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Heparanases and Tumor Metastasis

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The successful penetration of endothelial basement membranes is an important process in the formation of hematogenous tumor metastases. Heparan sulfate (HS) proteoglycan is a major constituent of endothelial basement membranes, and we have found that HS-degradative activities of metastatic B16 melanoma sublines correlate with their lung-colonizing potentials. The melanoma HS-degrading enzyme is a unique endo-β-D-glucuronidase (heparanase) that cleaves HS at specific intrachain sites and is detectable in a variety of cultured human malignant melanomas. The treatment of B16 melanoma cells with heparanase inhibitors that have few other biological activities, such as N-acetylated N-desulfated heparin, results in significant reductions in the numbers of experimental lung metastases in syngeneic mice, indicating that heparanase plays an important role in melanoma metastasis. HS-degrading endoglycosidases are not tumor-specific and have been found in several normal tissues and cells. There are at least three types of endo-β-D-glucuronidases based on their substrate specificities. Melanoma heparanase, an $M_r \sim 96{,}000$ enzyme with specificity for β-D-glucuronosyl-N-acetylglucosaminyl linkages in HS, is different from platelet and mastocytoma endoglucuronidases. Elevated levels of heparanase have been detected in sera from metastatic tumor-bearing animals and malignant melanoma patients, and a correlation exists between serum heparanase activity and extent of metastases. The results suggest that heparanase is potentially a useful marker for tumor metastasis.

Key words: endo-β-D-glucuronidase, heparan sulfate, melanoma, heparin, serum enzyme, basement membrane

Most cancer mortality is the result of metastasis of tumors to regional and distant sites. Metastasis formation occurs via a sequential and complex series of unique interactions between tumor cells and normal host cells and tissues. For example, during the process of metastasis formation migrating tumor cells are confronted by natural tissue barriers, such as connective stroma and basement membranes. The ability of malignant cells to penetrate these barriers is thought to depend on the presence of tumor and/or host enzymes capable of degrading stromal and basement membrane components—mainly collagenous and noncollagenous glycoproteins and proteoglycans. Recent progress on degradative enzymes secreted by invasive

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and metastatic tumors has been reviewed by Nicolson [1], Nicolson and Poste [2], Jones and De Clerck [3], Mullins and Rohrlick [4], and Pauli et al [5]; and by Terranova et al [6], Liotta et al [7], and Wooley [8] (collagenases); by Sloane and Honn [9] (cysteine proteinases); by Danø et al [10] (plasminogen activators); and by Bernacki et al [11] (exo- and endoglycosidases).

Basement membranes are continuous sheets of extracellular matrix that separate parenchymal cells from underlying interstitial connective tissue. They have characteristic permeabilities and play a role in maintaining normal tissue architecture [12,13]. Enzymatic degradation of basement membrane components by metastatic tumor cells has been observed by using intact basement membranes, their isolated components, or the extracellular matrices produced by endothelial or endodermal cells [14–22]. The activities of several cell-associated enzymes are thought to be involved in the destruction of basement membranes and their activities correlate with metastatic potential in several types of malignant cells [14,15,17,21–25] (Fig. 1). Here we focus on tumor endoglycosidases capable of degrading heparan sulfate (HS) proteoglycans and discuss their possible use as markers of tumor metastasis.

MOUSE B16 MELANOMA HEPARANASE

Due to their unique physical and chemical properties the HS proteoglycans are important structural elements of basement membranes [26,27]. For example, HS proteoglycan promotes basal lamina matrix assembly by enhancing the interactions of

