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Characterization of Tenascin Secreted by Human Melanoma Cells[1]

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ABSTRACT

Tenascin is a large glycoprotein of the extracellular matrix. It shows a site-restricted expression during embryogenesis and can be found in adult tissues during wound healing and tumorigenesis. Because of the potential involvement of tenascin in adhesion and invasion during metastasis, the study of the interactions of tumor cells with tenascin is of considerable interest. Using five anti-melanoma monoclonal antibodies to four different epitopes of human tenascin, we found that most melanoma cells secrete tenascin *in vitro* constitutively. Transforming growth factor β_1 in the medium increased secretion in tenascin-producing cells. Tenascin was present in sera of melanoma patients, with significantly elevated levels in patients with advanced melanomas as compared to patients with low tumor burden or to normal donors. Normal and malignant melanocytes did not attach to tenascin as substrate within 1 to 2 h and tenascin could also inhibit fibronectin-dependent adhesion. These results indicate that tenascin may play a critical role in cell-substrate interactions of melanoma cells.

INTRODUCTION

The extracellular matrix consists of a complex network of molecules that interact with cells to effect a wide range of cellular functions. During tumor proliferation and metastasis, extracellular matrix proteins act as substrates for tumor cell attachment and motility and as targets for tumor-derived proteolytic enzymes (1). One of these extracellular matrix proteins is tenascin (2), also known as hexabrachion (3), myotendinous antigen (4), glioma mesenchymal extracellular matrix antigen (5), J1 glycoprotein (6), cytotactin (7), GP-250 (8), and gp 150/225 (9). Human tenascin is a high mass oligomeric glycoprotein with disulfide-bonded M_r 32,000 subunits. It has a hexameric structure with two pairs of three arms connected to a central globule (10). The expression of tenascin shows an oncofetal predominance. During embryonic development it is found in the condensing mesenchyme of developing organs such as mammary glands, hair follicles, toothbuds, and kidneys (2). For neural crest cells it lines the future migratory pathways (11). Tenascin is largely absent in normal adult tissue but can be found during wound healing (12) and is expressed by stromal cells of epithelial tumors, including mammary carcinomas (2), lung carcinomas (13), and squamous cell carcinomas (14). Glioma (5) and melanoma (15) cells produce tenascin *in situ* and in culture.

The exact function of tenascin is not yet known, but it seems to play an important role in cell adhesion and spreading. While several reports indicate that tenascin itself has no cell adhesion activity (2, 15, 16), and may even inhibit cell attachment to other extracellular matrix proteins including fibronectin (17, 18), others have shown variable cell adhesion to tenascin (19).

In the present study, we describe five mAb[3] that were derived by immunizing mice with a high molecular weight fraction of culture supernatants of melanoma cells. These antibodies define 4 different epitopes on tenascin. Tenascin levels are increased in sera of patients with advanced melanoma and melanoma cells in culture secrete tenascin constitutively but do not attach to it.

MATERIALS AND METHODS

Origin and Culture of Human Cells and Sera. Normal human melanocytes were obtained from newborn foreskin (20, 21), nonmalignant nevus cells from common acquired and congenital nevi (22, 23), and melanoma cells from primary and metastatic lesions (24, 25). Methods for culturing melanocytic cells and production of serum-free spent medium containing soluble antigens have been described (26, 27). Medium for culturing melanoma cells was initially a 4:1 mixture of minimal essential medium and L15 medium; in later experiments minimal essential medium was replaced by MCDB 153 supplemented with 2 mM calcium (W489 medium). Undifferentiated keratinocytes were cultured as described (28). Other human cells in culture have been described (24). Sera of tumor patients were collected at the Cancer Center of the University of Pennsylvania. Control sera were randomly collected at the same institution from adult donors without evidence of disease.

Production of mAb to a High Molecular Weight Protein in Culture Supernatants of Melanoma Cells. Cationic proteins were isolated from the conditioned media of two metastatic melanoma cell lines (WM 239A and WM 266–4) derived from the same patient (25) by adhesion to sulfated dextran (29). The proteins were size fractionated by gel filtration chromatography on Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden), and mice were immunized with the high molecular weight fractions (>150,000) which were mixed with Lipid A of *Salmonella minnesota* as adjuvants. Selection of hybridomas for binding to culture supernatants of melanoma cells, growth of hybridomas, cloning by limited dilutions, and purification of antibodies from ascitic fluid of hybridoma-bearing mice were done following standard protocols (24).

