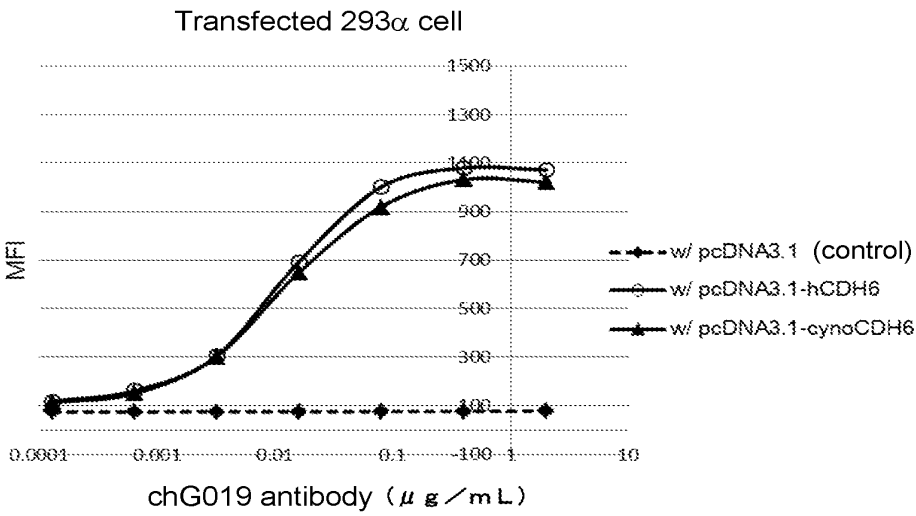
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[Figure 4]



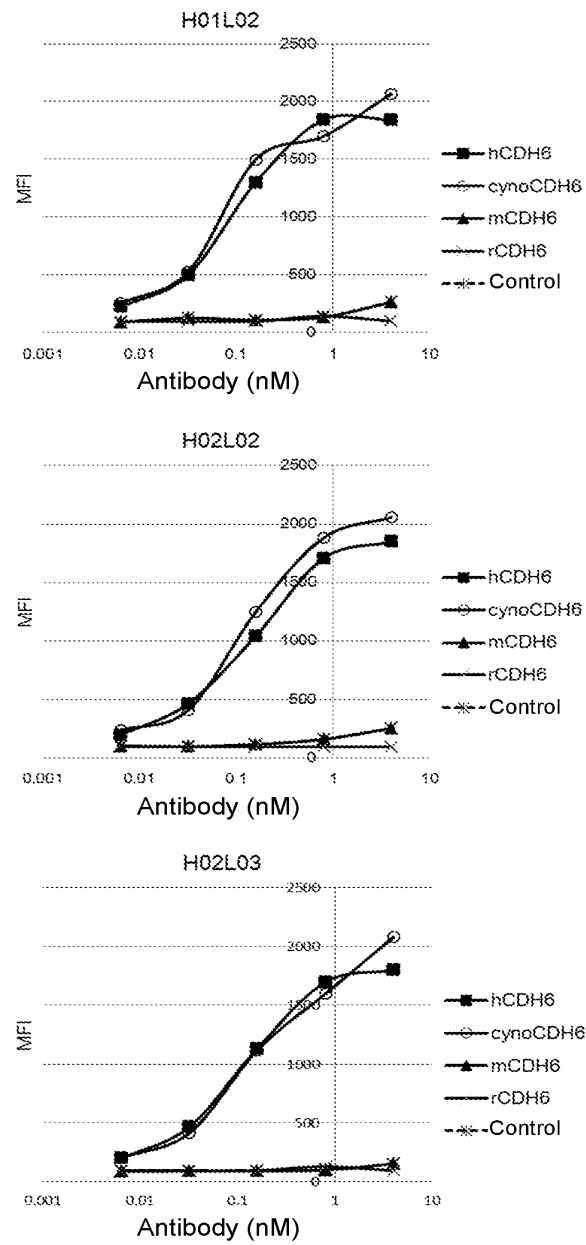
10/29

[Figure 5]



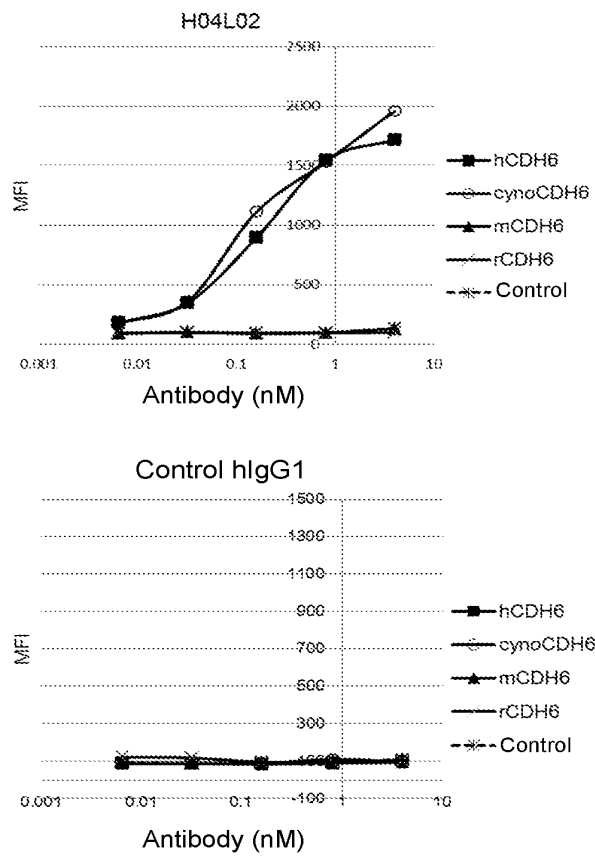
11/29

[Figure 6-1]



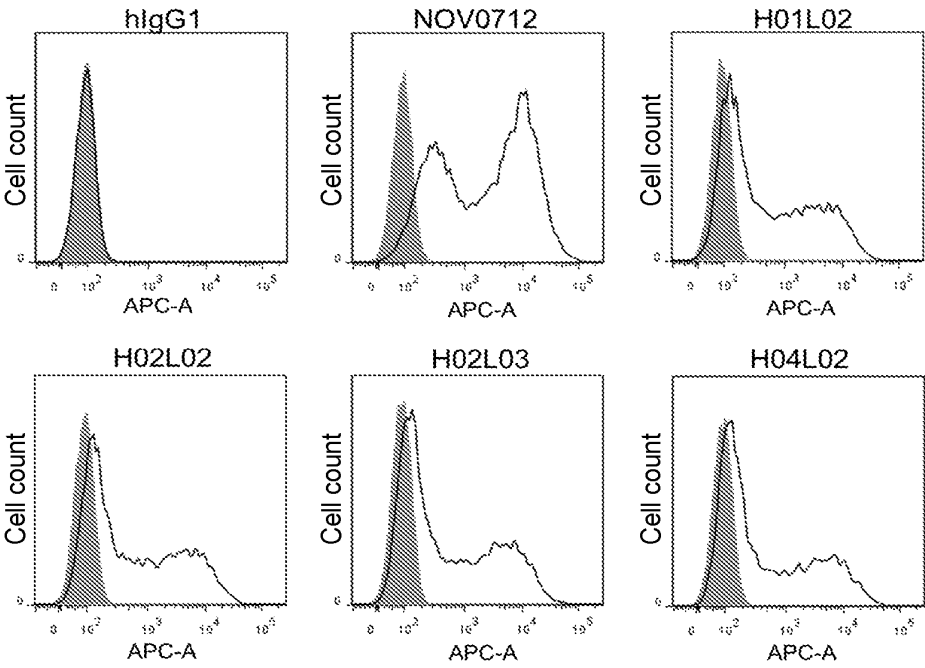
12/29

[Figure 6-2]



[Figure 7-1]

