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• *BRIEF REPORTS* •

Plasma von Willebrand factor level as a prognostic indicator of patients with metastatic colorectal carcinoma

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Abstract

AIM: To evaluate the correlations of plasma von Willebrand factor (vWF) level with the distant metastasis and prognosis of patients with colorectal cancer.

METHODS: A total of 86 patients with histologically confirmed metastatic colorectal cancers receiving treatment at Taipei Veterans General Hospital were enrolled. All patients had measurable metastatic lesions and life expectancies of more than 3 mo. Plasma vWF levels were measured by immuno-turbidimetric assay and compared with results from 40 non-metastatic colorectal cancer patients and 22 healthy controls. Patients with metastatic colorectal cancer were divided into two groups according to serum vWF levels and the differences between these two groups were analyzed using χ^2 test. Data on age, gender, performance status, location of primary tumor, extent of metastasis, site of metastases, histological differentiation, serum CEA and plasma vWF levels were analyzed to determine association with survival. Survival curves were constructed by Kaplan-Meier product limit method and the data was analyzed using log-rank test on a microcomputer. Multivariate analysis using the Cox's proportional hazards regression model was then performed to determine the independent prognostic indicators among all of the possible variables.

RESULTS: Colorectal cancer patients were identified as having significantly higher plasma vWF concentrations than healthy controls ($P<0.05$). Moreover, higher vWF plasma levels were associated with advanced tumor stage ($P<0.05$) and the presence of multiple metastases ($P = 0.014$). Patients with lower vWF plasma levels ($\leqslant 160\%$) survived significantly longer than those with a higher plasma vWF level (log-rank test, $P = 0.0043$). By multivariate analysis, plasma vWF levels ($P<0.001$), the extent of metastasis ($P = 0.012$), and the performance status ($P = 0.014$) were identified as independent prognostic factors.

CONCLUSION: Our data indicates that high plasma vWF concentrations correlate with advanced diseases and significantly poor prognosis of patients with metastatic colorectal carcinoma. It may serve as a potential biological marker of disease progression in these patients.

Key words: von Willebrand factor; colorectal carcinoma; prognosis

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INTRODUCTION

Recently, colorectal cancer has become one of the leading causes of cancer-related mortality in Taiwan and the incidence of colorectal cancer in Taiwan has increased over the past few decades. One possible reason is the introduction of Western foods to the local diets[1]. Blood-borne metastasis is the major cause of death from colorectal carcinoma[2]. The development of metastasis is a stepwise process that starts when cancer cells separate from a primary tumor, migrate across blood vessel walls into the bloodstream, and disperse throughout the body to generate new colonies. During the transit into the circulating system, tumor cells are exposed to fluid mechanical forces, plasma proteins, and vascular cells such as platelets. All of which may affect their survival and extravasations from the vasculature[3,4].

Von Willebrand factor (vWF) is a glycoprotein that synthesized mainly in endothelial cells and in megakaryocites[5]. It mediates the adherence of platelets to sub-endothelial matrices during vascular-endothelial damage and acts as a carrier protein for coagulation factor VIII[6]. Increased plasma vWF concentrations have been reported in various clinical conditions such as diabetes mellitus[7], myocardial infarction[8], liver diseases[9], connective tissue diseases[10], and acute infections, probably as a result of increased endothelial cell proliferation or as a part of the acute-phase reaction in response to vascular damage[11]. High plasma vWF concentrations have been reported in patients with various

types of cancer, such as prostate cancer[12], cervical and ovarian carcinoma[13], head and neck cancer[14,15], and colorectal cancer[16]. Moreover, high plasma vWF concentrations often correlate with advanced tumor staging and may have prognostic significance in these patients[12-16].

vWF plays a very important role in the pathogenesis of metastasis, by promoting the binding of tumor cells to platelets, and subsequently, to vascular subendothelium[17-20]. This interaction forms heterotypic cellular emboli, which are not easily recognized by the immune system and have more chance of attaching to the endothelial surfaces than single tumor cells[17-20]. Since blood-borne metastasis is the major cause of death from colorectal carcinoma and vWF is related to the process of metastatic dissemination of malignant cells, to evaluate its potential as a marker of tumor metastasis is warranted. In the current study we investigate the correlation of plasma vWF levels with the distant metastasis of colorectal cancer patients; furthermore, we determine the prognostic significance of plasma vWF levels in these patients.

