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Clinical

Increased pre-therapeutic serum vascular endothelial growth factor in patients with early clinical relapse of osteosarcoma

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To investigate the clinical significance of circulating angiogenic factors, especially in association with early relapse of osteosarcoma, we quantified pre-therapeutic levels of vascular endothelial growth factor, basic fibroblast growth factor and placenta growth factor in the sera of 16 patients with osteosarcoma using an enzyme-linked immunosorbent assay. After a 1-year follow-up, the serum level of angiogenic factors was analysed with respect to microvessel density of the biopsy specimen and clinical disease relapse. The serum vascular endothelial growth factor levels were positively correlated with the microvessel density with statistical significance (P=0.004; Spearman rank correlation) and also significantly higher in seven patients who developed pulmonary metastasis than the remaining nine patients without detectable disease relapse (P=0.0009; The Mann–Whitney U-test). In contrast, the serum levels of basic fibroblast growth factor or placenta growth factor failed to show significant correlation with the microvessel density or relapse of the disease. Although there was no significant correlation between serum vascular endothelial growth factor levels and the tumour volume, the serum vascular endothelial growth factor levels were significantly higher in patients with a vascular endothelial growth factor-positive tumour than those with a vascular endothelial growth factor-negative tumour. These findings suggest that the pre-therapeutic serum vascular endothelial growth factor level reflects the angiogenic property of primary tumour and may have a predictive value on early disease relapse of osteosarcoma.

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Angiogenesis is an absolute requirement for the neoplastic growth of solid tumours after they reach a critical size of 1–2 mm^3 (Folkman, 1995). It is also essential for tumour metastasis, facilitating the shedding of tumour cells into surrounding blood vessels (Folkman, 1971, 1972, 1990). Tumour cells have been shown to secrete a variety of angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and placenta growth factor (PlGF) and thereby induce the local formation of new blood capillaries (Kandel *et al*, 1991; Ferrara *et al*, 1992; Dvorak *et al*, 1995). An association between poor prognosis and increases in vascularity has been reported in a number of tumours, including breast carcinoma (Weidner *et al*, 1991), lung carcinoma (Weidner *et al*, 1993), prostate carcinoma (Macchiarini *et al*, 1992), cervical carcinoma (Smith-McCune and Weidner, 1994) and colon carcinoma (Takahashi *et al*, 1995).

Osteosarcoma is one of the most common malignant bone tumours. Despite recent advances in multimodality treatments consisting of chemotherapy and wide tumour resection, pulmonary metastasis occurs in approximately 50% of the patients with osteosarcoma and remains a major cause of fatal outcome (Rosen *et al*, 1974). Notably, such relapses with pulmonary metastasis or deaths most likely occur during the first year of treatment (Eilber *et al*, 1987; Pratt *et al*, 1990; Souhami *et al*, 1997). Therefore, it is particularly important for the treatment of osteosarcoma to predict the relapse of the tumour at the early phase and customise the protocols.

We previously demonstrated that VEGF expression in osteosarcoma tumour tissue is correlated with high microvessel density, metastatic spread and poor prognosis (Kaya *et al*, 2000). Recently, VEGF has been measured not only in tissues but also in sera by using an enzyme-linked immunosorbent assay (ELISA), and increased serum levels of VEFG have been reported to be correlated with a high incidence of remote metastasis and a poor prognosis in patients with various types of cancer (Salven *et al*, 1997, 1998; Hyodo *et al*, 1998; Jin-no *et al*, 1998; Kumar *et al*, 1998; Landriscina *et al*, 1998; Hara *et al*, 1999; Oehler and Caffier, 2000). Measurement of serum VEGF levels by ELISA appears to be a more promising procedure for quantification than immunohistochemical staining of tissue VEGF, although VEGF can be secreted by megakaryocytes and platelets other than tumour cells (Banks *et al*, 1998; Webb *et al*, 1998; Salven *et al*, 1999; George *et al*, 2000; Lee *et al*, 2000).

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In the present study, we investigated the clinical significance of serum VEGF, bFGF, and PlGF in osteosarcoma in a prospective manner, focusing on the correlation with early disease relapse and local angiogenesis. Serum VEGF levels were corrected by platelet counts and the relationship of serum VEGF with tissue VEGF expression as well as the volume of the primary tumours was also analysed.

MATERIALS AND METHODS

Patients and sample processing

This study was approved under the institutional guidelines for the use of human subjects in research. Between April 1998 and March 2000 (patients having presented in March 2001 could not be followed up for 1 year, thus the study duration should end in 2000 at latest), all consecutive patients with putative diagnosis of osteosarcoma gave informed consent to provide blood samples. Peripheral venous blood samples were taken from these patients before biopsy and the serum was stored at -80°C until the assays were performed. Among them, patients whose biopsy specimens exhibited histological features compatible to osteosarcoma were entered into the study. There were 16 patients (four women and 12 men) with the age ranging from 20 to 69 years (average 31.2 years). Nine of the tumours were located in the femur, four in the tibia, two in the humerus and one in the pelvis. All tumours were histologically high grade. None of the patients were associated with Paget's disease. Pretreatment work-up studies including palpation of the regional lymph nodes, plain chest X-rays, computed tomography of the lung and abdomen, and bone scintigraphy revealed the development of pulmonary metastasis in two patients. These 16 patients were enrolled into the treatment protocol consisting of neoadjuvant chemotherapy, wide tumour excision, and adjuvant chemotherapy, which was basically a combination of high-dose methotrexate, doxorubicine, cisplatin and ifosmaide. Wide resection margin was achieved in all patients.

