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ORIGINAL ARTICLE

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Circulating angiogenic growth factor levels in mice bearing human tumors using Luminex Multiplex technology

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Abstract Tumor angiogenesis is essential for tumor growth and metastasis formation. Luminex methodology was used to measure the levels of four angiogenic cytokines in cell culture medium and in the plasma of mice bearing human tumors. We obtained plasma and conditioned culture medium from 12 different human tumor cell lines. Tumor necrosis factor-alpha (TNF-α), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and transforming growth factor-beta (TGF-β) were determined by the Luminex FlowMetrix assay. VEGF, TNF-α, and bFGF were undetectable in non-tumor-bearing animals. HS746T gastric cancer and Caki-1 renal cell cancer cells in culture produced high levels of VEGF (1000 and 450 pg/10^6 cells, respectively). High levels of TGF-β were produced by HS746T gastric carcinoma and Calu-6 non-small-cell lung carcinoma (3000 and 1000 pg/10^6 cells, respectively). Caki-1 renal cell carcinoma and Calu-6 non-small-cell lung carcinoma cells in culture produced high levels of bFGF (42 and 10 pg/10^6 cells, respectively). Caki-1, SW2 SCLC, HCT-116 and HT-29 colon tumors produced high plasma levels of VEGF (200, 220, 42, and 151 pg/ml, respectively) and TGF-β (31, 36, 45, 32 pg/ml, respectively). A positive linear correlation was seen between tumor volume and VEGF in SW2 ($r = 0.87$) and Caki-1 ($r = 0.47$) tumors, and a moderate correlation in HCT116 tumors ($r = 0.3$). Angiogenic profiles in the plasma of nude mice bearing human tumors may be useful to identify appropriate biomarkers for antiangiogenic therapy, as diagnostic and prognostic tools, and to monitor the responses of individual tumors to antiangiogenic therapy.

Keywords Angiogenic growth factors · Tumor markers · Luminex methodology

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Introduction

Angiogenesis plays an important role in the process of growth and metastasis of solid tumors. Tumor angiogenesis is essential for tumor growth and metastasis formation [1, 2, 3]. Numerous angiogenic factors that regulate this complex process have been identified. Vascular endothelial growth factor (VEGF) which is also known as vascular permeability factor, is a potent, multifunctional and endothelial cell-specific growth factor. It stimulates vasodilation and cell proliferation, increases permeability and migration, and promotes endothelial cell survival through binding of the Flk1/KDR and Flt-1 receptors on endothelial cells [4, 5]. Other cytokines shown to regulate angiogenesis positively include transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α) and basic fibroblast growth factor (bFGF) [6].

Studies have been performed to profile these growth factors in patients with local and metastatic disease [7, 8, 9, 10]. Such profiles could provide diagnostic and prognostic parameters at initial presentation, assist in determining response to therapy and indicate recurrence in asymptomatic patients. Human tumor xenograft models could be useful in the design of antiangiogenic therapies and to understand the biology of malignant disease processes. The use of Luminex technology offers the advantage of measuring several cytokines within the same sample using a small sample volume. In the present study, the secretion of the angiogenic cytokines VEGF, TNF-α, bFGF, and TGF-β by numerous human tumor cell lines in culture and in the plasma of nude mice bearing human tumor xenografts, and their relationship to tumor burden, were examined.

Materials and methods

Cell culture

The SW2 human small-cell lung carcinoma cell line originated from the pleural fluid of a patient with small-cell lung cancer in 1979 [11]. Human umbilical vein endothelial cells (HUVEC) were purchased from Bio-Whittaker Technologies (Walkersville, Md.). All other cell lines were purchased from American Type Culture Collection (Manassas, Va.). SW2 cells were maintained in RPMI-1640 with 10% fetal bovine serum (FBS) and 2 g/l sodium bicarbonate. HUVEC were maintained in endothelial growth medium (EGM; Bio-Whittaker Technologies). Caki-1 human renal cell carcinoma cells were maintained in McCoy's medium with 10% FBS. HS746T human gastric cancer cells, NIH3T3 murine fibroblasts, and SW480 human colon carcinoma cells were maintained in Dulbecco's modified Eagle's medium with 10% FBS. Hep3B hepatocellular carcinoma cells were maintained in minimum essential medium with 10% FBS. MDA-MB468 and MX-1 breast carcinoma cells, Calu-6 and H460 non-small-cell lung carcinoma cells, H82 small-cell lung cancer cells, GC-3, HCT116, and HT29 colon carcinoma cells were maintained in RPMI-1640 with 10% FBS. Cells were grown to 80% confluency and the medium was replaced with serum-free medium for 24 h. After 24 h, the medium was collected for multiplex analysis and stored at −80°C until analysis.

