Significance of CD44 gene products for cancer diagnosis and disease evaluation

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With increasing emphasis on the early detection of cancer, the search is on for reliable markers that will be clinically helpful in the diagnosis of small tumours and in the assessment of their metastatic potential. This report presents evidence that an abnormal pattern of activity of the CD44 gene is a promising candidate for both of these purposes in various types of malignancy. By a mechanism known as alternative splicing this gene can produce different messenger RNA molecules (transcripts) which are detectable, after amplification, as separate bands in electrophoretic gels. In neoplasia many abnormal variant transcripts are produced. A previous finding in animal experiments, that one such variant might be important in metastasis, prompted our study of human tumour tissue, benign and malignant, and of corresponding normal tissues.

We studied tumour tissue from 34 patients with neoplastic disease (mostly breast or colon cancer) and normal or non-malignant diseased breast or colonic tissue from 11 patients and peripheral blood leucocytes from 4 healthy volunteers. CD44 gene activity was studied by amplifying messenger RNA with the polymerase chain reaction (PCR) followed by electrophoresis and blot hybridisation. In malignant tissues there was gross overproduction of each of 9 or more alternatively-spliced large molecular variants in all samples, whereas in the control samples only the standard product was routinely detected with occasional minimal quantities of one or two small variants. Furthermore, the band pattern permitted differentiation between the 23 cases with metastatic tumours of the breast or colon and the 8 with no detectable metastases. Calibration studies seeding blood with tumour cells showed that the technique can detect as few as 10 tumour cells among 10^7 leucocytes (1 ml of blood).

Analysis of CD44 splice variants may prove to have applications not just to the early detection of metastatic potential in surgical biopsy specimens but also, if our findings are confirmed, in readily available bodily fluids, to the early diagnosis of cancer in screening programmes, to the assessment of remaining disease in the body and to the early detection of recurrences.

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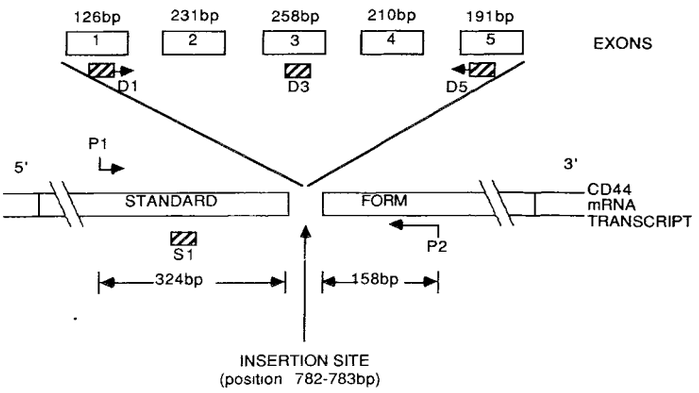
Introduction

In a recent *Lancet* review[1] it was noted that there is so far nothing that a malignant tumour cell can do that is not a routine activity of normal cells somewhere in the body. To illustrate the point the authors noted that metastatic tumour cells express a variant of glycoprotein CD44 "that is believed to play a part in normal lymphocyte migration and binding".

The human CD44 gene codes for a group of proteins originally defined as sharing determinants recognised by the CD44 monoclonal antibody (mAb). Our studies highlight the clinincal potential of the products of this gene. First of all, however, it is necessary to describe the CD44 gene and the functions of some of its products. The members (isoforms) of this group which have so far been purified and characterised are located at the cell surface and differ in properties such as size and glycosylation. This variation in the proteins produced from one gene is possible because the coding sequences (exons) in DNA are transcribed (expressed) in different combinations, according to the prevailing requirements of the cell. Messenger RNA (mRNA) consists of the transcribed sequences from the exons which can be spliced together in different combinations ("alternative splicing")[2] once the segments transcribed from non-coding regions have been excised.[7] A single cell may thus contain at the same time two or more variant mRNA from the same gene. These splice variants can be translated into proteins which are structurally related because they contain some sections with identical aminoacid sequences but whose functions may or may not be similar.



Standard (haemopoietic) form no insert

Epithelial form = standard form + products of exons 4 and 5

P1,P2,D1,D5 = primers

S1,D3 = probes

Fig 1—Map of CD44 gene products showing exons, probes, and primers.

