Deficient Activity of von Willebrand’s Factor-cleaving Protease in Patients with Disseminated Malignancies

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ABSTRACT

An aberrant platelet immunorelated glycoprotein Ib (GPIb) receptor expressed by human tumor cells appears to participate in primary adhesive interactions required for the metastatic process. Hence, we questioned whether plasma von Willebrand’s factor (vWF), its adhesive ligand, manifested comparable anomalies in patients with disseminated tumors. Plasma specimens from patients with disseminated metastases showed 68% (P < 0.013), 91% (P < 0.0009), and 207% (P < 0.0009) enhancements in FVIII:C activity, vWF-related antigen levels, and ristocetin cofactor activity, respectively, whereas their SDS-agarose electrophoretic analyses demonstrated a 165% (P < 0.001) increase in the highly polymeric forms of vWF compared to control preparations from patients with corresponding, localized solid tumors. Substantially reduced levels of vWF-cleaving protease activity were observed in study patient specimens, with no plasma inhibitors detectable. The clinical presence and absence of tumor metastases correlated significantly with vWF-cleaving enzyme activities of ≤15% and ≥88%, respectively (n = 20; P < 0.0001). Finally, with an in vitro model system, tumor-induced platelet aggregation was enhanced by 127% (P < 0.001) in study patient platelet-rich plasma (PRP) compared to control PRP and could be completely inhibited (P < 0.0009) when both tumor cells and their PRP substrates were incubated with monoclonal antibodies directed against the vWF binding epitope of GPIbα and against the GPIbs binding epitope of plasma vWF, respectively. Unusually large vWF multimers observed in patients with disseminated tumors probably result from deficient vWF-cleaving protease activity and may represent a novel mechanism regulating primary platelet-tumor adhesive interactions involved in the metastatic process.

INTRODUCTION

Cancer metastasis is a dynamic multistep process in which tumor cells undergo complex interactions with host platelets and the vascular endothelium prior to establishing a secondary colony. The same properties that confer the ability to arrest hemorrhage to platelets also render them with an increased affinity to assemble as pathological vascular thrombi. In the setting of circulating tumor cells, platelet-tumor adhesive interactions result in the generation of a thrombus with an enhanced facility for attachment to and invasion of the vascular subendothelium (1).

Mounting evidence supports the notion that a platelet-related GPIbα receptor, expressed by both cultured tumor cell lines (2) and fresh human carcinoma specimens (3, 4), participates in primary adhesive events required for the initiation of the metastatic process (3, 5). Additionally, recent reports demonstrate that the functional binding properties of these two homologous receptors also vary (5). In contrast to platelet GPIbα, which requires the nonphysiological cofactor, ristocetin, for in vitro binding under static conditions to its ligand, vWF, tumor GPIbα functions as a receptor for vWF in the absence of ristocetin (5). Hence, extrapolating to static flow conditions in the capillary or venule network, it is likely that spontaneous tumor GPIb binding to plasma vWF could initiate the process of tumor-induced platelet aggregation, thrombus formation, and the evolution of a metastatic colony.

Endothelial or megakaryocyte-derived vWF exists in the plasma as a series of multimers with molecular weights ranging up to 15 × 10⁶ (6). The distinctive property of the vWF molecule resides in its ability to regulate adhesive interactions with platelets depending upon its molecular size. Highly polymeric forms of vWF are the most effective in promoting platelet adhesion and aggregation (7). Although vWF is secreted from endothelial cells as an extremely large polymer (8), these molecules are converted in the plasma to a series of multimers (9) by a plasma vWF-cleaving protease (10, 11), which serves to limit their adhesive activity.

In view of the aberrant, metastasis-facilitating properties of tumor GPIbα, we wondered whether plasma vWF also manifested comparable structural anomalies in patients with metastatic carcinoma. Several hemostatic abnormalities result from perturbations in the multimeric organization of plasma vWF, including TTP (12), a microangiopathy characterized by disseminated intravascular platelet aggregation. This syndrome is associated with an increased predominance of high molecular weight vWF multimers resulting from either a deficiency of vWF-cleaving protease activity (13) or an IgG plasma inhibitor directed toward the enzyme (14). Although many of the pathoetiological features of TTP bear striking similarities to those involved in the metastatic cascade, vWF anomalies in cancer patients have yet to be reported, with the exception of elevated FVIII:Ag levels in patients with metastatic versus localized prostate carcinoma (15).

