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*Angiogenesis, Metastasis, and the Cellular Microenvironment*

Molecular Cancer Research

# Regional Control of Tumor Growth

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## Abstract

Tumors implanted near the scapulae have been shown to grow four times faster than the same tumors implanted at the iliac crest. Although there were marked differences in the vascularization of tumors from these two different sites, the mechanism controlling regional angiogenesis was not identified. Here, we show site-specific growth of intraperitoneal tumor implants in the mouse abdomen. Our data indicate that the angiogenic response of the host differs significantly between the upper and lower sites in the mouse abdomen and reveal that the expansion of tumor mass is restricted to sites with low angiogenic responses, such as the bowel mesentery in the lower abdomen. We show that, in this model, this suppression of angiogenesis is due to an expression gradient of thrombospondin-1 (TSP-1), a potent endogenous angiogenesis inhibitor. Mice with a targeted deletion of TSP-1 no longer show regional restriction of tumor growth. The physiologic relevance of these findings may be seen in patients with peritoneal carcinomatosis, whereby tumors spread within the peritoneal cavity and show differential growth in the upper and lower abdomen. We hypothesize that the difference in tumor growth in these patients may be due to a gradient of TSP-1 expression in stroma. Finally, our studies suggest that upregulation of TSP-1 in tumor cells is one method to suppress the growth of tumors in the upper abdomen. *Mol Cancer Res; 8(9); 1198–206. ©2010 AACR.*

## Introduction

Since the 1930s, there have been a number of studies that have documented regional differences in tumor growth in mice and rats. For example, a study done in mice showed variable sensitivity of the different regions of the skin to tumor-promoting carcinogenic agents. Repeated administration of a carcinogen to the interscapular and sacral regions led to a significant increase in the development of tumors specifically in the interscapular region. Interestingly, a high proportion of these tumors in the interscapular region converted to malignant tumors as compared with tumors in the sacral regions (1). These findings were later suggested to be the result of regional differences in mitotic activity, as shown by high mitotic activity in cells located in the anterior regions of the body and low mitotic activity in cells of the posterior-most parts of the body. This implicates the existence of a cranio-caudal mitotic gradient in the epidermis in response to various agonists and injury (2). Further studies showed that subcutaneous tumors implanted near the scapulae grew four times faster than the same tumors implanted at the iliac crest. Although there were marked differences in the vascularization of tumors from these two different sites, the mechanism controlling regional angiogenesis was not identified. Several hypotheses have been proposed to explain the anteroposterior differences in the growth of cells, including differences in local microcirculation as indicated by local skin temperature (3) and metabolic gradients as reflected by variable oxygen availability and consumption (1, 2).

The regional differences in tumor growth shown in mice have also been observed in cancer patients. Peritoneal carcinomatosis is a locoregional cancer that spreads within the peritoneal cavity, usually after tumor cells have escaped from their primary lesion, and is often considered a terminal feature of abdominal cancers (3, 4). Peritoneal carcinomatosis may occur as a result of a primary disease, such as peritoneal mesothelioma, or may be a result of regional spread of gastrointestinal, gynecologic, or other malignancies (5-7). Tumor cell spillage during curative gastric cancer surgeries, as well as during the dissection of lymph nodes in patients with positive lymph nodes, has also been

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A. Zaslavsky and C. Chen contributed equally to this work.

This work is dedicated to the memory of Dr. Judah Folkman who developed this model of differential tumor growth in the abdomen and whose original hypothesis was the basis of this work.

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implicated as the cause of peritoneal carcinomatosis (5-7). To date, the primary principle of cancer cell dissemination within the peritoneal cavity is believed to be based on the flow and absorption pattern of peritoneal fluid and gravity (3). In patients diagnosed with peritoneal carcinomatosis, tumor nodules are primarily found at the junction of the small bowel and the small bowel mesentery, within the greater and the lesser omentum, and beneath the right hemi-diaphragm (3). The growth and size of these tumor nodules vary significantly depending on their location within the upper or lower abdomen.

The description of peritoneal carcinomatosis as a locoregional condition in patients (4) and the observation of differential tumor growth based on anatomic location suggest that different levels of angiogenesis may exist in the upper versus lower abdomen of humans as well. Angiogenesis is a well-controlled process orchestrated by a balance between proangiogenic factors and inhibitors (8). The expression of endogenous angiogenesis inhibitors in tissues has been previously shown to have a direct effect on tumor growth and development (8). The most well-characterized endogenous angiogenesis inhibitors are thrombospondin-1 (TSP-1), angiostatin, endostatin, and tumstatin (9, 10). Mouse models with targeted deletions of either *Tsp-1* or tumstatin showed more rapid tumor growth as compared with wild-type littermate control mice (11, 12). In fact, reduction of TSP-1 in the tumor bed is suggested to be critical for the induction of vascularization, or what has been termed the angiogenic switch, preceding the expansion of a microscopic tumor into a large macroscopic, angiogenic tumor (13).

Because angiogenesis is a prerequisite for tumor growth beyond a microscopic size, we hypothesized that the regional tumor growth patterns observed in mouse models of intraperitoneal tumorigenesis, and possibly in patients with peritoneal carcinomatosis, may be due to an expression gradient of endogenous angiogenesis inhibitors. In this work, we show a model of tumor dormancy in which the dormancy is dictated by the site of implantation of tumor cells in the peritoneal cavity of mice. Our studies indicate that in this model, the endogenous angiogenesis inhibitor TSP-1 is differentially expressed in the stroma of the upper versus lower abdomen. This may underlie the differential tumor growth observed in the upper abdomen versus the bowel mesentery. Furthermore, our studies show that the restricted growth of tumors in the bowel mesentery is lost in mice with a targeted deletion of *Tsp-1*. Finally, we show that tumor cells overexpressing TSP-1 overrode this differential tumor growth in the abdomen of wild-type mice. These studies suggest that malignancies that originate or spread in the abdominal cavity may be more responsive to treatment by endogenous angiogenesis inhibitors, specifically TSP-1.

