A Subset of Metastatic Human Colon Cancers Expresses Elevated Levels of Transforming Growth Factor β1

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Abstract

Although transforming growth factor (TGF)-β1 is a potent growth inhibitor of normal epithelial cells including colonocytes, TGF-β1 has also been implicated as an enhancer of colon cancer metastasis. Decreasing TGF-β1 protein levels in the metastatic U9 colon cancer cell line by antisense methodology decreased both U9 cell metastasis to the liver and s.c. tumor formation in a nude mouse system, and the tumors that did arise had regained TGF-β1 expression (F. Huang et al., Cell Growth Differ., 6: 1635–1642, 1995). In addition, in a clinical immunohistochemistry study, colon cancers with elevated TGF-β1 protein levels were found to be 18 times more likely to recur as distant metastases than colon cancers expressing low TGF-β1 levels, after resection of the primary tumor (E. Friedman et al., Cancer Epidemiol. Biomark. Prev., 4: 549–554, 1995). Because both studies implicated TGF-β1 in colon cancer metastasis, we wished to know whether a selection bias for TGF-β1 was maintained in metastatic cells or was only a property of the primary site tumors that were likely to metastasize. TGF-β1 levels were measured using two different antibodies in paired primary site cancers and their metastases by immunohistochemistry and, in selected cases, by Western blot analysis. In 16 of 21 cases (76%) with antibody G and 23 of 31 cases (74%) with antibody P, higher expression of TGF-β1 was found in colon cancer cells invading local lymph nodes compared to the primary site cancer cells, or (2 and 6 cases, respectively) high TGF-β1 expression in the primary site cancer was maintained in invasive cells. Analysis by Western blotting using both antibodies also demonstrated that higher levels of TGF-β1 protein were found in metastases compared to the primary site tumor or normal tissue. Additional cases of paired primary site colon cancer, local lymph node metastases, and cancer cells metastasizing to distant sites were examined. In six of eight such cases (75%), TGF-β1 levels were increased in both invasive cell populations compared with the primary site cancer (five cases), or high levels in the primary site cancer were maintained in the metastatic cells (one case). These data suggest that TGF-β1 plays a role in promoting colon cancer metastasis throughout the metastatic process in roughly 75% of cases. TGF-β1 may increase metastasis by paracrine mechanisms, such as suppression of local immune response or increased angiogenesis, as was seen with the U9 cell line. In those cancers with nonmutated TGF-β receptors and nonmutated smad proteins like U9 cells, TGF-β1 could also act in an autocrine manner to increase invasion by increasing cell motility (Hsu et al., Cell Growth Differ., 5: 267–275, 1994).

Introduction

The TGF-β family of growth factors includes three highly homologous isoforms in mammals, TGF-β1, TGF-β2, and TGF-β3, whose mature forms exhibit nearly 80% identity. All three isoforms are synthesized as a single-chain pre-pro latent molecule. The C' terminal region is cleaved to form a 112-amino acid monomer, which subsequently dimerizes to an active Mr 25,000 form (1). As growth factors, TGF-β isoforms are unique because they inhibit the growth of normal epithelial cells. TGF-β1 inhibits the growth of normal intestinal epithelial cells in vivo, where it decreases both villus and crypt cell height and cellularity (2). Although TGF-β1 inhibits the growth of some less aggressive colon carcinoma cell lines in vitro, this growth-inhibitory response is lost with tumor progression. In ~20% of cases, TGF-β response is lost because of inactivating mutations in the TGF-β type II signaling receptor (3) or inactivating mutations in the TGF-β signaling molecules smad4 (4) and smad2 (5). No mutations were found in the other smad genes in colon cancers (6). Our previous studies have indicated that after loss of a growth-inhibitory response to TGF-β1, some malignant epithelial cells use TGF-β1 to stimulate their invasion (7). These aggressive colon cancers retain functional TGF-β receptors, but TGF-β signaling was associated with a new pattern of induction of immediate-early genes (7). Constitutive expression of a TGF-β1 antisense construct in these aggressive cells blocked tumor cell invasion and decreased the capacity of these cells to metastasize (8). Supporting these results in an experimental system, a strong correlation was shown in a clinical study between high expression of TGF-β1 protein in the primary site colorectal cancer and progression to metastases, suggesting that TGF-β1 produced by colon cancer cells promotes their metastasis (9). In the present study, we...
have asked whether a selection bias for TGF-β1 was maintained in metastatic cells or whether it was a property only of the primary site tumor.