On the basis of these observations, we hypothesized that similar pathological events with respect to adhesive interactions between tumor GPIb and plasma vWF might generate potent stimuli that are conducive to the initiation of the metastatic process. As such, in this study, we examined the quality, quantity, and functional activity of plasma vWF in patients with disseminated neoplasms. Additionally, we used an in vitro model system to investigate the participation of plasma vWF and its adhesive ligand, GPIbα, in tumor-platelet aggregation and thrombus formation, an early event required for the generation of a viable tumor metastasis (16). Prior studies assessing the predictive value of in vitro tumor-induced platelet aggregation have demonstrated its correlation with a tumor’s in vivo metastatic (1, 17, 18) and thrombogenic (19) potential.

MATERIALS AND METHODS

Patient Specimens. Twenty study patients with disseminated tumors and 15 control patients with corresponding, localized, nonmetastatic malignancies were included in this study (Table 1). Study patients were eligible only if they had widely metastatic solid tumors requiring a hematogenous route, as opposed to local spread. Control patients were required to have localized nonmetastatic disease at one site or at contiguous local sites. All eligible patients, based on protocol inclusion and exclusion criteria, were sequentially accrued. Patients...
MEM (Life Technologies, Inc., Grand Island, NY) supplemented with 10% MCF-7 cells were grown routinely as a monolayer in T80 flasks using Eagle’s 

were originally evaluated in a hospital inpatient setting or an outpatient clinic setting. Patients with a known history of coagulopathies, sepsis, or platelet disorders were excluded from accrual. For control patients, blood specimens were obtained prior to their surgeries' resection, and for study patients, blood specimens were obtained prior to any treatment. This protocol was approved by the Institutional Review Board, and all patients signed an informed consent prior to study entry. A second set of 10 control specimens were obtained prior to any treatment. This protocol was approved by the Institutional Review Board, and all patients signed an informed consent prior to study entry. A second set of 10 control specimens 

