Lu *et al. Molecular Biomedicine* (2025) 6:50
https://doi.org/10.1186/s43556-025-00299-y

Molecular Biomedicine

REVIEW

Open Access

Hyaluronidase: structure, mechanism of action, diseases and therapeutic targets

Jiamin Lu[1,2,3], Zheng Zhao[1,2,3], Lingli Pan[1,2,3], Hui Wu[4], Shibing Wang[1*], Xiangmin Tong[1*] and Shenghao Wu[5*]

Abstract

Hyaluronidase (HAase), a family of enzymes critical for regulating physiological and pathological states, catalyzes the degradation of hyaluronic acid (HA), a key component of the extracellular matrix (ECM). By modulating ECM composition and cellular signaling pathways, HAase plays a pivotal role in diverse biological processes, including wound healing, tissue regeneration, and tumor progression. This review systematically elucidates the classification, biological sources, structural diversity, and catalytic mechanisms of HAase, emphasizing its dynamic involvement in disease pathogenesis and diagnostic potential. Furthermore, the article explores innovative therapeutic strategies centered on HAase modulation. HAase inhibitors emerge as promising tools for maintaining HA homeostasis, with implications in anti-inflammatory, antimicrobial, and antitumor therapies by blocking excessive HA degradation. Concurrently, HAase-mediated drug delivery systems represent a paradigm shift in overcoming biological barriers, enhancing bioavailability, and optimizing therapeutic outcomes through ECM remodeling. Notably, the synergy between HAase and immunotherapeutic modalities, such as checkpoint inhibitors and adoptive cell therapies, demonstrates synergistic antitumor effects by reshaping the tumor microenvironment (TME) and augmenting immune cell infiltration. Nevertheless, numerous challenges persist in the clinical application of hyaluronidase, including its immunogenicity, safety, application limitations and ethical considerations. This review synthesizes current research advances and unresolved issues, integrating molecular insights with translational perspectives, aiming to provide a more comprehensive and in-depth understanding of hyaluronidase and to advance clinical therapeutic strategies for hyaluronidase.

Keywords Hyaluronidase, Hyaluronic acid, Tumor microenvironment, Extracellular matrix, Tumor immunotherapy

Introduction

Hyaluronic acid (HA) is a hydrophilic glycosaminoglycan, composed of unbranched repeating units of D-N-acetylglucosamine and D-glucuronic acid. As a major component of the extracellular matrix (ECM), it participates in fundamental biological processes including wound healing, tissue regeneration, and cell signaling [1–3]. Hyaluronidase (HAase) is a glycosidase that can degrade the glycosidic bonds in hyaluronic acid polymers.As the majority of hyaluronidases belong to glycoside hydrolase (GH) families, while a minority are classified within polysaccharide lyase (PL) families [4, 5], their classification within the CAZy database reflects evolutionary relationships and structural correlations at the molecular level [6]. Hyaluronidase is a key enzyme in the regulation of HA dynamic equilibrium [7]. In 1928, Duran-Reynals

*Correspondence:
Shibing Wang
wangshibing@hmc.edu.cn
Xiangmin Tong
tongxiangmin@163.com
Shenghao Wu
wushenghao30@gmail.com
[1] Department of Clinical Laboratory, School of Medicine, Affiliated Hangzhou First People's Hospital, Westlake University, Hangzhou 310014, China
[2] Zhejiang Provincial People's Hospital (Affiliated People's Hospital), Hangzhou Medical College, Hangzhou 310014, China
[3] Postgraduate Training Base Alliance of Wenzhou Medical University, Wenzhou 325035, China
[4] School of Basic Medical Sciences and Forensic Medicine, Hangzhou Medical College, Hangzhou 310014, China
[5] Department of Hematology, The Dingli Clinical College of Wenzhou Medical University, The Second Affiliated Hospital of Shanghai University, Wenzhou Central Hospital, Wenzhou 325000, China

Springer

© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

Halozyme EX2087
Merck v. Halozyme
PGR2025-00024

first discovered hyaluronidase in testicular extracts and bacterial filtrates, which was then referred to as the "diffusion factor" due to its role in promoting the diffusion of substances such as vaccines, dyes, and toxins [8]. In 1940, it was officially named hyaluronidase by Chain and Duthie [9].

As a matter of fact, hyaluronidase is abundant in nature and has been found from complex higher animals to lower microorganisms [10]. In recent years, the research on hyaluronidase has become a extensively studied, and its function and properties have been gradually revealed. HAase has applications in medical fields including drug permeation enhancement, cosmetic surgery, and tumor immunology [7]. This review comprehensively examines the structural diversity of hyaluronidase and its role in physiological and pathological processes, with an emphasis on the therapeutic application of hyaluronidase, in particular focusing on novel therapeutic strategies targeting hyaluronidase activity or the HA degradation pathway in tumor immunotherapy. At the same time, many challenges and thoughts on the clinical application of hyaluronidase are also discussed.

Classification, biological sources, and structure of hyaluronidase

Classification systems of hyaluronidase

There are many classification systems of hyaluronidase in the world, mainly including four mainstream methods according to the source of enzyme, optimal reaction pH, specificity and catalytic mode, and amino acid sequence homology. Origin-based classification categorizes these enzymes into three domains: eukaryotes, prokaryotes, and viruses [10]. PH-based classification can be divided into acid enzymes (pH 3.0–4.0, such as human liver, serum and some animal toxin sources) and neutral enzymes (pH 5.0–8.0, the majority) [11]. However, classification according to substrate specificity and catalytic mechanism holds fundamental significance for understanding functional divergence among hyaluronidases [12].

The most classical classification system, established by Meyer et al. in 1971 [12], categorizes hyaluronidases into three principal types based on substrate specificity toward HA and fundamental catalytic mechanisms (Fig. 1). Hyaluronoglucosaminidase (EC 3.2.1.35): A glycoside hydrolase (GH-class) predominantly sourced from mammals and venoms. It specifically hydrolyzes β−1,4-glycosidic bonds in HA, yielding even-numbered oligosaccharides (tetra-and hexa-saccharides) with N-acetylglucosamine as the reducing end [13, 14]. Hyaluronoglucosidase (EC 3.2.1.36): Represented by leech salivary enzymes [15], this GH-class hydrolase selectively hydrolyzes the β−1,3-glycosidic bond in HA, producing oligosaccharides with glucuronic acid as the reducing end, with hyaluronate tetra-saccharide as the major final product [16]. In stark contrast to the former two hydrolysis mechanisms, Hyaluronate lyase (EC 4.2.2.1): A polysaccharide lyase (PL-class) of microbial origin, which cleaves β−1,4-glycosidic bonds in HA through an elimination mechanism, generating non-reducing end products with unsaturated carbon−carbon bonds, as well as an unsaturated HA-disaccharide, namely 2-acetamido-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-D-glucose [17, 18]. This catalytic dichotomy—hydrolysis versus β-elimination, coupled with bond-specificity (β−1,4 vs. β−1,3)—reveals fundamentally distinct degradation pathways and product chemistries. Notably, CAZy database-based classifications (GH/PL family) are highly associated with this functional classification, EC 3.2.1.35 maps to GH16/GH56/GH84 families; EC 3.2.1.36 exclusively resides in GH79 family; and EC 4.2.2.1 distributes across PL8/PL16/PL31 families [19–22].

Common biological sources of hyaluronidase

Mammalian hyaluronidases

In the human genome, six different hyaluronidase genes have been identified, namely HYAL1, HYAL2, HYAL3, HYAL4, PH-20 (sperm adhesion molecule 1,SPAM1), and HYALP1(or HYAL6), with homology between 33 and 44% [4]. HYAL1 is widely present in plasma, urine, liver, kidneys, spleen, and heart. It is the main hyaluronidase that regulates the metabolism of hyaluronic acid in ECM. In addition, it is closely related to tumor biology, lysosomal storage diseases, and wound healing [23]. HYAL2 is primarily located in the liver, kidneys, spleen, and adult brain, where it is significantly expressed in lysosomes or on the cell membrane [24]. Moreover, HYAL2 is related to tumor biology and angiogenesis [23, 25]. PH-20 exhibits the strongest biological activity, existing in high concentrations in the testes, and is localized to the head and acrosome of human sperm, while there is no distribution in the midpiece and tail. PH-20 is a multifunctional membrane protein that not only degrades HA but also provides HA-induced signaling receptors [25, 26]. Compared to HYAL1, HYAL2 and PH-20, the biological activity and functional significance of HYAL3, HYAL4, and HYALP1 are relatively lower [27]. HYAL3 is primarily distributed in the testes and bone marrow, and is considered a non-enzymatic regulator of HYAL1, although its catalytic performance has not been clearly defined in current research [28]. HYAL4 is distributed in muscles and the placenta and is considered a chondroitinase, with no biological activity on hyaluronic acid [25]. HYAL6 is distributed in the testes and bone marrow, and due to the deletion of a segment of an exon during the human evolutionary process, the amino acid

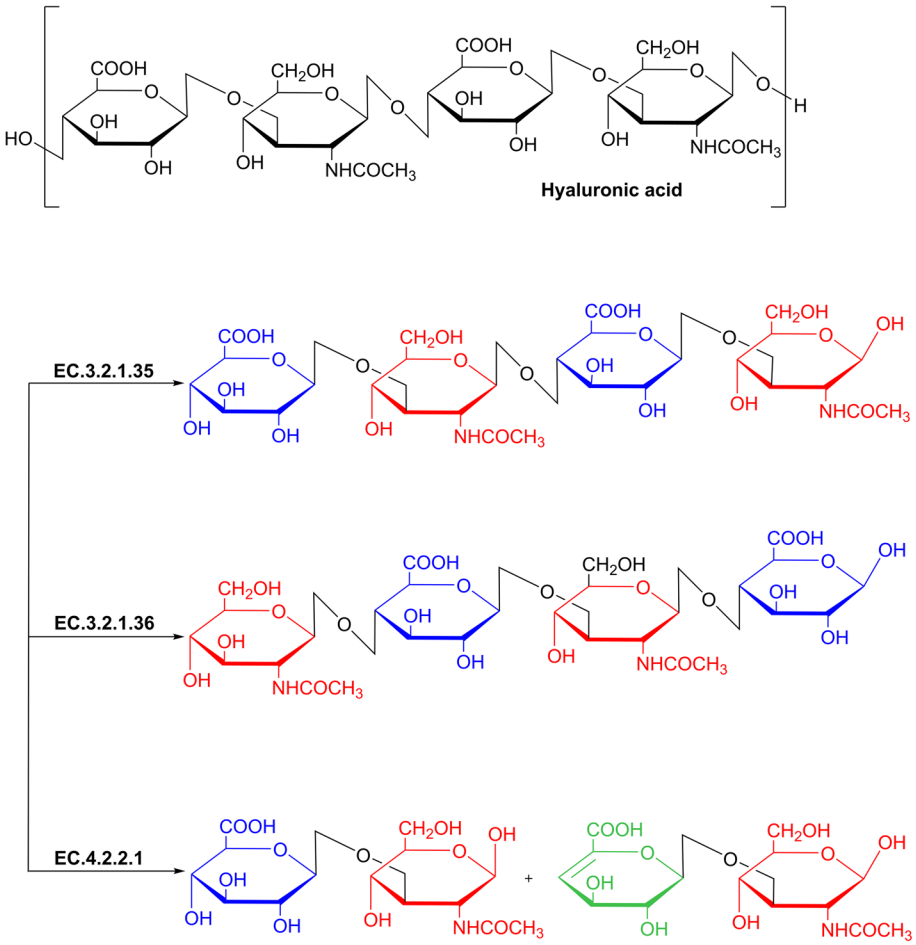


Fig. 1 Schematic of the chemical structure of hyaluronic acid, and the classification of hyaluronidase based on enzyme specificity and catalytic mode

translation process is terminated prematurely and only transcribed but not translated, rendering it a pseudogene [29].

In addition to the six canonical hyaluronidase gene, KIAA1199 and transmembrane protein 2 (TMEM2) show similar roles to hyaluronidase in the degradation of HA [18]. KIAA1199, a deafness gene of unknown function, is mainly expressed by dermal fibroblasts in normal skin and has been shown to have a key role in the depolymerization of HA [30]. In 2017, the hyaluronidase activity of TMEM2 was first identified [31], and TMEM2 has been linked to the catabolism of HA [19]. Recently, a major controversy has arisen about whether TMEM2 is a hyaluronidase, Niu et al. arguing that TMEM2 clearly lacks hyaluronidase activity [32]; Sato et al. arguing that human TMEM2 is not a hyaluronidase but a regulator of HA metabolism [33]; Takuma et al. overturning the above conclusions, arguing that TMEM2 is a bona fide hyaluronidase enzyme and has intrinsic catalytic activity [34]. Therefore, whether human TMEM2 is a hyaluronidase and its catalytic activity need to be further studied.

Unlike humans, mice have seven genes that encode hyaluronidase. In addition to the six genes already identified in the human genome, the seventh hyaluronidase gene is HYAL5, which shares high homology similarity with other types of hyaluronidase genes [35]. HYAL5 is closely related to the success of fertilization in the house mouse, and spermatozoa with deletion of the PH-20 gene can be successfully fertilized as long as they are normally expressed with HYAL5. In addition to the house mouse, HYAL5 is also found in other rodents [36–38].

Currently, most of the marketed hyaluronidases are derived from mammalian testis, especially testicular extracts from bovine and sheep show high enzyme activity [39]. Bovine testicular hyaluronidase (BTH) has the widest clinical application, with sequence homology to human hyaluronidase ranging from 22.9% to 25.2% [40].

Venom hyaluronidases

The presence of hyaluronidase has been found in the venom of many animals, such as bees, spiders, snakes and lizards [41–43]. Most of the animal venom hyaluronidases not only degrade HA, but also other glycosaminoglycans such as chondroitin and chondroitin sulfate [44]. The full gene sequence of venom hyaluronidase has now been described, which has led to further structural understanding of venom hyaluronidase [45]. Venom hyaluronidase is an allergen that causes IgE-mediated severe or fatal allergic reactions in humans [46, 47]. Because of its role as a "diffusion factor", hyaluronidase further exacerbates allergic reactions by facilitating the penetration of other harmful venom components and enhancing their entry into the bloodstream in various tissues [48]. Therefore, researchers have been working on the discovery of hyaluronidase inhibitors for use in antivenoms [49].

Leech hyaluronidases

Leech hyaluronidase (LHyal) was first discovered in leech extracts by Claude in 1937 [50]. The study of its enzymatic properties after isolation and purification revealed that LHyal has the advantages of high activity, high substrate specificity, no transglycosidase activity, and a more concentrated molecular weight distribution of its hydrolyzed products [51–53]. LHyal is sensitive to metal ions (Mn^{2+}, Cu^{2+}, and Fe^{3+}), and has an optimal temperature of 45 °C and an optimal pH of 6.5 [52]. Currently, it has been hypothesized that leech hyaluronidase has acetylheparinase activity, but the ability to cleave heparin or acetylheparin sulfate has not been found [4]. Recombinant leech hyaluronidase is of great significance in clinical therapy due to its substrate specificity and it can eliminate the risk of cross-infection in animals [16].

Microbial hyaluronidases

Hyaluronidase is widely distributed in microorganisms, and microbial hyaluronidase (mHyal) is present in bacteria, fungi, and phages. Jiang et al. [18] characterized microbial HAases (mHyals) as Mn^{2+}/Ni^{2+}-sensitive enzymes with optimal activity at 37–45 °C and pH 5.5–7.0 [54], mHyals serve as virulence factors that mediate bacterial invasion, tissue penetration, and dissemination [55, 56]. mHyal can reduce the hardness and density of ECM by degrading HA, thus breaking through the physical barrier and even directly contacting cellular tissues [57]; it can also utilize polysaccharide utilization loci (PULs) as a nutrient source for survival [58], and create a suitable environment for its own growth and reproduction through the immune-inflammatory response [59–61]. Gut microbial mHyal can remodel intestinal microbiota by enriching short-chain fatty acid (SCFA)-producing bacteria (e.g., Bifidobacterium), suggesting therapeutic potential for bacterial colitis [62–66]. In addition, mHyal plays an important role in inhibiting breast cancer growth [67, 68] and enhancing the permeability and targeting of tumor drugs [69, 70].

Structural features of hyaluronidase

In the previous section, we have already mentioned that the structure and catalytic mechanism of hyaluronidases are closely related to amino acid sequence homology, and the molecular structures of hyaluronidases from different species sources vary, but usually include catalytic domains and some auxiliary structural domains [71]. The overall three-dimensional folding, the key active site residues and the catalytic mechanism of the CAZy classification method are usually strictly conserved [22]. Therefore, this part is mainly based on CAZy, Protein Data Bank (PDB) database for the classification and elaboration of the structural relationships of different families of hyaluronidases, which are detailed in Table 1.

The catalytic structural domain of hyaluronidase is the key region for its core enzymatic function, which is responsible for directly recognizing and binding glycosaminoglycan substrates such as hyaluronic acid and catalyzing glycosidic bonds therein. The key structure of the

Table 1 The structures of hyaluronidases from different carbohydrate-active families

EC No	Family	Structure	PDB ID	Catalytic group	Catalytic mechanism
EC 3.2.1.35	GH16	β-jelly roll [72]	6XOF [73]	Glu/Glu	Retaining mechanism
	GH56	(β/α)7 [74]	2ATM	Glu/Asp	
	GH84	(β/α)8 [75]	8P0L [76]	Asp/Glu	
EC 3.2.1.36	GH79	(β/α)8 [77]	7EYO [78]	Glu/Glu	
EC 4.2.2.1	PL8	(α/α)6 toroid + anti-parallel β-sheet [79]	1C82 [80]	His/Tyr, Asn	cis-β-elimination reactions
	PL16	Triple strand β-helix	4UFQ [81]	Arg/Tyr, Gln	
	PL31	Parallel β-helix [82]	-	His/Tyr, Arg	

PDB ID RCSB protein data bank identity number, *Glu* Glutamic acid, *Asp* Aspartic acid, *His* Histidine, *Tyr* Tyrosine, *Asn* Asparagine, *Arg* Arginine, *Gln* Glutamin

catalytic domain, the catalytic group and the catalytic mechanism are the differences between hyaluronidases.

The overall structures of HA hydrolases are similar, but the catalytic mechanisms differ. Structurally, there are fewer reports on HA hydrolases belonging to the GH16 family, and only two GH16 family HA hydrolases derived from Penicillium spp. have been identified, and it is hypothesized that their structures are similar to the β-jelly roll [83]. Most HA hydrolases belong to GH56, GH79 and GH84, whose overall structures are similar to the TIM barrel fold, with differences only in the number of β/α folded sheets [16, 84, 85]. In terms of catalytic mechanism, the catalytic mechanism of all families of HA hydrolases belongs to the retention mechanism, but the types are slightly different. The GH16 and GH79 family of hyaluronanases is the classical retention mechanism, and the GH56 and GH84 family of hyaluronanases is the substrate-assisted retention mechanism [40, 85, 86].

The structures of HA lyases were significantly different, but the catalytic mechanism was the same. The PL8 family hyaluronidase structure consists of a torus (α/α)6/6 helical barrel and an antiparallel β-sheet 2 domains, while the PL16 family hyaluronidase structure consists of a three-stranded β-helix,PL30 family hyaluronidase structure has not been reported, PL31 family hyaluronidase is mainly composed of Parallel β-helix [79–82]. All HA lyases are catalyzed by cis-β-elimination reactions [22, 87, 88].

Mechanism of action

The core function of hyaluronidase depends on its specific recognition and catalytic degradation of HA substrate [89]. However, the non-catalytic functionalities of hyaluronidase warrant equal attention. The key to understanding these functions lies in elucidating their molecular mechanisms of action. Building upon the structural foundation delineated in Sect. "Classification, biological sources, and structure of hyaluronidase", this chapter systematically explores the enzymatic properties of hyaluronidase, the fine molecular processes of catalytic reactions, the regulatory mechanisms of its activity and the extracellular matrix (ECM)-modulating effects mediated by this enzyme. A comprehensive analysis of these aspects will provide critical insights into the significance of hyaluronidase beyond its conventional hydrolytic function.

Degradation pathways of hyaluronic acid

Hyaluronic Acid (HA) is a linear glycosaminoglycan formed by repeated N-acetyl Glucosamine and glucuronic Acid disaccharide units linked by β−1,3 and β−1,4 glycosidic bonds [1], different molecular weight of HA determines the difference in its function [3, 90]. There are three main mechanisms of HA degradation in humans: (1) hyaluronidase-mediated enzymatic degradation, (2) reactive oxygen species-mediated oxidative damage, and (3) systemic clearance in the liver and kidney [4].

Enzymatic degradation

EC 3.2.1.35 hydrolysis of β−1,4 glycosidic bond by retention mechanism, the β-configuration of the hydrolysate is consistent with the substrate [91], most of the reported animals and their venom-derived hyaluronidase belong to the GH56 family. The GH56 family HAases have many elongated clefts, known as binding clefts, which contain many positively charged hydrophobic amino acids [92]. Conserved in the binding cleft, the Glu catalytic residue acts as the proton donor, while the carbonyl oxygen atom of the acetylamino group in the substrate acts as the nucleophilic residue/base [93]. The specific steps are as follows (Fig. 2a): (1) HAase binds to the HA substrate. (2) Under the action of catalytic residues, the N-acetylglucosamine (GlcNAc) carbonyl oxygen of the substrate itself acts as an intramolecular nucleophile, attacking the heterologous carbon (C1) of the same sugar ring, forming a covalent oxonium ion intermediate accompanied by C1 configuration inversion, and completing the cleavage of the non-reducing side glycosidic bond of the glycosidic oxygen atom. (3) The protonated catalytic residue Glu transfers its proton to the glycosidic oxygen, releasing the substrate sugar chain on the reduced side of the broken glycosidic bond. (4) The water molecules in the active site hydrolyze the covalent intermediate, restore the catalytic residual matrix micronization state, prepare for the next round of catalysis, and trigger the second inversion of the C1 configuration. (5) The non-reducing end products of the broken glycosidic bond dissociate from the binding crack [6, 94–96].