Materials and Methods

Materials. 125I-labeled protein A was obtained from DuPont-New England Nuclear, polyvinylidene difluoride transfer paper Immobilon-P from Millipore, and the Vectastain Elite ABC kit from Vector Laboratories (Burlingame, CA). For antisera G, rabbit polyclonal antisera to TGF-β1 was developed by immunization with peptides representing amino acid residues 4–19 of the mature processed forms of TGF-β1. The specificity of this antibody was established by immunoblotting intact native and recombinant human TGF-β1 and by blocking immunoreactivity with the peptide used as immunogen (10). This antisera did not show cross-reactivity with TGF-β2 or TGF-β3 isoforms by Western blot analysis using recombinant TGF-β3 and porcine TGF-β2 (10). Antisera P (Promega Corp.) was raised in rabbits against acid-activated TGF-β1 from platelets. This antisera showed no cross-reactivity with recombinant TGF-β1 and porcine TGF-β2 (10). Antisera G (Promega Corp.) was raised in rabbits against peptide containing amino acid residues 4–19 of the TGF-β1 chain in tumor. The antisera showed no cross-reactivity with recombinant TGF-β2 and TGF-β3 by immunoblotting. All other reagents were purchased from Sigma Chemical Co.

Cell Culture. U4 human colon carcinoma cells were maintained in 7% fetal bovine serum supplemented with DMEM.

Western Blotting. Tissue specimens were homogenized using a Dounce homogenizer in 1.0 ml of RIPA buffer [137 mM NaCl, 20 mM Tris-HCl (pH 8.0), 1% NP-40, 0.1% SDS, and 0.1% sodium deoxycholate] containing 3 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 20 μM leupeptin, 5 mM NaVO4, and 0.15 unit/ml aprotinin. Extracts were adjusted to equal protein concentrations in SDS-sample buffer, heated for 5 min at 100°C, then electrophoresed in 15% acrylamide-SDS gels. Proteins separated in the gel were transferred by electrophoresis to a polyvinylidene difluoride membrane exactly as detailed (12). Proteins were detected using either 2 μg/ml of antisera P or 0.5 μg/ml antisera G, followed by 125I-labeled protein A and autoradiography. For U4 cell line studies, lysates were prepared identically. Fifty μg of cell lysate in SDS-sample buffer were heated for 5 min at 100°C, then electrophoresed in 15% acrylamide-SDS gels. Western blotting was as above. Densitometric scanning was performed for quantitation.

Tissues. Colon cancer tissue from the periphery of the tumor, normal colon mucosa dissected free of the muscle layer, liver metastases from the edge of the tumor, and fragments of normal liver were obtained at surgery and immediately frozen in liquid N2. Liver metastases were obtained from patients with synchronous or metachronous lesions. Patients were admitted for elective or emergency resection of colorectal cancer. For immunocytochemistry, sections from resected colon cancers were obtained from the Memorial Hospital pathology archives. All sections had been fixed in buffered formalin and stored in paraffin blocks. Patients’ slides were number coded, reacted with the TGF-β1 isomeric-specific antisera, processed for immunocytochemistry, scored by three independent observers (see sections below), and then uncoded. The entrance criteria for each patient were then reviewed by a Surgery Department data manager for accuracy.

Immunocytochemistry. For antisera G, number-coded tissue sections were deparaffinized in xylene, rehydrated in TBS, incubated with 1 mg/ml hyaluronidase in 0.1 M sodium acetate buffer, blocked with normal goat serum, and incubated with the primary antibody at 2.5 μg/ml in blocking solution for 16 h at 4°C, as detailed (9). Following the use of reagents of the Vectastain Elite ABC kit, the antibody reaction was detected with diaminobenzidine. Slides were reacted with each antisera at 4°C overnight in one lot using sections of the human colon carcinoma U4H cell line grown as a s.c. tumor in a nude mouse as the positive control and normal rabbit serum as the negative control. For antisera P, anti-human TGF-β1 polyclonal antisera was purchased from Promega and used at 13 μg/ml at 4°C overnight. Sections were incubated with 0.1% Pronase E (Sigma) for 8 min at room temperature before application of antisera. The degree of intensity of immunostaining was detected as above.