Primer P1 = 5'GACACATATTGCTTCAATGCTTCAGC
Primer P2 = 5'GATGCCAAGATGATCAGCCATTCTGGAAT
Primer D3 = 5'TGAGATTGGGTTGAAGAAATC-3.
Probe S1 = 5C'CTGAAGAAGATTGTACATCAGTCACAGAC.
Primer D1 = 5'GACAGACACCTCAGTTTTTCTGGA.
Primer D5 = 5'TTCCTTCGTGTGTGGGTAATGAGA

Fig 1, summarising what is known about alternatively-spliced products transcribed from the CD44 gene, shows that the basic or "standard" protein can be modified by the insertion of transcripts from any, some, or all of at least five extra exons.[3,4] 32 possible variants could be derived from these components alone. Some may be inadmissible combinations; on the other hand, differing degrees of modification of allowable ones, by attachment of carbohydrate, glycosyl side-chains, could create a much larger family of molecules. Two of the commonest proteins produced by this gene consist of a 90 kDa form with a heavily glycosylated central 37 kD protein core[5]

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TABLE I—CLINICAL AND PATHOLOGICAL DETAILS

Patient	Tumour size (cm)	Lymph nodes positive*	Histology†	Clinical Stage‡
Breast cancer, metastatic				
B1 (56, F)	2·5	8/11	IDC (2)	2
B2 (53, F)	3	1/8	IDC (2)	2
B3 (65, F)	3	4/11	IDC (1)	2
B4 (54, F)	5	10/10	IDC (mucinous) [1]	3
B5 (59, F)	5·5	3/15	IDC (2)	2
B6 (59, F)	3	4/7	IDC (3)	2
B7 (61, F)	4	17/17	ILC/IDC	3
B8 (38, F)	3·5	1/5	ILC	2
B9 (65, F)	1·8	5/6	ILC	2
B10 (61, F)	4·5	10/13	IDC (1)	2
B11 (80, F)	11	2/2	IDC (3)	3
B12 (68, F)	2·8	4/12	IDC (3)	2
B13 (47, F)	7	8/10	IDC (3)	2
B14 (65, F)	9	6/6	IDC/ILC	3
Breast cancer, not metastatic				
B15 (38, F)		0/7	DCIS	1
B16 (62, F)	3	0/4	IDC (3)	1
B17 (62, F)	3	0/16	IDC (2)	1
B18 (63, F)	3	0/6	IDC (2)	1
B19 (61, F)	3	0/6	IDC (3)	1
B20 (42, F)	4	0/6	IDC (2)	1
B21 (54, F)	6	0/4	IDC (3)	1
Fibroadenoma of breast				
B22 (49, F)	4	..	..	..
B23 (47, F)	3	..	..	..
Fibrocystic disease of breast				
B24 (29, F)	..	..	..	..
Colon cancer, metastatic				
C1 (72, F)	5	5/5	Adeno	3 (C)
C2 (60, M)	3	1/1	Adeno	3 (C)
C3 (65, M)	6·5	..*	(Adeno)	4 (D)
C4 (56, M)	7·8	8/10*	(Adeno)	4 (D)
C5 (71, F)	7·0	1/1	Adeno	3 (C)
C6 (57, F)	5	6/7	(Adeno)	3 (C)
C7 (74, F)	5·5	2/14	None	..
C8 (75, M)	6·5	..	(Adeno)	3 (C)
C9 (72, M)	5·5	4/10	(Adeno)	3 (C)
Colon cancer, not metastatic				
C10 (76, F)	4·5	0/8	Adeno	1 (B)
Thyroid cancer				
T1 (80, F)	..	..*	Follicular	..

*Liver metastasis in C3; nodes and liver in C4, lymph node in T1
†DCIS = ductal carcinoma-in-situ, IDC = infiltrating ductal carcinoma (mucinous in B4), ILC = infiltrating lobular carcinoma. Adeno = well-differentiated adenocarcinoma; (Adeno) = moderately differentiated adenocarcinoma
‡Letters refer to Dukes stage for colon carcinoma.

and a 180 kDa form with 135 extra aminoacids in the proximal extra-membrane domain, that is even more heavily glycosylated.[6] Both are widely distributed in different cells and tissues. The 90 kDa glycoprotein is the haemopoietic or standard form present on circulating leucocytes, bone marrow cells, and numerous other cell types.[7] The epithelial variant[6] (180 kDa) is detectable on several epithelial cell types and is formed by the intercalation of transcripts from exons 4 and 5. Both are thought to function as receptors mediating the adhesive interactions involved in attachment of cells to other cells or to the adjacent extracellular matrix.