EC 3.2.1.36 hydrolyzes the β−1, 3 glycosidic bond by the retention mechanism, and the heterocarbon configuration of its hydrolysis product is still retained. The catalytic process of the GH79 family hyaluronidase depends on the synergistic effect of two spatially separated conused Glu residues in the cleavage of the active site (Fig. 2b) [78]: One of them acts as a Nucleophile to directly attack the substrate hetero-carbon, forming a covalent glycosyl-enzyme intermediate; Meanwhile, another residue acts as an acidic carboxylate to provide a protonic group. In the subsequent hydrolysis step, the deprotonated acidic carboxylate acts as a base, launching a nucleophilic attack on the covalent intermediate by activating water molecules, triggering a secondary configuration inversion, and obtaining the hydrolysis product. This stepwise double substitution mechanism (nucleophilic substitution—hydrolysis) ultimately achieves the net retention of the heterocarbon configuration, and its

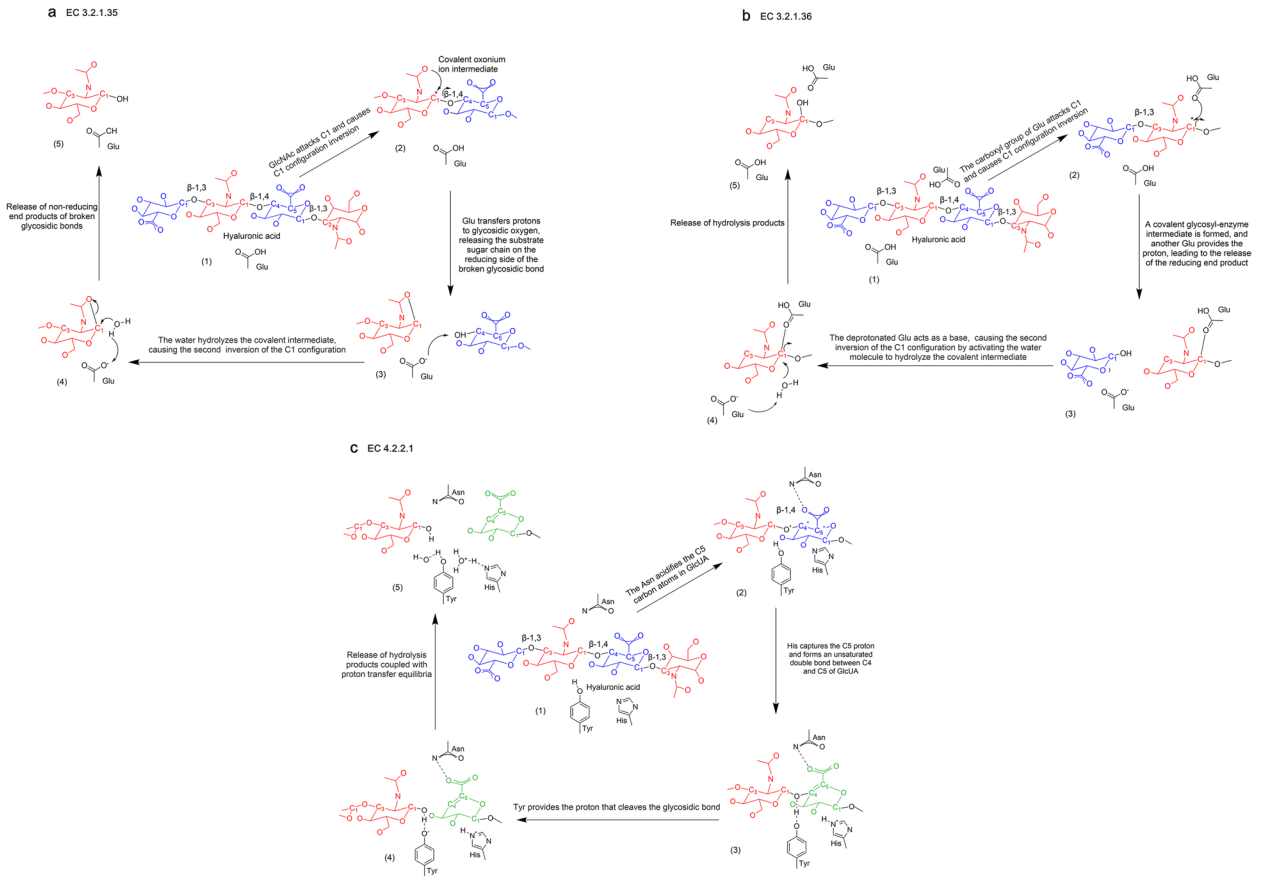


Fig. 2 Molecular mechanisms of hyaluronic acid degradation by three distinct classes of hyaluronidases. a. EC 3.2.1.35 hydrolyzes β−1,4-glycosidic bonds via a retention mechanism; b. EC 3.2.1.36 hydrolyzes β−1,3-glycosidic bonds via a retention mechanism;c. EC 4.2.2.1 cleaves β−1,4-glycosidic bonds through β-elimination

essence is the superimposed effect of a two-step single configuration inversion [96–99].

EC 4.2.2.1 cleaves the β−1,4-glycosidic bond using a β-elimination reaction, forming an unsaturated double bond between the C 4 and C5 atoms at the non-reducing end of the product [100]. The binding cleft of the PL8 family is located at the junction of these two domains, the N-terminal and c-terminal, where there are many positively charged amino acids that can bind to negatively charged HA substrates, and the binding cleft is located at the junction of the N-terminal and C-terminal domains, and through the dynamic inter-domain movement to open and close the substrate binding crack, and finally cleave hyaluronic acid [101, 102]. But despite extensive research, the specific catalytic mechanism is not yet fully understood [5, 11, 80]. It has been hypothesized that the catalytic mechanism proceeds as follows (Fig. 2c): (1) HA forms a catalytic pre-reaction conformation through electrostatic complementary binding to the substrate-binding cleft. (2) The Asn acidifies the C5 carbon atoms in Glucuronic acid (GlcUA) near the catalytic site, triggering an electronic rearrangement. (3) The key His residue at the active site captures the GlcUA C5 proton, this proton transfer drives the formation of unsaturated double bonds between the C4-C5 of GlcUA, generating the characteristic 4,5-unsaturated oligosaccharide products. (4) During the synchronous cooperative proton transfer process, the tyrosine residues donate protons to the oxygen atoms of the glycosidic bond, resulting in the cleavage of the β−1,4-glycosidic bond. (5) The hydrolysis product dissociates from the active cleft, and balances hydrogen ions with the aqueous environment [101, 103].

Free radical depolymerization

The degradation of hyaluronic acid can also be achieved by free radical-mediated depolymerization, including a series of free radicals and oxidative reactive species (ROS), such as carbonate radical, hydroxyl radical, dichloride radical anion, dibromide and peroxynitrite [104–106]. HA chains undergo oxidative cleavage, a process that involves hydroxyl radicals attacking the C-H bond of the sugar ring, triggering β-glycosidic bond cleavage and producing

polydisperse oligosaccharide fragments. In addition, high levels of ROS can induce hyaluronidase conformational changes or up-regulated expression in the inflammatory or tissue injury microenvironment, thereby inducing the enzymatic action of hyaluronidase on HA [107].

Clearance and metabolism of degradation products

Regardless of the degradation mechanism, the resulting HA fragments undergo efficient clearance. The generated HA fragments face two metabolic fates in the tissue microenvironment, 1. Local further decomposition: in the skin and other tissues, some small molecular fragments can continue to be degraded into monosaccharides; 2. Systemic lymphatic clearance: the vast majority of HA enters the lymphatic capillaries via interstitial fluid and is transported with the lymph to regional lymph nodes, where it is internalized and catabolized by lymphatic endothelial cells. Residual HA fragments enter the systemic circulation and are eventually degraded by liver endothelial cells. Multilevel scavenging systems ensure efficient metabolism of HA degradation products and maintain tissue homeostasis [108–113].

Regulatory factors influencing activity

The activity of Hyaluronidase is regulated by many physiological, biochemical and environmental factors, mainly including the following aspects: (1) PH and temperature: HYAL 1–4 is an acid Hyaluronidase with the highest activity at pH 3–4, neutral hyaluronidases such as PH 20 and venom hyaluronidase are most active at PH 5–8 [25]. Most hyaluronidases generally exhibit optimal activity near a physiological temperature of 37 °C, with a rapid decline in activity above 45 °C due to protein deformation. However, hyaluronidase from some bacteria can tolerate high temperature, and even the optimum reaction temperature can reach 70 °C [54, 114, 115]. (2) Ions and cofactors: part of the hyaluronidase activity requires $Ca2+$, $Mg2+$, $Zn2+$ as cofactors, and the activity is dependent on NaCl concentration [116, 117]. (3) Redox status: Dysregulation and excessive generation of ROS can induce oxidative stress, perturbing intracellular redox homeostasis and subsequently promoting the activation and upregulation of hyaluronidase expression [118]. Conversely, antioxidants such as N-acetylcysteine and glutathione demonstrate inhibitory effects on hyaluronidase activity [119]. (4) Endogenous regulators: The most critical endogenous regulatory factors are hyaluronidase inhibitors, notably members of the Inter-α-inhibitor (IαI) family [120]. These inhibitors exert their suppressive effects through specific binding to hyaluronidase, thereby blocking its interaction with the substrate hyaluronic acid and effectively attenuating enzymatic degradation. (5) Signaling pathways and inflammatory milieu: Hyaluronidase expression and activity are frequently modulated by cellular signaling pathways, particularly those associated with inflammatory responses, while its enzymatic activity reciprocally influences inflammatory progression. During the early phase of acute inflammation, pro-inflammatory cytokines such as TNF-α and IL-1β activate signaling cascades (e.g., NF-κB pathway), leading to upregulated expression of hyaluronidase isoforms including HYAL1, HYAL2, and HYAL3. This creates a self-perpetuating cycle characterized by "elevated enzyme activity → accelerated hyaluronic acid degradation → aggravated inflammatory response [121]. However, during the resolution phase of inflammation, anti-inflammatory cytokines like IL-10 counteract this process by suppressing hyaluronidase production [122].

Non-enzymatic functions of hyaluronidase

Beyond its canonical hyaluronan-degrading enzymatic activity, hyaluronidase exhibits diverse non-enzymatic functions that critically participate in physiological processes including cellular signaling, migration, proliferation, inflammatory responses, and immunomodulation [18]. It is noteworthy that these multifaceted non-enzymatic roles do not function in isolation, their mechanistic foundation lies in the direct regulation of extracellular matrix (ECM) physicochemical properties and signaling molecule homeostasis [7], with functional realization being highly dependent on this specific microenvironment. The ECM constitutes a highly specialized three-dimensional polymeric network, with collagen, fibronectin, laminin, proteoglycans, and glycosaminoglycans (GAGs) representing its principal components. Notably, HA serves as the predominant constituent of GAGs [123, 124].

By interacting with specific ECM components or anchoring directly to ECM structures, hyaluronidase effectively mediates cell-ECM communication and modulates downstream signaling cascades. Of particular relevance, the ECM not only provides structural support and mechanical integrity through components like HA but also actively participates in regulating cellular processes such as growth, differentiation, and dynamic remodeling [125, 126]. This bidirectional regulatory capacity enables hyaluronidase to exert broad physiological effects through alterations in ECM physicochemical characteristics and the generation of bioactive molecules. Consequently, the ECM serves as an indispensable integrative platform—combining physical, chemical, and signaling dimensions—for the manifestation of hyaluronidase's non-enzymatic functionalities.

HA is an important "space filler" in ECM, the high viscosity and hydration capacity of HMW-HA maintains the mechanical support and compression resistance of the tissues. After the degradation of HA by hyaluronidase, the mechanical properties of the ECM change,

the viscosity and elasticity decrease, which leads to the structural changes and affects the supportive role of the tissues [127–129]. In addition, the structure of ECM becomes loose, which leads to an increase in porosity and improves the permeability of ECM [130].

Under normal physiological conditions, ECM has a dense physical barrier that limits cell migration, but when hyaluronidase acts on ECM, its loose structure reduces cell adhesion and promotes cell migration [131]. HA and its degradation products bind to a variety of cell surface receptor, such as CD44, RHAMM, TLR2, TLR4, LYVE,LAYN and STAB2 (Fig. 3) [132], thereby activating various signaling pathways and regulating cell–matrix interactions [133]. HMW-HA has antiproliferative and antivascular effects, whereas the degradation product, low molecular weight hyaluronic acid (LMW-HA), has a distinct role in promoting angiogenesis and cell proliferation [133–136]. Degradation products can promote the binding of Toll-like receptors (TLR2, TLR4) or CD44 that promote inflammation, activate signaling pathways such as NF-κb, and promote the release of inflammatory factors such as TNF-α, IL-1β, and IL-6 [137, 138]. The ECM also limits the entry of inflammatory cells under physiological conditions, and when ECM permeability is increased, the recruitment of inflammatory cells such as neutrophils and macrophages is enhanced, further exacerbating the inflammatory response [136, 139, 140].

Role of hyaluronidases in disease

During physiological processes, hyaluronidase is involved in fertilization, adaptive immunity, platelet production, and remyelination [23]. Hyaluronidase plays a crucial role in the dynamic balance of HA. When the balance is disrupted, it will lead to a series of pathophysiological changes, which have an impact on the occurrence and development of the disease. In addition, hyaluronidase can also serve as a diagnostic marker for diseases and plays a significant role in the diagnosis of diseases.

Malignant tumors

The global incidence of malignant tumors has witnessed a continuous surge in recent years, with approximately 20 million newly diagnosed cancer cases and 9.7 million cancer-related deaths reported worldwide in 2022 [141]. The effect of hyaluronidase on tumor cells can cause changes in the ECM, which in turn affects the composition and functional status of the tumor microenvironment (TME) cells [142]. Currently, the mechanisms of ECM resistance are the focus of research [143]. In Table 2, we elaborate on the components that contribute to ECM resistance and their role in resistance.

The double-edged sword of tumor-promoting and anti-tumor effects

In the TME, hyaluronidase-mediated degradation of HA regulates tumor progression and immune response through dual mechanisms. On the one hand, HA degradation leads to ECM structural relaxation, reduced tissue stiffness and interstitial pressure, and HA degraders bind to receptors on the surface of immune cells, such as CD44 and TLR2, which are important targets of HA degradation, activation of immune cell migration and activation of signaling pathways that promote infiltration and activation of immune effector cells, including T cells, NK cells, and DCs [159–162]. and reduced the recruitment of immunosuppressive

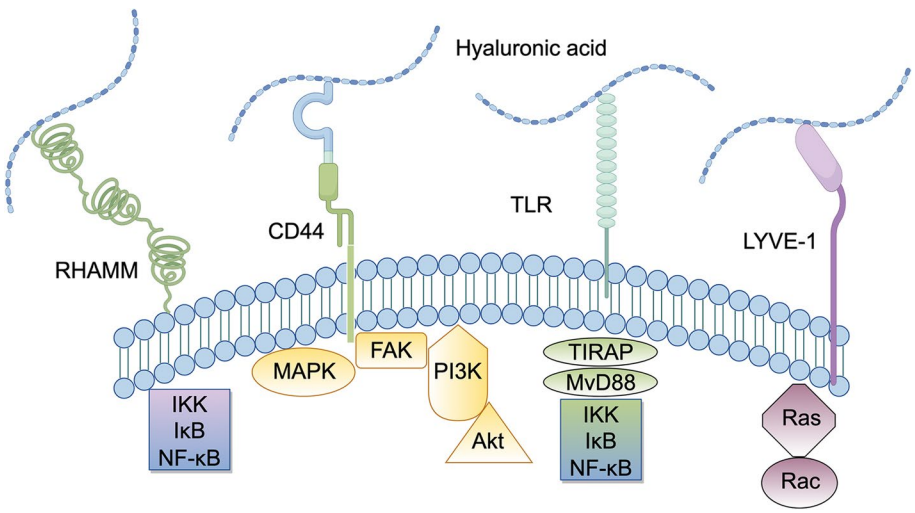


Fig. 3 Interaction of hyaluronic acid and cellular receptor swaying.CD44 and RHAMM signaling pathways mediate the most complex and important cell-substrate interactions

Table 2 The chemical components that lead to ECM resistance and their role in resistance

Chemical components	Mechanism of action	The impact on drug resistance
Hyaluronic acid	Forming physical barriers and regulate signaling pathways [144, 145]	Enhancing drug resistance and promote interstitial pressure [146]
Collagen	Regulating the integrin signaling pathway affects cell adhesion and migration [147]	Promoting the development of tumor stem cells leads to recurrence and resistance [148]
Fibronectin	Promoting resistance to nest loss apoptosis and activate pro-tumor signaling pathways [149–152]	Directly regulating cell adhesion-mediated resistance (CAM-DR) [153]
Laminins	Regulation of signaling pathways to alter cell adhesion, migration and differentiation [154]	Indirectly promoting resistance
Proteoglycans	Regulating the physical and chemical properties of ECM [155, 156]	Indirectly promoting resistance
Growth factors and cytokines	Regulating tumor growth, angiogenesis, and immune suppression [157, 158]	Enhancing the survival and proliferation ability of tumor cells

myeloid-derived suppressor cells (MDSCs). At the same time, Tumor-associated macrophage (TAMs) undergo a shift from an M2 anti-inflammatory phenotype to an M1 pro-inflammatory phenotype, accompanied by the release of inflammatory factors such as TNF-α, IL-1β, and IL-6. ECM remodelling also alleviates TME hypoxia, improves oxygenation and nutrient supply by promoting Neovascularization, and enhances immune cell accessibility [163, 164] (Fig. 4).ECM loosening can increase the permeability of anticancer drugs and improve efficacy [165].

On the other hand, ECM degradation has pro-tumor effects, high ECM stiffness promotes tumor growth compared with soft ECM [166], and degradation products bind to CD44 or RHAMM, activation of Ras-MAPK, PI3K-Akt, FAK and other signaling pathways to enhance tumor cell motility [165, 167], it can also activate TGF-β, and it can also activate TGF-β, NF-κb and other signaling to induce epithelial-mesenchymal transition (EMT) of tumor cells, to make them more invasive and migratory [168]. New Blood vessels also enhance the supply of

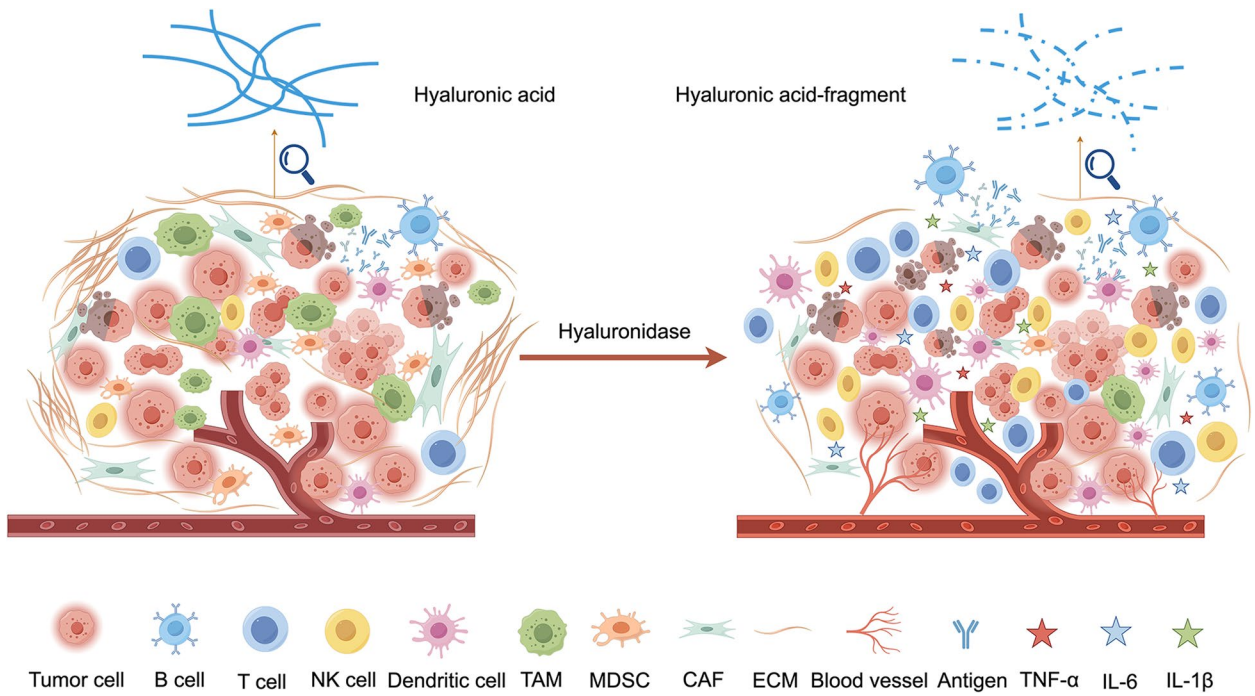


Fig. 4 Effect of hyaluronidase-mediated degradation of hyaluronan on tumor microenvironment. HA degradation leads to ECM structural relaxation, alleviates the TME hypoxia state, and improves oxygenation and nutrient supply by promoting Neovascularization formation, which is beneficial to the development of the TME, it promotes the infiltration and activation of immune effector cells, including T cells, NK cells, DCs, and reduces the recruitment of MDSCs, which is also accompanied by the release of inflammatory factors such as TNF-α,IL-1β, and IL-6

oxygen and nutrients to tumor cells and provide conditions for distant metastasis [169, 170].

Hyaluronidase as a biomarker for tumor diagnosis and prognosis

Among emerging biomarkers, hyaluronidases have garnered significant attention as research hotspots in tumor diagnosis due to their pivotal roles in shaping the TME. As a non-invasive diagnostic modality, combined urinary HAase activity and HA detection achieve 91% sensitivity and 83% specificity, outperforming conventional cytological examinations while enabling recurrence risk prediction [171, 172]. Elevated urinary HYAL1 levels in high-grade bladder cancer patients exhibit a 3–sevenfold increase compared to low-grade cases and healthy controls [173]. Recent studies further elucidate a dual functional role for HYAL3 in bladder cancer pathogenesis: its expression correlates significantly with immune cell infiltration status in the TME and serves as an independent prognostic factor for overall survival (OS) following multivariate analysis [174]. In prostate cancer, urinary HYAL1 emerges as a novel early diagnostic biomarker with independent predictive value [175], where elevated levels directly associate with increased disease probability and independently predict progression/recurrence risks [113, 176].

In breast cancer, augmented enzymatic activity of HYAL1 and HYAL2 correlates with aberrant signaling through HA receptors (CD44, RHAMM), thereby promoting tumor progression, metastasis, and recurrence. Notably, KIAA1199 (CEMIP) has been increasingly implicated in malignant progression across multiple carcinomas, including breast cancer, where its overexpression mediates invasive phenotypes [177–181]. TMEM2, transcriptionally activated by SOX4 in breast cancer, functions as a mediator of migratory and invasive processes, suggesting diagnostic potential for CEMIP and TMEM2 quantification [182].