Plasma collection

Nude mice of the same sex as the origin of the tumor were irradiated with 450 rad TBI. Human tumor cells (5×10^6) were suspended in 1:1 medium/Matrigel, and injected subcutaneously into the hind leg of the mouse. Tumor measurements were recorded twice weekly. To obtain plasma mice, were killed with CO_2 and blood was collected through cardiac puncture and placed into EDTA tubes on ice. The blood samples were centrifuged at 800 rpm for 30 min at 4°C, followed by 10 min at 3000 rpm. The plasma was collected and stored at −80°C until analysis.

Multiplex assay

A Multiplex kit was purchased from R&D Systems (Minneapolis, Minn.). Each 96-well filter plate (Millipore) was blocked with 100 µl blocking buffer for 30 min, followed by filtration under a vacuum at 2 psi. The 25× bead mix was vortexed for 1 min and sonicated for 30 s prior to diluting. The bead mix was diluted in wash buffer, and 50 µl was immediately added to each well. Wash buffer was vacuum-filtered. The standard was dissolved in medium supplied in the kit. The standards and samples were added to the filter plates containing bead mix in 50 µl in triplicate (plasma samples were diluted 1:2 with diluent provided in the kit). To measure TGF-β in cell culture, a 250-µl sample was acidified with 50 µl 1 *N* HCl and incubated for 10 min, then neutralized with 50 µl 1.2 *N* NaOH/0.5 *M* HEPES, and diluted with RD6 M for a 1:4 dilution. To measure TGF-β in plasma, a 10-µl sample was acidified with 10 µl 2.5 *N* acetic acid/10 *M* HEPES and incubated for 10 min, then neutralized with 10 µl 2.7 *N* NaOH/1 *M* HEPES, and diluted with 170 µl RD6 M for a 1:20 dilution. The bead mix is light sensitive and was thus stored in the dark. The plates were placed on a shaker at 4°C overnight to allow binding of growth factors to antibody-bound beads. The following day, the medium was vacuum-filtered, and 50 µl detection antibody was added to each well. The wells were washed four times with 100 µl wash buffer. The plates were incubated on a shaker at room temperature for 1 h in the dark. The plates were washed four times with 100 µl wash buffer. Streptavidin-PE (50 µl) was added to each well. The plate was shaken for 15 min at room temperature in the dark. The wells were washed four times with 100 µl wash buffer. The beads were resuspended in 150 µl wash buffer for analysis. Immediately prior to analysis the plates were shaken to resuspend the beads. Plates were read on a FACScan Luminex 100 (Luminex Corporation, Austin, Tx.). Data for 100 beads per cytokine were collected for each standard and sample dilution.

Statistical analysis

The correlation coefficient test was used for statistical analysis of both in vitro and in vivo data.

Results

VEGF production

VEGF, TNF-α, bFGF, and TGF-β secreted into the culture medium supernatant by human tumor cell lines and by normal cells found within tumor tissue were measured. TNF-α was undetectable in all the tumor cell line and the normal cell cultures tested. VEGF was secreted in the tumor cell line cultures studied (Fig. 1). In vitro VEGF levels were in the range 0–1064 pg/10^6 cells, with the highest levels secreted by HS746T gastric carcinoma cells (1064 pg/10^6 cells) and Caki-1 renal cell cancer cells (452 pg/10^6 cells). Those producing the lowest amount of VEGF into the cell culture medium were GC3 colon, SW480 colon, and SW2 SCLC cells (25 pg/10^6 cells).

We also determined the plasma levels of VEGF in mice bearing human tumor xenografts of the same tumor lines as in the cell cultures. Non-tumor-bearing nude mice did not show detectable plasma levels of VEGF. In vivo implantation of each tumor resulted in plasma VEGF levels ranging from 5 to 200 pg/ml, except MDA-MB468 breast carcinoma. The highest plasma VEGF levels were obtained in animals bearing Caki-1 renal cell, SW2 SCLC, HT-29 and HCT116 colon tumors. These data suggest there is no direct correlation between in vitro and in vivo tumor-induced VEGF expression ($r=-0.0056$).

bFGF production

bFGF was measured in the same plasma and cell culture samples as VEGF and TNF-α. In vivo bFGF was not detectable in the plasma of non-tumor-bearing control animals. The levels of bFGF secreted into culture were much lower than VEGF levels in the same cultures. All human tumor cell lines secreted detectable levels of bFGF from 0.5 to 42 pg/10^6 cells (Fig. 2). The highest bFGF levels were produced by Caki-1 renal cells (42 pg/10^6 cells), and Calu-6 NSCLC cells (10 pg/10^6 cells).

