Tumor Cell Invasion of Three-dimensional Matrices of Defined Composition: Evidence for a Specific Role for Heparan Sulfate in Rodent Cell Lines

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ABSTRACT

The abilities of rodent tumor cell lines; B16BL6, ND and LT dietary variants of B16BL6, +SA, RT7-4bs and RT7-4bLs to invade composite collagen I gels containing heparin, chemically modified heparins, heparan sulfate, chondroitin sulfate, hyaluronic acid, dextran, dextran sulfate, laminin and collagen IV were investigated, and compared to the invasion of plain collagen I gels. The presence of heparin or heparan sulfate most generally promoted tumor cell invasion of the gels, with more aggressive invasion being noted for the more metastatic variants examined. Of the chemically modified heparins tested, carboxyl-reduced heparin promoted matrix invasion by B16BL6 and +SA cells to the greatest degree. Hyaluronic acid marginally promoted invasion by +SA and RT7-4bs primary cells while, in these collagen I based gels laminin only promoted matrix invasion by primary +SA cells to a very limited degree. The tumor cell lines attached relatively poorly to heparan sulfate substrates compared to the other glycosaminoglycans tested, and the primary tumor cell lines also attached relatively poorly to collagen I. As expected, highly metastatic variants showed greater attachment to laminin than did their less metastatic counterparts. Apart from the negative correlation of cellular attachment to heparan sulfate substrates with invasiveness towards heparan sulfate containing gels, no other relationships emerged linking attachment rates with invasive activities for particular complex gel compositions. Our results suggest an important role for heparan sulfate, and possibly also tissue heparin, in promoting tumor cell invasion of extracellular matrices. Results from complex gels containing dextran or dextran sulfate failed to support the hypothesis that GAG sulfation is important to cellular invasion. The activity of the chemically modified heparins in promoting invasion, when present as components of these model matrices, suggests that part of the anti-metastatic activity of these compounds, when preincubated with tumor cells prior to intravenous inoculation, could result from interference with tumor cell extravasation.

INTRODUCTION

Tumor cells traverse extracellular matrices during the processes of invasion and metastasis. The ECM which is most relevant to metastasis is the basal lamina or basement membrane. Basement membranes are ubiquitous in an organism, including the vascular system where they separate the endothelium from extravascular and peripheral vascular elements. They form important physical barriers a tumor cell must traverse to go from one cell layer to another or to metastasize via the blood stream. Basement membranes are composed mainly of collagen type IV intermeshed with laminin, entactin, fibronectin, and proteoglycans, of which heparan sulfate is the principal glycosaminoglycan (2-4). Three-dimensional collagen I gels have been employed to study cellular invasion of biological matrices. Although the presence of enzymes active in matrix degradation is frequently found to correlate with metastatic ability, degradation of substrate components does not always correlate with invasion of ECM (5). Schor (5, 6) found considerable variation in the abilities of human and other mammalian normal and neoplastic cell lines to infiltrate collagen I gels. When these gels were overlaid with basement membrane matrix, infiltration by otherwise aggressively invasive hamster melanoma cells was found to be negligible, although basement membrane GAG and collagen destruction occurred. These findings implied that degradation of ECM components was not necessarily accompanied by invasion. Furthermore, penetration of collagen I gels by this tumor cell line did not involve collagen I degradation (5). Heparan sulfate and fibronectin present in basement membrane matrix synthesized in vitro by endothelial cells are degraded by B16BL6 mouse melanoma cells (7), and Nakajima and coworkers (8, 9) documented specific degradation of the heparan sulfate GAG. The possession of tumor cell metalloprotease activity specific for collagen IV correlates well with metastatic ability (10, 11), and this enzyme activity is augmented by cellular interaction with laminin (12). Tumor cells and conditioned media from the murine mammary tumor lines, +SA and -SA, the murine melanoma, B16BL6 and the rat hepatocarcinoma lines, RT7-4bs and RT7-4bLs have been shown to degrade collagen IV within bovine lens capsule basement membrane matrix (13), and heparan sulfate within this matrix has also been shown to be degraded by conditioned media from B16BL6 cultures (13). Overall, a majority of experimental evidence indicates a close relationship between ECM degradation and tumor cell invasion and metastasis. Nevertheless, other results (5, 6, 14) indicate that the relationship may be far from simple. In the current study, we determine whether the presence of laminin, collagen IV, or ECM GAG species enhance collagen gel infiltration by tumor lines with different capabilities for invasion and metastasis.