Some time ago, E. C. Butcher and colleagues (reviewed in Picker et al[7]) reported that some CD44 epitopes, recognised by the Hermes-3 monoclonal antibody, function as lymph node receptors or "homing" molecules which enable circulating lymphocytes to recognise regional lymph nodes. When, in 1989 Stamenkovic et al,[5] using a different mAb, isolated and cloned a complementary DNA (cDNA) sequence coding for the standard form of the molecule they found that the gene was expressed not only by lymphoid cells but also by a variety of carcinoma cell lines and by a representative sample of solid tumours. Two colonic carcinomas expressed more than normal colonic epithelium did. Further work by two other groups[8,9] indicated that the CD44 gene has a role in metastasis of certain human tumour cell lines when implanted in nude mice. Meanwhile. Gunthert et al[10] had obtained results indicating that a newly identified variant of cell surface glycoprotein CD44, is required for metastatic behaviour of rat pancreatic adenocarcinoma cells. Using an antibody to these metastatic tumour cells, they cloned a cDNA sequence corresponding to a new variant of rat CD44 glycoprotein. It contained previously unidentified exons. The introduction of a piece of DNA specially constructed to overexpress this unique sequence into non-metastatic rat tumour cells, appeared to induce metastatic behaviour.[10]

TABLE II—SUMMARY OF RESULTS

Type of tissue	No of patients/ volunteers	No showing increased splice variants
Neoplastic	34	34
Breast carcinoma with metastases	14	14
Breast carcinoma without metastases	7	7
Colon carcinoma with metastases	9	9
Colon carcinoma without metastases	1	1
Thyroid carcinoma	1	1
Fibroadenoma	2	2
Non-neoplastic	15	0
Cystic disease of breast	1	0
Normal breast	5	0
Normal colon	5	0
PBL	4	0

Therefore, might metastatic human tumours and ones without clinically detectable metastases, encountered in routine clinical practice, express CD44 products, particularly the new sequence mentioned above, differentially? An accurate and reliable way to study this question, when the tissue available is limited, would be to use the polymerase chain reaction (PCR) to amplify cDNA made from whole cellular mRNA. Publication of sequence data on further portions of the CD44 gene[3,4,6] allowed us to examine the expression of various parts of CD44, especially the portion (exon 3, fig 1) identified by Gunthert et al[10] as relevant to metastasis, in fresh surgical resection specimens from patients with benign and malignant tumours of the breast and colon and from metastases. We compared the findings with patterns of expression in normal tissues from the same and other patients and in blood from healthy volunteers. When results began to accumulate it became apparent that the appreciable differences in CD44 expression between metastatic tumours and locally invasive tumours with no clinical evidence of metastasis and benign tumours would be important for choice of treatment, while differences between locally or benign tumours and normal tissue would be important for early diagnosis and screening.

Materials and methods

Materials

Fresh tissue samples, 0·5–1 cm in diameter, were obtained from surgical resection specimens from 33 patients with breast or colon tumours and 1 with thyroid carcinoma. The samples were snap-frozen in liquid nitrogen within 10 min of arrival in the pathological specimen reception area and were kept frozen until use. Portions of any lymph node metastases or blood-borne metastases in the resected tissue were also collected. Normal breast tissue, colonic mucosa, lymph node and normal liver were obtained from the periphery of specimens surgically resected for treatment of cancer and from other specimens removed for non-neoplastic conditions, including fibrocystic disease of the breast, sigmoid, volvulus, and appendicitis. Normal peripheral blood leucocytes were obtained from 4 healthy volunteers. The patient details, the histological features of the tumours and the particulars of their clinical and pathological staging are described in table I.

