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Role of Proteoglycans in Tumor Progression*

József TIMÁR, András JENEY, Ilona KOVALSZKY, László KOPPER

1st Institute of Pathology and Experimental Cancer Research, Semmelweis University of Medicine, Budapest, Hungary

Data is now starting to accumulate on the differential expression of PGs in tumor cells of various invasive/metastatic potential. This is not so surprising if one considers the key functions that PGs play in the regulation of cell proliferation, adhesion and motility. However, characterization of PG expression in individual tumor types still awaits further detailed studies. Data on melanomas clearly indicate that PG phenotype is both specific and also promiscuous in a sense that ectopic expression of certain tissue specific PGs can occur in various tumors. Expression of a metastatic phenotype-specific splice variants of CD44 provides an example for the possible marker-function of PG. This also raises the hope that some PGs could be used as diagnostic/prognostic tools in pathology or even as a therapeutic targets against tumor dissemination. On the other hand, specific glycanation inhibitors may also be used for the modulation of tumor PG exist and the invasive phenotype. (Pathology Oncology Research Vol 1, No1, 85–93, 1995)

Key words: proteoglycan, metastasis, glycosaminoglycan, invasion

Introduction

Dissemination of malignant tumors (metastatization) is a cascade consisting of detachment of cells from the primary tumor, invasion of the surrounding extracellular matrix (ECM), intravasation, dissemination in the circulatory system, adhesion to vessel wall, extravasation and growth at secondary sites. It is clear that tumor cell-ECM interactions are extremely important and specific moments of the dissemination process.[1] Tumor cell-ECM interactions are mediated by adhesion receptors: integrins,[2,3] non-integrins,[4] proteoglycans,[5] motility-receptors and enzymes responsible for digestion. Cell proliferation and cell-cell/cell-matrix interactions are the two major aspects of tumor progression.[1] During tumor cell dissemination, adhesive processes become more significant than proliferative ones. The integrin receptors on tumor cells were shown to be involved in different steps of the metastatic cascade: in adhesion to and spreading on matrices,[6] in cellular migration[6] as well as in metastasis formation.[6] Among the different integrin receptors, $\beta1$[7] and $\beta3$ [8]were implicated in tumor cell-ECM interactions and thereby to influence the metastatic phenotype.

Although the regulation of the development and expression of the metastatic phenotype is not known yet, two sets of genes were discovered which may promote or inhibit this process. The gene product, nm23, inhibits the manifestation of the invasive/metastatic phenotype in several human tumor models – but most probably not in human melanoma[9] (Fodstad Ø. Personal communication). Two distinct genes were identified whose products promote the manifestation of the metastatic phenotype: a unique splice variant of the adhesion receptor CD44[10] and mts1, a Ca^{++}-binding cytoskeletal protein.[11] Unfortunately there is no data in the literature to support that either of these is involv-

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Correspondence: József TIMÁR, M.D., Ph.D.; 1st Institute of Pathology and Experimental Cancer Research, Semmelweis University of Medicine, Üllői út 26., 1085 Budapest, Hungary; Tel: (36)(1)133-5740; Fax: (36)(1)133-5784

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Abbreviation: CS: chondroitin sulphate; CSPG: chondroitin sulphate proteoglycan; DS: dermatan sulphate; DSPG: dermatan sulphate proteoglycan; ECM: extracellular matrix; FGF: fibroblast growth factor; GAG: glycosaminoglycan; galNac: N-acetylgalactosamine; glcA: glucosamine; glcNac: N-acetyl-glucosamine; glcUA: uronic acid; glcIA: iduronic acid; HA: hylauronic acid; HBP: heparin-binding protein; HCGF: hepatocyte growth factor; HS: heparan sulphate; HSPG: heparan sulphate proteoglycan; IL: interleukin; MAA: melanoma-associated antigen; PG: proteoglycan; TGF: transforming growth factor

ed in the regulation of the metastatic phenotype of malignant melanoma. Accordingly, in case of human melanoma the regulation of the expression of invasive/metastatic phenotype is not known yet and there are no specific invasion markers available either.