Scoring. Scoring was performed independently by three observers on number-coded slides on a scale of 1–4 using sections of the human colon carcinoma U4H cell line grown as a s.c. tumor in a nude mouse as the positive control at 1. Data were obtained by chart review, and patient data were verified by a Surgery Department data manager after the scoring of the slides was complete.

Results

Two Antibodies to TGF-β1 Identify Different TGF-β1 Species in Colon Carcinoma Cells by Western Blotting. TGF-β1 had been implicated as an enhancer of colon cancer metastasis in two ways: (a) decreasing expression of TGF-β1 protein levels in metastatic U9 colon cancer cells by an antisense expression plasmid decreased liver metastasis and s.c. tumor formation in a nude mouse system, and tumors that did not show expression of TGF-β1 expression (8); and (b) in a series of colorectal cancer specimens from patients whose disease recurrence or lack of recurrence had been documented, a strong correlation was shown between high expression of TGF-β1 protein in the primary site colorectal cancer and progression to metastases, suggesting TGF-β1 produced by colon cancer cells promotes their metastasis (9). In the present study, we have asked whether a selection bias for TGF-β1 was maintained in metastatic cells. The level of TGF-β1 protein in metastatic human colon cancer cells and their originating primary site cancer were compared by both Western blotting and immunocytochemistry.

Two TGF-β1 antibodies were used for this study. Antibody P was raised against the entire molecule of acid-activated natural TGF-β1 and therefore would be expected to recognize additional epitopes to those detected by an anti-peptide antibody such as antibody G. Treatment of U4 colon carcinoma cells with a differentiating agent such as sodium butyrate has been shown to increase TGF-β1 mRNA and active peptide levels (13). Treatment of U4 cells with 0.5–10 mm sodium butyrate for 24 h resulted in an increase in the abundance of the Mr 25,000 TGF-β1 dimer, the biologically active form (Fig. 1A). No other TGF-β1 species were identified. Similar data were seen when U4 cells were treated with other TGF-β1-inducing agents, sodium propionate and sodium isovalerate (data not shown). Nonnecrotic portions of five primary site colon cancers were resected by one of us within a 3-week period, were frozen in the operating room (see “Materials and Methods”), and were then used for immunoblotting for TGF-β1 species in human colon tumors. In marked contrast to the cell line studies, antibody P detected a Mr 110,000 protein complex in each of the five colon cancer lysates (Fig. 1B, 110 kDa complex seen in lysate #1 with longer exposure, data not shown) and a low abundance of the monomeric Mr 12,500 TGF-β1 chain in tumor 4. The Mr 25,000 form was not observed. A Mr 95,000–110,000 complex immunoreactive with TGF-β1 antisera has been detected in cell lysates by others in...
Fig. 1. Characterization of TGF-β1 species in colon carcinomas identified by antibodies P (A–C) and G (D and E) by Western blotting under nonreducing conditions. A, increasing abundance of TGF-β1 M, 25,000 dimer induced by 24-h treatment of U4 human colon carcinoma cells with a range of concentrations from 0.1 to 10 mM of the differentiation agent sodium butyrate. Dashes on right, molecular weight markers of M, 18,000 and M, 29,000 (in thousands). B, detection of TGF-β1 species in colon cancer lysates using antibody P. One hundred fifty µg of protein were analyzed per lane from specimens of five resected colon carcinomas. Lanes 1–5, fresh-frozen in liquid nitrogen in the operating room. The M, 110,000 TGF-β1 complex is detected in all lysates, with lysate 1 requiring more exposure of the autoradiogram (data not shown). Lysate 2 was rerun after freeze-thawing disrupted most of the M, 110,000 TGF-β1 complex into constituent mature M, 25,000 TGF-β1 dimer and the latent proTGF-β1 of M, 55,000 (rightmost lane). C, elevated abundance of M, 110,000 TGF-β1 complex in liver metastases. Samples of normal liver (L), primary site cancer (T), and liver metastases (M), fresh-frozen in liquid nitrogen immediately after surgery, are shown. Dashes on left, molecular weight markers of M, 68,000 and M, 97,000. D, mature TGF-β1 dimer of M, 25,000 detected by antibody G in the same five samples of human colon cancers analyzed in B. Reduced recombinant single-chain TGF-β1 of M, 12,500 was run in the molecular weight marker Lane M. E, elevated abundance of TGF-β1 M, 25,000 dimer in colon cancer metastases detected by antibody G. Samples of normal liver (L), primary site cancer (T), liver metastases (M), and normal colon mucosa (N) fresh-frozen in liquid nitrogen immediately after surgery are shown. These are identical samples to those analyzed in C with antibody P.