For head and neck squamous cell carcinoma (HNSCC) [183], HYAL1 demonstrates potential as a tissue-specific biomarker, with elevated HYAL1 and PH20 expression observed in laryngeal carcinoma specimens [184]. In pancreatic cancer, hypomethylation of HYAL2 in peripheral blood constitutes a valuable early diagnostic indicator [185]. Non-small cell lung cancer (NSCLC) studies confirm KIAA1199 overexpression in tumor tissues correlates with poor differentiation, lymph node metastasis, and advanced staging, establishing its role as an independent prognostic predictor [186].

Similarly, gastric cancer exhibits significant upregulation of KIAA1199 expression, which has been validated as a key independent predictor of clinical outcomes. Mechanistically, KIAA1199 activates the canonical Wnt/β-catenin signaling axis, upregulating matrix metalloproteinase (MMP) family proteolytic activity to induce epithelial-mesenchymal transition (EMT) phenotypic conversion—a process strongly associated with enhanced metastatic potential [187].

Infectious diseases

Hyaluronidase is an important "diffusion factor" for pathogens to invade the host [57]. As early as 1950, some researchers suggested that hyaluronidase could enhance the invasiveness and virulence of hemolytic streptococci [188]. Coleman et al. recently showed in the Macaca Nemestrina model that Group B streptococci (GBS), which has high expression of hyaluronidase, is more invasive during pregnancy and ultimately causes preterm birth [189]. Hyaluronidase also causes bacterial invasion in Streptococcus pneumoniae [88]. Hyaluronidase in Staphylococci is also an important causative agent of respiratory tract infections [190, 191].

Autoimmune and inflammatory diseases

Hyaluronidase also has a unique pathological role in autoimmune and inflammatory diseases. In patients with rheumatoid arthritis, HA and hyaluronidase activity and expression levels are significantly elevated [192, 193]. In scleroderma, patients have normal activity levels of HYAL1 in the early stages, but HYAL1 activity decreases in the late stages [194]. HYA1 deficiency has a beneficial role in the early stages of type 1 diabetes, which may be mediated by a variety of protective effects against early diabetes-induced damage to endothelial cells and the glycocalyx [195]. Petrey et al. found that mice deficient in HYAL2 were more susceptible to inflammatory bowel disease (IBD) and that HYAL2 could modulate the early inflammatory response in colitis by limiting leukocyte extravasation [196]. Serum hyaluronidase activity is also significantly elevated in patients with psoriasis [197].

Cardiovascular diseases

It is well known that NO reduces vascular stiffness, and in patients with coronary artery disease, the level of hyaluronidase rises in patients, and hyaluronidase is negatively correlated with NO production, a mechanism that is also closely related to the development of hypertension [198]. In patients with aortic disease, the ratio of collagen and Hyal to hyaluronidase is increased [199]. Researchers found that in HYAL2 KO mice, heart valve dilation, atrial dilation, cardiac hypertrophy, ECM deposition, and resulted in increased mortality [200]. In HYAL2 deficient mice, diastolic dysfunction and heart failure develop earlier [201]. In addition, HYAL2 deficiency in humans may be associated with a rare heart disease, Cor triatriatum

sinistrum, but the mechanisms involved still need to be further investigated [202]. In an IL-10-induced myocardial infarction model in mice, the reduction of HYAL3 levels promotes cardiac wound healing by inhibiting the inflammatory response [203].

Respiratory diseases

In the human airway epithelium, the expression and activity of HYAL1,2, and 3 are increased, as in allergic asthmatic responses [121]. In a rat model of radiation-induced lung injury, HYAL2 is involved in HA turnover during the early phase of lung injury and in the pathogenesis of pulmonary fibrosis [204]. Plasma HYAL1 levels are elevated in patients with Obstructive sleep apnoea (OSA) and further induce a systemic inflammatory response in OSA [205]. Elevated Hyal levels have been observed in patients with chronic obstructive pulmonary disease in both the stable and acute phases, and are particularly pronounced in the acute phase [206]. HYAL2 expression is elevated in both Idiopathic pulmonary fibrosis pulmonary fibrosis and pulmonary hypertension [207].

Digestive System diseases

In addition to the previously discussed roles in IBD and cancers, alterations in hyaluronidase activity play a significant role in other digestive system disorders. Elevated levels of HYAL1 have been shown to promote autophagic responses in pancreatic acinar cells, exacerbating pancreatic secretion and inflammation, ultimately leading to pancreatic leakage in patients undergoing duodenectomy [208]. In the very early stages of toxic liver injury, hyaluronidase levels begin to rise on the first day and peak by the second day [209]. Compared to healthy individuals, patients with acute and chronic hepatitis C exhibit increased serum hyaluronidase levels during the early phases of the disease [171]. Furthermore, both HYAL1 and 2 levels are elevated in liver pathologies such as steatosis, steatohepatitis, and cirrhosis [210].

Other diseases

In neurodegenerative diseases, hyaluronidase is an inhibitor in Alzheimer's disease that can reverse endolysosomal dysfunction and protein lesions, alleviate memory impairment, and prevent Alzheimer's disease, bring new tools to the treatment of Alzheimer's disease [211]. In skin disease, HYAL1 and 2 expression is reduced in keloid tissue compared with normal tissue [212]. The metabolic regulation of HYAL1 and 2 has also been linked to the effects of ultraviolet radiation (UVB) on the skin, with increased levels of HYAL1 and 2 in the skin after exposure to UVB [213].

Therapeutic strategy and application of hyaluronidase

Hyaluronidase targeting strategies

HAase exhibits multidimensional therapeutic potential through the regulation of HA degradation and ECM remodeling in disease treatment. Its therapeutic strategies can be categorized as follows: pathological process intervention via inhibitors and drug delivery optimization using enhancers. The following sections discuss target classification, innovations in tumor immunotherapy, and clinical applications in non-oncological diseases.

Inhibitors: restoring hyaluronic acid homeostasis

Hyaluronidase inhibitors maintain HA homeostasis—particularly the dynamic equilibrium between HMW-HA and LMW-HA—by blocking excessive HA degradation. These inhibitors play pivotal roles in anti-inflammatory, antimicrobial, antitumor, and contraceptive drug development [214]. Current research has identified diverse structurally heterogeneous compounds with HAase-inhibitory activity, encompassing proteinaceous inhibitors, glycosaminoglycan derivatives, polysaccharides, botanical-derived bioactives, and synthetic organic compounds [95].

As early as 1946, Haas et al. first reported a heat-labile glycoprotein HAase-circulating complex in human plasma [215]. Later identified in mammals including mice, these complex exhibits magnesium dependency and protease sensitivity. Elevated activity levels of this complex correlate with hepatopathy and malignant neoplasms [216]. Heparin, a glycosaminoglycan, non-competitively inhibits HAase through interactions with surface amino acid residues rather than catalytic sites, with inhibitory efficacy diminished by HAase surface amino modification [217, 218]. O-sulfated HA fragments represent another documented inhibitor class exhibiting dose-dependent, mixed-type inhibition with potent activity profiles [219]. Certain anti-inflammatory agents (e.g., indomethacin, dexamethasone, sodium salicylate, and sulodexide) demonstrate HAase-inhibitory properties [11]. Vitamin C, an antioxidant, suppresses streptococcal and bovine testicular HAase activity [220].

Natural products constitute a critical reservoir of HAase inhibitors, offering structural diversity and favorable safety profiles. Alkaloids, terpenoids, and flavonoids dominate this category. Aristolochic acid, an alkaloid, non-competitively modulates snake venom HAase activity through mechanisms analogous to heparin, primarily engaging exposed tyrosine and tryptophan residues [221]. Inhibitory outcomes vary among terpenoids and flavonoids depending on HAase isoform specificity and concentration gradients [222]. Quercetin and apigenin exemplify clinically validated compounds with

broad therapeutic applicability [223]. High-throughput screening by Jeremy et al. identified delphinidin, a natural anthocyanidin, as an HAase inhibitor that attenuates HMW-HA degradation, presenting novel antitumor opportunities [224]. Additionally, α-ketoglutarate derived from lactic acid bacteria exhibits anti-aging effects through HAase inhibition [225].

Enhancers: overcoming delivery barriers

The stratum corneum and ECM form dual barriers limiting drug penetration into deep tissues. HAase-mediated ECM remodeling represents a viable strategy to overcome these limitations. Currently, hyaluronidases used in clinical treatment include bovine-derived formulations, recombinant human hyaluronidase (rHuPH20), and PEGylated conjugates (PEGPH20) [226, 227]. The U.S. Food and Drug Administration (FDA) has approved nine HAase-containing drug formulations for enhanced delivery and large-volume injections [7].

Nanoparticles (NPs) are efficient local delivery systems owing to their nanoscale dimensions, biocompatibility, and surface modifiability [228]. These properties enable effective stratum corneum penetration, particularly for hydrophilic drugs and macromolecules [229]. Polymer-based nanocarriers, for example, encapsulate drugs to protect against environmental degradation while enabling controlled release [230, 231]. Synergistic strategies combining HAase with NPs include: 1) chemical conjugation of HAase to NPs for enzyme-carrier composite systems; 2) co-loading of HAase and drugs for enzymatic degradation-triggered release; and 3) pre-treatment of target sites with HAase to soften ECM and enhance NP penetration [232–234].

Nanocrystals (NCs), as submicron-sized carriers, significantly improve drug delivery by enhancing saturation solubility. Their application strengthens concentration gradients between skin and topical formulations, accelerating passive diffusion and prolonging tissue retention [235]. HAase-based liposomes (LPs) demonstrate clinical potential in intravenous delivery via two primary approaches: 1) HAase encapsulation on LP surfaces for composite systems, and 2) pre-injection of HAase at target sites for ECM remodeling [236, 237]. HAase-integrated NPs, NCs, and LPs represent promising strategies for drug delivery optimization. Beyond nanoscale systems, HAase research in non-nanoparticulate formulations—such as emulsions and microspheres—has advanced through matrix degradation kinetics, significantly improving transmembrane drug transport efficiency [238, 239].

Innovative applications in tumor immunotherapy

The high morbidity and mortality rates associated with malignant tumors necessitate the exploration of novel therapeutic strategies. In recent decades, our understanding of tumors has been revolutionized, and we have discovered that tumors are complex ecosystems, the concept of tumors has shifted from a purely confined disease centered on tumor cells to a disease in which a variety of non-tumor cells interact with each other within the tumor [142]. Tumor immunotherapy, which targets the TME, has emerged as a highly effective therapeutic modality. Notably, the combinatorial use of HAase with immunotherapeutic agents has demonstrated synergistic antitumor effects by modulating ECM composition and enhancing immune cell infiltration.

In recent years, the focus of tumor immunotherapy has shifted from directly using drugs to kill tumor cells to combating tumors by achieving a lasting immune response in cancer patients, while also preventing metastasis and recurrence, making the treatment of malignant tumors enter a new era of precision medicine [240, 241]. Compared with traditional treatments such as surgery, radiotherapy and chemotherapy, tumor immunotherapy has the advantages of strong specificity and few side effects [242].

According to the different mechanisms of action (Fig. 5), tumor immunotherapy is divided into six main categories. (1) Immune checkpoint inhibitor therapy, by blocking immune checkpoint molecules, reactivating the anti-tumor activity of T cells, and thereby enhancing the immune system's attack on tumor cells [243]. The U.S. Food and Drug Administration (FDA) approved immune checkpoint inhibitors include Cytotoxic T lymphocyte antigen 4 (CTLA-4), Programmed cell death 1 (PD-1), and its ligand Programmed cell death L1 (PD-L1) [244]. (2) Adoptive cell therapy (ACT), ACT is a form of passive tumor immunotherapy, in which immune cells originating from the patient's tumor are transformed, activated, and expanded in vitro and then re-infused back into the patient's body for the purpose of clearing the tumor [245–247]. (3) Cancer vaccines, cancer vaccines use tumor-associated autoantibodies (TAAS), tumor peptides or tumor cell lysis products to induce the body to produce tumor-specific immune responses, protect the body from tumor cell invasion, and achieve prevention and treatment of tumors [248]. (4) Oncolytic viruses (OVs), OVs can directly lyse tumor cells while retaining normal cells, and exert anti-tumor effects by releasing antigens and activating the tumor microenvironment [249]. (5) Bispecific antibodies: Bispecific antibodies can bind to two different antigens or epitopes at the same time. (6) Cytokine therapy: through the activation of natural immunity, enhancement of adaptive immunity,

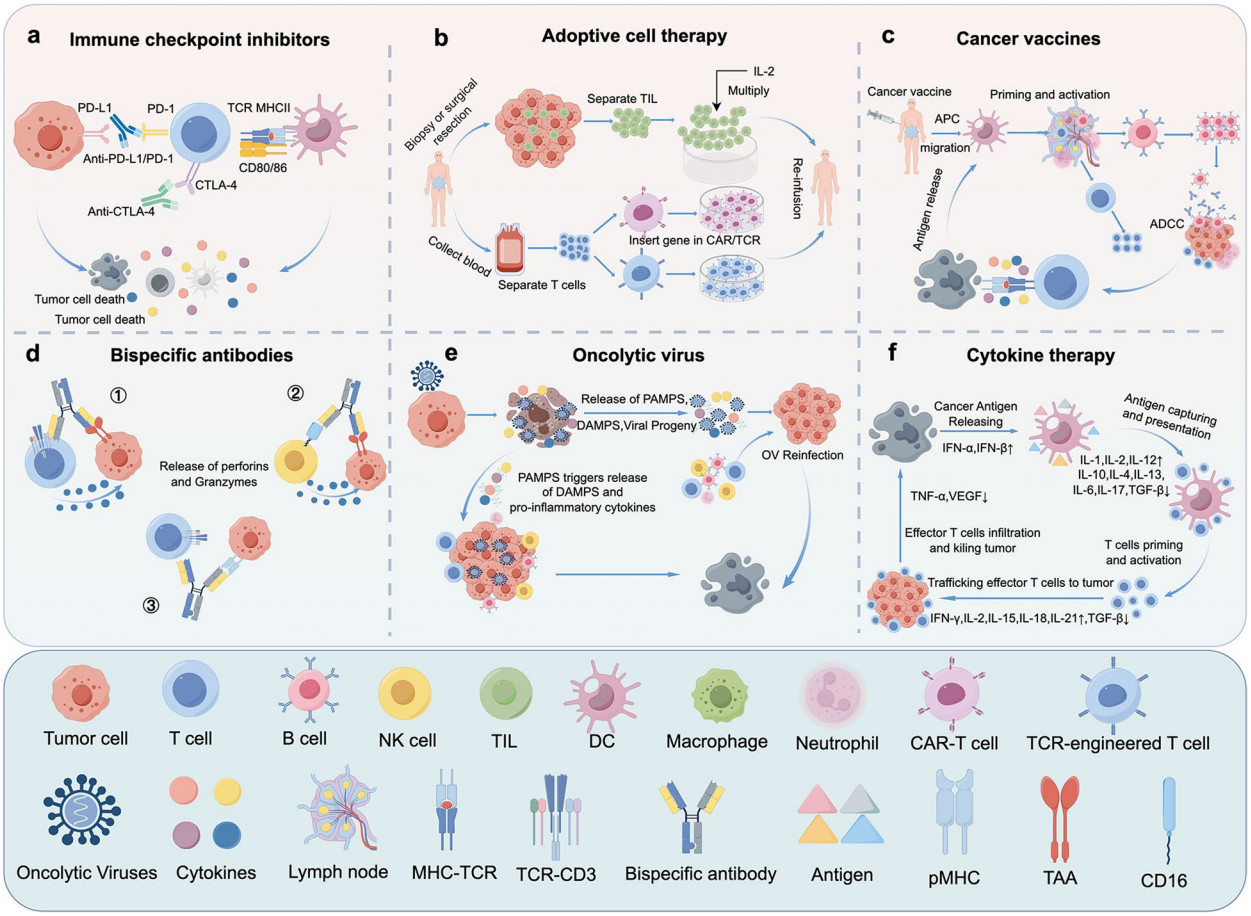


Fig. 5 Mechanism diagrams of various tumor immunotherapy methods.a. Immune checkpoint inhibitor therapy reactivates the antitumor activity of T cells, thereby enhancing the Immune system's attack on tumor cells; b. The patient's immune cells are transformed, activated, and expanded in vitro and then re-infused into the patient to clear the tumor, including CAR-T and TCR-T therapies, among others; c: Mechanism of action of cancer vaccines; d. Different kinds of Bispecific antibodies; e. OVs are generally thought to mediate anti-tumor activity through two different mechanisms of action, one is selective replication within tumor cells, which has a direct lytic effect on n tumor cells; The second is to induce systemic anti-tumor immunity and anti-immune escape, including promoting The release of pathogen-related molecular patterns (PAMPs), danger-associated molecular pattern signals (DAMPs), and cytokines from tumor cells, thereby activating antigen-specific CD4 + and CD8 + T cell responses to regulate TME; f. Cytokine therapy works by activating immune cells through antigen capture and presentation

enhancement of antigen presentation, which are the three main mechanisms to play an anti-tumor role, including interleukins (ILs), interferons (IFNs), tumor necrosis factor (TNF) [250].

Augmenting immune checkpoint inhibitions

In preclinical studies, PEGPH20 in combination with PD-L1 inhibitors can significantly increase the sensitivity of breast cancer to PD-L1 inhibitors and significantly inhibit tumor growth, and reduced resistance to PD-L1 inhibitor monotherapy [251]. In triple-negative breast cancer, the simultaneous targeted delivery of hyaluronidase and TGF-β siRNA modified the tumor microenvironment, effectively enhanced the T-cell-mediated anti-tumor immune response and enhanced the efficacy of PD-L1 inhibitors when used in combination, and brought hope to triple-negative breast cancer patients, who have the highest metastasis and recurrence rates [252]. Hydrogel-encapsulated anti-CTLA-4 antibody has enhanced target delivery and anti-tumor effects in tumor-draining lymph nodes (TDLN) after the addition of hyaluronidase [253]. In locally advanced or metastatic non-small cell lung cancer, a phase III clinical trial of atezolizumab in combination with rHuPH20 for subcutaneous administration has been completed and demonstrated similar pharmacokinetic, efficacy, and safety profiles to those of intravenous atezolizumab, which opens up the possibility of improving the ease of treatment for patients. Excitingly, Tecentriq Hybreza™ (atezolizumab-hyaluronidase-tqjs) has been approved

for marketing by the FDA in 2024 as the first subcutaneously administered PD-L1 inhibitor [254]. In advanced solid tumors such as metastatic melanoma, renal cell carcinoma, non-small cell lung cancer, hepatocellular carcinoma, colorectal cancer, and uroepithelial carcinoma, subcutaneous administration of nivolumab in combination with rHuPH20 has also yielded results that are not inferior to those of intravenous administration [255, 256]. In addition to in immune checkpoint inhibitors, subcutaneous formulations of hyaluronidase with other monoclonal agents such as trastuzumab-hyaluronidase-oysk [257], Darzalex Faspro (daratumumab-hyaluronidase-fihj) [258], Pertuzumab-Trastuzumab-Hyaluronidase-zzxf [259], rituximab and trastuzumab in combination with rHuPH20 [260] all showed similar efficacy and safety. rHuPH20 also increased the efficiency of absorption of cetuximab in subcutaneous administration [261].

Enhancing CAR-T cell infiltration

The combined use of hyaluronidase and adoptive cell therapy mainly focuses on CAR-T therapy. In existing preclinical studies, Xiong and colleagues found that the co-expression of IL-7 and PH20 in GPC3-targeted CAR-T (G3CAR-7×20) demonstrated significant anti-tumor effects in both in vivo and in vitro experiments, making it a potential treatment for hepatocellular carcinoma [262]. In addition, there are studies using bioorthogonal technology to couple α-PDL1 and HAase onto CAR-T cells, constructing H-P@CAR-T cells. Compared to traditional CAR-T cells, H-P@CAR-T cells exhibit better tumor infiltration ability, especially in mouse B-cell lymphoma (A20), where they enhance anti-tumor capabilities. In solid tumors, H-P@CAR-T cells have shown good therapeutic effects on mouse colon cancer [263].

Boosting cancer vaccine efficacy

Nanovaccines based on cancer vaccines are a hot research topic both domestically and internationally. Their smaller size makes them easier to be concentrated in lymph nodes, spleen and other lymphoid organs. Furthermore, dimensions comparable to pathogens promote efficient uptake by antigen-presenting cells (APCs), thereby improving the induction of anti-tumor immune responses [264]. The PEI/CpG/OVA (P/C/O) nanovaccine exemplifies a co-delivery system carrying both antigen and adjuvant. Within this construct, PEI enhances DCs internalization of the vaccine components. Concurrently, the CpG adjuvant is delivered to endosomes where it engages Toll-like receptor 9 (TLR9) [265, 266]. Critically, PEI mediates endosomal escape of the OVA antigen. This enables OVA processing and presentation via the major histocompatibility complex class I (MHC I) pathway, ultimately activating antigen-specific CD8 + T cells and amplifying the immune response [267]. By combining the PEI/CpG/OVA nano-vaccine with HAase, the infiltration of antigen-specific CD8 + T cells generated by the vaccine in tumor tissues has significantly enhanced the efficacy of in vivo anti-tumor immunotherapy. Hu et al. designed a new tumor nanovaccine and extracellular matrix scavenger. The main components of the nanovaccine are PEI/CaCO3/OVA/CpG (NVs), where PEI/CaCO3 nanoparticles not only efficiently deliver OVA and CpG to DCs, but also act as "autoluminescent" agonists to enhance the entire immune system [268]. PEG/PEI/pSpam1 (pSpam1@NPs), a new gene-mediated extracellular matrix scavenger, enables the isolation of polyethylene glycol by pH-responsiveness, which results in high expression of HAase in tumor tissues. Combining NVs with pSpam1@NPs promotes the infiltration of immune cells, especially CTLs, into tumors, and a stronger antitumor synergistic effect can be achieved [269]. It has been shown that by targeting the tumor extracellular matrix, hyaluronidase is able to activate tumor-draining lymph nodes (TDLNs), and the activated TDLNs can effectively enhance the immune response induced by tumor vaccines. When combined with a cryogel tumor vaccine, augmented systemic antigen-specific T-cell responses [270].

