Elevated Level of Plasma Basic Fibroblast Growth Factor in Multiple Myeloma Correlates with Increased Disease Activity

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Recent reports that bone marrow angiogenesis is increased in multiple myeloma prompted us to examine plasma concentrations of angiogenic growth factors and to elucidate their clinical and biological significance. In 45 cases including 36 cases of multiple myeloma and 9 cases of monoclonal gammopathies of undetermined significance (MGUS), plasma concentrations of basic fibroblast growth factor (FGF-2) and vascular endothelial growth factor (VEGF) were evaluated. FGF-2 was significantly elevated in 25 out of 45 (56%) of the patients with multiple myeloma compared with control subjects (median 9.01 pg/ml vs. 1.58 pg/ml, \( P \ll 0.0001 \)). The 25 cases were all active multiple myeloma, and none of the non-active myeloma and MGUS patients showed a high FGF-2 level. VEGF level was also elevated in 26 out of 45 patients (58%) compared with control subjects (median 42.0 pg/ml vs. 15.8 pg/ml, \( P \ll 0.0001 \) for VEGF). VEGF concentration was high in 20 active myelomas, but also in one non-active myeloma and five MGUS. Elevation of FGF-2 level was associated with β₂-microglobulin level, anemia and bone marrow plasma cell percentage, which represent disease activity. Interestingly, none of five Bence-Jones type myelomas, including four clinically active cases, revealed a high plasma FGF-2 level, while all of them showed a high VEGF level. In all five responders, the plasma FGF-2 levels were significantly decreased after chemotherapy. FGF-2 was immunohistochemically detected in the bone marrow myeloma cells of the patients with high plasma FGF-2 level. We conclude that plasma concentration of FGF-2 can be a useful indicator of disease activity.

Key words: Multiple myeloma — Basic fibroblast growth factor — Angiogenesis — Vascular endothelial growth factor — Disease activity

Multiple myeloma (MM) is one of the major hematologic malignancies, which mainly affects elderly people. In spite of intervention with conventional chemotherapy, the clinical course of the disease is progressive and exclusively fatal.1, 2) Growth factors are considered to play an important role in disease progression of patients with MM, and especially interleukin (IL)-6 has been identified as a crucial cytokine involved in expansion of the tumor clones.3) Recently, it was also reported that microvessel density (MVD) in the bone marrow of MM was increased, and bone marrow angiogenesis is assumed to play an important role in disease progression and clinical disease activity.4, 4) Fibroblast growth factors (FGFs) and vascular endothelial growth factor (VEGF) are pleiotropic growth factors that exert growth stimulation of normal and neoplastic cells. FGFs and VEGF are also known as major potent positive regulators of angiogenesis both in vitro and in vivo.7) These angiogenic factors have been indicated to play roles in the progression of several types of solid tumor. For example, elevated serum concentration of basic fibroblast growth factor or FGF-2 was reported to be associated with histological grade and stage of renal cell carcinoma.8) Recently, several reports have analyzed the biological role of FGF-2 and VEGF in hematologic malignancies.9–11) Aguayo et al. reported the elevated plasma concentration of these angiogenic factors and increased bone marrow angiogenesis in acute and chronic leukemias.12) Vacca et al. indicated association of increased bone marrow angiogenesis in chronic leukemias.12) Vacca et al. indicated association of increased bone marrow angiogenesis with disease activity and also showed elevated intracellular accumulation of FGF-2 in myeloma cells.13) It was also reported that VEGF was immunohistochemically detected in myeloma cells.14) More recently, simultaneous elevation of serum FGF-2 and VEGF levels was correlated with poor prognosis in non-Hodgkin’s lymphoma.15) Here we report that the plasma concentrations of FGF-2 and VEGF were frequently elevated in patients with MM. Elevation of FGF-2 level was observed only in clinically
active MM, but not in non-active MM and monoclonal gammopathies of undetermined significance (MGUS). We also found that FGF-2 is produced by myeloma cells.

