[CANCER RESEARCH 60, 4767–4770, September 1, 2000]

Advances in Brief

Astrocytes Contribute to the Brain-metastatic Specificity of Melanoma Cells by Producing Heparanase[1]

Dario Marchetti,[2] John Li, and Ruijun Shen

Department of Neurosurgery, The University of Texas-Houston Health Science Center [D. M., R. S.], and Laboratory Medicine, M. D. Anderson Cancer Center [J. L.], Houston, Texas 77030

Abstract

Neurotrophins (NTs) modulate the brain invasion of melanoma cells and the activity of an extracellular matrix degradative enzyme, heparanase, that has been recently cloned. Heparanase degrades the heparan sulfate proteoglycans (HSPGs) and is a critical mediator of tumor metastasis and angiogenesis. Because astrocytes are among the first brain cells encountered by extravasating melanoma cells, they may play important roles in the development of brain metastases. To test this hypothesis, we used purified *in vitro* astrocyte cultures and found that they express heparanase transcript and functional enzyme that were up-regulated by the prototypic NT, nerve growth factor. Coincubation of astrocytes (or their conditioned medium) with brain-metastatic cells resulted in a superadditive effect on heparanase activity and up to an 8-fold increase of *in vitro* chemoinvasion using purified HSPGs. These observations indicate that astrocytes significantly contribute to the brain colonization of melanoma cells via heparanase-driven modalities.

Introduction

Mechanisms responsible for the progression of malignant melanoma to highly aggressive brain-metastatic disease remain largely unknown (1). We have reported NT[3] effects on melanoma cell invasion and production of heparanase (2, 3). This enzyme is an endo-β-D-glucuronidase (4) that degrades the heparan sulfate chains of HSPGs, essential and ubiquitous macromolecules associated with the cell surface and ECM of a wide range of cells and tissues (5). Of relevance, human heparanase has been cloned recently as a single gene family (4, 6, 7) and found to be a potential target for antimetastasis drugs because of its relevant roles in angiogenic and invasive processes (8).

Astrocytes are among the brain cells encountered by extravasating melanoma cells that have breached the first two layers of the BBB, the brain microvessel endothelium, and the thick HSPG-enriched ECM. Injury-reacting astrocytes are frequently found in areas surrounding melanotic lesions and able to produce NGF, the prototypic NT (9). Furthermore, they are capable of binding NT because they express members of the Trk receptor family and the p75 low-affinity NT receptor ($p75^{NTR}$; Ref. 9). In this study, we have examined interactions between astrocytes and selected melanoma clones capable of brain colonization *in vivo* (10, 11). We report that astrocytes produce heparanase and potentiate melanoma-invasive properties as a result from the concerted action of heparanases of neoplastic and astrocytic origin.