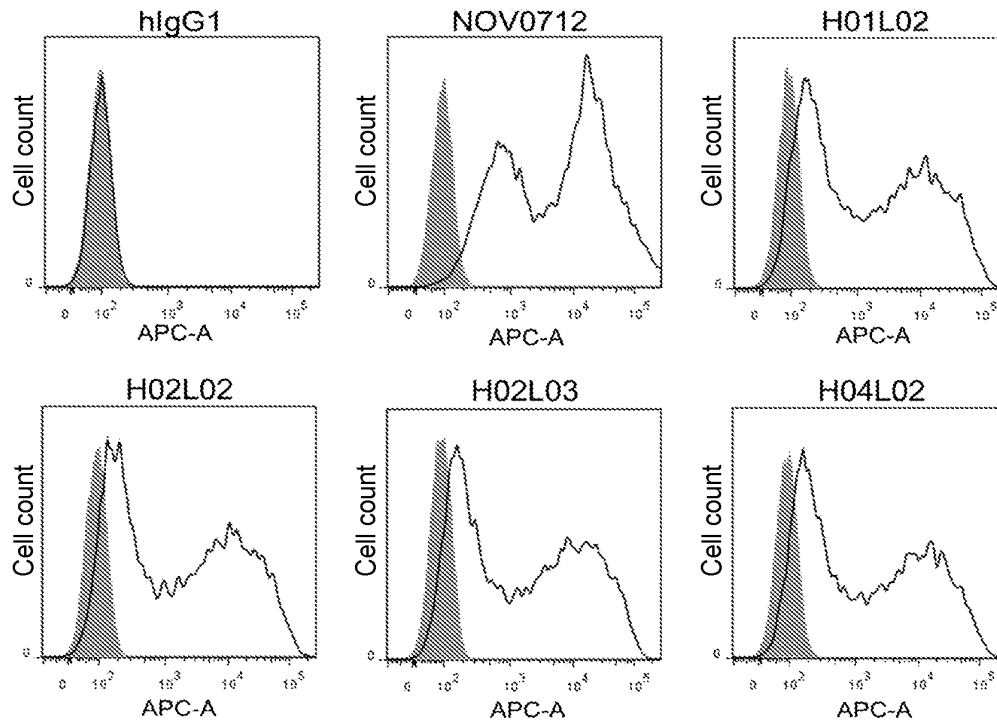
(1) Control cell or full-length hCDH6-transfected cell



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[Figure 7-2]

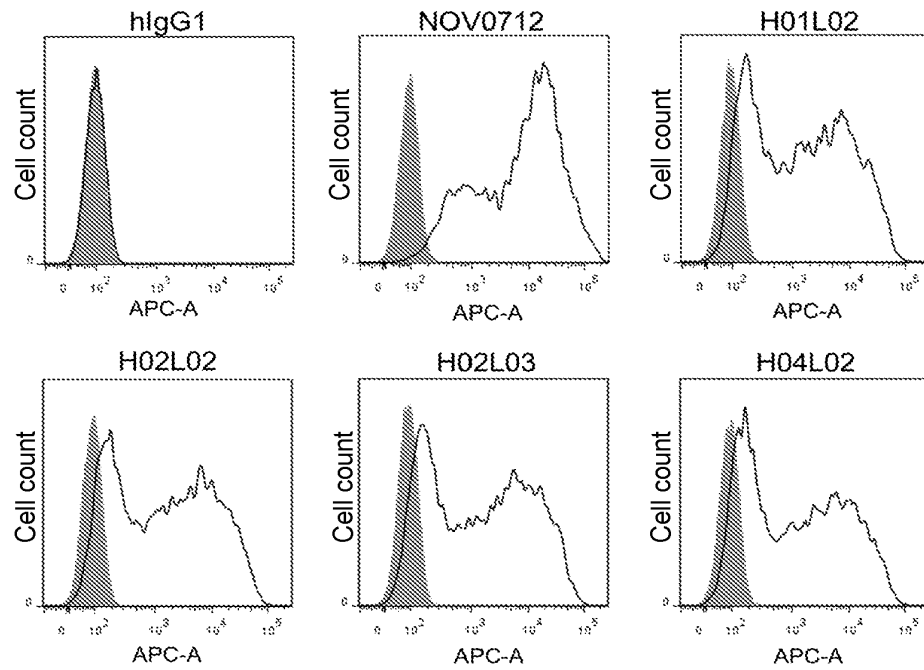
(2) Control cell or EC1-deleted hCDH6-transfected cell



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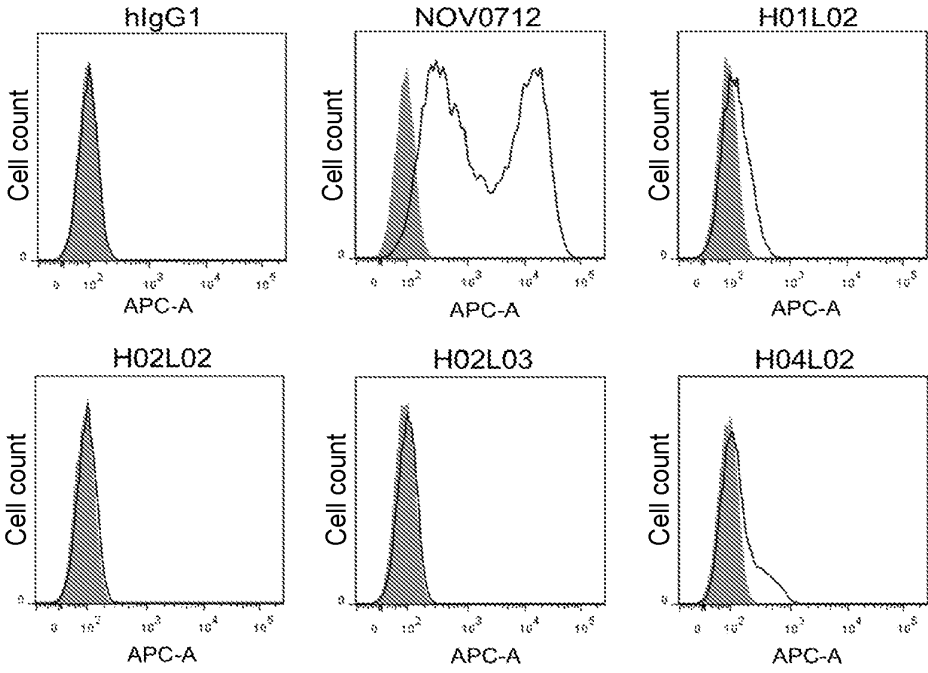
[Figure 7-3]

(3) Control cell or EC2-deleted hCDH6-transfected cell



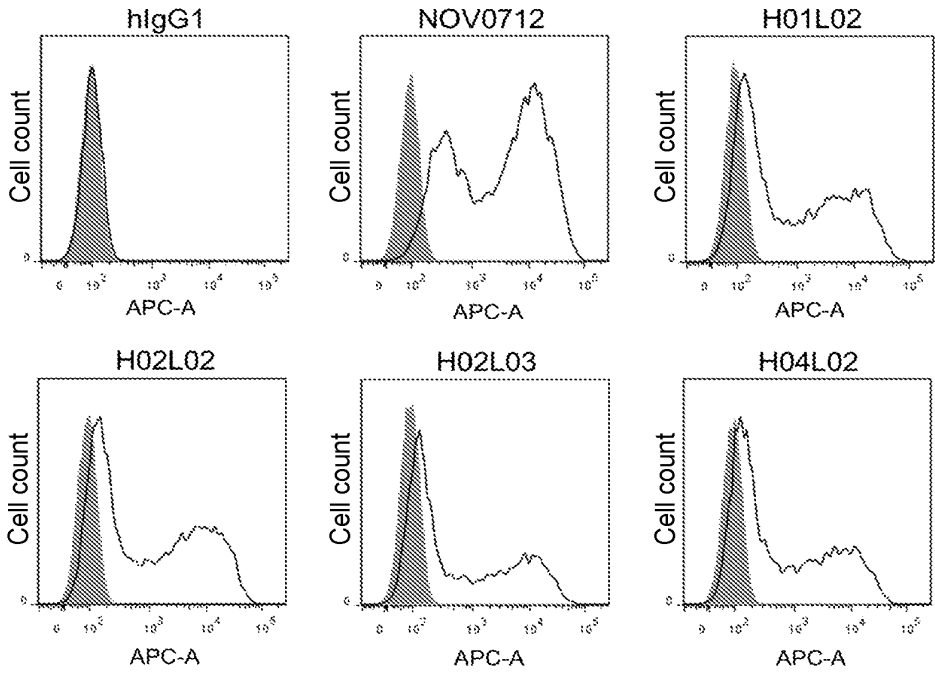
[Figure 7-4]