Amplifying oncolytic virus therapy

The combination of hyaluronidase and lysosomal viruses shows remarkable potential in tumor immunotherapy. Oncolytic adenoviruses armed with iRGD-modified hyaluronidase, have demonstrated strong tumor-specific CD8 + T cell activation and enhanced anti-tumor effects in immunocompetent mouse models [271]. The oncolytic adenovirus ICOVIR17 expressing hyaluronidase can also enhance the efficacy of oncolytic virus immunotherapy in refractory tumors such as glioblastoma by altering the extracellular matrix [272]. In other types of oncolytic adenoviruses, taking VCN-01 as an example, this oncolytic adenovirus, which combines hyaluronidase expression with RGD and HSG binding domain modifications, has shown significant anti-tumor effects across various types of cancer, such as pancreatic cancer, glioma, osteosarcoma, and melanoma, and has demonstrated good safety and efficacy in clinical studies [273–276]. It has also enhanced the effectiveness of chemotherapy using paclitaxel and gemcitabine [277]. In addition, the new generation of lysosomal adenovirus VCN-11 has hyaluronidase activity and is able to evade the attack of neutralizing antibodies, which further enhances the therapeutic potential of lysosomal viruses in the presence of neutralizing antibodies, and shows highly effective antitumor effects [278]. Previous studies have focused on lysogenic adenoviruses, and Wang et al. constructed a cowpox virus

(OVV- HYAL1) encoding the hyaluronidase HYAL1, which promotes viral dissemination, chemotherapeutic drug diffusion, leukocyte infiltration, and immune activation in the TME. It showed promising anti-tumor effects and significantly prolonged survival in mouse models of pancreatic, breast and colorectal cancer [279].

Combination of hyaluronidase and other immunotherapeutic drugs

Although the clinical application of immune checkpoint inhibitors is in full swing, there are still many tumors for which immunotherapy still fails to achieve satisfactory therapeutic effects, the emergence of small molecules has made up for some of the shortcomings of immunotherapy [280]. Indoleamine 2,3-dioxygenase (IDO) is highly expressed in most human tumor tissues [281], and IDO activity is closely related to the regulation of cancer immune escape mechanisms and T-cell activity [282], therefore, IDO inhibitors have become small molecule drugs with therapeutic potential. Li et al. constructed a Sono-activatable semiconducting polymer nanoreshapers (SPNDNH) loaded with an IDO inhibitor (NLG919) and modified with hyaluronidase, which could be used as an anti-inflammatory agent for the treatment of cancer, promoting nanoparticle enrichment and immune cell infiltration by degrading abundant HA in the pancreatic cancer ECM, and also blocking IDO activity by NLG919, remodeling the tumor immune microenvironment, as well as the tumor immune microenvironment, tumor in situ growth and distant metastasis were suppressed in a mouse model of pancreatic cancer [283]. Induction of immunogenic cell death (ICD) is an effective method to activate TME, which can release antigens and DAMP, transforming "cold tumors" into "hot tumors" and enhancing immunotherapy [284]. Drugs capable of activating ICD such as doxorubicin (DOX), mitoxantrone, oxaliplatin, etc. have been extensively studied in tumor immunity [285]. A hyaluronidase-modified DOX-loaded silica nanocarriers (DOX@HMSPHs) have been designed, and the hyaluronidase on the surface of this nanomaterial promotes drug diffusion by degrading the HA in the ECM and decreasing the tumor mesenchymal pressure. Moreover, drug-loaded nanoparticles can release chemotherapeutic drug DOX continuously in acidic microenvironment, induce ICD of tumor cells, promote the maturation of DCs, and ultimately achieve anti-tumor therapeutic effect. In addition, the nanocarrier HMSPs can be used as an immune adjuvant to further promote the activation of tumor-infiltrating T lymphocyte in mice [286]. Dox@pssp-hh NPS is also a novel nano-delivery system that, in addition to promoting drug diffusion by reducing tumor stromal pressure, the highly expressed Glutathione within tumors can disrupt disulfide bonds in nanoparticles, thereby increasing drug delivery and drug resistance, promote structural changes in nanoparticles to release Dox, which is also associated with the production of reactive oxygen species, further inducing oxidative stress in tumor cells [287]. ZnO-DOX@HAase PEG-PAH-DMMA (ZDHD) NPs, also a nanocarrier that can deliver antitumor drugs deep into tumors and elicit ICD, was validated for antitumor effects in a mouse 4T1 tumor model. There was no histological damage to these organs, heart, liver, spleen, lungs and kidneys, after ZDHD treatment compared to the DOX alone treatment group, providing a new idea to improve the efficacy and reduce the adverse effects of chemoimmunotherapy for tumors [288]. Pts@DOXHANG@Gal is an engineered platelets (Pts)-based nano-aircraft, which is armed with Dox and the TGF-βri inhibitor GALUNISERTIB (Gal), redox-sensitive nanospheres (HANG) composed of hyaluronidase and bis-N-hydroxy succinimide are shed from the structure along with the GAL. Dox and Gal exerted antitumor effects through ICD and TGF-β inhibition and induced long-term immune memory [289]. Fluorouracil, folinic acid, irinotecan and oxaliplatin's regimen (FOLFIRINOX) is an important treatment for metastatic pancreatic cancer, and a phase IB/II trial evaluated PEGPH20 in combination with FOLFIRINOX, surprisingly poor outcomes were seen, including greater toxicity and shorter median survival, so greater caution is needed when using hyaluronidase in combination with Oxaliplatin [290].

As of now, clinical trials combining bispecific antibodies with hyaluronidase are in full swing, particularly the star product Rybrevant (amivantamab) from Johnson & Johnson, which is being administered in a fixed subcutaneous combination with recombinant human hyaluronidase. These trials are currently recruiting patients with non-small cell lung cancer (NCT05498428). However, there have been no reports on the study of the combined use of cytokine therapy and hyaluronidase to enhance anti-tumor effects. In Table 3, based on data from ClinicalTrials.gov, we have listed representative clinical trial developments of hyaluronidase in tumor immunotherapy.

Current research findings indicate that the combined use of hyaluronidase with tumor immunotherapy in most clinical trials can enhance anti-tumor effects, increase patient survival, and improve prognosis. However, some clinical trials failed to show the superiority of hyaluronidase in enhancing the clinical activity of therapeutic antibodies, such as two MORPHEUS trials(NCT03193190 and NCT03281369). This discrepancy stems from three interconnected dimensions. (1) Trial design heterogeneity: insufficient sequential timing of key drugs. (2) Unstratified patient cohorts: Inclusion

Table 3 Clinical trial progress of hyaluronidase in Tumor Immunotherapy

NCT	Combined treatment programmes	Cancer Type	Phase	Location
NCT03719131	Rituximab and hyaluronidase human (Rituxan Hycela)	Advanced Melanoma Undergoing Nivolumab and Ipilimumab Therapy	II	United States
NCT03656718	Nivolumab and rHuPH20	Non-Small Cell Lung Cancer(NSCLC), Renal Cell Carcinoma(RCC), Hepatocellular Carcinoma(HCC), unresectable or metastatic melanoma, MSI-H/dMMR CRC, melanoma	I/II	United States, Argentina, Brazil, Chile, France, Italy, Mexico, Netherlands, New Zealand, Poland, Spain, United, Kingdom
NCT03446040	Nivolumab and rHuPH20	Solid Cancers That Are Advanced or Have Spread [RCC, CRC, NSCLC, Triple Negative Breast Cancer (SCCHN), Squamous Cell Carcinoma of the Head and Neck (TNBC)]	I/II	United States, Australia, Canada, Japan
NCT06504394	Pembrolizumab and Hyaluronidase	Relapsed or Refractory Classical Hodgkin Lymphoma (rrcHL) or Relapsed or Refractory Primary Mediastinal Large B-cell Lymphoma (rrPMBCL)	II	United States
NCT06212752	Pembrolizumab and Hyaluronidase	NSCLC	III	Japan
NCT06099782	Pembrolizumab and Hyaluronidase	RCC, NSCLC, Melanoma	II	United States, Argentina, Australia, Chile, France, Japan, New Zealand, Poland, South Africa, Turkey
NCT05722015	Pembrolizumab and Hyaluronidase	Metastatic Non-small Cell Lung Cancer	III	United States, Argentina, Brazil, Chile, China, France, Guatemala, Hungary, Japan, Poland, Romania, South Africa , Spain, Thailand, Turkey
NCT04058964	Pembrolizumab and PEGPH20	Metastatic Pancreatic Cancer	II	United States
NCT03634332	Pembrolizumab and PEGPH20	Metastatic Pancreatic Cancer	II	United States
NCT02563548	Pembrolizumab and PEGPH20	NSCLC, Gastric Cancer	I	United States
NCT05340309	Atezolizumab and Recombinant Human Hyaluronidase	NSCLC	II	United States
NCT06099782	Atezolizumab and rHuPH20	NSCLC	II	Argentina, Brazil, Chile, China, Costa Rica, Greece, Guatemala, Hungary, Italy, Republic of Korea, Latvia, Mexico , New Zealand, Peru, Poland, Russian Federation, South Africa
NCT03267940	Atezolizumab and PEGPH20	Cholangiocarcinoma, Gallbladder Adenocarcinoma	I	United States, Republic of Korea, Thailand
NCT02045589	VCN-01(Genetically modified human adenovirus encoding human PH20 hyaluronidase) and Abraxane®/Gemcitabine	Pancreatic Adenocarcinoma	I	Spain
NCT02045602	VCN-01 and Abraxane®/ Gemcitabine	Locally Advanced Solid Tumors, Metastatic Solid Tumors	I	Spain
NCT03284268	VCN-01	Rrefractory Retinoblastoma	I	Spain
NCT03799744	VCN-01 and Durvalumab	TNBC	I	Spain
NCT05057715	VCN-01 and Human Chimeric Antigen Receptor Modified T Cells (huCART-meso)	Pancreatic Cancer, Serous Ovarian Cancer	I	United States
NCT05673811	VCN-01 and Nab-Paclitaxel, Gemcitabine	Metastatic Pancreatic Cancer	II	United States, Spain
NCT05498428	Amivantamab and rHuPH20	NSCLC	II	United States, Brazil, China, France, Germany, Israel, Italy, Japan, Republic of Korea, Malaysia, Spain, United Kingdom

Table 3 (continued)

NCT	Combined treatment programmes	Cancer Type	Phase	Location
NCT05388669	Recombinant Human Hyaluronidase and Amivantamab, Lazertinib	NSCLC	III	United States, Argentina, Australia, Brazil, Canada, China, France, Germany, Israel, Italy, Japan, Republic of Korea, Malaysia, Poland, Portugal, Spain, Taiwan, Thailand, United Kingdom,
NCT04606381	Amivantamab and rHuPH20	Advanced Solid Malignancie	I	United States, Canada, Republic of Korea, United Kingdom,

of HA-low tumors obscuring response in HA-high subsets. (3) Dynamic TME compensation: Rapid HA regeneration via HAS2 upregulation and collagen crosslinking [291–293]. Although the combination of atezolizumab and PEGPH20 failed to achieve the primary endpoint of improved objective response rate (ORR) compared to chemotherapy regimens in both studies, the signal-seeking trial design accelerated proof-of-concept evaluation while minimizing patient exposure to suboptimal therapies. The MORPHEUS platform further enabled randomized efficacy assessment of this novel combination within an adaptive framework [294].

Clinical applications in non-oncological diseases

Hyaluronidase is widely used in medicine, enhancing the absorption and distribution of other medications. In addition to its applications in the field of oncology,it is valuable in ophthalmology, cosmetic surgery, anesthesia assistance [23]. Additionally, its low incidence of clinical adverse reactions has allowed hyaluronidase to enter the fast lane in clinical applications in recent years [18].

Enhance local infiltration anesthesia

The use of hyaluronidase to enhance surgical infiltration anesthesia is one of its most well-known effects. In 1951, Thorpe was the first to apply hyaluronidase in dermatological surgery [295], When hyaluronidase is used in combination with local anesthetic, the diffusion rate of local anesthetic is proportional to the amount of hyaluronidase injected, which accelerates the onset time of anesthesia and reduces the swelling caused by local infiltration [296]. This ensures the effectiveness and safety of local anesthesia, and now hyaluronidase has been widely used as an adjunct to local anesthetics, such as ophthalmology, dermatology, trauma surgery, and stomatology [23, 297].

Promote drug permeation and enhance drug bioavailability

Hyaluronidase acts as a "Diffusion factor" catalyzing the degradation of HA in the ECM, enhancing tissue permeability, thereby enhancing drug penetration and improving the bioavailability of injected drugs [130], it can also promote the dissipation of local accumulations of blood or fluid to eliminate hematoma and edema, and relieve pain at the injection site [298]. In addition to its use as a local anesthetic, hyaluronidase is also useful for the subcutaneous administration of drugs such as insulin, morphine, antibiotics, therapeutic monoclonal antibody, and immunoglobulins, accelerated circulation of drugs in synovial fluid speeds up pharmacokinetics, improves drug availability, and increases patient satisfaction with drug response [23, 299].

Application in the field of cosmetic plastic surgery

HA is a widely used soft tissue filler in the field of cosmetic surgery, applicable for facial remodeling, tissue enhancement, and deep skin hydration. However, improper operation can lead to side effects such as vascular occlusion, blindness, nodules, granulomas, delayed hypersensitivity reactions, and poor cosmetic results [300]. Alam et al. confirmed that hyaluronidase can remove nodules caused by hyaluronic acid [301]. When hyaluronic acid enters the ophthalmic artery, it is easy to cause embolism of the central retinal artery, and the injection of hyaluronidase from the back of the eye can degrade the hyaluronic acid in the blood vessel and surrounding tissues, so as to achieve the therapeutic effect [302]. Recently, it has been found that pretreatment with hyaluronidase at the recipient site of fat transplantation can improve the survival rate of fat transplantation [303].

Challenges and considerations

Potential side effects and safety concerns

In clinical application, the adverse reaction rate of hyaluronidase is low, and the most common side effect is anaphylaxis, but the incidence of anaphylaxis is only 0.05%–0.69% [304]. Hyaluronidase from different biological sources is one of the causes of allergic reactions [305]. Animal-derived hyaluronidase has the problems of low purity, high miscellaneous protein content, and strong immunogenicity, which can increase IgE-mediated immunogenic allergic reactions [23]. However, with the use of human recombinant hyaluronidase rHuPH20, the number of allergic reactions due to origin has been greatly reduced [306]. In allergic reactions, immediate hypersensitivity reactions are the most common, but delayed hypersensitivity reactions may also occur. The clinical manifestations of immediate hypersensitivity after hyaluronidase application include erythema, edema, urticaria, and in severe cases, can lead to anaphylaxis, treatment may include topical steroid creams or oral steroids or antihistamines [307]. Different injection sites and injection doses lead to different allergic reactions, and allergic reactions after ocular injections are the most reported, which may be related to the long residence time due to the dense blood vessels in the eye, and the main symptoms include edema, pain, and itching, etc., but there are also serious cases related to loss of vision, and even death, have been reported [308, 309]. When the local injection dose is less than 1500 IU, the allergic reaction is limited to the local area, but when high doses of hyaluronidase (200,000 IU) or intravenous injections are given, the risk of allergic reaction can rise to 31.3% [310]. Therefore, in order to avoid adverse reactions, an allergy skin test seems to be effective, especially in patients allergic to bee stings, where hyaluronidase is likely to be the

allergen [311]. In addition, we should avoid injecting at the site of inflammation, adhere to strict hyaluronidase injection formulation and dosage control, and consider drug interactions, appropriate post-injection care can reduce the incidence of adverse reactions [312].

Resistance and limitations in therapeutic applications

Animal-derived hyaluronidase can cause human immune response, lead to the production of neutralizing antibodies, affect the activity of hyaluronidase, and then cause allergic reactions and reduce drug permeability. In a related study of recombinant human hyaluronidase, treatment-induced rHuPH20 antibody responses could be observed, but no neutralizing antibodies were observed. It also has a low immunogenic potential in terms of CD4+T cell activation and B cell cross-reactivity, so that long-term use is also well tolerated when rHuPH20 is used in combination with therapeutic agents [313, 314]. Because rHuPH20 has a short half-life and is easily degraded, repeated administration may be required, increasing the risk of local irritation and immunity, and reducing the risk of infection, the successful development of Polyethylene glycol recombinant human hyaluronidase PEGPH20 has extended the half-life of rHuPH20 from less than 3 min to 10.3 h, providing a new option for therapeutic modalities that require prolonged hyaluronidase maintenance [315]. Hyaluronidase may have the limitation of insufficient tissue selectivity for the unspecific recognition of normal and diseased tissues, especially in tumor immunotherapy, where combination with therapeutic modalities that can target tumor tissue is essential. In reversing hyaluronan complications, there are case reports of injections of high doses of hyaluronidase leading to total degradation of HA, which ultimately led to loss of therapeutic efficacy [316]. This also gives us the insight that multiple smaller doses of hyaluronidase can be used for treatment of non-urgent complications.

Ethical considerations in medical use

There are many ethical issues to be concerned about in the medical use of hyaluronidase, and the safety risk and informed consent need to be considered first, the possible allergic reactions caused by animal-derived hyaluronidase, whether long-term application of hyaluronidase can degrade hyaluronan in normal tissues, such as skin laxity, decreased joint stability, etc. is not yet clear [309], patients should be fully informed of the risks and give informed consent before use. The ethical controversy surrounding animal-derived products can not be ignored. Bovine hyaluronidase may be involved in animal slaughter, may violate animal welfare, and may touch on certain religious and cultural taboos [317]. There have been no clinically confirmed cases, although cross-species infections with products of animal origin are thought to be possible and concerns have been raised about the carrying of unknown viruses such as prions [318]. So using rHuPH20 instead is a good choice. In addition, there are also ethical challenges with novel technologies, nanocarriers or gene-editing delivery systems may introduce unknown toxicities, and patients participating in early-phase clinical trials may bear high risks but limited benefits, which may increase the risk of adverse effects, strict Declaration of Helsinki is therefore required to ensure that trial designs are consistent with the risk–benefit ratio and that reversible interventions are prioritized [319].

Conclusion

Hyaluronidase, a pivotal enzyme in the metabolism of HA, plays multifaceted roles in both physiological and pathological processes. Its structural diversity, derived from variations in catalytic domains and sequence homology across species, underpins its distinct mechanisms of action—ranging from hydrolytic cleavage to β-elimination. The dynamic degradation of HA by hyaluronidase profoundly impacts the ECM, including modulatiion of tissue permeability, immune cell infiltration, and cell–matrix interactions, which are critical in wound healing, inflammation, and tumor progression.

In oncology, hyaluronidase emerges as a double-edged sword that plays both pro- and anti-tumor roles, strategic exploitation of its ECM-remodeling ability offers promising therapeutic avenues. Innovations in tumor immunotherapy highlight hyaluronidase as a key adjuvant, enhancing drug permeability and immune cell infiltration. rHuPH20 and PEGPH20 have revolutionized subcutaneous drug delivery, improving the efficacy of immune checkpoint inhibitors and adoptive cell therapies. Furthermore, hyaluronidase-engineered oncolytic viruses and nanovaccines demonstrate synergistic anti-tumor effects by reshaping the immunosuppressive TME.

Despite these advances, challenges persist. Safety concerns, including allergenicity and off-target ECM degradation, necessitate rigorous dosing protocols and the development of species-specific recombinant enzymes. Resistance mechanisms, such as neutralizing antibodies against animal-derived hyaluronidases, underscore the importance of humanized formulations. Ethical considerations surrounding animal-derived products further advocate for synthetic alternatives. Future research should prioritize designing tumor-targeted hyaluronidase delivery systems to minimize systemic toxicity and enhance antitumor effects.

In conclusion, hyaluronidase stands at the nexus of ECM biology and translational medicine. Its dual role as

a pathogenic driver and therapeutic ally underscores the need for precision in clinical applications. By harnessing its ECM-remodeling potential while mitigating adverse effects, hyaluronidase-based strategies may redefine standards in tumor immunotherapy.

Acknowledgements
The Fig 1, 2 were created by ChemDraw, Figs 3, 4 and 5 were created by an online drawing tool website named "Figdraw".

Authors' contributions
All authors have significant contributions to the conception of the work. Jiamin Lu drafted the manuscript. Zheng Zhao, Lingli Pan and Hui Wu revised manuscript, Shibing Wang, Xiangmin Tong and Shenghao Wu provided final approval of the submitted manuscript. All authors have read and approved the final manuscript.

Funding
This study was supported by the National Natural Science Foundation of China (No. 82470182), Funds of the Natural Science Foundation of Hangzhou (No. 2024SZRYBC080002).

Data availability
The results/data/figures in this manuscript have not been published elsewhere, nor are they under consideration by another publisher.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
All authors state that there are no potential competing interests.