Materials and Methods

Cell Culture. The human melanoma MeWo cellular system was obtained from Dr. Robert S. Kerbel (Sunnybrook Health Science Center, Toronto, Ontario, Canada). The MeWo parental line was propagated from a lymph node metastasis and subjected to wheat germ agglutinin selection in tissue culture (10). The MeWo brain-metastatic variant, named 70W, arose after multiple tissue culture passages in graded concentrations of wheat germ agglutinin (10). 70W cells are highly aggressive, with the capacity to colonize various organs and to form brain colonies in nude mice (10). As such, target organ site colonization by the 70W line is similar to the clinical presentation of human melanoma metastasis in advanced disease stages. Murine brain-metastatic melanoma B16B15b and parental B16F1 cells (11) were received from Dr. Garth L. Nicolson (Institute for Molecular Medicine, Huntington Beach, CA). Purified astrocytes were prepared from newborn rat or mouse cerebral cortices using a modification of the method of McCarthy and deVellis (12). Briefly, cerebral cortices were isolated, meningeal tissue was removed, and tissue was incubated in calcium-free buffer (Hanks' CMF) containing 0.025% trypsin for 30 min at 37°C. Cells were dissociated by trituration through a fire-polished Pasteur pipette, spun at 1000 × *g* for 5 min, and resuspended in 5 ml of DMEM (Life Technologies, Inc., Grand Island, NY) containing 20% fetal bovine serum (Life Technologies) and 2 mM L-glutamine. After centrifugation, cells were filtered through a 140- and 20-μm nylon filters, plated onto polylysine-coated (0.1 mg/ml) 75-cm^2 tissue culture flasks at a density of 2.0×10^7 cells/flask, and incubated at 37°C with 5% CO_2 overnight. Primary cultures were then enriched in astrocytes by removing the less adherent oligodendrocytes, oligodendrocyte-type 2 astrocyte progenitor cells, and microglia (which grow on top of astrocyte monolayers) by shaking at 250 rpm for 18 h at 37°C. The detached cells were removed and discarded. Cellular confluence was reached 10 days after plating the cultures, showing a polygonal flat cell morphology. The purity of the primary astrocyte cultures was confirmed by immunostaining. The following antibodies were used: a rabbit MAb against GFAP (Boehringer Mannheim, Indianapolis, IN) that is a specific reactive astrocyte marker; a rabbit polyclonal antibody against myelin basic protein (Dakopatts, Copenhagen, Denmark) that recognizes oligodendrocytes; the antibody A2B5 (Chemicon International, Temecula, CA), which binds to gangliosides and recognizes oligodendrocyte-type 2 astrocytes; and a MAb against vimentin (Santa Cruz Biotechnology, Santa Cruz, CA) that identifies fibroblast contamination (13). Experiments were performed with a nonspecific immune stimulation of the astrocytes in cross species *versus* same species cocultures of astrocytes and melanoma cells and as controls for possible effects attributable to xenogeneic differences. Syngeneic astrocytes were used in experiments involving B16 murine cell lines. To obtain ACM, astrocytes from subconfluent primary cultures were seeded at 5×10^5 cells/well into 24-well plates (Costar, Cambridge, MA) in complete medium. After 48 h, cells were washed four times with serum-free medium and subsequently cultured for 48 h in 1.5 ml of serum-free medium supplemented with 5 μg/ml bovine insulin, 5 μg/ml human transferrin, and 5 ng/ml sodium selenate (ITS). The ACM was centrifuged at 5000 × *g* to remove any detached cells and cellular debris and concentrated using Centricon-30 microconcentrator units (Millipore, Bedford, MA).

Astrocyte Visualization by Epifluorescence Microscopy. Confluent astrocytes monolayers were fixed in 4% paraformaldehyde in 0.15 M phosphate

Received 4/27/00; accepted 7/20/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by Grant NIH R-29 CA64178 (to D. M.) and by the Vivian L. Smith Foundation for Neurological Research.

[2] To whom requests for reprints should be addressed, at Department of Neurosurgery, University of Texas-Houston Health Science Center, MSB 7.136, 6431 Fannin Street, Houston, TX 77030. Phone: (713) 500-6137; Fax: (713) 500-7787; E-mail: Dario.Marchetti@uth.tmc.edu.

[3] The abbreviations used are: NT, neurotrophin; NGF, nerve growth factor; HSPG, heparan sulfate proteoglycan; BBB, blood brain barrier; ECM, extracellular matrix; ACM, astrocyte conditioned medium; GFAP, glial fibrillary acidic protein; HPLC, high-pressure liquid chromatography; MAb, monoclonal antibody; CM, conditioned medium.

buffer, and coverslips were incubated in rabbit anti-GFAP MAb (1:200 dilution), followed by a rhodamine-conjugated secondary antibody (1:150 dilution; Zymed Laboratories, Inc., South San Francisco, CA). Control experiments included deletion or substitution of the primary antibody in the labeling procedure. Astrocytes were then examined and photographed using an inverted epifluorescence microscope (Diaphot-TMD, Nikon, Japan).