**MATERIALS AND METHODS**

**Patients** A total of 45 Japanese patients at Keio University Hospital who fulfilled the South West Oncology Group (SWOG) diagnostic criteria for MM (*n*=36) and MGUS (*n*=9) were studied. Twenty-six cases were untreated, and the other 19 cases were treated, but blood and bone marrow samplings were conducted more than one month after the previous treatment. The patients were defined as active (*n*=33) or non-active (*n*=12), using the criteria of Vacca et al. Briefly, active were those: (a) at diagnosis, with symptomatic disease and an increase in the M-component level; (b) at relapse; (c) with rapidly progressive disease, characterized by severe bone pain, hypercalcemia and pancytopenia. Non-active were those in the off-treatment plateau phase of stable M-component levels with no clinical symptoms. All patients gave informed consent for the use of peripheral blood and bone marrow biopsy specimens.

**Blood sampling and measurement of plasma growth factors** Platelets contain large amounts of FGF-2 and VEGF, and these growth factors may be released during platelet activation. Venous blood samples were collected in tubes containing EDTA and were centrifuged (2000 g, 10 min) within 2 h after blood sampling and stored at −80°C until assayed. Plasma concentrations of FGF-2 and VEGF were analyzed using a sandwich ELISA technique (R&D Systems). Each value was calculated by averaging results for triplicate samples.

**Measurement of FGF-2 in the conditioned medium** Cells (5×10^5) were incubated with 1 ml of RPMI-1640 medium supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, UT) at 37°C in a humidified atmosphere with 5% CO₂.

**Reverse transcriptase-polymerase chain reaction (RT-PCR)** One microgram of total cellular RNAs were used for cDNA synthesis using reverse transcriptase (Super Script, Life Technologies) under the conditions recommended by the manufacturer. The primer sequences used for FGF-2 amplification were as follows. P1: (sense) CAAGCGGCTGTACTGCAA, and P2: (anti-sense) CCTTCATAGCCAGGTAAACGG. PCR reaction was performed using recombinant *Thermus aquaticus* DNA polymerase (Toyobo Co., Tokyo). Reaction conditions were 35 cycles of denaturation (94°C for 1 s), annealing (58°C for 15 s) and extension (72°C for 30 s). As an internal control, β-actin cDNA was also amplified simultaneously using a specific primer set (Toyobo). All products were separated on 1.5% agarose gel.

**Western blotting** Cell pellets of myeloma cell line were lysed in the sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 80 mM dithiothreitol, 0.01% bromophenol blue). Lysates (50 μg) were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto polyvinylidene fluoride membrane (Immobilon-P, Millipore Corp., Bedford, MA). The
membrane was probed with rabbit antihuman-FGF-2 polyclonal antibody (Santa Cruz) diluted 1:200 with phosphate-buffered saline (PBS)/0.1% Tween 20. Detection was performed using the enhanced chemiluminescence system ECL-plus (Amersham Pharmacia Biotech, Buckinghamshire, UK) according to the manufacturer’s instructions.

**Statistical analyses** For evaluation of plasma concentration of FGF-2 and VEGF, the Mann-Whitney U test was used. Association of elevated growth factor level with clinical parameters was evaluated by the Fisher exact test and by the $\chi^2$ test. Effects of treatment on growth factor concentrations were evaluated by the Wilcoxon test. The software package Stat-View 5.0 (SAS Institute, Inc., Cary, NC) was used for statistical analysis. Multivariate analysis for evaluation of correlations between FGF-2 level and clinical parameters, including age, sex, VEGF level, stage, disease activity, hemoglobin level, $\beta_2$M, bone marrow plasma cell percentage and serum calcium level was performed by logistic regression analysis using the SAS package. $P$ values $<0.05$ were considered as statistically significant.

