Effect of Thrombin Treatment of Tumor Cells on Adhesion of Tumor Cells to Platelets in Vitro and Tumor Metastasis in Vivo

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ABSTRACT

Seven different tumor cell lines (human melanoma SK MEL 28; hamster melanoma HM29; murine melanomas B16F10 and amelanotic melanoma B16a; human colon carcinoma HT28; murine colon carcinoma CT26; and murine Lewis lung carcinoma) were treated with thrombin at 0.5-1 unit/ml and examined for their ability to bind to adherent platelets; HM29 was studied for its ability to bind to fibronectin and von Willebrand factor; CT26, B16F1, B16F10, and B16a were studied for their ability to form pulmonary metastasis after i.v. injection of thrombin-treated tumor cells; CT26 was studied for its ability to grow s.c. Five of 7 thrombin-treated tumor cell lines increased their adhesion to adherent platelets 2- to 3-fold. HM29 increased its adherence to fibronectin and von Willebrand factor 2- to 3-fold. CT26, B16F1, B16F10, and B16a increased experimental pulmonary metastasis 10- to 150-fold. Thrombin-treated CT26 cells demonstrated 2-fold greater growth in vitro after s.c. injection. The mechanism of enhanced adhesion of thrombin-treated tumor cells to platelets required the platelet integrin GPIIb-GPIIIa since it could be inhibited by agents known to block adhesion of ligands to GPIIb-GPIIIa (monoclonal antibody 10E5, tetrapeptide RGDS, disintegrin Albolabrin); as well as a "GPIIb-GPIIIa-like" structure on tumor cells since it could be inhibited by treatment of thrombin-treated tumor cells with 10E5 and RGDS. The thrombin effect on tumor cells was optimum at 1 h of incubation with thrombin, did not require active thrombin on the tumor cell surface, and did not require protein synthesis (not inhibited by cycloheximide). Thus, thrombin-treated tumor cells markedly enhance pulmonary metastasis. It is suggested that this may be secondary to thrombin-induced enhanced adhesion as well as growth of tumor cells.

INTRODUCTION

The requirement of platelets for experimental as well as spontaneous tumor metastasis is well established in the murine system (1, 2). Thrombocytopenia results in an 85-95% inhibition of pulmonary metastasis (2). We have recently demonstrated that it is the adhesive function of platelets via the platelet GPIIb-GPIIIa adhesive ligand receptor that is important for tumor platelet interaction in vitro and tumor metastasis in vivo (3). Agents which inhibit binding of GPIIb-GPIIIa to fibronectin or von Willebrand factor such as monoclonal antibody 10E5, tetrapeptide RGDS, disintegrin Albolabrin; as well as a "GPIIb-GPIIIa-like" structure on tumor cells since it could be inhibited by treatment of thrombin-treated tumor cells with 10E5 and RGDS. The thrombin effect on tumor cells was optimum at 1 h of incubation with thrombin, did not require active thrombin on the tumor cell surface, and did not require protein synthesis (not inhibited by cycloheximide). Thus, thrombin-treated tumor cells markedly enhance pulmonary metastasis. It is suggested that this may be secondary to thrombin-induced enhanced adhesion as well as growth of tumor cells.

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MATERIALS AND METHODS

Tumor Cell Lines and Tissue Culture Media. CT26, N-nitroso-N-methylurethane-induced mouse undifferentiated colon carcinoma cells were obtained through the courtesy of Dr. M. H. Goldrosen, Roswell Park Memorial Institute, Buffalo, NY. HT28 spontaneous human colon adenocarcinoma cells were obtained through the courtesy of Dr. E. Cadman, Yale University Medical School, New Haven, CT. HM29 hamster malignant melanoma cells were obtained through the courtesy of Dr. C. Maniglia, Yale University Medical School. B16F1 and B16F10 mouse melanoma cells were obtained through the courtesy of Dr. I. J. Fidler, University of Texas, M. D. Anderson Cancer Center, Houston, TX, and grown in minimal essential media supplemented with 5% fetal bovine serum, 2% glutamine, 2% penicillin-streptomycin (GIBCO, Grand Island, NY), and 1% sodium pyruvate (100 mM), 1% vitamins (100x) and 1% nonessential amino acids (Sigma, St. Louis, MO). All other cell lines were maintained and grown in tissue culture as described previously (2-4).