MATERIALS AND METHODS

Cell Lines, Culture Conditions, and Animals. The cell lines used in this study, chosen for having spontaneous metastatic abilities, were: the highly invasive metastatic B16BL6 murine melanoma line (15), two lung colonizing variants of the B16BL6 line derived from tumor-bearing mice fed experimental diets (16), the metastatic murine mammary adenocarcinoma line +SA (17) and the highly invasive and metastatic rat hepatocarcinoma cell lines, RT7-4bs and RT7-4bLs (18, 19). The B16BL6 variants were isolated from spontaneous lymph node metastases of tumor-bearing mice fed either a diet low in tyrosine and phenylalanine (B16BL6-LT variant) or fed a nutritionally adequate diet (B16BL6-ND variant) (16, 20, 21). B16BL6-LT is much less lung colonizing than B16BL6-ND (Table 1) (1). The B16BL6-ND and B16BL6-LT cell populations used in the studies described herein are two in vitro passages later than those assayed for lung colonizing ability in Table 1. The B16BL6 line was kindly supplied by Dr. I. J. Fidler, Frederick Cancer Research Institute, National Cancer Institute, Frederick, MD. The +SA line, generously provided by Dr. H. L. Hosick, Zoology, Washington State University, Pullman, WA, is highly lung colonizing and exhibits anchorage-independent growth in vitro (17). The RT7-4bs and RT7-4bLs metastatic variants were derived from the IAR6-1-RT7 cell line, a chemically transformed epithelial parenchymal liver cell line from the BD-IV rat (18, 19). The RT7-4bLs cell line

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1 Supported by NIH Grant CA39611 and Grant 85B79 from the American Institute for Cancer Research.
2 To whom requests for reprints should be addressed.
3 The abbreviations used are: ECM, extracellular matrix; GAG, glycosaminoglycan; FBS, fetal bovine serum; EHS, Englebreth-Holm-Swarm.
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Table 1. Lung colonization abilities of metastatic variants

<table>
<thead>
<tr>
<th>Variant</th>
<th>Number of animals</th>
<th>Number of tumor lung colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT7-4bs</td>
<td>20</td>
<td>45</td>
</tr>
<tr>
<td>RT7-4bLs</td>
<td>10</td>
<td>75</td>
</tr>
<tr>
<td>B16BL6-LT</td>
<td>12</td>
<td>89</td>
</tr>
<tr>
<td>B16BL6-ND</td>
<td>12</td>
<td>206</td>
</tr>
</tbody>
</table>

* Value different from that for RT7-4bs (P = 0.014) by Wilcoxon's two sample rank sum test.

** Value different from that for B16BL6-ND (P < 0.01) by Wilcoxon's two sample rank sum test.

Data previously published in extended form in Ref. 16.

is more highly lung colonizing than the RT7-4bs line (Table 1). The RT7-4bLs and RT7-4bs cell populations used in the studies described herein are from the same passages as those assayed for lung colonization in Table 1.

Primary cultures were prepared from tumor tissue as described previously (18). Briefly, nonneoplastic tumor tissue was harvested from tumor-bearing animals and passed through a 70-mesh stainless steel screen in serum containing medium. The cells were then pelleted by centrifugation, washed twice in complete RPMI medium, and aliquots were used for cell culture.