Table 1 Patient characteristics*  

<table>
<thead>
<tr>
<th>Study patients (no./sex/age)</th>
<th>Diagnosis</th>
<th>Metastatic sites</th>
<th>Control patients (no./sex/age)</th>
<th>Diagnosis</th>
<th>Site of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/71</td>
<td>Prostate carcinoma</td>
<td>Widely disseminated osseous metastases</td>
<td>1/M/75</td>
<td>Prostate carcinoma</td>
<td>Clinically inapparent T1b lesion</td>
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<tr>
<td>2/M/66</td>
<td>Prostate carcinoma</td>
<td>Regional lymph nodes, osseus metastases</td>
<td>2/M/87</td>
<td>Prostate carcinoma</td>
<td>T2a, prostatic carcinoma</td>
</tr>
<tr>
<td>3/M/52</td>
<td>Renal cell carcinoma</td>
<td>Pulmonary metastases, large abdominal soft tissue mass</td>
<td>3/M/55</td>
<td>Renal cell carcinoma</td>
<td>Stage II renal cell carcinoma with 4-cm left renal mass</td>
</tr>
<tr>
<td>4/M/51</td>
<td>Renal cell carcinoma</td>
<td>Disseminated pulmonary nodules, large abdominal soft tissue mass</td>
<td>4/M/49</td>
<td>Renal cell carcinoma</td>
<td>T3b,N1a renal cell carcinoma of the left kidney</td>
</tr>
<tr>
<td>5/M/45</td>
<td>Renal cell carcinoma</td>
<td>Pulmonary metastases, multiple hepatic metastases</td>
<td>5/F/51</td>
<td>Renal cell carcinoma</td>
<td>T2 mixed clear and granular cell carcinoma of the right kidney</td>
</tr>
<tr>
<td>6/M/30</td>
<td>Testicular carcinoma</td>
<td>Retroperitoneal adenopathy, lung metastases</td>
<td>6/M/26</td>
<td>Testicular carcinoma</td>
<td>Stage I seminoma</td>
</tr>
<tr>
<td>7/F/72</td>
<td>Squamous carcinoma of the head and neck</td>
<td>Nasopharyngeal carcinoma with bilateral cervical nodes, peri- esophageal soft tissue mass and pulmonary metastases</td>
<td>7/M/58</td>
<td>Squamous carcinoma of the head and neck</td>
<td>Tumor of the left tonsillar pillar</td>
</tr>
<tr>
<td>8/M/58</td>
<td>Colorectal carcinoma</td>
<td>Large abdominal soft tissue mass, hepatic metastases</td>
<td>8/F/61</td>
<td>Colorectal carcinoma</td>
<td>Partially obstructing mass of the ascending colon (Dukes C2)</td>
</tr>
<tr>
<td>9/M/42</td>
<td>Colorectal carcinoma</td>
<td>5 × 5 cm retroperitoneal mass, hepatic metastases</td>
<td>9/M/66</td>
<td>Colorectal carcinoma</td>
<td>Dukes B2 lesion of the large bowel</td>
</tr>
<tr>
<td>10/M/69</td>
<td>Rectal carcinoma</td>
<td>Right lobe pulmonary metastatic nodules and left adrenal mass</td>
<td>10/F/64</td>
<td>NSCLC</td>
<td>T2 superior sulcus tumor of the right upper lobe</td>
</tr>
<tr>
<td>11/M/70</td>
<td>NSCLCb</td>
<td>Right middle lobe mass and brain metastases</td>
<td>10/F/64</td>
<td>NSCLC</td>
<td>4-cm right middle lobe mass with obstructive pneumonitis extending to the hilar region</td>
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<tr>
<td>12/M/50</td>
<td>NSCLC</td>
<td>Left upper lobe mass, mediastinal, hilar and retroperitoneal adenopathy, and splenic metastases</td>
<td>11/M/76</td>
<td>NSCLC</td>
<td>6-cm gastric mass, with direct extension into the body of the pancreas</td>
</tr>
<tr>
<td>13/M/49</td>
<td>Gastric carcinoma</td>
<td>Hepatic nodules and gastric mass</td>
<td>12/M/85</td>
<td>Gastric carcinoma</td>
<td>6-cm gastric metastases, with direct extension into the body of the pancreas</td>
</tr>
<tr>
<td>14/M/45</td>
<td>Metastatic melanoma</td>
<td>Widely disseminated subcutaneous soft tissue metastases, hepatic and splenic metastases, mediastinal and hilar adenopathy, multiple pulmonary metastases</td>
<td>13/F/52</td>
<td>Metastatic melanoma</td>
<td>Left leg lesion, Clark’s level III</td>
</tr>
<tr>
<td>15/M/58</td>
<td>Metastatic melanoma</td>
<td>Bilateral pulmonary metastases, right axillary nodes, mass at the base of skull</td>
<td>15/F/61</td>
<td>Metastatic melanoma</td>
<td>T3b,N1a invasive ductal carcinoma</td>
</tr>
<tr>
<td>16/M/69</td>
<td>Adenocarcinoma, unknown primary</td>
<td>Liver and lung metastases</td>
<td>16/F/69</td>
<td>Adenocarcinoma, unknown primary</td>
<td>T1b,N1a invasive ductal carcinoma</td>
</tr>
<tr>
<td>17/F/48</td>
<td>Breast carcinoma</td>
<td>Liver, lung, and osseous metastases</td>
<td>17/F/48</td>
<td>Breast carcinoma</td>
<td>T2a,N1b invasive ductal carcinoma</td>
</tr>
<tr>
<td>18/F/55</td>
<td>Breast carcinoma</td>
<td>Pulmonary metastases and malignant pleural effusions</td>
<td>18/F/61</td>
<td>Breast carcinoma</td>
<td>T2b,N1a invasive ductal carcinoma</td>
</tr>
<tr>
<td>19/F/63</td>
<td>Breast carcinoma</td>
<td>Pulmonary metastases, osseous metastases, and brain metastases</td>
<td>19/F/63</td>
<td>Breast carcinoma</td>
<td>T2b,N1a invasive ductal carcinoma</td>
</tr>
<tr>
<td>20/F/80</td>
<td>Breast carcinoma</td>
<td>Bilateral axillary nodes, osseous metastases of the thoracic spine</td>
<td>20/F/80</td>
<td>Breast carcinoma</td>
<td>T2b,N1a invasive ductal carcinoma</td>
</tr>
</tbody>
</table>

