Differential Growth Factor Production, Secretion, and Response by High and Low Metastatic Variants of B16BL6 Melanoma

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ABSTRACT

Low levels of tyrosine and phenylalanine alter the metastatic phenotype of B16BL6 murine melanoma. In this study, we investigated expression and secretion of fibroblast growth factor-like (FGF-like) and transforming growth factor β-like (TGFβ-like) molecules as well as the biological effect of basic FGF (bFGF) and TGFβ1 on high (NDP) and low (LTP) metastatic variants of B16BL6 melanoma. Both NDP and LTP cells expressed bFGF-like and TGFβ-like polypeptides as detected by Western blot analysis. An Mr 29,000 bFGF-like form eluted from heparin-Sepharose by 0.6 M NaCl was found in extracts of both NDP and LTP cells. Elution at 0.6 M NaCl suggested that this Mr 29,000 form might be more closely related to FGF-5 than to bFGF. In addition, cell extracts of LTP, but not NDP cells, contained an Mr 47,000 monomeric bFGF-like form that was not retained on heparin-Sepharose. Three major specific immunoreactive forms of Mr 24,400, 36,000, and 29,000 were present in conditioned medium from NDP cells. The Mr 29,000 form present in the conditioned medium of NDP cells was retained on heparin-Sepharose. Only the Mr 24,400 and 36,000 FGF-like molecules were detected in conditioned medium from LTP cells, and they were also not retained on heparin-Sepharose. Anti-TGFβ antibody that recognized both TGFβ1 and TGFβ2 detected 3 different TGFβ-like forms (Mr 25,000, 23,000 and 22,000) in NDP and LTP cell extracts. Conditioned medium from NDP cells contained an Mr 38,000 form of TGFβ; however, no immunoreactive forms were found in conditioned medium from LTP cells. Thus, the NDP-LTP differences in this melanoma system were primarily in growth factor secretion, not expression. The effect of exogenous bFGF and TGFβ1 on proliferation of LTP and NDP cells was determined by [methyl-3H]thymidine uptake. bFGF stimulated proliferation of NDP cells; whereas, LTP cells exhibited no increase in proliferation. Both NDP and LTP cells responded to TGFβ1. Proliferation of NDP cells was inhibited more by this growth factor than was proliferation of LTP cells. When NDP and LTP cells were incubated with 5 ng/ml TGFβ1 and various amounts of bFGF, the effect of TGFβ1 was masked. Antibody depletion of bFGF-like molecules from NDP conditioned medium resulted in the decreased proliferation of NDP cells but not LTP cells. Depletion of TGFβ-like molecules resulted in increased proliferation of LTP cells but did not affect NDP cells. These data suggest (a) that bFGF-like and/or TGFβ-like molecules are important markers of the metastatic phenotype of murine B16BL6 melanoma and (b) that the inability of B16BL6 melanoma cells to secrete FGF-like and TGFβ-like molecules may contribute to a decreased metastatic capability.

INTRODUCTION

Progress during the past 10 years has increased understanding of the different mechanisms that contribute to the initiation and growth of cancer, such as oncogene overexpression (1, 2), alterations in growth factor expression (3), dietary factors (4–6), and host selection pressures (7, 8). Tumor cell–tumor cell and tumor–host cell interactions can alter tumor growth as well as metastasis (1, 6, 9–12). These cellular interactions are modulated by cell contact and/or soluble factors (12, 13). Soluble growth factors are known to affect tumor growth (13, 14–18) and metastasis (13). Growth factors of the FGFs3 (19, 20) and TGFβs (15) affect not only tumor growth (14, 15) and metastasis (13) but also tumor angiogenesis (21, 22). Some tumors have specific amino acid requirements that differ from those of normal cells (23, 24). Previous studies show that metastatic potential can be suppressed by diet (4). Tyrosine and phenylalanine restriction suppresses growth and spontaneous metastasis of B16BL6 melanoma (25). To better understand how amino acid restriction suppresses metastasis of the highly invasive B16BL6 melanoma (6), in this study we compared differences in growth factor secretion between highly metastatic tumor cells (NDP) isolated from mice fed a normal diet and variants (LTP) isolated from mice fed a low phenylalanine and tyrosine diet for which the metastatic phenotype is suppressed (6).