Received: 8 October 2024 Revised: 28 June 2025 Accepted: 30 June 2025
Published online: 12 July 2025

References

1. McAtee CO, Barycki JJ, Simpson MA. Emerging roles for hyaluronidase in cancer metastasis and therapy. Adv Cancer Res. 2014;123:1–34. https://doi.org/10.1016/B978-0-12-800092-2.00001-0.
2. Iaconisi GN, Lunetti P, Gallo N, Cappello AR, Fiermonte G, Dolce V, et al. Hyaluronic Acid: A Powerful Biomolecule with Wide-Ranging Applications—A Comprehensive Review. Int J Mol Sci. 2023;24(12):10296. https://doi.org/10.3390/ijms241210296.
3. Garantziotis S, Savani RC. Hyaluronan biology: A complex balancing act of structure, function, location and context. Matrix Bio. 2019;78–79:1–10. https://doi.org/10.1016/j.matbio.2019.02.002.
4. Sindelar M, Jilkova J, Kubala L, Velebny V, Turkova K. Hyaluronidases and hyaluronate lyases: From humans to bacteriophages. Colloids Surf B Biointerfaces. 2021;208: 112095. https://doi.org/10.1016/j.colsurfb.2021.112095.
5. Lombard V, Bernard T, Rancurel C, Brumer H, Coutinho PM, Henrissat B. A hierarchical classification of polysaccharide lyases for glycogenomics. Biochem J. 2010;432(3):437–44. https://doi.org/10.1042/BJ20101185.
6. Stern R, Jedrzejas MJ. Hyaluronidases: Their genomics, structures, and mechanisms of action. Chem Rev. 2006;106(3):818–39. https://doi.org/10.1021/cr050247k.
7. Zhao J, Chen J, Li C, Xiang H, Miao X. Hyaluronidase overcomes the extracellular matrix barrier to enhance local drug delivery. Eur J Pharm Biopharm. 2024;203: 114474. https://doi.org/10.1016/j.ejpb.2024.114474.
8. Duran-Reynals F. Exaltation de l'activité du virus vaccinal par les extraits de certains organes. Compt Rend Soc biol. 1928;9:6–7. n.d.
9. Chain E, Duthie ES. Identity of hyaluronidase and spreading factor. Br. J. Exp. Pathol. 1940;21(6):324. n.d.
10. Wang W, Wang J, Li F. Hyaluronidase and Chondroitinase. In: Atassi MZ, editor. Protein Rev. 2016; p. 75–87. https://doi.org/10.1007/5584_2016_54.
11. Girish KS, Kemparaju K. The magic glue hyaluronan and its eraser hyaluronidase: A biological overview. Life Sci. 2007;80(21):1921–43. https://doi.org/10.1016/j.lfs.2007.02.037.
12. Meyer K. 11 Hyaluronidases. The Enzymes, vol. 5, Elsevier; 1971; p. 307–20. https://doi.org/10.1016/S1874-6047(08)60094-3.
13. Cramer JA, Bailey LC, Bailey CA, Miller RT. Kinetic and mechanistic studies with bovine testicular hyaluronidase. Biochim Biophys Acta. 1994;1200(3):315–21. https://doi.org/10.1016/0304-4165(94)90173-2.
14. Meyer K, Rapport MM. Hyaluronidases. Adv Enzymol Relat Subj Biochem. 1952; p. 199–236. https://doi.org/10.1002/9780470122587.ch6.
15. Linker A, Hoffman P, Meyer K. The hyaluronidase of the leech: an endoglucuronidase. Nature. 1957;180(4590):810–1. https://doi.org/10.1038/180810b0.
16. Jin P, Kang Z, Zhang N, Du G, Chen J. High-yield novel leech hyaluronidase to expedite the preparation of specific hyaluronan oligomers. Sci Rep. 2014;4:4471. https://doi.org/10.1038/srep04471.
17. Rigden DJ, Littlejohn JE, Joshi HV, de Groot BL, Jedrzejas MJ. Alternate structural conformations of Streptococcus pneumoniae hyaluronan lyase: insights into enzyme flexibility and underlying molecular mechanism of action. J Mol Biol. 2006;358(4):1165–78. https://doi.org/10.1016/j.jmb.2006.02.066.
18. Zhang Y-S, Gong J-S, Yao Z-Y, Jiang J-Y, Su C, Li H, et al. Insights into the source, mechanism and biotechnological applications of hyaluronidases. Biotechnol Adv. 2022;60: 108018. https://doi.org/10.1016/j.biotechadv.2022.108018.
19. Henrissat B. A classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem J. 1991;280(2):309–16. https://doi.org/10.1042/bj2800309.
20. Henrissat B, Bairoch A. Updating the sequence-based classification of glycosyl hydrolases. Biochem J. 1996;316(Pt 2):695–6. https://doi.org/10.1042/bj3160695.
21. Pluvinage B, Massel PM, Burak K, Boraston AB. Structural and functional analysis of four family 84 glycoside hydrolases from the opportunistic pathogen Clostridium perfringens. Glycobiology. 2020;30(1):58–68. https://doi.org/10.1093/glycob/cwz069.
22. Garron M-L, Cygler M. Structural and mechanistic classification of uronic acid-containing polysaccharide lyases. Glycobiology. 2010;20(12):1547–73. https://doi.org/10.1093/glycob/cwq122.
23. Weber GC, Buhren BA, Schrumpf H, Wohlrab J, Gerber PA. Clinical applications of hyaluronidase. In: Labrou N, editor. Therapeutic Enzymes: Function and Clinical Implications, vol. 1148, Adv Exp Med Biol. 2019, p. 255–77. https://doi.org/10.1007/978-981-13-7709-9_12.
24. Lepperdinger G, Müllegger J, Kreil G. Hyal2–less active, but more versatile? Matrix Biol. 2001;20(8):509–14. https://doi.org/10.1016/s0945-053x(01)00170-6.
25. Bohaumilitzky L, Huber A-K, Stork EM, Wengert S, Woelfl F, Boehm H. A Trickster in Disguise: Hyaluronan's Ambivalent Roles in the Matrix. Front Oncol. 2017;7:242. https://doi.org/10.3389/fonc.2017.00242.
26. Vines CA, Li MW, Deng X, Yudin AI, Cherr GN, Overstreet JW. Identification of a hyaluronic acid (HA) binding domain in the PH-20 protein that may function in cell signaling. Mol Reprod Dev. 2001;60(4):542–52. https://doi.org/10.1002/mrd.1119.
27. Cherr GN, Yudin AI, Overstreet JW. The dual functions of GPI-anchored PH-20: Hyaluronidase and intracellular signaling. Matrix Biol. 2001;20(8):515–25. https://doi.org/10.1016/s0945-053x(01)00171-8.
28. Atmuri V, Martin DC, Hemming R, Gutsol A, Byers S, Sahebjam S, et al. Hyaluronidase 3 (HYAL3) knockout mice do not display evidence of hyaluronan accumulation. Matrix Biol. 2008;27(8):653–60. https://doi.org/10.1016/j.matbio.2008.07.006.
29. Chi A, Shirodkar SP, Escudero DO, Ekwenna OO, Yates TJ, Ayyathurai R, et al. Molecular characterization of kidney cancer: Association of hyaluronic acid family with histological subtypes and metastasis. Cancer. 2012;118(9):2394–402. https://doi.org/10.1002/cncr.26520.

30. Yoshida H, Nagaoka A, Kusaka-Kikushima A, Tobiishi M, Kawabata K, Sayo T, et al. KIAA1199, a deafness gene of unknown function, is a new hyaluronan binding protein involved in hyaluronan depolymerization. Proc Natl Acad Sci U S A. 2013;110(14):5612–7. https://doi.org/10.1073/pnas.1215432110.
31. Yamamoto H, Tobisawa Y, Inubushi T, Irie F, Ohyama C, Yamaguchi Y. A mammalian homolog of the zebrafish transmembrane protein 2 (TMEM2) is the long-sought-after cell-surface hyaluronidase. J Biol Chem. 2017;292(18):7304–13. https://doi.org/10.1074/jbc.M116.770149.
32. Niu M, McGrath M, Sammon D, Gardner S, Morgan RM, Di Maio A, et al. Structure of the transmembrane protein 2 (TMEM2) ectodomain and its apparent lack of hyaluronidase activity. Wellcome Open Res. 2023;8:76. https://doi.org/10.12688/wellcomeopenres.18937.2.
33. Sato S, Miyazaki M, Fukuda S, Mizutani Y, Mizukami Y, Higashiyama S, et al. Human TMEM2 is not a catalytic hyaluronidase, but a regulator of hyaluronan metabolism via HYBID (KIAA1199/CEMIP) and HAS2 expression. J Biol Chem. 2023;299(6): 104826. https://doi.org/10.1016/j.jbc.2023.104826.
34. Narita T, Tobisawa Y, Bobkov A, Jackson M, Ohyama C, Irie F, et al. TMEM2 is a bona fide hyaluronidase possessing intrinsic catalytic activity. J Biol Chem. 2023;299(9): 105120. https://doi.org/10.1016/j.jbc.2023.105120.
35. Kim E, Baba D, Kimura M, Yamashita M, Kashiwabara S, Baba T. Identification of a hyaluronidase, Hyal5, involved in penetration of mouse sperm through cumulus mass. Proc Natl Acad Sci U S A. 2005;102(50):18028–33. https://doi.org/10.1073/pnas.0506825102.
36. Reitinger S, Laschober GT, Fehrer C, Greiderer B, Lepperdinger G. Mouse testicular hyaluronidase-like proteins SPAM1 and HYAL5 but not HYALP1 degrade hyaluronan. Biochem J. 2007;401(1):79–85. https://doi.org/10.1042/BJ20060598.
37. Kimura M, Kim E, Kang W, Yamashita M, Saigo M, Yamazaki T, et al. Functional Roles of Mouse Sperm Hyaluronidases, HYAL5 and SPAM1, in Fertilization1. Biol Reprod. 2009;81(5):939–47. https://doi.org/10.1095/biolreprod.109.078816.
38. Park S, Kim Y, Jeong P, Park C, Lee J, Kim J, et al. SPAM1/HYAL5 double deficiency in male mice leads to severe male subfertility caused by a cumulus-oocyte complex penetration defect. FASEB J. 2019;33(12):14440–9. https://doi.org/10.1096/fj.201900889RRR.
39. Modarressi SM, Koolivand Z, Akbari M. Enhancing hyaluronidase enzyme activity: Insights from advancement in bovine and ovine testicular hyaluronidase purification. Chromatogr B Analyt Technol Biomed Life Sci. 2024;1234: 124031. https://doi.org/10.1016/j.jchromb.2024.124031.
40. Jedrzejas MJ, Stern R. Structures of vertebrate hyaluronidases and their unique enzymatic mechanism of hydrolysis. Proteins. 2005;61(2):227–38. https://doi.org/10.1002/prot.20592.
41. Khan N, Niazi ZR, Rehman FU, Akhtar A, Khan MM, Khan S, et al. Hyaluronidases: A Therapeutic Enzyme. Protein Pept Lett. 2018;25(7):663–76. https://doi.org/10.2174/0929866525666180629121823.
42. Barth T, Mandacaru SC, Charneau S, Souza MVD, Ricart CAO, Noronha EF, et al. Biochemical and structural characterization of a protein complex containing a hyaluronidase and a CRISP-like protein isolated from the venom of the spider Acanthoscurria natalensis. J Proteomics. 2019;192:102–13. https://doi.org/10.1016/j.jprot.2018.08.012.
43. Frangieh J, Salma Y, Haddad K, Mattei C, Legros C, Fajloun Z, et al. First Characterization of The Venom from Apis mellifera syriaca, A Honeybee from The Middle East Region. Toxins. 2019;11(4):191. https://doi.org/10.3390/toxins11040191.
44. Ferrer VP, De Mari TL, Gremski LH, Trevisan Silva D, Da Silveira RB, Gremski W, et al. A Novel Hyaluronidase from Brown Spider (Loxosceles intermedia) Venom (Dietrich's Hyaluronidase): From Cloning to Functional Characterization. PLoS Negl Trop Dis. 2013;7(5): e2206. https://doi.org/10.1371/journal.pntd.0002206.
45. Justo Jacomini DL, Campos Pereira FD, Aparecido Dos Santos Pinto JR, Dos Santos LD, Da Silva Neto AJ, Giratto DT, et al. Hyaluronidase from the venom of the social wasp Polybia paulista (Hymenoptera, Vespidae): Cloning, structural modeling, purification, and immunological analysis. Toxicon. 2013;64:70–80. https://doi.org/10.1016/j.toxicon.2012.12.019.
46. Antolín-Amérigo D, Ruiz-León B, Boni E, Alfaya-Arias T, Álvarez-Mon M, Barbarroja-Escudero J, et al. Component-resolved diagnosis in hymenoptera allergy. Allergol Immunopathol (Madr). 2018;46:253–62. https://doi.org/10.1016/j.aller.2017.05.003.
47. Ruëff F, Bauer A, Becker S, Brehler R, Brockow K, Chaker AM, et al. Diagnosis and treatment of Hymenoptera venom allergy: S2k Guideline of the German Society of Allergology and Clinical Immunology (DGAKI) in collaboration with the Arbeitsgemeinschaft für Berufs- und Umweltdermatologie e.V. (ABD), the Medical Association of German Allergologists (AeDA), the German Society of Dermatology (DDG), the German Society of Oto-Rhino-Laryngology, Head and Neck Surgery (DGHNOKC), the German Society of Pediatrics and Adolescent Medicine (DGKJ), the Society for Pediatric Allergy and Environmental Medicine (GPA), German Respiratory Society (DGP), and the Austrian Society for Allergy and Immunology (ÖGAI). Allergol Select. 2023;7:154–90. https://doi.org/10.5414/ALX02430E.
48. Sivaramakrishnan V, Ilamathi M, Girish KS, Kemparaju K, Rangappa KS, Dhananjaya BL. Viper venom hyaluronidase and its potential inhibitor analysis: a multipronged computational investigation. J Biomol Struct Dyn. 2017;35(9):1979–89. https://doi.org/10.1080/07391102.2016.1203820.
49. Silva De França F, Tambourgi DV. Hyaluronan breakdown by snake venom hyaluronidases: From toxins delivery to immunopathology. Front Immunol. 2023;14:1125899. https://doi.org/10.3389/fimmu.2023.1125899.
50. Claude A. SPREADING PROPERTIES OF LEECH EXTRACTS AND THE FORMATION OF LYMPH. J Exp Med. 1937;66(3):353–66. https://doi.org/10.1084/jem.66.3.353.
51. Kang Z, Zhang N, Zhang Y. Enhanced production of leech hyaluronidase by optimizing secretion and cultivation in Pichia pastoris. Appl Microbiol Biotechnol. 2016;100(2):707–17. https://doi.org/10.1007/s00253-015-7056-5.
52. Huang H, Liang Q, Wang Y, Chen J, Kang Z. High-level constitutive expression of leech hyaluronidase with combined strategies in recombinant Pichia pastoris. Appl Microbiol Biotechnol. 2020;104(4):1621–32. https://doi.org/10.1007/s00253-019-10282-7.
53. Lv M, Wang M, Cai W, Hao W, Yuan P, Kang Z. Characterisation of separated end hyaluronan oligosaccharides from leech hyaluronidase and evaluation of angiogenesis. Carbohydr Polym. 2016;142:309–16. https://doi.org/10.1016/j.carbpol.2016.01.052.
54. Jiang J-Y, Xue D, Gong J-S, Zheng Q-X, Zhang Y-S, Su C, et al. A comprehensive review on microbial hyaluronan-degrading enzymes: from virulence factors to biotechnological tools. Bioresour Bioprocess. 2024;11(1):114. https://doi.org/10.1186/s40643-024-00832-x.
55. Coleman M, Orvis A, Brokaw A, Furuta A, Sharma K, Quach P, et al. GBS hyaluronidase mediates immune suppression in a TLR2/4- and IL-10-dependent manner during pregnancy-associated infection. mBio. 2023;14(5):e02049–23. https://doi.org/10.1128/mbio.02049-23.
56. Dokoshi T, Zhang L, Li F, Nakatsuji T, Butcher A, Yoshida H, et al. Hyaluronan Degradation by Cemip Regulates Host Defense against Staphylococcus aureus Skin Infection. Cell Rep. 2020;30(1):61-68.e4. https://doi.org/10.1016/j.celrep.2019.12.001.
57. Zamboni F, Wong CK, Collins MN. Hyaluronic acid association with bacterial, fungal and viral infections: Can hyaluronic acid be used as an antimicrobial polymer for biomedical and pharmaceutical applications? Bioact Mater. 2023;19:458–73. https://doi.org/10.1016/j.bioactmat.2022.04.023.
58. Feng J, Qian Y, Zhou Z, Ertmer S, Vivas EI, Lan F, et al. Polysaccharide utilization loci in Bacteroides determine population fitness and community-level interactions. Cell Host Microbe. 2022;30(2):200-215.e12. https://doi.org/10.1016/j.chom.2021.12.006.
59. Osman N, Mohammed M, Omolo CA, Ibrahim UH, Gafar MA, Rambharose S, et al. Hyaluronidase inhibitor surface-modified niosomes: An efficient strategy for antibacterial and anti-biofilm drug delivery. Materialia. 2023;30: 101826. https://doi.org/10.1016/j.mtla.2023.101826.
60. Wang Z, Guo C, Xu Y, Liu G, Lu C, Liu Y. Two Novel Functions of Hyaluronidase from Streptococcus agalactiae Are Enhanced Intracellular Survival and Inhibition of Proinflammatory Cytokine Expression. Infect Immun. 2014;82(6):2615–25. https://doi.org/10.1128/IAI.00022-14.
61. Hajam IA, Katiki M, McNally R, Lázaro-Díez M, Kolar S, Chatterjee A, et al. Functional divergence of a bacterial enzyme promotes healthy or acneic skin. Nat Commun. 2023;14(1):8061. https://doi.org/10.1038/s41467-023-43833-8.

62. Mao T, Su C-W, Ji Q, Chen C-Y, Wang R, Vijaya Kumar D, et al. Hyaluronan-induced alterations of the gut microbiome protects mice against Citrobacter rodentium infection and intestinal inflammation. Gut Microbes. 2021;13(1):1972757. https://doi.org/10.1080/19490976.2021.1972757.
63. Zhao R, Zhang C, Yu L, Zhang C, Zhao J, Narbad A, et al. In Vitro Fermentation of Hyaluronan with Different Molecular Weights by Human Gut Microbiota: Differential Effects on Gut Microbiota Structure and Metabolic Function. Polymers. 2023;15(9):2103. https://doi.org/10.3390/polym15092103.
64. Ndeh D, Baslé A, Strahl H, Yates EA, McClurgg UL, Henrissat B, et al. Metabolism of multiple glycosaminoglycans by Bacteroides thetaiotaomicron is orchestrated by a versatile core genetic locus. Nat Commun. 2020;11(1):646. https://doi.org/10.1038/s41467-020-14509-4.
65. Pan L, Ai X, Fu T, Ren L, Shang Q, Li G, et al. In vitro fermentation of hyaluronan by human gut microbiota: Changes in microbiota community and potential degradation mechanism. Carbohydr Polym. 2021;269: 118313. https://doi.org/10.1016/j.carbpol.2021.118313.
66. Zheng X, Wang B, Tang X, Mao B, Zhang Q, Zhang T, et al. Absorption, metabolism, and functions of hyaluronic acid and its therapeutic prospects in combination with microorganisms: A review. Carbohydr Polym. 2023;299: 120153. https://doi.org/10.1016/j.carbpol.2022.120153.
67. Lee JH, Moore LD, Kumar S, Pritchard DG, Ponnazhagan S, Deivanayagam C. Bacteriophage hyaluronidase effectively inhibits growth, migration and invasion by disrupting hyaluronan-mediated Erk1/2 activation and RhoA expression in human breast carcinoma cells. Cancer Lett. 2010;298(2):238–49. https://doi.org/10.1016/j.canlet.2010.07.011.
68. Thirumurthy K, Kaliyamoorthy K, Kandasamy K, Ponnuvel M, Viyakarn V, Chavanich S, et al. Antioxidant and Anti-Breast Cancer Properties of Hyaluronidase from Marine Staphylococcus aureus (CASMTK1). JMSE. 2023;11(4):778. https://doi.org/10.3390/jmse11040778.
69. Ebelt ND, Zuniga E, Passi KB, Sobocinski LJ, Manuel ER. Hyaluronidase-Expressing *Salmonella* Effectively Targets Tumor-Associated Hyaluronic Acid in Pancreatic Ductal Adenocarcinoma. Mol Cancer Ther. 2020;19(2):706–16. https://doi.org/10.1158/1535-7163.MCT-19-0556.
70. Thomas SC, Madaan T, Kamble NS, Siddiqui NA, Pauletti GM, Kotagiri N. Engineered Bacteria Enhance Immunotherapy and Targeted Therapy through Stromal Remodeling of Tumors. Adv Healthc Mater. 2022;11(2):2101487. https://doi.org/10.1002/adhm.202101487.
71. Marković-Housley Z, Miglierini G, Soldatova L, Rizkallah PJ, Müller U, Schirmer T. Crystal structure of hyaluronidase, a major allergen of bee venom. Structure. 2000;8(10):1025–35. https://doi.org/10.1016/s0969-2126(00)00511-6.
72. Viborg AH, Terrapon N, Lombard V, Michel G, Czjzek M, Henrissat B, et al. A subfamily roadmap of the evolutionarily diverse glycoside hydrolase family 16 (GH16). J Biol Chem. 2019;294(44):15973–86. https://doi.org/10.1074/jbc.RA119.010619.
73. Liberato MV, Teixeira Prates E, Gonçalves TA, Bernardes A, Vilela N, Fattori J, et al. Insights into the dual cleavage activity of the GH16 laminarinase enzyme class on β-1,3 and β-1,4 glycosidic bonds. J Biol Chem. 2021;296: 100385. https://doi.org/10.1016/j.jbc.2021.100385.
74. Skov LK, Seppälä U, Coen JJF, Crickmore N, King TP, Monsalve R, et al. Structure of recombinant Ves v 2 at 2.0 Angstrom resolution: structural analysis of an allergenic hyaluronidase from wasp venom. Acta Crystallogr D Biol Crystallogr. 2006;62(Pt 6):595–604. https://doi.org/10.1107/S0907444906010687.
75. Çetinbaş N, Macauley MS, Stubbs KA, Drapala R, Vocadlo DJ. Identification of Asp^{174} and Asp^{175} as the Key Catalytic Residues of Human *O*-GlcNAcase by Functional Analysis of Site-Directed Mutants. Biochemistry. 2006;45(11):3835–44. https://doi.org/10.1021/bi052370b.
76. Calvelo M, Males A, Alteen MG, Willems LI, Vocadlo DJ, Davies GJ, et al. Human O-GlcNAcase Uses a Preactivated Boat-skew Substrate Conformation for Catalysis. Evidence from X-ray Crystallography and QM/MM Metadynamics. ACS Catal. 2023; 13(20):13672–8. https://doi.org/10.1021/acscatal.3c02378.
77. Michikawa M, Ichinose H, Momma M, Biely P, Jongkees S, Yoshida M, et al. Structural and Biochemical Characterization of Glycoside Hydrolase Family 79 β-Glucuronidase from Acidobacterium capsulatum. J Biol Chem. 2012;287(17):14069–77. https://doi.org/10.1074/jbc.M112.346288.
78. Huang H, Hou X, Xu R, Deng Z, Wang Y, Du G, et al. Structure and cleavage pattern of a hyaluronate 3-glycanohydrolase in the glycoside hydrolase 79 family. Carbohydr Polym. 2022;277: 118838. https://doi.org/10.1016/j.carbpol.2021.118838.
79. Kelly SJ, Taylor KB, Li S, Jedrzejas MJ. Kinetic properties of Streptococcus pneumoniae hyaluronate lyase. Glycobiology. 2001;11(4):297–304. https://doi.org/10.1093/glycob/11.4.297.
80. Ponnuraj K, Jedrzejas MJ. Mechanism of hyaluronan binding and degradation: structure of Streptococcus pneumoniae hyaluronate lyase in complex with hyaluronic acid disaccharide at 1.7 A resolution. J Mol Biol. 2000; 299(4):885–95. https://doi.org/10.1006/jmbi.2000.3817.
81. Messina L, Gavira JA, Pernagallo S, Unciti-Broceta JD, Sanchez Martin RM, Diaz-Mochon JJ, et al. Identification and characterization of a bacterial hyaluronidase and its production in recombinant form. FEBS Lett. 2016;590(14):2180–9. https://doi.org/10.1002/1873-3468.12258.
82. Helbert W, Poulet L, Drouillard S, Mathieu S, Loiodice M, Couturier M, et al. Discovery of novel carbohydrate-active enzymes through the rational exploration of the protein sequences space. Proc Natl Acad Sci U S A. 2019;116(13):6063–8. https://doi.org/10.1073/pnas.1815791116.
83. Bakke M, Kamei J, Obata A. Identification, characterization, and molecular cloning of a novel hyaluronidase, a member of glycosyl hydrolase family 16, from *Penicillium* spp. FEBS Lett. 2011;585(1):115–20. https://doi.org/10.1016/j.febslet.2010.11.021.
84. Rungsa P, Janpan P, Saengkun Y, Jangpromma N, Klaynongsruang S, Patramanon R, et al. Heterologous expression and mutagenesis of recombinant Vespa affinis hyaluronidase protein (rVesA2). J Venom Anim Toxins Incl Trop Dis. 2019;25: e20190030. https://doi.org/10.1590/1678-9199-JVATITD-2019-0030.
85. Zhang R, Zhou J, Song Z, Huang Z. Enzymatic properties of β-N-acetylglucosaminidases. Appl Microbiol Biotechnol. 2018;102(1):93–103. https://doi.org/10.1007/s00253-017-8624-7.
86. Planas A. Bacterial 1,3–1,4-beta-glucanases: structure, function and protein engineering. Biochim Biophys Acta. 2000;1543(2):361–82. https://doi.org/10.1016/s0167-4838(00)00231-4.
87. Jedrzejas MJ, Mello LV, de Groot BL, Li S. Mechanism of hyaluronan degradation by Streptococcus pneumoniae hyaluronate lyase. Structures of complexes with the substrate. J Biol Chem. 2002; 277(31):28287–97. https://doi.org/10.1074/jbc.M112009200.
88. Li S, Kelly SJ, Lamani E, Ferraroni M, Jedrzejas MJ. Structural basis of hyaluronan degradation by Streptococcus pneumoniae hyaluronate lyase. EMBO J. 2000;19(6):1228–40. https://doi.org/10.1093/emboj/19.6.1228.
89. Garantziotis S. Modulation of hyaluronan signaling as a therapeutic target in human disease. Pharmacol Ther. 2022;232: 107993. https://doi.org/10.1016/j.pharmthera.2021.107993.
90. Salih ARC, Farooqi HMU, Amin H, Karn PR, Meghani N, Nagendran S. Hyaluronic acid: comprehensive review of a multifunctional biopolymer. Futur J Pharm Sci. 2024;10(1):63. https://doi.org/10.1186/s43094-024-00636-y.
91. Martinez-Fleites C, Korczynska JE, Davies GJ, Cope MJ, Turkenburg JP, Taylor EJ. The crystal structure of a family GH25 lysozyme from Bacillus anthracis implies a neighboring-group catalytic mechanism with retention of anomeric configuration. Carbohydr Res. 2009;344(13):1753–7. https://doi.org/10.1016/j.carres.2009.06.001.
92. El-Safory NS, Fazary AE, Lee C-K. Hyaluronidases, a group of glycosidases: Current and future perspectives. Carbohydr Polym. 2010;81(2):165–81. https://doi.org/10.1016/j.carbpol.2010.02.047.
93. Kakizaki I, Ibori N, Kojima K, Yamaguchi M, Endo M. Mechanism for the hydrolysis of hyaluronan oligosaccharides by bovine testicular hyaluronidase. FEBS J. 2010;277(7):1776–86. https://doi.org/10.1111/j.1742-4658.2010.07600.x.
94. Amin M, Barzegari E, Pourshohod A, Zeinali M, Jamalan M. 3D structure prediction, dynamic investigation and rational construction of an epitope-masked thermostable bovine hyaluronidase. Int J Biol Macromol. 2021;187:544–53. https://doi.org/10.1016/j.ijbiomac.2021.07.098.
95. Chao KL, Muthukumar L, Herzberg O. Structure of human hyaluronidase-1, a hyaluronan hydrolyzing enzyme involved in tumor growth and angiogenesis. Biochemistry. 2007;46(23):6911–20. https://doi.org/10.1021/bi700382g.
96. Yuan F, Yang Y, Zhou H, Quan J, Liu C, Wang Y, et al. Heparanase in cancer progression: Structure, substrate recognition and therapeutic potential. Front Chem. 2022;10: 926353. https://doi.org/10.3389/fchem.2022.926353.