Early relapse was defined as the detectable tumour developing in remote sites within 1 year from the onset of the treatments. The cases in which metastatic disease was evident at the onset of the treatments were also defined as early relapse. During a 1-year period, patients were taken plain chest X-rays every month and computed tomography of the lung every 3 months. Bone scintigraphy was taken at 1 year. Consequently, seven out of 16 patients showed early relapse of the disease, all of them being pulmonary metastasis. These patients were divided into two groups, the relapse (seven patients) and no-relapse group (nine patients).

Immunohistochemical staining

The expression of VEGF and CD34 in the biopsy specimens was determined using the avidin-biotin complex method as described previously (Kaya *et al*, 2000). The primary antibody for VEGF was a rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1 : 200 dilution, and the antibody for CD34 was a mouse monoclonal antibody (Nichirei, Tokyo, Japan) at 1 : 100 dilution. The polyclonal antibody reactivity for VEGF with individual tissue sections was considered positive if equivalent staining was seen either in the membrane or the cytoplasm of more than 30% of the tumour cells. The number of CD34-positive vessels was counted in four randomly selected areas of a 1-mm^2 field and the average number was referred as the microvessel density.

Evaluation and statistical analysis

Concentration of VEGF, bFGF and PlGF in the sera of patients taken before biopsy was assessed by a commercially available sandwich ELISA (VEGF, IBL, Fujioka, Japan; bFGF and PlGF, R&D Systems, Minneapolis, MN, USA). The serum levels of each angiogenic factor were analysed with respect to (i) the correlation with microvessel density in the biopsy specimen and (ii) the difference between the relapse group and the no-relapse group. The serum VEGF level corrected for the number of platelet before biopsy was also included in the analysis. In addition, the correlation of the serum level of VEGF with (i) volume of the primary tumour and (ii) expression of VEGF in the biopsy specimens were analysed. The volume of the primary tumour was assessed upon the longitudinal and transverse images of magnetic resonance imaging that had been taken before biopsy, and calculated with the following formula: $\pi/6 \times \text{height} \times \text{width} \times \text{depth}$.

Spearman rank correlation test was used for the analysis of the correlation between the serum levels of each angiogenic factor and the microvessel density, between the serum VEGF level and the tumour volume, and between the serum VEGF level and patient age. The Mann–Whitney *U*-test was used for the comparative analysis of each angiogenic factor levels between the relapse group and no-relapse group and between the VEGF-positive group and the VEGF-negative group in evaluation of the biopsy specimen. Statistical significance was defined as $P<0.05$.

RESULTS

Clinical parameters of 16 patients with osteosarcoma

Table 1 summarises the clinical parameters of individual patients. The average serum levels and the 95% confidence interval (CI) of each angiogenic factor were following, VEGF; 1069.4 pg ml^{-1} (95% CI=551.0–2075.5 pg ml^{-1}), bFGF; 109.7 pg ml^{-1} (95% CI=61.1–196.8 pg ml^{-1}), PlGF; 60.0 pg ml^{-1} (95% CI=32.2–111.9 pg ml^{-1}). To determine the distribution of values, means and confidence intervals on the log-transformed data have been back-transformed to give estimates on the untransformed scale.

Two out of 16 patients had pulmonary metastases at the time of the diagnosis and five patients relapsed with pulmonary meta-stasis within 1 year after the onset of the treatments.

Serum angiogenic factors concentration and microvessel density

We first analysed the correlation between the serum levels of angiogenic factors and the microvessel density in the biopsy specimen of the primary tumour. As shown in Figure 1A, there was a significant correlation between serum VEGF level and vessel counts within the osteosarcoma tumour (Figure 1A, P=0.004; Spearman rank correlation). The correlation remained significant when the serum VEGF levels were corrected by platelet counts (Figure 1B, P=0.0057). In contrast, no statistically significant correlation was observed between the serum PlGF or serum bFGF level and microvessel density (Figure 1C, D, PlGF: P=0.169; bFGF: P=0.529). These results indicate that the serum VEGF level reflects the microvessel density of the primary osteosarcoma tumour. There was no correlation between the serum VEGF level and patient age (P=0.898).

Serum angiogenic factors concentration and early disease relapse

We next assessed the association between the serum angiogenic factor levels and early relapse of osteosarcoma. The serum VEGF level was significantly higher in the relapse group than that in the no-relapse group (Figure 2A, P=0.0009; Mann–Whitney *U*-test). The abovementioned mean value of serum VEGF (1069.4 pg ml^{-1}) was chosen as the cut-off category, yielding the sensitivity of 100% and the specificity of 88.9% for detecting early disease relapse. The significance was retained after the serum VEGF level was corrected for platelet counts (Figure 2B, P=0.0018). In contrast, there was no significant differences in the serum PlGF or serum bFGF level between the two groups (Figure 2C, D, PlGF: P=0.458; bFGF: P=0.397).