earlier studies (11, 14) and found to contain mature TGF-β1 covalently linked by disulfide bonds to M, 55,000 unprocessed pro-TGF-β1 and to the associated protein portion of the precursor. To determine whether the large molecular weight complexes detected by antisera P contained known TGF-β1 species, lysate from carcinoma 2 was freeze-thawed and reanalyzed by SDS-PAGE and Western blotting. Freeze-thawing released the M, 25,000 TGF-β1 dimer and the M, 55,000 unprocessed pro-TGF-β1 from the M, 110,000 complex (Fig. 1A, right, Lane 2R). We next assayed TGF-β1 levels in two cases of paired metastases and normal tissue (Fig. 1C). In case 18, an increase in TGF-β1 levels was seen in liver metastases when compared with the primary site tumor or paired normal mucosa. In case 15, an increase in TGF-β1 levels was seen in liver metastases when compared with uninvolved liver. In both cases, the only TGF-β1 species identified by antibody P was the M, 110,000 TGF-β1 complex. Therefore, although antibody P could detect the M, 25,000 dimer form in established colon cancer cell lines, the major TGF-β1 species it detected in human colon cancers resected from patients was a larger complex.

Antibody G was raised to a synthetic peptide corresponding to residues 4–19 of mature TGF-β1. Antibody G has been used extensively by several groups to quantitate TGF-β1 expression within various tumor types and in mammalian development (15–18). This is an isotype-specific antibody that does not cross-react with TGF-β2 or TGF-β3 and the reactivity of which is blocked by the synthetic peptide used as immunogen (10).

Antibody G recognized both the monomeric M, 12,500 chain of recombinant TGF-β1 used as a marker and the M, 25,000 dimer form of mature TGF-β1 (Fig. 1D) within each of the five human colon cancer lysates blotted previously with antibody P (Fig. 1B). No M, 110,000 TGF-β1 complex was detected by antibody G. The same two paired cases of colon
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Fig. 2. Detection of TGF-β1 in sectioned colon carcinoma cells by immunocytochemistry using antisera G. Upper panel left, P4: primary colon cancer. Upper panel right, P4 met: concurrent liver metastases from primary colon cancer P4. Lower panel left, P1: primary colon. Lower panel right, P1 LN: regional lymph node resected concurrently with primary colon cancer P1. Arrow, colon cancer cells arising around a vessel within the lymph node. ×109. No sections were counterstained; therefore, the specimens in the negative control slides are not shown (they did not develop any benzidine stain; no cellular morphology could be seen).

cancer metastases and normal tissues tested previously with antibody P (Fig. 1C) were then assayed by Western blotting with antibody G (Fig. 1E). In both cases, the only TGF-β1 species identified by antibody G was the Mr 25,000 TGF-β1 complex. In case 18, an increase in TGF-β1 protein levels was seen in liver metastases when compared with the primary site tumor, normal uninvolved liver, or paired normal mucosa. In case 15, an increase in TGF-β1 levels was seen in liver metastases when compared with uninvolved liver. Thus, colon cancers with elevated expression of TGF-β1 Mr 25,000 dimer detected by antibody G also exhibited elevated expression of the Mr 110,000 TGF-β1 complex detected by antibody P. Both antibodies gave the same results: enhanced levels of TGF-β1 protein within metastases of tumors 15 and 18 compared with normal tissues, and in case 18, the primary site tumor (Fig. 1, compare C and E). In addition, tumor 4, a mucinous tumor, exhibited the greatest abundance of TGF-β1 species of the five human primary site cancers compared by both antibodies (Fig. 1, compare B and D). This result is consistent with a recent study (19) in which a mucinous colon cancer exhibited a TGF-β1 mRNA level higher than colon cancers of other differentiation classes. In addition, this study demonstrated that high levels of TGF-β1 mRNA levels in colon cancers correlated with disease progression (19).