We demonstrate that NDP cells secrete (a) FGF-like molecules with Mr 24,400, 36,000, and 29,000, but only the Mr 29,000 form was retained on the heparin-Sepharose column and was eluted with 0.6 M NaCl and (b) TGFβ-like molecules with Mr 38,000. The low metastatic LTP variant did not secrete FGF- or TGFβ-like molecules, suggesting an important role for these growth factors in metastasis. Both FGF- and TGFβ-like molecules were present intracellularly in the NDP and LTP cells. However, a major bFGF-like protein with Mr 47,000 was found only in LTP cells. bFGF induced cell proliferation of NDP cells but did not affect proliferation of LTP cells. The growth of both NDP and LTP cells was inhibited by TGFβ1 in the concentration range of 1–100 ng/ml, and the NDP cells were more affected than the LTP cells. Depletion of these growth factors from NDP-conditioned medium induced significant changes in [methyl-3H]thymidine uptake in NDP and LTP cells.

MATERIALS AND METHODS

Culture of NDP and LTP Tumor Cells. Normal B16BL6 tumor cells (NDP) and variant cells exhibiting a suppressed metastatic phenotype (LTP) were isolated and characterized as previously described (25). Briefly, B16BL6 melanoma cells were inoculated s.c into the dorsal hip of C57BL/6 X DBA/2 mice consuming either a normal diet (NDP variant) or a diet restricted in tyrosine and phenylalanine (LTP variant). Tumors were removed after one in vivo passage and grown in vitro in DMEM (Gibco, Grand Island, NY), pH 7.4, containing 10% heat-inactivated fetal bovine serum (HyClone, Salt Lake City, UT), 2.4 g/liter N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 3.7 g/liter sodium bicarbonate, 100 mg sodium pyruvate, 15 ml/liter minimum essential medium vitamin solution (100×), 10 ml/liter nonessential amino acids (100×), 5 mg/ml insulin, 20,000 units/ml penicillin-G, and 20,000 μg/ml streptomycin sulfate. Cultures were incubated at 37°C in a humidified atmosphere of 95% air/5% CO2. Cells were harvested with 10× trypsin (Sigma, St. Louis, MO) diluted to 1× with 20 ml phosphate-buffered saline, pH 7.5. Cell numbers were assessed using an hemocytometer, and viability was determined by trypan blue exclusion. The metastatic phenotype of the NDP and LTP cells is stable during in vitro culture in DMEM for up to 10 passages (25). NDP and LTP cells from two separate in vivo isolations were compared and found to exhibit the same growth factor characteristics as described herein. Additionally,

Received 2/22/93; accepted 6/28/93.

The costs of publication of this article were defrayed in part by the payment of page charges. The costs of this work were supported by National Cancer Institute grant CA 42465.

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2 The abbreviations used are: FGF, fibroblast growth factor; BSA, bovine serum albumin; CS, culture supernatant; DMEM, Dulbecco's modified Eagle's medium; ECL, Enhanced Chemiluminescence; bFGF, basic fibroblast growth factor; NR, nonretained fraction; TGFβ, transforming growth factor β.

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in vitro growth of the variants was not significantly different (P > 0.05) between the two isolates or between the NDP and LTP cells (data not shown).