Table 1 Patients and clinical parameters

Case	Age	Gender	VEGF (pg ml^{-1})	Plt (10^4 p ml^{-1})	VEGF/Plt (pg per 10^6 Plt)	bFGF (pg ml^{-1})	PlGF (pg ml^{-1})	Vessel counts	Tumour volume (cm^3)	Pulmonary metastasis[a]
1	60	M	1200.0	17.9	1.34	11.6	17.2	84	150.8	–
2	10	F	503.5	24.8	0.25	153.7	187.0	53	134.3	–
3	25	F	498.5	20.3	0.24	321.4	35.6	33	88.0	–
4	10	M	521.3	20.4	0.25	41.2	324.5	18	380.1	–
5	20	F	412.3	21.7	0.19	175.4	124.8	52	329.7	–
6	9	F	250.1	24.2	0.1	300.8	62.4	24	49.9	–
7	13	F	156.2	54.9	0.02	314.5	18.7	28	67.7	–
8	52	M	253.2	22.7	0.11	156.2	22.7	30	162.8	–
9	14	M	512.3	45.4	0.11	177.4	270.3	28	120.8	–
10	18	M	1250.0	32	0.39	17.8	15.6	88	146.5	+ (10 months)[b]
11	16	M	3900.0	22.5	1.73	19.4	17.8	84	628.8	+ (11 months)[b]
12	10	F	4700.5	17.1	2.74	82.3	127.4	49	234.4	+ (0 months)[b]
13	11	M	5200.6	38.5	1.35	153.7	187.0	100	143.9	+ (4 months)[b]
14	14	M	5700.1	25.6	2.22	162.3	27.6	98	231.7	+ (5 months)[b]
15	60	M	4100.3	27.6	1.48	334.5	17.9	140	189.9	+ (3 months)[b]
16	14	M	3200.7	27.8	1.15	169.7	262.7	85	453.3	+ (0 months)[b]

[a]Relapse with pulmonary metastasis within 1 year after the onset of the treatment. [b]Relapse time with pulmonary metastasis from the onset of the treatment.