Antibodies and Extracellular Matrix Proteins. Other mAb used were No. 3, binding to human collagen type IV (kindly provided by Dr. E. Engvall, Cancer Research Foundation, La Jolla, CA); 0762, anti-human amniotic fibronectin (Boehringer Mannheim Biochemicals, Indianapolis, IN); and 428006, anti-human laminin type 1 (Calbiochem, San Diego, CA); anti-tenascin mAb 81C6 (kindly provided by Dr. C. Wikstrand, Duke University, Durham, NC); and anti-melanocyte mAb 487 (20). For controls, culture supernatants of mouse myeloma P3X63Ag8 or purified IgG of nonrelated mAb were used. Human basement membrane proteins included were collagen types I-IV (Southern Biotechnology Associates, Birmingham, AL), placental laminin (kindly provided by Dr. M. Ohno, Connective Tissue Research Institute, Philadelphia, PA), fibrinogen (Sigma), plasma fibronectin (Boehringer Mannheim), and tenascin from human glioma cells (Telios Pharmaceuticals, Inc., San Diego, CA). Fibronectin from culture supernatants of melanoma cell line WM 9 was isolated by absorption to gelatin-Sepharose 4B (Pharmacia) and elution with 8 M urea in 0.1 M citric acid, pH 4.7 (30).

Binding Studies. Binding of mAb to antigen released by cultured cells was tested in solid phase RIA. Culture supernatants of confluent

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[3] The abbreviations used are: mAb, monoclonal antibody; RIA, radioimmunoassay; BSA, bovine serum albumin; TGF, transforming growth factor.

cultures were absorbed overnight at room temperature to polyvinyl chloride microtiter wells. Nonspecific binding of antibodies was blocked by absorption of wells with 2% gelatin, and antigens were cross-linked before assay with 0.05% glutaraldehyde. Binding of mAb was tested with ^{125}I-labeled goat IgG anti- F(ab')$_2$. Binding inhibition assays (31) were done to determine the presence of soluble antigen in sera of patients. For this, serum or antigen preparations were premixed with a dilution of mAb adjusted to give 60% of its maximal binding to the immobilized antigen. After incubation overnight at 4°C, reactivity of unbound antibody was then tested in solid phase RIA. Results of inhibition assays were subjected to statistical analysis using a two-tailed Student *t* test to compare the mean percentage of inhibition in various test groups. Quantitative double determinant RIA with purified tenascin as standard were done to determine tenascin secretion in spent medium of serum-free cultures. Microtiter plates with 96 wells were precoated with 300–3, an anti-tenascin mAb (1 μg/ml). After incubation overnight and blocking of unspecific binding with BSA buffer (1% BSA plus 0.05% Tween plus 0.02% sodium azide), wells were incubated with conditioned medium or purified tenascin for 4 h. Bound antigen was detected with ^{125}I-labeled 302–9, another anti-tenascin mAb, in solid phase RIA. Additive competition assays (32) were done to determine whether mixed mAb would show additive binding in solid phase RIA which would indicate binding to a different determinant. In this assay, saturating quantities of ^{125}I-labeled rabbit IgG anti-mouse F(ab')$_2$ were used. In direct competition RIA, binding of one radiolabeled mAb to target was inhibited by a 100-fold excess of cold purified antibody. Both competition assays gave comparable results with the antibodies included in this study. Mixed hemadsorption assays for binding of mAb to cell surfaces of cultured cells were done as described (33).

Modulation of Tenascin Secretion by TGF-β_1. Melanocytes from 14 different melanoma cell lines were plated in 6-well 35-mm culture plates in 3 ml of W489 medium supplemented with insulin (5 μg/ml), and 0.2% BSA at 3×10^5 cells/well. After 3 h, medium with or without TGF-β_1 (R-D Systems, Minneapolis, MN) at 10 ng/ml (final TGF-β_1 concentration of 2.5 ng/ml) was added to cells. Four days later, conditioned medium was collected and assayed for tenascin content by quantitative double determinant RIA, and cell numbers were counted (Coulter Counter) to adjust tenascin secretion to the actual number of cells per well. Tenascin secretion was expressed as ng/ml/10^5 cells.