97. Davies G, Henrissat B. Structures and mechanisms of glycosyl hydrolases. Structure. 1995;3(9):853–9. https://doi.org/10.1016/S0969-2126(01)00220-9.
98. Schmerling C, Sewald L, Heilmann G, Witfeld F, Begerow D, Jensen K, et al. Identification of fungal lignocellulose-degrading biocatalysts secreted by Phanerochaete chrysosporium via activity-based protein profiling. Commun Biol. 2022;5(1):1254. https://doi.org/10.1038/s42003-022-04141-x.
99. Vuong TV, Wilson DB. Glycoside hydrolases: Catalytic base/nucleophile diversity. Biotechnol Bioeng. 2010;107(2):195–205. https://doi.org/10.1002/bit.22838.
100. Pilgaard B, Vuillemin M, Holck J, Wilkens C, Meyer AS. Specificities and Synergistic Actions of Novel PL8 and PL7 Alginate Lyases from the Marine Fungus Paradendryphiella salina. J Fungi (Basel). 2021;7(2):80. https://doi.org/10.3390/jof7020080.
101. Elmabrouk ZH, Vincent F, Zhang M, Smith NL, Turkenburg JP, Charnock SJ, et al. Crystal structures of a family 8 polysaccharide lyase reveal open and highly occluded substrate-binding cleft conformations. Proteins. 2011;79(3):965–74. https://doi.org/10.1002/prot.22938.
102. Smirnou D, Krčmář M, Kulhánek J, Hermannová M, Bobková L, Franke L, et al. Characterization of Hyaluronan-Degrading Enzymes from Yeasts. Appl Biochem Biotechnol. 2015;177(3):700–12. https://doi.org/10.1007/s12010-015-1774-0.
103. Zhu L, Li S, Jiang J-Y, Yao Z-Y, Li Q, Lian S-J, et al. High-Level Extracellular Expression of Hyaluronate Lyase HylP in Bacillus subtilis for Hyaluronan Degradation. Appl Biochem Biotechnol. 2024;196(10):6782–801. https://doi.org/10.1007/s12010-024-04883-w.
104. Al-Assaf S, Navaratnam S, Parsons BJ, Phillips GO. Chain scission of hyaluronan by peroxynitrite. Arch Biochem Biophys. 2003;411(1):73–82. https://doi.org/10.1016/s0003-9861(02)00724-5.
105. Al-Assaf S, Navaratnam S, Parsons BJ, Phillips GO. Chain scission of hyaluronan by carbonate and dichloride radical anions: potential reactive oxidative species in inflammation? Free Radic Biol Med. 2006;40(11):2018–27. https://doi.org/10.1016/j.freeradbiomed.2006.01.033.
106. Parsons BJ. Oxidation of glycosaminoglycans by free radicals and reactive oxidative species: A review of investigative methods. Free Radic Res. 2015;49(5):618–32. https://doi.org/10.3109/10715762.2014.985220.
107. Sibanda S, Parsons BJ, Houee-Levin C, Marignier J-L, Paterson AWJ, Heyes DJ. One-electron oxidation and reduction of glycosaminoglycan chloramides: A kinetic study. Free Radic Biol Med. 2013;63:126–34. https://doi.org/10.1016/j.freeradbiomed.2013.05.012.
108. Noble PW. Hyaluronan and its catabolic products in tissue injury and repair. Matrix Biol. 2002;21(1):25–9. https://doi.org/10.1016/s0945-053x(01)00184-6.
109. Tammi MI, Oikari S, Pasonen-Seppänen S, Rilla K, Auvinen P, Tammi RH. Activated hyaluronan metabolism in the tumor matrix - Causes and consequences. Matrix Biol. 2019;78–79:147–64. https://doi.org/10.1016/j.matbio.2018.04.012.
110. Avenoso A, Bruschetta G, D'Ascola A, Scuruchi M, Mandraffino G, Gullace R, et al. Hyaluronan fragments produced during tissue injury: A signal amplifying the inflammatory response. Arch Biochem Biophys. 2019;663:228–38. https://doi.org/10.1016/j.abb.2019.01.015.
111. Fakhari A, Berkland C. Applications and emerging trends of hyaluronic acid in tissue engineering, as a dermal filler and in osteoarthritis treatment. Acta Biomater. 2013;9(7):7081–92. https://doi.org/10.1016/j.actbio.2013.03.005.
112. Triggs-Raine B, Natowicz MR. Biology of hyaluronan: Insights from genetic disorders of hyaluronan metabolism. World J Biol Chem. 2015;6(3):110–20. https://doi.org/10.4331/wjbc.v6.i3.110.
113. Karbownik MS, Nowak JZ. Hyaluronan: towards novel anti-cancer therapeutics. Pharmacol Rep. 2013;65(5):1056–74. https://doi.org/10.1016/s1734-1140(13)71465-8.
114. Wang X, Wei Z, Wu H, Li Y, Han F, Yu W. Characterization of a Hyaluronic Acid Utilization Locus and Identification of Two Hyaluronate Lyases in a Marine Bacterium Vibrio alginolyticus LWW-9. Front Microbiol. 2021;12: 696096. https://doi.org/10.3389/fmicb.2021.696096.
115. Li Y, Zhang S, Wu H, Wang X, Yu W, Han F. Biochemical characterization of a thermophilic hyaluronate lyase TcHly8C from Thermasporomyces composti DSM22891. Int J Biol Macromol. 2020;165(Pt A):1211–8. https://doi.org/10.1016/j.ijbiomac.2020.10.011.
116. Pritchard DG, Trent JO, Li X, Zhang P, Egan ML, Baker JR. Characterization of the active site of group B streptococcal hyaluronan lyase. Proteins. 2000;40(4):675.
117. Giubertoni G, Pérez de Alba Ortíz A, Bano F, Zhang X, Linhardt RJ, Green DE, et al. Strong Reduction of the Chain Rigidity of Hyaluronan by Selective Binding of Ca2+ Ions. Macromolecules. 2021;54(3):1137–46. https://doi.org/10.1021/acs.macromol.0c02242.
118. Nantarat N, Mueller M, Lin W-C, Lue S-C, Viernstein H, Chansakaow S, et al. Sesaminol diglucoside isolated from black sesame seed cake and its antioxidant, anti-collagenase and anti-hyaluronidase activities. Food Biosci. 2020;36: 100628. https://doi.org/10.1016/j.fbio.2020.100628.
119. Sunitha K, Suresh P, Santhosh MS, Hemshekhar M, Thushara RM, Marathe GK, et al. Inhibition of hyaluronidase by N-acetyl cysteine and glutathione: Role of thiol group in hyaluronan protection. Int J Biol Macromol. 2013;55:39–46. https://doi.org/10.1016/j.ijbiomac.2012.12.047.
120. van der Heijden J, Kolliopoulos C, Skorup P, Sallisalmi M, Heldin P, Hultström M, et al. Plasma hyaluronan, hyaluronidase activity and endogenous hyaluronidase inhibition in sepsis: an experimental and clinical cohort study. Intensive Care Med Exp. 2021;9(1):53. https://doi.org/10.1186/s40635-021-00418-3.
121. Monzón ME, Manzanares D, Schmid N, Casalino-Matsuda SM, Forteza RM. Hyaluronidase Expression and Activity Is Regulated by Pro-Inflammatory Cytokines in Human Airway Epithelial Cells. Am J Respir Cell Mol Biol. 2008;39(3):289–95. https://doi.org/10.1165/rcmb.2007-0361OC.
122. Jung M, Ma Y, Padmanabhan Iyer R, Yabluchiansky A, Garrett MR, Lindsey ML. Abstract 142: IL-10 Regulates Inflammation to Improve LV Physiology After Myocardial Infarction by Stimulating M2 Macrophage Polarization and Fibroblast Activation. Circ. Res. 2017; 121(suppl_1). https://doi.org/10.1161/res.121.suppl_1.142.
123. Lampi MC, Reinhart-King CA. Targeting extracellular matrix stiffness to attenuate disease: From molecular mechanisms to clinical trials. Sci Transl Med. 2018; 10(422):eaao0475. https://doi.org/10.1126/scitranslmed.aao0475.
124. Theocharis AD, Manou D, Karamanos NK. The extracellular matrix as a multitasking player in disease. FEBS J. 2019;286(15):2830–69. https://doi.org/10.1111/febs.14818.
125. Brown BN, Badylak SF. Extracellular matrix as an inductive scaffold for functional tissue reconstruction. Transl Res. 2014;163(4):268–85. https://doi.org/10.1016/j.trsl.2013.11.003.
126. Manou D, Caon I, Bouris P, Triantaphyllidou I-E, Giaroni C, Passi A, et al. The Complex Interplay Between Extracellular Matrix and Cells in Tissues. Methods Mol Biol. 2019;1952:1–20. https://doi.org/10.1007/978-1-4939-9133-4_1.
127. Fallacara A, Baldini E, Manfredini S, Vertuani S. Hyaluronic Acid in the Third Millennium. Polymers (Basel). 2018;10(7):701. https://doi.org/10.3390/polym10070701.
128. Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. J Cell Sci. 2010;123(Pt 24):4195–200. https://doi.org/10.1242/jcs.023820.
129. Chaudhuri O, Cooper-White J, Janmey PA, Mooney DJ, Shenoy VB. Effects of extracellular matrix viscoelasticity on cellular behaviour. Nature. 2020;584(7822):535–46. https://doi.org/10.1038/s41586-020-2612-2.
130. Elosegui-Artola A. The extracellular matrix viscoelasticity as a regulator of cell and tissue dynamics. Curr Opin Cell Biol. 2021;72:10–8. https://doi.org/10.1016/j.ceb.2021.04.002.
131. Yamada KM, Sixt M. Mechanisms of 3D cell migration. Nat Rev Mol Cell Biol. 2019;20(12):738–52. https://doi.org/10.1038/s41580-019-0172-9.
132. Liu Y, Li L, Wang L, Lu L, Li Y, Huang G, et al. 'Two-faces' of hyaluronan, a dynamic barometer of disease progression in tumor microenvironment. Discov Onc. 2023;14(1):11. https://doi.org/10.1007/s12672-023-00618-1.
133. Cyphert JM, Trempus CS, Garantziotis S. Size Matters: Molecular Weight Specificity of Hyaluronan Effects in Cell Biology. Int J Cell Biol. 2015;2015: 563818. https://doi.org/10.1155/2015/563818.
134. Fisher GJ. Cancer resistance, high molecular weight hyaluronic acid, and longevity. J Cell Commun Signal. 2015;9(1):91–2. https://doi.org/10.1007/s12079-015-0278-6.
135. Buhren BA, Schrumpf H, Hoff N-P, Bölke E, Hilton S, Gerber PA. Hyaluronidase: from clinical applications to molecular and cellular mechanisms. Eur J Med Res. 2016;21(1):5. https://doi.org/10.1186/s40001-016-0201-5.

136. Tavianatou AG, Caon I, Franchi M, Piperigkou Z, Galesso D, Karamanos NK. Hyaluronan: molecular size-dependent signaling and biological functions in inflammation and cancer. FEBS J. 2019;286(15):2883–908. https://doi.org/10.1111/febs.14777.
137. Collins SL, Black KE, Chan-Li Y, Ahn Y-H, Cole PA, Powell JD, et al. Hyaluronan Fragments Promote Inflammation by Down-Regulating the Anti-inflammatory A2a Receptor. Am J Respir Cell Mol Biol. 2011;45(4):675–83. https://doi.org/10.1165/rcmb.2010-0387OC.
138. Marinho A, Nunes C, Reis S. Hyaluronic Acid: A Key Ingredient in the Therapy of Inflammation. Biomolecules. 2021;11(10):1518. https://doi.org/10.3390/biom11101518.
139. Campo GM, Avenoso A, D'Ascola A, Prestipino V, Scuruchi M, Nastasi G, et al. mer hyaluronan oligosaccharides stimulate inflammation response in synovial fibroblasts in part via TAK-1 and in part via p38-MAPK. Curr Med Chem. 2013;20(9):1162–72. https://doi.org/10.2174/0929867311320090005.
140. Rayahin JE, Buhrman JS, Zhang Y, Koh TJ, Gemeinhart RA. High and low molecular weight hyaluronic acid differentially influence macrophage activation. ACS Biomater Sci Eng. 2015;1(7):481–93. https://doi.org/10.1021/acsbiomaterials.5b00181.
141. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229–63. https://doi.org/10.3322/caac.21834.
142. Chao Y, Liu Z. Biomaterials tools to modulate the tumour microenvironment in immunotherapy. Nat Rev Bioeng. 2023;1(2):125–38. https://doi.org/10.1038/s44222-022-00004-6.
143. LeSavage BL, Zhang D, Huerta-López C, Gilchrist AE, Krajina BA, Karlsson K, et al. Engineered matrices reveal stiffness-mediated chemoresistance in patient-derived pancreatic cancer organoids. Nat Mater. 2024;23(8):1138–49. https://doi.org/10.1038/s41563-024-01908-x.
144. Xu Z. CRISPR/Cas9-mediated silencing of CD44: unveiling the role of hyaluronic acid-mediated interactions in cancer drug resistance. Naunyn Schmiedebergs Arch Pharmacol. 2024;397(5):2849–76. https://doi.org/10.1007/s00210-023-02840-8.
145. Dhar D, Antonucci L, Nakagawa H, Kim JY, Glitzner E, Caruso S, et al. Liver Cancer Initiation Requires p53 Inhibition by CD44-Enhanced Growth Factor Signaling. Cancer Cell. 2018;33(6):1061-1077.e6. https://doi.org/10.1016/j.ccell.2018.05.003.
146. Salwowska NM, Bebenek KA, Żądło DA, Wcisło-Dziadecka DL. Physiochemical properties and application of hyaluronic acid: a systematic review. J Cosmet Dermatol. 2016;15(4):520–6. https://doi.org/10.1111/jocd.12237.
147. Gong L, Zhang Y, Yang Y, Yan Q, Ren J, Luo J, et al. Inhibition of lysyl oxidase-like 2 overcomes adhesion-dependent drug resistance in the collagen-enriched liver cancer microenvironment. Hepatol Commun. 2022;6(11):3194–211. https://doi.org/10.1002/hep4.1966.
148. Song Y, Kim J-S, Choi EK, Kim J, Kim KM, Seo HR. TGF-β-independent CTGF induction regulates cell adhesion mediated drug resistance by increasing collagen I in HCC. Oncotarget .2017; 8(13):21650–62. https://doi.org/10.18632/oncotarget.15521.
149. Topalovski M, Brekken RA. Matrix control of pancreatic cancer: New insights into fibronectin signaling. Cancer Lett. 2016;381(1):252–8. https://doi.org/10.1016/j.canlet.2015.12.027.
150. Bae YK, Kim A, Kim MK, Choi JE, Kang SH, Lee SJ. Fibronectin expression in carcinoma cells correlates with tumor aggressiveness and poor clinical outcome in patients with invasive breast cancer. Hum Pathol. 2013;44(10):2028–37. https://doi.org/10.1016/j.humpath.2013.03.006.
151. Chang L, Hu Y, Fu Y, Zhou T, You J, Du J, et al. Targeting slug-mediated non-canonical activation of c-Met to overcome chemo-resistance in metastatic ovarian cancer cells. Acta Pharm Sin B. 2019;9(3):484–95. https://doi.org/10.1016/j.apsb.2019.03.001.
152. Ye Y, Zhang R, Feng H. Fibronectin promotes tumor cells growth and drugs resistance through a CDC42-YAP-dependent signaling pathway in colorectal cancer. Cell Biol Int. 2020;44(9):1840–9. https://doi.org/10.1002/cbin.11390.
153. Farooq F, Amin A, Wani UM, Lone A, Qadri RA. Shielding and nurturing: Fibronectin as a modulator of cancer drug resistance. Cell Physiol. 2023;238(8):1651–69. https://doi.org/10.1002/jcp.31048.
154. Li L, Li Z, Lu C, Li J, Zhang K, Lin C, et al. Ibrutinib reverses IL-6-induced osimertinib resistance through inhibition of Laminin α5/FAK signaling. Commun Biol. 2022;5(1):155. https://doi.org/10.1038/s42003-022-03111-7.
155. Wang X, Zuo D, Chen Y, Li W, Liu R, He Y, et al. Shed Syndecan-1 is involved in chemotherapy resistance via the EGFR pathway in colorectal cancer. Br J Cancer. 2014;111(10):1965–76. https://doi.org/10.1038/bjc.2014.493.
156. Jurj A, Ionescu C, Berindan-Neagoe I, Braicu C. The extracellular matrix alteration, implication in modulation of drug resistance mechanism: friends or foes? J Exp Clin Cancer Res. 2022;41(1):276. https://doi.org/10.1186/s13046-022-02484-1.
157. Kumari N, Dwarakanath BS, Das A, Bhatt AN. Role of interleukin-6 in cancer progression and therapeutic resistance. Tumour Biol. 2016;37(9):11553–72. https://doi.org/10.1007/s13277-016-5098-7.
158. Jones VS, Huang R-Y, Chen L-P, Chen Z-S, Fu L, Huang R-P. Cytokines in cancer drug resistance: Cues to new therapeutic strategies. Biochim Biophys Acta. 2016;1865(2):255–65. https://doi.org/10.1016/j.bbcan.2016.03.005.
159. Misra S, Heldin P, Hascall VC, Karamanos NK, Skandalis SS, Markwald RR, et al. Hyaluronan–CD44 interactions as potential targets for cancer therapy. FEBS J. 2011;278(9):1429–43. https://doi.org/10.1111/j.1742-4658.2011.08071.x.
160. Jiang D, Liang J, Noble PW. Hyaluronan in tissue injury and repair. Annu Rev Cell Dev Biol. 2007;23:435–61. https://doi.org/10.1146/annurev.cellbio.23.090506.123337.
161. Pp P, Sr H. Hyaluronan, fluid pressure, and stromal resistance in pancreas cancer. Br J Cancer. 2013; 108(1). https://doi.org/10.1038/bjc.2012.569.
162. Whatcott CJ, Han H, Posner RG, Hostetter G, Von Hoff DD. Targeting the tumor microenvironment in cancer: Why hyaluronidase deserves a second look. Cancer Discov. 2011;1(4):291–6. https://doi.org/10.1158/2159-8290.CD-11-0136.
163. Spinelli FM, Vitale DL, Sevic I, Alaniz L. Hyaluronan in the Tumor Microenvironment. In: Birbrair A, editor. Tumor Microenvironment, vol. 1245, Adv Exp Med Biol.Cham: Springer International Publishing; 2020, p. 67–83. https://doi.org/10.1007/978-3-030-40146-7_3.
164. Chanmee T, Ontong P, Itano N. Hyaluronan: Cancer and Cancer Metastasis. In: Taniguchi N, Endo T, Hart GW, Seeberger PH, Wong C-H, editors. Glycoscience: Biology and Medicine, Tokyo: Springer Japan; 2015, p. 1411–7. https://doi.org/10.1007/978-4-431-54841-6_191.
165. Huang J, Zhang L, Wan D, Zhou L, Zheng S, Lin S, et al. Extracellular matrix and its therapeutic potential for cancer treatment. Signal Transduct Target Ther. 2021;6(1):153. https://doi.org/10.1038/s41392-021-00544-0.
166. Kaushik N, Kim S, Suh Y, Lee S-J. Proinvasive extracellular matrix remodeling for tumor progression. Arch Pharm Res. 2019;42(1):40–7. https://doi.org/10.1007/s12272-018-1097-0.
167. Misra S, Hascall VC, Markwald RR, Ghatak S. Interactions between Hyaluronan and Its Receptors (CD44, RHAMM) Regulate the Activities of Inflammation and Cancer. Front Immunol. 2015;6:201. https://doi.org/10.3389/fimmu.2015.00201.
168. Mai Z, Lin Y, Lin P, Zhao X, Cui L. Modulating extracellular matrix stiffness: a strategic approach to boost cancer immunotherapy. Cell Death Dis. 2024;15(5):307. https://doi.org/10.1038/s41419-024-06697-4.
169. Winkler J, Abisoye-Ogunniyan A, Metcalf KJ, Werb Z. Concepts of extracellular matrix remodelling in tumour progression and metastasis. Nat Commun. 2020;11(1):5120. https://doi.org/10.1038/s41467-020-18794-x.
170. Semenza GL. The hypoxic tumor microenvironment: A driving force for breast cancer progression. Biochim Biophys Acta. 2016;1863(3):382–91. https://doi.org/10.1016/j.bbamcr.2015.05.036.
171. Isman FK, Kucur M, Baysal B, Ozkan F. Evaluation of serum hyaluronic acid level and hyaluronidase activity in acute and chronic hepatitis C. J Int Med Res. 2007;35(3):346–52. https://doi.org/10.1177/147323000703500309.
172. Liang Z, Zhang Q, Wang C, Shi F, Cao H, Yu Y, et al. Hyaluronic acid/ Hyaluronidase as biomarkers for bladder cancer: a diagnostic meta-analysis. Neoplasma. 2017;64(6):901–8. https://doi.org/10.4149/neo_2017_612.
173. Pham HT, Block NL, Lokeshwar VB. Tumor-derived hyaluronidase: a diagnostic urine marker for high-grade bladder cancer. Cancer Res. 1997;57(4):778–83.
174. Liu J-P, Fang Y-T, Jiang Y-F, Lin H. HYAL3 as a potential novel marker of BLCA patient prognosis. BMC Genom Data. 2022;23(1):63. https://doi.org/10.1186/s12863-022-01070-w.