## Materials and Methods

### Cell culture

CT26 mouse colon carcinoma parental cells and TSP-1–overexpressing cells have been previously described (14). Lewis lung carcinoma cells were obtained from the American Type Culture Collection. All cells were maintained in DMEM (Cambrex Bioscience) supplemented with 10% FCS (Life Technologies, Inc.), 100 units/mL penicillin, and 100 μg/mL streptomycin (Cambrex) at 37°C with 5% $CO_2$. The M5076 cell line was cultured in RPMI 1640 supplemented with 15% equine serum, 2 mmol/L L-glutamine, and antibiotics. The P210-32D murine leukemia cell line, a gift of Dr. J. Griffin (Dana-Farber Cancer Institute, Boston, MA), was maintained in RPMI 1640 with 10% FCS, 2 mmol/L L-glutamine, and antibiotics. The murine K1000 tumor cell line, generated by transfection of basic fibroblast growth factor into a murine 3T3 fibroblast cell line and a gift of Dr. A. Hori (Takeda Chemical Industries, Osaka, Japan), was maintained in DMEM supplemented with 10% FCS (FCS), 2 mmol/L L-glutamine, 100 units/mL penicillin/streptomycin. The murine M5076 reticulum cell sarcoma cell lines, a gift of Dr. I.J. Fidler (M.D. Anderson Cancer Center, Houston, TX), were maintained in RPMI 1640 supplemented with 15% equine serum, 2 mmol/L L-glutamine, and antibiotics. The human MDA-MB-435 breast carcinoma cell line, a gift of Dr. B. Zetter (Children's Hospital, Boston, MA), was cultured in DMEM with 10% FCS, 2 mmol/L L-glutamine, and antibiotics.

### Animals

All animal experiments were done according to protocols approved by Children's Hospital Boston Institutional Animal Care and Use Committee. Six- to eight-week-old male C57Bl/6 animals were purchased from Charles River. *Tsp-1*$^{-/-}$ mice on a C57Bl/6 background were provided by Dr. Jack Lawler (Beth Israel Deaconess Medical Center, Boston, MA; ref. 15). Six-week-old male NCR nude, 6-week-old male Swiss white background, and 6-week-old male severe combined immunodeficient mice were purchased from Massachusetts General Hospital. Six-to eight-week-old male natural killer cell–defective beige mice (C57B16/bgj), C3H mice, and BALB/c mice were purchased from The Jackson Laboratory. Six- to eight-week-old male NIH-3 mice deficient in T, B, and natural killer cells (Tac:NIHS-bg nu xidfDF) were purchased from Taconic.

### Tumor growth

Six- to eight-week-old mice received i.p. injections of $1 \times 10^6$ tumor cells resuspended in 100 μL of PBS. Mouse body weights were measured daily, and their general health conditions were monitored closely. Mice were sacrificed 7 to 14 days after tumor cell inoculation, depending on their health condition, and tumors were excised, weighed, and fixed.

### Immunohistochemistry

Formalin-fixed, paraffin-embedded tumor sections were deparaffinized by successive incubations in xylene, 100% ethanol, 90% ethanol, 70% ethanol followed by PBS. Epitopes were unmasked with 10 μg/mL proteinase K in PBS

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at 37°C for 40 minutes and rinsed twice in PBS with 0.3% Triton X-100. Sections were immunostained with anti-rat CD31 monoclonal antibody (1:50; BD Pharmingen) or anti-mouse TSP-1 monoclonal antibody (1:500; Neomarkers) overnight at room temperature, followed by incubation for 1 hour with goat anti-rat or goat anti-mouse Alexa 594–conjugated secondary antibody (1:500; Invitrogen). The mean of five to six fields was computed for each tumor section and microvessel densities (MVD) were calculated for the peripheral and central sections of each tumor. The MVD of each tumor nodule was then computed by calculating the mean of all counts and was expressed as MVD ± SD.

For proliferating cell nuclear antigen (PCNA) staining, formaldehyde-fixed sections (5 μm) were pretreated two times for 5 minutes each in a microwave oven in 6.0 mmol/L citrate buffer. Immunohistochemical staining was done with anti-PCNA murine monoclonal antibodies (Signet Laboratories), and positive staining was detected as described above. The PCNA labeling index was determined by counting the percentage of stained cells under a light microscope in selected 63× fields. A minimum of 1,000 cells were counted for each tumor specimen.

For staining of apoptotic cells, formaldehyde-fixed sections (5 μm) were stained with Apoptag (Oncor). After TdT labeling of specimens, positive staining was detected with peroxidase-labeled antibody against dUTP-digoxigenin, followed by development with diaminobenzidine peroxidase substrate. Sections were counterstained with Mayer's hematoxylin (Sigma). The apoptotic index was determined by counting the percentage of stained cells under a light microscope. A minimum of 2,000 cells were counted for each tumor specimen.