Cell Attachment Assays. For attachment assays, cells were trypsinized 1 day before experiments and reseeded in medium containing [*methyl*-^{3}H]thymidine at 2 μCi/ml. Plates with 24 wells (2 cm^2) were coated with extracellular matrix proteins at 10 μg/ml for 1 h at 37°C and then incubated for 30 min with 4% BSA in Dulbecco's modification of phosphate-buffered saline. The radiolabeled cells were then trypsinized and seeded at 2×10^4/cm^2 in serum-free medium. After incubation for 30 and 90 min at 37°C, medium was aspirated and cells were washed twice for removal of nonadherent cells. Attached cells were then lysed in 300 μl of 1% sodium dodecyl sulfate in 0.5% Triton X-100 and transferred into an aqueous counting cocktail (CYTOSCINT ES; ICN Biomedicals, Irvine, CA) for counting the radioactivity. The percentage of attachment was calculated by dividing the number of counts of the attached cells by the number of counts for total cells seeded.

Biochemical Analyses. For immunoprecipitation of mAb-defined antigens under reducing and nonreducing conditions, subconfluent monolayers of melanoma cells were biosynthetically labeled with [^{35}S]methionine (1213 Ci/mmol) at 25 μCi/ml for 4 h, or with D-[6-^{3}H]glucosamine hydrochloride (39 Ci/mmol) at 8 μCi/ml for 48 h following a procedure as described (34). For analysis by Western blotting, melanoma cells were lysed with 0.5% Nonidet P-40 (80 μl), and the lysate was mixed with 20 μl of 50% glycerol:10% sodium dodecyl sulfate (1:1) and applied to a 10% Laemmli gel. Western blotting was carried out essentially as described by Towbin *et al.* (35). Isolation of mAb-defined antigen was done by either applying detergent-solubilized melanoma cell extract or spent culture medium to a mAb-Sepharose 4B column.

RESULTS

Binding Specificity. Five mAb (300–1, 300–2, 300–3, 302–1, 302–9), all of IgG1 isotype, from two independent fusion experiments, were reactive with spent medium of melanoma cells. The mAb did not bind to plasma or melanoma-derived fibronectin (as 34 mAb from the same fusion experiments did) or to collagen types I-IV, or laminin (Table 1). In contrast, strong binding was seen to glioma-derived purified tenascin. Control anti-tenascin mAb 81C6 showed similar binding. Competitive binding assays indicated the presence of at least 5 noncompeting determinants that were detected with antibodies 300–1, 300–2, 300–3, 302–9, and 81C6. Binding of mAb 302–1 was inhibited by 302–9, but 302–1 was unable to inhibit binding of 302–9 indicating that these epitopes partially overlapped (results not shown).

The majority of cultures of metastatic melanomas (86%), advanced primary melanomas (86%), gliomas (100%), and normal skin fibroblasts (100%) secreted tenascin at detectable levels (Table 2). On the other hand, cultures of early primary melanomas (3 of 4), carcinomas (13 of 16 cultures), lymphomas, leukemias, and normal keratinocytes did not secrete tenascin or secreted it at low levels. Culture supernatants of 3 of 15 melanocyte cultures and 2 of 8 nevus cultures bound anti-tenascin mAb at low but detectable levels. All five mAb (300–1, 300–2, 300–3, 302–1, 302–9) showed a binding pattern comparable to that of anti-tenascin mAb 81C6 known to bind to tenascin (not shown). In quantitative double determinant RIA with purified tenascin as standard, the level of secretion between different primary and metastatic melanoma cell lines varied and secretion ranged between 10 and 910 ng/ml (Fig. 1). Primary and metastatic melanomas showed significantly higher levels of tenascin secretion when compared to normal melanocytes ($t = 2.1$ and 3.7, respectively). All of 34 melanoma cultures tested also secreted fibronectin (results not shown). Fibronectin secretion, when tested in solid phase RIA, was at similar levels to tenascin secretion in 13 of 16 cultures (81%). Collagen type IV and laminin secretion by 15 melanoma cultures, on the other hand, was at or below the detection level in solid phase RIA. Tenascin could not be detected by mixed hemadsorption assays or fluorescence-activated cell sorting on the cell surface of melanomas (13 cultures tested) or gliomas (4 cultures tested).

Biochemical Analysis of Melanoma-derived Tenascin. Radiolabeled tenascin could be specifically precipitated from culture supernatants of melanoma cells which were grown in the presence of [^{35}S]methionine (Fig. 2). In addition, although the antigen was not detected on the surface of the cells, it could be immunoprecipitated from cell lysates. The antigen could also be immunoprecipitated from solubilized melanoma cells extracted after biosynthetic labeling with [^{3}H]glucosamine (data not shown). Presumably the cell-associated population of antigen represents internal, mature molecules which are probably fully glycosylated and ready for secretion. The inability to detect the antigen on the cell membrane suggests that its residence time and/or concentration at the cell surface during secretion is negligible.