Table 1. **Hyaluronic acid binding proteoglycans (hyaloadherins)**

ECM cartilage	*ECM non-cartilage*	*Plasma membrane*
aggrecan (CSPG)	PG-M (CSPG) versican (CSPG) hyaluronectin (CSPG) neurocan (CSPG)	CD44

Proteoglycans are a special class of glycoconjugate molecules. The core protein is glycosylated on serine/threonine residues with glycosaminoglycan (GAG) chains. GAGs are negatively charged sugar-polymers in which the negative charges are carried by carboxyl and sulphate residues. Hyaluronate (HA) is the only GAG which is not connected to a core protein. Other GAGs are chondroitin-(CS), dermatan-(DS), heparan (HS) and keratan sulphates (KS). These are attached to specific core proteins to form proteoglycans (PG). HA, DS and HS contains gleA and gleNac while CS and KS contain gleA and galNac with alternate gleUA (HA, CS) or gleIA (DS,HS).

HA is one of the best known ECM molecule and can be considered a ubiquitous component of the matrix. HA is also the best known glycosaminoglycan which differs from others by the lack of a protein core. The molecular weight of HA is considerably higher than that of other PGs, providing a unique feature for HA to be distinguished from the other GAGs. *Table 1.* shows current examples of HA-binding molecules involved in the cell-HA interactions.[12]

Classification of proteoglycans is based on the type of sugar chains attached to the core protein: CSPG, HSPG, DSPG and KSPG. However, it turned out that this classification is too rigid, because some of PGs, if not the majority, are hybrids, i.e. the core protein can be glycanated with either CS or HS chains or even with both. PGs are one of the main components of the ECM. CSPGs such as aggrecan and versican are the high molecular weight representatives while decorin and biglycan are the small molecular weight members. To date only perlecan was found in the ECM from the group of HSPGs. The cell surface also contains several members of the PG family of both CS- hybrid- and HSPG classes. Among CSPGs, CD44, the melanoma associated antigen and thrombomodulin are transmembrane proteins. Interestingly, the hybrid PGs at the cell surface were proved to be involved in the binding and signalling of growth factors. Syndecans (bFGF) and betaglycan (TGFβ) are coupled to the tyrosine kinase and serine/threonine kinase pathways, respectively. Syndecans have other important functions, too. They have supportive roles in cell adhesion to matrix proteins via the HS chains of the core protein. Syndecan-2 (fibroglycan) is the most abundant membrane HSPG in fibroblasts and hepatocytes. Glypican is a unique membrane HSPG because the core protein is not a transmembrane protein and this molecule is linked to the lipid bilayer through an inositolphosphate-HS linkage. A class of PGs is intracellular and these PGs are predominantly CSPGs. Prototype of these PGs is ser-glycin, found in NK cell, macrophage and mast cell granules; and chromogranin-A, a component of neurosecretory granules *(Table 2)*. This diversity of structure and function of PGs may justify the studies on the role of tumor cell PGs in the invasive/metastatic cascade.[3,4]

Table 2. **Proteoglycans**

Sugar chain	*Intracellular*	*Plasma membrane*	*Extracellular*
KS			fibromodulin corneal-KSPG
CS	chromogranin-A	CD44 i-chain of HLA-DR melanoma associated antigen (MAA) neurocan NG2 phosphocan thrombomodulin	aggrecan biglycan decorin versican collagen-IX
CS/HS	ser-glycin	betaglycan syndecan-1 syndecan 3 syndecan 4 (amphiglycan; ryudocan)	
HS		glypican syndecan 2 (fibroglycan) transferrin-receptor	perlecan

Proteoglycans and rodent tumor metastases

Initially, studies on the role of glycoconjugates in tumor metastasis concentrated on glycolipids and glycoproteins. We were among the first trying to reveal the role of PGs in the invasiveness of tumors. We used 3LL tumor lines with different (high versus low) liver metastatic potentials.[15] Studies on the surface glycoconjugates of these tumor lines indicated decreased gal-lectin binding and MHC-KbDb expression and increased acridine orange binding due to increased sulphated moieties on the surface of highly metastatic (hm) 3LL-HH cells.[16] Biochemical studies further substantiated the cytochemical observation on the accumulation of GAGs in the microenvironment of highly metastatic 3LL-HH cells.[17] Analysis of the extra-

cellular and surface GAG pattern of the 3LL cells indicated that in the low metastatic cells CS, while in the highly metastatic cells HS was the predominant form of GAGs. This phenotypic characteristic was stable and was maintained in cell lines derived from the solid tumors.[18] Further studies indicated that besides the increased biosynthesis an elevated secretion and decreased degradation of GAGs were the characteristics of the highly metastatic cells.[18] Studies on the core protein expression of PGs in these tumor lines resulted in a controversial observation: both the CS- as well as the HS-PG antigen expression decreased on the surface of highly metastatic cells.[19] We have suggested that this can be explained by (1) the increased glycanation of PGs masking the core antigen; (2) the expression of different core epitopes on the cell lines; (3) the loss of certain core protein epitopes in the highly metastatic cells.