**Partial Purification of Heparin-binding Growth Factors from NDP Cells, LTP Cells, and Conditioned Media.** Cells from *in vitro* passages 2–6 were grown to subconfluence. Medium was discarded, and cells were washed once in 50 ml phosphate buffer, pH 7.5 (buffer A), containing 150 mM NaCl (buffer B) before the addition of serum-free DMEM containing 0.01% bovine serum albumin (BSA). Cells were subsequently incubated at 37°C for 48 h. Conditioned media and cells were harvested, stored separately, and frozen at -70°C until use. After thawing, cells were disrupted with a dounce homogenizer (70 strokes) in buffer B, containing 1 mM EDTA, 1 µg/ml phenylmethylsulfonyl fluoride, and 1 µg/ml leupeptin (Sigma). Cells were then sonicated twice for 10 s at 60 Hz. Homogenates and conditioned media were centrifuged at 10,000 × g for 30 min at 4°C. Supernatants were each loaded onto heparin-Sepharose (Pharmacia LKB, Uppsala, Sweden) columns (bed volume, 1 ml). The heparin-Sepharose was first rehydrated in buffer C (buffer A containing 2.5 mM NaCl) and rinsed with 50 bed volumes of buffer B. After the columns were loaded, they were rinsed with 50 ml of buffer B. Molecules possessing an affinity for heparin were sequentially eluted into fractions with buffer A containing 0.3, 0.6, 1.15, and 2 mM NaCl. The fraction not retained on heparin-Sepharose is termed the NR fraction. The other fractions are labeled according to the saline concentration used for elution: 0.3, 0.6, 1.15, and 2 mM. Determination of protein concentrations of the conditioned media, cell extracts, and different fractions obtained from heparin-Sepharose chromatography were determined using the method of Bradford (26) with BSA as the standard.

**Gel Electrophoresis and Western Blots.** Aliquots of the different fractions obtained after heparin-Sepharose chromatography were precipitated overnight with 20% (v/v) trichloroacetic acid (Sigma) at 4°C. Each sample was collected on a glass filter (Whatman, Maidstone, England). The filters were transferred to an Eppendorf tube to which 50 µl of double distilled water and 25 µl of sample buffer [60 mM Tris, pH 6.8, containing 20% sucrose, 2% sodium dodecyl sulfate (SDS)] with or without 1% β-mercaptoethanol (Sigma) were added. Samples were boiled for 3 min and centrifuged before loading onto 15% polyacrylamide gels. Gels were electrophoresed according to Laemmli (27). Following electrophoresis, gels were incubated for 30 min in transfer buffer (Trisglycine, pH 8.9, containing 15% methanol and 0.01% sodium dodecyl sulfate). Concurrently, Hibond-ECL membranes (Amersham, Arlingtom Heights, IL) were also incubated in transfer buffer. Electrotransfer was performed as detailed elsewhere (28). After electrophoresis, the membranes were incubated in buffer D (50 mM phosphate buffer, pH 7.5, 0.5 mM NaCl) containing 0.01% amido-black dye. After stained proteins were examined, membranes were loaded, they were rinsed with 50 ml of buffer B. Molecules possessing an affinity for heparin were sequentially eluted into fractions with buffer A containing 0.3, 0.6, 1.15, and 2 mM NaCl. The fraction not retained on heparin-Sepharose is termed the NR fraction. The other fractions are labeled according to the saline concentration used for elution: 0.3, 0.6, 1.15, and 2 mM. Determination of protein concentrations of the conditioned media, cell extracts, and different fractions obtained from heparin-Sepharose chromatography were determined using the method of Bradford (26) with BSA as the standard.

**RESULTS**

**NDP and LTP Cells Differ in Protein Secretion.** The LTP variant secreted less protein into conditioned media than did an equivalent number of NDP cells, as shown in Fig. 1. However, when equal amounts of protein from the conditioned media were loaded onto heparin-Sepharose affinity columns, no difference in the amount of protein bound to heparin-Sepharose was observed between NDP and LTP cells.