The cells were then plated in 96-well microtiter plates at 10,000 cells per well, and the gels allowed to set at 24°C for 2 h before being preincubated for 2 h with 2 ml of complete RPMI medium in a 37°C humidified incubator with a 7% CO2 in air atmosphere. Isolation procedures were designed to avoid premature gelling which altered the collagen so that it no longer gelled in the presence of some matrix components such as heparin. Collagen which had prematurely gelled was not used in subsequent experiments.

Heparan Sulfate Preparation. Anterior lens capsules were obtained from adult bovine eyes procured from local slaughterhouses as described in Ref. 18. The capsules were lyophilized and weighed. Heparan sulfate was then isolated, quantified, and checked for purity as described elsewhere (13). A mix of isomers of heparan sulfate was also obtained from the EHS tumor using two methods. The first method employed pronase digests of EHS proteoglycans obtained from cesium chloride gradients of guanidinium chloride extracted material (27). When analyzed by cellulose acetate electrophoresis in barium acetate buffer (7%) as well as by degradation by both nitrous acid (28), and crude Flavobacterium heparinase (29), this material yielded at least two kinds of heparan sulfate chains. Secondly, EHS heparan sulfate was also prepared from tumor tissue as follows: To 100 mg of tissue was added 200 ml methanol, and the mixture was homogenized with a Virtis apparatus, followed by addition of 100 ml chloroform. The homogenate was delipidated according to the protocol of Svennerholm et al. (30). The chloroform-methanol insoluble residue was air dried, weighed, and resuspended in 60 ml 0.2 M Tris buffer (pH 8.0), with 0.5 mg Pronase (Calbiochem, La Jolla, CA) added. Pronase treatment was for 72 h at 55°C with three additions of fresh enzyme. After all enzyme treatments, undigested material was collected using the ethanol precipitation method of Doctor et al. (31). Enzymes were inactivated by boiling for 5 min. The ethanol precipitate was then treated with deoxyribonuclease I (Sigma) under a layer of toluene for 24 h, using the protocol described in the "Worthington Enzymes" handbook (Worthington Biochemical Corporation, Freehold, NJ, 1977). The ethanol precipitate was further treated with bovine pancreatic ribonuclease (Calbiochem, La Jolla, CA) for 24 h under toluene using the Worthington procedures. Removal of these enzymes at the end of the digests and also after digestion of isolated EHS GAG was by means of TCA precipitation (31). Cellulose acetate electrophoresis revealed the presence of contaminating hyaluronic acid, and chondroitin and dermatan sulfates. The latter were eliminated using chondroitin sulfate lyase ABC digestion (Sigma) for 24 h. Since this does not remove all of the hyaluronate under the conditions ideal for complete digestion of the other two GAG, the residual GAG were further digested with Streptomyces hyaluronidase (33) at 55°C for 2.5 h. The residual GAG was shown to be heparan sulfate by cellulose acetate electrophoresis of aliquots treated with Flavobacterium heparinase. The amount of heparan sulfate in EHS and lens capsule GAG was assayed by uronic acid analysis (34) using...
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D-glucurononolactone as a standard. Similar heparan sulfate preparations were obtained from the EHS tumor regardless of which protocol was used.