(4) Control cell or EC3-deleted hCDH6-transfected cell



[Figure 7-5]

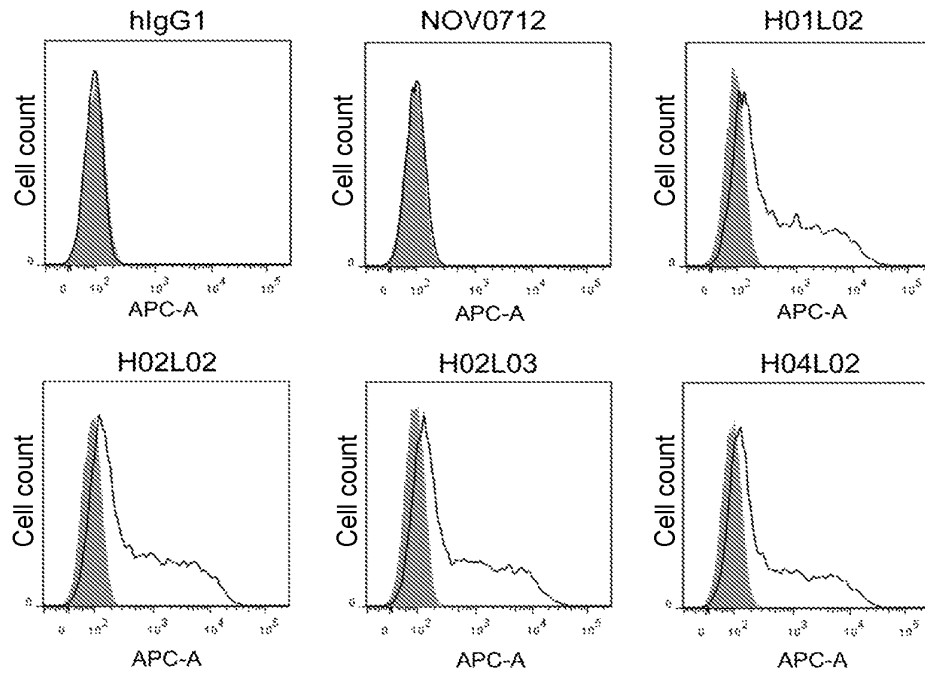
(5) Control cell or EC4-deleted hCDH6-transfected cell



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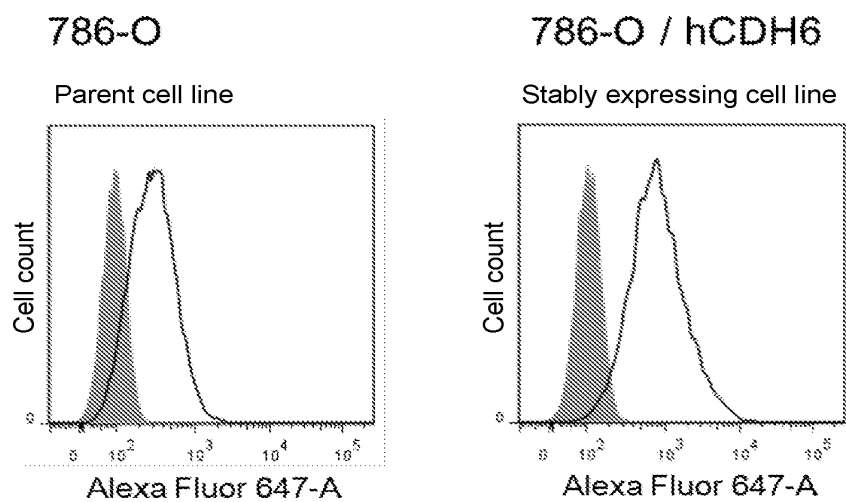
[Figure 7-6]