MATERIALS AND METHODS

Patients

A total of 86 patients with histologically confirmed metastatic colorectal carcinoma receiving treatment at Taipei Veterans General Hospital were enrolled between 2002 and 2003. All patients had measurable metastatic lesions and life expectancies of more than 3 mo. They all received 5-fluorouracil (5-FU)-based systemic chemotherapy and the treatments were continued until disease progression or intolerable toxicity appeared. Salvage chemotherapy with CPT-11, oxaliplatin or capecitabine was allowed. Data on age, gender, performance status, location of primary tumor, extent of metastases, site of metastases, histological differentiation, serum CEA level, and plasma vWF levels was analyzed to determine the association with survival. The characteristics of these patients are shown in Table 1.

Methods

Plasma vWF concentrations were measured using immuno-turbidimetric assay according to manufacturer's instruction (DIAGNOSTICA STAGO, France). Briefly, this assay is based on the change in turbidity of a microparticle suspension that is measured by photometry. A suspension of latex microparticles, coated by covalent bonding with antibodies specific for vWF, is mixed with the test plasma whose vWF antigen level is to be assayed. An antigen-antibody reaction leads to an agglutination of the latex microparticles which induces an increase in turbidity of the reaction medium. The increase in turbidity is reflected by an increase in absorbance which can be measured photometrically. The cut-off level of vWF was set at 160%. The data was compared with results from 40 non-metastatic colorectal cancer patients and 22 healthy controls.

Patients with metastatic colorectal cancer were divided into two groups according to plasma vWF levels. Stratification and the differences between these two groups were analyzed using the χ^2 test[21]. Survival curves were constructed by the Kaplan-Meier product limit method and the data was analyzed by log-rank test on a microcomputer[22]. Multivariate analysis using the Cox's proportional hazards regression model was then performed to determine the independent prognostic indicators among all of the possible variables[23]. All statistical analyses were carried out with SPSS statistical software package.

RESULTS

High plasma vWF level is associated with advanced stage of colorectal carcinoma

The mean plasma level of vWF was 241.3±68.2% in patients with colorectal cancer and 110.1±27.0% in healthy controls ($P<0.001$). vWF measurements according to Dukes' stage are presented in Figure 1. In patients with tumors invading regional lymph nodes (Dukes' C, $n = 26$), the mean plasma vWF level was similar to those without regional lymph node invasion (Dukes' B, $n = 14$) (211.7±40.0% *vs* 207.9±36.0%). However, the plasma vWF level was 266.1±91.3% in patients with metastatic diseases (Dukes' D, $n = 86$), which was significantly higher than those without distant metastasis (Dukes' B or C, $P = 0.001$). As shown in Table 1, there was no significant correlation between plasma vWF concentrations and age, gender, performance status, location of tumor, histological grading, or serum carcinoembryonic antigen level. However, higher vWF plasma levels were correlated with multiple metastatic sites ($P = 0.014$) and, to some extent, trend of liver metastasis ($P = 0.110$).

Table 1 Patients' characteristics according to different plasma vWF levels

Characteristics	vWF>160%	vWF≤160%	*P*
n	53	33	
Age (yr)			0.986
<50	23	14	
50–70	18	11	
>70	12	8	
Gender			0.496
Male	36	20	
Female	17	13	
Performance status			0.656
0	32	18	
1. 2	21	15	
Primary tumor			0.825
Colon	28	16	
Rectum	25	17	
Extent of metastases			0.014
One site	20	22	
Multiple sites	33	11	
Site of metastases			0.110
Liver	37	17	
Lung	34	20	
Lymph nodes	6	3	
Peritoneum	8	5	
Others	3	1	
Histology			0.558
Well-differentiated	8	5	
Moderately-differentiated	25	19	
Poorly-differentiated	13	4	
Unknown	7	5	
Serum CEA level			0.646
≤20ng/mL	19	10	
≤20ng/mL	34	23	

CEA: Carcinoembryonic antigen; vWF: Von Willebrand factor.