Analysis of Gel Matrix Invasion. Cellular invasion of the collagen I gels was determined by focusing below the surface of the gels using a Nikon phase contrast inverted tissue culture microscope. The optical distortion created by the meniscus of the gel at the dish periphery limited counting to those cells that penetrated the flat central portion of the gel. A grid within a circle of 2.5 cm diameter was drawn, having 69 complete squares, 2.5 by 2.5 mm each. Gels were centered on this grid, and cells present within the gel overlying these squares were counted. In this manner, the entire central area (55% of the total area) of each gel was evaluated for cellular invasion. Infiltrating cells were tallied according to aggregate size, with four categories being defined: single cells, two to four cells, five to eight cells, and ≥9 cells. Cells within the gels were first enumerated when at least 100 single cells had infiltrated one of the gels of an experimental set. Over the next 2 days additional cells infiltrated the gel after which time the counts of cells within the gels neither increased nor decreased. A second count of cells within the gels was made when this “plateau” was reached. The length of time elapsing before cells started to invade the gels varied most obviously with the method of collagen I preparation. For collagen gels formed from nonlyophilized collagen, the initial counts were done 4 days after plating and the final counts at 7 days after plating. Collagen which had been lyophilized prior to use delayed cellular invasion so that significant infiltration was not seen until 30 days postplating if at all. A grid within a circle of 2.5 cm diameter was drawn, having 69 complete squares, 2.5 by 2.5 mm each. Gels were centered on this grid, and cells present within the gel overlying these squares were counted. In this manner, the entire central area (55% of the total area) of each gel was evaluated for cellular invasion. 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Table 2: Invasion of collagen I gels by long term tissue culture passaged cells

<table>
<thead>
<tr>
<th>Secondary ECM gel component</th>
<th>Invasion of gels by single cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B16BL6</td>
</tr>
<tr>
<td>None</td>
<td>1.00</td>
</tr>
<tr>
<td>Collagen IV</td>
<td>2.07 (P = 0.09)</td>
</tr>
<tr>
<td>Laminin</td>
<td>0.28</td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>0.41</td>
</tr>
<tr>
<td>Chondroitin sulfate</td>
<td>1.24</td>
</tr>
<tr>
<td>Heparin</td>
<td>15.86 (P = 0.0001)</td>
</tr>
</tbody>
</table>

* Pooled weighted variance of data shown (log_{10} scale) = 0.015.
* Statistical analysis was carried out using Student’s t test applied to the log_{10} data values. All probabilities <0.099 are shown. Results from complex gels are compared to those from plain gels. *P value for comparison of invasion of heparin containing gels by B16BL6-ND cells and B16BL6-LT cells = 0.06.
* ND, not done.

Table 3: Invasion of gels prepared from nonlyophilized collagen I by cells isolated from primary tumor cell cultures

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B16BL6</td>
</tr>
<tr>
<td>None</td>
<td>1.00</td>
</tr>
<tr>
<td>Collagen IV</td>
<td>0.34</td>
</tr>
<tr>
<td>Laminin</td>
<td>0.54</td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>0.45</td>
</tr>
<tr>
<td>Chondroitin sulfate</td>
<td>0.58</td>
</tr>
<tr>
<td>Lens capsule heparan sulfate</td>
<td>3.69 (P = 0.049)</td>
</tr>
<tr>
<td>EHS heparan sulfate</td>
<td>6.19 (P = 0.015)</td>
</tr>
<tr>
<td>Dextran</td>
<td>1.92</td>
</tr>
<tr>
<td>Dextran sulfate</td>
<td>1.68</td>
</tr>
</tbody>
</table>

* Pooled weighted variance of the data shown (log_{10} scale) = 0.05.
* Statistical analysis was carried out using Student’s t test applied to the log_{10} data values. All probabilities <0.099 are shown. Results from complex gels are compared to those from plain collagen I gels. *P value for comparison of invasion of complex gels containing lens capsule heparan sulfate by RT7-4bs cells and RT7-4bLs cells = 0.044. P value for a comparison of invasion of complex gels containing EHS heparan sulfate by RT7-4bs and RT7-4bLs cells = 0.013.