In the plasma of tumor-bearing animals, bFGF levels were measurable in mice bearing each human tumor except H82 SCLC, with levels in the range 1–100 pg/ml. The highest bFGF plasma levels in vivo were found in HT29 and SW480 colon tumor-bearing animals and reached 80–100 pg/ml. Again there was no direct correlation between in vitro and in vivo data ($r=0.24$), nor

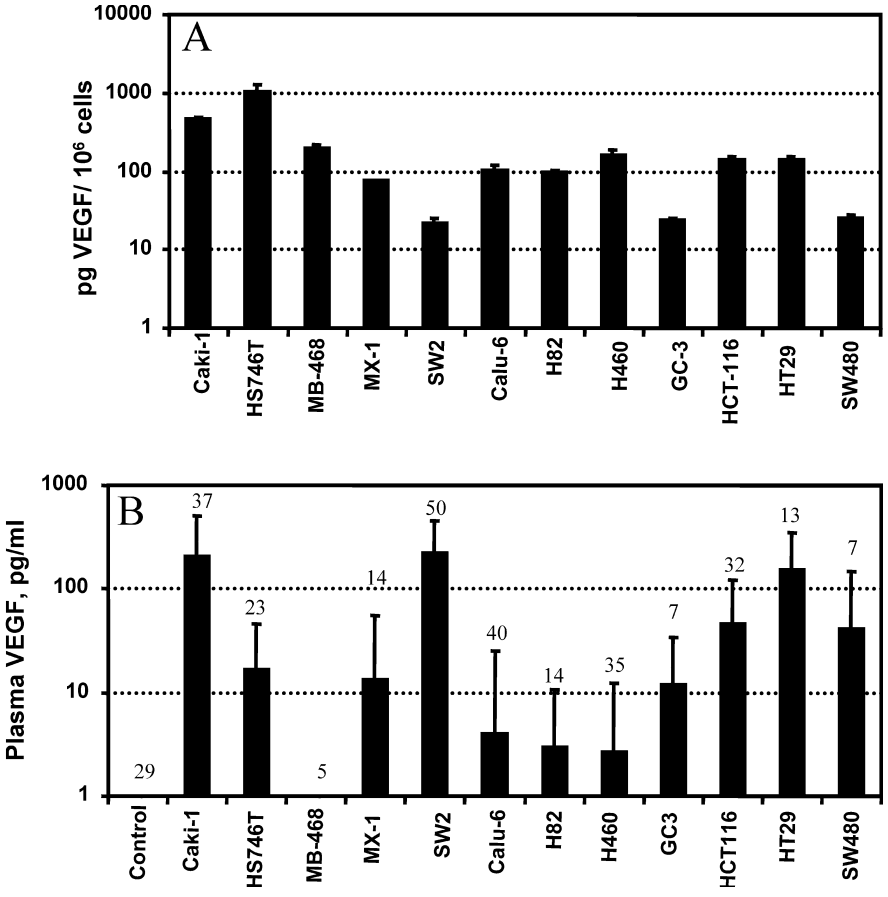

Fig. 1 **A** Mean levels of VEGF secretion by human tumor cell lines into the cell culture medium. **B** Mean plasma levels of VEGF from nude mice bearing human tumors. The *numbers* indicate the sample size analyzed for each group. The *bars* represent the means + SEM from three individual experiments

was there a correlation between VEGF expression and bFGF expression.

TGF-β production

TGF-β levels, both in culture medium and in plasma were much higher than those of VEGF and bFGF (Fig. 3). In vitro, TGF-β was secreted by all of the cells studied except HT-29 colon carcinoma cells. TGF-β levels were in the range 0–2961 pg/10^6 cells, with the highest levels secreted by HS746T gastric carcinoma (2961 pg/10^6 cells) and Calu-6 NSCLC cells (1053 pg/10^6 cells) cells. Non-tumor-bearing animals had plasma TGF-β levels in the range 6–20 ng/ml. All tumor-bearing animals produced plasma levels equal to or greater than non-tumor-bearing controls. High plasma levels of TGF-β occurred in nude mice bearing SW2 SCLC (4–72 ng/ml), Caki-1 renal cell (4–72 ng/ml), and HCT116 colon carcinoma (7–87 ng/ml) cells. Again there was no direct correlation between in vitro and in vivo data, nor was there a correlation between TGF-β expression and bFGF production or VEGF production in the plasma. However, a correlation was found between VEGF and TGF-β secretion in cell culture ($r = 0.80$).

To determine the contribution of normal cells found in the tumor tissue to angiogenic growth factor production, we examined the secretion of the four growth factors by murine RAW264.7 macrophages, NIH3T3 fibroblasts and HUVEC into cell culture medium. As with the tumor lines, none of these normal cell types secreted detectable levels of TNF-α. In contrast to the tumor lines, the normal cells examined produced no detectable levels of VEGF (Fig. 4). This suggests that the high VEGF levels found in the circulation of tumor-bearing mice result from secretion of the cytokine by the tumor cells. Only HUVEC secreted measurable levels of bFGF into the culture medium. All three normal cell lines secreted TGF-β at levels similar to or above those detected in the culture medium with most of the tumor cell lines.