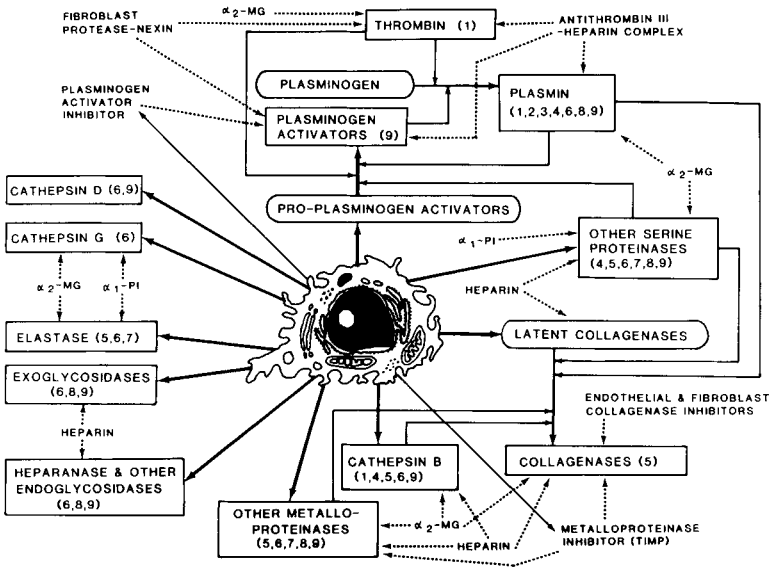

Fig. 1. Tumor-associated degradative enzymes and plasma proteinases: their possible roles in metastatic processes and their regulation by inhibitors derived from tumor and normal cells. The bold solid lines represent the release of enzymes or activation of proenzymes. The broken lines represent possible inhibitory mechanisms. The numbers represent the possible roles of degradative enzymes: **(1)** platelet aggregation, **(2)** fibrinolysis, **(3)** lamininolysis, **(4)** fibronectinolysis, **(5)** collagenolysis, **(6)** proteoglycanolysis, **(7)** elastinolysis, **(8)** host cell lysis, **(9)** other activities. Abbreviations: α_1PI, α_1-proteinase inhibitor; α_2-MG, α_2-macroglobulin.

collagenous and noncollagenous protein components while protecting them against proteolytic attack [28,29]. Thus, the destruction of the HS proteoglycan barrier could be important in basement membrane invasion by tumor cells.

The interactions between malignant cells and vascular endothelium have been studied by using monolayers of cultured vascular endothelial cells that synthesize an extracellular matrix resembling a basement membrane [15–17]. With this model, Kramer et al [16] found that metastatic B16 melanoma cells degrade matrix glycoproteins such as fibronectin and sulfated glycosaminoglycans, but predominantly HS. Since HS was released in solution as fragments approximately one-third their original size, it was proposed that metastatic tumor cells have an HS endoglycosidase [16]. We have examined the relationship between metastatic properties and the ability of the five B16 melanoma sublines of various implantation and invasion characteristics to enzymatically degrade subendothelial extracellular matrix and have found that highly invasive and metastatic B16 sublines degrade sulfated glycosaminoglycans faster than did sublines of lower metastatic potential [17].

Using purified lung HS, intact B16 cells (or their cell extracts) with a high potential for lung colonization degrade HS at higher rates than B16 cells with a poor potential for lung colonization [17]. We then examined the abilities of B16 cells to degrade HS from various origins and other purified glycosaminoglycans such as heparin, chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate, keratan sulfate and hyaluronic acid [18,30,31]. HS purified from basement-membrane producing EHS sarcoma [18] and PYS-2 carcinoma [30], lung, kidney, and extracellular matrices of bovine aortic endothelial [18], and corneal endothelial cells [31] was degraded into HS fragments of characteristic molecular weight, in contrast to other glycosaminoglycans listed above, which were essentially undegraded [18]. Interestingly, heparin but not other glycosaminoglycans inhibited HS degradation [18]. The time dependence of HS degradation into particular molecular weight fragments indicated that melanoma heparanase cleaves HS at specific intrachain sites.

In order to determine the heparanase-specific HS cleavage points, the newly formed reducing termini of HS fragments were investigated. From this analysis the HS-degrading enzyme was identified as an endo-β-D-glucuronidase that cleaves β-D-glucuronosyl-N-acetylglucosaminyl linkages of the HS molecule [18]. To distinguish the melanoma HS-degrading endoglucuronidase from HS-specific elimination enzymes, such as heparitinase (EC 4.2.2.8) from *Flavobacterium heparinum*, we proposed that the melanoma enzyme should be called a heparanase (Fig. 2) [18].