### TSP-1 expression

Total RNA was isolated from the liver, pancreas, spleen, duodenum, colon, mesentery, and diaphragm, using TRIzol reagent (Invitrogen) according to the manufacturer's protocol, and treated with DNase I (NEB). RNA samples (1 μg) were reverse transcribed using Invitrogen Superscript

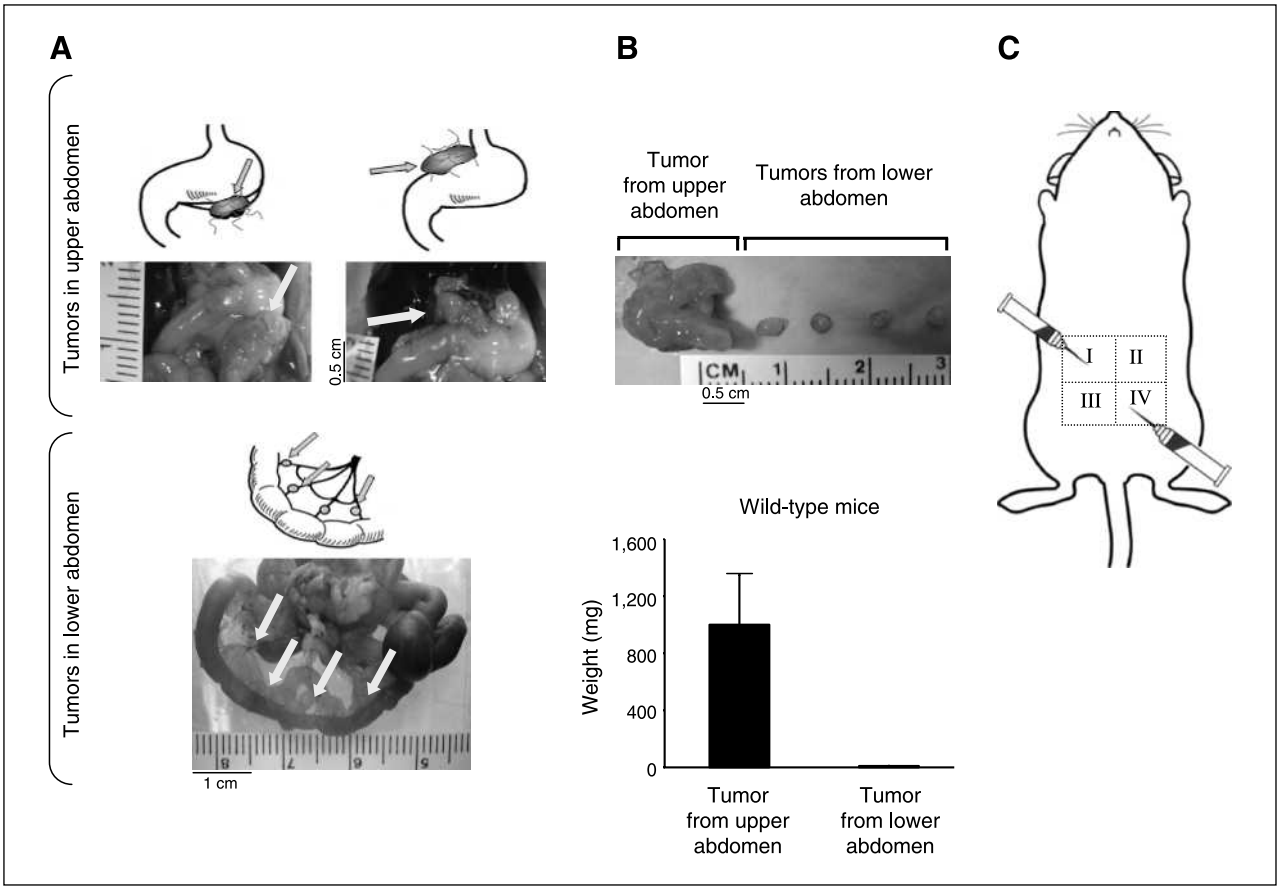


**FIGURE 1.** Inoculation of CT26 tumor cells into the peritoneal cavity leads to differential tumor growth in the upper versus lower abdomen. A, top, CT26 carcinoma cells form large, angiogenic tumors in the upper region of the peritoneal cavity and small, nonangiogenic tumors on the mesentery in the lower abdomen. Arrows point to large angiogenic tumors located on or below the stomach in the top image and to small nonangiogenic tumors in the lower abdomen ($n$ = 10 mice per group). B, CT26 tumors isolated from the upper abdomen are significantly larger in size and weight than the tumors from the lower abdomen. Data are represented as mean ± SEM. C, schematic depicting the sites of tumor cell inoculation (quadrants I and IV) and the direction of the needle.

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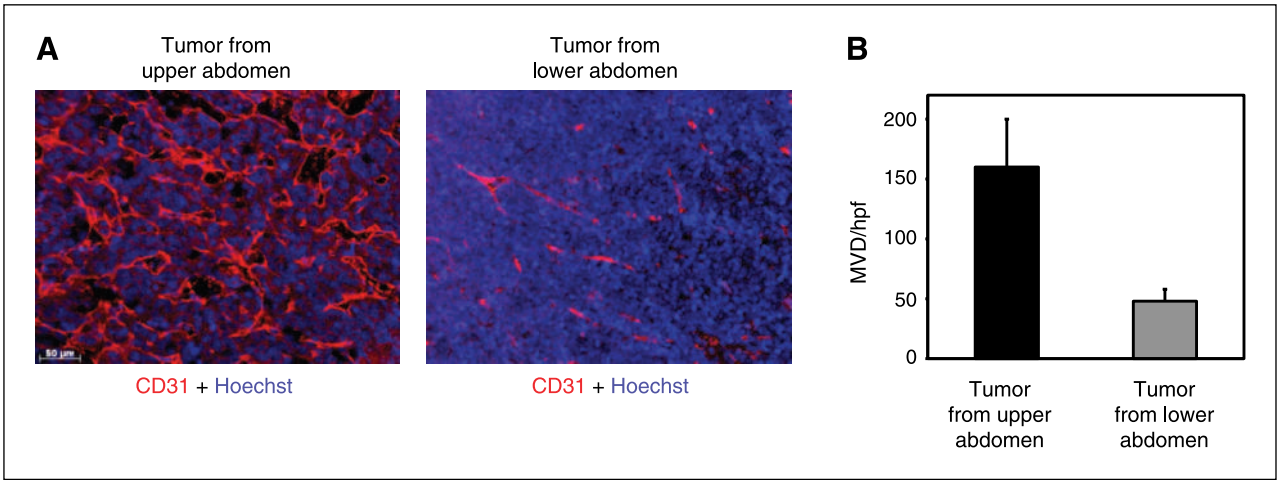