**RESULTS**

**Elevated plasma concentration of angiogenic growth factors in multiple myeloma** Plasma concentrations of FGF-2 and VEGF were examined by ELISA in 45 MM and MGUS patients (Fig. 1). Median values of FGF-2 and VEGF levels are also shown in Fig. 1. Plasma concentration of FGF-2 in cases was significantly higher than that of normal control subjects ($P<0.0001$). The FGF-2 level of clinically active MM was significantly higher than that of the control group ($P<0.0001$) and that of non-active MM and MGUS ($P=0.0003$). There was no statistically significant difference of FGF-2 concentrations between non-active cases, including non-active MM and MGUS, and the control group ($P=0.18$). Plasma VEGF concentrations were also elevated in all cases compared with the control group ($P<0.0001$). The VEGF level in active MM was significantly higher than that of healthy control subjects ($P<0.0001$), but there was no statistically significant difference between active and non-active cases ($P=0.086$). The VEGF level of non-active MM and MGUS was significantly higher than that of the control group ($P=0.015$).

**Correlation between FGF-2 level and clinical parameters** To investigate the clinical significance of the elevated plasma FGF-2 level, the relationship between FGF-2 concentration and clinical parameters was analyzed (Table I). When the cut-off value was taken as 7.67 pg/ml, which is the highest value in the control group, plasma FGF-2 concentration was elevated in 25 of 45 cases (57%). In 33 cases with active MM, anemia, $\beta_2$M, immunoglobulin (Ig) subclass and bone marrow plasma cell percentage were also statistically significant factors in all cases. In 33 cases with active MM, anemia, $\beta_2$M and bone marrow plasma cell percentage were not statistically significant factors. Therefore, these factors were considered to relate to disease activity. Multivariate analysis by using a logistic regression model also indicated that disease activity was an independent factor correlating with plasma FGF-2 level ($P=0.0013$). Interestingly, plasma FGF-2 level was within...
### Table 1. Correlation between Plasma FGF-2 Concentration and Clinical Parameters

| Parameters | Total No. | High FGF-2 | Low FGF-2 | P value$^b$ |
|------------|-----------|------------|-----------|-------------|
| Overall    | 45        | 25         | 20        |             |
| Age        |           |            |           |             |
| 60 yr≤     | 17        | 11         | 6         | 0.37        |
| 60 yr>     | 28        | 14         | 14        |             |
| Gender     |           |            |           |             |
| male       | 25        | 12         | 13        | 0.37        |
| female     | 19        | 13         | 7         |             |
| MGUS or    |           |            |           |             |
| multiple myeloma | 36 | 25 | 11 | 0.0002 |
| Durie-Salmon stage |   |       |       |            |
| I+II       | 10        | 5          | 5         | 0.22        |
| III        | 26        | 20         | 6         |             |
| Disease activity$^a$ |   |       |       | >0.0001    |
| active     | 33        | 25         | 8         |             |
| non-active | 12        | 0          | 12        |             |
| Anemia     |           |            |           |             |
| Hb<10 g/dl | 20        | 16         | 4         | 0.006       |
| Hb≥10 g/dl | 25        | 9          | 16        |             |
| Serum creatinine |       |       |       |             |
| >2.0 mg/dl | 6         | 4          | 2         | 0.68        |
| ≤2.0 mg/dl | 39        | 21         | 18        |             |
| β₂-microglobulin |       |       |       |             |
| 2.3 mg/liter< | 29 | 21 | 8 | 0.001 |
| 2.3 mg/liter≥ | 15 | 3 | 12 | |
| Serum calcium |       |       |       |             |
| 9.8 mg/dl< | 11        | 9          | 2         | 0.086       |
| 9.8 mg/dl≥ | 32        | 16         | 16        |             |
| C-reactive protein |       |       |       | >0.99       |
| 0.15 mg/liter< | 19 | 12 | 7 | |
| 0.15 mg/liter≥ | 21 | 13 | 8 | |
| Immunoglobulin subclass |       |       |       |             |
| IgG        | 28        | 17         | 11        | 0.03        |
| IgA        | 7         | 6          | 1         |             |
| IgD        | 4         | 2          | 2         |             |
| IgM        | 1         | 0          | 1         |             |
| Light chain |           |            |           |             |
| κ          | 27        | 12         | 15        | 0.06        |
| λ          | 18        | 13         | 5         |             |
| Bone marrow plasma cell |       |       |       |             |
| 10%≤       | 26        | 20         | 6         | 0.004       |
| 10%>       | 17        | 5          | 12        |             |
| Bone marrow micro vessel density |       |       |       | >0.99       |
| 120/mm²≤  | 10        | 7          | 3         |             |
| 120/mm²>  | 10        | 7          | 3         |             |

*a* Vacca’s criteria. See “Materials and Methods.”