HEPARAN-SULFATE-DEGRADING ENDOGLYCOSIDASES IN NORMAL AND TUMOR CELLS

The enzymatic degradation of HS proteoglycan by tumor cells has now been observed by several laboratories [19,22,24,32–34]. Vlodavsky et al [24] found that highly metastatic ESb T-lymphoma cells degrade HS proteoglycans present in the subendothelial matrix into low-molecular-weight HS fragments at faster rates than the less-invasive Eb subline. Becker et al [22] reported the degradation of HS proteoglycans in bovine corneal endothelial cell extracellular matrix by metastatic variants of rat rhabdomyosarcoma cells and a correlation between their enzyme activity and spontaneous metastatic potential was established. Ricoveri and Cappelletti [32] noted that cell extracts or intact metastatic mouse fibrosarcoma and B16 melanoma degraded

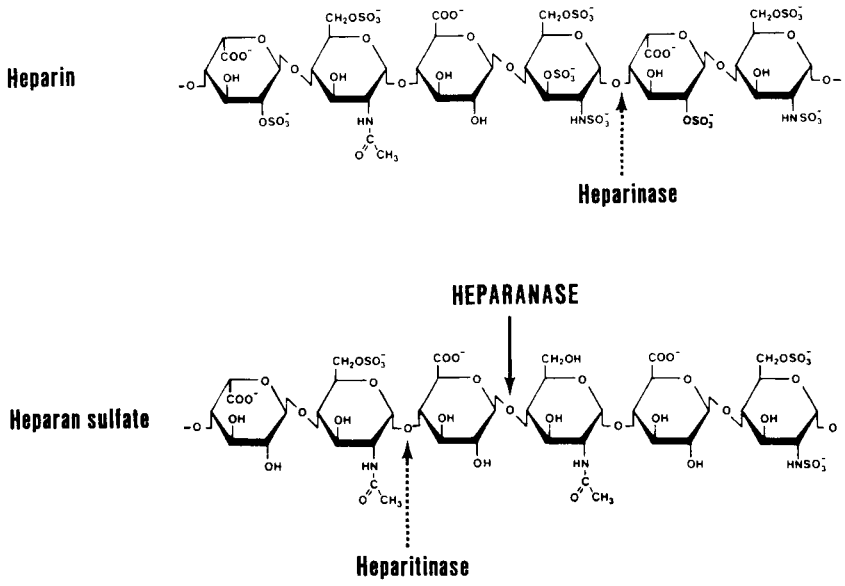

Fig. 2. Specificities of melanoma heparanase (endo-β-D-glucuronidase) and Fravobacter eliminases such as heparinase (EC 4.2.2.7) and heparitinase (EC 4.2.2.8).

HS faster than did their nonmetastatic counterparts. The optimum pH for HS degradation by HS endoglycosidases of mouse fibrosarcoma and B16 melanoma cells was about 5.6, but significant activity is still present at physiological pH [17,18,32].

To perform rapid microquantitative assays of large sample numbers of heparanase, we recently developed a solid-phase HS substrate by crosslinking of partially N-desulfated and N-[^{14}C or ^{3}H]acetylated HS onto agarose beads via one covalent linkage [35]. With this solid-phase substrate, several malignant human melanoma cell lines and normal cells such as fibroblasts and endothelial cells were assayed for HS-degrading activity [35,36]. All of the 15 human melanoma cells tested had HS-degradative activity, and some possessed activities comparable to that of mouse B16-F10 melanoma cells [36]. However, normal mouse skin fibroblasts and aortic endothelial cells showed very low activities [35] (Table I). These results suggest that melanoma cells generally possess heparanase and that this enzyme may perform an important role in human melanoma metastasis.

As stated above, HS-degrading endoglycosidase activity (Table II) is not unique to metastatic tumor cells, and the following normal tissues and cells have been found to possess HS-degrading endoglycosidases: liver [37,38], spleen [39], skin fibroblasts [40], placenta [41], platelets [42–45], T-lymphocytes [46,47], and inflammatory macrophages [47,48]. Enzymes present in rat liver, human skin fibroblasts, human placenta, and human platelets have also been identified as endoglucuronidases [38,40,41,43,44]. In some cases these normal cell enzymes have been shown to be different from tumor enzymes. For example, a platelet endo-β-D-glucuronidase different from melanoma heparanase have been purified by Oosta et al [44]. This normal cell enzyme is capable of degrading both HS and heparin and has an M_r of 134,000. In contrast, heparin is a potent inhibitor of melanoma heparanase [18].