**FIGURE 2.** MVD is significantly increased in tumors isolated from the upper abdomen. A, sections from CT26 colon carcinomas isolated from the upper and lower abdomen were immunostained with anti-CD31 antibody to detect endothelial cells and with Hoechst dye to detect nuclei. Bar, 40 μm. B, quantification of MVD in CT26 tumors isolated from the upper and lower abdomen per high-power field (hpf; $n$ = 20 tumors per group). Data are represented as mean ± SEM.

III reverse transcriptase according to the manufacturer's instructions. Amplification was done in a volume of 25 μL containing 8 μL of template cDNA, 10 μL of real-time Dynamo HS SYBR Green master mix (Finzymes, Inc.), 5 μL of $H_2O$, and 2 μL of reverse transcription primer set. Amplification was done for 40 cycles (95°C for 15 seconds, 60°C for 1 minute) on the DNA Engine Opticon 2 System (MJ Research, Inc.). The following primers were used: *Tsp-1*, sense, 5′-TCCCCTATTCTGGAGGGTTC-3′, and antisense, 5′-TCCCTGGAAATAGGCACAAG-3′; *GAPDH*, sense, 5′-ACCACAGTCCATGCCATCAC-3′, and antisense, 5′-TCCACCACCCTGTTGCTGTA-3′. For data analysis, the $2^{-\Delta\Delta C}$T method (16) was used with normalization of raw data to the housekeeping gene *GAPDH*.

## Results

### Regional differences in the growth of tumor cells in the peritoneal cavity of mice

Differential tumor growth has been well characterized in patients with peritoneal carcinomatosis. The peritoneal surface malignancies located in the perigastric region are often found as large macroscopic tumors, whereas tumors in the lower abdomen within the umbilical and hypogastric regions are small microscopic tumors (17). To confirm whether regional differences in tumor growth occurred in the peritoneal cavity of mice, we inoculated syngeneic murine CT26 colon carcinoma cells into the peritoneal cavity of wild-type BALB/c mice (Fig. 1) and Lewis lung carcinoma cells into the peritoneal cavity of wild-type C57Bl/6 mice (Supplementary Fig. S1). The mice were sacrificed 14 days after tumor cell injection, and the dissemination and growth of CT26 carcinoma cells were examined (Fig. 1). The location of tumor implants in the upper and lower abdomen is shown schematically in Fig. 1A. Implants in the bowel mesentery were most often found as cuffs of tumor cells around preexisting host mesenteric arterioles. The upper abdominal site is distinct and separate from the stomach, pancreas, spleen, and liver. We consistently observed macroscopic, highly vascularized tumors, which grew on the surface of the mouse pancreas, and similar angiogenic tumors could also be seen attached to the liver and above the stomach (Fig. 1A). In contrast, in the lower abdomen, we observed primarily small, nonvascularized tumors that grew on the mesentery and serosal surface of the small and large bowel (Fig. 1A).

There was up to a 140-fold difference between the tumor volume of the larger tumors isolated from the upper abdomen and that of the smaller avascular tumors isolated from the lower abdomen (Fig. 1B; Supplementary Fig. S1). Similar differences were found regardless of host gender, the absence of an intact immune system, the tumor cell line used, or when tumor cells were injected in a cranial or caudal direction in any quadrant of the abdomen (Fig. 1C; Supplementary Fig. S1). The mesenteric tumor implants in the lower abdomen generally formed small, avascular, dormant tumors. In contrast, in the upper abdomen, the large red vascularized tumors grew progressively until they eventually killed the host, on average, 18 days after tumor cell inoculation.

### Characterization of site-specific differences in intraperitoneal tumor implants

To investigate whether differences in tumor angiogenesis may be responsible for this differential tumor growth, we assessed the microvessel density (MVD) of tumors isolated from the upper and lower abdomen using endothelial specific markers. Immunohistochemical analysis with an antibody against von Willebrand factor (data not shown) and immunofluorescence analysis with an anti-CD31 antibody

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of both CT26 tumors (Fig. 2A) and Lewis lung tumors (Supplementary Fig. S1A) showed significant differences in MVD. Comparison of the degree of vascularity between tumor implants in the upper versus lower abdomen illustrated a significant 3- to 5-fold increase in MVD in the upper abdominal tumors as compared with the lower abdomen (Fig. 2B).

Suppression of tumor angiogenesis may be secondary to increased tumor cell apoptosis. Therefore, we examined proliferation and apoptosis in tumor implants isolated from the upper and lower abdomen. Proliferation of tumor cells was assessed by immunohistochemical analysis with an anti-PCNA antibody (Supplementary Fig. S2A). These studies showed that there was no significant difference in proliferation between the large upper abdominal and small lower abdominal tumors (Supplementary Fig. S2A). In contrast, tumor cell apoptosis was more than 2-fold higher in tumors in the lower abdomen than in tumors isolated from the upper abdominal sites (Supplementary Fig. S2B). However, this difference in apoptosis would not be sufficient to explain the dramatically different tumor growth in the upper versus lower abdomen.