**NDP and LTP Cells Differ in Growth Factor Secretion.** Analysis of NDP-conditioned media (Fig. 2A, lane 1) showed the presence of several immunoreactive forms as detected by polyclonal anti-bFGF antibody. These polypeptides possessed Mr, 66,000, 44,000, 36,000, and 29,000. The Mr, 66,000, 44,000, and 36,000 immunoreactive forms were not retained on heparin-Sepharose (Fig. 2A, lane 2). Sequential elution with 0.3 (Fig. 2A, lane 3), 0.6 (Fig. 2A, lane 4), 1.15 (Fig. 2A, lane 5), and 2 M NaCl (Fig. 2A, lane 6), followed by PAGE analysis, showed that an Mr, 29,000 polypeptide was maximally eluted with 0.6 M NaCl. Conditioned medium of the LTP variant, as shown on Fig. 2B, possessed only an Mr, 36,000 bFGF-like molecule which was not retained on heparin-Sepharose and a polypeptide of Mr, 66,000.

Dot blots of 4 mg protein of conditioned media from NDP or LTP cells were probed using a polyclonal antibody that recognizes both TGFβ1 and TGFβ2 (Fig. 3A). TGFβ-like proteins are secreted by NDP cells. Most immunoreactivity is located in the conditioned medium under reducing conditions. The immunoreactivity is present in the 0.3 M NaCl fraction. Comparable TGFβ-like forms were not detected in conditioned medium from LTP cells. Immunodetection by Western blot after electrophoresis under nonreducing conditions showed that the apparent Mr of the immunoreactive forms are 66,000 and 38,000 (Fig. 3B). TGFβ-like proteins are secreted by NDP cells. Most immunoreactivity is located in the conditioned medium under reducing conditions. The immunoreactivity is present in the 0.3 M NaCl fraction. Comparable TGFβ-like forms were not detected in conditioned medium from LTP cells. Immunodetection by Western blot after electrophoresis under nonreducing conditions showed that the apparent Mr of the immunoreactive forms are 66,000 and 38,000 (Fig. 3B).
molecular weight of the TGFβ-like polypeptide(s) that bound weakly to the affinity column (data not shown).

**NDP and LTP Cell Extracts Differ in FGF-like but not TGFβ-like Expression.** Cell extracts from both NDP and LTP cells expressed an Mr 29,000 bFGF-like molecule which was eluted by 0.6 M NaCl following heparin-Sepharose chromatography, as well as two other bFGF-like polypeptides that possessed Mr 26,000 and 25,000 (Fig. 4). Additionally, the intensity of the signal in NDP and LTP cells for the Mr 29,000 bFGF-like form appeared similar and suggested no quantitative difference in protein synthesis for this bFGF-like form. However, differences were observed between the NDP cell extract (Fig. 4, lane 1) and the LTP cell extract (Fig. 4, lane 3). The LTP cell extract also contained an Mr 47,000 bFGF-like form. This high molecular weight bFGF-like form was not found in NDP cell extract. There were no differences between gels run under reducing and nonreducing conditions (data not shown).

Several TGFβ-like forms were detected in NDP and LTP cell extracts (Fig. 5). These forms possessed Mr 25,000, 23,000, and 22,000. Three bands of Mr 45,000, 40,000, and 38,000 were detected following electrophoresis under nonreducing conditions (data not shown).

**Effect of bFGF and TGFβ1 on the Proliferation of NDP and LTP Cells.** No difference between NDP and LTP growth rates was observed when NDP and LTP cells were cultured in complete DMEM with 10% heat-inactivated fetal calf serum (25). However, in these experiments NDP and LTP cells differed markedly in their response to different growth factors (Fig. 6). NDP cells proliferated in response to bFGF in a dose-dependent manner, while LTP cells did not respond to the addition of bFGF under identical conditions (Fig. 6A). The mitogenic effect of bFGF on NDP cells was maximal with doses of 0.1–1 ng/ml. The mitogenic effect of TGFβ1 followed a similar dose-effect pattern for both NDP and LTP cells with several differences (Fig. 6B). Low concentrations of TGFβ1 of 0.001–0.1 ng/ml induced a weak [methyl-3H]-thymidine uptake that was higher for LTP than for NDP cells. For higher concentrations of TGFβ1 of 1 ng-100 ng/ml, inhibition by TGFβ1 was greater for NDP cells than for LTP cells. When 5 ng/ml of TGFβ1, a concentration found to inhibit [methyl-3H]-thymidine uptake of NDP cells, were incubated with different concentrations of bFGF, 0.001–1000 ng/ml (Fig. 6C), the [methyl-3H]-

