# Expression of Angiogenic Factors in Myeloma Cells

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**ABSTRACT.** To clarify the cellular biological roles of angiogenic factors in myeloma cells, we studied the gene expression of various angiogenic factors, including vascular endothelial growth factors (VEGFs), in 10 human myeloma cell lines (MMCLs) using the multiplex-reverse transcriptase-polymerase chain reaction (MP-RT-PCR). The VEGF-A secretion into culture medium, the effects of anti-VEGF-monoclonal antibody (MoAb) and interferon (IFN)-$\alpha$ on myeloma cells were also studied. The following results were obtained. (1) VEGF-A, -B, and -D were expressed in all the lines studied and that the expression levels of VEGF-A, and -B were significantly higher in the MMCLs than in the non-myelomatous hematological cell lines (nMCLs). Three out of ten MMCLs showed VEGF-C expression, but none of the nMCLs did. VEGF receptor-1 (VEGFR1) was expressed in all the lines, VEGF receptor-2 (VEGFR2) was found in three of the ten MMCLs, and two of the nMCLs. (2) VEGF-A production measured by the enzyme-linked immunosorbent assay (ELISA) was significantly higher in the MMCLs than in the nMCLs. (3) Anti-VEGF-MoAb resulted in growth inhibition in some myeloma cell lines. (4) IFN-$\alpha$ caused growth inhibition in KMS-21BM cells *in vitro*, but VEGF-A secretion increased. Based on these results, it is suggested that the angiogenic factors studied play important roles in myeloma cell proliferation, and that anti-angiogenic therapies may be useful not only for solid tumors but also for hematological malignancies, especially myelomas.

**Key words: myeloma — angiogenic factors — VEGF — IFN-$\alpha$**

Angiogenesis is a necessary step in the progression of tumor growth, invasion, and metastasis.[1-4] Although the relation between tumors and angiogenesis has been thoughly investigated, little is known about angiogenesis in response to hematological malignancies. Recently, Bellamy *et al* reported that the vascular endothelial growth factor (VEGF) gene was expressed in human hematopoietic tumor cell lines derived from leukemia, lymphoma and multiple myeloma.[5] In addition, the gene expression level of VEGF has been found to be closely associated with the induction of neovascularization and to correlate with tumor growth, metastatic potential and vascular permeability in experimental mouse tumors.[6]

As multiple myeloma often manifests nodular progression in bone marrow and metastasis to other organs and tissues, such as the pleural and abdominal cavities, bone, skin, and breast, myeloma cells may possess cellular characteristics similar to those of solitary tumor cells. Regarding myeloma and angiogenesis, there have been several studies reporting that bone marrow

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neoangiogenesis in patients with myeloma paralleled tumor progression and was associated with a poor prognosis, suggesting an angiogenesis-dependent regulation of disease activity.[7–10]

In this study, we examined the gene expression of four VEGF families (VEGF-A, -B, -C, and -D) and their receptors in human myeloma cell lines and compared it with that of other hematological malignant cell lines. In addition, we analyzed the effects of anti-VEGF-A monoclonal antibody (MoAb) on the proliferation of myeloma cells and the effects of interferon (IFN)-$\alpha$ on production of VEGF-A from myeloma cells.

## MATERIAL AND METHODS

### *Cell lines and cultivation.*

Ten human myeloma cell lines (MMCLs) and seven non-myelomatous hematological cell lines (nMCLs) were used in this study. Eight of the MMCLs, excluding the u266 and RPMI 8226 lines, were established at Kawasaki Medical School between 1982 and the present.[11–12] All the cell lines were cultured in RPMI1640 medium with 10% fetal bovine serum and 100 $\mu$g/ml of kanamycin in a humidified atmosphere with 5% $CO_2$ at 37°C.

### *Total RNA extraction and first strand cDNA synthesis*

Total cellular RNAs from each cell line were extracted using a TRIzol™ reagent (GIBCO BRL Life Technologies, Gaithersburg, MD), according to the manufacturer's instructions. Then 1 $\mu$g of total RNA and oligo(dT)18 primer (final concentration 1 $\mu$M) in 12.5 $\mu$l of diethyl-pyrocarbonate (DEPC)-treated $H_2O$ were heated to 70°C for 2 min followed by cooling on ice for 1 min. cDNA synthesis was initiated using 200 units of recombinant MMLV (Molodey-Murine Leukemia Virus) reverse transcriptase (Clontech Lab. Inc., Palo Alto, CA) under conditions recommended by the manufacturer, and the reaction was allowed to proceed at 42°C for 60 min. The reaction was terminated by heating at 94°C for 5 min, and then it was diltued to a final volume of 100 $\mu$l by adding 80 $\mu$l of DEPC-treated $H_2O$.