Based on these studies we have concluded that HS or HSPG may play important role in shaping the metastatic phenotype of 3LL-HH cells. Therefore we have analyzed the interaction of exogenous HS with the low and highly metastatic 3LL cells. We found that HS but not other GAGs has stimulated the proliferation of highly metastatic cells but did not the low metastatic variants.[20] Both cell lines expressed HS-receptors ($Kd=10^{-8}M$) and were able to internalize the bound ligand. However, in the low metastatic cell line, 3LL, the higher rate of internalization was accompanied by a higher intracellular degradation in contrast to the highly metastatic cells, which expressed a surface HS-degrading endoglycosidase.[20] The expression of this enzyme was found to be associated with increased metastatic phenotype in different experimental model systems.[21] Biochemical studies indicated a highly significant increase in the expression of HS-binding proteins on the surface and in the nucleus of highly metastatic cells.[20] Similar association of HS binding protein expression and increased metastatic potential was found in rat rhabdomyosarcoma cell lines as well.[22] Acording to our interpretation, the most important finding was the fact that HS (but not other GAGs) stimulated the proliferation of highly metastatic 3LL cells while it was ineffective on low metastatic cells. A group of cytokine growth factors (such as TGFβ, a/bFGF, HCGF, IL-4 etc.; *Table 3)* have the capacity to bind heparin, an analogue of HS.[23,24,25] In case of these cytokines HS(PG)s serve as receptors for these ligands and present them for the signaling receptor.[23] On the other hand some heparin-binding growth factors (like bFGF) is stored in the basement membrane bound to HSPG.[26] Cells exhibiting surface HS-degrading endoglycosidase activity would be able to release heparin-binding cytokines from this store.[26] It is noteworthy that highly metastatic tumor cells exhibit high surface expression of HS(PG),[17,18] high HS binding potential and surface HS-endoglycosidase.[20] Biochemical analysis of the HS-binding proteins indicated that they are heterogenous proteins: including the HS-binding growth factor bFGF[27] and other proteins (such as proteolytic enzymes), too *(Table 3)*. It is important to stress that there was no difference detected in the *in vitro/in vivo* proliferation of 3LL cell lines characterized by different liver metastatic potential.[28] Therefore, differences in cell proliferation, if any, may occur only in the metastases themselves regulated by the local growth factors/cytokines. We suggest that the local (organ-derived) HS-binding growth factors/cytokines are good candidates for such a regulatory role *(Table 3)*.

Table 3. **Heparin/heparan sulphate binding proteins**

ECM proteins	fibronectin, laminin, vitronectin, collagen I-IV, thrombospondin, elastin
Cytokines/growth factors	bFGF, TGFβ, HGF, hEGF, GM-CSF, hAMF, IL-5, IL-8, IL-12
Enzymes	mast cell protease, lipoprotein lipase, elastase, SOD
Other proteins	histons, N-CAM, viral capsid proteins (HSV), thrombocyte factor 4

HA and tumor progression

Wilm's tumor is not only characterized by increased PG content, but due to excessive production, the patient's sera and urine also contain elevated levels of hyaluronic acid, which can be diagnostic for the tumor recurrence after surgery. Wilm's tumor contains 7-9 fold more GAG than the normal kidney, comprising mainly hyaluronic acid and HS.[29]

Malignant mesotheliomas, fibrosarcomas and liposarcomas are also characterized by high hyaluronic acid content.[30] In case of mesotheliomas, HA can also be detected in the pleural transsudate.[30] The predominant PG of all of these tumors, however, is CSPG.

Tumor cells containing HA-receptors are able to establish a HA-rich pericellular matrix.[31,32] High affinity/high specificity HA-receptors are overexpressed in invasive human bladder carcinoma cells.[33] Metastatic rat carcinoma cells express the CD44 variant, which is absent from non-metastatic clones.[34] Transfection of this CD44v variant into lymphoma cells increases tumorigenicity as well as metastatic potential.[35]

In summary, it seems that HA and the expression of HA-receptor on the invading tumor cells is an important phenotypic marker. HA and HA-receptors provide a structural basis for tumor cell locomotion and also an effective pericellular defense-sheet against attacks of anti-tumoral defense mechanisms. Based on the experimental data it seems that expression of HA-receptors by tumor cells provides a selectional advantage for those cells which are able to invade distant organs. Therefore, it seems that the HA-HA-receptor interaction may be a potential target for an anti-metastatic intervention.