TABLE I. Heparan Sulfate (HS) Degradation Activity in Mouse and Human Malignant Melanoma Cells [35,36]

Cell line	HS degradation activity[a]	
	Mean cpm ± SD	Ratio[b]
Mouse		
B16-F1	341 ± 32	1.00
B16-F10	510 ± 34	1.50
B16-BL6	758 ± 58	2.22
B16-B15b	716 ± 71	2.10
Human		
SK-MEL-19	379 ± 40	1.11
SK-MEL-23	397 ± 29	1.16
SK-MEL-93 (D×1)	625 ± 36	1.83
SK-MEL-93 (D×3)	381 ± 25	1.12
SK-MEL-93 (D×6)	703 ± 19	2.06
Hs 294T	381 ± 44	1.12
Hs 852T	202 ± 16	0.59
Hs 939	619 ± 44	1.82
T294	366 ± 15	1.07
M40	787 ± 75	2.31
RON	457 ± 27	1.34
BMCL	118 ± 31	0.35
A375 parent	392 ± 38	1.15
A375 Met Mix	659 ± 22	1.93
A375 M6	612 ± 48	1.79
Mouse skin fibroblasts	58 ± 17	0.17
Bovine aortic endothelia cells	42 ± 18	0.12

[a]Heparanase assay was carried out by the incubation of a Triton X-100 cell extract (2.4×10^5 cells) with PNDS-N[^{14}C]Ac-HS immobilized on agarose beads (4,500 cpm) at 37°C for 12 hr. The amount of radioactivity released in the presence of heat-inactivated enzymes (52 ± 12 cpm) was subtracted from the raw data (n = 3).
[b]Activity relative to the activity of B16-F1 (1.00).

We have purified melanoma heparanases from mouse B16-F10, human A375 Met, and Hs939 melanoma cells by using heparin-Sepharose, concanavalin A–Sepharose, and N-acetylated N-desulfated heparin-Sepharose affinity column chromatography. The human and mouse melanoma heparanases are of M_r ~96,000, as determined by SDS–polyacrylamide gel electrophoresis, and their pI is 5.2. At neutral pH such heparanases are highly active, while they are totally inactive at pH less than 5 and above 8.

The results of the studies on enzyme substrate specificities [18,37,38,45] suggest that there are at least three different types of mammalian cell endo-β-D-glucuronidases. Melanoma and liver glucuronidases appear to degrade HS but not heparin [18,37,38]. The human platelet enzyme depolymerizes both HS and heparin and cleaves the β-glucuronidic linkage in the antithrombin-binding octasaccharide of heparin molecules [45]. Another endoglucuronidase from mouse mastocytoma catalyzes the depolymerization of macromolecular heparin proteoglycans into fragments similar in size to commercial heparin [49,50]. The mastocytoma enzyme has little or no activity against HS and does not cleave the antithrombin-binding regions of the heparin [45,49].

TABLE II. HS-Degrading Endoglycosidases in Mammalian Cells

Normal cells	
Rat liver cells	Höök et al [37]
	Kjellén et al [38]
Rat spleen cells	Höök et al [39]
Human skin fibroblasts	Klein et al [40]
Human placenta	Klein and von Figura [41]
Human platelets	Wasteson et al [42]
	Oldberg et al [43]
	Oosta et al [44]
	Thunberg et al [45]
Rat activated T-lymphocytes	Naparstek et al [46]
Murine-activated T-lymphocytes	Savion et al [47]
Murine inflammatory macrophages	Savion et al [47]
Rat resident, alveolar, intratumoral, and inflammatory macrophages	Nakajima et al [48]
Tumor cells	
Murine melanoma cells	Kramer et al [16]
	Nakajima et al [17,18]
	Vlodavsky et al [19]
	Ricoveri and Cappelletti [32]
Murine T-lymphoma cells	Vlodavsky et al [24]
	Bar-Ner et al [34]
Human melanoma cells	Nakajima et al [35,36]
Rat rhabdomyosarcoma cells	Becker et al [22]
Murine fibrosarcoma cells	Ricoveri and Cappelletti [32]
Human colon carcinoma cells	Iozzo [33]
Rat mammary adenocarcinoma cells	Nakajima et al (unpublished)

HEPARANASE INHIBITORS

To confirm the biological significance of heparanases in metastatic processes, inhibitors against these enzymes have been used. Heparin is a potent inhibitor of heparanase; however, it has a variety of other biological activities, including anticoagulation [51,52] (Fig. 1). In certain experimental tumor systems heparin protects against metastases [53–55], while in others enhancement of tumor cell dissemination occurs [56–58]. The inhibitory effects of heparin on metastasis are thought to be mainly a consequence of the inhibition of blood coagulation and platelet aggregation that are known to facilitate implantation of tumor cells in organ capillaries. Most heparins are actually mixtures of polysaccharides that have intrinsic heterogeneity and molecular diversity; thus it is difficult to determine the role of particular structures in their complex biological activities.