175. Skarmoutsos I, Skarmoutsos A, Katafigiotis I, Tataki E, Giagini A, Adamakis I, et al. Hyaluronic acid and hyaluronidase as possible novel urine biomarkers for the diagnosis of prostate cancer. Med Oncol. 2018;35(7):97. https://doi.org/10.1007/s12032-018-1157-9.
176. Ekici S, Cerwinka WH, Duncan R, Gomez P, Civantos F, Soloway MS, et al. Comparison of the prognostic potential of hyaluronic acid, hyaluronidase (HYAL-1), CD44v6 and microvessel density for prostate cancer. Int J Cancer. 2004;112(1):121–9. https://doi.org/10.1002/ijc.20368.
177. Spataro S, Guerra C, Cavalli A, Sgrignani J, Sleeman J, Poulain L, et al. CEMIP (HYBID, KIAA1199): structure, function and expression in health and disease. FEBS J. 2023;290(16):3946–62. https://doi.org/10.1111/febs.16600.
178. Banach A, Jiang Y-P, Roth E, Kuscu C, Cao J, Lin RZ. CEMIP upregulates BiP to promote breast cancer cell survival in hypoxia. Oncotarget. 2019;10(42):4307–20. https://doi.org/10.18632/oncotarget.27036.
179. Dong X, Yang Y, Yuan Q, Hou J, Wu G. High Expression of CEMIP Correlates Poor Prognosis and the Tumur Microenvironment in Breast Cancer as a Promisingly Prognostic Biomarker. Front Genet. 2021;12: 768140. https://doi.org/10.3389/fgene.2021.768140.
180. Siiskonen H, Poukka M, Tyynelä-Korhonen K, Sironen R, Pasonen-Seppänen S. Inverse expression of hyaluronidase 2 and hyaluronan synthases 1–3 is associated with reduced hyaluronan content in malignant cutaneous melanoma. BMC Cancer. 2013;13:181. https://doi.org/10.1186/1471-2407-13-181.
181. Parnigoni A, Moretto P, Viola M, Karousou E, Passi A, Vigetti D. Effects of Hyaluronan on Breast Cancer Aggressiveness. Cancers (Basel). 2023;15(15):3813. https://doi.org/10.3390/cancers15153813.
182. Lee H, Goodarzi H, Tavazoie SF, Alarcón CR. TMEM2 Is a SOX4-Regulated Gene That Mediates Metastatic Migration and Invasion in Breast Cancer. Cancer Res. 2016;76(17):4994–5005. https://doi.org/10.1158/0008-5472.CAN-15-2322.
183. Franzmann EJ, Schroeder GL, Goodwin WJ, Weed DT, Fisher P, Lokeshwar VB. Expression of tumor markers hyaluronic acid and hyaluronidase (HYAL1) in head and neck tumors. Int J Cancer. 2003;106(3):438–45. https://doi.org/10.1002/ijc.11252.
184. İnan HC, Yener M, Buyru N, Çelebi A, Yilmaz M, Çomunoğlu N. The investigation of hyaluronic acid and hyaluronidase-1 levels as tumour marker in larynx cancer. Clin Otolaryngol. 2019;44(6):914–8. https://doi.org/10.1111/coa.13390.
185. Schott S, Yang R, Stöcker S, Canzian F, Giese N, Bugert P, et al. HYAL2 methylation in peripheral blood as a potential marker for the detection of pancreatic cancer: a case control study. Oncotarget. 2017;8(40):67614–25. https://doi.org/10.18632/oncotarget.18757.
186. Deng F, Lei J, Zhang X, Huang W, Li Y, Wu D. Overexpression of KIAA1199: An independent prognostic marker in nonsmall cell lung cancer. J Cancer Res Ther. 2017;13(4):664–8. https://doi.org/10.4103/jcrt.JCRT_61_17.
187. Jia S, Qu T, Wang X, Feng M, Yang Y, Feng X, et al. KIAA1199 promotes migration and invasion by Wnt/β-catenin pathway and MMPs mediated EMT progression and serves as a poor prognosis marker in gastric cancer. PLoS ONE. 2017;12(4): e0175058. https://doi.org/10.1371/journal.pone.0175058.
188. Sallman B, Birkeland JM. THE ROLE OF HYALURONIDASE IN HEMOLYTIC STREPTOCOCCAL INFECTION. Ann Ny Acad Sci. 1950;52(7):1062–9. https://doi.org/10.1111/j.1749-6632.1950.tb54007.x.
189. Coleman M, Armistead B, Orvis A, Quach P, Brokaw A, Gendrin C, et al. Hyaluronidase Impairs Neutrophil Function and Promotes Group B Streptococcus Invasion and Preterm Labor in Nonhuman Primates. mBio. 2021; 12(1):e03115–20. https://doi.org/10.1128/mBio.03115-20.
190. Devriese LA. Staphylococci in healthy and diseased animals. Soc Appl Bacteriol Symp Ser. 1990;19:71S-80S. https://doi.org/10.1111/j.1365-2672.1990.tb01799.x.
191. Ibberson CB, Parlet CP, Kwiecinski J, Crosby HA, Meyerholz DK, Horswill AR. Hyaluronan Modulation Impacts Staphylococcus aureus Biofilm Infection. Infect Immun. 2016;84(6):1917–29. https://doi.org/10.1128/IAI.01418-15.
192. Nagaya H, Ymagata T, Ymagata S, Iyoda K, Ito H, Hasegawa Y, et al. Examination of synovial fluid and serum hyaluronidase activity as a joint marker in rheumatoid arthritis and osteoarthritis patients (by zymography). Ann Rheum Dis. 1999;58(3):186–8. https://doi.org/10.1136/ard.58.3.186.
193. Sasaki Y, Uzuki M, Nohmi K, Kitagawa H, Kamataki A, Komagamine M, et al. Quantitative measurement of serum hyaluronic acid molecular weight in rheumatoid arthritis patients and the role of hyaluronidase. Int J Rheum Dis. 2011;14(4):313–9. https://doi.org/10.1111/j.1756-185X.2011.01683.x.
194. Neudecker BA, Stern R, Connolly MK. Aberrant serum hyaluronan and hyaluronidase levels in scleroderma. Br J Dermatol. 2004;150(3):469–76. https://doi.org/10.1046/j.1365-2133.2004.05805.x.
195. Dogné S, Rath G, Jouret F, Caron N, Dessy C, Flamion B. Hyaluronidase 1 Deficiency Preserves Endothelial Function and Glycocalyx Integrity in Early Streptozotocin-Induced Diabetes. Diabetes. 2016;65(9):2742–53. https://doi.org/10.2337/db15-1662.
196. Petrey AC, Obery DR, Kessler SP, Zawerton A, Flamion B, de la Motte CA. Platelet hyaluronidase-2 regulates the early stages of inflammatory disease in colitis. Blood. 2019;134(9):765–75. https://doi.org/10.1182/blood.2018893594.
197. Kunder EV. Criteria for the differential diagnosis of spondyloarthropathies, by determining serum hyaluronidase activity. Klin Lab Diagn. 2010: (4)10–2.
198. Karadag B, Ikitimur B, Firinciogullari H, Cakmak HA, Cosansu K, Yuksel H. The relationship between aortic stiffness and serum hyaluronidase levels in patients with diabetes mellitus and hypertension. Blood Press. 2015;24(1):35–40. https://doi.org/10.3109/08037051.2014.940708.
199. Krishnamurthy VK, Stout AJ, Sapp MC, Matuska B, Lauer ME, Grande-Allen KJ. Dysregulation of hyaluronan homeostasis during aortic valve disease. Matrix Biol. 2017;62:40–57. https://doi.org/10.1016/j.matbio.2016.11.003.
200. Chowdhury B, Hemming R, Hombach-Klonisch S, Flamion B, Triggs-Raine B. Murine hyaluronidase 2 deficiency results in extracellular hyaluronan accumulation and severe cardiopulmonary dysfunction. J Biol Chem. 2013;288(1):520–8. https://doi.org/10.1074/jbc.M112.393629.
201. Chowdhury B, Xiang B, Liu M, Hemming R, Dolinsky VW, Triggs-Raine B. Hyaluronidase 2 Deficiency Causes Increased Mesenchymal Cells, Congenital Heart Defects, and Heart Failure. Circ Cardiovasc Genet. 2017;10(1): e001598. https://doi.org/10.1161/CIRCGENETICS.116.001598.
202. Al Kindi HN, Shehata M, Ibrahim AM, Roshdy M, Simry W, Aguib Y, et al. Cor Triatriatum Sinister (Divided Left Atrium): Histopathologic Features and Clinical Management. Ann Thorac Surg. 2020;110(4):1380–6. https://doi.org/10.1016/j.athoracsur.2020.01.025.
203. Jung M, Ma Y, Iyer RP, DeLeon-Pennell KY, Yabluchanskiy A, Garrett MR, et al. IL-10 improves cardiac remodeling after myocardial infarction by stimulating M2 macrophage polarization and fibroblast activation. Basic Res Cardiol. 2017;112(3):33. https://doi.org/10.1007/s00395-017-0622-5.
204. Li Y, Rahmanian M, Widström C, Lepperdinger G, Frost GI, Heldin P. Irradiation-Induced Expression of Hyaluronan (HA) Synthase 2 and Hyaluronidase 2 Genes in Rat Lung Tissue Accompanies Active Turnover of HA and Induction of Types I and III Collagen Gene Expression. Am J Respir Cell Mol Biol. 2000;23(3):411–8. https://doi.org/10.1165/ajrcmb.23.3.4102.
205. Meszaros M, Kis A, Kunos L, Tarnoki AD, Tarnoki DL, Lazar Z, et al. The role of hyaluronic acid and hyaluronidase-1 in obstructive sleep apnoea. Sci Rep. 2020;10(1):19484. https://doi.org/10.1038/s41598-020-74769-4.
206. Papakonstantinou E, Roth M, Klagas I, Karakiulakis G, Tamm M, Stolz D. COPD Exacerbations Are Associated With Proinflammatory Degradation of Hyaluronic Acid. Chest. 2015;148(6):1497–507. https://doi.org/10.1378/chest.15-0153.
207. Collum SD, Chen N-Y, Hernandez AM, Hanmandlu A, Sweeney H, Mertens TCJ, et al. Inhibition of hyaluronan synthesis attenuates pulmonary hypertension associated with lung fibrosis. Br J Pharmacol. 2017;174(19):3284–301. https://doi.org/10.1111/bph.13947.
208. Li L, Tian F-Y, Yuan Y, Zhang T, Yang W-B, Kong R, et al. HYAL-1-induced autophagy facilitates pancreatic fistula for patients who underwent pancreaticoduodenectomy. FASEB J. 2020;34(2):2524–40. https://doi.org/10.1096/fj.201901583R.
209. George J, Stern R. Serum hyaluronan and hyaluronidase: very early markers of toxic liver injury. Clin Chim Acta. 2004;348(1–2):189–97. https://doi.org/10.1016/j.cccn.2004.05.018.
210. Mustonen A-M, Salvén A, Kärjä V, Rilla K, Matilainen J, Nieminen P. Hyaluronan histochemistry-a potential new tool to assess the progress

of liver disease from simple steatosis to hepatocellular carcinoma. Glycobiology. 2019; 29(4:298–306. https://doi.org/10.1093/glycob/cwz002.
211. Kim S-H, Cho Y-S, Kim Y, Park J, Yoo S-M, Gwak J, et al. Endolysosomal impairment by binding of amyloid beta or MAPT/Tau to V-ATPase and rescue via the HYAL-CD44 axis in Alzheimer disease. Autophagy. 2023;19(8):2318–37. https://doi.org/10.1080/15548627.2023.2181614.
212. Sidgwick GP, Iqbal SA, Bayat A. Altered expression of hyaluronan synthase and hyaluronidase mRNA may affect hyaluronic acid distribution in keloid disease compared with normal skin. Exp Dermatol. 2013;22(5):377–9. https://doi.org/10.1111/exd.12147.
213. Averbeck M, Gebhardt CA, Voigt S, Beilharz S, Anderegg U, Termeer CC, et al. Differential regulation of hyaluronan metabolism in the epidermal and dermal compartments of human skin by UVB irradiation. J Invest Dermatol. 2007;127(3):687–97. https://doi.org/10.1038/sj.jid.5700614.
214. Girish KS, Kemparaju K, Nagaraju S, Vishwanath BS. Hyaluronidase inhibitors: a biological and therapeutic perspective. Curr Med Chem. 2009;16(18):2261–88. https://doi.org/10.2174/092986709788453078.
215. Haas E. On the mechanism of invasion; antinvasin I, an enzyme in plasma. J Biol Chem. 1946;163:63–88.
216. Mio K, Carrette O, Maibach HI, Stern R. Evidence that the serum inhibitor of hyaluronidase may be a member of the inter-alpha-inhibitor family. J Biol Chem. 2000;275(42):32413–21. https://doi.org/10.1074/jbc.M005428200.
217. Wolf RA, Glogar D, Chaung LY, Garrett PE, Ertl G, Tumas J, et al. Heparin inhibits bovine testicular hyaluronidase activity in myocardium of dogs with coronary artery occlusion. Am J Cardiol. 1984;53(7):941–4. https://doi.org/10.1016/0002-9149(84)90529-0.
218. Maksimenko AV, Petrova ML, Tischenko EG, Schechilina YV. Chemical modification of hyaluronidase regulates its inhibition by heparin. Eur J Pharm Biopharm. 2001;51(1):33–8. https://doi.org/10.1016/s0939-6411(00)00136-3.
219. Toida T, Ogita Y, Suzuki A, Toyoda H, Imanari T. Inhibition of hyaluronidase by fully O-sulfonated glycosaminoglycans. Arch Biochem Biophys. 1999;370(2):176–82. https://doi.org/10.1006/abbi.1999.1395.
220. Spickenreither M, Braun S, Bernhardt G, Dove S, Buschauer A. Novel 6-O-acylated vitamin C derivatives as hyaluronidase inhibitors with selectivity for bacterial lyases. B Bioorg Med Chem Let. 2006;6(20):5313–6. https://doi.org/10.1016/j.bmcl.2006.07.087.
221. Girish KS, Kemparaju K. Inhibition of Naja naja venom hyaluronidase: Role in the management of poisonous bite. Life Sci. 2006;78(13):1433–40. https://doi.org/10.1016/j.lfs.2005.07.015.
222. Kuppusamy UR, Khoo HE, Das NP. Structure-activity studies of flavonoids as inhibitors of hyaluronidase. Biochem Pharmacol. 1990;40(2):397–401. https://doi.org/10.1016/0006-2952(90)90709-t.
223. Cho KHT, Hounsell N, McClendon E, Riddle A, Basappa, Dhillon SK, et al. Postischemic Infusion of Apigenin Reduces Seizure Burden in Preterm Fetal Sheep. IJMS. 2023; 24(23):16926. https://doi.org/10.3390/ijms242316926.
224. McGuire J, Taguchi T, Tombline G, Paige V, Janelsins M, Gilmore N, et al. Hyaluronidase inhibitor delphinidin inhibits cancer metastasis. Sci Rep. 2024;14(1):14958. https://doi.org/10.1038/s41598-024-64924-6.
225. Sato T, Matsuda T, Tagawa K, Segawa S. α-ketoglutarate produced by lactic acid bacteria inhibits hyaluronidase activity. Biosci Microbiota Food Health. 2024; 43(4):391–400. https://doi.org/10.12938/bmfh.2024-017.
226. De Vos A, Van Landuyt L, Van Ranst H, Vandermonde A, D'Haese V, Sterckx J, et al. Randomized sibling-oocyte study using recombinant human hyaluronidase versus bovine-derived Sigma hyaluronidase in ICSI patients. Hum Reprod. 2008;23(8):1815–9. https://doi.org/10.1093/humrep/den212.
227. Lee JH, Yoo M, Lee SM, Park S-J, Kil TY, Kim MK. Toxicity of the recombinant human hyaluronidase ALT-BC4 on embryonic development. J Anim Sci Technol. 2021;63(2):272–80. https://doi.org/10.5187/jast.2021.e34.
228. Chen Y, Feng X. Gold nanoparticles for skin drug delivery. Int J Pharm. 2022;625: 122122. https://doi.org/10.1016/j.ijpharm.2022.122122.
229. Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. Nat Rev Drug Discov. 2021;20(2):101–24. https://doi.org/10.1038/s41573-020-0090-8.
230. Devi LS, Casadidio C, Gigliobianco MR, Di Martino P, Censi R. Multifunctionality of cyclodextrin-based polymeric nanoparticulate delivery systems for chemotherapeutics, combination therapy, and theranostics. Int J Pharm. 2024;654: 123976. https://doi.org/10.1016/j.ijpharm.2024.123976.
231. Meng Y, Qiu C, Li X, McClements DJ, Sang S, Jiao A, et al. Polysaccharide-based nano-delivery systems for encapsulation, delivery, and pH-responsive release of bioactive ingredients. Crit Rev Food Sci Nutr. 2024;64(1):187–201. https://doi.org/10.1080/10408398.2022.2105800.
232. Chen E, Han S, Song B, Xu L, Yuan H, Liang M, et al. Mechanism Investigation of Hyaluronidase-Combined Multistage Nanoparticles for Solid Tumor Penetration and Antitumor Effect. Int J Nanomedicine. 2020;15:6311–24. https://doi.org/10.2147/IJN.S257164.
233. Soundararajan R, Wang G, Petkova A, Uchegbu IF, Schätzlein AG. Hyaluronidase Coated Molecular Envelope Technology Nanoparticles Enhance Drug Absorption via the Subcutaneous Route. Mol Pharm. 2020;17(7):2599–611. https://doi.org/10.1021/acs.molpharmaceut.0c00294.
234. Yin S, Gao Y, Zhang Y, Xu J, Zhu J, Zhou F, et al. Reduction/Oxidation-Responsive Hierarchical Nanoparticles with Self-Driven Degradability for Enhanced Tumor Penetration and Precise Chemotherapy. ACS Appl Mater Interfaces. 2020;12(16):18273–91. https://doi.org/10.1021/acsami.0c00355.
235. Parmar PK, Wadhawan J, Bansal AK. Pharmaceutical nanocrystals: A promising approach for improved topical drug delivery. Drug Discovery Today. 2021;26(10):2329–49. https://doi.org/10.1016/j.drudis.2021.07.010.
236. Kasinathan N, Volety SM, Josyula VR. Application of experimental design in preparation of nanoliposomes containing hyaluronidase. J Drug Deliv. 2014;2014: 948650. https://doi.org/10.1155/2014/948650.
237. Ikeda-Imafuku M, Gao Y, Shaha S, Wang LL-W, Park KS, Nakajima M, et al. Extracellular matrix degrading enzyme with stroma-targeting peptides enhance the penetration of liposomes into tumors. J Control Release. 2022;352:1093–103. https://doi.org/10.1016/j.jconrel.2022.11.007.
238. Favorito LA, Gallo CBM, Costa WS, Sampaio FJB. Ultrastructural Analysis of the Foreskin in Patients With True Phimosis Treated or Not Treated With Topical Betamethasone and Hyaluronidase Ointment. Urology. 2016;98:138–43. https://doi.org/10.1016/j.urology.2016.07.007.
239. Ohta S, Matsuura M, Kawashima Y, Cai X, Taniguchi M, Okochi H, et al. Facile fabrication of PEG-coated PLGA microspheres via SPG membrane emulsification for the treatment of scleroderma by ECM degrading enzymes. Colloids Surf B Biointerfaces. 2019;179:453–61. https://doi.org/10.1016/j.colsurfb.2019.04.028.
240. Debela DT, Muzazu SG, Heraro KD, Ndalama MT, Mesele BW, Haile DC, et al. New approaches and procedures for cancer treatment: Current perspectives. SAGE Open Med. 2021;9:20503121211034370. https://doi.org/10.1177/20503121211034366.
241. Oli AN, Adejumo SA, Rowaiye AB, Ogidigo JO, Hampton-Marcell J, Ibeanu GC. Tumour Immunotherapy and Applications of Immunological Products: A Review of Literature. J Immunol Res. 2024;2024:8481761. https://doi.org/10.1155/2024/8481761.
242. Zhang Y, Zhang Z. The history and advances in cancer immunotherapy: understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. Cell Mol Immunol. 2020;17(8):807–21. https://doi.org/10.1038/s41423-020-0488-6.
243. Hargadon KM, Johnson CE, Williams CJ. Immune checkpoint blockade therapy for cancer: An overview of FDA-approved immune checkpoint inhibitors. Int Immunopharmacol. 2018;62:29–39. https://doi.org/10.1016/j.intimp.2018.06.001.
244. Vaddepally RK, Kharel P, Pandey R, Garje R, Chandra AB. Review of Indications of FDA-Approved Immune Checkpoint Inhibitors per NCCN Guidelines with the Level of Evidence. Cancers (Basel). 2020;12(3):738. https://doi.org/10.3390/cancers12030738.
245. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. Science. 2015;348(6230):62–8. https://doi.org/10.1126/science.aaa4967.
246. Baker DJ, Arany Z, Baur JA, Epstein JA, June CH. CAR T therapy beyond cancer: the evolution of a living drug. Nature. 2023;619(7971):707–15. https://doi.org/10.1038/s41586-023-06243-w.
247. Li D, Li X, Zhou W-L, Huang Y, Liang X, Jiang L, et al. Genetically engineered T cells for cancer immunotherapy. Signal Transduct Target Ther. 2019;4:35. https://doi.org/10.1038/s41392-019-0070-9.