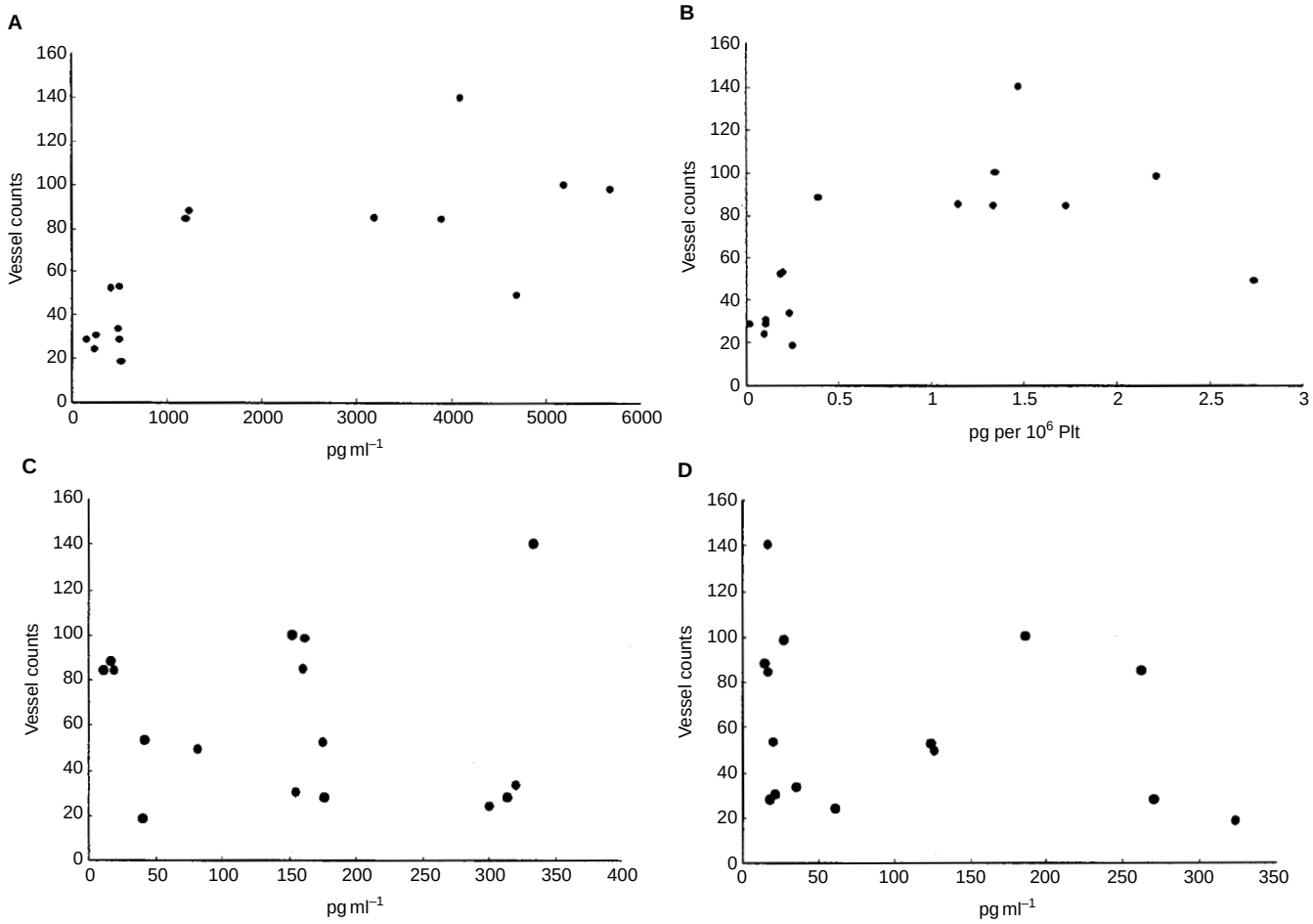

Figure 1 Relationship between microvessel count and serum angiogenic factors levels. Serum angiogenic factors levels against the vessel counts in 16 patients with osteosarcoma. (**A**) VEGF, (**B**) VEGF corrected for platelet counts (**C**) bFGF and (**D**) PlGF. The microvessel counts of the primary osteosarcoma tumours correlated with serum VEGF level (P=0.004). and serum VEGF/Plt level (P=0.0057). In contrast, no statistically significant correlation was observed between the serum PlGF or serum bFGF level and microvessel density.

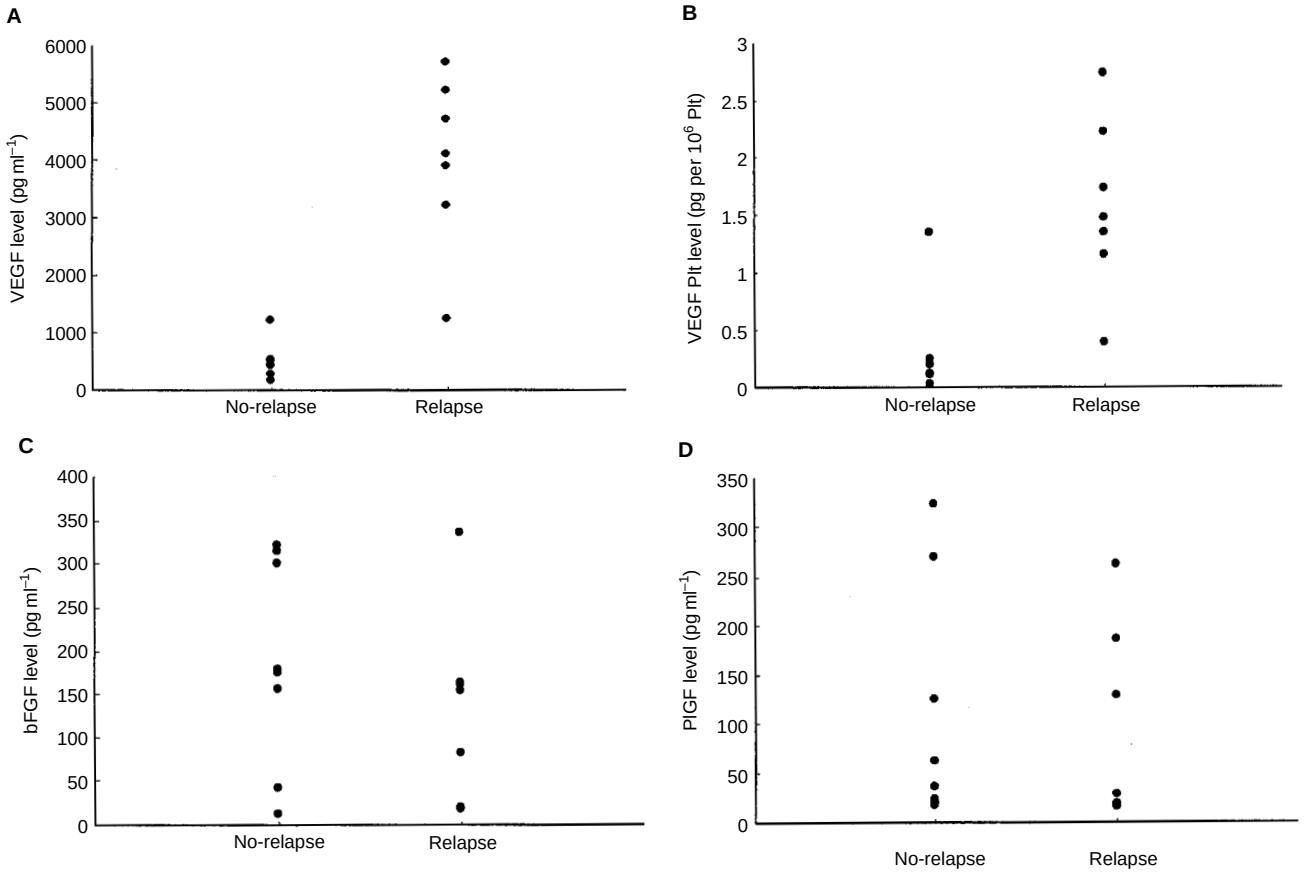

Figure 2 Relationship between the disease relapse and serum concentration of each angiogenic factor. The differences in each angiogenic factor level between the no-relapse and relapse group. The serum levels of VEGF and the serum levels of VEGF corrected for the count of platelet were significantly higher in the patients of the relapse group. In contrast, there was no significant differences in the serum PlGF or serum bFGF level between the two groups.