Isolation of RNA, Heparanase Primers, and Semiquantitative RT-PCR. Astrocyte preparations were divided in two aliquots and incubated (or not) with pure and biologically active recombinant rat NGF (R & D Systems, Minneapolis, MN). Poly(A)$^+$ RNA was obtained from subconfluent cells using a mRNA purification kit (Qiagen, Valencia, CA) according to the manufacturer's instructions and quantitated by ultraviolet absorption. RNA was reverse-transcribed with avian myeloblastosis virus reverse transcriptase (Life Technologies) and used for PCR with sense (5′CTTCTAAGAAAGTCCACCTTC-3′) and antisense (5′-AAACTATATGAGAAAGCTGGC-3′) oligonucleotide primers (Integrated DNA Technologies, Coralville, IA) designed to amplify a 535-bp PCR product specific for human heparanase (4). DNA sequence analysis of this PCR product confirmed identity with the heparanase sequence. All PCR reactions (25 μl) were performed with an annealing temperature of 68°C, standardized over a wide range of cycles (20–40) to avoid plateau effects and to allow a semiquantitative comparison. Aliquots of 10 μl of amplification products were separated by 1% TBE (Tris-borate-EDTA) agarose gel electrophoresis and bands visualized by ethidium bromide staining. PCR primers (5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′, forward; 5′-CATGTGGGCCATGAGGTCCACCAC-3′, reverse) designed to amplify a 983-bp fragment of glyceraldehyde-3-phosphate dehydrogenase were used to normalize the cDNA preparation. Quantitation of signal expression was performed by densitometric analysis and computer integration of normalized data.

Chemoinvasion and Heparanase Assays. Melanoma cell invasion was assayed by cell culture chambers (Costar) as reported (2, 3). ACM (100 μl) was applied to high-density (2.5×10^4 cells/filter) 70W and B16B15b cultures in chemoinvasion assays, the Transwell filters (6.5-mm diameter; 8-μm pore size) of which were coated previously with purified HSPGs (50 μg/filter). Invasion was monitored by fluorescence using a multiwell plate reader (CytoFluor Series 4000; PE Biosystems, Foster City, CA; Refs. 2, 3), taking into consideration both background and map reading area of the instrument. ECM-derived HSPG preparations were pure, as determined by 4–20% gradient SDS-PAGE and Western blotting using HSPG MAb. Heparanase activity was determined by degradation of purified ECM [^{35}S]HS using high-speed gel permeation chromatography or by agarose gel electrophoretic analyses. We have demonstrated previously that: (*a*) B16B15b and 70W brain-metastatic clones possess higher heparanase content than their respective parental counterparts (B16F1 and MeWo; Ref. 2); and (*b*) the heparanase activity is indistinguishable in these two cellular sources by the two heparanase assays used (3, 14). Only data within the linear range for relative activity measurements were taken into account, with relative degradation activity determined by the amount of radiolabeled HS degraded/min/μg of protein.

Results

Heparanase Expression and Its Functionality in Astrocytes. We investigated heparanase presence in astrocyte cultures. Primary glial cells were obtained from newborn rat or mouse cerebra, and their identification as astrocytes was established by positive immunoreactivity with an antibody against the astrocyte-specific intermediate filament GFAP (Fig. 1*A*). Neither vimentin, myelin basic protein, nor A2B5 was expressed by the astrocyte cultures, confirming their purity (data not shown). A specific heparanase transcript (5.0 kb) amplification was detected by semiquantitative RT-PCR and up-regulated in astrocytes incubated previously with purified and biologically active NGF (Fig. 1*B*). Similar results were obtained in human brain-metastatic 70W cells (Fig. 1*B*) that are known to possess NGF-regulated heparanase activity (2). Densitometric analyses from four independent experiments revealed that NGF caused a 1.5–2.3-fold increase of heparanase RT-PCR product in astrocytes, whereas up to almost a 4-fold increase was obtained after NGF treatment of 70W cells. Heparanase is a hydrolase that differs from heparinases or HS-specific