### Differential expression of TSP-1 may regulate tumor growth in the upper and lower abdomen

Because of the differences observed in MVD in tumor implants from the upper and lower abdomen, we wanted

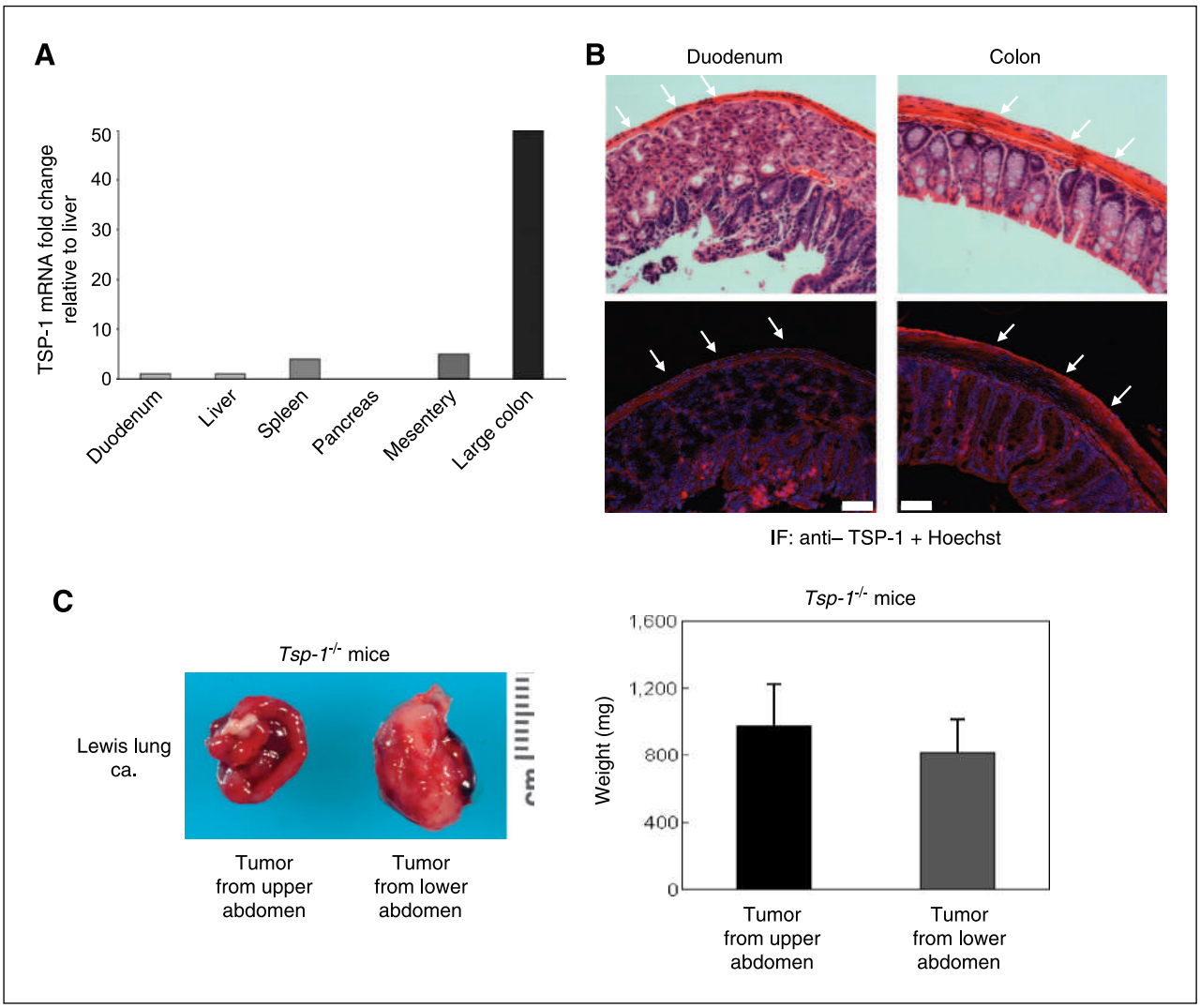


**FIGURE 3.** Differential expression of TSP-1 in the stroma of the upper and lower abdomen. A, *Tsp-1* mRNA expression in organs from the upper and lower abdomen. The fold change is relative to liver and normalized to *GAPDH*. B, TSP-1 immunofluorescence of the serosal surface of organs in the upper (duodenum) versus lower (colon) abdomen. White arrows identify the serosal surfaces. Top, H&E. Bottom, anti–TSP-1 (red) immunofluorescence and Hoechst dye (blue). Pictures are taken at ×63 magnification. Bar, 50 μm. C, the differential growth of Lewis lung tumors in the upper versus lower abdomen is lost in *Tsp-1*$^{-/-}$ mice. The weight of tumors isolated from the upper (small intestine) and lower (large colon) abdomen was measured ($n$ = 10 mice per group). Data are represented as mean ± SEM.