3B, lanes 1 and 2) and confirmed that the LTP variant did not secrete the Mr 38,000 TGFβ-like form into the conditioned medium (Fig. 3B, lanes 3 and 4). Because of the small amount of immunoreactive material in the 0.3 M NaCl fraction, we were unable to determine the...
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thymidine uptake for NDP cells was maximal with 0.1 ng/ml of bFGF and decreased to a plateau in the presence of higher concentrations of bFGF. The LTP cells did not respond to bFGF under identical conditions. The response obtained with TGFβ1 and bFGF was the same as obtained with bFGF alone.

Depletion of Growth Factors from NDP-conditioned Medium by Immunoprecipitation: Effect on Growth of NDP and LTP Cells. As shown in Fig. 7, there was no difference in [methyl-³H]-thymidine uptake between NDP and LTP cells when incubated with the NDP-conditioned medium control. Significant inhibition of proliferation (P < 0.05) was observed when NDP cells, but not LTP cells, were incubated with conditioned medium treated with anti-bFGF antibody.

When conditioned medium was depleted with anti-TGFβ antibody, the [methyl-³H]-thymidine incorporation by NDP cells did not differ; however, incorporation was significantly increased in LTP cells (P < 0.05). Since LTP cells either did not secrete growth factor or only secreted growth factors in very small amounts, LTP-conditioned medium was not depleted of growth factors by immunoprecipitation and used for similar experiments.

DISCUSSION

Previously, we found that, when the highly invasive, metastatic B16BL6 murine melanoma is exposed to continuous low dietary levels of tyrosine and phenylalanine, tumor heterogeneity and metastatic potential are dramatically suppressed (6). It is important to note that continuous exposure to low levels of tyrosine and phenylalanine is not necessary to maintain the altered phenotype in vitro (LTP variant) since the suppressed metastatic potential of the LTP cells is stable during continuous culture in medium complete in tyrosine and phenylalanine (25). Herein, we report differences between the highly metastatic NDP cells and the low metastatic LTP variant in FGF and TGFβ growth factor secretion under these culture conditions.

Both NDP and LTP cells expressed bFGF-like molecules (Fig. 4) of Mt, 29,000, 26,000, and 25,000. Only LTP cells expressed an Mt, 47,000 bFGF-like form. It was previously shown that bFGF exists in endothelial cells in an NH2-terminal extended Mt, 25,000 form (30) and in bovine tissues in forms ranging from Mt, 18,000 to 60,000 (31). The Mt for bFGF-like polypeptides that we describe for NDP and LTP melanoma cells are well within the Mt, range of bFGF forms previously described for other systems.

bFGF (FGF-2) does not possess a signal peptide that permits its secretion outside the cell (30). However, conditioned medium from NDP cells contained bFGF-like molecules (Fig. 1A) with Mt, 29,000, 36,000, and 44,000. Conditioned medium from LTP cells possessed only an Mt, 36,000 bFGF-like form that might be a degradation product of the Mt, 47,000 bFGF-like form present within the cells (Fig. 1B). In the FGF/oncogene family, only acidic FGF and bFGF are not secreted; whereas, int-2 (32), hst/kFGF (33), FGF-5 (34), FGF-6 (35), and FGF-7 (36) are secreted. In NDP and LTP cell extracts, an Mt, 29,000 bFGF-like form possessing an affinity for heparin which was eluted with 0.6 M NaCl during heparin-Sepharose chromatography was present. This Mt, 29,000 bFGF-like molecule was secreted by highly metastatic NDP cells (Fig. 2A) but was not detected in LTP-conditioned medium (Fig. 2B). In NDP-conditioned medium, Mt, 36,000 and 44,000 bFGF-like forms not retained on heparin-Sepharose were present. Since the Mt, 26,000 and 25,000 forms...
Histocompatibility regulation in melanomas (47). Although the Mr 32,500-38,500 (38). In NDP-conditioned medium, perhaps the M, core of M, 28,500 (32) and was glycosylated up to M, 31,500 (39). MGF-5 elutes from heparin-Sepharose at 1.0 M NaCl and has an M, 29,500 core protein (34) which is further glycosylated into forms of M, 32,500-38,500 (38). In NDP-conditioned medium, perhaps the M, 36,000 and 44,000 forms are derived from the M, 29,000 bFGF-like form by glycosylation. It is plausible that glycosylation might affect the heparin-binding properties.

