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ORIGINAL PAPER

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Serum levels of basic fibroblast growth factor reflect disseminated disease in patients with testicular germ cell tumors

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Abstract The potential role of angiogenesis stimulators in the pathogenesis of different tumor entities has been confirmed in several studies. We measured the serum levels of basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) in 51 patients with testicular germ cell tumors and in 39 healthy volunteers. Serum concentrations of bFGF, VEGF and PDGF-AB were determined by enzyme-linked immunosorbent assay. The median serum bFGF level for tumor patients was 3.46 pg/ml (range 0–61.6) compared to 0.7 pg/ml (0–11) in the control group ($P < 0.01$). In patients with metastatic disease, the median serum bFGF level was 10.3 pg/ml (0–61.6) in contrast to 2.8 pg/ml (0–50) in patients with localized disease ($P < 0.01$). The median serum VEGF and PDGF levels were 270 pg/ml (0–1,903) and 37,837 pg/ml (9,075–108,800), respectively, for tumor patients and 200 pg/ml (44–585) and 23,000 pg/ml (4,250–70,650) in the control group ($P < 0.05$). Our data suggest that angiogenesis, as reflected by serum concentrations of bFGF, VEGF and PDGF, plays a functional role in the growth and progression of testicular germ cell tumors.

Keywords Testicular germ cell tumor · Angiogenesis · Angiogenic factors

Introduction

Angiogenesis, the formation of new blood vessels from pre-existing capillaries, is fundamental in reproduction, development and wound repair [7]. Furthermore, angiogenesis has been established as a basic feature in tumor development, growth and spread beyond regional borders [6]. The degree of neovascularization within a primary tumor is of prognostic significance for different tumor entities [4]. Normally, angiogenesis is under tight regulatory control, consisting of both angiogenic and antiangiogenic factors. In malignant tumors, this control is lost and the production of angiogenic molecules exceeds that of endogenous angiogenic inhibitors. Sustained angiogenesis is essential for tumor growth beyond a tumor size of 1–2 mm^3 [10]. Among several identified peptides with angiogenic properties, basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) are thought to play a major role in tumor angiogenesis [14, 17, 20]. bFGF, VEGF and PDGF can be detected in human serum by ELISA, and elevated serum concentrations have been reported in patients with various types of tumor, implying a significant role for these factors in tumor growth and progression [13,22].

Although angiogenesis has been studied extensively in other urological tumors [11], there are only very few and controversial data on the significance of angiogenesis in testicular tumors and there are no studies investigating the circulating levels of angiogenic factors. Therefore, we determined the serum levels of bFGF and VEGF in 51 patients and PDGF in 20 patients with testicular cancer prior to surgery and correlated the results to clinicopathological parameters. In addition we detected serum levels of the above angiogenesis stimulators in 39 age matched volunteers without any signs of neoplastic disease.

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Material and methods

Fifty-one patients who underwent orchiectomy for a newly diagnosed testicular germ cell tumor at the University Hospital Frankfurt/Main between 1995 and 1999 and 39 age matched volunteers without any signs of neoplastic diseases (mainly patients who were admitted for the treatment of small kidney stones) were

recruited in this study. None of the tumor patients had received prior chemotherapy or irradiation. All experiments were performed after obtaining informed consent according to the institutional guidelines. In the tumor group, sera were drawn from all patients prior to surgery and clinical information was extracted from the clinical records. Histopathological staging and classification were assessed according to the criteria of the Union Internationale Contre le Cancer (UICC) from 1997 and the World Health Organization from 1998, clinical staging was assessed according to the Lugano Classification.

Serum was collected from clotted blood by centrifugation (1,600 *g*), aliquoted and stored at –70°C until tested. Serum concentrations of bFGF, VEGF and PDGF-AB were measured with Quantikine human immunoassay kits (R&D Systems, Minneapolis, Minn.). According to the manufacturer's protocols, serum for the VEGF- and bFGF-assays were not diluted, while serum for the PDGF-AB-assay was diluted 1:50.

The Mann-Whitney U-test was used to compare the serum levels of patients with those of controls. The Kruskal-Wallis test and Mann-Whitney U-test were used for relating serum levels with tumor status.

Results

The median age was 29 years (range 16–63) in tumor patients and 31 years (17–66) in the control group. The histological review of the primary tumors revealed 23 (45%) seminomas, 8 (16%) embryonal cell carcinomas, 11 (21%) teratocarcinomas, 1 (2%) mature teratoma, 1 (2%) chorioncarcinoma and 7 (14%) mixed germ cell tumors.

Among the 51 tumor patients, 34 (67%) (19 seminomas and 15 non-seminomatous germ cell tumors) were considered to have localized disease according to clinical examinations, such as serum tumor marker assays, chest radiographs, ultrasonographs, computed tomography scans (abdomen, chest) and histology in the case of retroperitoneal lymph node dissection. Metastatic disease was revealed in 17 (33%) patients (4 seminomas and 13 non-seminomatous germ cell tumors). The exact clinical and pathological stage distribution of all tumor patients is shown in Table 1.