Anti-Platelet-adhesive Protein Receptor and Adhesive Protein Reagents. Monoclonal antibodies 10E5 and 6D1 were provided through the courtesy of Dr. Barry Coller, State University of New York, Stony Brook, NY. 10E5 binds to the GPIIb-GPIIIa complex and inhibits the binding of fibronectin, fibronectin, and von Willebrand factor to activated platelets (6); 6D1, used as a control monoclonal antibody, binds to GPIb and inhibits ristocetin-induced platelet aggregation (7). Monospecific anti-human fibronectin antibody was raised in rabbits immunized with human fibronectin purified from Sepharose 4B gel filtration and gelatin-Sepharose 4B affinity chromatography. The details of fibronectin purification and antibody production have been described previously (3). Human von Willebrand factor was purified from Peninsula Laboratories, Inc., Belmont, CA. Albolabrin, the disintegrin from viper venom is a potent RGDS-containing peptide isolated from Trimeresurus albolabrin which inhibits metastasis (9), kindly supplied by Dr. [CANCER RESEARCH 52. 3267-3272, June 15, 1992]
THROMBIN-TREATED TUMOR CELLS ENHANCE METASTASIS

Stefan Niewiarowski, Temple University Medical School, Philadelphia, PA.

Thrombin and Anti-Thrombin Reagents. Human thrombin (3000 NIH units/mg) was purchased from Sigma, DAPA, a potent, highly specific competitive inhibitor of thrombin (10) was a gift of Dr. Michael Nesheim, Queens University, Ontario, Canada.

Preparation of Platelets. Human platelets were separated from citrated platelet-rich plasma (0.38% final concentration) on a 10 and 20% discontinuous Stractan gradient (arabino-galactan polymer) dissolved in an isotonic balance salt solution, as described in detail, previously (3, 4).

In Vitro Platelet-Tumor Cell Adhesion Assay. Platelets (3 x 10^9) were applied to 96-well flat bottomed microtitre plates (Falcon Labware, 3072, Oxnard, CA) for 1 h at room temperature to develop a platelet lawn as described previously (3, 4). Platelets were then treated with buffer, thrombin, DAPA, or their combination and incubated overnight at 4°C. Plates were then incubated with 0.01% PBS plus 1% BSA for 1 h at 37°C, washed once with the above, and three times with PBS-BSA plus 0.9 mm CaCl_2, 0.9 mm MgCl_2 before the addition of 1 x 10^4 naive or thrombin-treated tumor cells in PBS-BSA-Ca-Mg for 1 h at 37°C. In some experiments, blocked and washed platelets were treated with various monoclonal antibodies and inhibitors prior to the addition of tumor cells. Adherent tumor cells were removed with trypsin-EDTA (GIBCO Laboratories) and enumerated in a hemocytometer by phase microscopy.

Tumor cells were incubated with or without thrombin for 1 h at 37°C with gentle rocking on a shaker prior to their addition to the platelet lawn. In some experiments, thrombin or sham-treated tumor cells were sedimented and resuspended with RGDS or monoclonal antibody 10E5 for 1 h at 37°C and then washed in PBS-BSA-Ca-Mg prior to their addition to the platelet lawn.

In Vitro Tumor Cell Adhesion to Fibronectin and von Willebrand Factor. Microtitre plates were coated with optimum concentrations of fibronectin (0.6 µg/well) or von Willebrand factor (1.2 µg/well) overnight at 4°C for maximum adhesion. They were then washed and blocked with PBS-BSA-Ca-Mg prior to the addition of tumor cells for 1 h at 37°C. Non-adherent tumor cells were removed by washing with PBS-BSA-Ca-Mg. Adherent cells were removed with trypsin-EDTA and enumerated by phase microscopy.

Protein Synthesis Inhibition with Cycloheximide. Tumor cells were incubated as described above in the presence and absence of 5 µCi/ml [3H]leucine (New England Nuclear NET-460, 37 MBq/ml) in leucine-free RPMI (GIBCO). Following incubation, cells were washed 3 times with PBS-BSA-Ca-Mg. Cells were then centrifuged, the pellet was lysed with 1 ml of N NaOH, 4 ml of 5% trichloroacetic acid at -20°C for 10 min. The supernatant was removed, the pellet was dissolved in 0.5 ml of N NaOH, 4 ml of scintillation fluid were added, and radioactivity was monitored.

