Serum Concentrations of Vascular Endothelial Growth Factor in Vulvar Cancer

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ABSTRACT

The aim of the present study was to evaluate serum concentrations of vascular endothelial growth factor (VEGF) in patients with vulvar cancer and healthy female controls with respect to correlation of VEGF with clinicopathological parameters and impact on the patients’ prognosis. Serum concentrations of VEGF were measured using a commercially available ELISA. Results were correlated to clinical data. Median serum concentrations of VEGF in patients with vulvar cancer (n = 41) and healthy female controls (n = 130) were 260 (range, 33–1216) pg/ml and 216 (range, 0–777) pg/ml, respectively (Mann-Whitney U test, P = 0.048). Serum concentrations of VEGF significantly correlated with tumor stage (Mann-Whitney U test, P = 0.02) but not with histological grade (Mann-Whitney U test, P = 0.2). In a univariate analysis, elevated pretreatment serum concentrations of VEGF were significantly correlated with a shortened disease-free and overall survival (Wilcoxon test, P = 0.03; and Wilcoxon test, P = 0.04, respectively). A multivariate Cox regression model considering tumor stage and serum concentrations of VEGF revealed, however, that serum concentrations of VEGF did not confer additional prognostic information to that already obtained by the established prognosticator tumor stage (multivariate Cox regression model; P = 0.9 and P = 0.8, respectively). Our data indicate that angiogenesis, as reflected by serum concentrations of VEGF, plays a functional role in vulvar carcinogenesis. VEGF seems to be a mediator of vulvar tumor growth but not of tumor cell dedifferentiation. Although associated with impaired disease-free and overall survival, pretreatment serum concentrations of VEGF are not an independent predictor of outcome in patients with vulvar cancer.

INTRODUCTION

Angiogenesis, the formation of new blood vessels, is considered essential for wound healing, placental growth, and the female reproductive system (1). Furthermore, angiogenesis has been established as a basic feature of tumor development, growth, and spread beyond regional borders (2). Supporting that hypothesis, various angiogenic molecules, e.g., VEGF, have been shown to parallel selective steps of tumor growth and the development of metastases (3, 4).

VEGF, which is also known as vascular permeability factor, stimulates angiogenesis by increasing vascular permeability and by acting as an endothelial cell mitogen (5, 6). VEGF is a dimeric glycoprotein with four spliced variants containing of 121, 165, 189, and 206 amino acid residues, expressing almost identical biological activities by binding to specific class III receptor tyrosine kinases, i.e., flt-1 and KDR (7, 8).

VEGF expression has been investigated in various human malignancies, e.g., esophageal, gastric, colorectal, hepatocellular, pancreatic, lung, breast, endometrial, cervical, and ovarian cancer (9–18). Regarding vulvar lesions, it has been suggested that VEGF might be involved in promoting a new vascular network as a basic condition for the progression of vulvar intraepithelial neoplasia, indicating a possible role of VEGF in the progression of premalignant vulvar lesions into invasive vulvar cancer (19, 20). Furthermore, immunohistochemically detected VEGF overexpression has been demonstrated to be associated with poor survival of patients with vulvar cancer (21).

The aim of the present study was to evaluate serum concentrations of VEGF in patients with vulvar cancer and healthy female controls. Furthermore, we correlated serum concentrations of VEGF with clinicopathological parameters and evaluated the prognostic value of pretreatment serum concentrations of VEGF regarding disease-free and overall survival.

MATERIALS AND METHODS

Patients. Clinical data were obtained from files at the University Hospital of Vienna, Department of Gynecology and Obstetrics. Forty-one consecutive patients with squamous cell vulvar cancer treated at our department were included in the study. Vulvar cancer FIGO Stages I, II, and III were seen in 18, 16, and 7 cases, respectively. Histologically, 17 tumors were graded as well differentiated and 24 tumors as moderately differentiated. Median age at the time of diagnosis of vulvar

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¹The abbreviations used are: VEGF, vascular endothelial growth factor; FIGO, International Federation of Gynecology and Obstetrics.

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cancer was 75 (range, 42–92) years. The median duration of follow-up was 46 (range, 0.5–70) months. Eighteen patients (44%) developed recurrent disease after primary therapy with a median time to recurrence of 35 months; nine patients (22%) died of cancer-related death. For all patients, five-year disease-free and overall survival was 49 and 68%, respectively. Additionally, serum concentrations of VEGF were evaluated in a panel of 130 healthy female controls.