* Demographic and clinical characteristics of patients with disseminated metastatic disease (study patients) and those with localized, nonmetastatic solid tumors (control patients).

b NSCLC, non-small cell lung carcinoma.

vWF-CLEAVING PROTEASE IN CANCER PATIENTS were carried out with cells grown at 37°C in a humidified atmosphere of 5% CO2 in air. Cells were harvested with 5 mM EGTA, 5 μM leupeptin, and 0.5 mM phenylmethylsulfonyl fluoride in HBSS (Life Technologies, Inc.), enumerated in a Nageotte counting chamber and adjusted with HBSS to a concentration of −2.5 × 106 cells/ml for aggregation experiments, as described previously (2). Viability, as determined by trypan blue exclusion, was >80%.

Blood obtained from study and control patients was drawn through a 19-gauge needle, into a plastic syringe containing 3.8% trisodium citrate, was centrifuged at room temperature for 20 min at 70°C. Tumor-induced platelet aggregation experiments were performed using optical aggregometry, as outlined in prior reports (2, 3, 5), using a dual-channel optical aggregometer (Chrono-Log model 560Ca).

For aggregation experiments with MoAbs, MCF-7 cells or PRP was preincubated for 30 min at 37°C with the following MoAbs: SZ2 (anti-GP Ibα, mouse IgG1, directed against the vWF binding epitope of the α subunit of GP Ib, Immunotech, Westbrook, ME), P2 (anti-αIIbβ3, mouse IgG1, reacts
with α1dβ1 complex, precluding its activated state, and preventing ligand binding; Immunotech), AMF-7 (anti-vitronectin receptor, mouse IgG1, recognizes the M1, 120,000 α chain of the vitronectin receptor, precluding ligand binding; Immunotech), and/or RRF VIII R1/ (anti-vWF, mouse IgG1x, specific for the GPIb binding epitope of human vWF; Harlan Sera Laboratory, Sussex, United Kingdom). SZ2, P2, and AMF-7 were used at a final concentration of 80 μg/ml, whereas the final concentration of RRF VIII R1 was 24 μg/ml, as described previously (2, 3, 5). For assays requiring an isotype-matched non-specific MoAb, mouse IgG1 (MOPC-21; Sigma Chemical Co., St. Louis, MO) served as a control. These tumor preparations were washed with HBSS, pelleted, resuspended in HBSS, and then added at a final concentration of 2.5 × 10^6 tumor cells/ml to PRP that had previously equilibrated at 37°C in the aggregometer cuvette. The subsequent change in absorbance was registered on the chart recorder. For each specimen, the maximum aggregation were calculated by a computer interfaced with a Chrono-Log integrator.

**vWF Multimer Analysis.** vWF Multimer analysis was performed in SDS-0.8% agarose gels [SeaKem HGT(P); FMC, Rockland, ME] according to Ruggeri and Zimmerman (20). Plasma samples containing 0.0025 units of vWF:Ag were electrophoretically separated. These preparations were subsequently subjected to Western blot analysis using an anti-vWF MoAb (A0082; Dako, Carpenteria, CA) and an alkaline phosphatase labeled γ-chain specific for antihuman IgG (D0478, Dako). Analysis of subunit composition and proteolytic fragments of plasma vWF was performed by electrophoresis on SDS-7% polyacrylamide gels. To quantify the vWF multimers, we scanned the blots with a densitometer (EC Densitometer; EC Apparatus, St Petersburg, FL) attached to a Hewlett Packard Integrator (HP 3396A, Purchase, NY). The proportion of large multimers was determined by the fraction consisting of the intensity of all but the five smallest bands, divided by that of all of the bands, as described previously (21).