Proteoglycans and human tumor metastases

CD44-CSPG was found to be expressed by a wide variety of human cell types including epithelia, mesenchyme and lymphoid cells. The importance of CD44 was recognized when it was detected that some of the splice variants (v5/v6) were exclusively expressed in experimental metastatic tumors. Further analysis indicated, that in human tumors, such as large cell lung cancer,[85] colon carcinoma,[87,88] neuroblastoma,[89] aggressive non-Hodgkin lymphoma[90] the CD44-v6 variant was overexpressed.

Proteoglycans were isolated from several human tumors. The best characterized among them is the CSPG of melanomas having a 250 kD protein core.[91] This protein is expressed in transformed melanocytes.[92] It is important to consider that this CSPG may be present in other tumor types like fibrosarcoma[93] and can also be present in lymphoid tissues.[92] Analysis of human melanoma lines with various metastatic/invasive phenotype indicated that the expression of melanoma associated antigen does not correlate with the invasive behaviour of tumor cells.[45] It was shown however, that CSPG was involved in the motility of human melanoma cells.[46]

The variety of HBPs *(Table 3)* calls the attention on the search for the role of HSPGs in tumor progression. These PGs are able to interact with HBPs, transduce signals generated by them and several HBPs are involved in invasion and metastasis. The best known HSPG of human tumors is unquestionably the perlecan-like HSPG of colon carcinoma.[47] Both CSPG (a homologue of melanoma CSPG)[45] and HSPG were isolated from human gliomas[94] where the expression of the latter was increased during malignant transformation. HSPGs first were isolated from human melanoma by Roberts et al.[54] using the A-2058 cell line. In these cells two types of HSPGs were found: a transmembrane protein with thrombospondin-binding potential and a basement membrane-like HSPG with fibronectin-binding characteristics. The role of PGs in the invasiveness of human tumors wasn't known at that time.

We used several human melanoma lines derived from the parent A-2058 cell line[51,52] and the HT18 tumor.[51] In this panel HT18 proved to be the least metastatic according to *in vivo* tests, followed by HT168. The most metastatic melanoma was the M1 variant of the HT168 line[51] followed by the parent A-2058 line. The model system in which we first found PG alterations in tumor cells provided a unique selection mechanism by which high liver metastatic tumor cells and lines could be established using intrasplenic inoculation in immunosuppressed mice.[15,51,52] We suggested that such phenotypic alterations may take place during the first sequence of spleen-liver transition of tumor cells. Therefore, we compared the spleen primary tumor of the low metastatic HT168 melanoma to the metastatic tumor cells derived from the corresponding liver metastases using ^{3}HgleN and ^{35}S precursor labelings. The liver-derived human melanoma cells were characterized by a higher intra- and pericellular sulphated GAG biosynthetic potential compared to the spleen-derived melanoma cells S1.[53] We suggested that the increased cellular accumulation of sGAGs in liver derived tumor cells resulted in a decreased GAG release to the microenvironment. Analysis of the cell-associated GAGs revealed that the liver-derived human melanoma cells contained two GAG fractions compared to a single one in the primary tumor derived cells.[53] The first one contained HA, undersulphated CS and HS while the second one proved to be a new GAG-fraction containing two bands of sulphated CS and a heavily sulphated HS.[53] This data indicated that the low metastatic human melanoma cells in liver metastases expressed a new GAG phenotype which is not detectable in the corresponding primary tumor. We concluded that either the microenvironment (liver) affected the biosythesis of GAGs in melanoma cells or the tumor cell population derived from the liver was a result of clonal expansion of a melanoma cell population present in an undetectable proportion in the primary tumor.

Next we studied the GAG content of independent human melanoma xenografts with different liver metastatic potentials. HT18 anf HT168 primary tumors exhibited similar GAG contents, even though an analysis of the individual components CS,DS,HS indicated a different pattern. The relatively more metastatic tumor (HT168) had a higher relative amount of HS compared to CS than the low metastatic HT18.[54] Since the growth rate of the tumors differed significantly, therefore it was not clear whether the altered proliferation capacity or the metastatic potential was associated with the different GAG-pattern.[54] Another fact complicated the interpretation of these findings namely that the primary tumor xenografts might have contain stromal elements contributing to the alterations in the GAG pattern.