*b* The χ² test was used for immunoglobulin subclass, and the Fisher exact test was used for other clinical parameters.
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the normal range in all of the Bence-Jones protein (BJP)-
type MM.

Plasma VEGF concentration was also elevated in 26 out
of 45 of the patients (58%), in 20 out of 33 active MM
(60.6%) and in 6 of 12 non-active MM and MGUS (50%).
No statistically significant correlation was seen with any
of the clinical parameters using the Fisher exact test and
the χ² test (data not shown). As mentioned earlier, elevation
of plasma VEGF level likely occurs in the very early
stages of myelomagenesis, and therefore it is not surpris-
ing that no significant difference was seen among MM
patients. Contrary to the case of FGF-2, all of the patients
with BJP-type MM showed elevated VEGF. Therefore,
VEGF may be a key factor that regulates the progression
of BJP-type myeloma cells.

Change of plasma growth factor concentrations of five
responders after chemotherapy is shown in Fig. 2. FGF-2
level was clearly decreased in all five responders after
chemotherapy. However, the VEGF level was not signifi-
cantly decreased after treatment.

An increased activity of angiogenesis in the bone mar-
rrow of the patients with MM has been reported (Refs. 6
and 18 and Wenlin et al., in preparation). It is speculated
that up-regulation of plasma growth factor concentration
may lead to increased marrow angiogenesis. In our study,
bone marrow biopsy specimens from twenty patients were
available. As shown in Table I, however, there is no sig-
nificant relationship between plasma FGF-2 level and
MVD. No correlation of plasma VEGF level with bone
marrow MVDs was observed, either (data not shown).

**Product of FGF-2 by myeloma cells** The next impor-
tant question is, which cells produce FGF-2? It is espe-
cially of interest whether myeloma cells produce FGF-2.
Expression of FGF-2-gene product was examined using
four multiple myeloma lines. As shown in Fig. 3A, FGF-2
transcripts were detected in three myeloma cell lines,
U266, KMS12-BM and RPMI8226 cells, by RT-PCR
analysis. Immunoblot analysis also revealed a 22.5-kDa
band in U266 cells. FGF-2 does not contain a signal pep-
tide sequence, and the mechanism of excretion of FGF-2
from the cell is still unclear. To elucidate whether FGF-2
produced by myeloma cells is secreted, the concentration
of FGF-2 in the culture medium was also examined. FGF-
2 was detected in the conditioned medium of U266 cells,
but it was below the limit of detection in the medium
of HS-Sultan cells, which do not produce FGF-2 (Fig. 3, A
and B). To examine production of FGF-2 by primary
myeloma cells, FGF-2 was immunohistochemically stained
using bone marrow biopsy specimens. Representative
cases are shown in Fig. 4, in which FGF-2 was detected
in the myeloma cells of the patients with high plasma
FGF-2 level (Fig. 4c). There was no significant staining
in this case using non-immune rabbit serum (data not
shown). When the bone marrow specimens of active MM
patients with low FGF-2 level were also examined, no
significant staining of FGF-2 was detected in myeloma
cells (Fig. 4d).