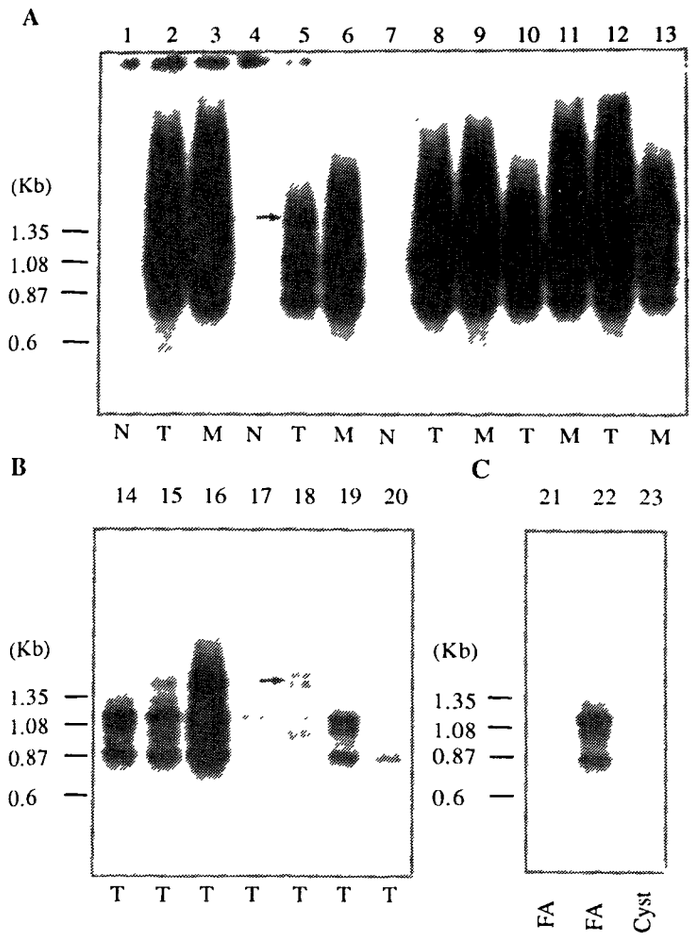

Fig 2—Breast tissue probed with D3 (10 h exposure).

(A) malignant primary breast carcinomas with metastases. Tracks 1, 2, and 3 = patient B1; 4, 5, and 6 = B2; 7, 8, and 9 = B3; 10 and 11 = B4; 12 and 13 = B5 Compared with normal breast tissue, primary breast carcinomas and metastatic deposits overexpress several splice-variants. Doublet (arrow in track 5) at 1500 and 1650 bp is present in all tumours and metastases but is fogged in other tracks by this time of exposure; it is not detectable in any normal sample, even at exposures of 23 h.

(B) breast carcinomas with no clinical evidence of metastasis (tracks 14–20 are from patients B15–B21): all overexpress several variants but show fewer bands and the signal intensity is less (except track 16, see text). The doublet (arrow) is easily recognisable in tracks 15 and 18 (it is blurred in 16 at this length of exposure) but became detectable in all other tracks on longer exposure, by which time there is fogging of tracks with stronger signals.

(C) fibroadenomas (FA) and fibrocystic disease of the breast (Cyst). Benign tumours (samples B22 and 23) express more than non-neoplastic sample (B24).

PCR method

Total cellular RNA was extracted[11] and cDNA was synthesised with viral reverse transcriptase followed by amplification by PCR (Superscript preamplification system; BRL Life Technologies UK). For the 30 cycles of PCR the conditions were: 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. Negative controls, with no template cDNA in the reaction mix, were run with every batch. We devised the primers and probes using information from the sequence for human CD44 cDNA[2-4] (fig 1). 10 µl PCR product was electrophoresed in a 1·2% agarose gel and transferred to Hybond N^+ (Amersham UK) nylon membranes for hybridisation with ^{32}P-labelled oligonucleotide probe D3. Blotting and autoradiography according to standard protocols were performed to improve sensitivity of detection and resolution. Filters were exposed to Kodak X-ray film for 2–16 h and then boiled in 0·5% SDS before rehybridisation with another radiolabelled probe (S1) we designed to anneal to the standard portion of the CD44 molecule. The conditions for hybridisation, washing, and autoradiography were as above.