In Vivo Metastatic Studies. Experiments were performed as described previously (2-4). Tumor cells were removed from subconfluent tissue culture dishes with trypsin-EDTA, washed in PBS-BSA-Ca-Mg, and examined for viability with trypan blue. Tumor cells (1 x 10^6) were suspended in 500-1,000 milliunits of thrombin in PBS-BSA-Ca-Mg, or PBS-BSA-Ca-Mg vehicle, incubated for 1 h at 37°C, sedimented, washed twice in PBS-BSA-Ca-Mg, and 25,000-50,000 viable tumor cells were injected i.v. into the tail vein of syngeneic BALB/c or C57B1/6J mice in a volume of 200 µl. In one experiment, thrombin-treated tumor cells were incubated with hirudin, 1 anti-thrombin unit/ml for 15 min, washed once with the above, and then incubated for 1 h at 37°C, followed by washing. Right, thrombin-treated platelets were incubated with thrombin-treated HM29 cells as in Left and Middle. In all experiments cells were washed with buffer, eluted with trypsin-EDTA, and enumerated under phase microscopy. Columns, mean of 4 experiments performed in triplicate; bars, SEM.

RESULTS

Comparison of Effect of Thrombin Treatment of HM29 Tumor Cells versus Thrombin Treatment of Platelets on Adhesion of HM29 Tumor Cells to Platelets

Fig. 1 compares the effect of thrombin treatment of HM29 melanoma tumor cells on their adhesion to platelets with thrombin treatment of platelets on their adhesion to tumor cells. Note the 2.7-fold enhancement of adhesion of thrombin-treated tumor cells to naive platelets (Fig. 1, center) versus the 3.1-fold enhancement of adhesion of untreated tumor cells to thrombin-treated platelets (Fig. 1, left). Note the lack of an additive effect of the combination of thrombin-treated tumor cells plus thrombin-treated platelets on tumor platelet adhesion (Fig. 1, right, 2.8-fold enhancement).

Effect of Thrombin Treatment of Other Tumor Cells on Their Adhesion to Platelets

Fig. 2 demonstrates the enhanced adhesion effect of thrombin-treated tumor cells on naive platelets with 5 of 7 different tumor cell lines from 3 different species.
THROMBIN-TREATED TUMOR CELLS ENHANCE METASTASIS

Fig. 2 displays the effect of seven different thrombin-treated tumor cells on adhesion to naive platelets. Tumor cells (1 x 10^6) were incubated with buffer or 1 unit/ml thrombin for 1 h at 37°C, washed, and incubated with naive platelets as in Fig. 1. Number of experiments is given in parentheses.

Effect of Thrombin-treated Tumor Cells on Their Adhesion to Fibronectin and von Willebrand Factor

Fig. 3 demonstrates the enhanced adhesion effect of thrombin-treated tumor cells on fibronectin (P < 0.001) and von Willebrand factor-coated plates (P = 0.002), adhesive ligands which bind to platelet GPIIb-GPIIIa.

Kinetics of Thrombin Preincubation of Tumor Cells on Their Adhesion to Platelets

Fig. 4 demonstrates a time course for the enhanced adhesion of thrombin-activated tumor cells to platelets with the HM29 tumor cell line. Note the optimum effect at 1 h with HM29 (>control at 60 min, P < 0.05; 60 min >15 min, P < 0.04; 60 min >30 min, P = 0.05; 60 min >120 min, P < 0.03, one-tailed t test).

Effect of Cycloheximide. Table 1 demonstrates that de novo protein synthesis is not responsible for the thrombin effect. With these experimental conditions, cycloheximide at 5 and 10 μg/ml inhibited protein synthesis 88 and 92%, respectively.

Specificity of Thrombin Reaction with Tumor Cells and Lack of Requirement of Active Thrombin on Tumor Cell Surface Once Tumor Cells Have Been Exposed to Thrombin

Fig. 5 demonstrates the specificity of the thrombin effect on tumor cells as well as the lack of requirement of active thrombin on the tumor surface, once the tumor cells have been treated with thrombin. Note inhibition of the thrombin effect if thrombin is preincubated with DAPA (specific competitive inhibitor of thrombin) and lack of effect of DAPA if added 1 h after preincubation of tumor cells with thrombin (probability of DAPA inhibiting after 1 h (P > 0.1)).

Role of Platelet GPIIb-GPIIIa Integrin in Adhesion of Thrombin-treated Tumor Cells to Naive Platelets

Fig. 6 demonstrates inhibition of thrombin-activated tumor cell binding to platelets with previously determined, optimum concentrations of inhibitors of the platelet GPIIb-GPIIIa integrin receptor: RGDS (P = 0.03), monoclonal antibody 10E5 (P = 0.02), and the disintegrin, albolabrin (P = 0.04). Isotype-specific control murine IgG2a and irrelevant peptide GRGES had no effect.