**Assays Measuring vWF-cleaving Protease Activity.** The activity of vWF-cleaving protease in patients’ plasma samples was assayed as described previously (10). Briefly, 100 μl of an incubation mixture consisting of dilutions of control or patient’s plasma containing the protease inhibitor, Prefabloc SC (Boehringer, Mannheim, Germany), at a final concentration of 10 mmol/l were added to 50 μl of the purified vWF substrate (American Bioproducts Co., Parsippany, NJ). The resulting solution was transferred onto a hydrophilic filter membrane (VSWP, 25-mm diameter; Millipore, Bedford, MA) floating on the surface of 50 ml of dialysis buffer [1.5 mol/liter urea-5 mmol/liter Tris-HCl (pH 8.0)] in a screw-cap plastic tube and incubated for 24 h in a dry oven at 37°C. The multimeric size distribution of vWF and its proteolytic fragments was assayed by electrophoresis on SDS-7% polyacrylamide gels. To quantify the vWF multimers, we scanned the blots with a densitometer (EC Densitometer; EC Apparatus, St Petersburg, FL) attached to a Hewlett Packard Integrator (HP 3396A, Purchase, NY). The proportion of large multimers was determined by the fraction consisting of the intensity of all but the five smallest bands, divided by that of all of the bands, as described previously (21).

**Screening for Inhibitors.** Protease activity in patient plasma specimens was assayed by measuring vWF cleaving in 1:1 (v/v) mixtures of control plasma from hospital personnel and patients’ test plasma. Additionally, dot blot analysis was performed to explore the possibility of an autoantibody directed against a putative vWF proteolytic cleaving site. Five μl of a 0.5 mg/ml vWF solution were applied onto nitrocellulose and overlaid for 4 h at room temperature with plasma dilutions of 1:500 or 1:50, and bound antibodies were detected with alkaline phosphate-labeled goat antihuman IgG. The result was expressed as a function of the mean size of multimers (defined as the distance between the peak of the tracing and the lowest molecular weight band) from scans of the autoradiographs.

**Statistical Analysis.** The Student’s t test (P) was used for comparisons of vWF parameters and aggregation measurements in study versus control patient samples. The Mann-Whitney and Fisher’s exact tests were used to determine the significance of the association between the clinical presence of metastases and the semi-quantitative t-dimer values (P) and between metastases and the level of vWF cleaving activity (P), respectively. All values are expressed as mean ± SD, unless otherwise indicated.

**RESULTS**

Table 1 lists clinical characteristics of 20 patients with widely disseminated solid tumors and 15 corresponding control patients with localized malignancies. Although about one-quarter of these patients manifested mild thrombocytopenia (platelet count, <150,000 cells/μl) as well as a modest prolongation in their partial thromboplastin or prothrombin times, these abnormalities were not statistically significant. A significant increase, however, was observed in their mean D-dimer levels (P < 0.023) in comparison to control patients. The study patients demonstrated no abnormalities in their fibrinogen levels relative to controls. Fig. 1 displays the study patients’ mean levels of FVIII:C activity, vWF FVIII-related antigen activity (vWF:Ag), and ristocetin cofactor activity (vWF:RCoF) in plasma samples from study and control patients. No significant differences were observed in the vWF profile of control patients versus the control hospital personnel. n = 20 for study patients; n = 15 for control patients.

![Fig. 1. A comparison of FVIII:C activity, vWF:Ag concentration, and ristocetin cofactor activity (vWF:RCoF) in plasma samples from study and control patients.](image-url)
confirmed that the disappearance of the large multimers results from proteolytic degradation of the vWF substrate rather than cleavage of the disulfide bridges linking the protomeric subunits of vWF.

In screening tests designed to explore the possibility of an autoantibody directed against the cleaving protease or the vWF molecule, study patient’s plasma was mixed with an equal volume of normal human plasma and subjected to the vWF protease cleaving assay. As Fig. 4C illustrates, the pattern of digested vWF in this mixture is comparable to that observed with diluted control plasma and is consistent with deficient activity of the vWF-cleaving enzyme opposed to a plasma inhibitor. A dot blot analysis also aimed at detecting the presence of possible autoantibodies, demonstrated no binding of any such antibodies to immobilized vWF (data not shown).