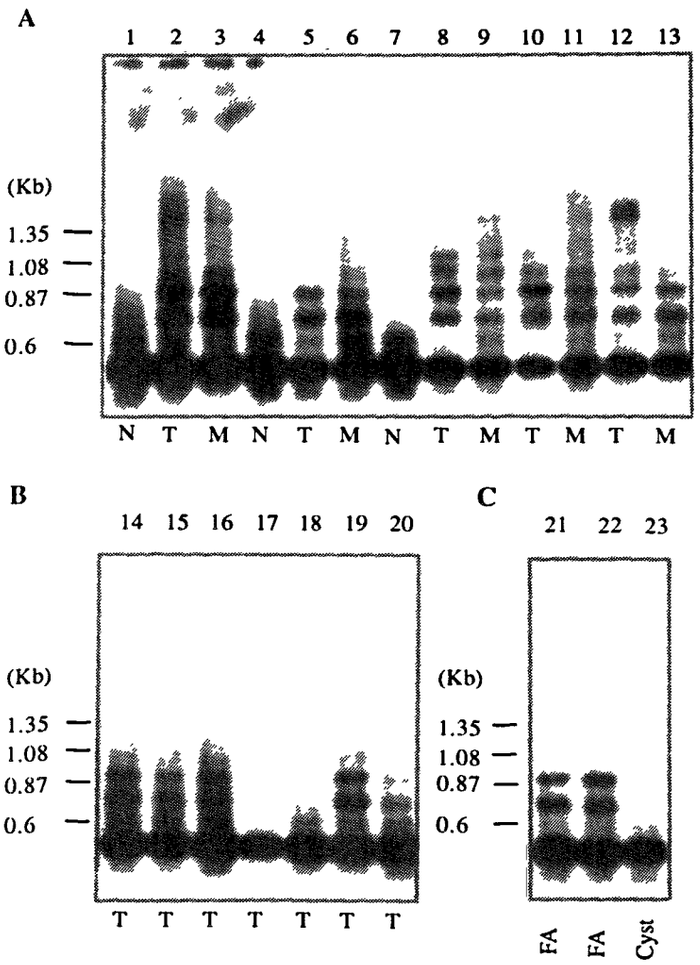

Fig 3—Breast tissue probed with probe S1 (1·5 h exposure).

Filter used in fig 1 after probe D3 had been stripped off shows that differences in fig 1 are not due to unequal loading; that expression of the standard form is quantitatively greater than any of the variants; that the standard form is expressed in all tissues examined and that variants which do not contain exon 3 transcripts are also overexpressed in tumours. (Doublet recognisable in panel A but needed longer exposure in panels B and C)

Calibration of sensitivity

Peripheral blood leucocytes (PBL) were purified from 20 ml whole blood by ammonium chloride lysis of packed red cells and centrifugation. The white cell pellet was divided into four tubes which were seeded with 0, 1, 10, and 100 µl of a suspension of ZR75-1 breast carcinoma cells (5000 cells per ml). Total RNA was then extracted and dissolved in 20 µl water. cDNA synthesis was done on 4 µl samples of RNA representing 0, 1, 10, and 100 tumour cells. PCR was done on these samples and on positive (tumour cells only) controls using primers D1 and D5, we designed to anneal specifically to exons 1 and 5. ZR75-1 cells express both these exons (and others) in a pattern easily distinguishable from PBL, and we chose these primers to increase sensitivity by shortening the segment to be amplified. PCR conditions were as above, except that the reaction was continued for 35 cycles and 2·5 units of AmpliTaq were added to each tube at the beginning and a further 2·5 units at the 15th cycle. The oligonucleotide sequence used for probing was ^{32}P-labelled D3.

Results

Overview (table II)

Because primers P1 and P2 amplify across the site in the mRNA at which extra segments may be inserted to produce different forms of CD44, the intervening "standard" portion of the molecule will be amplified along with any transcripts from exons 1–5 spliced into it. P1 has its

origin 324 bp upstream from the insertion site in the cDNA of standard CD44 molecule[5] and P2 is 158 bp downstream. Thus the amplified fragment will be 482 bp if a sample expresses standard (haemopoietic) CD44 only; a fragment of 878 bp will be produced if it is the epithelial form of CD44; and there will be several other fragments if a sample contains alternatively spliced transcripts. These appear as bands of differing molecular size when resolved by gel electrophoresis and blot hybridisation with CD44 probes. The total number of different products theoretically detectable by a probe such as S1 to the standard part of the molecule considering all possible combinations is calculated to be 32. Probe D3 will pick up at most only the 16 that contain D3 transcripts from exon 3. In practice, the full range of possible combinations was not detected, but 9 or more bands were seen in PCR products of neoplastic tissues hybridised with these probes. Normal breast, colon, and lymph nodes did very weakly express some small D3-containing transcripts besides the standard molecule but peripheral blood leucocytes and liver seemed only to express the latter with this combination of probes and primers.

Breast tissue (figs 2 and 3)

Metastatic tumour deposits and their corresponding primary tumours from all cases over-expressed several bands (alternatively spliced products) containing transcripts from exon 3 (fig 2A), reported by Gunthert et al[10] to be relevant to metastasis. Nine bands were frequently seen, including a doublet at 1500 and 1650 bp that was present in all tumours. Normal breast tissue and normal lymph node produced two bands (1150 bp and 860 bp) with this probe; the doublet was not seen. Differences in the number and size of the bands and the intensity of signal from the bound probe, between tissues in normal and malignant categories, were obvious in all samples examined. For occasional tumour samples it was necessary to expose the filter to the photographic film for longer to see the distinctive differences, but this finding was confirmed in every case studied.