Effect of Monoclonal Antibody 10E5 and Tetrapeptide RGDS on Thrombin-treated Tumor Cells

Fig. 7 demonstrates the inhibitory effect of 10E5 (P = 0.02) and RGDS (P = 0.002) on thrombin-treated tumor cells prior to their incubation with platelets, suggesting the presence of a “GPIIb-GPIIIa-like” integrin on thrombin-activated tumor cells.

Effect of Thrombin-treated Tumor Cells on in Vivo Metastasis

Table 2 demonstrates the effect of thrombin-treated tumor cells on experimental pulmonary metastasis. Four different tumor cell lines increased metastasis (pulmonary tumor mass) 10- to 156-fold by increasing both their mean nodule number and volume. Thus CT26 (2 experiments), B16F1, B16F10, and B16a increased their mean nodule number 1.5-, 1.5-, 7.6-, 7.4-, and 7.0-fold, respectively, as well as their mean nodule volume 6.5-, 30-, 22-, 9.2-, and 5.4-fold, respectively. Hirudin did not inhibit the effect of thrombin-treated tumor cells on enhanced metastasis, indicating that active thrombin was not required on the tumor cell surface (P > 0.1).

Effect of Thrombin-treated Tumor Cells on in Vivo Growth

Because of the markedly 10- to 156-fold enhancement effect of thrombin-treated tumor cells on in vivo metastasis compared to its 2- to 3-fold effect in vitro on tumor-platelet adhesion, we postulated that thrombin may be affecting tumor growth as well. Accordingly, 1 x 10^6 CT26 cells, treated and untreated with 1 unit/ml thrombin for 1 h at 37°C, followed by washing, were injected s.c. into the flank of BALB/c mice, and their
cells to enhance their binding to adherent platelets, fibronectin, and von Willebrand factor in vitro and enhance their development of pulmonary metastasis in vivo. In a previous study (5) we noted the ability of thrombin to activate platelets, rendering their surface 2- to 5-fold more adherent to 6 different tumor cell lines. Thrombin injected in vivo enhanced pulmonary metastasis 4- to 413-fold with 2 different tumor cell lines. The mechanism for the enhanced in vitro adhesiveness required active thrombin on the platelet surface, and was shown to require the interaction of tumor cells to the platelet integrin GPIIb-GPIIIa, presumably via fibronectin and von Willebrand factor bridging.

The direct adhesive affect of thrombin on tumor cells appears to be via both a similar as well as dissimilar mechanism as its action on platelets. It is similar with respect to the requirement of the platelet integrin GPIIb-GPIIIa for binding to thrombin-activated tumor cells (i.e., inhibition of enhanced adhesion by preincubation of platelets with monoclonal antibody 10E5 but not 6D1; inhibition by the tetrapeptide RGDS but not GRGES; inhibition by the disintegrin, albolabrin), as well as the enhanced adhesion of thrombin-activated tumor cells to the adhesive ligands fibronectin and von Willebrand factor. Indeed, thrombin-activated tumor cells appear to have a GPIIb-GPIIIa-like receptor on their surface, since their binding to platelets is blocked by preincubating tumor cells with monoclonal antibody 10E5 or the tetrapeptide RGDS. This observation is compatible with reports of other investigators who have noted GPIIb-GPIIIa-like integrins on tumor cells [i.e., reactivity with monoclonal antibody 10E5 (12, 13)], as well as other integrins capable of binding fibronectin, von Willebrand factor, vitronectin, lam-

### Table 1 Effect of cyclohexamide on binding of thrombin-treated HM29 tumor cells to naive platelets

<table>
<thead>
<tr>
<th>Cyclohexamide (µg/ml)</th>
<th>Control buffer</th>
<th>Thrombin (1 unit/ml)</th>
<th>Fold increase</th>
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<tr>
<td>0</td>
<td>1.6</td>
<td>5.0</td>
<td>3.1</td>
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<td>1.4</td>
<td>4.3</td>
<td>3.1</td>
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<td>10.0</td>
<td>2.1</td>
<td>6.2</td>
<td>3.1</td>
</tr>
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</table>

Fig. 4. Effect of time on thrombin pretreatment of tumor cells prior to their incubation with adherent platelets. SK-Mel HM29 tumor cells (1 x 10⁶) were preincubated with buffer or 0.5 unit/ml thrombin for various time intervals prior to their incubation with adherent platelets on microtiter plates for 1 h at 37°C, as in Fig. 1. Except for the 120-min thrombin preincubation, thrombin-treated tumor cells were first incubated with buffer for various intervals of time, prior to the addition of thrombin at various time intervals, so that all tumor cells were incubated with or without thrombin for the same period of time (120 min). Adherent tumor cells were enumerated as in Fig. 1. Data are from three experiments performed in triplicate.