Tumor cells took very much longer to invade gels made from collagen I which had been lyophilized prior to use, and some tumor cell lines failed to invade these gels at all. Lyophilization appeared to have altered the collagen I to the extent that it was no longer conducive to cellular infiltration. Cells derived from primary B16BL6 and +SA cultures actively invaded collagen I gels containing kidney heparan sulfate (Table 4). B16BL6 cells, however, did not invade gels containing kidney heparan sulfate as aggressively as they did similar gels containing lens capsule or EHS heparan sulfates (Tables 3 and 4). All three chemically modified heparins promoted invasion by +SA cells when present as components of the collagen I matrix gel. The enhancement of invasion over plain collagen I gels was modest for N-resulfated-N,O-desulfated heparin and for N-acetylated-N,O-desulfated heparin, while carboxyl-reduced heparin promoted invasion to the greatest extent. N-Acetylated-N,O-desulfated heparin and carboxyl-reduced heparin both promoted invasion of B16BL6 cells to a modest degree.

Tumor Cell Attachment to Extracellular Matrix Components. Attachment of cells to the various GAG species should be compared against attachment to tissue culture plastic as well as to plastic coated with polylysine. We used 50 µg/well polylysine and 200 µg/well GAG under the assumption that this 1:4 ratio would result in saturation or masking of all available polylysine attachment sites. This condition was probably achieved since

Fig. 1. Invasion of collagen I gels by metastatic tumor cells. A, light micrograph of living B16BL6 cells within a complex collagen I gel containing heparin (×320, phase-contrast); B, light micrograph of RT7-4bs cells invading a complex collagen I gel containing lens capsule heparan sulfate. The gel surface is to the top of the figure. Arrows, positions of tumor cells within the gel. Embedded in JB-4 and stained with hematoxylin & eosin (×300); C, electron micrograph of cells from a B16BL6 aggregate within a complex collagen I gel containing heparin (×25,000).
DISCUSSION

The major finding from these studies is that the presence of heparan sulfate or heparin GAGs promotes invasion of three-dimensional collagen I gels. Although only two sets of metastatic variants were examined, the extent of this selective invasion appears to be positively correlated with metastatic ability (B16BL6-ND versus B16BL6-LT and RT7-4bLs versus RT7-4bs). The two highly invasive and metastatic lines, B16BL6 and RT7-4bLs, preferentially invaded gels containing lens capsule derived heparan sulfate, and invaded those containing the EHS derived GAG to a lesser extent (Table 3).

Because the EHS heparan sulfate GAG is a tumor-derived matrix product, it may not be a completely normal basement membrane heparan sulfate, and it is possible that tumor cell lines respond differently to any abnormal structure.

Heparin-containing gels favor invasion to a degree similar to that noted for heparan sulfate (Table 2). It was much harder, however, to get heparin containing gels to set well routinely. This problem was also reported by Guidry and Grinnell (36), and may be related to the fact that the addition of heparin was found to prevent cell-associated gel contraction (36). Addition of dextran polysaccharides to the gels also enhanced invasion of B16BL6 to a modest degree. GAG sulfation, however, may not be relevant to invasion because no consistent pattern was noted comparing the invasion of dextran to dextran sulfate-containing gels by the spectrum of tumor lines tested (Table 3).

It is more likely that interchain bonding characteristics and the chair configurations (which give rise to the three-dimensional polymer morphology and make heparin/heparan sulfate different from the other GAG and the dextrans (37)) are causally related to gel invasion characteristics. The postulated structural requirement could be related to direct cellular interaction with the GAG or function via macromolecular interaction of the GAG with collagen I, as reported for instance in (36).

The B16BL6 cell line degrades heparan sulfate GAG exclusively (7–9), and the endoglycosidase is released into the environment where it can digest the GAG within basement membrane matrix (13). Since these highly invasive and metastatic cell lines also invade gels containing heparin, which they do not degrade, degradation per se may not be a prerequisite for invasion of our complex collagen I gels. Although the composition of some of the GAG made by +SA and B16BL6 is known (38, 39), there is little known about cell surface GAG receptors, and even less about possible functional interactions during cell locomotion. Recently, membrane bound heparan sulfate was shown to be endocytosed by some cells (40). This endocytosis, particularly if linked with tumor endoglycosidase activity, could theoretically facilitate locomotion.