Samples from locally invasive tumours with no associated clinical evidence of metastasis (fig 2B) and from the 2 fibroadenomas (fig 2C), also overexpressed products containing transcripts from domain 3 relative to normal tissues, but the extent of this was easily distinguished from that in the tumours with metastases. Distinction from the patterns seen in normal tissues was also easy. However, a single sample gave a similar result to malignant tumours with metastases (lane 16, fig 2B). The two fibroadenomas showed band patterns that were similar to those from non-metastatic carcinomas and the sample from a case of fibrocystic disease of the breast resembled the pattern for normal non-neoplastic breast tissue (fig 2C). This is the first instance of definitive diagnosis by this method. The piece of tissue was handed to us by the duty pathologist as being from a benign tumour (fibroadenoma) on the basis of inspection with the naked eye. We characterised it as definitely non-neoplastic after PCR amplification of its cDNA (lane 23, fig 2C), and subsequent microscopic examination confirmed this.

To ensure direct comparability of results, all samples from lanes 1–23, were dealt with in exactly the same manner and all PCR products were run in the same gel and blotted and probed on the same piece of filter membrane. Hence the differences seen between the groups of samples in fig 2A, B, and C are reliable, and every effort has been made to exclude variation due to differences in processing.

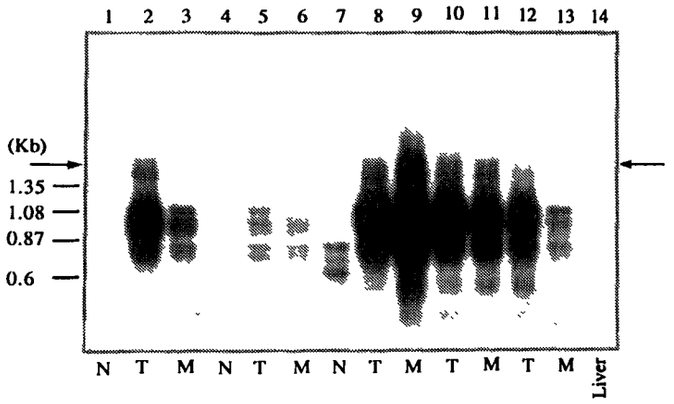

Fig 4—Colon tissue samples probed with D3 (10 h exposure).

Tracks 1, 2 and 3 = patient C1; tracks 4, 5, and 6 = patient C2; tracks 7, 8, and 9 = patient C3; tracks 10 and 11 = patient C4; tracks 12 and 13 = patient C5; track 14 = normal liver sample. Picture shows features described in legend to fig 1—ie, findings apply to carcinoma of colon also. 1500/1650 bp doublet (arrow) is easily recognisable in seven tumour tracks (2 and 8–12) and faint signal in corresponding position in tracks 3, 5, 6, and 13 became stronger on longer exposure. However none appeared in this vicinity in tracks 1, 4, 7 or 14 (normal tissue).

To confirm that the differences seen with probe D3 are not technical artifacts the same filter was hybridised with probe S1. We found that all tissues examined expressed the standard form of the gene equally; splice variants not containing transcripts from domain 3 were present in tumours and metastases (ie, bands became visible with probe S1 that were not visible with probe D3), and the differences described above were not due to unequal loading of tracks in the panels and lanes on this composite filter but resulted from alternative splicing. All conditions in this experiment were the same as those in hybridisation with D3 except for exposure time of filter to photographic film (10 h for fig 2, 1·5 h for fig 3). This difference in exposure time is necessary to avoid fogging the film completely in circumstances where more radioactive probe is bound to the filter.

Colon samples

The findings in colon carcinoma were identical to those in breast carcinoma (figs 4 and 5). In all cases the colon carcinoma tissues showed an increased number of more intensely labelled, larger molecular weight bands with probe D3 than normal colonic mucosa and other normal tissues.

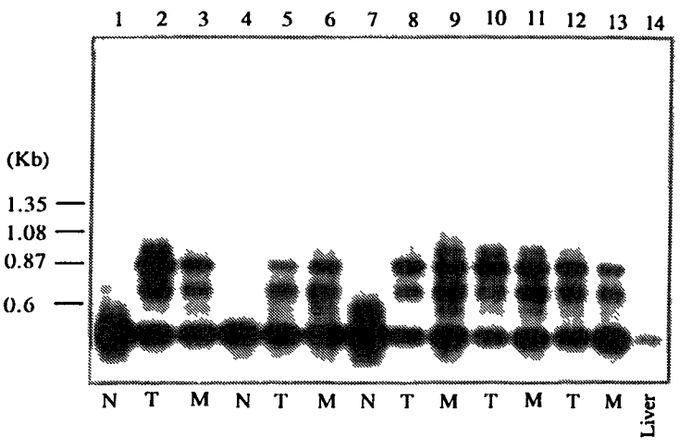

Fig 5—Colon tissue samples probed with S1 (1·5 h exposure).

This confirms that equal loading tracks and other points illustrated in fig 2 apply to colon carinomas Normal liver expresses standard form of CD44.