### *Multiplex-reverse transcriptase-polymerase chain reaction (MP-RT-PCR) and measurement of relative gene expression levels for angiogenic factors.*

The MP-RT-PCR was applied to examine the gene expression levels for the angiogenic factors and their receptor genes listed in Table 1. To amplify $\beta$-actin, the housekeeping control gene, and the target gene in a single reaction, the MP-RT-PCR was performed. The ratios of primer sets between the target gene and $\beta$-actin are also shown in Table 1. These ratios and PCR cycles were determined so as to amplify both products logarithmically and in relatively similar amounts. The detailed MP-RT-PCR procedure has been reported previously.[13] After visualization of MP-RT-PCR product electrophoresis on a 1.2% agarose gel stained with ethidium bromide, gel images were obtained using the FAS-II UV image analyzer (TOYOBO Co.Ltd., Tokyo, Japan), and the densities of the products were quantitated using Quantity One version 2.5 (PDI Inc., Huntington Station, NY). The relative expression level (REL) of each target gene in individual cell lines was calculated as the density of the product of the target gene divided by the density of the product of the $\beta$-actin gene derived from the same MP-RT-PCR.

TABLE 1. The primer sequences and PCR strategy used in this study

| Gene | Primer sequences (F, forward primer ; R, reverse primer) | Temperature (°C) | Product size (bp) | β-actin : target gene ratio |
|---|---|---|---|---|
| VEGF-A | F 5'-GCAGAATCATCACGAAGTGG-3' ; R 5'-GCATGGTGATGTTGGACTCC-3' | 58 | 212 | 0.8:2.0 |
| VEGF-B | F 5'-CCTTGACTGTGGAGCTCATG-3' ; R 5'-TGTCTGGCTTCACAGCACTG-3' | 60 | 246 | 0.7:2.0 |
| VEGF-C | F 5'-AGACTCAATGCATGCCACG-3' ; R 5'-TTGAGTCATCTCCAGCATCC-3' | 57 | 435 | 0.3:2.0 |
| VEGF-D | F 5'-GCTGTTGCAATGAAGAGAGC-3' ; R 5'-TCTTCTGTTCCAGCAAGTGG-3' | 56 | 313 | 0.2:2.0 |
| HGF | F 5'-GATCATCAGACACCACACCG-3' ; R 5'-CAACGCTGACATGGAATTCC-3' | 57 | 281 | 0.4:2.0 |
| FGF-2 | F 5'-CAAGCGGCTGTACTGCAA-3' ; R 5'-CCTTCATAGCCAGGTAACGG-3' | 58 | 173 | 0.4:2.0 |
| FGF-4 | F 5'-CTCTCGGCTAGCATCCTCAG-3' ; R 5'-GAGGACTTCAACACCGCAC-3' | 55 | 105 | 0.3:2.0 |
| Ang-1 | F 5'-AACACGATGGCAACTGTCG-3' ; R 5'-GGTTCCTATCTCCAGCATGG-3' | 56 | 251 | 0.5:2.0 |
| Ang-2 | F 5'-GCCACAACCATGATGATCC-3' ; R 5'-TTCTTGGTTGTGACAGCAGC-3' | 58 | 340 | 0.4:2.0 |
| VEGFR-1/Flt1 | F 5'-AAGAGAGCTTCCGTAAGGCG-3' ; R 5'-GCATCCTCTTCAGTTACGTCC-3' | 56 | 380 | 0.2:2.0 |
| VEGFR-2/KDR | F 5'-CCTTCTTCGAAGCATCAGC-3' ; R 5'-AGAGATTCCATGCCACTTCC-3' | 56 | 230 | 0.2:2.0 |
| VEGFR-3/Flt4 | F 5'-AGTCACACGTCATCGACACC-3' ; R 5'-CTTCCTGTTGACCAAGAGCG-3' | 61 | 344 | 0.4:2.0 |
| Tie1 | F 5'-GTTCAGTGGTTGTGTCTGCC-3' ; R 5'-TCAGCTGTGGTCTTCTCTGG-3' | 60 | 243 | 0.4:2.0 |
| Tek | F 5'-TCTCTGTGGAGTCAGCTTGC-3' ; R 5'-AGGAAGGAAGCTTGTTGACG-3' | 57 | 351 | 0.2:2.0 |
| CD31 | F 5'-CAAGAGTGAACTGGTCACCG-3' ; R 5'-ACTTTGCACGTGTAGTTGCC-3' | 58 | 352 | 0.2:2.0 |
| β-actin | F 5'-TGACGGGGTCACCCACACTGTGCCCATCTA-3'<br>R 5'-CTAGAAGCATTTGCGGTGGACGATGGAGGG-3' | | 661 | |