Toole and coworkers (41) have shown that hyaluronic acid production in the immediate vicinity of the V2 carcinoma is elevated in the rabbit where this tumor is invasive, but not in the nude mouse, where it is noninvasive. Elevated hyaluronate synthesis by fibroblasts has also been reported to be stimulated by an invasive human carcinoma cell line but not by a noninvasive one, supporting the belief that invasive cells could stimulate production of this GAG by host cells to aid invasive locomotion (42). In the current study, we did note a weak positive effect of hyaluronic acid in invasion by some, but not all routes.
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Table 6 Attachment of the B16BL6 dietary variants to extracellular matrix components

<table>
<thead>
<tr>
<th>Substrate</th>
<th>B16BL6-ND</th>
<th>B16BL6-LT</th>
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<tbody>
<tr>
<td>Collagen I</td>
<td>266 ± 5</td>
<td>286 ± 7</td>
</tr>
<tr>
<td>Collagen IV</td>
<td>139 ± 21</td>
<td>138 ± 27</td>
</tr>
<tr>
<td>Laminin</td>
<td>218 ± 11</td>
<td>89 ± 7</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>275 ± 7</td>
<td>352 ± 13</td>
</tr>
<tr>
<td>Tissue culture plastic</td>
<td>161 ± 29</td>
<td>276 ± 97</td>
</tr>
</tbody>
</table>

* Substrates were prepared as described in "Materials and Methods" with the exception that 500 μg collagen I and 250 μg collagen IV were used per well.
* Pooled weighted variance of the data shown (log10 scale) = 0.013.
* Statistical analysis was carried out using Student’s t test applied to the log10 data values. Attachment results to matrix substrates are compared to those for tissue culture plastic. P value for a comparison of the attachment of B16BL6-ND cells and B16BL6-LT cells to laminin = 0.014.

all, tumor cell lines tested. Heparin and heparan sulfate have varying effects on normal cells. Heparin enhances capillary endothelial cell motility (43) and is also chemotactic for these cells (44). In contrast, the migration of smooth muscle cells on two-dimensional plastic substrates is inhibited by heparan and dextran sulfates (45). Several different matrix components have been implicated in determining pathways of embryonic cell migrations (46-52). In general, however, tumor cell invasion by the rodent lines we examined in this study appears not to be facilitated by the same matrix components as those active in embryonic cell migrations. Tumor cell invasion may utilize nonembryonic systems or, alternatively, embryonic systems may be altered to convert an inhibitory state to a facilitatory one. It is also possible that the appropriate embryonic migratory systems have not yet been described.

As expected from a review of the literature (4, 12, 53-58), the most metastatic tumor cell lines, B16BL6, B16BL6-ND, and RT7-4bLs, all showed preferential attachment to laminin, most likely reflecting the presence of the cell surface laminin receptor. These cell lines, however, did not exhibit enhanced invasion of our laminin containing complex collagen I based gels. We did not examine the effect of complex gels containing both collagen IV and laminin on tumor cell invasion. Since cell surface interaction with laminin is associated with increased collagen IV degradation (12), the presence of laminin in such a gel might well lead to increased invasion. Whole laminin and a specific laminin fragment have been shown to have chemotactic activities for tumor cells (53, 59). Also, in two-dimensional systems, laminin has been shown to promote cell movement (4, 60). Given these results, we feel that under the correct conditions, the presence of laminin within a matrix should promote tumor cell invasion of that matrix. It is possible that we had an insufficient concentration of laminin in our test matrices. Perhaps more likely is the hypothesis that laminin interacts differently with collagen I than it does with basement membrane matrix components, and that this prevents it from taking part in chemotactic or other facilitation of cell movement.