We were also interested in examining the relationship between tumor burden and cytokine levels in the plasma of mice bearing these human tumors. We observed a linear correlation between plasma VEGF levels and tumor volume. As shown in Fig. 5, plasma VEGF levels increased with tumor volume for five tumor lines which produced varying plasma levels of VEGF: SW2 SCLC, HCT116 colon tumor, Caki-1 renal cell tumor, Calu-6 NSCLC, and HS746T gastric carcinoma ($r = 0.87$, 0.30, 0.47, 0.56, and 0.50, respectively). We also found a correlation between tumor burden and TGF-β in GC3 colon carcinoma, HS746T gastric carcinoma, and MX-1 breast carcinoma ($r = 0.83$, 0.50, and 0.75, respectively; Fig. 6). We did not find a relationship between tumor volume and bFGF. We also found that in many of the

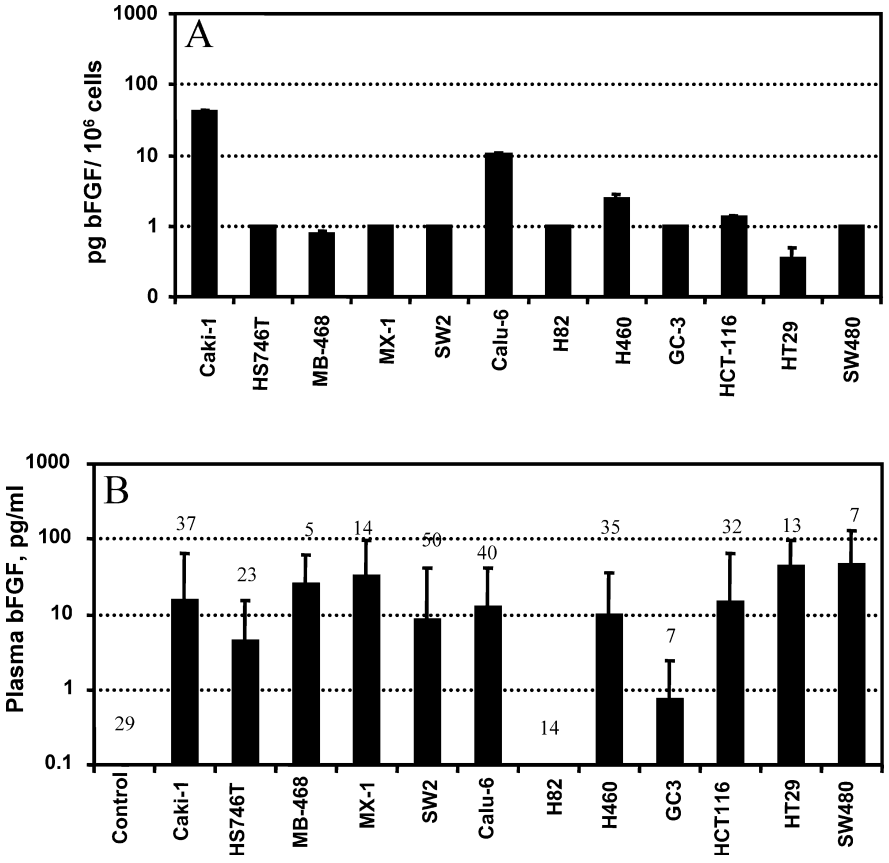

Fig. 2 **A** Mean levels of bFGF secretion by human tumor cell lines into the cell culture medium. **B** Mean plasma levels of bFGF from nude mice bearing human tumors. The *numbers* indicate the sample size analyzed for each group. The *bars* represent the means + SEM from three individual experiments

tumors studied, VEGF was not detectable in plasma until the tumor volume reached 1000 mm^3.

Discussion

Analysis of circulating angiogenic tumor markers may play an important role in the evaluation of prognosis at initial presentation as well as tumor metastasis, disease-free and overall survival. Knowledge of circulating angiogenic factors may influence drug selection for adjuvant chemotherapy, and aid in the understanding of response to therapy. Many studies have evaluated the clinical significance of increased angiogenic growth factor levels in patients with various cancers to be used as a predictor for such indicators as outcome after adjuvant therapy, metastasis, tumor size, and recurrence in asymptomatic patients [8, 9, 12, 13]. In many of these studies the focus was on plasma or serum cytokine levels, while in others increases in tumor cytokine levels were evaluated [10, 14, 15].