**Clinical Management.** Diagnosis of vulvar cancer was established preoperatively by punch biopsy. Treatment of patients with vulvar cancer was performed according to Kucera and Weghaupt (22). Patients with a depth of invasion of more than 1 mm and clinically negative lymph nodes underwent adjuvant postoperative groin irradiation. In cases with a strictly lateral location of the tumor (confined to the labium major), only the unilateral groin was irradiated. Groin lymph node dissection was performed in cases with clinically suspicious groin lymph nodes. In cases of positive lymph nodes, postoperative irradiation was applied. Histological staging was performed according to the current International Union Against Cancer classification and clinical staging according to FIGO classification. For the aim of this study, an experienced pathologist, blinded to the clinical data, reviewed all histological specimens.

**Serum Assay.** Blood samples were obtained 24–48 h before surgery by peripheral venous puncture and immediately centrifuged at 3000 × g for 15 min. The serum was frozen at −80°C until examination. For the measurement of serum VEGF, a commercially available ELISA was used (Quantikine Human VEGF Immunoassay; R&D Systems, Minneapolis, MN). All serum VEGF analyses were performed at the same time and in the same batch. The intraassay variability was 5.1% at a VEGF concentration of 512 pg/ml. Immunoassays were performed as described previously (15). According to the manufacturer, the assay measures the isoform VEGF165, containing 165 amino acid residues. The assay recognizes both natural human VEGF and recombinant VEGF and does not exhibit cross-reactivity with a series of cytokines and growth factors. The manufacturer claims a sensitivity of <5.0 pg/ml.

**Statistical Analysis.** Because of the skewed distribution of serum concentrations of VEGF, median and range of serum concentrations of VEGF are given. Comparisons between unpaired groups were made using the Mann-Whitney U test. Survival probabilities were calculated by the product limit method of Kaplan and Meier. Differences between groups were tested using the Wilcoxon test. The results were analyzed for the end point of disease-free and overall survival. Because groin lymph node dissection was not performed on a regular basis, lymph node involvement was not considered as an end point of statistical analysis. Survival times of patients still alive or disease-free were censored with the last follow-up date. Because of the low number of recurrences and to prevent an overfit of the Cox model, the number of covariates included into the Cox model was restricted to tumor stage and serum concentrations of VEGF. The correlation between serum concentrations of VEGF and platelet count was assessed by Kendall’s τ-b correlation coefficient. Ps of <0.05 were considered statistically significant. We used the SAS statistical software system (SAS Institute Inc., Cary, NC) to do the calculations.

**RESULTS**

**Serum Concentrations of VEGF and Platelet Counts in Patients with Vulvar Cancer and Healthy Female Controls.** Median serum concentrations of VEGF in patients with vulvar cancer prior to therapy (n = 41) and of healthy female controls (n = 130) were 260 (range, 33–1216) pg/ml and 216 (range, 0–777) pg/ml, respectively. Serum concentrations of VEGF were significantly elevated in patients with vulvar cancer compared with healthy female controls (Mann-Whitney U test, P = 0.048). Median platelet count in patients with vulvar cancer prior to therapy was 264,000 (range, 129,000–778,000)/μl. We found a significant correlation between platelet count and serum concentrations of VEGF (Kendall’s τ-b correlation coefficient; r = 0.34, P = 0.005).

**Correlation of Serum Concentrations of VEGF with Tumor Stage and Histological Grade in Patients with Vulvar Cancer.** When serum concentrations of VEGF were grouped by tumor stage and histological grade, we found a significant correlation between serum concentrations of VEGF and tumor stage (251 pg/ml versus 560 pg/ml for FIGO stages I and II versus III; Mann-Whitney U test, P = 0.006), whereas no correlation was found between serum concentrations of VEGF and histological grade (Mann-Whitney U test, P = 0.2).

**Correlation of Serum Concentrations of VEGF with Disease-free and Overall Survival of Patients with Vulvar Cancer.** Serum concentrations of VEGF lack a clearly defined cutoff level. Because of the skewed distribution of serum concentrations of VEGF, the 75th quantile of serum concentrations (445 pg/ml) of VEGF in the panel of patients with vulvar cancer was chosen as the cutoff level. In a univariate analysis, tumor stage and serum concentrations of VEGF, but not histological grade, were significantly associated with disease-free and overall survival (Table 1). In a multivariate Cox regression model, serum concentrations of VEGF did not correlate with a shortened disease-free and overall survival (multivariate Cox regression, P = 0.9; and Wilcoxon test, P = 0.8, respectively; Table 1).