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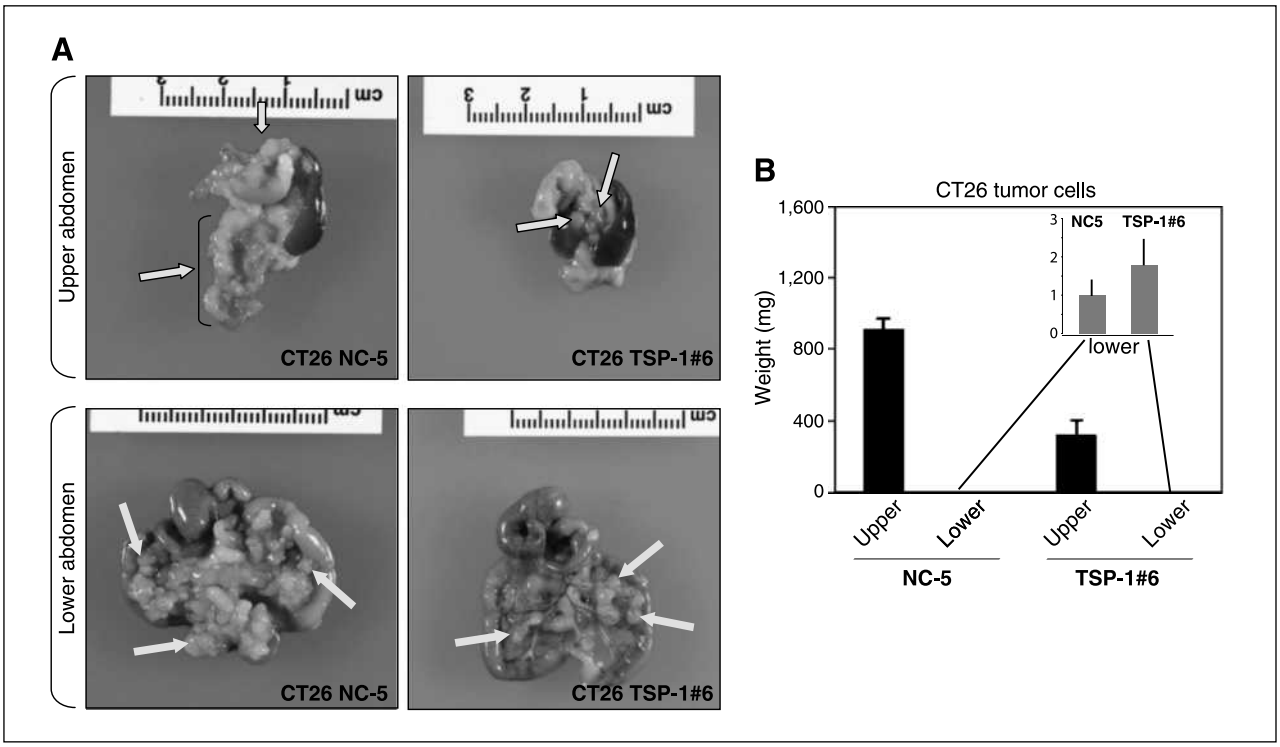


**FIGURE 4.** Tumor growth is suppressed in the upper abdomen on overexpression of TSP-1 in CT26 colon carcinoma cells. A, vector-transfected CT26 control (CT26 NC-5) tumor cells showed differential tumor growth in the upper and lower abdomen. However, TSP-1–overexpressing CT26 tumor cells (CT26 TSP-1#6) showed limited tumor growth in the upper abdomen. Arrows point to large and small tumors in the upper abdomen and small nonangiogenic tumors in the lower abdomen. B, the weight of tumors from the upper abdomen derived from CT26 TSP#6 tumor cells was significantly decreased in comparison with that of tumors derived from CT26 NC-5 control tumor cells. Inset, the weights of CT26 TSP-1#6 and CT26 NC-5 tumors from the lower abdomen show no significant difference. Data are represented as mean ($n$ = 5 mice per group) ± SEM.

to examine whether tumor angiogenesis was differentially regulated in the upper and lower abdomen. Initially, we examined vascular endothelial growth factor levels and found similar expression in tumors from the upper and lower abdominal regions (data not shown). Therefore, we chose to examine the expression of the endogenous angiogenesis inhibitor TSP-1. To determine whether TSP-1 expression varied in the upper versus lower abdomen, we isolated organs from the upper abdomen (liver, pancreas, spleen, and small bowel) and the lower abdomen (large bowel and mesentery) and analyzed *Tsp-1* mRNA by quantitative PCR. *Tsp-1* mRNA levels seemed to be the highest in the organs isolated from the lower abdomen, whereas the organs from the upper abdomen showed significantly lower *Tsp-1* mRNA expression (Fig. 3A).

To determine whether TSP-1 protein showed a similar pattern of differential expression, we performed immunofluorescence staining on tissues isolated from the upper and lower abdomen of the peritoneal cavity. As shown in Fig. 3B, TSP-1 expression on the surface of the large colon was significantly higher than that on the surface of the duodenum.

To validate the physiologic relevance of variable TSP-1 expression in the abdomen, we used mice with a targeted deletion of *Tsp-1* on a C57Bl/6 background and investigated the growth of intraperitoneal tumor implants in these mice. Lewis lung tumor cells were inoculated into the peritoneal cavity of *Tsp-1*$^{-/-}$ mice and tumor growth was examined after 10 to 14 days. Tumors grew much more rapidly in *Tsp-1*$^{-/-}$ mice as compared with littermate controls, with a significant increase in the numbers of tumors in the *Tsp-1*$^{-/-}$ mice in comparison with littermate control mice (data not shown). In contrast to the differential growth of tumors observed in wild-type mice, large angiogenic tumors were identified throughout the peritoneal cavity of *Tsp-1*–null mice (Fig. 3C). These data indicate that TSP-1 expression may be a critical determinant of differential tumor growth in the peritoneal cavity.