Serum VEGF levels, tumour volume and tissue VEGF expression

To clarify whether the serum level of VEGF is dependent on the quantitative or qualitative aspects of the primary tumour, we assessed the relationship between the serum VEGF level and the tumour volume as well as the tissue VEGF expression. As shown in Figure 3A, there was no significant correlation between the serum VEGF level and the tumour volume. In contrast, the serum VEGF levels were significantly higher in patients with a VEGF-positive tumour than those with a VEGF-negative tumour (Figure 3B, P=0.005). There was no significant differences in the tumour volume between the relapse and no-relapse groups (Figure 3C, P=0.064).

DISCUSSION

In the current study, we have found that increased pre-therapeutic serum levels of VEGF in 16 patients with osteosarcoma correlate with (i) high microvessel density of the primary tumour, (ii) relapse with pulmonary metastasis during the first year of treatment, and (iii) positive expression of tissue VEGF. In contrast, serum bFGF or PlGF failed to show a positive correlation with the microvessel density or a significant association with relapse of the disease. These findings suggest that the pre-therapeutic serum VEGF levels reflect the angiogenic property of primary tumour and may have a predictive value on early disease relapse of osteosarcoma.

It is known that the majority of patients with osteosarcoma have micrometastatic diseases on initial presentation (Goorin *et al*, 1985; Eliber and Rosen, 1989), leading to early clinical relapse of the tumour in approximately 50% of the patients despite introduction of neoadjuvant chemotherapy (Rosen *et al*, 1974; Eilber *et al*, 1987; Pratt *et al*, 1990; Souhami *et al*, 1997). This fact implies the importance of identifying patients at high risk of relapse early in the course of disease, to whom a more intense chemotherapy or other therapeutic modalities are applied. Accordingly, an attempt has been made to switch the chemotherapy regimen into an alternative one with intensification of reagents before surgery in patients who exhibit progressive disease during neoadjuvant chemotherapy (Wada *et al*, 1996; Meyer *et al*, 2001). It appears to be more ideal to find a biologic profile of osteosarcoma, available at diagnosis, which is linked to relapse of the disease and poor prognosis. Measurement of pre-therapeutic serum VEGF is advantageous for its simple and rapid procedure and provides a prognostic value consistent with immunohistochemical (Kaya *et al*, 2000) or genetical detection (Lee *et al*, 1999) of tissue VEGF in biopsy specimens.

Recently, expression of VEGF has been examined in malignant bone and soft tissue tumours other than osteosarcoma and there have been contradictory results. Whereas Yudoh *et al* (2001) found a significant correlation between tissue VEGF expression and poor prognosis in soft tissue sarcomas, Chao *et al* (2001) and Kuhnen *et al* (2000) failed to find such significance. Kawauchi *et al* (1999) documented no prognostic significance of VEGF expression in synovial sarcoma. In chondrosarcomas, expression of VEGF was associated the histological grade (Ayala *et al*, 2000). Because histological grade is the most important, generally accepted, prognostic factor in soft tissue sarcomas as well as in chondrosarcoma, the predictive value of VEGF may be less important in those tumours than that in conventional osteosarcomas that are exclusively high grade in histology.