When attachment behavior and invasive behavior in complex gels are compared, the only consistent correlation noted is for poor attachment to heparan sulfate with enhanced invasion of heparan sulfate-containing complex collagen I gels. This may provide a starting point in elucidating the mechanisms whereby heparan sulfates and heparin facilitate invasion of these three dimensional gels. It is of interest to speculate on how far the results presented in this manuscript can be extrapolated to invasion and metastasis in the animal. Heparin may be elevated in the tissues surrounding an invasive tumor due to degradation of tumor-associated mast cells, and such a situation could aid not only tumor angiogenesis but also local tumor cell invasion. With specific reference to metastasis, heparan sulfate proteoglycan is the predominant, often the only, proteoglycan present in basement membranes. An ability to use heparan sulfate to facilitate movement through basement membrane matrices would clearly promote intravasation and extravasation of tumor cells.

Heparin has been found by a number of workers, for instance, Coombe et al. (61), to inhibit lung metastasis. Heparin would be expected to have an inhibitory effect on hematogenous metastasis by interfering with tumor cell arrest in the blood stream or by interfering with extravasation via inhibition of tumor heparanase. Good correlations of anticoagulant activity with antimetastatic activity have not been reported for antimeastatic sulfated polysaccharides (61) suggesting that anticoagulant activity alone cannot explain their mechanism of action. Should tumor cell membrane interaction with basement membrane matrix heparan sulfate be critical to extravasation, prior saturation of membrane sites with heparin or related sulfated polysaccharides could interfere with subsequent invasion. This postulate fits most easily with our results in this study. However, using a rosetting assay to detect cell surface receptors for sulfated polysaccharides, Coombe et al. (61) found an inverse correlation of antimetastatic activity with cell binding. These workers did not, however, examine compounds closely related to heparin and heparan sulfate such as the chemically modified heparins examined in this study. Although it is tempting to ascribe the activity of all these antimetastatic GAG species to the same mechanism of action, there may well be more than one important pathway. Two of the chemically modified heparins which we examined as components of our complex collagen I gels, are active in inhibiting lung colonization of the B16BL6 cell line but to different degrees (62). The N-sulfated-N,O-desulfated heparin is the most active followed by the carboxyl-reduced heparin (62). The antimetastatic activity of the N-acetylated-N,O-desulfated heparin is not reported. The N-sulfated-N,O-desulfated heparin exhibits the greatest activity in inhibiting the tumor heparanase, the carboxyl-reduced heparin exhibits slightly less inhibitory activity against this enzyme while the N-acetylated-N,O-desulfated heparin is essentially inactive (62). All of these modified heparins can promote tumor cell invasion of complex collagen I gels when present as components of the matrix. The carboxyl-reduced heparin exhibits greater promotion of invasion than does renal heparan sulfate while the other two exhibit a promotion of cellular invasion which is slightly less than that seen for renal heparan sulfate. Thus, no clear correlation of antiheparanase activity with promotion of cellular matrix invasion is evident. Our results with these modified heparins suggest that they can substitute for heparan sulfate in promoting cellular invasion of matrix. At this time the mechanism is unclear although the close structural relationship of these compounds should greatly help in elucidating this. Turley et al. (63) have recently reported that pre-treatment of human amnion with either heparin or hyaluronate enhanced invasion of that structure by SV40-transformed 3T3 cells. Changes were seen in both the overlying epithelial layer and the extracellular matrices. Since the effect of these glycosaminoglycans on denuded amnion was not reported, it is difficult to relate these findings to the current study. When attempting to analyze cell mobility in model extracellular matrices, the physical properties of the matrix should not be ignored. For instance, Comper et al. (64) have demonstrated that cells may...
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be passively transported by countercurrent convection in mixtures of extracellular matrix molecules and their degradative products. Relatively small concentration gradients were sufficient to produce these effects, and these studies serve to underline the importance of local matrix composition and also to emphasize physical effects which local cell induced matrix degradation could have.

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Tumor Cell Invasion of Three-dimensional Matrices of Defined Composition: Evidence for a Specific Role for Heparan Sulfate in Rodent Cell Lines

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