To clarify these points we used another approach: we studied the PGs produced by human melanoma cell lines with different metastatic potential derived from the same parent line A-2058. We compared the relatively low metastatic HT168 line to the more metastatic A-2058 and highly metastatic HT168-M1. All these cell lines exhibited similar *in vitro/in vivo* proliferation capacity, therefore differences in PG biosynthesis may be associated with their invasive phenotype.

Both in the HT168 and its highly metastatic variant M1 we found similar amount of cellular sGAG by metabolic labeling.[54] However, the high metastatic line M1 accumulated CS intracellularly, and as a consequence, in these cells HS dominated over CS at the cell surface. Next we studied the localization of CS and HS in human melanoma lines with different metastatic potential by immunocytochemistry. Adherent tumor cells were fixed and labelled with anti-CS and anti-HS monoclonal antibodies. It is evident that CS is heavily expressed at the apical surface of low

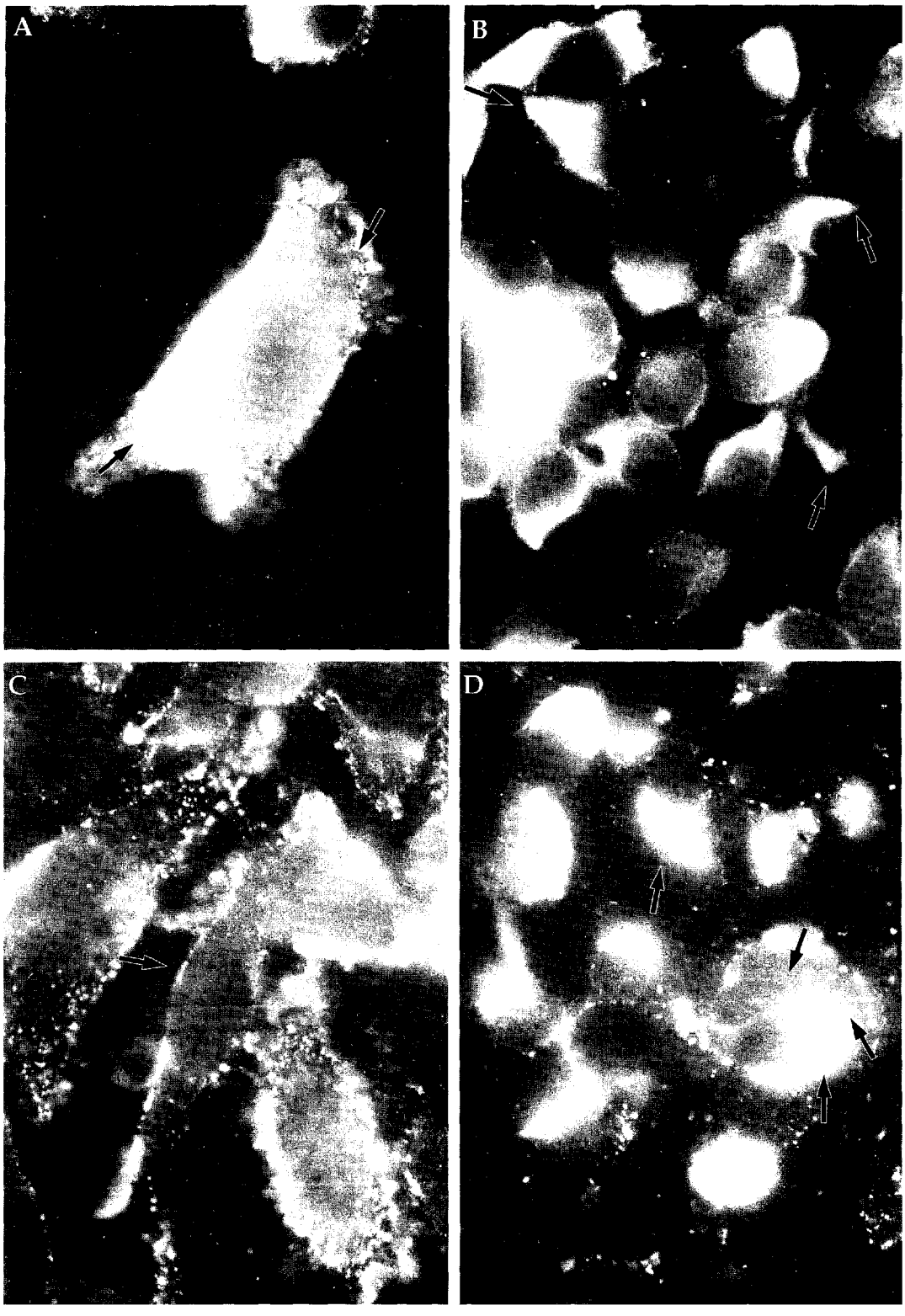

Figure 1. *Localization of glycosaminoglycans in fibronectin-adherent human melanoma cells with different metastatic potential (immunofluorescence). – A: Cellular distribution of CS in HT168 low metastatic melanoma cell. Note the diffuse cytoplasmic label as well as the even staining at the plasma membrane (arrow) using monoclonal anti-CS antibody and Streptavidin-Texas Red. 3000 x. – B: Cellular distribution of CS in high metastatic HT168-M1 cells. Note the clear polarized distribution of CS concentrated at leading edges (arrow). 3000 x. – C: Cellular distribution of HS in low metastatic HT168 human melanoma cells. Note the patchy labeling along the plasma membrane. HS was detected with anti-HS monoclonal antibody and Streptavidin-Texas Red. 3000 x. – D: Cellular distribution of HS in high metastatic HT168-M1 cells. Note the accumulation of HS at or under the leading edges (arrow) of the cells. 3000 x.*

metastatic HT168 human melanoma cells *(Fig.1 a)* while its distribution became highly polarized on the surface of highly metastatic HT168-M1 cells *(Fig.1 b)*. The subcellular distribution of HS-chains is different from that of CS on human melanomas. HS expression is low and diffuse on low metastatic HT168 melanoma cells *(Fig.1 c)*, while the HS-reaction is high on highly metastatic melanoma cells and can be localized to attachment membranes *(Fig.1 d)*. This indicates that the CS- and HS-PGs are localized to different surface domains on human melanoma cells. The polarized distribution of CS suggest its involvement in cell movement, as it was recently suggested,[46] while the accumulation of HS at attachment membranes indicates the involvement of some HS-PGs in tumor cell adhesion.