**DISCUSSION**

Recent advances in genetic as well as biological analy-
oses have revealed that multiple myeloma evolves via a
multistep process involving alterations in various onco-

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Fig. 2. Plasma FGF-2 and VEGF in serial samples from five
responding patients. Plasma concentrations before and after che-
motherapy are shown. Median pre-treatment and post-treatment
levels of FGF-2 were 27.1 pg/ml and 3.19 pg/ml, respectively
(\(P=0.080\) by the Wilcoxon test). Those of VEGF were 48.0 pg/
ml and 80.6 pg/ml, respectively (\(P=0.89\) by the Wilcoxon test).
genes and tumor suppressor genes. Cytokines and growth factors also play important roles in myeloma cell growth in an autocrine or a paracrine manner. Recently, it has also been reported that bone marrow MVD significantly increases in active MMs. Thus, we examined the plasma concentrations of angiogenic growth factors, and found significant elevations of FGF-2 and VEGF in 56–58% of active MMs. As shown in Fig. 1, elevation of FGF-2 level is observed specifically in active MM, while increase of VEGF concentration occurs not only in active MM, but also in non-active MM and MGUS. Therefore, it is speculated that FGF-2 may predominantly increase at the transition from non-active to active disease, while VEGF may be elevated in the earlier clinical course, such as MGUS or non-active MM. As shown in Table I, elevation of FGF-2 concentration was associated with advanced anemia, high B2M level and high plasma cell percentage in the bone marrow, which also indicate disease activity. In addition, multivariate analysis showed the association of disease activity with elevated FGF-2 level. As shown in Fig. 2, plasma FGF-2 levels were significantly decreased after chemotherapy in responders. This fact also indicates the association of FGF-2 levels with disease activity. In our study, FGF-2 level was low in all of the BJP-type MMs (Table I). Although the reason is unclear, the association between low FGF-2 level and BJP-type does not relate to disease activity, because the FGF-2 level was low even in all four clinically active BJP-type MMs.

The exact role of FGF-2 in the progression of MM is not clear. To elucidate the effect of FGF-2 on bone marrow angiogenesis, the relationship between plasma growth factor level and MVD in the bone marrow was examined (Table I). However, we could not find a significant correlation. In addition, the MVD of two active MMs was within the normal range, despite their high plasma FGF-2 level (data not shown). It was also reported that the patients with chronic lymphocytic leukemia show a high plasma FGF-2 level, but their bone marrow MVD is com-

Fig. 4. Immunohistochemical detection of FGF-2 in the bone marrow of active myeloma cases. H&E staining (a, b) and immunohistochemical staining for FGF-2 (c, d) of the two representative positive and negative cases were shown. (a, c) An IgA-type active myeloma patient who revealed high plasma FGF-2 level (278 pg/ml). Myeloma cells were positively stained with anti-human FGF-2 polyclonal antibody. (b, d) A Bence-Jones-type active myeloma whose plasma concentration of FGF-2 was 1 pg/ml. There was no significant staining of myeloma cells.
parable with that of control subjects.\textsuperscript{12} These results suggested that elevated plasma FGF-2 may contribute to the progression of B-cell malignancies not only by increasing bone marrow angiogenesis, but also via some other unknown mechanism. A recent report showed that 20–30\% of MM patients carry chromosomal translocations of the immunoglobulin heavy-chain (IgH) locus on chromosome 14q32 with FGF receptor-3 (fgfr3) on 4p16 locus. Some of the translocated fgfr3 have activating mutations within their coding sequence.\textsuperscript{21, 22} These data provide a strong link between previous reports of the expression of the gene for FGF receptor-1, a high-affinity receptor for FGF-2, in myeloma cell lines.\textsuperscript{23} Activation of FGF receptors on myeloma cells may be important for progression of myeloma cells. The origin of FGF-2 is of great interest. FGF-2 is ubiquitously expressed in various tissues, especially in mesenchymal cells. The concentration of FGF-2 is much higher in bone marrow than in peripheral blood.\textsuperscript{24} Therefore, it is speculated that FGF-2 is released from bone marrow interstitial cells or bone matrix.\textsuperscript{20} In our study, FGF-2-gene products were also detected in three myeloma cell lines and also in bone marrow biopsy specimens. Otsuki et al. reported the expression of FGF-2 transcripts in all six cell lines which they established.\textsuperscript{25} Recently, it has been reported that myeloma cells produce VEGF and hepatocyte growth factor (HGF).\textsuperscript{14, 26} Therefore, myeloma cells per se are considered to be one of the sources for these growth factors.