The volume was measured at 21 days. Thrombin-treated tumor cells had a 2.1-fold increased in mean volume compared to sham-treated tumor cells. (6.4 ± 1.3 versus 3.1 ± 0.75, cm³, n = 9, P = 0.02).

### DISCUSSION

These data indicate that thrombin can directly activate tumor cells to enhance their binding to adherent platelets, fibronectin, and von Willebrand factor in vitro and enhance their development...
in, and collagen type IV (14–19). Using specific monoclonal antibodies, we have identified integrin receptors for fibronectin (α3β1) and vitronectin (αvβ3) (5).

The mechanism is dissimilar with respect to the lack of requirement of active thrombin on the tumor cell surface as is the case with active thrombin on the platelet surface (5). We have demonstrated that thrombin binds to the tumor cell surface in a saturation-dependent manner.* The absence of active thrombin on tumor cells rendered more adhesive implies a secondary event on the tumor cell surface. This is supported by the presence of a lag period of approximately 1 h before achieving the optimum thrombin effect. The secondary event does not require protein synthesis as it is not inhibited by cycloheximide. We postulate the formation of a more adhesive integrin either by an increase in density or change in conformation of a GPIIb-GPIIIa-like integrin already present on the tumor cell surface or the development of a new adhesive receptor, perhaps secondary to thrombin proteolysis.

The requirement of the platelet integrin GPIIb-GPIIIa as well as RGD ligands (fibronectin and von Willebrand factor) for both the thrombin-activated platelet (5) as well as the thrombin-activated tumor cell arm of the enhanced platelet-tumor cell interaction suggests that thrombin may be having the same effect on both platelets and tumor cells (i.e., mobilization and/or conformational activation of GPIIb-GPIIIa and GPIIb-GPIIIa-like integrins on platelets and tumor cells with RGD ligand bridging). This is supported by our observation that enhanced adhesion of thrombin-activated platelets and thrombin-activated tumor cells is not additive. Thus, we propose that active thrombin on the platelet surface interacts with a thrombin receptor on tumor cells to enhance tumor cell integrin reactivity. This is supported by the well-described greater efficiency and reactivity of procoagulants on the platelet membrane surface (20), as well as the greater thrombin sensitivity of thrombin-activated platelet adhesion to naive tumor cells [10–100 milliunit reactivity (5)] than thrombin-activated tumor cell adhesion to naive platelets (500–1000 milliunit reactivity).

The 10–156-fold increase in pulmonary metastasis of 4 different thrombin-activated tumor cells is particularly impressive. This indicates that thrombin activation of tumor cells induces a metastatic phenotype by enhancing tumor cell adhesion to platelets, subendothelial matrix (fibronectin, von Willebrand factor, other RGD ligands, collagens), and as we have recently noted, endothelial cells; or that thrombin renders the tumor cell more oncogenic.

The in vivo enhancement of pulmonary metastases induced by i.v. thrombin (4- to 413-fold) (5) or thrombin-activated tumor cells (10- to 156-fold) is considerably greater than the in vitro 2- to 5-fold enhanced adhesion (5) of platelet-tumor cell or adhesive ligand interaction. Indeed two tumor cell lines responsive to thrombin in vivo (CT26 and B16F10) did not enhance their in vitro adhesion to platelets or fibronectin following thrombin activation. These observations would suggest that thrombin may be having an additional effect on tumor cells, possibly unrelated to platelet or adhesive ligand interaction, at least with some tumor cell lines. This is supported by the enhanced s.c. growth of thrombin-treated tumor cells versus naive cells. We propose that thrombin may enhance the metastatic phenotype by activating the oncogenic potential of tumor cells.