High plasma vWF level is associated with poor prognosis of patients with metastatic colorectal carcinoma

As shown in Table 2, by univariate analysis the plasma vWF levels ($P<0.001$), performance status ($P = 0.022$), and extent of metastasis ($P = 0.028$) were identified as survival predicting factors, while age, gender, location of primary tumor, site of metastasis, histological differentiation, and pre-treatment serum CEA levels were not statistically significant. By multivariate analysis, plasma vWF levels ($P<0.001$), performance status ($P = 0.014$), and extent of metastasis ($P = 0.012$) were identified as independent prognostic factors. As shown in Figure 2, the survival curves plotted by Kaplan-Meier method and analyzed by log-rank test revealed that patients with plasma vWF level of less than 160% survived significantly longer than those with plasma vWF of more than 160% ($P = 0.0043$).

DISCUSSION

Metastasis is a multi-step process involved in the alterations of cell-cell adhesion, angiogenesis, degradation of extracellular matrix, escape of immune surveillance, and cell-matrix adhesion[24]. Cell-matrix adhesive interaction plays an important role in the normal organization and stabilization of the cell layer in epithelial tissue. However, in tumor cells the adhesive interaction for these cells and the subendothelial matrices is essential for their metastasis, and molecules that mediate this adhesive process may facilitate tumor cells to metastasize. vWF appears to play an important role in this process, and higher plasma vWF levels have indeed been reported in various types of cancer[12-16]. Evidence has shown that this phenomenon is related to the accelerated endothelial synthesis associated with tumor-dependent angiogenesis[3,4]. In addition, the release of thrombin by tumor cells may induce vWF production in endothelial cells and enhance the adhesion of tumor cells[25,26]. A deficiency of vWF-cleaving activity by its protease control system has recently been identified in colorectal cancer patients, and this deficiency was associated with the progression of the disease[27]. Moreover, a high vWF-positive microvessel number was also identified as an unfavorable prognostic marker for patients with stage-II and stage-III colorectal cancer[28].

The binding of vWF to several types of collagen may contribute to the attachment of platelets to the extracellular matrices of subendothelium; furthermore, a direct interaction between vWF and neoplastic cells has been demonstrated[29]. The expression of surface glycoprotein GpIb and GpIIb-IIIa complex, the adhesive ligands for vWF, has been reported in tumor cells[25,26], which may contribute to the metastatic process by promoting the binding of tumor cells to platelets via plasma vWF. Moreover, such interaction results in heterotypic cell aggregates, which are not easily recognized by the immune system and are more capable of producing adherence to endothelial surface than single tumor cells[25,26,29]. Interestingly, experiments on animals have shown that anti-platelet and anti-vWF antibodies can substantially reduce the occurrence and number of metastasis[25-27,29], indicate that plasma vWF indeed plays a crucial role in the process of metastasis of tumor cells.

A positive correlation between Dukes' stage and plasma vWF concentrations in colorectal cancer patients was recently reported[16]. Our study demonstrated a similar result that plasma vWF levels in colorectal cancer patients were significantly higher than in healthy controls, and the highest vWF plasma levels were observed in patients with metastatic diseases (Figure 1). Moreover, a higher vWF plasma level was shown to be associated with multiple metastases ($P = 0.014$) in our study (Table 1). Elevations in vWF plasma levels of patients with disseminated diseases reflect the enhancement

Table 2 Analysis of prognostic factors on survival

Variables	*n*	Univariate(*P*)	Multivariate(*P*)
Age (yr)			
<50	37	0.528	–
⩾50	49		
Gender			
Male	56	0.986	–
Female	30		
Performance status			
0	50	0.022	0.014
1. 2	36		
Site of primary tumor			
Colon	44	0.430	–
Rectum	42		
Extent of metastases			
One site	42	0.028	0.012
Multiple sites	44		
Liver metastases			
Present	54	0.658	–
Absent	32		
Lung metastases			
Present	54	0.443	–
Absent	32		
Histological differentiation			
Well/moderate	57	0.718	–
Poor/unknown	29		
Pre-treatment CEA			
⩽6 ng/mL	29	0.213	–
>6 ng/mL	57		
Pre-treatment vWF			
⩽160%	33	<0.001	<0.001
>160%	53		

CEA, carcinoembryonic antigen; vWF: von Willebrand factor; –: no significance.