In conclusion, we found that plasma level of FGF-2 is significantly elevated in clinically active MM, and this should be a good indicator for disease activity.

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REFERENCES

1) Oken, M. M. Multiple myeloma: prognosis and standard treatment. Cancer Invest., 15, 57–64 (1997).

2) Alexanian, R. and Dimopoulos, M. The treatment of multiple myeloma. N. Engl. J. Med., 330, 484–489 (1994).

3) Kawano, M., Hirano, T., Matsuda, T., Taga, T., Horii, Y., Iwato, K., Asaoku, H., Tang, B., Tanabe, O., Tanaka, H., Kuramoto, A. and Kishimoto, T. Autocrine generation and essential requirement of BSF-2/IL-6 for human multiple myeloma. Nature, 332, 83–85 (1988).

4) Vacca, A., Ribiatti, D., Roncali, L., Ranieri, G., Serio, G., Silvestris, F. and Dommacco, F. Bone marrow angiogenesis and progression in multiple myeloma. Br. J. Haematol., 87, 503–508 (1994).

5) Vacca, A., Ribiatti, D., Roncali, L. and Dommacco, F. Angiogenesis in B cell lymphoproliferative diseases. Biological and clinical studies. Leuk. Lymphoma, 20, 27–38 (1995).

6) Vacca, A., Loreto, M. D., Ribiatti, D., Stefano, R. D., Gadaleta-Caldarola, G., Lodice, G., Caloro, D. and Dommacco, F. Bone marrow of patients with active myeloma: angiogenesis and plasma cell adhesion molecules LFA-1, VLA-4, LAM-1, and CD44. Am. J. Hematol., 50, 9–14 (1995).

7) Asahara, T., Bauters, C., Zheng, L. P., Takeshita, S., Bunting, S., Ferrara, N., Symes, J. F. and Isner, J. M. Synergistic effect of vascular endothelial growth factor and basic fibroblast growth factor on angiogenesis in vivo. Circulation, 92, 365–371 (1995).

8) Fujimoto, K., Ichimori, Y., Kakizoe, T., Okajima, E., Sakamoto, H., Sugimura, T. and Terada, M. Increased serum levels of basic fibroblast growth factor in patients with renal cell carcinoma. Biochem. Biophys. Res. Commun., 180, 386–392 (1991).

9) Dirix, L. Y., Vermeulen, P. B., Pawinski, A., Prove, A., Benoy, I., De Pooter, C., Martin, M. and Van Oosterom, A. Elevated levels of the angiogenic cytokines basic fibroblast growth factor and vascular endothelial growth factor in sera of cancer patients. Br. J. Cancer, 76, 235–243 (1997).

10) Menzel, T., Rahman, Z., Calleja, E., White, K., Wilson, E. L., Wieder, R. and Gabrilove, J. Elevated intracellular level of basic fibroblast growth factor correlates with stage of chronic lymphocytic leukemia and is associated with resistance to fludarabine. Blood, 87, 1055–1063 (1996).

11) Salven, P., Teerenhovi, L. and Joensuu, H. A high pretreatment serum basic fibroblast growth factor concentration is an independent predictor of poor prognosis in non-Hodgkin’s lymphoma. Blood, 94, 3334–3339 (1999).

12) Aguayo, A., Kantarjian, H., Manshouri, T., Gidel, C., Estey, E., Thomas, D., Koller, C., Estrov, Z., O’Brien, S., Keating, M., Freireich, E. and Albitar, M. Angiogenesis in acute and chronic leukemias and myelodysplastic syndromes. Blood, 96, 2240–2245 (2000).

13) Vacca, A., Ribiatti, D., Presta, M., Minischetti, M., Iurlaro, M., Ria, R., Albini, A., Bussolino, F. and Dommacco, F. Bone marrow neovascularization, plasma cell angiogenic potential, and matrix metalloproteinase-2 secretion parallel progression of human multiple myeloma. Blood, 93, 3064–3073 (1999).