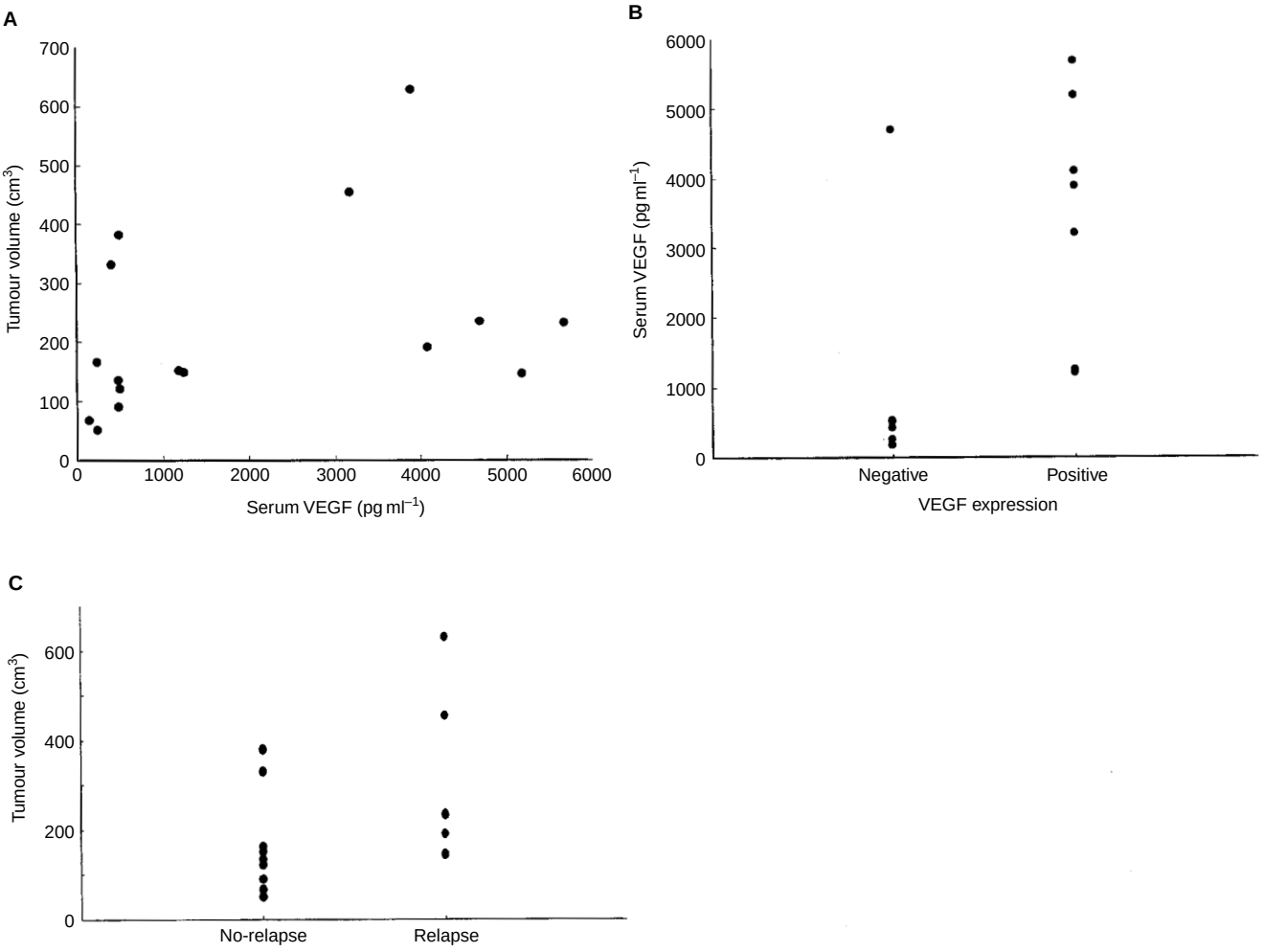

Figure 3 Relationship between the tumour volume or tumour VEGF expression serum VEGF level and disease relapse. (**A**) Relationship between the serum VEGF level and tumour volume. (**B**) Relationship between serum concentration of VEGF and *in vivo* expression of VEGF protein in the primary osteosarcoma tumour. (**C**) Relationship between the disease relapse and tumour volume.

Contrary to VEGF, serum bFGF or PlGF measurements failed to show a significant correlation with the microvessel density or association with relapse of the disease, emphasising the critical role of VEGF in angiogenesis of osteosarcoma. Such a predominant role of VEGF than other angiogenic factors has been shown by comparative studies in gastric carcinoma (Yoshikawa *et al*, 2000) and renal cell carcinoma (Edgren *et al*, 1999). On the other hand, bFGF but not VEGF has shown prognostic relevance for head and neck cancer, suggesting that the dependency of tumoral neovascularisation on angiogenic factors may vary between tumour types (Dietz *et al*, 2000).

Since the values of serum VEGF in drawn blood samples can be increased during clot formation (Banks *et al*, 1998; Webb *et al*, 1998; Salven *et al*, 1999; George *et al*, 2000; Lee *et al*, 2000), it is possible that increased serum VEGF levels seen in the present study may not be a true reflection of tumour angiogenic activity. However, correction of the VEGF levels by platelet counts did not impair the significance in correlation with microvessel density or association with early disease relapse. In addition, the increased serum VEGF levels reflected well with tissue expression of VEGF protein within the primary tumour. It should be noted, in this regard, that one patient exhibited high serum VEGF despite negative tissue VEGF expression. In this patient, we have observed significant reduction of serum VEGF after removal of the primary tumour (Kaya M *et al*, unpublished observation), suggesting a problem in tissue staining procedure or the quality of the tissue sections examined.

Because of the rarity of osteosarcoma, there is a difficulty in designing a prospective study with a large number of participants. Although the median value of serum VEGF (1069.4 pg ml^{-1}) chosen as the cut-off category yielded the sensitivity of 100% and the specificity of 88.9% for detecting early disease relapse, generalisation of this value requires additional prospective studies with large sample size as well as the sequential analysis of serum VEGF levels in individual patients at various points including the timing of disease relapse. In this sense, the present analysis serves as a pilot study with the strength in its prospective design and the uniformity of participants and treatment protocol.