(6) Control cell or EC5-deleted hCDH6-transfected cell

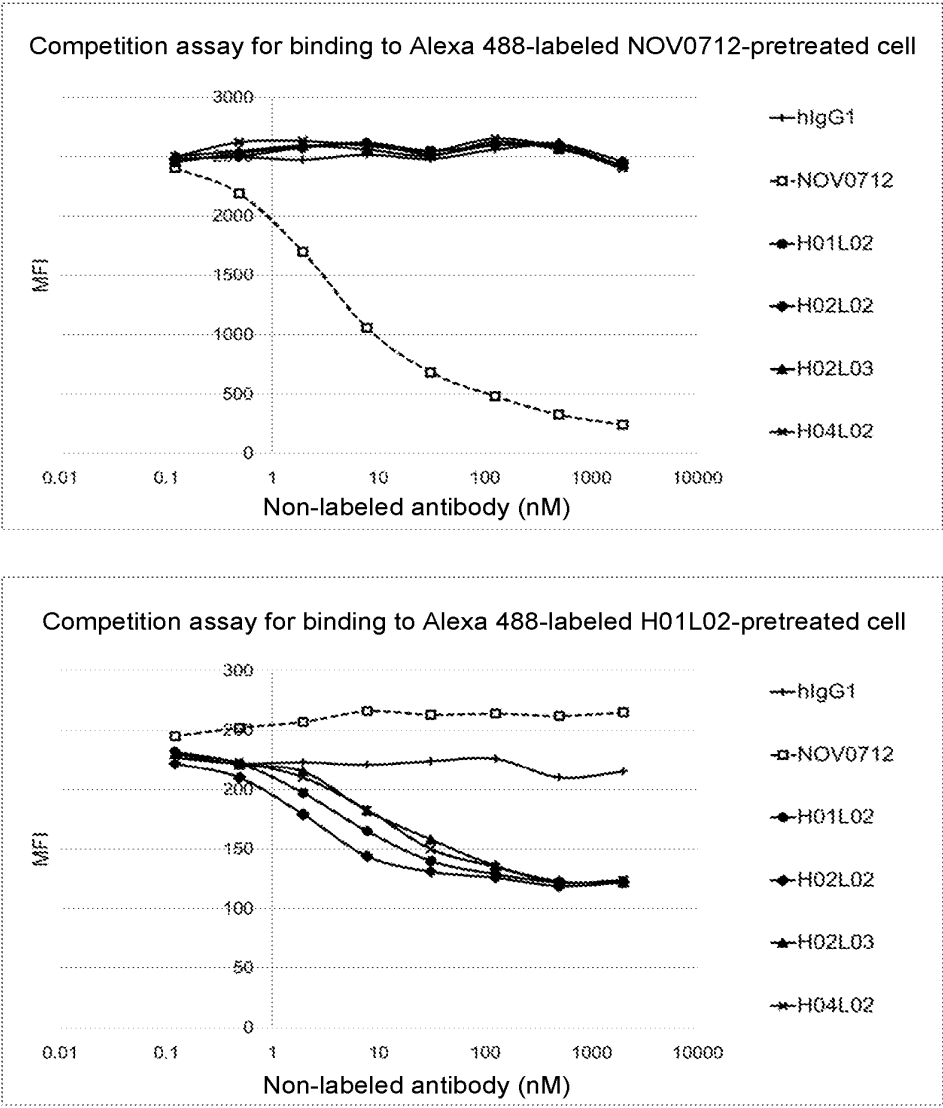


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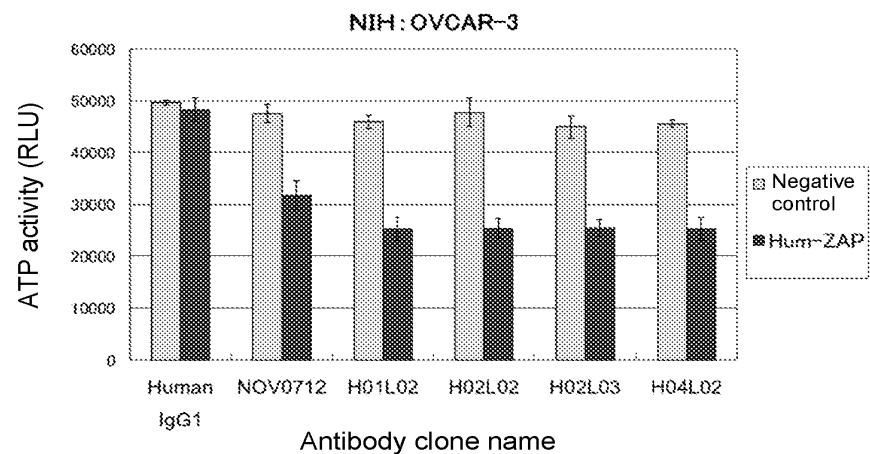
[Figure 8]



[Figure 9]



[Figure 10-1]

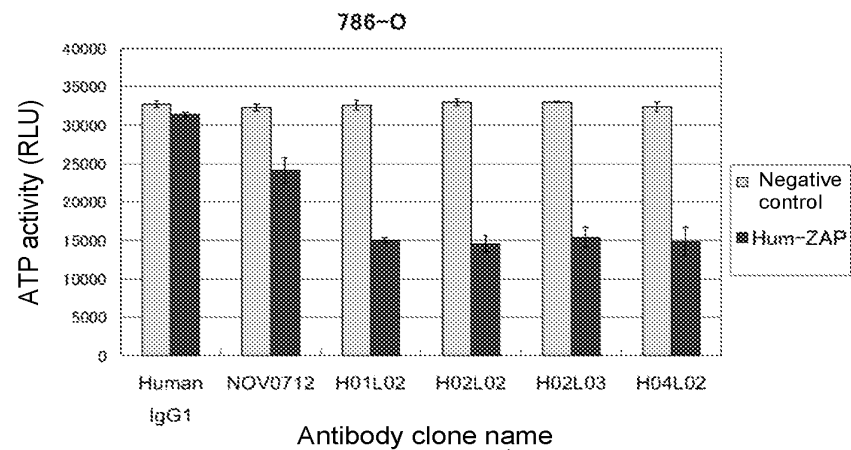


NIH:OVCAR-3 cell survival rate using Hum-ZAP (%)

Human IgG1	NOV0712	H01L02	H02L02	H02L03	H04L02
97.3	67.0	55.1	53.1	56.7	55.6

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[Figure 10-2]

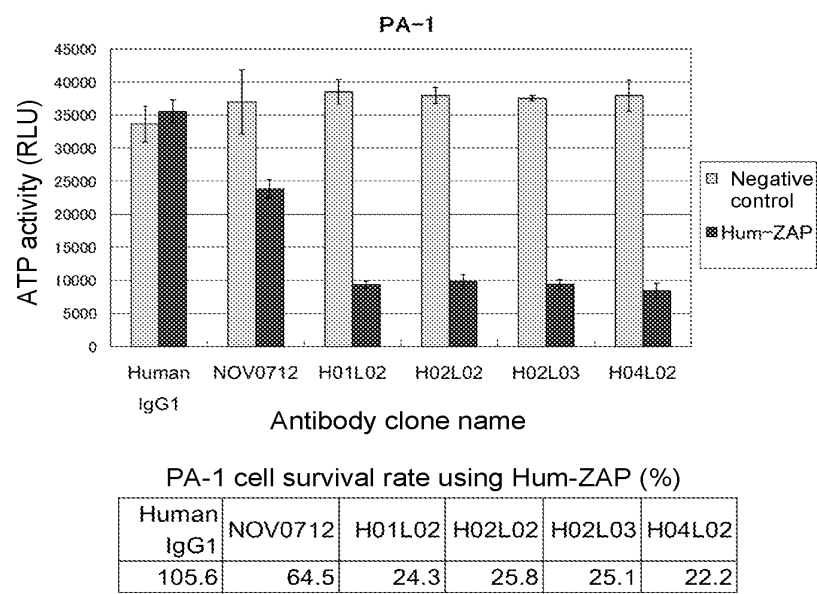


786-O cell survival rate using Hum-ZAP (%)

Human IgG1	NOV0712	H01L02	H02L02	H02L03	H04L02
95.9	74.8	46.0	44.1	46.5	45.9

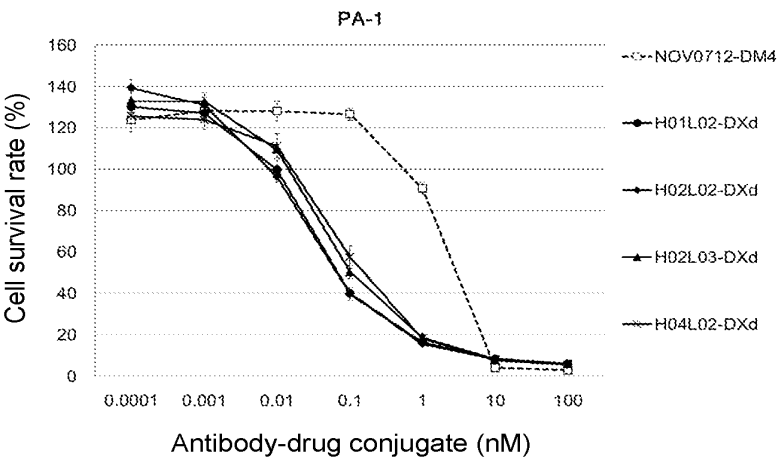
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[Figure 10-3]



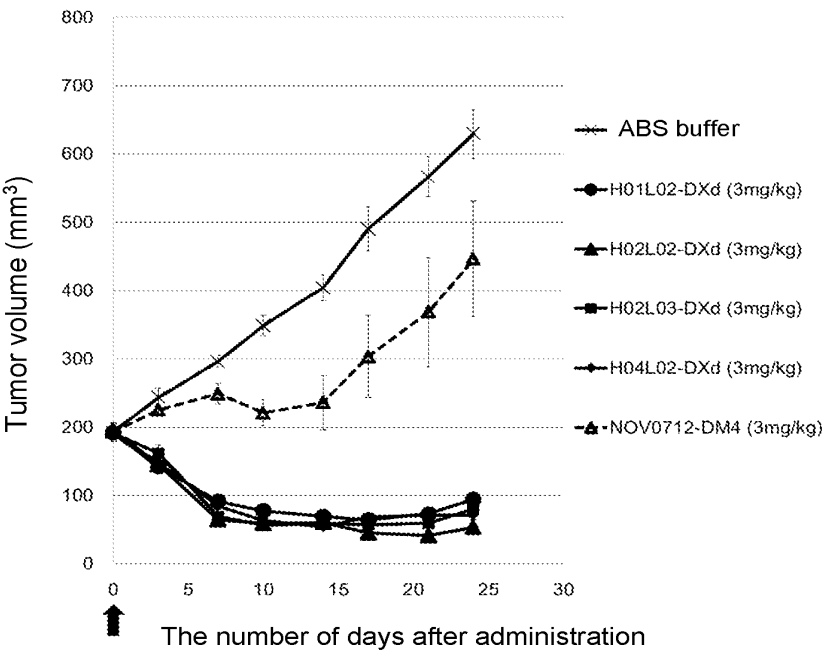
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[Figure 11]