The antigen defined by the melanoma-derived mAb was further compared to authentic human glioma-derived tenascin using Western blot analysis with mAb 300–3 (Fig. 3). The immunoreactive band in both unfractionated melanoma culture supernatant and antigen that was affinity purified from the same culture medium was indistinguishable from commercial human glioma tenascin (Fig. 3*A*). The major immunoreactive band in all cases was at a molecular weight of approximately 320,000 and was coincident with the major band detected after either silver staining or Coomassie blue staining of duplicate lanes containing glioma tenascin. An identical band was also

Table 1 *Binding of monoclonal antibodies to purified human basement membrane proteins*

Target proteins were applied to polyvinylpyrollidone microtiter plates and, after blocking nonspecific binding of proteins, binding of monoclonal antibodies was tested in an indirect radioimmunoassay with ^{125}I-goat IgG anti-mouse F(ab')$_2$.

	cpm in solid phase radioimmunoassay[b]							
			Collagen[e]					
Monoclonal antibody[a]	Plasma fibronectin[c]	Melanoma fibronectin[d]	Type I	Type II	Type III	Type IV	Laminin[f]	Tenascin[e]
302-1	0	0	0	0	0	0	202	7,870
300-3	0	0	0	0	0	0	560	8,272
300-2	0	0	0	0	0	0	486	7,925
302-9	0	0	148	0	0	0	318	8,158
300-1	0	0	0	0	0	0	358	8,089
Anti-collagen type IV	0	0	0	0	0	3,315	210	NT[g]
Anti-fibronectin	18,370	7,091	0	0	0	0	4,888	NT
Anti-laminin	0	0	0	0	0	0	10,174	0
Anti-tenascin	NT	NT	NT	NT	NT	NT	NT	5,220

[a] Results are shown for antibody concentrations of 10–15 μg/ml in tissue culture supernatants (300–302 series), ascites (anti-collagen IV), or purified antibody (anti-fibronectin, anti-laminin).
[b] cpm after subtraction of cpm obtained with 10 μg/ml of control antibody. All assays were done in duplicate or triplicate with less than 7% deviation from mean.
[c] One μg/ml; similar results were obtained with 10 μg/ml.
[d] Urea eluate (8 M) of gelatin-Sepharose 4B column, using serum-free medium, of WM 9 cell line.
[e] 1 μg/ml.
[f] 2.5 μg/ml; prepared from human placenta.
[g] NT, not tested.

Table 2 *Binding of monoclonal antibody 300-3 to serum-free supernatants of cultured cell lines*

Confluent cultures grown in the presence of serum were washed twice and maintained for 3 to 5 days in the absence of serum. After removal of cellular debris, culture supernatants were tested in indirect solid phase radioimmunoassay for binding of monoclonal antibody.

			No. of supernatants of cell lines binding antibody in indirect radioimmunoassay			
Tissue type	Cells	No. of cell lines tested	Negative (<500 cpm)[a]	Weak (500–2500 cpm)	Moderate (2500–5000 cpm)	Strong (>5000 cpm)
Tumor	Metastatic melanoma	30	4	5	12	9
	Primary melanoma, advanced	7	1	4	0	2
	Primary melanoma, early	4	3	1	0	0
	Glioma	6	0	0	0	6
	Carcinoma	16	13	1	2	0
	Lymphoma/leukemia	6	6	0	0	0
Nonmalignant lesion	Nevus	8	6	2	0	0
Normal	Melanocytes[b]	15	12	3	0	0
	Keratinocytes	4	4	0	0	0
	Skin fibroblasts	9	0	4	4	1

[a] A 500 cpm value was arbitrarily chosen as cutoff between negative and positive.
[b] Melanocytes were grown in the presence of the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate.

detected by Western blot analysis of the same samples with the anti-tenascin mAb 81C6 (data not shown). Also, fractionation of spent culture medium from WM 373 melanoma cells showed that the melanoma antigen could be precipitated between 35% and 40% saturation with ammonium sulfate (Fig. 3*B*). This observation compares well with the expected precipitation of tenascin at 37% saturation (36).