The median serum bFGF level for tumor patients was 3.46 pg/ml (range: 0–61.6; $n = 51$) compared to a median bFGF level of 0.7 pg/ml (0–11; $n = 39$) in the control group ($P < 0.01$; Mann-Whitney U-test; Fig. 1). We did not find any significant statistical difference in serum bFGF levels when comparing seminomas [median 2.85 pg/ml (0–61.6); $n = 23$] with non-seminomatous testicular germ cell tumors [median 4.35 pg/ml (0–50.5); $n = 28$]. There were also no statistical differences in bFGF levels in the various entities of non-seminomatous tumors, or when comparing embryonal cell carcinomas with all other germ cell tumors. According to tumor stage, the median serum bFGF levels were 10.3 pg/ml (0–61.6; $n = 17$) in patients with metastatic and 2.8 pg/ml (0–50; $n = 34$) in patients with localized disease ($P < 0.01$; Mann-Whitney U-test; Fig. 2). The median serum VEGF level was 270 pg/ml (0–1,903; $n = 51$) for tumor patients and 200 pg/ml (44–585; $n = 39$) in the control group. This was also statistically significant ($P < 0.05$; Mann-Whitney U-test).

Table 1 Clinical and pathological stage distribution for all tumor patients

Clinical stage	*n* (%) patients	Pathological stage	*n* (%) patients
I	34 (67%)	pT1	37 (72%)
IIa	3 (6%)	pT2	10 (20%)
IIb	5 (10%)	pT3	3 (6%)
IIc	3 (6%)	pT4	1 (2%)
III	6 (11%)		

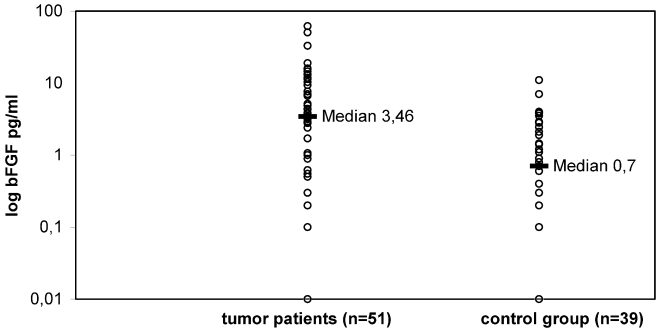

Fig. 1 Serum bFGF levels in tumor patients and in the control group

The median preoperative serum levels for PDGF were 37,837 pg/ml (9,075–108,800; $n = 20$) in patients with malignant germ cell tumors, in contrast to 23,000 pg/ml (4,250–70,650; $n = 39$) in the controls ($P < 0.05$; Mann-Whitney U-test). Within the group of patients with malignant germ cell tumors, we did not observe any significant statistical relationship between serum VEGF or PDGF levels and histopathological or clinical findings. The median levels of bFGF, VEGF and PDGF in the different clinical and pathological stages are shown in Tables 2 and 3.

In ten (20%) patients, histopathological examination of the tumor revealed vascular invasion. There was no significant relationship between the presence or absence of vascular invasion and the serum levels of bFGF, VEGF or PDGF.

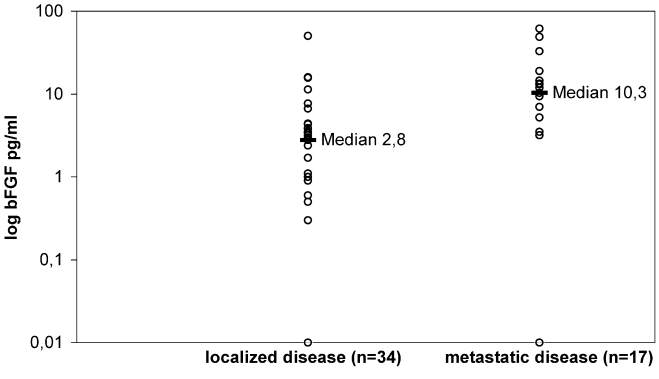

Fig. 2 Serum bFGF levels in tumor patients with localized and metastatic disease

Table 2 Median bFGF, VEGF and PDGF levels in clinical stages I-III

Clinical stage	Median bFGF pg/ml (*n* patients)	Median VEGF pg/ml (*n* patients)	Median PDGF pg/ml (*n* patients)
I	2.8 (34)	273 (34)	39,500 (15)
IIa	5.8 (3)	88 (3)	95,150 (1)
IIb	13.3 (5)	222 (5)	25,500 (1)
IIc	9.3 (3)	318 (3)	–
III	13.2 (6)	430 (6)	46,400 (3)

Table 3 Median bFGF, VEGF and PDGF levels in pathological stages pT1–4

Pathological stage	Median bFGF pg/ml (*n* patients)	Median VEGF pg/ml (*n* patients)	Median PDGF pg/ml (*n* patients)
pT1	3.5 (37)	276 (37)	38,287 (12)
pT2	3.9 (10)	171 (10)	25,500 (5)
pT3	0.6 (3)	314 (3)	39,500 (3)
pT4	33 (1)	486 (1)	–

Twenty-one (75%) of 28 patients with non-seminomatous testicular germ cell tumor and eight (35%) of 23 patients with seminoma had elevated serum levels for either alpha-fetoprotein, human chorionic gonadotrophin or beta-human chorionic gonadothrophin. There was no statistically significant relationship with tumor stage. In addition, we did not find a association with the serum expression of bFGF, VEGF or PDGF.