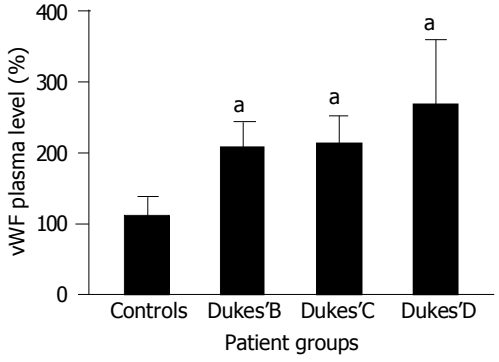

Figure 1 Plasma vWF levels according to Dukes' stages of colorectal cancer. Elevated plasma vWF levels were observed in colorectal cancer patients in a stage-dependent manner. (Data are means±SD. $^aP<0.05$ *vs* the control group).

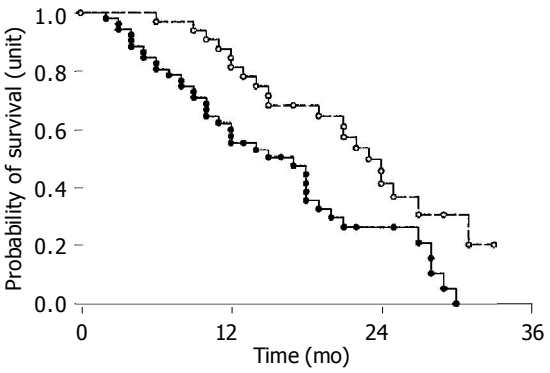

Figure 2 Survival curves according to plasma vWF levels. -○-, vWF ≤160% (n=33); -●-, vWF>160% (n = 53). Patients with plasma vWF levels of less than 160% survived significantly longer than those with vWF of more than 160% (P = 0.0043; log-rank test).

of angiogenic activity to sustain a larger tumor cell burden and the metastatic progression[16]. Furthermore, the metastatic status of these patients may represent an effect of the adhesive property of vWF, which seems to play a crucial role during the course of hematological spread[16].

Recently, a high vWF-positive microvessel number was identified as an independent prognostic marker of patients with stage-II/III colorectal carcinoma[28]. However, the relationship between plasma vWF levels and the survival of patients with metastatic colorectal cancer remains unclear. Since high plasma vWF concentrations were associated with advanced stage and multiple metastasis of colorectal cancer patients in our study, its impact on survival is of interest. In the current study, by log-rank test, patients with lower vWF plasma levels (≤160%) survived significantly longer than those with higher plasma vWF levels (Figure 2). By multivariate analysis, plasma vWF level (P<0.001) was also identified as an independent prognostic factor (Table 2).

Serum carcinoembryonic antigen (CEA) level is the most widely used marker for both prognostic predicting and post-treatment monitoring of patients with colorectal cancer[30,31]. Furthermore, serum CEA levels could also be used in the monitoring of response to systemic chemotherapy in patients with metastatic colorectal carcinoma[32]. In addition to CEA, CA19-9 is another useful serum marker for predicting the prognosis of metastatic colorectal cancer patients[33]. However, in spite of higher plasma vWF levels correlating with both advanced stage and poor prognosis of colorectal patients in our study, a higher plasma vWF level was not identified to be associated with higher serum CEA level (Table 1).

In summary, our data indicates that plasma vWF levels are elevated in colorectal cancer patients in a stage-dependent manner, and a high plasma vWF level correlates with significantly poor prognosis of patients with metastatic diseases. Plasma vWF level may serve as a potential biological marker of disease progression in colorectal cancer patients.

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