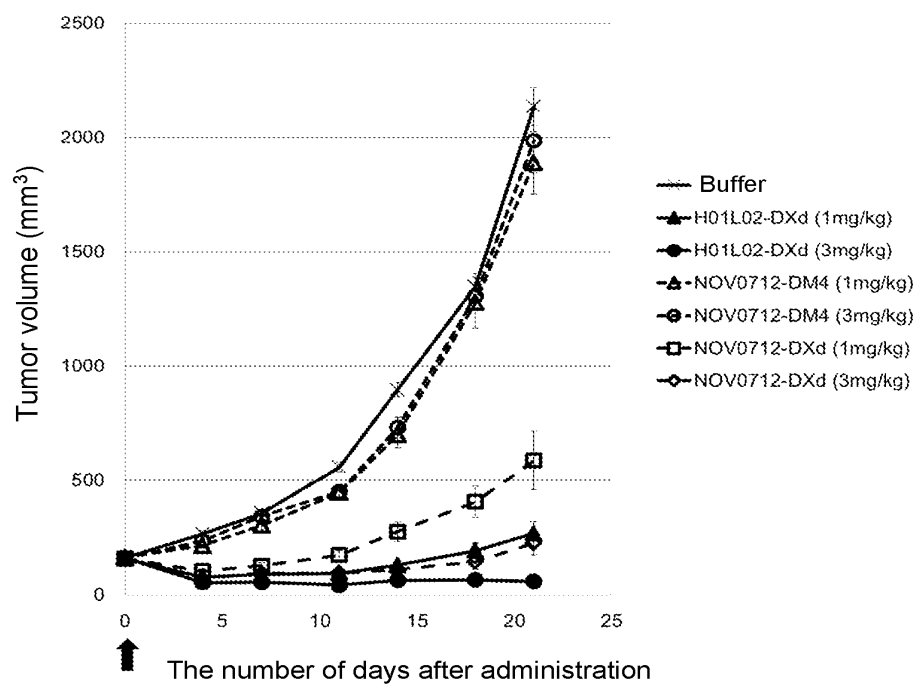
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[Figure 12]



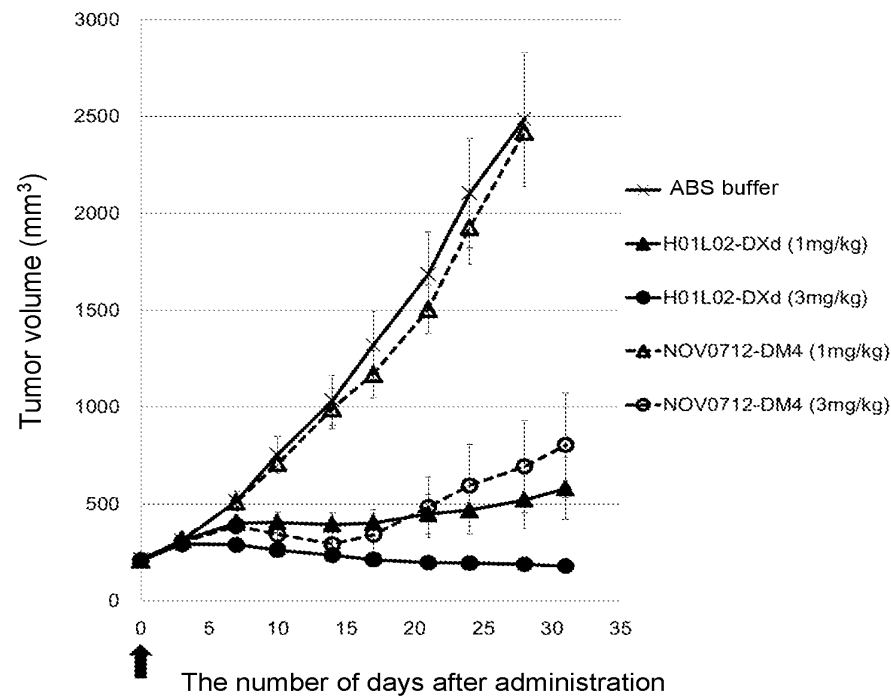
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[Figure 13]



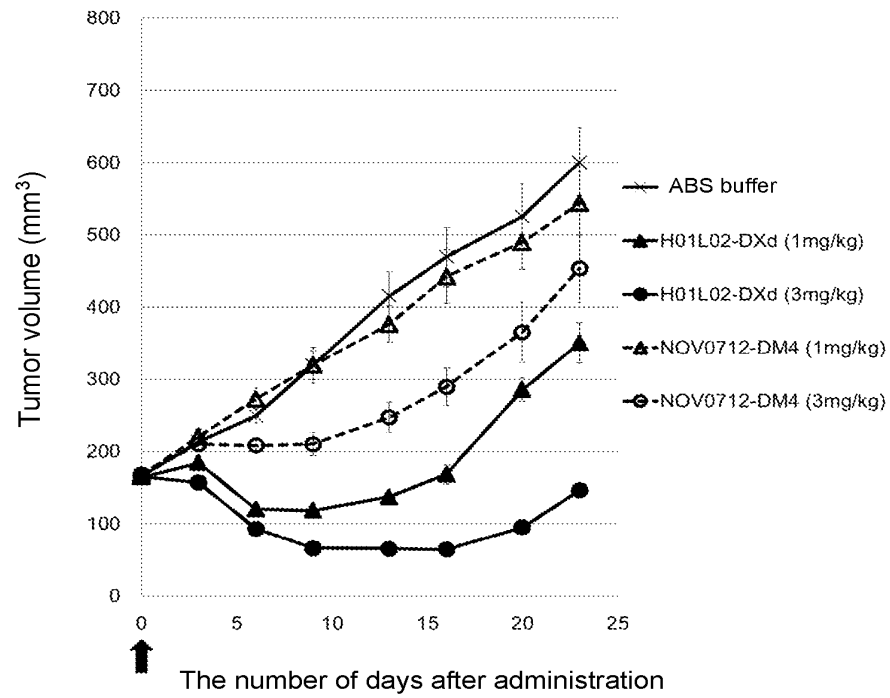
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[Figure 14]



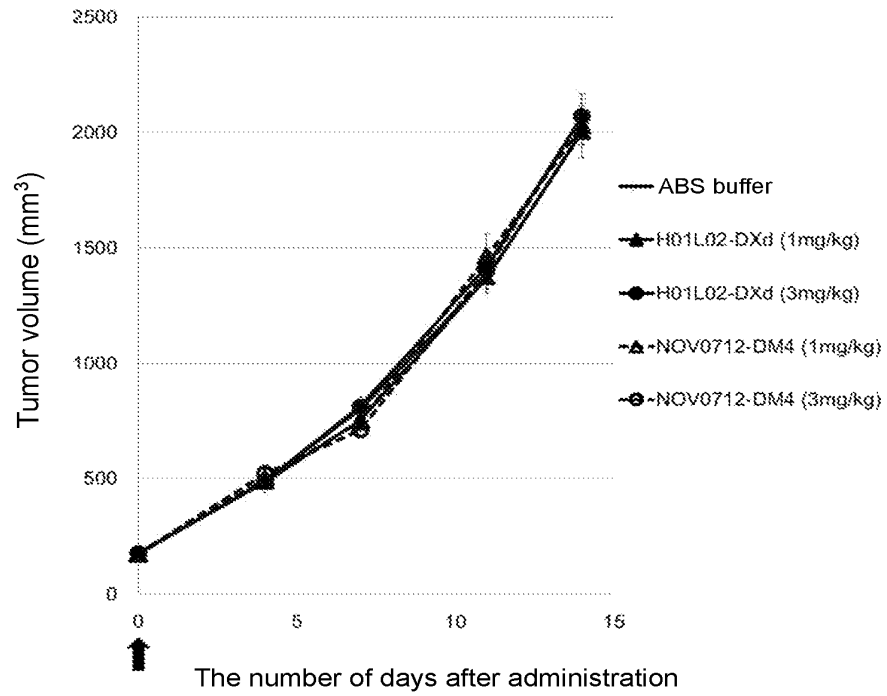
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[Figure 15]



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[Figure 16]



Atty. Dkt. No. 098065-0249

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Inventor Name: Atsuko SAITO

Title: ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG
CONJUGATE

Appl. No.: Not Yet Assigned

International Filing Date: May 14, 2018

371(c) Date: Herewith

Examiner: Not Yet Assigned

Art Unit: Not Yet Assigned

Confirmation Number: Not Yet Assigned

PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.115

Mail Stop AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

Prior to examination of the present Application, Applicant respectfully requests that the application be amended as follows:

Amendments to the Specification begin on page 2 of this document.