Studies in which the role of increased cytokine levels in breast cancers has been evaluated have shown that elevated tumor cytosolic content of VEGF is a possible predictor of survival in patients with node-positive breast cancer [14], disease-free interval and post-relapse overall survival, poor rate of response compared with patients with low tumor VEGF levels, and poor response to tamoxifen and chemotherapy [15]. However, in a recent review of surrogate markers for angiogenesis in breast cancer [12], only in 5 of 12 studies were serum VEGF levels found to correlate with overall survival, while in 7 of these 12 trials serum VEGF was correlated with relapse-free survival [12]. Serum bFGF was found to correlate with relapse-free survival in only 1 of 10 studies, and overall survival in 2 of 10 studies [12]. When evaluating the predictive value of serum TGF-β, higher serum TGF-β levels have been found in patients with more advanced TNM stages [13], while serum TGF-β levels in breast cancer patients have been found not to differ significantly from those in healthy women [16].

In colorectal carcinoma patients, high serum VEGF levels have been found to correlate with advanced disease [17, 18, 19, 20], high risk of early aggressive metastasis [17, 20], and a shorter survival period [17, 18, 19], and predict patients who would benefit from adjuvant chemotherapy [17], or have a poor treatment outcome [19]. In colorectal carcinoma patients, post-operative levels of TGF-β have been found to be highly predictive of liver metastasis after curative resection [21].

In renal cell carcinoma patients, serum VEGF levels have been found to be significantly correlated with tumor stage and grade, and overall survival, but not with progressive disease state [10]. Gene expression of VEGF and bFGF has been found to be higher in the primary tumors of patients with metastasis compared with the primary tumors of disease-free patients at 48 months of

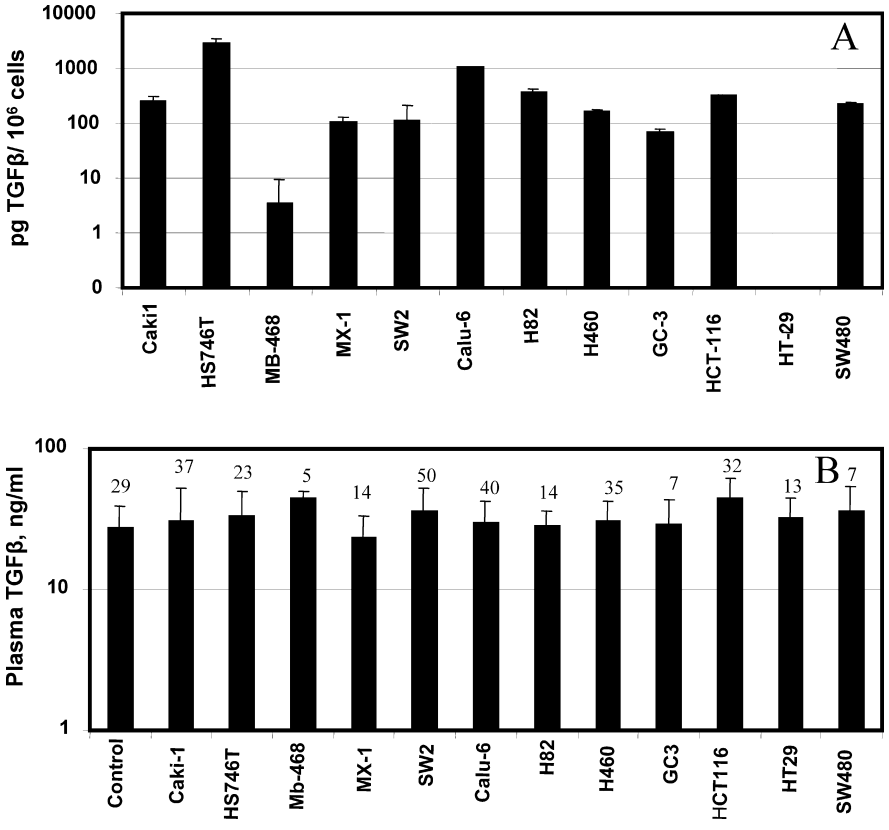

Fig. 3 **A** Mean levels of TGF-β secretion by human tumor cell lines into the cell culture medium. **B** Mean plasma levels of TGF-β from nude mice bearing human tumors. The *numbers* indicate the sample size analyzed for each group. The *bars* represent means + SEM from three individual experiments