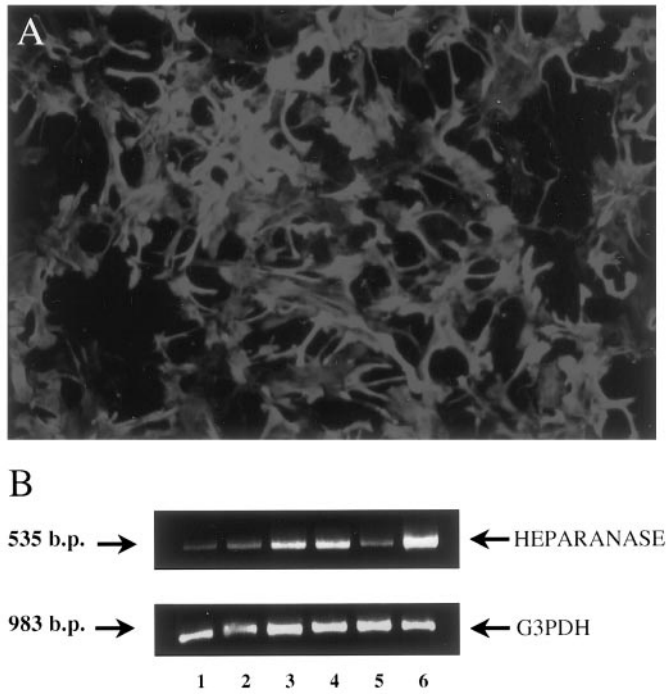

Fig. 1. *A*, fluorescence micrography of isolated cerebral cortical astrocytes immunostained for the astrocyte-specific intermediate filament GFAP. *B*, semiquantitative PCR analysis for heparanase expression from rat cortical astrocytes and human brain-metastatic 70W melanoma. Independent cortical astrocytes preparations from rat cerebra were not exposed (*Lanes 1* and *2*) or exposed (*Lanes 3* and *4*) to NGF (4 nM for 6 h at 37°C). Similarly, the presence and up-regulation of the human heparanase transcript were analyzed in brain-metastatic 70W not exposed (*Lane 5*) or exposed to NGF (*Lane 6*). See "Materials and Methods" for experimental details.

elimination enzymes, cleaving HS into characteristic large molecular weight fragments, approximately one-third of the original HS size (15). Heparanase activity was detectable and NGF regulated in cellular extracts from purified astrocytes. Distinct HS degradation products were detected by gel shift assays (Fig. 2) or by HPLC analysis (Fig. 3).

Cooperativity Between Melanoma and Astrocytic Cell Heparanases. We analyzed heparanase activity for brain-metastatic and astrocytic cell populations in logarithmic growth. We obtained HPLC-derived elution profiles of HS-digested products by heparanase assays performed at various incubation times (2). Highly brain-metastatic 70W cells produced a gradual and time-dependent increase of heparanase activity for up to 72 h. The same was found when astrocytes were used (Fig. 3). However, coincubation of 70W and astrocytes in equicellular amounts, followed by heparanase assays at defined time intervals, resulted in a superadditive increase of enzymatic activity (Fig. 3). These results were confirmed coincubating the murine B16B15b cell line, a brain-metastatic B16F1-variant (11), with syngeneic astrocytes. Heparanase levels were as much as 65% lower at confluency in melanoma and astrocytic cells, alone or together (data not shown).

ACM Enhances Invasion via Heparanase-driven Mechanisms. We wanted to determine the relevance of melanoma and astrocytic cell heparanases in invasion. We incubated ACM with 70W and B16B15b and examined ACM effects on their invasive behavior. Consistent increases in invasive values after ACM exposure to 70W and B16B15b were found. Invasion was most pronounced using CM from NGF-treated astrocytes and completely abrogated in presence of an antibody against human heparanase in its activated form (HEP; Ref. 16; Table 1). The invasion enhancement caused by this NGF treatment was also abolished in presence of a neutralizing NGF MAb

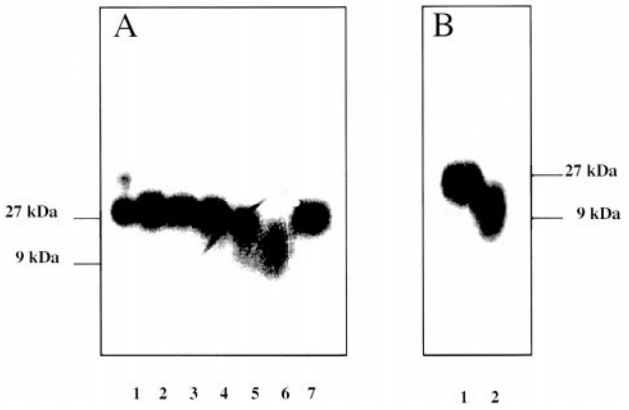

Fig. 2. Purified [^{35}S]HS from brain subendothelial ECM HSPGs was incubated with astrocyte cell extracts at indicated times: *A:* 0 (*Lane 1*), 1 h (*Lane 2*), 3 h (*Lane 3*), 6 h (*Lane 4*), 12 h (*Lane 5*), 24 h (*Lane 6*); extract treated with the heparanase inhibitor suramin (100 μM; Ref. 14; *Lane 7*). *B:* 0 (*Lane 1*); 6 h, extract from NGF-treated astrocytes (*Lane 2*). Isolation and characterization of [^{35}S]HS and heparanase assays were performed as reported (Ref. 3; see also "Materials and Methods"). Estimation of molecular sizes is based on elution profiles from Superose 6 column calibrated with standard glycans (14). The resulting products were separated by agarose gel electrophoresis and visualized by autoradiography.