Modulation of Tenascin Secretion by TGF-β_1. Cells from tenascin-secreting ($n = 11$) and nonsecreting ($n = 3$) melanoma cell lines were grown in serum-free medium with or without TGF-β_1. Quantitative double determinant RIA with purified tenascin as standard showed an average increase of tenascin secretion of 200% (range, 18–545%) for all tenascin-secreting melanoma cell lines (Fig. 4). The supernatants of nonsecreting melanoma cells did not contain detectable amounts of tenascin before or after incubation of cells with TGF-β_1 for 4 days. Induction of fibronectin secretion by TGF-β_1 was also tested in the same cell lines by solid phase RIA. All tenascin-secreting cell lines also secreted fibronectin, which was increased after TGF-β_1 treatment of cells by 30–200% (data not shown). Cell lines not secreting tenascin also did not secrete fibronectin, nor could they be induced by TGF-β_1 to secrete fibronectin.

Detection of Serum Antigen. The ability of antigen in patients' sera to inhibit the binding of mAb 302–9 and 300–3 to serum-free conditioned medium of metastatic melanoma cell line WM 9 was determined by binding inhibition assays (Table 3). Normal sera inhibited binding of both mAb [means, 17 ± 3.2% (SD) and 13.6 ± 4.1%, respectively], suggesting the presence of low levels of circulating antigen. In studies with mAb 302–9 (Table 3), sera from melanoma patients with minimal or no apparent tumor burden (designated A, B, and C) and evident metastatic tumor burden (stage D) both showed significantly elevated levels of circulating antigen as compared to controls ($P = 0.003$ and $P < 0.001$, respectively). Furthermore, sera from metastatic melanoma patients with evident tumor burden had more circulating antigen than sera from patients with low tumor burden ($P = 0.001$). Sera of patients with other cancers also had significantly elevated levels of tenascin antigen compared to normal control sera ($P = 0.048$). In studies with mAb 300–3, patients with advanced melanoma had significantly elevated circulating tenascin serum levels as compared to normal donors ($P = 0.048$), whereas patients with minimal or no evident melanoma and patients with other cancers did not differ significantly from normal donors ($P = 0.989$ and $P = 0.213$, respectively).

Inhibition of Cell Attachment by Tenascin. Cells from primary and metastatic melanomas were tested for attachment to fibronectin, collagen types I and IV, laminin, fibrinogen, and tenascin. With the exception of tenascin, all tested extracellular matrix proteins allowed attachment of melanoma cells (Table

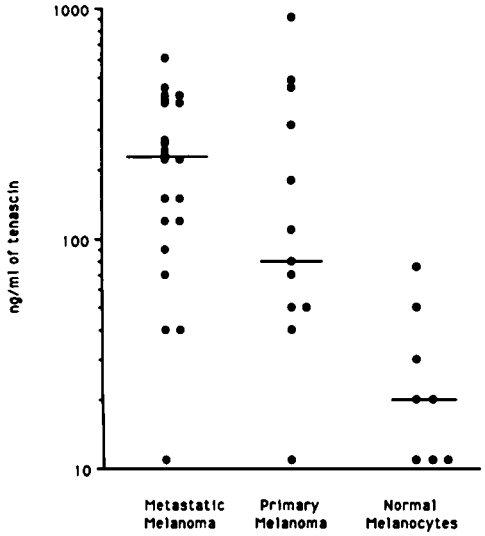

Fig. 1. Secretion of tenascin by confluent cultures of primary and metastatic melanoma cells and normal melanocytes was determined by double determinant assays using antibody 300–3 as antigen catcher and ^{125}I-labeled antibody 302–9 as antigen tracer. Purified tenascin was used as standard. *Bars*, median.