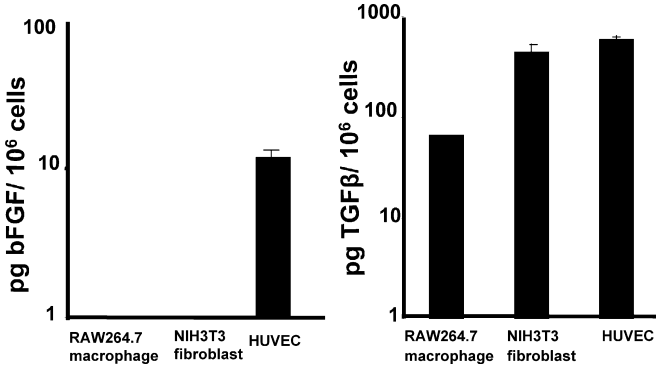

Fig. 4 Mean angiogenic growth factor secretion by normal cell types found in tumor tissue in cell culture medium. The *bars* represent means + SEM from three individual experiments

follow-up [22]. TGF-β has also been found to be elevated in pre- and postoperative renal cell carcinoma patients compared with healthy patients [23].

In evaluating circulating cytokines in lung carcinoma patients as predictors of response, significant changes have been shown to occur in serum VEGF levels in lung cancer patients who respond to therapy compared to nonresponders [24]. Tumor VEGF expression has been found to correlate with poor prognosis in non-small-cell lung cancer [25]. However, in a similar study no prognostic value of tumor VEGF expression in non-small-cell lung cancer was shown [26]. Serum VEGF levels have been found not to have potential for predicting survival of patients with ovarian cancer [8, 27]. High tumor VEGF expression [28] and high plasma VEGF levels [29] have been found to correlate with poorer response to therapy in gastric carcinoma patients than lower levels of VEGF. In general, gastric cancer patients with high plasma VEGF levels have been found to have shorter overall survival, increased metastasis and disease progression, poorer prognosis, and non-response to therapy compared to patients with low VEGF expression.

In the current study the secretion of four angiogenic cytokines was evaluated in a variety of malignant and normal cell types found in tumor tissue, both in cell culture and in the plasma of mice bearing human tumors. The four growth factor profiles evaluated were VEGF, bFGF, TNF-α, and TGF-β. We used plasma level as indicator of tumor-induced cytokine production in tumor-bearing animals because of the simplicity in obtaining plasma samples from cancer patients compared with tumor biopsies. These studies focused on identifying a relationship between cytokine production and tumor burden, determining whether elevated cytokine production relates to tissue type in human cancers, as well as evaluating plasma cytokine production in human tumor xenografts to identify tumor models for the evaluation of antiangiogenic agents.

We observed no direct correlation between growth factor levels in plasma of tumor-bearing nude mice and conditioned medium of tumor cells in culture. This was expected since the microenvironment in cell culture may

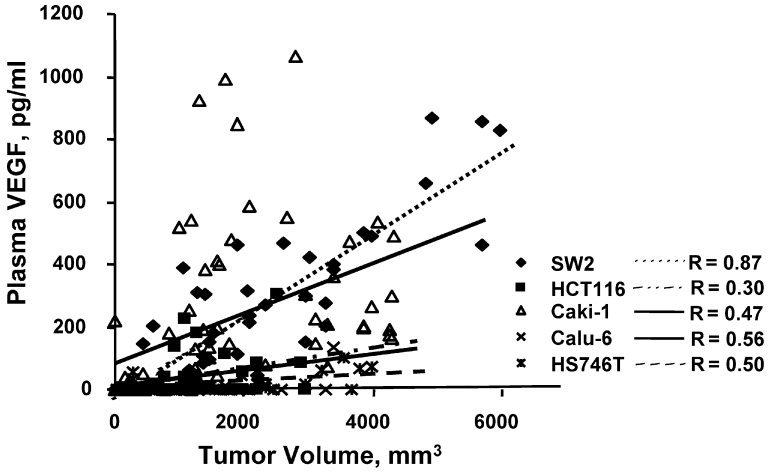

Fig. 5 Relationship between tumor volume and VEGF secretion into the plasma of mice bearing SW2 SCLC, HCT116 colon, Caki-1 renal cell, Calu-6 NSCLC, and HS746T gastric tumors. the *lines* indicate the linear trend. The *R* value indicate the linear correlations

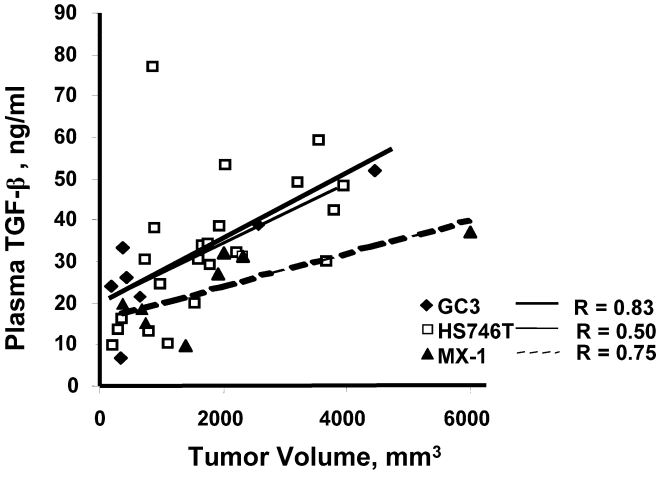

Fig. 6 Relationship between tumor volume and TGF-β secretion into the plasma of mice bearing GC3 colon, HS746T gastric, and MX-1 breast tumors. The *lines* indicate the linear trend. The *R* values indicate the linear correlations