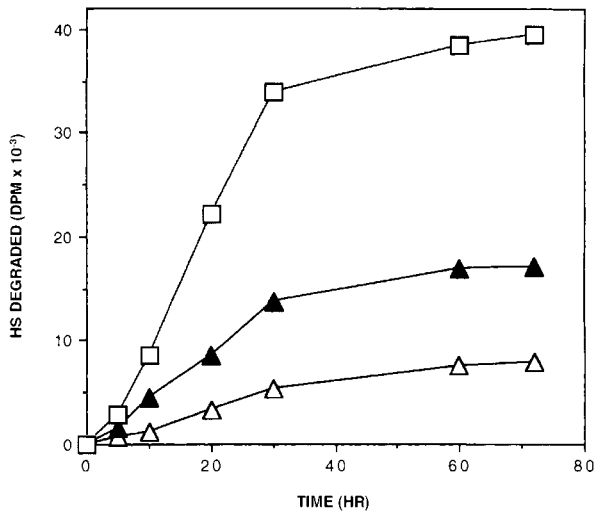

Fig. 3. Heparanase activity of astrocyte cell monolayers (△), brain-metastatic melanoma cells (70W; ▲), or a combination of the two in equicellular amounts (□). Cells were preincubated at indicated times before obtaining cell extracts. Lysates were then incubated for 18 h at 37°C with purified brain subendothelial [^{35}S]HS, followed by HPLC analysis of HS elution profiles (14). Control values (no cell extract) were subtracted from values from test samples as decrease in area of the high molecular weight half of the HS peak. Each *point* represents the average of three experiments, where SD was <10% of raw data. These results were confirmed coincubating the murine B16B15b cell line, a brain-metastatic B16F1-variant (11), with syngeneic astrocytes.

(17). Controls for chemoinvasion specificity consisted in seeding the invasive but non-brain-metastatic parental cells (human MeWo and murine B16F1). In both cases, invasion rates were significantly less pronounced when compared with brain-metastatic 70W and B16B15b cells (data not shown; Ref. 2).

Discussion

Because the brain microvasculature represents an exceedingly small fraction of the total microvasculature, much smaller than the proportion of tumors that metastasize to the brain, the process of brain metastasis is thought to be selective and nonrandom (1). Neoplastic cells with the potential to colonize the brain may express unique molecular determinants and may also respond to brain-derived factors important for their invasion in the brain (18). Malignant melanoma is the cancer type with the highest increase of cases among young adults and one with the highest frequencies of brain metastases formation (1). Melanoma cells that have successfully penetrated the first two zones of the BBB, the brain microvessel endothelium and the thick basement membrane, must then establish interactions with, and respond to, astroglial cells. Among the different cytokines that are produced by astrocytes, NGF play a specific role on brain-metastatic cells. Recent studies have indicated that melanoma cell-surface receptor p75^{NTR} and its ligand, NGF, are excellent candidates as determinants of melanoma brain metastasis (2, 17, 20). For example, p75^{NTR} strongly correlates with brain colonization potential and is capable of signaling in response to NGF independently of its high-affinity counterpart TrkA (19). Moreover, NGF is produced by normal brain tissue at the invasion front of melanoma lesions growing in the brain (20). Exogenously supplied NGF, and possibly other NTs, may stimulate melanoma cells to invade further into the brain parenchyma by augmenting the production of heparanase, which degrades the glycosaminoglycan side chains of HSPGs (3, 4) and play important roles in tumor metastasis (15). Interestingly, brain-metastatic melanoma cells express transforming growth factor-β1, interleukin 1β, and basic fibroblast growth factor, factors that have been shown to stimulate NGF synthesis by astrocytes (18, 21). Therefore, brain metastasis outcome can depend on interactions with, and responsiveness to, astrocyte-released NGF that aid in the survival, growth, and invasion of p75^{NTR}-expressing melanoma cells in the central nervous system.