Remarks begin on page 6 of this document.

Please amend the application as follows:

Atty. Dkt. No. 098065-0249

Amendments to the Specification:

Please amend the specification as follows:

Please insert the following new paragraph after the Title of the Invention and before the “TECHNICAL FIELD” of the invention:

CROSS-REFERENCE TO RELATED APPLICATIONS

[0000]

The present application claims priority under 37 U.S.C. § 371 to International Patent Application No. PCT/JP2018/018572, filed May 14, 2018, which claims priority to and the benefit of Japanese Patent Application No. 2017-096749, filed on May 15, 2017. The contents of these applications are hereby incorporated by reference in their entireties.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, is named 098065-0249_SL.txt and is 107 kb in size.

Please amend the Paragraph [0057] of the Specification as follows:

[0057] (2) Production of anti-CDH6 monoclonal antibody

The anti-CDH6 antibody used in the present invention is not particularly limited. For example, an antibody specified by an amino acid sequence shown in the sequence listing of the present application can be suitably used. The anti-CDH6 antibody used in the present invention is desirably an antibody having the following properties:

(1) an antibody having the following properties:

(a) specifically binding to CDH6, and

(b) having the activity of being internalized into CDH6-expressing cells by binding to CDH6;

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(2) the antibody according to the above (1) ~~or the aforementioned antibody~~, wherein the CDH6 is human CDH6; or

(3) the antibody according to the above (1) or (2), wherein the antibody specifically recognizes EC3 of human CDH6, and having internalization activity.

The method for obtaining the antibody against CDH6 of the present invention is not particularly limited as long as an anti-CDH6 antibody can be obtained. It is preferred to use CDH6 retaining its conformation as an antigen.

Please amend the Paragraph [0077] of the Specification as follows:

[0077] The amino acid sequence of the light chain variable region of the chG019 antibody is identical to the amino acid sequence of the light chain variable region of the rG019 antibody, and consists of the amino acid sequence shown in SEQ ID NO: 10. The light chain of the chG019 antibody has CDRL1 consisting of the amino acid sequence shown in SEQ ID NO: 12, CDRL2 consisting of the amino acid sequence shown in SEQ ID NO: 13, and CDRL3 consisting of the amino acid sequence shown in SEQ ID NO: 14, which are identical to the light chain CDRL1, CDRL2 and CDRL3, respectively, of rG019. The amino acid sequence of the light chain variable region of the chG019 antibody is encoded by the nucleotide sequence shown in SEQ ID NO: 55.

Please amend the Paragraph [0223] of the Specification as follows:

[0223] Using each cDNA moiety of the rat CDH6 protein (NP_037059)-encoding cDNA expression vector (OriGene Technologies Inc., RN211850), the cDNA was incorporated into a vector for mammalian expression according to a method known to a person skilled in the art to produce ~~human-rat~~ CDH6 expression vectors pcDNA3.1-rCDH6 and p3xFLAG-CMV-9-rCDH6. The amino acid sequence of the rat CDH6 ORF is shown in SEQ ID NO: 8.

Please amend the Paragraph [0259] of the Specification as follows:

[0259] 4)-3 Evaluation of binding activity of human chimeric anti-CDH6 antibody chG019

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The CDH6-binding activity of the human chimeric anti-CDH6 antibody chG019 purified in 4)-2 was confirmed by flow cytometry. Using Lipofectamine 2000, pcDNA3.1-hCDH6 or pcDNA3.1-cynoCDH6 produced in Example 1)-1, or pcDNA3.1 was transiently introduced into 293 α cells. The cells were cultured overnight under conditions of 37°C and 5% CO₂, and thereafter, a cell suspension was prepared. chG019 was added to the suspension of each of these cells. The cells were left standing at 4°C for 1 hour. Thereafter, the cells were washed twice with PBS supplemented with 5% FBS, and then suspended by the addition of PE-labeled F(ab')₂ Fragment anti-human IgG, Fc γ antibody (Jackson ImmunoResearch Laboratories, Inc.) that had been 500-fold diluted with PBS supplemented with 5% FBS. The cells were left standing at 4°C for 1 hour. The cells were washed twice with PBS supplemented with 5% FBS, and then re-suspended in PBS supplemented with 5% FBS, followed by detection using a flow cytometer (Canto II; BD Biosciences). The data was analyzed using FlowJo (Tree Star, Inc.). As shown in Figure 5, chG019 did not bind to the ~~293 α 293T~~ cells transfected with pcDNA3.1 as a negative control, but did bind to the ~~293 α 293T~~ cells transfected with pcDNA3.1-hCDH6 or pcDNA3.1-cynoCDH6 in an antibody concentration-dependent manner. In Figure 5, the abscissa depicts antibody concentration, and the ordinate depicts the amount of the antibody bound, based on mean fluorescence intensity. It is evident from this result that chG019 specifically binds to human CDH6 and cynomolgus monkey CDH6 with almost equivalent binding activity.

Please amend the Paragraph [0278] of the Specification as follows:

[0278] [Table 2]

	Antibody	KD(M)
1	H02L02 <u>H01L02</u>	1.5E-09
2	H02L02	1.1E-09
3	H02L03	1.4E-09
4	H04L02	1.1E-09

Please amend the Paragraph [0282] of the Specification as follows:

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[0282] 6)-2-2-2 Binding competition assay using labeled H01L02 and labeled NOV0712

Labeled H01L02 and labeled NOV0712 were produced using an Alexa Fluor 488 Monoclonal Antibody Labeling Kit (Thermo Fisher Scientific Inc.). The cell suspension of the 786-O/hCDH6 stably expressing cell line produced in 76)-2-2-1 was centrifuged, and the supernatant was then removed. Thereafter, the cells were suspended by the addition of labeled NOV0712 or labeled H01L02 (final concentration: 5 nM) and, further, the addition of each of the 4 humanized hG019 antibodies (clone Nos: H01L02, H02L02, H02L03 and H04L02), which had been prepared in Example 5)-5-3, or the anti-CDH6 antibody NOV0712, which had been prepared in Reference Example 1, or human IgG1 (Calbiochem) as a negative control (final concentration: as shown in the abscissa of Figure 9). The cells were left standing at 4°C for 1 hour. Thereafter, the cells were washed twice with PBS supplemented with 5% FBS, followed by detection using a flow cytometer (Canto II; BD Biosciences). The data was analyzed using FlowJo (Tree Star, Inc.). The results are shown in Figure 9. The abscissa depicts the final concentration of the added unlabeled antibody, and the ordinate depicts the amount of the antibody bound based on mean fluorescence intensity. When unlabeled NOV0712 is added to cells supplemented with labeled NOV0712, the amount of the labeled antibody bound is decreased by replacement with the unlabeled antibody in an addition concentration-dependent manner because they compete with each other for binding to the same epitope. On the other hand, even if each of the 4 humanized hG019 antibodies or human IgG1 as a negative control is added to cells supplemented with labeled NOV0712, there is no change in the amount of the labeled antibody bound, indicating that these antibodies differ in epitope and thus do not compete with each other for binding. Likewise, when each of the 4 unlabeled humanized hG019 antibodies is added to cells supplemented with labeled H01L02, the amount of the labeled antibody bound is decreased by replacement with the unlabeled antibody in an addition concentration-dependent manner because they compete with each other for binding to the same epitope. On the other hand, even if NOV0712 or human IgG1 as a negative control is added to cells supplemented with labeled H01L02, there is no change in the amount of the labeled antibody bound, indicating that these antibodies differ in epitope and thus do not compete with each other for binding.