We have attempted to ascertain the structural requirement for heparanase inhibition and find potent heparanase inhibitors that lack anticoagulation activity by chemically modifying heparin molecules [59]. Heparin modifications such as desulfation, deacetylation, sulfation, acetylation, and carboxyl-reduction were performed, and among several modified heparins, N-acetylated N-desulfated heparin, N-resulfated N- and O-desulfated heparin, and carboxyl-reduced heparin were found to maintain their heparanase inhibitory activity (Table III). These substrates also inhibited the degradation of mouse lung endothelial cell extracellular matrix by B16 melanoma cells. Treatment of B16-BL6 melanoma cells with the heparanase inhibitors resulted in significant reductions in the numbers of experimental melanoma lung

TABLE III. Effects of Heparin Derivatives on Heparanase Activity and Blood-Borne Lung Colonization of B16-BL6 Melanoma Cells in Mice [59]

Treatment	Heparanse inhibition[a]	Lung colonies[b]	
		No.	Median
None	0	0, 1, 26, 48, 75, 163, 193, 200+, 200+	75
Heparin	100.7	0, 0, 0, 0, 0, 1, 1, 2, 12	0
N-acetylated N-desulfated heparin	88.5	0, 1, 2, 3, 5, 5, 25, 37, 200+	5
N-resulfated N-, O-desulfated heparin	40.3	0, 0, 2, 5, 8, 13, 20, 90, 200+	8
Carboxyl-reduced heparin	53.2	7, 13, 42, 49, 51, 55, 58, 89, 120	51

[a]Heparanase activity was measured by incubating B16-BL6 melanoma cell lysates equivalent to 10^6 cells with a suspension of [^{3}H]HS-agarose beads and 1.25 mg/ml heparanase inhibitor at 37°C for 24 hr.
[b]The cells were incubated with chemically modified heparins (500 μg/ml) at 4°C for 2 hr before intravenous injection of 5×10^4 cells/0.1 ml into C57BL/6 mice. Experimental blood-borne metastasis to lung and other organs was determined after 20 days.

metastases in syngeneic mice (Table III) [59], supporting our hypothesis that heparanase plays an important role in melanoma metastasis. It is known that surgery, chemotherapy, and radiotherapy may enhance tumor cell dissemination. Therefore, antimetastasis treatments employing degradative enzyme inhibitors such as heparanase inhibitors may be useful in combination with other therapies to prevent further tumor spread.

SERUM ENZYME LEVELS AND TUMOR METASTASIS

The expression of certain serum proteins, such as carcinoembryonic antigen [60], α-fetoprotein [61], and placentalike alkaline phosphatase [62], is associated with some human cancers, and their immunologic detection has been used diagnostically for neoplastic disease. High levels of glycosyltransferases, such as sialyl- and galactosyltransferases, have been found in the sera of animals bearing spontaneously metastasizing tumors [63–65]. There is no clear functional relationship, however, between elevated serum levels of such components and metastatic disease.

Since tissue-degrading enzymes are secreted in high amounts from invasive tumor cells, the levels of such enzymes in body fluids could be useful as diagnostic markers of tumor invasion and metastasis. In fact, high levels of β-N-acetylglucosaminidase and β-glucuronidase were found in the sera of animals and patients with various types of tumors [11]. The release of these exoglycosidases from lysosomes has been related to the ability of Lewis lung carcinoma cells to form lung metastases [66], and changes in dipeptidase and acid proteinase activities in blood plasma of mice were associated with the presence of ascites tumors [67]. Pietras et al [68] investigated hydrolytic enzyme activities in sera of 121 women with invasive cancer and found that cathepsin B1–like activity in sera before therapy was an average of 45 times greater than that of normal controls, and cathepsin B1–like activity increased