Comparing the amount of HS in A-2058 cells to that in the lower metastatic HT168 line, we found an elevated amount of extracellular HS in the A-2058 cells and an intracellular accumulation of HS in the low metastatic HT168 cells, though in a highly degraded form (8-10 Kd versus 30-40 kD). Besides the difference in the intracellular HS-degrading potential, the more metastatic cells were characterized by a more dynamic HS secretion contributing to the establishment of an HS-rich microenvironment.[55] By comparing the two more metastatic lines A-2058 and HT168-M1 to HT168 low metastatic cells we concluded that the development of the altered GAG phenotype was achieved in two ways. In A-2058 cells a decreased intracellular degradation and increased secretion of HS was associated with the more invasive phenotype while in the HT168-M1 variant an intracellular accumulation of CS resulted in a predominance of HS at the cell surface.

Next, we studied the expression of PG antigens in melanoma lines with different metastatic phenotypes. There was no difference in mel-CSPG expression between the melanoma lines studied which is in aggreement with previous reports that showed the mel-CSPG did not play a role in the invasive phenotype of melanoma cells.[54] It was also interesting that aggrecan antigens were expressed by almost all of the melanoma lines[103] detected by monoclonal antibodies against fetal (HFPG529) and adult (MK172) cartilage CSPG. In the highly metastatic HT168-M1 cells the disappearence of HFPG epitopes was observed with preservation of the MK172-types.[54] In the metastatic A-2058 cells the expression of mel-CSPG did not change compared to HT168, while the expression of HFPG epitopes decreased.[53] Based on this data we can conclude that the increased liver metastatic phenotype does not correlate with a well-defined CSPG antigen pattern. Biochemical studies indicated that A-2058 variants contain a range of CSPGs with molecular sizes of 400; 500; 600 and 1000 kD.[56] In the A-2058 cells the 1000 kD band disappeared in parallel with a loss of HA-binding sites.[56] We therefore conclude that the expression of aggrecan-type CSPG and mel-CSPG are regulated differently in melanoma cells.