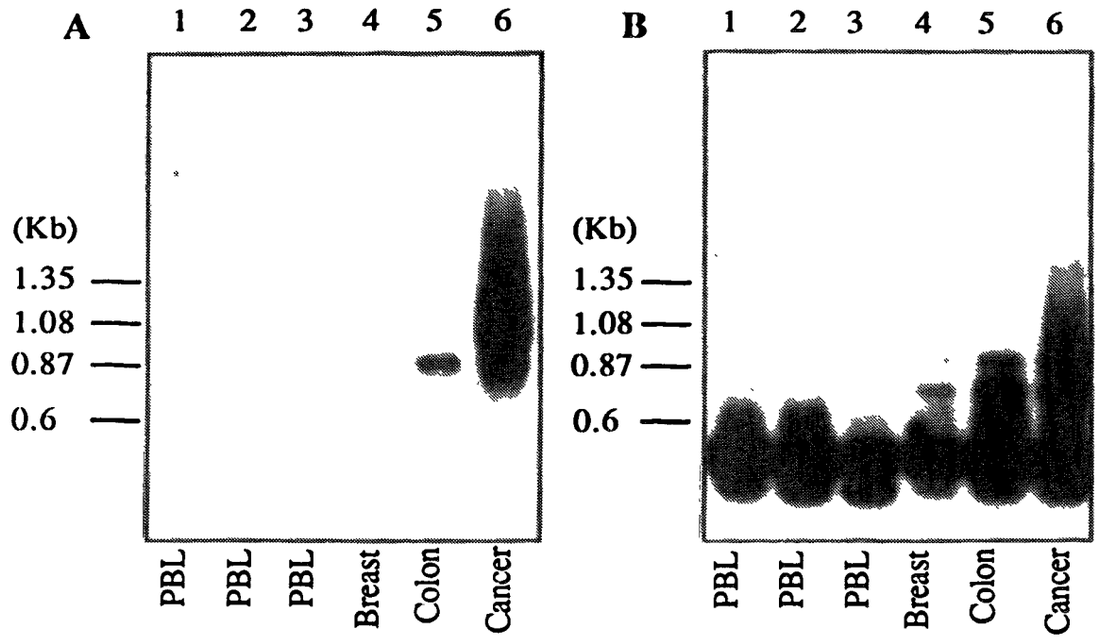

Fig 6—Normal PBL (from 3 different persons) and other normal tissues probed with D3 (panel A; 8 h exposure) or S1 (panel B, 5 h exposure).

Track 6 contains PCR product from breast cancer (B1) as positive control. With this combination of primers and probes, leucocytes express standard CD44 but not splice variants. Track 4 = breast tissue obtained at necropsy from the body of a woman who died of bacterial endocarditis; track 5 = colon resected for volvulus.

Again the 1500/1650 bp was seen in all tumours and not in any normal colonic mucosal sample. As with breast carcinomas, hybridisation with probe S1 showed no differences in the degree of expression of the standard form of the molecule between the various samples.

PBL and other normal tissues

PBL and liver expressed the standard form of CD44 without splice variants (fig 6). Breast, colon and lymph nodes co-expressed the standard form and a trace of small D_3 containing variant or two.

Calibration of sensitivity

Examination of autoradiograms of PCR products of peripheral blood leucocytes seeded with known numbers of ZR75-1 colon carcinoma cells showed the presence of additional bands characteristic of the tumour cells, down to a level of 10 tumour cells in a sample of 10^7 leucocytes.

Discussion

All samples of neoplastic tissue showed overexpression of many alternatively-spliced products of the CD44 gene whilst none of those from non-neoplastic tissue did so. The differences in pattern between metastatic tumours and ones without any clinical indication of metastasis also seemed distinct. This feature may provide a useful indicator of prognosis and deserves further evaluation. In one instance, a tumour from a patient with no clinical evidence of metastasis was found to have a pattern of expression indicating metastatic capability. We do not know yet whether this is a false-positive result, or a sign of imminent metastasis.