TABLE IV. HS-Degrading Activities in the Sera From Rats Subcutaneously Injected With Highly Metastatic 13762NF Mammary Adenocarcinoma Cells [70]

Days after injection of cells (or DPBS)	HS-degrading activity in the sera, U/ml[a] (control rats injected with DPBS)	
0	0.12	(0.12)
9	0.10	(0.10)
16	0.46	(0.11)
23	0.96	(0.12)
30	2.01	(0.12)

No. of metastases[b]	(No. of rats)	HS-degrading activity in the sera, U/ml[a]
Lung		
0	(8)	0.47
1–10	(3)	0.44
>250	(10)	1.91
Lymph nodes		
0	(4)	0.10
1–2	(5)	0.49
>4	(12)	1.77

[a]HS-degrading activity was measured by incubation of ^{3}H-labeled solid-phase heparanase substrates with the serum diluted tenfold with 0.1 M sodium acetate, 0.15 M sodium chloride, pH 6.0. One unit of activity refers to the amounts of enzyme that liberates 1 μg of HS per minute.

[b]Rats were killed at various periods after subcutaneous injection of 1×10^6 MTLn3 (T44).5 cells in 0.1 ml of Dulbecco's phosphate-buffered saline (DPBS) and the numbers of metastases and serum enzyme activities were measured.

progressively during the transition from preinvasive to invasive cervical carcinoma. Ishihara et al [69] found increased neutral proteinase activity in the sera of rats bearing AH109A ascites hepatoma cells. The partially purified serum protease fraction contained both serine and cysteine proteinase activities and degraded pepsin-treated chains of basement membrane type IV collagen [69].

We have found that HS-degrading endoglycosidase is released from metastatic tumor cells, and this enzyme can circulate in the body fluids of tumor-bearing animals. We have assessed the sera from rats bearing mammary adenocarcinomas of differing metastatic potentials for this enzyme [70]. HS-degradative activity in sera diluted tenfold increased with time after subcutaneous injection of highly metastatic MTLn3 mammary adenocarcinoma cells in female F344 rats (Table IV). In contrast, sera from rats bearing mammary adenocarcinoma of low metastatic potential, such as clone MTC, possessed low levels of enzyme, even 30 days after tumor cell injection. In contrast to MTLn3 cells, clone MTC cells remained localized at the injection site and did not metastasize to regional lymph nodes or distant sites. Enzyme activities in the sera of rats bearing subcutaneous MTLn3 tumors correlated with extent of metastases. Rats with large numbers of metastases in the lung and/or lymph nodes had much higher activities of HS-degradative enzymes in their sera than rats with few or no metastases (Table IV). These results suggest that the activity of heparanase in

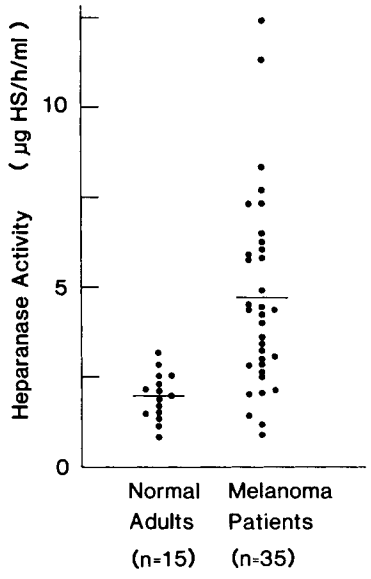

Fig. 3. Heparan sulfate degrading activity in sera of normal adults and malignant melanoma patients. The enzyme activity was measured by using solid-phase assay substrates (Table I).

serum is related to the occurrence of metastases to lung and lymph nodes. Thus, HS-degradative enzymes may be useful serum markers of tumor metastasis.

Since human malignant melanoma tissues have high heparanase activities and the heparanase is released from melanoma cells, we tested sera from malignant melanoma patients and normal adults for heparanase (Fig. 3). The average serum heparanase activities of melanoma patients (n=35) and normal adults (n=15) were 3.72 and 1.98 U/ml serum, respectively ($P < .05$). The sera from the patients having metastases in lymph nodes, liver, lung, and brain showed more than four times the level of heparanase activity than normal controls ($P < .001$). The source of HS-degradative activity in the normal adult serum includes platelets and other blood cells, because heparin-degrading activity was also detected. Therefore, the difference in serum heparanase may be much greater in patients with metastatic disease than we have estimated. A more specific enzyme assay for tumor-associated heparanaase should lead to more accurate analyses of clinical samples.