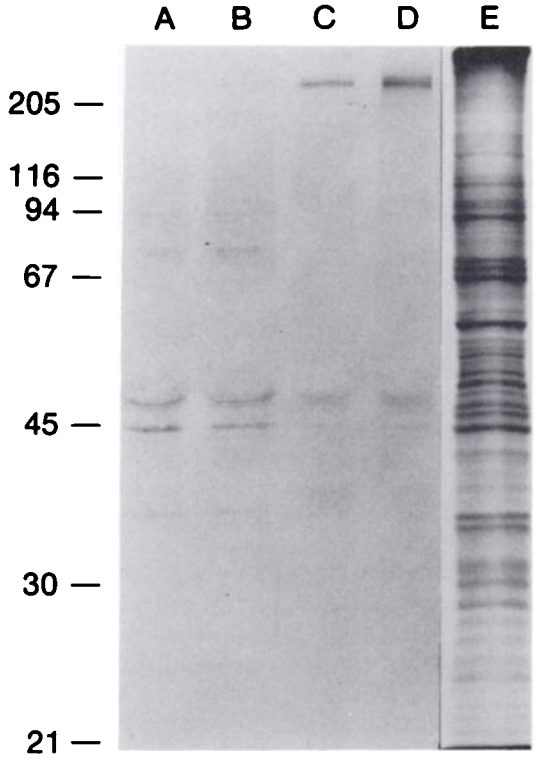

Fig. 2. Immunoprecipitation of tenascin from [^{35}S]methionine-labeled melanoma cells by mAb 300–3. *A*, spent medium collected from labeled WM 9 cells maintained for 48 h in serum-free medium, concentrated and immunoprecipitated with a non-specific mAb, P3X63Ag8; *B*, cell lysate of WM 9 cells from the same experiment and immunoprecipitated with control mAb; *C*, spent medium from WM 9 cells immunoprecipitated with mAb 300–3; *D*, WM 9 cell lysate immunoprecipitated with mAb 300–3; *E*, silver stain of the WM 9 cell lysate.

4). Highest attachment was seen for fibronectin, laminin, and collagen type IV. Collagen type I promoted attachment only of the primary melanoma cell line WM 793 and fibrinogen supported attachment at relatively low levels. Tenascin inhibited attachment also of normal melanocytes (not shown). When wells were first coated with fibronectin (25 μg/ml) and then with tenascin (20 μg/ml), cell attachment was reduced by more that 80% as compared to attachment to fibronectin alone. Cells adhering to a mixture of fibronectin and tenascin were more rounded and showed less spreading than cells attached to fibronectin alone.

DISCUSSION

Interactions between extracellular matrix proteins of basement membranes and tumor cells play a pivotal role in tumor invasion and metastasis (1). The role of extracellular matrix proteins secreted by tumor cells is less clear. In order to further characterize matrix proteins released by human tumor cells, we have produced mAb to a high M_r fraction found in spent medium of cell cultures of melanomas. As a result of this approach, we have described five anti-melanoma mAb, which were shown to bind to the extracellular matrix protein tenascin. Competition binding studies showed that these mAb recognize four different epitopes on the human tenascin molecule and do not compete for binding with the only other anti-human tenascin mAb 81C6.

Tenascin can be found in the stroma of several malignant tumors (2, 5, 13, 14, 15), but with the exception of glioma and melanoma cells, human tumor cells appear not to produce tenascin themselves. Therefore, we further studied tenascin secretion by melanocytes from different stages of melanoma progression, other tumor cells and by normal human skin cells, including fibroblasts, keratinocytes, and melanocytes. Cells expressing high amounts of tenascin were advanced primary and metastatic melanomas, gliomas, and normal skin fibroblasts. Fibroblasts and gliomas are known to produce tenascin at 200–

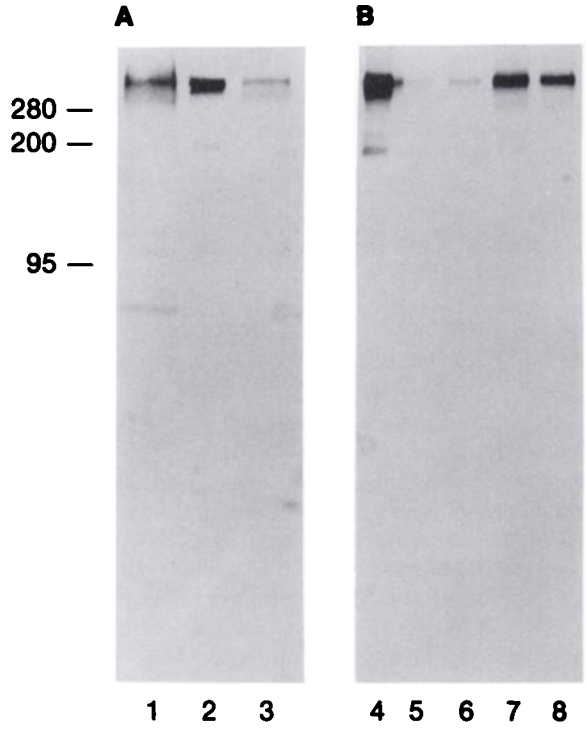

Fig. 3. Western blot comparison of human melanoma- and glioma-derived tenascin using mAb 300–3. Samples were separated using reducing conditions on a 7% Laemmli gel. *A: Lane 1*, antigen purified from culture supernatants of WM 373 cells by affinity chromatography on a Sepharose 4B containing about 1 mg/ml of mAb 300–3; *Lane 2*, 0.5 μg of human glioma-derived tenascin; *Lane 3*, 125 μl of spent culture supernatant from WM 373 cells. *B: Lane 4*, human glioma tenascin; *Lanes 5* to *8*, ammonium sulfate fractionation of spent melanoma culture medium; *Lane 5*, 40% supernatant; *Lane 6*, 35% supernatant; *Lane 7*, 40% pellet; *Lane 8*, 35% pellet.