Atty. Dkt. No. 098065-0249

REMARKS

Applicant respectfully requests that the foregoing amendments be made prior to examination of the present application.

Status to the Specification

The specification is amended merely to add the domestic benefit and priority claim of the present application, to add a reference statement to the Sequence Listing, and to amend Paragraphs [0057], [0077], [0223], [0259], [0278], and [0282].

No new matter has been added.

Concluding Remarks

Applicant believes that the application is in condition for allowance. Favorable consideration is respectfully requested. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance prosecution.

The Commissioner is hereby authorized to charge any additional fees, **except for excess claims fees**, which may be required for this application to Deposit Account No. 19-0741.

Respectfully submitted,

Date November 13, 2019

By /Michael D. Kaminski/

FOLEY & LARDNER LLP
Customer Number: 22428
Telephone: (202) 672-5490
Facsimile: (202) 672-5399

Michael D. Kaminski
Attorney for Applicant
Registration No. 32,904

PATENT COOPERATION TREATY

PCT/JP2018/018572

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From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION,
OBTENTION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

ISHIBASHI Koki
c/o DAIICHI SANKYO COMPANY, LIMITED, 1-2-58,
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Date of mailing (day/month/year) 28 May 2018 (28.05.2018)	
Applicant's or agent's file reference DSPCT-FP1814	IMPORTANT NOTIFICATION
International application No. PCT/JP2018/018572	International filing date (day/month/year) 14 May 2018 (14.05.2018)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 15 May 2017 (15.05.2017)
Applicant DAIICHI SANKYO COMPANY, LIMITED	

The applicant is hereby notified of the date of receipt (or of obtaining by the International Bureau) of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to the date of receipt, **the priority document concerned was submitted or transmitted to or obtained by the International Bureau in compliance with Rule 17.1(a), (b) or (b-bis)**. This Form replaces any previously issued notification concerning submission, transmittal or obtaining of priority documents.

<u>Priority date</u>	<u>Priority application No.</u>	<u>Country or regional Office or PCT receiving Office</u>	<u>Date of receipt of priority document</u>
15 May 2017 (15.05.2017)	2017-096749	JP	24 May 2018 (24.05.2018)

The letters "NR" denote a priority document which, on the date of mailing of this Form, had not yet been received or obtained by the International Bureau in compliance with Rule 17.1(a), (b) or (b-bis). Where the applicant has failed to either submit, request to prepare and transmit, or to request the International Bureau to obtain the priority document within the applicable time limit under that Rule, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

An asterisk "*" next to a date of receipt, denotes a priority document submitted or transmitted to or obtained by the International Bureau but not in compliance with Rule 17.1(a), (b) or (b-bis) (the priority document was received after the time limit prescribed in Rule 17.1(a); the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b) or the request to the International Bureau to obtain the priority document was made after the applicable time limit under Rule 17.1(b-bis)). Even though the priority document was not furnished in compliance with Rule 17.1(a), (b) or (b-bis), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as the priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Mineko Mohri e-mail pct.team8@wipo.int Telephone No. +41 22 338 74 08
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Form PCT/IB/304 (July 2012)

1/7G3DKFNJ7DZC0

Atty. Dkt. No. 098065-0249

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Inventor Name: Atsuko SAITO

Title: ANTI-CDH6 ANTIBODY AND ANTI-CDH6 ANTIBODY-DRUG
CONJUGATE

Application No.: Not Yet Assigned

International Filing Date: May 14, 2018

371(c) Date: Herewith

Examiner: Not Yet Assigned

Art Unit: Not Yet Assigned

Confirmation No.: Not Yet Assigned

INFORMATION DISCLOSURE STATEMENT
UNDER 37 CFR §1.56

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

Applicant submits herewith documents for the Examiner's consideration in accordance with 37 CFR §§1.56, 1.97 and 1.98.

Applicant respectfully requests that each listed document be considered by the Examiner and be made of record in the present application and that an initialed copy of Form PTO/SB/08 be returned in accordance with MPEP §609.

Applicant includes copies of all Foreign and Non-Patent Literature reference documents cited on Form PTO/SB/08.

The submission of any document herewith is not an admission that such document constitutes prior art against the claims of the present application or that such document is considered material to patentability as defined in 37 CFR §1.56(b). Applicant does not waive

Atty. Dkt. No. 098065-0249

any rights to take any action which would be appropriate to antedate or otherwise remove as a competent reference any document submitted herewith.

CONCISE EXPLANATION OF RELEVANCE

References A2 and A3 were cited in an International Search Report issued in the corresponding International Patent Application No. PCT/JP2018/018572, dated August 7, 2018. English language Abstracts of the references are provided.

Copies of the International Search Report with its English language translation and the Written Opinion are submitted herewith as references A4 and A5.

References A1-A3 and A6-A18 are cited in the Specification as filed.

TIMING OF THE DISCLOSURE

The listed documents are being submitted in compliance with 37 CFR §1.97(b), within three (3) months of the filing date of the application.

Although Applicant believes that no fee is required, the Commissioner is hereby authorized to charge any additional fees, **except for excess claims fees**, which may be due to Deposit Account No. 19-0741.

Respectfully submitted,

Date November 13, 2019

By /Michael D. Kaminski/

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Michael D. Kaminski
Attorney for Applicant
Registration No. 32,904

PTO/SB/08 (modified)

Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT Date Submitted: November 13, 2019 <i>(use as many sheets as necessary)</i>				Complete if Known	
				Application Number	Not Yet Assigned
				Filing Date	Herewith
				First Named Inventor	Atsuko SAITO
				Art Unit	Not Yet Assigned
				Examiner Name	Not Yet Assigned
Sheet	1	of	2	Attorney Docket Number	098065-0249

U.S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	Document Number Number-Kind Code ² (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	A1.	2016/0297890-A1	10-13-2016	Agatsuma et al.	

UNPUBLISHED U.S. PATENT APPLICATION DOCUMENTS					
Examiner Initials*	Cite No. ¹	U.S. Patent Application Document Serial Number-Kind Code ² (if known)	Filing Date of Cited Document MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document Country Code ³ -Number ⁴ -Kind Code ⁵ (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Documents	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T ⁶
	A2.	WO-2014/057687-A1	04-17-2014	DAIICHI SANKYO CO LTD		ABS
	A3.	WO-2016/024195-A1	02-18-2016	NOVARTIS AG et al.		ABS

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.) date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁶
	A4.	International Searching Authority, "International Search Report," issued in connection with International Patent Application No. PCT/JP2018/018572, dated August 7, 2018.	✓
	A5.	International Searching Authority, "Written Opinion," issued in connection with International Patent Application No. PCT/JP2018/018572, dated August 7, 2018.	
	A6.	SHIMOYAMA et al., "Isolation and Sequence Analysis of Human Cadherin-6 Complementary DNA for the Full Coding Sequence and Its Expression in Human Carcinoma Cells," Cancer Research, Vol. 55, May 15, 1995, pp. 2206-2211.	
	A7.	INOUE et al., "Cadherin-6 Expression Transiently Delineates Specific Rhombomeres, Other Neural Tube Subdivisions, and Neural Crest Subpopulations in Mouse Embryos," Developmental Biology, Vol. 183, 1997, pp. 183-194.	
	A8.	OSTERHOUT et al., "Cadherin-6 Mediates Axon-Target Matching in a Non-Image-Forming Visual Circuit," Neuron Report, Vol. 71, August 25, 2011, pp. 632-639.	
	A9.	CHO et al., "Differential expression and function of cadherin-6 during renal epithelium development," Development, Vol. 125, 1998, pp. 803-812.	
	A10.	MAH et al., "Kidney Development in Cadherin-6 Mutants: Delayed Mesenchyme-to-Epithelial Conversion and Loss of Nephrons," Developmental Biology, Vol. 223, 2000, pp. 38-53.	
	A11.	PAUL et al., "Cadherin-6, a Cell Adhesion Molecule Specifically Expressed in the Proximal Renal Tubule and Renal Cell Carcinoma," Cancer Research, Vol. 57, July 1, 1997, pp. 2741-2748.	