To investigate the adhesive interactions between tumor cells and plasma vWF, we carried out tumor-induced platelet aggregation as
Fig. 5. SDS-PAGE/Western blot analysis of vWF-cleaving protease activity in study and control patient plasma samples. Incubation mixtures, consisting of plasma specimens and purified vWF, were subjected to SDS-PAGE under reduced conditions, followed by immunostaining. Plasma specimens from study patients: Lanes A–C, metastatic breast carcinoma, patient 18 (A); metastatic colorectal carcinoma, patient 8 (B); and metastatic renal cell carcinoma, patient 4 (C). Plasma specimens from control patients: Lanes D–F, localized breast carcinoma, patient 14 (D); localized colorectal carcinoma, patient 9 (E); and localized renal cell carcinoma, patient 4 (F). Plasma specimens from control hospital personnel: Lanes G and H. Note the enhanced quantities of immunoreactive fragments at Mr 170,000 and 140,000 resulting from proteolysis of the vWF monomeric subunit (Mr 170,000) and those observed in study patient specimens.

DISCUSSION

Significantly elevated levels of highly polymeric forms of vWF were observed in patients with metastatic disease but not in control patients with localized tumors. Furthermore, deficient plasma activity of the vWF-cleaving protease was detected in patients with metastatic tumor, and results from mixing experiments and the dot blot analysis support either a deficiency or a functional aberration of this enzyme, as opposed to a plasma inhibitor. Finally, adhesive interactions between these highly polymeric vWF molecules and tumor cells occurred via their platelet immuno-related GPIbα and αmβ3 receptors. A recent study establishing platelet GPIbα and αmβ3 receptors as receptors for these unusually large vWF multimers (22) lends support to the observations reported herein. Also supportive are the findings by Grossi et al. (23, 24), who have identified immunorelated GPIbα and αmβ3 receptors on a variety of cultured tumor cell lines. When these tumor receptors were simultaneously blocked with MoAbs, tumor-induced platelet aggregation was completely inhibited.

Although the vitronectin receptor did not appear to participate in MCF-7 tumor-induced platelet aggregation, it is possible that a heretofore undescribed vWF receptor expressed by tumor cells may addi-
functionally augment primary GPIb-mediated vWF adhesive attachments. Hence, extrapolating from our in vitro model, hemostatically active, highly polymeric vWF molecules, under static conditions, may function as a potent nidus for adhesive interactions with both platelets and circulating tumor cells, resulting in tumor-induced platelet aggregation, thrombus formation, and the presumptive development of a metastatic colony (16-19). Also, constitutive expression of both GPIbα and αvβ3 by circulating tumor cells could result in their interaction with other plasma proteins, triggering platelet aggregation and tumor-induced thrombus formation. Therefore, the abnormal function of these receptors could also facilitate the hemostatic spread of tumors.

vWF is secreted from endothelial cells by either a constitutive or a regulated pathway (25). The most biologically potent molecules are released from endothelial Weibel-Palade bodies in response to thrombin, vasoactive amines, purine nucleotides, and a variety of cytokines (26). Because enhanced quantities of platelet-tumor aggregates were generated using study patient PRP in our *in vitro* assays, these thrombi could conceivably constitute potent sources of vWF secretagogues (27), sequentially catalyzing the release of high molecular weight vWF multimers from stimulated endothelium. Notwithstanding, our findings reveal deficient activity of the vWF-cleaving enzyme, which regulates the size and, hence, the adhesive activity of plasma vWF. The extent to which tumor-platelet aggregates themselves might contribute to augmented secretion of highly polymeric vWF is unclear and will require further study using different approaches. It is clear, however, that increased quantities of highly polymeric vWF were associated with enhanced *in vitro* generation of tumor-platelet aggregates, as well as being highly correlated with the clinical presence of metastatic disease in patients bearing malignancies.