provide different signals for cytokine production from those in the tumor microenvironment in vivo. All of the 12 tumor cell lines secreted VEGF into the culture medium. However, in vivo, only 4 of 12 tumors resulted in high plasma VEGF levels, while several had very low or undetectable plasma VEGF levels (4 of 12). In cell culture Caki-1 renal cell carcinoma cells secreted very high amounts of bFGF, but few Caki-1 tumor-bearing mice had detectable amounts of plasma bFGF levels.

When evaluating the relationship of plasma cytokine levels with tumor burden, we observed a positive linear correlation between tumor volume and plasma VEGF levels in animals bearing SW2 SCLC, HCT116 colon, Caki-1 renal, HS746T gastric, and Calu-6 NSCLC tumors. In most animals, VEGF was not detectable when tumor volumes were below 800 mm^3. There was also a positive linear correlation with TGF-β and tumor volume in animals bearing GC3 colon, HS746T gastric and MX-1 breast tumors. Side by side analysis of the TGF-β ELISA and Luminex assays consistently revealed threefold higher TGF-β levels in the Luminex assay (data not shown). It is possible that there is a correlation between those tumors producing high levels of VEGF and those producing high levels of TGF-β. Plasma VEGF levels continued to increase as the tumor grew. In mice bearing bFGF-secreting tumors, plasma bFGF did not appear to be dependent on tumor volume. None of the tumors consistently resulted in high plasma bFGF levels even in animals with a large tumor burden. All of these determinations were made with human tumor xenografts growing subcutaneously, it is possible that tumors growing in the orthotopic tissue or in key sites for metastasis would have different angiogenic profiles. For this initial study, the subcutaneous site of implantation allowed a head-to-head comparison of a variety of tumor lines without the confounding effects of wound healing from surgical orthotopic implantation.

In conclusion, there was a linear correlation between plasma VEGF levels as well as TGF-β and tumor volume in some human tumor xenografts, in good agreement with clinical studies suggesting a relationship between these growth factors and tumor burden and disease stage. However, this observation was tumor-specific, not tissue type-specific, suggesting that VEGF may not be a predictive tumor marker for all patients with cancer of the same tissue type. This study identified several human tumor lines that could be useful in future studies as models for studying antiangiogenic therapies, and the effects of those therapies on cytokine production.

References

1. Folkman J (1995) Angiogenesis in cancer, vascular, rheumatoid and other diseases. Nat Med 1:27–3
2. Folkman J (1989) What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 82:4–6
3. Teicher BA (ed) (1999) Antiangiogenic agents in cancer therapy. Humana Press, New Jersey
4. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z (1999) Vascular endothelial growth factor (VEGF) and its receptors. FASEB J 13:9–22