Moreover, we found that age had no influence on the expression of serum bFGF, VEGF or PDGF, and we also did not find any relationship with the patient's erythrocyte, leukocyte or platelet counts.

Discussion

The formation of new microvessels from the pre-existing vascular bed is known as angiogenesis and is normally under the tight control of angiogenic mediators. This regulation is lost in malignant tumors [9]. In testicular germ cell tumors, only a few studies refer to the role of angiogenesis on tumor biology either by investigating the microvascular density (MVD) of the tumor as a measure of the degree of angiogenesis or by quantifying VEGF levels within tumor specimens.

In stage I non-seminomatous germ cell tumors, MVD was found to correlate with occult metastasis in retroperitoneal lymph nodes by univariate analysis, but not by multivariate analysis [16]. Viglietto et al. demonstrated that VEGF mRNA expression within the tumor, measured by polymerase chain reaction (PCR), was significantly higher in primary germ cell tumors than in the normal testis and was significantly correlated with microvascular density [21]. Another study, based on immunohistochemical analysis, showed that VEGF expression within testicular germ cell tumors was significantly correlated by multivariate analysis with MVD density and metastatic disease [8]. In summary, these results indicate that VEGF expression may play a significant role in the progression of germ cell tumors, and inhibitors of VEGF and VEGF-receptors might act to suppress tumor growth.

On the other hand, there are controversial data demonstrating no relationship between microvessel density and VEGF as well as MVD, VEGF and relapse free survival [12]. According to Maher et al., MVD does not correlate with metastatic disease [15], and other authors have revealed that tumor angiogenesis is not predictive of metastatic status [1]. Concerning PDGF, there is only one recent study showing that PDGF, through complex interactions, could play a leading role in ontogenesis and testicular pathophysiology, as well as in tumorigenesis or tumor progression in testicular Leydig cell tumors [2]. However, as there are no studies analyzing circulating angiogenic promoters in patients with testicular germ cell tumors, we measured serum bFGF, VEGF and PDGF levels and correlated the results with clinical and histological parameters. As has been observed in various tumor types [18], patients with testicular germ cell tumors had significantly higher serum concentrations of VEGF, PDGF and particularly bFGF compared to age-matched male controls without evidence for malignant disease, suggesting that angiogenesis may have a role in the tumor biology of testicular germ cell tumors. No significant relationship was found for serum concentrations of bFGF, VEGF and PDGF and tumor type. Thus, our results could indicate that elevated serum concentrations of bFGF, VEGF and PDGF in patients with testicular germ cell tumors are indicative of testicular tumor growth but not of tumor cell differentiation. On the other hand, the discovery of a relationship may well have been missed due to the wide variety of tumors used in this study, resulting in only small groups of identical tumor types. Most interestingly, patients with metastatic testicular germ cell tumors had higher serum bFGF levels than patients with localized disease or controls. This correlates with the results of other groups, showing an association of serum bFGF levels with tumor stage and prognosis in numerous human malignancies [19]. Despite this significant difference, the standard deviations were very large, resulting in a significant overlap of the groups (Figs. 1, 2) so that a larger prospective study is needed to confirm whether bFGF can be of clinical utility for the individual patient. Although serum levels of VEGF and PDGF in cancer patients were significantly higher than those in healthy controls, no significant differences were observed for localized and disseminated disease. This has also been described in some other tumor entities supposing that angiogenic factors, other than VEGF and PDGF, are more important in testis tumor biology [3].

In summary, our data support the hypothesis that angiogenesis, as reflected by serum concentrations of bFGF, VEGF and PDGF, plays a functional role in the growth of testicular germ cell tumors. Furthermore,

while bFGF seems to have a great influence in the pathogenesis of testicular germ cell tumors, the impact of VEGF and PDGF seems to be secondary. As there are no further data, particularly with regard to bFGF and other angiogenic promoters, the role of angiogenesis in testicular germ cell tumors remains unclear and further studies are needed, as long as there are indications that bFGF and PDGF expression quantified by immunohistochemistry or PCR within different tumor types, e.g., bladder cancer, correlate significantly with tumor progression and metastasis [5]. Moreover, further prospective series are needed to confirm whether circulating bFGF, VEGF and PDGF levels can be used as an additional tool in the management of patients with testicular germ cell tumors and as a measure of treatment response and tumor aggressiveness. An angiogenic profile might also be useful in the design of specific anti-angiogenic therapies, i.e., to tailor the therapy to the needs of the individual tumor based on the profile of positive and negative regulators of angiogenesis. Finally, measuring the levels of angiogenic mediators may provide useful information with respect to understanding tumor biology. Furthermore, the identification of bFGF as a mediator of metastasis in testicular germ cell tumor should be investigated as a potential target for systemic therapy.

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