248. Melief CJM, van Hall T, Arens R, Ossendorp F, van der Burg SH. Therapeutic cancer vaccines. J Clin Invest. 2015;125(9):3401–12. https://doi.org/10.1172/JCI80009.
249. Wang X, Maeng HM, Lee J, Xie C. Therapeutic implementation of oncolytic viruses for cancer immunotherapy: Review of challenges and current clinical trials. J Biomed Sci Res. 2022; 4(2):164. https://doi.org/10.36266/JBSR/164.
250. Waldmann TA. Cytokines in Cancer Immunotherapy. Cold Spring Harb Perspect Biol. 2018;10(12): a028472. https://doi.org/10.1101/cshperspect.a028472.
251. Clift R, Souratha J, Garrovillo SA, Zimmerman S, Blouw B. Remodeling the tumor microenvironment sensitizes breast tumors to anti-programmed death-ligand 1 immunotherapy. Cancer Res. 2019;79(16):4149–59. https://doi.org/10.1158/0008-5472.CAN-18-3060.
252. Yang M, Qin C, Tao L, Cheng G, Li J, Lv F, et al. Synchronous targeted delivery of TGF-β siRNA to stromal and tumor cells elicits robust anti-tumor immunity against triple-negative breast cancer by comprehensively remodeling the tumor microenvironment. Biomaterials. 2023;301: 122253. https://doi.org/10.1016/j.biomaterials.2023.122253.
253. Harui A, Roth MD. Hyaluronidase enhances targeting of hydrogel-encapsulated anti-CTLA-4 to tumor draining lymph nodes and improves anti-tumor efficacy. Gels. 2022;8(5):284. https://doi.org/10.3390/gels8050284.
254. Moeller J, Green MD, Ramnath N. Pros and cons of subcutaneous (SC) versus intravenous (IV) administration of immune checkpoint inhibitors in non-small cell lung cancer. Transl Lung Cancer Res. 2024;13(6):1444–1449. https://doi.org/10.21037/tlcr-24-111.
255. Zhao Y, Sanghavi K, Roy A, Murthy B, Bello A, Aras U, et al. Model-Based Dose Selection of Subcutaneous Nivolumab in Patients with Advanced Solid Tumors. Clin Pharmacol Ther. 2024;115(3):488–97. https://doi.org/10.1002/cpt.3148.
256. Albiges L, Bourlon MT, Chacón M, Cutuli HJ, Chuken Y a. L, Żurawski B, et al. Subcutaneous versus intravenous nivolumab for renal cell carcinoma. Ann Oncol. 2025; 36(1):99–107. https://doi.org/10.1016/j.annonc.2024.09.002.
257. Duco MR, Murdock JL, Reeves DJ. Trastuzumab/Hyaluronidase-oysk: A New Option for Patients With HER2-Positive Breast Cancer. Ann Pharmacother. 2020;54(3):254–61. https://doi.org/10.1177/1060028019877936.
258. Sonneveld P, Dimopoulos MA, Boccadoro M, Quach H, Ho PJ, Beksac M, et al. Daratumumab, Bortezomib, Lenalidomide, and Dexamethasone for Multiple Myeloma. N Engl J Med. 2024;390(4):301–13. https://doi.org/10.1056/NEJMoa2312054.
259. Gao JJ, Osgood CL, Gong Y, Zhang H, Bloomquist EW, Jiang X, et al. FDA Approval Summary: Pertuzumab, Trastuzumab, and Hyaluronidase-zzxf Injection for Subcutaneous Use in Patients with HER2-positive Breast Cancer. Clin Cancer Res. 2021;27(8):2126–9. https://doi.org/10.1158/1078-0432.CCR-20-3474.
260. Shpilberg O, Jackisch C. Subcutaneous administration of rituximab (MabThera) and trastuzumab (Herceptin) using hyaluronidase. Br J Cancer. 2013;109(6):1556–61. https://doi.org/10.1038/bjc.2013.371.
261. Gao X, Lee J, Deshpande K, Kang DW, Fathallah AM, Kagan L. Mechanistic Modeling of the Effect of Recombinant Human Hyaluronidase (rHuPH20) on Subcutaneous Delivery of Cetuximab in Rats. Pharm Res. 2022;39(8):1867–80. https://doi.org/10.1007/s11095-022-03294-y.
262. Xiong X, Xi J, Liu Q, Wang C, Jiang Z, Yue S-Y, et al. Co-expression of IL-7 and PH20 promote anti-GPC3 CAR-T tumour suppressor activity in vivo and in vitro. Liver Int. 2021;41(5):1033–43. https://doi.org/10.1111/liv.14771.
263. Zhao Y, Dong Y, Yang S, Tu Y, Wang C, Li J, et al. Bioorthogonal equipping CAR-T cells with hyaluronidase and checkpoint blocking antibody for enhanced solid tumor immunotherapy. ACS Cent Sci. 2022;8(5):603–14. https://doi.org/10.1021/acscentsci.2c00163.
264. Chow EK-H, Ho D. Cancer nanomedicine: From drug delivery to imaging. Sci Transl Med. 2013; 5(216):216rv4. https://doi.org/10.1126/scitranslmed.3005872.
265. Krieg AM. Toll-like receptor 9 (TLR9) agonists in the treatment of cancer. Oncogene. 2008;27(2):161–7. https://doi.org/10.1038/sj.onc.1210911.
266. Wang C, Sun W, Wright G, Wang AZ, Gu Z. Inflammation-triggered cancer immunotherapy by programmed delivery of CpG and anti-PD1 antibody. Adv Mater. 2016;28(40):8912–20. https://doi.org/10.1002/adma.201506312.
267. Guan X, Chen J, Hu Y, Lin L, Sun P, Tian H, et al. Highly enhanced cancer immunotherapy by combining nanovaccine with hyaluronidase. Biomaterials. 2018;171:198–206. https://doi.org/10.1016/j.biomaterials.2018.04.039.
268. Hu Y, Lin L, Chen J, Maruyama A, Tian H, Chen X. Synergistic tumor immunological strategy by combining tumor nanovaccine with gene-mediated extracellular matrix scavenger. Biomaterials. 2020;252: 120114. https://doi.org/10.1016/j.biomaterials.2020.120114.
269. Guan X, Guo Z, Wang T, Lin L, Chen J, Tian H, et al. A pH-responsive detachable PEG shielding strategy for gene delivery system in cancer therapy. Biomacromol. 2017;18(4):1342–9. https://doi.org/10.1021/acs.biomac.7b00080.
270. Najibi AJ, Shih T-Y, Zhang DKY, Lou J, Sobral MC, Wang H, et al. Targeting tumor extracellular matrix activates the tumor-draining lymph nodes. Cancer Immunol Immunother. 2022;71(12):2957–68. https://doi.org/10.1007/s00262-022-03212-6.
271. Al-Zaher AA, Moreno R, Fajardo CA, Arias-Badia M, Farrera M, De Sostoa J, et al. Evidence of Anti-tumoral Efficacy in an Immune Competent Setting with an iRGD-Modified Hyaluronidase-Armed Oncolytic Adenovirus. Mol Ther Oncolytics. 2018;8:62–70. https://doi.org/10.1016/j.omto.2018.01.003.
272. Kiyokawa J, Kawamura Y, Ghouse SM, Acar S, Barçın E, Martínez-Quintanilla J, et al. Modification of extracellular matrix enhances oncolytic adenovirus immunotherapy in glioblastoma. Clin Cancer Res. 2021;27(3):889–902. https://doi.org/10.1158/1078-0432.CCR-20-2400.
273. Vera B, Martínez-Vélez N, Xipell E, Acanda de la Rocha A, Patiño-García A, Saez-Castresana J, et al. Characterization of the antiglioma effect of the oncolytic adenovirus VCN-01. PLoS One. 2016; 11(1):e0147211. https://doi.org/10.1371/journal.pone.0147211.
274. Rodríguez-García A, Giménez-Alejandre M, Rojas JJ, Moreno R, Bazan-Peregrino M, Cascalló M, et al. Safety and efficacy of VCN-01, an oncolytic adenovirus combining fiber HSG-binding domain replacement with RGD and hyaluronidase expression. Clin Cancer Res. 2015;21(6):1406–18. https://doi.org/10.1158/1078-0432.CCR-14-2213.
275. N M-V, E X, B V, A A de la R, M Z, L M, et al. The oncolytic adenovirus VCN-01 as therapeutic approach against pediatric osteosarcoma. Clin Cancer Res. 2016; 22(9). https://doi.org/10.1158/1078-0432.CCR-15-1899.
276. Bazan-Peregrino M, Garcia-Carbonero R, Laquente B, Álvarez R, Mato-Berciano A, Gimenez-Alejandre M, et al. VCN-01 disrupts pancreatic cancer stroma and exerts antitumor effects. J Immunother Cancer. 2021;9(11): e003254. https://doi.org/10.1136/jitc-2021-003254.
277. Garcia-Carbonero R, Bazan-Peregrino M, Gil-Martín M, Álvarez R, Macarulla T, Riesco-Martinez MC, et al. Phase I, multicenter, open-label study of intravenous VCN-01 oncolytic adenovirus with or without nab-paclitaxel plus gemcitabine in patients with advanced solid tumors. J Immunother Cancer. 2022;10(3): e003255. https://doi.org/10.1136/jitc-2021-003255.
278. Mato-Berciano A, Morgado S, Maliandi MV, Farrera-Sal M, Gimenez-Alejandre M, Ginestà MM, et al. Oncolytic adenovirus with hyaluronidase activity that evades neutralizing antibodies: VCN-11. J Control Release. 2021;332:517–28. https://doi.org/10.1016/j.jconrel.2021.02.035.
279. Wang S, Li Y, Xu C, Dong J, Wei J. An oncolytic vaccinia virus encoding hyaluronidase reshapes the extracellular matrix to enhance cancer chemotherapy and immunotherapy. J Immunother Cancer. 2024;12(3): e008431. https://doi.org/10.1136/jitc-2023-008431.
280. Zhang J, Yu J, Liu M, Xie Z, Lei X, Yang X, et al. Small-molecule modulators of tumor immune microenvironment. Bioorg Chem. 2024;145: 107251. https://doi.org/10.1016/j.bioorg.2024.107251.
281. Platten M, Nollen EAA, Röhrig UF, Fallarino F, Opitz CA. Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond. Nat Rev Drug Discov. 2019;18(5):379–401. https://doi.org/10.1038/s41573-019-0016-5.
282. Xue C, Li G, Zheng Q, Gu X, Shi Q, Su Y, et al. Tryptophan metabolism in health and disease. Cell Metab. 2023;35(8):1304–26. https://doi.org/10.1016/j.cmet.2023.06.004.
283. Li M, Liu Y, Zhang Y, Yu N, Li J. Sono-Activatable Semiconducting Polymer Nanoreshapers Multiply Remodel Tumor Microenvironment for Potent Immunotherapy of Orthotopic Pancreatic Cancer. Adv Sci (Weinh). 2023;10(35): e2305150. https://doi.org/10.1002/advs.202305150.

284. Li Z, Lai X, Fu S, Ren L, Cai H, Zhang H, et al. Immunogenic Cell Death Activates the Tumor Immune Microenvironment to Boost the Immunotherapy Efficiency. Adv Sci (Weinh). 2022;9(22): e2201734. https://doi.org/10.1002/advs.202201734.
285. Zhou J, Wang G, Chen Y, Wang H, Hua Y, Cai Z. Immunogenic cell death in cancer therapy: Present and emerging inducers. J Cell Mol Med. 2019;23(8):4854–65. https://doi.org/10.1111/jcmm.14356.
286. Liu Q, Sun Y, Yin X, Li J, Xie J, Xie M, et al. Hyaluronidase-Functionalized Silica Nanocarrier for Enhanced Chemo-Immunotherapy through Inducing Immunogenic Cell Death. ACS Appl Bio Mater. 2020;3(5):3378–89. https://doi.org/10.1021/acsabm.0c00299.
287. Wang Z, Xu Y, Wu G, Zuo T, Zhang J, Yang J, et al. Dual-Responsive and Deep-Penetrating Nanomicelles for Tumor Therapy via Extracellular Matrix Degradation and Oxidative Stress. ACS Biomater Sci Eng. 2021;7(1):166–79. https://doi.org/10.1021/acsbiomaterials.0c01394.
288. Yang X, Zhang C, Song M, Zhang Z, Zhou J, Zhang H, et al. Enzyme-Silenced Nanosponges Prolong Intratumoral Lifetime to Facilitate Intercellular Relay Drug Delivery and Treatment Efficacy. ACS Nano. 2023;17(23):23568–83. https://doi.org/10.1021/acsnano.3c06544.
289. Geng M, Du H, Wei X, Chen S, Cheng J, Meng S, et al. Engineered platelets-based nano-aircraft system for precise tumor chemo-immunotherapy with graded drug delivery and self-recognized tumor targeting. Sci Bull (Beijing). 2025;S2095–9273(25):00164–71. https://doi.org/10.1016/j.scib.2025.02.005.
290. Ramanathan RK, McDonough SL, Philip PA, Hingorani SR, Lacy J, Kortmansky JS, et al. Phase IB/II Randomized Study of FOLFIRINOX Plus Pegylated Recombinant Human Hyaluronidase Versus FOLFIRINOX Alone in Patients With Metastatic Pancreatic Adenocarcinoma: SWOG S1313. J Clin Oncol. 2019;37(13):1062–9. https://doi.org/10.1200/JCO.18.01295.
291. Brennan L, Brouwer-Visser J, Nüesch E, Karpova M, Heller A, Gaire F, et al. T-Cell Heterogeneity in Baseline Tumor Samples: Implications for Early Clinical Trial Design and Analysis. Front Immunol. 2022;13: 760763. https://doi.org/10.3389/fimmu.2022.760763.
292. Wang J, Ruan S, Yu T, Meng X, Ran J, Cen C, et al. Upregulation of HAS2 promotes glioma cell proliferation and chemoresistance via c-myc. Cell Signal. 2024;120: 111218. https://doi.org/10.1016/j.cellsig.2024.111218.
293. Wong KM, Horton KJ, Coveler AL, Hingorani SR, Harris WP. Targeting the Tumor Stroma: the Biology and Clinical Development of Pegylated Recombinant Human Hyaluronidase (PEGPH20). Curr Oncol Rep. 2017;19(7):47. https://doi.org/10.1007/s11912-017-0608-3.
294. Ko AH, Kim K-P, Siveke JT, Lopez CD, Lacy J, O'Reilly EM, et al. Atezolizumab Plus PEGPH20 Versus Chemotherapy in Advanced Pancreatic Ductal Adenocarcinoma and Gastric Cancer: MORPHEUS Phase Ib/II Umbrella Randomized Study Platform. Oncologist. 2023;28(6):553-e472. https://doi.org/10.1093/oncolo/oyad022.
295. Thorpe JN. Procaine with hyaluronidase as local anesthetic. Lancet. 1951;1(6648):210–1. https://doi.org/10.1016/s0140-6736(51)93361-2.
296. Lee A, Grummer SE, Kriegel D, Marmur E. Hyaluronidase. Dermatol Surg. 2010;36(7):1071–7. https://doi.org/10.1111/j.1524-4725.2010.01585.x.
297. Remy M, Pinter F, Nentwich MM, Kampik A, Schönfeld C-L. Efficacy and safety of hyaluronidase 75 IU as an adjuvant to mepivacaine for retrobulbar anesthesia in cataract surgery. J Cataract Refract Surg. 2008;34(11):1966–9. https://doi.org/10.1016/j.jcrs.2008.07.022.
298. Han JH, Kim J, Yoon KC, Shin HW. Treatment of post-traumatic hematoma and fibrosis using hyaluronidase injection. Arch Craniofac Surg. 2018;19(3):218–21. https://doi.org/10.7181/acfs.2017.01396.
299. Bookbinder LH, Hofer A, Haller MF, Zepeda ML, Keller G-A, Lim JE, et al. A recombinant human enzyme for enhanced interstitial transport of therapeutics. J Control Release. 2006;114(2):230–41. https://doi.org/10.1016/j.jconrel.2006.05.027.
300. Borzabadi-Farahani A, Mosahebi A, Zargaran D. A scoping review of hyaluronidase use in managing the complications of aesthetic interventions. Aesth Plast Surg. 2024;48(6):1193–209. https://doi.org/10.1007/s00266-022-03207-9.
301. Alam M, Hughart R, Geisler A, Paghdal K, Maisel A, Weil A, et al. Effectiveness of low doses of hyaluronidase to remove hyaluronic acid filler nodules: A randomized clinical trial. JAMA Dermatol. 2018;154(7):765–72. https://doi.org/10.1001/jamadermatol.2018.0515.
302. Teng Y, Chen Y, Tang X, Wang S, Yin K. PAD2: A potential target for tumor therapy. Biochim Biophys Acta Rev Cancer. 2023;1878(5): 188931. https://doi.org/10.1016/j.bbcan.2023.188931.
303. Kim SK, Yang JY, Kim CW, Baek SH, Kim U, Hwang E. The Effect of Hyaluronidase on the Fat Graft. J Craniofac Surg. 2020;31(3):618–21. https://doi.org/10.1097/SCS.0000000000006042.
304. Jung H. Hyaluronidase: An overview of its properties, applications, and side effects. Arch Plast Surg. 2020;47(4):297–300. https://doi.org/10.5999/aps.2020.00752.
305. Murray G, Convery C, Walker L, Davies E. Guideline for the Safe Use of Hyaluronidase in Aesthetic Medicine, Including Modified High-dose Protocol. J Clin Aesthet Dermatol. 2021;14(8):E69-75.
306. Kim MS, Youn S, Na CH, Shin BS. Allergic reaction to hyaluronidase use after hyaluronic acid filler injection. J Cosmet Laser Ther. 2015;17(5):283–5. https://doi.org/10.3109/14764172.2015.1007069.
307. Sharma DSC, Lahiri MA. Use of hyaluronidase in plastic surgery: A review. J Plast Reconstr Aesthet Surg. 2021;74(7):1610–4. https://doi.org/10.1016/j.bjps.2021.03.125.
308. Bailey SH, Fagien S, Rohrich RJ. Changing Role of Hyaluronidase in Plastic Surgery. Plast Reconstr Surg. 2014;133(2):127e-e132. https://doi.org/10.1097/PRS.0b013e3182a4c282.
309. Guliyeva G, Huayllani MT, Kraft C, Lehrman C, Kraft MT. Allergic Complications of Hyaluronidase Injection: Risk Factors, Treatment Strategies, and Recommendations for Management. Aesthetic Plast Surg. 2024;48(3):413–39. https://doi.org/10.1007/s00266-023-03348-5.
310. Cavallini M, Gazzola R, Metalla M, Vaienti L. The Role of Hyaluronidase in the Treatment of Complications From Hyaluronic Acid Dermal Fillers. Aesthet Surg J. 2013;33(8):1167–74. https://doi.org/10.1177/1090820X13511970.
311. Keller EC, Kaminer MS, Dover JS. Use of hyaluronidase in patients with bee allergy. Dermatol Surg. 2014;40(10):1145–7. https://doi.org/10.1097/DSS.0000000000000123.
312. Kroumpouzos G, Treacy P. Hyaluronidase for Dermal Filler Complications: Review of Applications and Dosage Recommendations. JMIR Dermatol. 2024;7: e50403. https://doi.org/10.2196/50403.
313. Rosengren S, Dychter SS, Printz MA, Huang L, Schiff RI, Schwarz H-P, et al. Clinical Immunogenicity of rHuPH20, a Hyaluronidase Enabling Subcutaneous Drug Administration. AAPS J. 2015;17(5):1144–56. https://doi.org/10.1208/s12248-015-9782-0.
314. Printz MA, Sugarman BJ, Paladini RD, Jorge MC, Wang Y, Kang DW, et al. Risk Factors, Hyaluronidase Expression, and Clinical Immunogenicity of Recombinant Human Hyaluronidase PH20, an Enzyme Enabling Subcutaneous Drug Administration. AAPS J. 2022;24(6):110. https://doi.org/10.1208/s12248-022-00757-3.
315. Thompson CB, Shepard HM, O'Connor PM, Kadhim S, Jiang P, Osgood RJ, et al. Enzymatic depletion of tumor hyaluronan induces antitumor responses in preclinical animal models. Mol Cancer Ther. 2010;9(11):3052–64. https://doi.org/10.1158/1535-7163.MCT-10-0470.
316. Hilton S, Schrumpf H, Buhren BA, Bölke E, Gerber PA. Hyaluronidase injection for the treatment of eyelid edema: a retrospective analysis of 20 patients. Eur J Med Res. 2014;19(1):30. https://doi.org/10.1186/2047-783X-19-30.
317. Tolba RH. Current regulations in the Animal Welfare Act and the significance for animal research. Orthopadie (Heidelb). 2024;53(5):336–40. https://doi.org/10.1007/s00132-024-04493-8.
318. Dunn AL, Heavner JE, Racz G, Day M. Hyaluronidase: a review of approved formulations, indications and off-label use in chronic pain management. Expert Opin Biol Ther. 2010;10(1):127–31. https://doi.org/10.1517/14712590903490382.
319. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191–4. https://doi.org/10.1001/jama.2013.281053.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.