We report that astrocytes contribute to the invasive capabilities of brain-colonizing melanoma cells. Importantly, our laboratory is the first to provide evidence that astrocytes are *per se* a source of heparanase, able to produce the active enzyme and modulate cell invasion. We suggest that astrocytes contribute to the microenvironment that selectively supports the growth of metastatic cells once they have crossed the BBB, thus being directly involved in the development of brain metastases. Our data support the concept that melanoma brain invasion results from establishing reciprocal circuits between the tumor cells and the normal glial cells present in the central nervous system. After mechanical/chemical brain insults, increased NGF presence is imperative for regeneration events of injured areas (18). These changes may be paralleled by brain invasive melanoma cells, the colonization of which can trigger NGF and NGF-regulated heparanase secretion by brain cells as a response to the invasion event. Melanoma cells, known to overexpress p75^{NTR} (19), can benefit from such a synergistic microenvironment and further invade into the brain parenchyma.

Questions related to the benefit of therapeutic suppression of the NGF/heparanase axis in brain-metastatic melanoma remain unanswered. Further studies will be useful to address these questions.

Table 1 *Invasion of HSPG-coated filters in brain-metastatic melanoma cells: effects of cortical astrocytes CM*

Conditions	Time (h)	Brain-metastatic melanoma cells	
		70W	B16B15b
Cells alone	0	1.00 ± 0.1[a]	1.13 ± 0.2
	72	2.84 ± 0.9	3.02 ± 0.7
+ ACM	0	1.10 ± 0.5	1.08 ± 0.3
	72	6.81 ± 0.7	7.83 ± 0.3
+ CM, NGF-treated astrocytes	0	1.02 ± 0.2	1.10 ± 0.5
	72	20.7 ± 0.6	24.3 ± 0.2
+ CM, HEP-treated astrocytes	0	1.08 ± 0.3	1.07 ± 0.2
	72	1.02 ± 0.2	1.02 ± 0.1
		$n = 4$[b]	$n = 4$

[a] The values given are arbitrary fluorescence units. The fluorescence units at time 0 were set to 1.00. Chemoinvasion assays were performed as reported (2).

[b] Data are the means of four independent experiments with quadruplicate assays performed in each experiment; $P < 0.01$.

Acknowledgments

We would like to thank Dr. Philip Tofilon, M. D. Anderson Cancer Center, for comments and help in the initial stages of our studies, Drs. Motono Nakajima and Jian Dong, Novartis Pharma K. K. Takarazuka, Japan for helpful discussion, and Dr. Robert L. Heinrikson, Research Operations, Pharmacia and Upjohn, Inc., Kalamazoo, MI, for making available antibodies against human active heparanase.