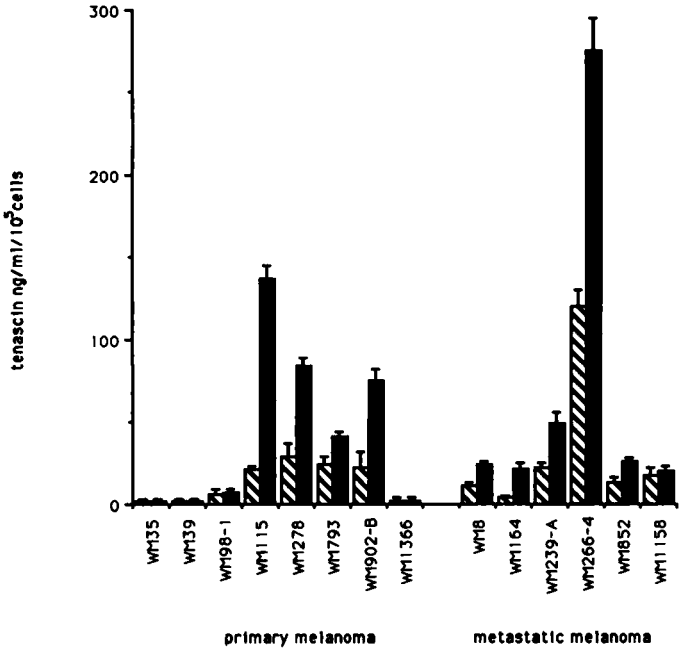

Fig. 4. Effect of TGF-β_1 on secretion of tenascin by primary and metastatic melanoma cells. Cells were incubated for four days in the presence (■) or absence (▨) of 2.5 ng/ml TGF-β_1. Tenascin in culture supernatants was quantitated in double determinant RIA with purified tenascin as standard.

500 ng/ml and 5–10 μg/ml, respectively (36, 37). Here we report that melanoma cells are able to secrete tenascin in amounts comparable to those produced by fibroblasts. It is interesting to note that melanoma cells showed tenascin secretion even under growth factor-free culture conditions, indicating a constitutive tenascin production in these cells. Our quantitative studies on tenascin secretion of *in vitro* cultures of melanocytes from different stages of melanoma progression showed an increase with malignant progression. Melanocytes from metastatic melanomas produced the highest concentration of tenascin, whereas normal melanocytes showed no or only marginal tenascin secretion. The increased expression of tenascin by premalignant and malignant melanocytes within the dermal portion of skin (15) suggests that nevus and melanoma cells produced tenascin for decreased substrate adhesion for migration during invasion. In the normal skin, tenascin, which localizes within the lamina lucida of the basement membrane (14), is apparently synthesized by fibroblasts and not by melanocytes or keratinocytes.

To determine whether an increase in tenascin production correlates also *in vivo* with tumor progression, we tested serum from normal donors and melanoma patients for soluble tenascin. In patients with melanomas, there was a correlation between tumor burden and tenascin secretion, showing the highest levels of tenascin in sera from patients with evident melanoma metastases. Our results also show that tenascin, which is highly expressed in the stroma of several epithelial tumors, can be found in sera from such tumor patients. However, differences in the relative levels of tenascin in sera of normal donors compared to tumor patients are not striking and possibly are too low for this assay to be used as a general diagnostic tumor marker.

Several authors have studied the effect of tenascin on cell-matrix interactions (2, 15–19). These experiments suggest that tenascin is involved in cell adhesion, migration, and probably also cell growth (2). For the cell-matrix adhesion, tenascin seems to have inhibitory functions which can vary for different cell types. Bourdon and Ruoslahti (19) reported no cell adhesion to tenascin for the human melanoma cell line M21 but some adhesion for the tenascin-producing glioma cell line U251-MG. Using an adhesion assay comparable to ours, Lightner *et al.* (14) could not detect cell adhesion to tenascin for human skin fibroblasts and several others cells, including the U251-MG cells. Our results show that normal human melanocytes and melanocytes from melanomas, regardless of whether they secrete tenascin or not, do not adhere to tenascin.