A precedent for thrombin-induced enhanced oncogenesis is supported by the following observations. Thrombin can act as a mitogenic agent (21–27). Thrombin stimulates cell division in nonproliferating fibroblast cultures of chick and mouse embryo, human diploid foreskin, and Chinese hamster lung in the absence of added serum or additional growth factors (21, 22, 24). Thrombin potentiates the response to fibroblast growth factor of human vascular endothelium (23). Thrombin promotes cell transformation in Balb 3T3/a31-1-13 cells (27). The proliferative events initiated by thrombin require receptor binding as well as proteolysis (25, 26, 28).

Thrombin generates some of the same signals induced by oncogenes. Thrombin activates phosphoinositol production via a specific phospholipase C through a pertussis toxin-sensitive GTP-binding regulatory G-protein. Several laboratories have recently established that activated ras oncogenes, transfected into NIH-3T3 cells, can induce pulmonary metastasis of the nonmetastatic parental line (29–32). The ras gene encodes a M1, 21,000 protein on the inner surface of the plasma membrane with structural similarity to the G-proteins. Thus, it is likely that the ras protein initiates aberrant signals and/or interferes with normal signal transduction across the cell membrane, presumably by binding to GTP. A recent report has demonstrat-

### Table 2 Effect of washed thrombin-treated tumor cells on pulmonary metastasis

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Group</th>
<th>Mean no. of nodules</th>
<th>Mean nodule volume</th>
<th>Tumor mass</th>
<th>Fold increase</th>
<th>P</th>
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<tbody>
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<td>CT26</td>
<td>Control 12</td>
<td>8.0 ± 0.8</td>
<td>6.9 ± 1.6</td>
<td>55.2</td>
<td>9.7</td>
<td>0.008</td>
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<td>Thrombin 9</td>
<td>11.9 ± 2.1</td>
<td>44.9 ± 14.9</td>
<td>534</td>
<td>30</td>
<td>0.045*</td>
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<tr>
<td>CT26</td>
<td>Control 5</td>
<td>2.2 ± 0.5</td>
<td>1.1 ± 0.5</td>
<td>2.1</td>
<td>41</td>
<td>0.040*</td>
</tr>
<tr>
<td></td>
<td>Thrombin 5</td>
<td>3.2 ± 1.0</td>
<td>19.6 ± 7.9</td>
<td>62.7</td>
<td>156</td>
<td>0.011</td>
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<tr>
<td></td>
<td>Thrombin + hirudin 5</td>
<td>4.2 ± 0.6</td>
<td>20.4 ± 8.6</td>
<td>85.7</td>
<td>0.5</td>
<td>0.099</td>
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<td>B16F1</td>
<td>Control 11</td>
<td>0.7 ± 0.3</td>
<td>0.8 ± 0.4</td>
<td>0.6</td>
<td>78</td>
<td>0.046</td>
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<td>Thrombin 12</td>
<td>5.3 ± 1.3</td>
<td>17.7 ± 6.3</td>
<td>93.8</td>
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<td>B16F10</td>
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<td>0.1 ± 0.1</td>
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<td>9.2 ± 4.9</td>
<td>34.0</td>
<td>78</td>
<td>0.046</td>
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</table>

*a Paired one-tailed t test.

*b Difference in total tumor area of control and thrombin-treated tumor cells on a slide section.
stromed that thrombin induced c-fos mRNA expression in cultured human umbilical vein endothelial cells (33). c-fos and c-jun can form a heterodimer complex corresponding to the transcription factor AP1 which regulates transcription of the ras gene through an AP1 site (34). Thus thrombin could be activating ras through the induction of c-fos and other possible oncoproteins.

In summary, thrombin directly activates four different tumor cell lines to induce a markedly enhanced metastatic phenotype in vivo. The precise mechanism(s) for this induction remains to be established. In at least 5 of 7 tumor cell lines it may be related to the in vitro enhancement of adhesion of tumor cells to platelets, subendothelial matrix, or endothelial cells; in other cell lines it may be acting as a growth factor.

Extrapolating from these data, we propose that low grade thrombin generation may foster metastatic progression in some patients with some tumors. Indeed, many tumor cells activate the coagulation system with generation of thrombin (35). Low-grade intravascular coagulation has been observed in most patients with solid tumors (36–39). One study reported elevated fibrinopeptide A levels in 60% of cancer patients at time of presentation with increasing levels noted with progression of disease. Persistent elevation was associated with a poor prognosis (39). Thus, it is proposed that anti-thrombin and anti-platelet adhesive agents may be helpful in the prevention of tumor metastasis.

REFERENCES

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