In NDP cells, the M, 29,000 bFGF-like form is secreted into conditioned medium. Among the FGF family, only int-2 and FGF-5 share similar molecular weights, but they differ in elution properties. int-2 is not efficiently secreted (39); whereas, FGF-5 possesses a conventional signal peptide (34). int-2 is not expressed in human melanoma; however, expression of bFGF has been observed in all melanoma cell lines studied (40). Additionally, FGF-5 is present only in a subset of melanoma cell lines (40). It is possible that the M, 29,000 bFGF-like form corresponds to FGF-5. This growth factor is often associated with transformation (34, 41) and can serve as a marker of melanoma metastatic phenotype (40). Since the same M, 29,000 bFGF-like molecule was present in cell extracts of the LTP variant, the decreased metastatic phenotype may be due to a lack of growth factor secretion. Both NDP- and LTP-conditioned media contained an M, 66,000 protein that reacted with the polyclonal bFGF antibody. This M, 66,000 signal was due to nonspecific binding of the antibody to BSA: the serum-free medium was supplemented with BSA, and the antibody bound to the BSA molecular weight marker (data not shown).

Both NDP and LTP cells contained M, 25,000, 23,000, and 22,000 TGFβ-like forms detected under both reducing and nonreducing conditions. Only NDP cells secreted an M, 38,000 TGFβ-like form into the medium. Like the FGF family, the TGFβ family also displays considerable molecular weight heterogeneity, because TGFβ is often associated with other proteins to form inactive complexes (42). The molecular weights obtained in our NDP-LTP melanoma system are higher than TGFβ1 and TGFβ2 M, 25,000 homodimers (43, 44). It has been shown that human melanoma cells and melanocytes possessed TGFβ1, but only TGFβ2 was expressed in melanoma cells (40). TGFβ2 possesses immunosuppressive properties (45) that might contribute to tumor growth by enabling these tumors to escape immune surveillance (46); whereas, TGFβ1 has been shown to affect class II histocompatibility regulation in melanomas (47). Although the antibody used recognized both TGFβ1 and TGFβ2, it is not possible at this time to state whether TGFβ1 and TGFβ2 are coexpressed, or whether only TGFβ1 or TGFβ2 is expressed in this murine melanoma system. If only one TGFβ-like polypeptide is expressed, the presence of three bands might be due to protein degradation or maturation of a precursor form as was previously reported for TGFβ1 (48). Both NDP- and LTP-conditioned media contained an M, 66,000 immunoreactive form. This M, 66,000 signal was due to nonspecific binding of the antibody to BSA, similar to what we observed for the anti-bFGF antibody.