Elevated Levels of TGF-β1 Were Found in Colon Cancer Cells Invasive to Regional Lymph Nodes and to Distant Metastatic Sites in a Subset of Colon Cancers. These two antibodies gave us a novel opportunity to compare expression of both TGF-β1 species, the Mr 25,000 biologically active dimer and the Mr 110,000 complex, by immunocytochemistry in a series of fixed colon tumors. We first needed to confirm that quantitation of TGF-β1 abundance in colon cancer tissues by immunocytochemistry was valid. In parallel to the samples of tumors 1–5 used for immunoblotting in Fig. 1, other samples were fixed by routine methods for immunocytochemistry. TGF-β1 protein levels within each tumor were then determined using both antibodies by comparing sections of the human colon carcinoma cell line U4H, which has a known high level of expression of TGF-β1 protein.
Colon Cancer Cases

Fig. 4. TGF-β1 abundance assayed by immunocytochemistry with antisera P in eight cases of number-coded paired primary site colon cancers, regional lymph nodes, and distant metastases.

Fig. 1. TGF-β1 abundance assayed by immunocytochemistry in number-coded paired nodes and primary site colon cancers, 21 cases with antisera G (A) and 31 paired cases with antisera P (B).

(13), as the positive control and normal rabbit serum as the negative control. Antibody binding was detected by the diaminobenzidine reaction without counterstaining; thus, the intensity of the color reflects the antibody binding and thus the abundance of TGF-β1. Tumor 4, which had displayed the highest level of TGF-β1 protein by Western blotting, also exhibited the highest levels of TGF-β1 by immunohistochemistry of the five cancers, clearly higher than tumor 1 (Fig. 2, antibody G sections shown; similar data for antibody P). Because there was a correlation between the results obtained by Western blotting and those from immunohistochemistry, quantitation of the level of TGF-β1 protein in colon cancer specimens could be obtained by immunohistochemical methods.

TGF-β1 levels were measured by immunocytochemistry as above in a coded series of primary site colon carcinomas and their corresponding regional lymph nodes. All sections were number-coded and scored by three reviewers without the knowledge of the case number. Primary site cancer and nodes were scored independently. A range from + to +++ was observed within each primary site colon cancer, whereas lymph nodes scored from + to ++++ compared with the U4H tumor positive control. With antibody G, the protein level of TGF-β1 was higher in the cancer cells in the local lymph nodes than in the primary site cancer in the same patient in 14 cases (Fig. 3A). For example, colon cancer cells with high levels of TGF-β1 were evident surrounding vessels within the regional lymph nodes resected with tumor P1 (Fig. 2, arrow, lower right panel tumor #1 LN). These invasive colon cancer cells exhibited more TGF-β1 protein than the primary site colon cancer 1 (Fig. 2, compare two lower panels). Only in four cases (19%) was there a decrease in TGF-β1 levels in the cancer cells in the lymph nodes compared with the primary site cancer. Our original observation (9) was that high levels of TGF-β1 in a primary site colon cancer made the tumor more likely to recur as distant metastases. High levels of TGF-β1, as judged by immunohistochemistry compared with a positive control xenograph known to synthesize TGF-β1, were +3/+4. In the current study, two of the cases exhibited +3 or +4 TGF-β1 levels in both primary site and local lymph nodes (Fig. 3A). In these two tumors, high levels of TGF-β1 in the primary were maintained in the metastatic cells. Therefore, in 16 of 21 cases (76%), either increased TGF-β1 protein levels were found in the invasive cells or high levels in the primary site colon cancers were maintained in the cells invading the local lymph nodes.