HSPG of A-2058 melanoma cells was found in two forms: a small and a high mw forms[54] the former corresponding to a transmembrane-type HSPG and the latter to a perlecan-type HSPG. Both the high-met HT168-M1[53] and the metastatic A-2058 cells exhibited increased perlecan-like HSPG-antigen expression (BN42) compared to the low metastaticy HT168 line whereas HT168-M1 cells, unlike A-2058 ones, overexpressed a small mw fibroblast-type HSPG (FW16).[54] These findings, together the previously described biochemical studies indicated that in A-2058 cells, HSPG secretion while in the M1 cells the surface localization of HS(PG) were predominant. In M1 cells HSPG was present exclusively in adhesion plaques indicating their role in adhesion.[54] This observation was further substantiated by ultrastructural cytochemistry. The cell surfaces of both HT168 and M1 cells contained Cuprolinic Blue-positive granules indicating the presence of highly sulphated PG expression.[53] Unlike in HT168 cells however, in highly metastatic HT168-M1 cells highly sulphated PG was detected under the plasma membrane in contact with the substrate.[53] Further studies showed that the HS chains of HT168 cells were able to interact with fibronectin and laminin.[55] By immuno electron microscopy the fibroblast-HSPG epitopes were rarely found at the surface of HT168 and more frequently on M1 cell[53] with no difference in the intracellular distribution.

The PG phenotype of human melanoma lines was recently analyzed by using molecular biology and PG specific antibodies (J.Tímár, I.Kovalszky: unpublished observations). These studies indicated that (1) among known HSPGs only perlecan can be identified in both HT168 and HT168-M1 lines; (2) four different CSPG species can be identified in these tumor lines: decorin, mel-CSPG, aggrecan and CD44 *(Table 4)*. At least in our model system the highly metastatic melanoma cells are characterized by high surface expression of HS(PG), CS(PG) and CD44.

***Table 4.* Expression of proteoglycans at the cell surface of human melanoma lines with different metastatic potential**

Cell line	*HSPG*	*decorin*	*aggrecan*	*CD44*	*mel-PG*
HT168	32.5	92.4	10.2	39.9	70.8
HT168-M1	80.9	89.9	80.3	79.8	80.3

Data represent percent of positive cells in the cell population determined by immunocytochemistry and flow cytometry (mean of three samples; SD < 10%)

A role for HS-proteoglycans in tumor progression was recently indicated in experimental model systems *(Table 5)*. Analysis of the expression of syndecan-1 in tumors indicated that in both experimental and human epithelial tumors this HSPG is lost during malignant transformation.[57] This statement can be corroborated by our recent studies on human melanoma lines with different metastatic potential

Table 5. **Alteration of HS/CS ratio in more metastatic variants of experimental tumor model systems**

Tumor type	*Lines*	*HS/CS ratio*	*Reference*
mouse melanoma	B16F1/F10	up	59
	B16YL1/YM1,H1	up	60
mouse lung carcinoma	LLT/LLT-HH	up	17
	LM/HM	up	18
mouse T lymphoma	Eb/EsB	down	61
rat rhabdomyosarcoma	RMS8/RMS0, RMS6	up	62,63
human melanoma	HT18/HT168	up	51
	HT168/HT168-M1	up	54
	M4Be,IC8/7Gp122	down	64
	skin	up	58

(J.Tímár, I.Kovalszky: unpublished observations). More recently, studies were performed on the expression of another HSPG, perlecan, in human melanoma lines, and it was found that in skin melanoma, perlecan expression is dramatically increased.[58] Furthermore, these studies also suggested that neutrophins regulate the expression of perlecan which in turn may be responsible for the release of heparanase activity.[58]

The widespread involvement of tumor cell HSPG in tumor progression raised the possibility to use these PGs as therapeutic target to inhibit metastasis formation. The biosynthesis of tumor cell HSPG can be selectively inhibited by a newly discovered inhibitor of HS, HUdR.[16,65] These studies indicated that *in vivo* inhibition of tumor cell HS biosynthesis inhibits both lung and liver colony formation without a significant effect on the growth of the primary tumor, suggesting that tumor cell HSPG is necessary for dissemination.[63] Analyzing the effect of HUdR it was found that the inhibition of HSPG biosynthesis inhibits both tumor cell ECM interaction (adhesion and spreading) as well as microinvasiveness on fibroblast monolayers.[65]

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