NDP and LTP cell growth rates do not differ in vitro (25). However, the growth rate data obtained by testing bFGF and TGFβ1 showed that NDP cells increased proliferation in response to bFGF (Fig. 6A) but were inhibited by TGFβ1 (Fig. 6B). When these two factors were tested together (Fig. 6C), they did not act synergistically as shown in other systems (49). LTP cells did not proliferate in response to bFGF (Fig. 6A) but their growth was inhibited by TGFβ1. In the presence of bFGF, they were not responsive to TGFβ1 (Fig. 6B). The fact that LTP cells are nonresponsive to bFGF is intriguing because both NDP and LTP cells possess high-affinity binding sites for bFGF. The lack of LTP cell response to bFGF might be due to an alteration of the receptor tyrosine kinase domain, a possibility currently under investigation. Additionally, the immunoprecipitation of bFGF-like molecules from NDP-conditioned medium induced a decrease in [methyl-3H]thymidine uptake by NDP cells; whereas, no difference was found in LTP cells. This observation suggests that bFGF-like molecules are biologically active and can target NDP cells but not LTP cells. In contrast, no differences were observed due to the depletion of TGFβ-like molecules when tested on NDP cells. An increased proliferation of LTP cells was, however, observed. It is possible that NDP cells are unable to activate latent TGFβ as has been shown in the A549 human lung carcinoma cell line (42).

The mechanisms by which tyrosine and phenylalanine restriction suppresses the secretion of growth factors by the tumor cells still remain unclear. There are several possibilities: (a) restriction of tyrosine and phenylalanine might stimulate expression of a molecule that affects the role of signal peptides, (b) amino acid restriction might change the biochemical fate of some protein, i.e., dimerization, compartmentalization, and/or storage in the cell, (c) tyrosine and phenylalanine restriction might cause changes in hydrophobicity or in cellular membrane moieties, or (d) tyrosine and phenylalanine restriction might influence intracellular protein-folding pathways, as was recently shown with amino acid substitutions (50).

The role of growth factors in angiogenesis is well known. FGFs and TGFβs act as angiogenic factors permitting blood vessel growth into a tumor (14, 20). Additionally, growth factors act as paracrine and/or autocrine factors promoting cell division (51). Little is known about the role of growth factors during metastasis. However, some clues are available from other systems such as embryogenesis and carcinogenesis. During embryogenesis, protooncogenes of the FGF family such as FGF-5 (34) or int-2 (32) and of the TGFβ family such as TGFβ2 (44) are involved in cell migration and/or cell differentiation (52, 53). During carcinogenesis, a process that resembles embryogenesis, these growth factors are able to transform normal cells (32, 41). This suggests that these growth factors might also act during cell invasion and intravasation of tumor cells into a vessel as well as in extravasation, early invasion, and proliferation within the tissue.

It has been shown that FGFs induce the production of plasminogen activator, an enzyme that degrades the extracellular matrix (54). In addition, enzymes such as cathepsin B, type IV collagenase, elastase, and/or cathepsin B, type IV collagenase, elastase.
heparitinase, and plasminogen activator are released by highly metastatic tumor cells but not by nonmetastatic cells (55). The fact that FGFs are able to increase the production of enzymes capable of degrading the extracellular matrix might also result in the release of other stored components which could serve as chemotaxins or mitogens of tumor cells (56, 57). Generally, TGFβs suppress the production of enzymes that might degrade the extracellular matrix (58, 59) and/or act on cell proliferation (42, 60) and angiogenesis (20, 21, 58). Thus, the idea that growth factors, particularly FGF, might be involved prior to the metastatic event correlates well with our knowledge of the different processes occurring during the establishment of secondary tumors. In this case, the highly metastatic cell line NDP possesses FGF molecules that could participate in (a) transformation of the cells, (b) the production of enzymes degrading the extracellular matrix, (c) implantation of the secondary tumor (blood vessels formation), and (d) tumor cell growth. The LTP variant lacking secretion of these growth factors should be less able to invade preferential tissues.

In conclusion, we have shown differences between NDP cells and LTP melanoma metastatic variants in growth factor production, secretion, and response. FGF and TGFβ are produced by both NDP and LTP cells, but these growth factors were only found in NDP-conditioned medium. bFGF induced the growth of NDP cells but not the LTP low metastatic variant. The fact that LTP cells show important changes in FGF production and response when compared to the NDP cells indicates that FGF may play an important role during invasion and metastasis.

ACKNOWLEDGMENTS

The authors wish to thank Ms. Dawn J. Cardeiro for her excellent technical assistance and Dr. Jeannette Huijzer for critical review of the manuscript.

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