### Ectopic TSP-1 overexpression in tumor cells overrides differential tumor growth in the abdomen

Because host expression of TSP-1 seemed to suppress tumor growth in the lower abdomen, we next examined whether ectopic overexpression of TSP-1 by tumor cells could override the macroscopic tumor growth observed in the upper abdomen. Murine colon carcinoma cells CT26 were transfected with TSP-1 (CT26-TSP-1; ref. 14) and inoculated into the peritoneal cavity of syngeneic wild-type mice, and tumor growth was examined 10 to 14 days after inoculation. As expected, there

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was a significant reduction in the overall total number of tumors throughout the peritoneal cavity of the mice injected with overexpressing CT26-TSP-1 cells as compared with that in mice injected with mock-infected CT26 cells (Fig. 4A). Tumors overexpressing TSP-1 in the upper abdomen were considerably smaller than the mock-infected tumors (Fig. 4B). CT26-TSP-1 tumors isolated from the upper abdomen were small and avascular as compared with CT26 mock-infected tumors, which were large, macroscopic, and highly vascularized as assessed by anti-CD31 immunostaining (Fig. 5A-E). Anti–TSP-1 immunofluorescence showed high levels of TSP-1 expression in CT26-TSP-1 tumors as compared with CT26 mock-infected tumors (Fig. 5A-D). Taken together, these data suggest that regional tumor growth in the peritoneal cavity may be regulated by host expression of the endogenous angiogenesis inhibitor TSP-1, and that this differential tumor growth in the upper abdomen may be responsive to therapy with recombinant TSP-1.

## Discussion

Patients with peritoneal carcinomatosis often show differential tumor growth in the upper and lower abdominal cavity as a consequence of serosal invasion, perforation of the viscus by a primary tumor, or surgical interventions (3). To determine the mechanism behind this differential growth in the abdomen, we developed a mouse tumor model to recapitulate this disparate growth in the upper versus lower abdomen. After inoculating syngeneic tumor cells into the peritoneal cavity of wild-type mice, we observed regional differences in tumor growth. We found a striking and reproducible pattern of tumor growth in the mice with multiple different tumor cell lines and murine host backgrounds. Large tumors were found primarily in the epigastric or upper abdominal region, whereas only small tumors were observed within the lower abdominal region or pelvic cavity. There were no differences in location or size of tumor implants when tumor cells were injected in a cranial or caudal direction, extending previous observations (1, 2) and providing further anatomic evidence for anteroposterior differences in the growth of tumor cells and normal cells. The anatomic correlate of the murine upper abdominal site in humans seems to be the mesenchymal tissue surrounding the left and right gastroepiploic arteries or the greater omentum.

Although dissemination of tumor cells throughout the peritoneal cavity is dependent on several factors including drainage by lymphatic vessels, gravity, movement of the