5. Senger D, Galli S, Dvorak A, Perruzzi C, Harvey V, Dvorak H (1983) Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 219:983–985
6. Poon RT, Fan S, Wong J (2001) Clinical implications of circulating angiogenic factors in cancer patients. J Clin Oncol 19:1207–1225
7. Fuhrmann-Benzakein E, Ma M, Rubbia-Brandt L, Mentha G, Ruefenacht D, Sappino A, Pepper M (2000) Elevated levels of angiogenic cytokines in the plasma of cancer patients. Int J Cancer 85:40–45
8. Oehler MK, Caffier H (2000) Prognostic relevance of serum vascular endothelial growth factor in ovarian cancer. Anticancer Res 20:5109–5112
9. Salven P, Orpana A, Teerenhovi L, Joensuu H (2000) Simultaneous elevation in the serum concentrations of the angiogenic growth factors VEGF and bFGF is an independent predictor of poor prognosis in non-Hodgkin lymphoma: a single-institution study of 200 patients. Blood 96:3712–3718
10. Jacobsen J, Rasmuson T, Grankvist K, Ljungberg B (2000) Vascular endothelial growth factor as prognostic factor in renal cell carcinoma. J Urol 163:343–347
11. Francis J, Bernal SD, Gazdar AF, Thompson R, Baylin S (1980) L-Dopa decarboxylase activity (DCC): a distinguishing biomarker for the growth of small cell lung cancer (SCCL) in tissue culture. Proc Am Assoc Cancer Res 21:52
12. Gasparini G (2001) Clinical significance of determination of surrogate markers for angiogenesis in breast cancer. Crit Rev Oncol Hematol 37:97–114
13. Sheen-Chen S, Chen H, Sheen C, Eng H, Chen W (2001) Serum levels of transforming growth factor β1 in patients with breast cancer. Arch Surg 136:937–940
14. Linderholm B, Grankvist K, Wilking N, Johansson M, Tavelin B, Henriksson R (2000) Correlation of vascular endothelial growth factor content with recurrences, survival, and first relapse site in primary node-positive breast carcinoma after adjuvant chemotherapy. J Clin Oncol 18:1423–1431
15. Foekens J, Peters H, Grebenchtchikov M, Look M, Meijer-van Gelder M, Geurts-Moespot A, van der Kwast T, Sweep C, Klijn G (2001) High tumor levels of VEGF predict poor response to systemic therapy in advanced breast cancer. Cancer Res 61:5407–5414
16. Kajaniuk D, Marek B, Swietochowska E, Ostrowska Z, Glogowska-Szelag J, Kos-Kudla B, Ciesielska N, Wieloszyski T (2000) Plasma transforming growth factor β1 in breast cancer patients treated with CMF chemotherapy. J Clin Pharm Ther 25:291–294
17. Chin K, Greenman J, Gardiner E, Kumar H, Topping K, Monson J (2000) Pre-operative serum vascular endothelial growth factor can select patients for adjuvant treatment after curative resection in colorectal cancer. Br J Cancer 11:1425–1431
18. Broll R, Erdmann H, Duchrow M, Oevermann E, Schwandner O, Merkert U, Bruch H, Windhovel U (2001) Vascular endothelial growth factor (VEGF)—a valuable serum tumor marker in patients with colorectal cancer? Eur J Surg Oncol 27:37–42
19. Werther K, Christensen I, Brunner N, Nielsen H, and the Danish RANX05 Colorectal Cancer Study Group (2000) Soluble vascular endothelial growth factor levels in patients with primary colorectal carcinoma. Eur J Surg Oncol 26:657–662
20. Takeda A, Shimada H, Imaseki H, Okazumi S, Natsume T, Suzuki T, Ochiai T (2000) Clinical significance of serum vascular endothelial growth factor in colorectal cancer patients: correlation with clinicopathological factors and tumor markers. Oncol Rep 7:333–338
21. Tsushima H, Ito N, Tamura S, Matsuda Y, Inada M, Yabuuchi I, Imai Y, Nagashima R, Misawa H, Takeda H, Matsuzawa Y, Kawata S (2001) Circulating transforming growth factor β1 as a predictor of liver metastasis after resection in colorectal cancer. Clin Cancer Res 7:1258–1262
22. Slaton J, Inoue K, Perrotte P, El-Naggar A, Swanson D, Fidler I, Dinney C (2001) Expression levels of genes that regulate metastasis and angiogenesis correlate with advanced pathological stage of renal cell carcinoma. Am J Pathol 158:735–743
23. Junker U, Haufe C, Nuske K, Rebstock K, Stiener T, Wunderlich H, Junker K, Reinhold D (2000) Elevated plasma TGF-β1 in renal diseases: cause or consequence? Cytokine 12:1084–1091
24. Kido Y (2001) Vascular endothelial growth factor (VEGF) serum concentration changes during chemotherapy in patients with lung cancer. Kurume Med J 48:43–47
25. Han H, Silverman J, Santucci T, Macherey R, dAmato T, Tung M, Weyant R, Landreneau R (2001) Vascular endothelial growth factor expression in stage I non-small cell lung cancer correlates with neoangiogenesis and a poor prognosis. Ann Surg Oncol 8:72–79
26. Baillie R, Carlile J, Pendleton N, Schor A (2001) Prognostic value of vascularity and vascular endothelial growth factor expression in non-small cell lung cancer. J Clin Pathol 54:116–120
27. Abendstein B, Daxenbichler G, Windbichler G, Zeimet A, Geurts A, Sweep F, Marth C (2000) Predictive value of uPA, PAI-1, HER-2, and VEGF in the serum of ovarian cancer patients. Anticancer Res 20:569–572
28. Hyodo I, Doi T, Endo H, Hosokawa Y, Nishikawa Y, Tanimizu M, Jinno K, Kotani Y (1998) Clinical significance of plasma vascular endothelial growth factor in gastrointestinal cancer. Eur J Cancer 34:2041–2045
29. Takiuchi H, Hirata I, Kawabe S, Egashira Y, Katsu K (2000) Immunohistochemical expression of vascular endothelial growth factor can predict response to 5-fluorouracil and cisplatin in patients with gastric adenocarcinoma. Oncol Rep 7:841–846