CONCLUSION

The development of sensitive methods to diagnose and predict metastasis and to monitor the therapy of metastases is an important goal. Studies on the role of basement-membrane degrading enzymes in tumor metastasis led to our hypothesis that such tumor enzymes may be useful markers for tumor metastasis and also possible targets to block the process of metastasis. Although basement-membrane degradative enzymes are not unique to malignant tumor cells; elevated levels of these enzymes in serum have been found to be associated with many types of metastatic tumors, and certain enzyme inhibitors were shown to have antimetastatic effects. Future research on metastasis-associated-degradative enzymes should allow the development of new approaches to diagnose and monitor tumor metastases, and this could lead to the synthesis of unique inhibitors of metastatic disease.

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REFERENCES

1. Nicolson GL: Biochim Biophys Acta 695:113, 1982.
2. Nicolson GL, Poste G: Int Rev Exp Pathol 25:77, 1983.
3. Jones PA, De Clerck YA: Cancer Metastasis Rev 1:289, 1982.
4. Mullins DE, Rohrlich ST: Biochim Biophys Acta 695:177, 1983.
5. Pauli BU, Schwartz DE, Thonar EJ-M. Kuettner KE: Cancer Metastasis Rev 2:129, 1983.
6. Terranova VP, Hujanen ES, Martin GR: J Natl Cancer Inst 77:311, 1986.
7. Liotta LA, Thorgeirsson UP, Garbisa S: Cancer Metastasis Rev 1:277, 1982.
8. Wooley DE: Cancer Metastasis Rev 3:361, 1984.
9. Sloane BF, Honn KV: Cancer Metastasis Rev 3:249, 1984.
10. Danø K, Andreasen PA, Grondahl-Hansen J. Kristense P, Nielsen LS, Skriver L: Adv Cancer Res 44:139, 1985.
11. Bernacki RJ, Niedbala MJ, Korytnyk W: Cancer Metastasis Rev 4:81, 1985.
12. Vracko R: Am J Pathol 77:313, 1974.
13. Kefalides NA, Alper R, Clark CC: Int Rev Cytol 61:167, 1979.
14. Liotta LA, Abe S, Robey PG, Martin GR: Proc Natl Acad Sci USA 76:2268, 1979.
15. Nicolson GL: J Histochem Cytochem 30:214, 1982.
16. Kramer RH, Vogel KG, Nicolson GL: J Biol Chem 257:2678, 1982.
17. Nakajima M, Irimura T, Di Ferrante D, Di Ferrante N, Nicolson GL: Science 220:611, 1983.
18. Nakajima M, Irimura T, Di Ferrante N, Nicolson GL: J Biol Chem 259:2283, 1984.
19. Vlodavsky I, Ariav Y, Atzmon R, Fuks Z: Exp Cell Res 140:145, 1982.
20. Kramer RH, Vogel KG: J Natl Cancer Inst 72:889, 1984.
21. Starkey JR, Hosick HL, Stanford DR, Liggitt HD: Cancer Res 44:1585, 1984.
22. Becker M, Moczar M, Poupon MF, Moczar E: J Natl Cancer Inst 77:417, 1986.
23. Liotta LA, Tryggvason K, Garbisa S, Hart I, Foltz CM, Shafie S: Nature (Lond) 284:67, 1980.
24. Vlodavsky I, Fuks Z, Bar-Ner M, Ariav Y, Schirrmacher V: Cancer Res 43:2704, 1983.
25. Sloane BF, Dunn JR, Honn KV: Science (Wash) 212:1151, 1981.
26. Parthasarathy N, Spiro RG: J Biol Chem 256:507, 1981.
27. Kanwar YS, Linker A, Farquhar MG: J Cell Biol 86:688, 1980.
28. Hascal VC: In Ginsburg V (ed): "Biology of Carbohydrates." New York: John Wiley and Sons, 1981, pp 1–49.
29. Chiarugi VP: Anticancer Res 2:275, 1982.
30. Irimura T, Nakajima M, Di Ferrante N, Nicolson GL: Anal Biochem 130:461, 1983.
31. Wang Z-W, Irimura T, Nakajima M, Belloni PN, Nicolson GL: Eur J Biochem 153:125, 1985.
32. Ricoveri W, Cappelletti R: Cancer Res 46:3855, 1986.
33. Iozzo RV: J Biol Chem 262:1888, 1987.
34. Bar-Ner M, Kramer MD, Schirrmacher V, Ishai-Michaeli R, Fuks Z, Vlodavsky I: Int J Cancer 35:483, 1985.
35. Nakajima M, Irimura T, Nicolson GL: Anal Biochem 157:162, 1986.
36. Nakajima M, Irimura T. Nicolson GL: Cancer Lett 31:277, 1986.
37. Höök M, Wasteson Å, Oldberg Å: Biochem Biophys Res Commun 67:1422, 1975.
38. Kjellén L, Pertoft H, Oldberg Å, Höök M: J Biol Chem 260:8416, 1985.
39. Höök M, Pettersson I, Ögren S: Thromb Res 10:857, 1977.
40. Klein U, Kresse H, von Figura K: Biochem Biophys Res Commun 69:158, 1976.
41. Klein U, von Figura K: Biochem Biophys Res Commun 73:569, 1976.
42. Wasteson Å, Glimelius B, Busch C, Westermark B, Heldin C-H, Norling B: Thromb Res 11:309, 1977.
43. Oldberg ÅA, Heldin C-H, Wasteson Å, Busch C, Höök M: Bioichemistry 19:5755, 1980.