In conclusion, the present study revealed the clinical significance of pre-therapeutic serum VEGF levels that were significantly higher in patients with osteosarcoma who relapsed during the first year of treatment, and also provided the basis to establish the anti-angiogenic principles for further therapy targeting patients at high risk of angiogenesis-dependent relapse of osteosarcoma.

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REFERENCES

Ayala G, Liu C, Nicosia R, Horowitz S, Lackman R (2000) Microvasculature and VEGF expression in cartilaginous tumors. *Hum Pathol* **31:** 341 – 346

Banks RE, Forbes MA, Kinsey SE, Stanley A, Ingham E, Walters C, Selby PJ (1998) Release of the angiogenic cytokine vascular endothelial growth factor (VEGF) from platelets: significance for VEGF measurements and cancer biology. *Br J Cancer* **77:** 956 – 964

Chao C, Al-Saleem T, Brooks JJ, Rogatko A, Kraybill WG, Eisenberg B (2001) Vascular endothelial growth factor and soft tissue sarcomas: tumor expression correlates with grade. *Ann Surg Oncol* **8:** 260 – 267

Dietz A, Rudat V, Conradt C, Weidauer H, Ho A, Moehler T (2000) Prognostic relevance of serum levels of the angiogenic peptide bFGF in advanced carcinoma of the head and neck treated by primary radiochemotherapy. *Head Neck* **22:** 666 – 673

Dvorak HF, Brown LF, Detmar M, Dvorak AM (1995) Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am J Pathol* **146:** 1029 – 1039

Edgren M, Lennernas B, Larsson A, Nilsson S (1999) Serum concentrations of VEGF and b-FGF in renal cell, prostate and urinary bladder carcinomas. *Anticancer Res* **19:** 869 – 873

Eilber F, Giuliano A, Eckardt J, Patterson K, Moseley S, Goodnight J (1987) Adjuvant chemotherapy for osteosarcoma: a randomized prospective trial. *J Clin Oncol* **5:** 21 – 26

Eliber FR, Rosen G (1989) Adjuvant chemotherapy of osteosarcoma. *Semin Oncol* **16:** 312 – 323

Folkman J (1971) Tumor angiogenesis: therapeutic implications. *N Engl J Med* **285:** 1182 – 1186

Folkman J (1972) Anti-angiogenesis: new concept for therapy of solid tumors. *Ann Surg* **175:** 4 – 6

Folkman J (1990) What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* **82:** 4 – 6

Folkman J (1995) Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med* **1:** 27 – 31

Ferrara N, Houck L, Jakeman L, Leung DW (1992) Molecular and biological properties of the vascular endothelial cell growth factor family of proteins. *Endoc Rev* **13:** 18 – 32

George ML, Eccles SA, Tutton MG, Abulafi AM, Swift RI (2000) Correlation of plasma and serum vascular endothelial growth factor levels with platelet count in colorectal cancer: clinical evidence of platelet scavenging? *Clin Cancer Res* **6:** 3147 – 3152

Goorin AM, Abelson HT, Frei III E (1985) Osteosarcoma: fifteen years later. *New Engl J Med* **313:** 1637 – 1642

Hara I, Miyake H, Yamanaka K, Hara S, Arakawa S, Kamidono S (1999) Expression of CD44 adhesion molecules in nonpapillary renal cell carcinoma and normal kidneys. *Urology* **54:** 562 – 566

Jin-no K, Tanimizu M, Hyodo I, Nishikawa Y, Hosokawa Y, Doi T, Endo H, Yamashita T, Okada Y (1998) Circulating vascular endothelial growth factor (VEGF) is a possible tumor marker for metastasis in human hepatocellular carcinoma. *J Gastroenterol* **33:** 376 – 382

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

Kandel J, Bossy-Wetze E, Radvanyi F, Klagsbrun M, Folkman F, Hanahan D (1991) Neovascularization is associated with a switch to the export of bFGF in the multistep development of fibrosarcoma. *Cell* **66:** 1095 – 1104

Kawauchi S, Fukuda T, Tsuneyoshi M (1999) Angiogenesis does not correlate with prognosis or expression of vascular endothelial growth factor in synovial sarcomas. *Oncol Report* **6:** 959 – 964

Kaya M, Wada T, Akatsuka T, Kawaguchi S, Nagoya S, Shindoh M, Higashino F, Mezawa F, Okada F, Ishii S (2000) Vascular endothelial growth factor (VEGF) expression in untreated osteosarcoma is predictive of pulmonary metastasis and poor prognosis. *Clin Cancer Res* **6:** 572 – 577

Kuhnen C, Lehnhardt M, Tolnay E, Muehlberger T, Vogt PM, Muller KM (2000) Patterns of expression and secretion of vascular endothelial growth factor in malignant soft-tissue tumours. *J Cancer Res Clin Oncol* **126:** 219 – 225

Kumar H, Heer K, Lee PW, Duthie GS, MacDonald AW, Greenman J, Kerin MJ, Monson JR (1998) Preoperative serum vascular endothelial growth factor can predict stage in colorectal cancer. *Clin Cancer Res* **4:** 1279 – 1285