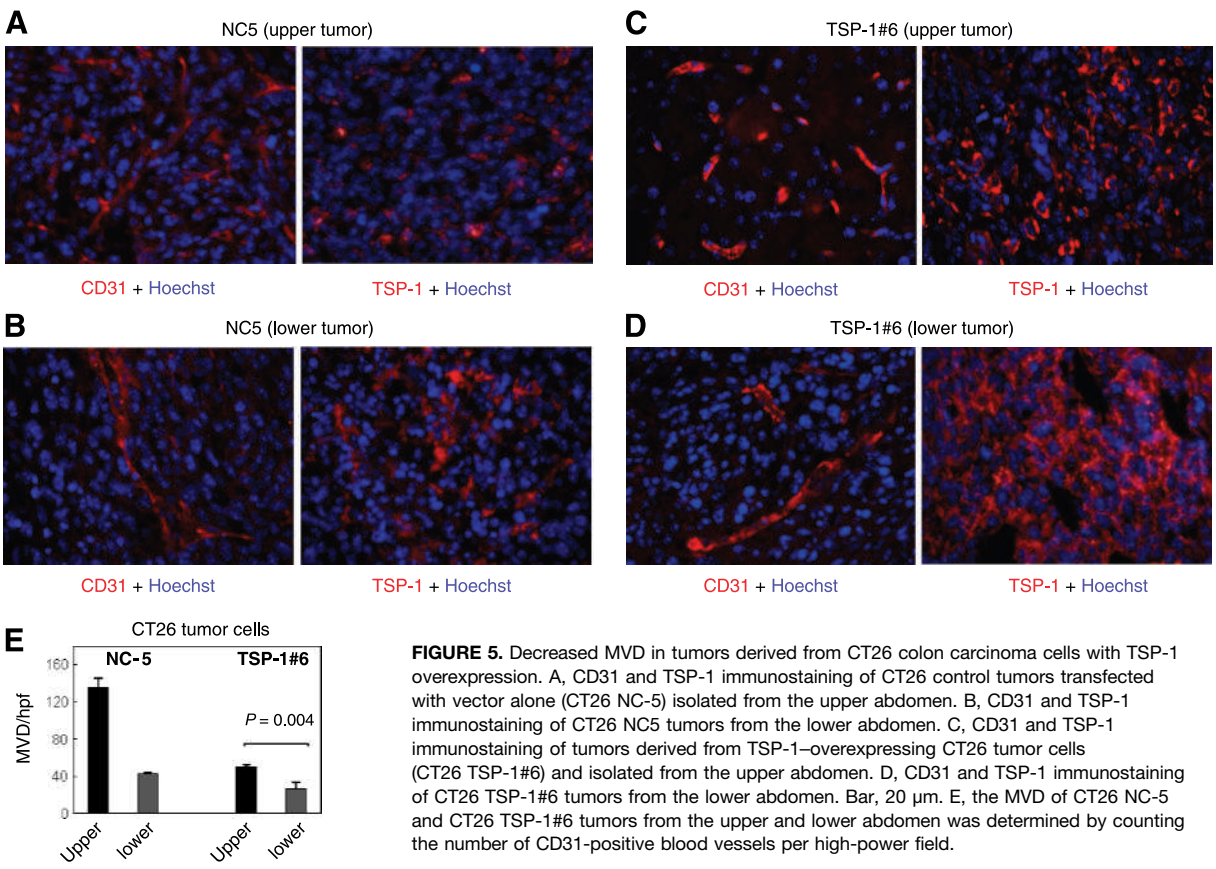


**FIGURE 5.** Decreased MVD in tumors derived from CT26 colon carcinoma cells with TSP-1 overexpression. A, CD31 and TSP-1 immunostaining of CT26 control tumors transfected with vector alone (CT26 NC-5) isolated from the upper abdomen. B, CD31 and TSP-1 immunostaining of CT26 NC5 tumors from the lower abdomen. C, CD31 and TSP-1 immunostaining of tumors derived from TSP-1–overexpressing CT26 tumor cells (CT26 TSP-1#6) and isolated from the upper abdomen. D, CD31 and TSP-1 immunostaining of CT26 TSP-1#6 tumors from the lower abdomen. Bar, 20 μm. E, the MVD of CT26 NC-5 and CT26 TSP-1#6 tumors from the upper and lower abdomen was determined by counting the number of CD31-positive blood vessels per high-power field.

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viscera, and flow of ascitic fluid, our data suggest that the microenvironment also plays a role. Our studies show that regional tumor growth in this model is mediated by an expression gradient of the endogenous angiogenesis inhibitor TSP-1 within the peritoneal surface of the lower abdomen. Several lines of evidence support this conclusion. First, we detected a significant difference in the MVD of tumors isolated from the upper versus lower abdomen. Second, TSP-1 expression inversely correlated with tumor growth such that regions with low levels of TSP-1 showed macroscopic tumor growth (i.e., upper abdomen) and vice versa. Third, our studies showed that the loss of TSP-1 in the murine host led to rapid tumor growth in both the upper and lower abdomen. These data implicate that the expression of TSP-1 is an important regulator of the differential tumor growth observed in wild-type animals. Finally, our studies show that tumor cells with ectopic overexpression of TSP-1 inoculated into wild-type mice leads to minimal tumor growth in the upper abdomen, suggesting that TSP-1 upregulation in tumor cells may override the host gradient expression of TSP-1. Taken together, these data indicate that differential expression of endogenous angiogenesis inhibitors in the abdominal microenvironment may be a novel mechanistic explanation responsible for the progression of peritoneal carcinomatosis. This disease is a local-regional cancer that results from tumor cell dissemination in the peritoneal cavity and is the most frequent cause of death in patients affected with colon adenocarcinoma due to the rapid outgrowth of disseminated tumor cells (18). Although the distribution and growth of cancer cells within the abdominal cavity is no longer considered a random process, there are still very few models that explain the differential growth characteristics of tumors disseminated in the abdominal cavity (17). In our experimental model, we observed that tumor growth patterns seemed to be independent of tumor cell type because multiple tumor cell lines all followed the same growth and distribution pattern within the peritoneal cavity. Taken together, these data suggest that differential tumor growth is a consequence of host regulation of tumor growth.

It is now well accepted that tumor growth beyond 1 to 3 $mm^3$ requires the formation of new blood vessels, a process known as angiogenesis (19, 20). Angiogenesis is regulated by a balance of proangiogenic and antiangiogenic regulatory proteins. However, most antiangiogenic therapies have targeted the proangiogenic protein vascular endothelial growth factor and its receptors (21, 22). There have been a number of endogenous angiogenesis inhibitors that have been discovered both in the circulation and in the extracellular matrix, such as TSP-1, endostatin, and tumstatin (23). Several studies have shown a direct role for these angiogenesis inhibitors in regulating tumor growth by examining tumorigenesis in mice with a targeted deletion of each of these genes; results showed that tumors grew at much faster rates than the wild-type controls. In our study, we observed that the lack of *Tsp-1* in transgenic mice altered the tumor growth pattern, and large tumors grew in abdominal regions previously protected from the growth of large angiogenic tumors. In fact, tumors isolated from the lower abdomen seemed to be even larger and more angiogenic than those that grew in the upper abdomen of the same animals. Interestingly, when we inoculated syngeneic tumor cells overexpressing TSP-1, the regional tumor growth was very different than that of the parental, mock-infected tumor cells. It has been previously suggested that the tumor cell overexpression of TSP-1, or even prolonged exposure to TSP-1, may force tumor cells to increase the production of proangiogenic factors to override its inhibitory effect and promote angiogenesis (14, 24).

To date, the treatment options for peritoneal carcinomatosis include cytoreductive surgery and heated intraoperative intraperitoneal chemotherapy, followed by systemic chemotherapy (25). However, the high level of recurrence after initial treatment of peritoneal carcinomatosis remains a significant problem (26). The changes in tumor growth pattern and angiogenic phenotype that we observed in *Tsp-1*–null animals indicate that TSP-1 is a major regulator of tumor angiogenesis within the lower region of the peritoneal cavity. Our data also suggest that a decrease in the expression of endogenous TSP-1 may provide an environment suitable for tumor growth and thus may be a prerequisite for the progression of peritoneal carcinomatosis. Therefore, it is possible that, in addition to surgery, antiangiogenic therapy using recombinant antiangiogenic regulators, such as TSP-1, in combination with chemotherapy may offer another treatment modality and further increase the survival of patients with peritoneal carcinomatosis.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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