44. Oosta GM, Favreau LV, Beeler DL, Rosenberg RD: J Biol Chem 257:11249, 1982.
45. Thunberg L, Bräckström G, Wasteson Å, Robinson HC, Ögren S, Lindahl U: J Biol Chem 257:10278, 1982.
46. Naparstek Y, Cohen IR, Fuks Z, Vlodavsky I: Nature 310:241, 1984.
47. Savion N, Vlodavsky I, Fuks Z: J Cell Physiol 118:169, 1984.
48. Nakajima M, North S, Irimura T, Nicolson GL: J Cell Biol 101:215a, 1985.
49. Ögren S, Lindahl U: J Biol Chem 250:2690, 1976.
50. Robinson HC, Horner AA, Höök M, Ögren S, Lindahl U: J Biol Chem 253:6687, 1978.
51. Jaques LB: Pharmacol Rev 31:99, 1980.
52. Casu B: Adv Carbohydr Chem Biochem 43:51, 1985.
53. Hagmar B, Norrby K: Int J Cancer 5:72, 1970.
54. Tsubura E, Yamashita T, Kobayashi M, Higuchi Y, Isobe J: Gann Monogr Cancer Res 20:147, 1977.
55. Hilgard P: In Nicolson GL, Milas L (eds): "Cancer Invasion and Metastasis: Biologic and Therapeutic Aspects." New York: Raven Press, 1984, pp 353–360.
56. Maat B: Br J Cancer 37:369, 1978.
57. Hagmar B, Boeryd B: Pathol Eur 4:274, 1969.
58. Chan SY, Pollard M: J Natl Cancer Inst 64:1121, 1980.
59. Irimura T, Nakajima M, Nicolson GL: Biochemistry 25:5322, 1986.
60. Gold, P, Freedman SO: J Exp Med 121:439, 1965.
61. Abelev GI: Adv Cancer Res 14:295, 1971.
62. Kellen JA, Bush RS, Malkin A: Cancer REs 36:269, 1976.
63. Chatterjee SK: Eur J Cancer 15:1351, 1979.
64. Bernacki RJ, Kim U: Science 195:577, 1977.
65. Kondo Y, Sato K, Ueyama Y, Ohsawa N: Cancer Res 41:2912, 1981.
66. Dobrossy L, Pavelic ZP, Vaughan M, Porter N, Bernacki RJ: Cancer Res 40:3281, 1980.
67. Ottoson R, Sylvén B: Arch Biochem Biophys 87:41, 1960.
68. Pietras RJ, Szego CM, Mangan CE, Seeler BJ, Burtnett MM: Gynecol Oncol 7:1, 1979.
69. Ishihara A, Nabeshima K, Koono M: Invasion Metastasis 6:225, 1986.
70. Nakajima M, Welch DR, Irimura T, Nicolson GL: Prog Clin Biol Res 212:113, 1986.