In a subset of patients, disseminated carcinoma is believed to function as the chief stimulus for TTP, generating a “carcinoma-associated microangiopathy” that is characterized by widespread endothelial-adherent platelet-tumor emboli occluding the microcirculation (28). Although most of these patients display mucinous adenocarcinomas, recent studies have also linked almost all other common tumors with this TTP-related process (29, 30), with unusually large vWF multimers detected in the few cancer patients examined (30). Curiously, a similar array of hemostatic abnormalities are observed in both patients with disseminated metastases and the carcinoma-associated microangiopathies. Previously recognized as a hypercoagulable state and attributed to an acute phase reaction in patients with disseminated metastases, these hemostatic abnormalities characteristically consist of elevated fibrin degradation products (Ref. 31 and this study), platelet activation (32), coagulopathies (33), and increased thrombotic tendencies (34). Hence, it seems reasonable to speculate that similar pathoetiologic stimuli may contribute to a common mechanistic process both in the TTP subgroup of carcinoma-associated microangiopathies and in the clinical setting of widely disseminated tumor.

Although the mechanism whereby the reduced functional activity of the vWF-cleaving enzyme developed in these cancer patients is speculative, several recent observations may provide clues to its etiopathogenesis. A frequent event accompanying cellular neoplastic transformation involves altered expression of extracellular proteins, which could directly result in perturbations in vWF-cleaving activity. Various oncogenes have been shown to regulate the expression of proteins such as the matrix-degrading metalloproteinases and plasminogen activators, consistent with the transforming and metastatic response induced by oncogenes (35). Alternately, protein phosphorylation, modulated by a network of oncogene-mediated kinases, could directly or indirectly impact on the functional properties of the vWF-cleaving enzyme (36). Conceivably, the activity of the enzyme itself or associated regulatory molecules, cofactors, or docking-type receptors could be altered by phosphorylation, either positively or negatively (37). Further studies will be required to identify the process through which the vWF-cleaving enzyme is reduced or impaired in patients with metastatic tumors.

In conclusion, high concentrations of vWF antigen, organized in unusually large multimers, were observed in plasma samples from patients with disseminated neoplasms. The functional activity of this aberrant vWF was significantly enhanced, as demonstrated by ristocetin cofactor and tumor-induced platelet aggregation assays. Tumor-induced platelet aggregation and subsequent thrombus formation could be completely inhibited by simultaneously blocking the vWF epitope of tumor GPIbα and the GPIbα epitope of plasma vWF. These unusually large vWF multimers appear to result from either a deficiency or a functional aberration of the vWF-cleaving protease, similar to the pathogenesis of TTP and other microangiopathies. Not only do these laboratory findings have important implications with regard to the formation of tumor metastases, but they also have significance with regard to their prognostic utility. Elevated levels of highly polymeric vWF may constitute a novel biomarker indicative of the presence or risk of metastatic disease.

**REFERENCES**


**Table 2** Mixing studies for the detection of possible plasma inhibitors: tumor-induced platelet aggregation

<table>
<thead>
<tr>
<th>Assay</th>
<th>Platelet concentration (cells/μl)</th>
<th>Dilution</th>
<th>Maximum aggregation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP+ + PPP</td>
<td>600,000</td>
<td>1:1 (autologous plasma)</td>
<td>2</td>
</tr>
<tr>
<td>PRP+ + PPP</td>
<td>600,000</td>
<td>1:1 (control plasma)</td>
<td>31</td>
</tr>
<tr>
<td>PRP+ + PPP</td>
<td>400,000</td>
<td>3:1 (autologous plasma)</td>
<td>1</td>
</tr>
<tr>
<td>PRP+ + PPP</td>
<td>400,000</td>
<td>3:1 (control plasma)</td>
<td>1</td>
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<tr>
<td>PRP+ + PPP</td>
<td>750,000</td>
<td>2:3 (autologous plasma)</td>
<td>1</td>
</tr>
<tr>
<td>PRP+ + PPP</td>
<td>750,000</td>
<td>2:3 (control plasma)</td>
<td>1</td>
</tr>
</tbody>
</table>

*a* Four study patients with platelet counts ≥300,000 cells/μl were candidates for mixing studies. Study patients’ PRP was diluted with either autologous or control PPP to yield a final platelet concentration of 300,000 cells/μl for tumor-induced aggregation assays. n, no. of patients. All assays were performed in duplicate. PRP, study patient PRP. PPP, study patient PPP; PPP+, control patient PPP. Note that the reductions in tumor-induced maximum aggregation were proportional to the dilution when control plasma was used as the diluent.


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