The immunohistochemical survey was then repeated using antibody P on the same 21 cases shown in Fig. 3A and an additional 10 cases. Antisera P gave identical results to antisera G in the majority of cases (85%), when the results from both antisera were uncoded and compared (data not shown). In 17 cases, TGF-β1 protein expression was higher in the cancer cells in the local lymph nodes than in the primary site cancer from the same patient (Fig. 3B). In six cases, high levels of TGF-β1 (+3/+4) found in the original tumor were maintained in the lymph nodes. Only in five cases (16%) did the invasive cancer
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cells in the lymph nodes exhibit less TGF-β1 than the primary site cancer. Thus, in 23 of 31 cases (74%), the data supported the interpretation that elevated levels of TGF-β1 were a selection criteria for metastatic colon cancer cells.

We next determined whether the elevated expression of TGF-β1 protein found in many colon cancer cells invasive to local lymph nodes would be maintained when these cells disseminated to distant metastatic sites. It was difficult to obtain many cases for this study because the original resection tended to be performed at a local hospital, whereas the metastases were removed at Memorial Hospital, a regional cancer center. Eight cases of paired primary site, local lymph nodes, and distant metastases were examined for TGF-β1 expression by immunocytochemistry (Fig. 4). In five cases (63%), the abundance of TGF-β1 protein was higher in lymph nodes than in the primary site colon cancer, and this elevated level was maintained or increased in distant metastases. For example in case P16, the colon carcinoma cells within the lymph node (Fig. 5, P16LN, center) and within the liver could be clearly detected by their abundant quantities of TGF-β1 protein compared with the surrounding tissues (Fig. 5, P16met, right; arrows point to the invading cancer cells detected with antisera G). In one case, the primary site cancer exhibited high levels of TGF-β1, and this high level was maintained in the cells invading the lymph nodes and those metastatic cells reaching distant sites. An example of the latter is colon cancer case 4, with synchronous liver metastases. The primary site cancer exhibited high levels of TGF-β1, and this high level was maintained in the metastases, as shown by immunohistochemistry (Fig. 2, compare upper panels). Thus, the results of immunocytochemistry (Fig. 4) and Western blotting gave similar results, elevated levels of TGF-β1 protein within invasive and metastatic cells.

Discussion

In a prior study, we examined the expression of TGF-β1, TGF-β2, and TGF-β3 isoforms using immunocytochemistry in a series of colorectal cancer specimens from patients whose disease recurrence or lack of recurrence had been documented (9). A strong correlation was shown between high expression of TGF-β1 protein in the primary site colorectal cancer and progression to metastases, suggesting that TGF-β1 produced by colon cancer cells promotes their metastasis (9). TGF-β1 was also found to be an independent prognostic marker for shorter postoperative survival in a second study by another group of investigators (20), confirming our observations. These studies suggested that one selection criteria for invasive colon cancer cells was their level of TGF-β1. In the current study, TGF-β1 levels were measured by immunocytochemistry in a number-coded series of primary site colon carcinomas and their corresponding regional lymph nodes, and where possible, in metastases from the same patient. In roughly 75% of cases, metastatic cells either in local lymph nodes or at distant sites exhibited elevated levels of TGF-β1 compared with the primary site cancer, or in a few cases where the primary site cancer itself exhibited high levels of TGF-β1, this high level was maintained in the metastatic cells.

Three hypotheses, not mutually exclusive, may explain the presence of high levels of TGF-β1 within invasive colon cancers:

(a) We propose that high levels of TGF-β1 within a colon cancer may increase the likelihood of metastases by locally suppressing immune function. In syngeneic mice with an intact immune system, Meth A sarcoma cells overexpressing TGF-β1 were more tumorigenic than transfectants expressing lower levels of TGF-β1 (21). Because Meth A sarcoma cell growth was strongly inhibited by TGF-β1 in vitro in an autocrine manner, tumorigenicity in vivo may be enhanced by TGF-β1 through host immunosuppressive activities. Thus, the presence of high levels of TGF-β1 in cells that were proliferating to form enlarging, invasive, and finally metastatic tumors in vivo is inconsistent with the growth inhibition function of TGF-β1 in vitro. TGF-β1 generally functions as an immunosuppressive
agent by affecting the function of various target immune cells. This includes inhibiting B-cell, thymocyte, T-lymphocyte, and large granular lymphocyte proliferation and inhibiting the generation of CTLs and natural killer cells (22). In this way, increased expression and release of TGF-β1 from a primary site or metastatic colon cancer cell could impair a patient’s local immune response, thereby promoting tumor growth.