References

1. Wright, D. C., and Delaney, T. F. Treatment of metastatic cancer to the brain. *In:* V. T. DeVita, S. Hellmann, and S. A. Rosenberg (eds.), Cancer: Principals and Practice of Oncology, pp. 2245–2261. New York: J. B. Lippincott, 1989.
2. Marchetti, D., Menter, D. G., Jin, L., Nakajima, M., and Nicolson, G. L. Nerve growth factor effects on human and mouse melanoma cell invasion and heparanase production. Int. J. Cancer, *55:* 692–699, 1993.
3. Marchetti, D., McQuillan, D. J., Spohn, W. C., Carson, D. D., and Nicolson, G. L. Neurotrophin stimulation of human melanoma cell invasion: selected enhancement of heparanase activity and heparanase degradation of specific heparan sulfate subpopulations. Cancer Res., *56:* 2856–2863, 1996.
4. Toyoshima, M., and Nakajima, M. Human Heparanase. J. Biol. Chem., *274:* 24153–14160, 1999.
5. Iozzo, R. V., and Murdoch, A. D. Proteoglycans for the extracellular environment: clues from the gene and protein side offer new perspectives in molecular diversity and function. FASEB J., *10:* 598–614, 1996.
6. Vlodavsky, I., Friedmann, Y., Elkin, M., Aingorn, H., Atzmon, R., Ishai-Michaeli, R., Bitan, M., Pappo, O., Peretz, T., Michal, I., Spector, L., and Pecker, I. Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis. Nature Med., *5:* 793–802, 1999.
7. Hulet, M. D., Freeman, C., Hamdorf, B. J., Baker, R. T., Harris, M. T., and Parish, C. E. Cloning of mammalian heparanase, an important enzyme in tumor invasion and metastasis. Nature Med., *5:* 803–809, 1999.
8. Parish, C. R., Freeman, C., Brown, K. J., Francis, D. J., and Cowder, W. B. Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using a novel *in vitro* assay for angiogenesis and heparanase activity. Cancer Res., *14:* 3433–3441, 1999.
9. Condorelli, D. F., Dell'Albani, P., Mudo', G., Timmusk, T., and Belluardo, N. Expression of neurotrophins and their receptors in primary astroglial cultures: induction by cyclic AMP-elevating agents. J. Neurochem., *63:* 509–516, 1994.
10. Ishikawa, M., Fernandez, B., and Kerbel, R. S. Highly pigmented human melanoma variant which metastasizes widely in nude mice, including skin and brain. Cancer Res., *48:* 4897–4903, 1988.
11. Miner, K. M., Lotan, R., and Nicolson, G. L. Metastatic and melanogenic properties of *in vivo* selected B16 melanoma sublines and their clonal derivatives. Cancer Res., *42:* 4631–4638, 1982.
12. McCarthy, K. D., and deVellis, J. Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. J. Cell. Biol., *85:* 890–902, 1980.
13. Raff, M. C., Fields, K. L., Hakomori, S. I., Mirski, R., Pruss, R. M., and Winter, J. Cell-type-specific markers for distinguishing and studying neurons and the major classes of glial cells in culture. Brain Res., *174:* 283–308, 1979.
14. Marchetti, D., Liu, S., Spohn, W. C., and Carson, D. D. Heparanase and a synthetic peptide of heparan sulfate-interacting protein recognize common sites on cell surface and extracellular matrix heparan sulfate. J. Biol. Chem., *272:* 15891–15897, 1997.
15. Nakajima, M., Irimura, T., and Nicolson, G. L. Heparanase and tumor metastasis. J. Cell. Biochem., *36:* 157–167, 1986.
16. Fairbanks, M. B., Mildner, A. M., Leone, J. W., Cavey, G. S., Mathews, R., Dong, R. F., Slighton, J. L., Bienkowski, M. J., Smith, C. W., Bannow, C. A., and Heinrikson, R. L. Processing of the human heparanase precursor and evidence that active enzyme is a heterodimer. J. Biol. Chem., *274:* 29587–29590, 1999.
17. Walch, E. T., and Marchetti, D. Role of neurotrophins and neurotrophin receptors in the *in vitro* invasion and heparanase production of human prostate cancer cells. Clin. Exp. Metastasis, *17:* 307–314, 1999.
18. Nicolson, G. L., Menter, D. G., Herrmann, J. L., Yun, Z., Cavanaugh, P., and Marchetti, D. Brain metastasis: role of trophic, autocrine and paracrine factors in tumor invasion and colonization of the central nervous system. *In:* U. Gunthert and W. Birchmeier (eds.), Attempts to Understand Metastasis Formation II, pp. 90–115. Berlin: Springer-Verlag, 1996.
19. Herrmann, J. L., Menter, D. G., Hamada, J., Marchetti, D., Nakajima, M., and Nicolson, G. L. Mediation of NGF-stimulated extracellular matrix invasion by the human melanoma low-affinity p75 neurotrophin receptor: melanoma p75 functions independently of *trkA.* Mol. Biol. Cell, *4:* 1205–1216, 1993.
20. Marchetti, D., McCutcheon, I., Ross, M., and Nicolson, G. L. Inverse expression of neurotrophins and neurotrophin receptors at the invasion from of human melanoma brain metastases. Int. J. Oncol., *7:* 87–94, 1995.
21. Yoshida, K., and Gage, F. H. Fibroblast growth factors stimulate nerve growth factor synthesis and secretion by astrocytes. Brain Res., *538:* 118–126, 1991.

Astrocytes Contribute to the Brain-metastatic Specificity of Melanoma Cells by Producing Heparanase

Dario Marchetti, John Li and Ruijun Shen

Cancer Res 2000;60:4767-4770.