Our findings relate to three organ systems and are not necessarily representative of tumours of other tissues but there is indirect evidence that changes in CD44 expression may occur in several types of cancer. Signs of overexpression of some parts of this gene can be seen in northern blots of RNA from pulmonary adenocarcinoma, colonic carcinoma, oesophageal carcinoma, mesothelioma, and melanoma, hybridised with appropriate CD44 probes.[5,6] However, northern blotting requires the use of much more RNA and hence larger amounts of tissue (and time) than are practical for diagnostic purposes and is not nearly as sensitive as our cDNA/PCR/blot-hybrisation technique for detecting small amounts of diverse and unusual transcripts. With our different approach, we were able to examine a large series of tumours rapidly and have shown that analysis of CD44 activity in small specimens is not only feasible but also provides information of clinical interest. PCR amplification of cDNA alone would not have revealed the same information. Many of the amplified PCR products resolved by electrophoresis were invisible in ethidium bromide-stained gels, and the subsequent hybridisation with various radioactive oligonucleotide probes enormously increased the sensitivity. This development opens the way to further work, using PCR, on patterns of CD44 expression in biopsy material from other types of common human tumour, to clarify the clinical significance of the abnormal expression of this gene.

The possibility that some process other than neoplasia may sometimes produce similar changes in CD44 expression necessitates caution in interpretation. The specificity of the correlation between overexpression of alternatively spliced CD44 transcripts and neoplasia will only emerge after exhaustive clinical testing and it would be wrong to draw more general conclusions or make extrapolations at this stage.

In both normal colonic epithelium and bone marrow (unpublished) there is substantial cell turnover but we did not observe the band pattern seen with tumours, which suggests that overexpression observed in neoplasia is probably not simply reflecting a state of rapid cell proliferation.

The strong association between altered CD44 expression and neoplasia should not be taken to imply that any of the individual exons are expressed only in neoplasia or in

progression to metastasis. Instead, we think that an abnormal pattern of gene activity (overexpression and inappropriate splicing) such as that affecting the CD44 locus could play a part in malignancy and be a useful marker of that state. However, the finding that there is marked increase in the number and signal intensity of bands hybridising with radiolabelled probe D_3 in the PCR products from human tumours capable of metastasis supports the findings of Gunthert et al,[10] that exons 2 and 3 (fig 1) of the gene may be involved in this process. To know whether expression of splice variants containing transcripts from these exons actively contributes to or only incidentally accompanies metastatic behaviour of human tumour cells, further work is needed. The diagnostic significance of the findings reported here is unaffected by this issue.

The test described is sensitive enough to detect as few as 10 tumour cells in 1 ml of blood. With the right choice of primers and PCR conditions, it should be possible to detect a single tumour cell among 10^7 normal leucocytes. If this sort of sensitivity were to be achievable with sputum, urine, stools, blood, cervical smears, or other body fluids naturally seeded with small numbers of tumour cells, the observations and techniques reported would have wide, general applicability to cancer prevention and screening. We are now trying to find out if these techniques will facilitate the very early detection of common cancers.

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Continuous intravenous famotidine for haemorrhage from peptic ulcer

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Peptic ulcer bleeding often stops spontaneously but rebleeding may be catastrophic. Emergency surgery carries risks so safe medical therapies are needed. Since platelet function and plasma coagulation are both pH sensitive and since pepsin lyses clot at low pH the maintenance of gastric pH close to neutrality might influence rebleeding rates. Previous trials with H_2 antagonists have been inadequate although a 1985 meta-analysis did support an important clinical effect. We report here a large multicentre trial of famotidine in ulcer bleeding.

1005 patients admitted to one of sixty-seven hospitals in the UK or Eire with haemorrhage from peptic ulcer with endoscopic signs of oozing, black slough, fresh clot or visible vessel were randomly allocated to famotidine (10 mg bolus followed by 3·2 mg/h intravenously) or matching placebo for 72 h. This famotidine regimen had previously been shown to maintain pH near 7 in such patients. 497 patients received famotidine and 508 placebo. The treatment groups were similar in respect of age, sex, ulcer site, and signs and severity of bleeding. Case fatality (6·2% famotidine *vs* 5·0% placebo), rebleeding (23·9% *vs* 25·5% placebo), and surgery (15·5% *vs* 17·1% placebo) rates were not significantly different between the two groups. This trial suggests that potent inhibition of gastric secretion does not influence the natural history of peptic ulcer haemorrhage.

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Introduction

Gastrointestinal haemorrhage carries a substantial risk of fatal outcome despite advances in surgery and endoscopy, and clinicians have been hoping for a simple medical therapy that might influence prognosis. One possibility is antisecretory therapy but few clinical trials have had sufficient statistical power to produce a clear-cut result: some have reported significant benefits from H_2 antagonists while others have not.[1-6] A meta-analysis on trials of H_2 receptor antagonists in bleeding gastric ulcer indicates that this treatment might reduce rebleeding by 32%, operation rates by 39%, and case fatality rates by 47%; a benefit in duodenal ulcer haemorrhage was less clear.[7] We have shown that bolus doses of cimetidine and ranitidine produce only a modest increase in gastric pH in patients admitted with

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