Landriscina M, Cassano A, Ratto C, Longo R, Ippoliti M, Palazzotti B, Crucitti F, Barone C (1998) Quantitative analysis of basic fibroblast growth factor and vascular endothelial growth factor in human colorectal cancer. *Br J Cancer* **78:** 765 – 770

Lee JK, Hong YJ, Han CJ, Hwang DY, Hong SI (2000) Clinical usefulness of serum and plasma vascular endothelial growth factor in cancer patients: which is the optimal specimen?. *Int J Oncol* **17:** 149 – 152

Lee YH, Tokunaga T, Oshika Y, Suto R, Yanagisawa K, Tomisawa M, Fukuda H, Nakano H, Abe S, Tateishi A, Kijima H, Yamazaki H, Tamaoki N, Ueyama Y, Nakamura M (1999) Cell-retained isoforms of vascular endothelial growth factor (VEGF) are correlated with poor prognosis in osteosarcoma. *Eur J Cancer* **35:** 1089 – 1093

Macchiarini P, Fontanini G, Hardin MJ, Squartini F, Angeletti CA (1992) Relation of neovascularisation to metastasis of non-small-cell lung cancer. *Lancet* **340:** 145 – 146

Meyer WH, Pratt CB, Poquette CA, Rao BN, Parham DM, Marina NM, Rappo AS, Mahmoud HH, Jenkins JJ, Harper J, Meel M, Fletcher BD (2001) Carboplatin/Isofamide window therapy for osteosarcoma: results of the St Jude Children's Research Hospital OS-91 trial. *J Clin Oncol* **19:** 171 – 182

Oehler MK, Caffier H (2000) Prognostic relevance of serum vascular endothelial growth factor in ovarian cancer. *Anticancer Res* **20:** 5109 – 5112

Pratt CB, Champion JE, Fleming ID, Rao B, Kumar AP, Evans WE, Green AA, George S (1990) Adjuvant chemotherapy for osteosarcoma of the extrimity: long-term results of two consecutive prospective protocol studies. *Cancer* **65:** 439 – 445

Rosen G, Suwansirikul S, Kwon C, Tan C, Wu SJ, Beattie Jr EJ, Murphy ML (1974) High-dose methotrexate with citrovorum factor rescue and Adriamycin in childhood osteogenic sarcoma. *Cancer* **33:** 1151 – 1163

Salven P, Teerenhovi L, Joensuu H (1997) A high pretreatment serum vascular endothelial growth factor concentration is associated with poor outcome in non-Hodgkin's lymphoma. *Blood* **90:** 3167 – 3172

Salven P, Ruotsalainen T, Mattson K, Joensuu H (1998) High pre-treatment serum level of vascular endothelial growth factor (VEGF) is associated with poor outcome in small-cell lung cancer. *Int J Cancer* **79:** 144 – 146

Salven P, Orpana A, Joensuu H (1999) Leukocytes and platelets of patients with cancer contain high levels of vascular endothelial growth factor. *Clin Cancer Res* **5:** 487 – 491

Smith-McCune KK, Weidner N (1994) Demonstration and characterization of the angiogenic properties of cervical dysplasia. *Cancer Res* **54:** 800 – 804

Souhami RL, Craft AW, Van der Eijken JW, Nooij M, Spooner D, Bramwell VHC, Wierzbicki R, Malcolm AJ, Kirkpatrick A, Uscinska BM, Van Glabbeke M, Machin D (1997) Randomised trial of two regimens of chemotherapy in operable osteosarcoma: a study of the European osteosarcoma intergroup. *Lancet* **350:** 911 – 917

Takahashi Y, Kitadai Y, Bucana CD, Cleary KR, Ellis LM (1995) Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. *Cancer Res* **55:** 3964 – 3968

Wada T, Isu K, Takeda N, Usui M, Ishii S, Yamawaki S (1996) A preliminary report of neoadjuvant chemotherapy NSH-7 study in osteosarcoma: preoperative salvage chemotherapy based on clinical tumor response and the use of granulocyte colony-stimulating factor. *Oncology* **53:** 221 – 227

Webb NJ, Bottomley MJ, Watson CJ, Brenchley PE (1998) Vascular endothelial growth factor (VEGF) is released from platelets during blood clotting: implications for measurement of circulating VEGF levels in clinical disease. *Clin Sci (Lond)* **94:** 395 – 404

Weidner N, Semple JP, Welch WR, Folkman J (1991) Tumor angiogenesis and metastasis correlation in invasivebreast carcinoma. *N Engl J Med* **324:** 1 – 8

Weidner N, Carroll PR, Flax J, Blumenfeld W, Folkman J (1993) Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. *Am J Pathol* **143:** 401 – 409

Yoshikawa T, Tsuburaya A, Kobayashi O, Sairenji M, Motohashi H, Yanoma S, Noguchi Y (2000) Plasma concentrations of VEGF and bFGF in patients with gastric carcinoma. *Cancer Lett* **29:** 7 – 12

Yudoh K, Kanamori M, Ohmori K, Yasuda T, Aoki M, Kimura T (2001) Concentration of vascular endothelial growth factor in the tumour tissue as a prognostic factor of soft tissue sarcomas. *Br J Cancer* **84:** 1610 – 1615