Examiner Signature		Date Considered	
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PTO/SB/08 (modified)

Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT Date Submitted: November 13, 2019 <i>(use as many sheets as necessary)</i>				Application Number	Not Yet Assigned
				Filing Date	Herewith
				First Named Inventor	Atsuko SAITO
				Art Unit	Not Yet Assigned
				Examiner Name	Not Yet Assigned
Sheet	2	of	2	Attorney Docket Number	098065-0249

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.) date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁶
	A12.	SHIMAZUI et al., "The Level of Cadherin-6 mRNA in Peripheral Blood Is Associated with the Site of Metastasis and with the Subsequent Occurrence of Metastases in Renal Cell Carcinoma," Cancer, Vol. 101, No. 5, September 1, 2004, pp. 963-968.	
	A13.	KOEBEL et al., "Ovarian Carcinoma Subtypes Are Different Diseases: Implications for Biomarker Studies," PLoS MEDICINE, Vol. 5, Issue 12, e232, December 2008, pp. 1749-1760.	
	A14.	GUGNONI et al., "Cadherin-6 promotes EMT and cancer metastasis by restraining autophagy," Oncogene, Vol. 36, 2017, pp. 667-677.	
	A15.	POLAKIS, Paul, "Antibody Drug Conjugates for Cancer Therapy," Pharmacological Reviews, Vol. 68, January 2016, pp. 3-19.	
	A16.	PETERS et al., "Antibody-drug conjugates as novel anti-cancer chemotherapeutics," Bioscience Reports, Vol. 35, Issue 4, August 2015, pp. 1-20.	
	A17.	GOEPPERT et al., "Cadherin-6 is a putative tumor suppressor and target of epigenetically dysregulated miR-429 in cholangiocarcinoma," Epigenetics, Vol. 11, No. 11, 2016, pp. 780-790.	
	A18.	YOKOI et al., "A Novel Target Gene, SKP2, within the 5p13 Amplicon That Is Frequently Detected in Small Cell Lung Cancers," The American Journal of Pathology, Vol. 161, Issue 1, July 2002, pp. 207-216.	

Examiner Signature		Date Considered	
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A61K 39/395 (2006.01) A61K 31/48 (2006.01)
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(71) 出願人: 第一三共株式会社 (DAIICHI SANKYO COMPANY, LIMITED) [JP/JP]; 〒1038426 東京都中央区日本橋本町三丁目5番1号 Tokyo (JP).

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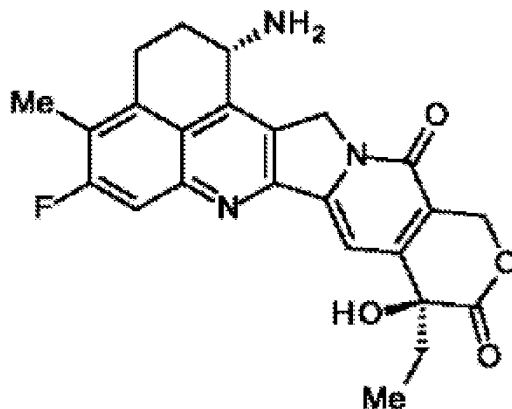
(74) 代理人: 大野 聖二, 外(OHNO, Seiji et al.); 〒1000005 東京都千代田区丸の内1丁目6番5号丸の内北口ビル21階 大野総合法律事務所 Tokyo (JP).

(81) 指定国 (表示のない限り、全ての種類の国内保護が可能): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NL, NO, NZ, OM, PA, PE, PG, PH,

[続葉有]

(54) Title: ANTIBODY-DRUG CONJUGATE

(54) 発明の名称: 抗体-薬物コンジュゲート



(57) Abstract: Provided is an antibody-drug conjugate which can be used as an anti-tumor agent that has an excellent anti-tumor effect and is highly safe, said conjugate being characterized by being produced by binding an anti-tumor compound represented by formula (1) to an antibody through a linker having a structure represented by the formula: $-L^1-L^2-L^3-NH-(CH_2)_m-L^4-L^5-L^6-$ (wherein the antibody is bound at the terminal of L^1 , and the anti-tumor compound is bound at the terminal of L^6 in which the binding site of the anti-tumor compound is a nitrogen atom in an amino group located at position-1).

(57) 要約: 抗腫瘍効果と安全性面に優れた抗腫瘍薬として、次式で示される抗腫瘍性化合物と抗体とを、次式: $-L^1-L^2-L^3-NH-(CH_2)_m-L^4-L^5-L^6-$ で示される構造のリンカーを介して結合させたことを特徴とする抗体-薬物コンジュゲート (抗体は L^1 の末端において結合し、抗腫瘍性化合物は、1位のアミノ基の窒素原子を結合部位として、 L^6 の末端において結合する) を提供する。



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PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK,
SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA,
UG, US, UZ, VC, VN, ZA, ZM, ZW.

MY, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TG,
OAPl (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM,
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- (84) 指定国 (表示のない限り、全ての種類の広域保
護が可能): ARIPO (BW, GH, GM, KE, LR, LS, MW,
MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), ユーラ
シア (AM, AZ, BY, EG, KZ, RU, TJ, TM), ヨーロ
ッパ (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK,

添付公開書類:

- 国際調査報告 (条約第 21 条(3))
- 明細書の別個の部分として表した配列リスト
(規則 5.2(a))

WO 2014/057687

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PCT/JP2013/006069

明 細 書

発明の名称：抗体－薬物コンジュゲート

技術分野

〔0001〕 本発明は、腫瘍細胞を標的にできる抗体と抗腫瘍性薬物とをリンカー構造部分を介して結合させた、抗腫瘍薬として有用な抗体－薬物コンジュゲートに関する。

背景技術

〔0002〕 癌細胞表面に発現し、かつ細胞に内在化できる抗原に結合する抗体に、細胞毒性のある薬物を結合させた抗体－薬物コンジュゲート（Antibody-Drug Conjugate；ADC）は、癌細胞に選択的に薬物を送達できることによって、癌細胞内に薬物を蓄積させ、癌細胞を死滅させることが期待できる（非特許文献1～3参照）。ADCとして例えば、抗CD33抗体にカリチアマイシンを結合させたMylotarg（ゲムツズマブオゾガマイシン）は急性骨髄性白血病の治療薬として認可されている。また、抗CD30抗体にオーリスタチンEを結合させたAdcetris（ブレンツキシマブベドティン）はホジキンリンパ腫と未分化大細胞リンパ腫の治療薬として最近認可された（非特許文献4参照）。これまでに認可されたADCに含有される薬物は、DNA、又はチューブリンを標的としている。

〔0003〕 抗腫瘍性の低分子化合物としてトポイソメラーゼⅠを阻害して抗腫瘍作用を発現する化合物であるカンプトテシン誘導体が知られている。その中で下式

〔0004〕 [化1]