Chiquet-Ehrismann *et al.* (17) could demonstrate that cells grow on tenascin-containing substrata in a more dispersed manner and have a less flattened morphology as compared to growth on fibronectin. Our results show a similar effect for melanocytes and melanoma cells when seeded on a mixture of fibronectin and tenascin, but considerably more tenascin than fibronectin had to be used since melanocytes have a high affinity for fibronectin. Tenascin is expressed along the migratory pathways of neural crest cells during embryonal development (11, 37, 39) for cell dispersion and motility and it is interesting to note that glioma and melanoma cells, which both derive from the neural crest, are high producers of tenascin. The fact that migration along tenascin belongs to the functional repertoire of neural crest cells may be one reason why glioma and melanoma cells, in contrast to other tumor cells, produce tenascin.

Table 3 *Binding of anti-tenascin antibodies to circulating antigen in patients' sera*

Sera were diluted by mixing with equal quantity of monoclonal antibody which was prediluted to ~60% of maximal binding. Inhibition of binding of antibodies was tested in indirect radioimmunoassays on culture supernatants of melanoma WM 9.

Donor disease	No. of sera	% of inhibition of binding of mAb to target	
		302-9	300-3
Melanoma D[a]	65	31.5 ± 3.4[b]	21.3 ± 5.1
Melanoma A, B, C[c]	32	22.8 ± 1.8	13.5 ± 5.7
Other cancers[d]	14	23.1 ± 5.1	18.8 ± 8.2
Normal donors	35	17.0 ± 3.2	13.6 ± 4.1

[a] "Tumor burden" D, evident metastatic disease.
[b] Mean ± SEM (99% confidence interval).
[c] "Tumor burden" A–C, either no apparent disease after resection of primary lesion (A, 2 sera), regional lymph node metastasis (B, 8 sera), or intact primary tumor before resection (C, 22 sera).
[d] Sera of patients (numbers in parentheses) with either breast carcinoma (2), pancreas carcinoma (2), lung carcinoma (2), colorectal carcinoma (4), renal cell carcinoma (1), or lymphoma (3).

Table 4 *Attachment of primary (WM 793) and metastatic (Lu451) melanoma cells to different extracellular matrix proteins*

Radiolabeled cells were seeded at 2 × 10^4/cm² in serum-free medium on different substrates. After 30 min incubation at 37°C, the cells were washed twice, attached cells were lysed, and radioactivity was counted. The percentage of attachment was calculated by dividing the number of counts of attached cells by the number of counts for total cells seeded.

Attachment proteins[a]	% of attached cells after 30 min ± SD	
	WM 793	Lu451
Fibronectin	42 ± 6.3	39 ± 12
Collagen type I	36 ± 6.5	3 ± <0.1
Collagen type IV	33 ± 5.2	32 ± 8
Laminin	39 ± 2.6	84 ± 9
Fibrinogen	18 ± <0.1	22 ± 7.8
Tenascin	<0.1 ± <0.01[b]	<0.1 ± <0.01
4% bovine serum albumin (control)	0.7 ± <0.01	1.6 ± <0.01

[a] Wells coated with 10 μg/ml of attachment protein for 1 h (except bovine serum albumin control) at 37°C, washed twice with Dulbecco's modification of phosphate-buffered saline and blocked with 4% bovine serum albumin in Dulbecco's modification of phosphate-buffered saline for 30 min.
[b] Similar results were obtained with 20 and 30 μg/ml of tenascin.

The majority of tumors which express tenascin in their stroma do not seem to secrete tenascin, but they release factors that induce tenascin production in surrounding fibroblasts. One candidate for this function is TGF-β, which has been shown to increase the tenascin expression in cultured fibroblasts (40). During embryogenesis the distribution of tenascin is almost completely correlated with the presence of TGF-β_1 RNA (41, 42). Using serum-free cultures we were able to demonstrate enhancement of tenascin secretion for melanoma cells by TGF-β_1. This effect could be observed only in cells that normally produced tenascin, indicating that TGF-β_1 can up-regulate but not initiate tenascin secretion. Melanoma cells are known to produce TGF-β (43), which may account for an autocrine stimulation of tenascin to facilitate cell dispersion and metastasis.

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