**DISCUSSION**

In this study, we found serum concentrations of VEGF to be markedly elevated in patients with vulvar cancer compared with healthy female controls. Furthermore, serum concentrations VEGF were shown to be associated with tumor stage but not with tumor dedifferentiation. Although associated with a shorter disease-free and overall survival in a univariate analysis, VEGF did not provide additional prognostic information when considering tumor stage and serum concentrations of VEGF simultaneously in a multivariate model.

The role of angiogenesis in vulvar cancer has been discussed controversially. Some authors have shown that immunohistochemically detected VEGF overexpression was associated with a poor survival of patients with vulvar cancer (21, 23). However, other authors have found that tumor angiogenesis in vulvar cancer does not correlate positively with stage, survival, or pattern of invasion and cannot be used as a prognostic
have been described, it has to be stated that other possible sources of serum VEGF are due to tumor cell production, caution. Although it is reasonable to speculate that elevated serum concentrations of VEGF have to be interpreted with promoter of malignant growth. It has to be noted, however, that serum concentrations of VEGF have to be interpreted with caution. Although it is reasonable to speculate that elevated serum concentrations of VEGF are due to tumor cell production, it has to be stated that other possible sources of serum VEGF have been described, e.g., platelets during platelet aggregation, activated human neutrophils, T lymphocytes, and blood mononuclear cells (25–29).

It has been shown that serum concentrations of VEGF correlate with platelet counts in various human malignancies (25). Furthermore, serum concentrations of VEGF have been demonstrated to be elevated compared with plasma concentrations, indicating VEGF release during blood coagulation (26). Our results are in accordance with these data showing that serum concentrations of VEGF of patients with vulvar cancer prior to therapy were significantly associated with platelet counts. No data are available to date on plasma concentrations of VEGF in patients with vulvar cancer. This should be investigated in further studies involving serum and plasma analyses and a large number of patients, allowing for multivariate analysis of both variables.

Serum concentrations of VEGF have been investigated in numerous human malignancies, including melanoma, non-Hodgkin’s lymphoma, brain, renal, colorectal, small cell lung, and ovarian cancer (11, 14, 30–34). As to the prognostic value of serum VEGF, it has been shown that elevated serum concentrations of VEGF are correlated with poor prognosis in non-Hodgkin’s lymphoma and colorectal, ovarian, and small cell lung cancer (14, 18, 31, 35). To the our knowledge, no data have been reported on the prognostic value of serum concentrations of VEGF in patients with vulvar cancer. In our series, patients with vulvar cancer with elevated serum concentrations of VEGF had a significantly shorter disease-free and overall survival. Our results are in accordance with data published previously reporting immunohistochemical evidence of a correlation between VEGF overexpression and adverse outcome in patients with vulvar cancer (21). However, in the multivariate analysis including tumor stage and serum concentration of VEGF, only tumor stage was found to be an independent prognosticator.

The correlation between serum concentrations of VEGF and clinico-pathological parameters has been discussed controversially in various human malignancies. In our study, we found that serum concentrations of VEGF correlate significantly correlate with tumor stage, whereas no significant correlation was seen between serum concentrations of VEGF and tumor grade. Thus, our results indicate that serum concentrations of VEGF in patients with vulvar cancer are indicative of the tumor bulk rather than the differentiation of tumor cells.

It is a shortcoming of this study that the correlation between serum concentrations of VEGF and metastatic spread to the groin lymph nodes was not evaluated. Therefore, it cannot be ruled out that micrometastases may have been present in patients included in the study. This has to be taken into account when interpreting the results of this study. Furthermore, we recognize that serum concentrations of VEGF may fluctuate in healthy women according to their menstrual status (36, 37). However, reports on serum concentrations of VEGF are inconsistent. It has been shown that serum concentrations of VEGF were significantly lower in the luteal phase (36). It has also been found that serum concentrations during the periovulatory phase were lower compared with the early follicular and luteal phase (37).

In summary, we found that angiogenesis, as reflected by serum concentrations of VEGF, plays a functional role in vulvar carcinogenesis. VEGF seems to be a mediator of vulvar tumor growth but not of tumor cell dedifferentiation. Although associated with impaired disease-free and overall survival, pretreatment serum concentrations of VEGF are not an independent predictor of outcome in patients with vulvar cancer.

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