(b) A second hypothesis is that TGF-β1 may have a biphasic effect on tumor growth, becoming growth promoting at later stages in progression. Although TGF-β1 was shown to inhibit benign tumor formation, TGF-β1 enhanced progression of skin tumors to invasive spindle carcinomas in carcinogen-treated transgenic mice with keratinocyte-targeted expression of TGF-β1 (23). TGF-β1 appeared to have a biphasic action during colon tumor progression, acting on well-differentiated cancers as a tumor suppressor and growth inhibitor, but at later stages in tumor progression acting to enhance malignancy.

TGF-β1 is a growth inhibitor for normal epithelial cells and certain highly differentiated colon cancers in vitro. However, upon progression of colon cancer to more invasive phenotypes, colon cancer cells become autocrine growth stimulated by TGF-β1, possibly because they use a different TGF-β1 signal transduction pathway. Resected poorly differentiated colon cancers and resected metastatic colon cancers in primary culture were stimulated to proliferate by TGF-β1 and by hexamethylene bisacetamide, which induced TGF-β1. This increase in tumor growth was measured by cumulative [3H]thymidine incorporation and by direct cell counting (24, 25). These data obtained from primary cultures of resected cancers were similar to those obtained from HT29 colon carcinoma sublines. Colon carcinoma cell lines that respond to exogenous TGF-β1 by growth stimulation are highly invasive in vitro and are highly tumorigenic in athymic mice (7). These cells also synthesize TGF-β1 mRNA and secrete TGF-β1 as a bioactive dimer. Neutralizing antibody to TGF-β1 and constitutive expression of a TGF-β1 antisense construct blocked both cell growth and invasion in vitro and growth in athymic mice, thereby demonstrating the autocrine stimulating activity of TGF-β1 in highly aggressive colon cancer cells (7, 26). Thus, elevated levels of TGF-β1 may provide a selective advantage for aggressive colon cancer cells by directly stimulating their growth and invasiveness.

(c) A third function for elevated levels of TGF-β1 in colon tumor cells is to increase the expression of PDGF-B chain in invasive, undifferentiated colon carcinoma cells but not in differentiated cells (27). There is evidence linking the production of PDGF-B in human colon cancers with increased angiogenic potential. The PDGF-β receptor was found in microvascular pericytes, not in the epithelial cells, in each of a series of 210 colorectal cancers by immunohistochemistry (28, 29). Colon cancer cells that produce PDGF-B in response to TGF-β1 could stimulate the growth or function of microvascular pericytes expressing PDGF-β receptors, leading to an increase in tumor vascularization.

Evidence has accumulated that many carcinomas respond to TGF-β1 with disease progression. Examples are numerous. Increased expression of TGF-β1 and to a lesser extent TGF-β2 and TGF-β3 correlated with disease progression to complex hyperplasia and adenocarcinoma of the endometrium (16). Increased mRNA and protein expression of TGF-β isoforms in pancreatic cancer correlated with decreased survival (18). Increased levels of TGF-β1 protein in breast carcinomas was positively correlated with disease progression (17). Intense immunoreactivity for TGF-β1, not TGF-β2 or TGF-β3, in tumor cells was statistically significant (P = 0.009) for recurrence or progression in breast cancer, independent of age, stage, nodal status, or estrogen receptor status (17). The metastatic potential of mammary adenocarcinoma cells was increased by in vitro treatment with TGF-β1 before injection into syngeneic rats. TGF-β1 pretreatment increased the number of metastases at the surface of the lung 2–3-fold and increased in vitro invasion 2–3-fold by increasing type IV collagenase and heparanase activities (30). In other studies, breast cancer cells that overexpressed TGF-β1 exhibited estrogen-independent tumorigenicity in athymic mice (31). Moreover, elevated levels of TGF-β1 protein have been found in prostate cancer (32). TGF-β1 has been shown to be a marker for malignancy in astrocytomas because normal astrocytes do not express this isoform (15). Thus, TGF-β1 has been associated with increased tumor progression in several human cancers in addition to colon cancer. Because elevated levels of TGF-β1 correlate with aggressive and metastatic behavior of tumor cells in a variety of cancers, TGF-β1 may also be considered a prognostic marker of malignant progression.

References


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