14) Bellamy, W. T., Richter, L., Frutiger, Y. and Grogan, T. M.
Expression of vascular endothelial growth factor and its receptors in hematopoietic malignancies. *Cancer Res.*, **59**, 728–733 (1999).

15) Salven, P., Orpana, A., Teerenhovi, L. and Joensuu, H. Simultaneous elevation of 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 (2000).

16) Durie, B. G. M. and Salmon, S. E. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. *Cancer*, **36**, 842–854 (1975).

17) Banks, R. E., Forbes, M. A., Kinsey, S. E., Stanley, A., Ingham, E., Walters, C. and Selby, P. J. 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 (1998).

18) Perez-Atyde, A. R., Sallan, S. E., Tedrow, U., Connors, S., Allred, E. and Folkman, J. Spectrum of tumor angiogenesis in the bone marrow of children with acute lymphoblastic leukemia. *Am. J. Pathol.*, **150**, 815–821 (1997).

19) Tricot, G. New insights into role of microenvironment in multiple myeloma. *Lancet*, **355**, 248–250 (2000).

20) Rajkumar, S. V., Leong, T., Roche, P. C., Fonseca, R., Dispensieri, A., Lacy, M. Q., Lust, J. A., Witzig, T. E., Kyle, R. A., Gertz, M. A. and Greipp, P. R. Prognostic value of bone marrow angiogenesis in multiple myeloma. *Clin. Cancer Res.*, **6**, 3111–3116 (2000).

21) Chesi, M., Nardini, E., Brents, L. A., Schrock, E., Ried, T., Kuehl, M. W. and Bersagel, P. L. Frequent translocation t(4;14)(p16.3;q32.3) in multiple myeloma is associated with increased expression and activating mutations of fibroblast growth factor receptor 3. *Nat. Genet.*, **16**, 260–264 (1997).

22) Ploiwright, E. E., Li, Z., Bergsagel, L., Chesi, M., Barber, D. L., Branch, D. R., Hawley, R. G. and Stewart, A. K. Ectopic expression of fibroblast growth factor receptor 3 promotes myeloma cell proliferation and prevents apoptosis. *Blood*, **95**, 992–998 (2000).

23) Hattori, Y., Odagiri, H., Katoh, O., Sakamoto, H., Morita, T., Shimotohno, K., Tobinai, K., Sugimura, T. and Terada, M. K-sum-related gene, N-sum, encodes fibroblast growth factor receptor and is expressed in T-lymphocytic tumors. *Cancer Res.*, **52**, 3367–3371 (1992).

24) Raimondo, F. D., Azzaro, M. P., Palumbo, G. A., Bagnato, S., Giustolisi, G., Floridia, P. M., Sortino, G. and Giustolisi, R. Angiogenic factors in multiple myeloma: higher levels in bone marrow than in peripheral blood. *Haematologica*, **85**, 800–805 (2000).

25) Otsuki, T., Yamada, O., Yata, K., Sakaguchi, H., Kurebayashi, J., Nakazawa, N., Taniwaki, M.,Yawata, Y. and Ueki, A. Expression of fibroblast growth factor and FGFR-receptor family genes in human myeloma cells, including lines possessing t(4;14)(q16.3;q32.3) and FGFR3 translocation. *Int. J. Oncol.*, **15**, 1205–1212 (1999).

26) Borset, M., H.-Hansen, H., Seidel, C., Sundan, A. and Waage, A. Hepatocyte growth factor and its receptor c-MET in multiple myeloma. *Blood*, **88**, 3998–4004 (1996).

27) Sezer, O., Jakob, C., Eucker, J., Niemoller, K., Gatz, F., Wernicke, K.-D. and Possinger, K. Serum levels of the angiogenic cytokines basic fibroblast growth factor, vascular endothelial growth factor and hepatocyte growth factor in multiple myeloma. *Eur. J. Haematol.*, **66**, 83–88 (2001).