The abbreviations used in this table are : VEGF,vascular endothelial growth factor; HGF,hepatocyte growth factor; FGF,fibroblast growth factor; Ang,angiopoetin; VEGFR,vascular endothelial growth factor receptor.

### *Measurement of VEGF-A in cultured medium*

The concentration of VEGF-A in cultured medium was measured by the enzyme-linked immunosorbent assay (ELISA) method (Otsuka Assay Laboratory, Tokushima, Japan), which has been reported previously.[14] After cultivation of $50\times10^4$ cells in a 35 mm culture dish with 2 ml medium for 48 hours, cells were counted and supernatant was applied for the ELISA assay. The production of VEGF-A was calculated as the elevation of the VEGF-A concentration for 48 hours divided by increasing cell numbers for 48 hours (expressed as "pg/$10^4$ proliferated cells/48 hours"). Then the production between MMCLs and nMCLs was compared.

### *Effects of anti-VEGF-A-MoAb*

The anti-VEGF-A MoAb was kindly provided by the Bioscience Research Department, Tsukuba Research Laboratory, Toagosei Co., Ltd., Tsukuba, Ibaraki, Japan.[15-16] First, 10 to $30\times10^4$ cells from the KMM-1, KMS-18, KMS-20, KMS-21PE and KMS-21BM cell lines were cultured with 0 (control), 50, 100 and 200 μg/ml of anti-VEGF-A MoAb for four days. Then, cells were counted afer staining with trypan-blue. The growth rate was expressed as the percentage of the control. In addition, the correlation between the growth rate of 200 μg/ml of anti-VEGF-A MoAb and the REL of the VEGF receptors (VEGFRs) was statistically examined.

### *Effects of IFN-α on cell growth and VEGF-A production*

KMS-21BM myeloma cell lines were cultured with 1,000 U/ml of recombinant IFN-α-2b, Intron A (kindly provided by Schering-Plough K.K., Osaka, Japan) for two days, and cell numbers were expressed as the percentage of the control. The viability of cells was determined by trypan blue exclusion. VEGF-A was measured using the method described above.

### *Statistical analysis*

The Mann-Whiney test was used to analyze statistically the RELs of angiogenic factor-genes and their receptor genes, and the production and release of VEGF-A into the culture medium by MMCLs and nMCLs. The growth

inhibitory effects of anti-VEGF-A MoAb on the myeloma cell lines were analyzed by Fisher's PLSD test. The growth inhibitory effects of IFN-$\alpha$ on these cells were analyzed by Student's F test.

## RESULTS

### *gene expression of the angiogenic factors and their receptors*

As shown in Fig 1, which shows a gel image of the MP-RT-PCR, all of the lines studied expressed VEGF-A, -B and -D gene. Interestingly, although VEGF-C was expressed in three out of the ten MMCLs studied, it was not detected in the nMCLs. The expression of VEGF-B was significantly higher in the MMCLs than in the nMCLs. The expression of VEGF-A and -D tended to be higher in the MMCLs, although the expression was not statistically significant. However, the expression of three VEGFRs varied from line to line. Angiopoietin (Ang)-1, Ang-2 and CD31 gene expression were detected in all the lines studied. Tie-1 and Tek, receptors for Ang-1 and Ang-2, were sporadically expressed in the MMCLs and nMCLs. Fig 2 shows that VEGF-B and -C were highly expressed in the MMCLs when the RELs of the genes in the MMCLs were compared with those of the nMCLs. These results suggested

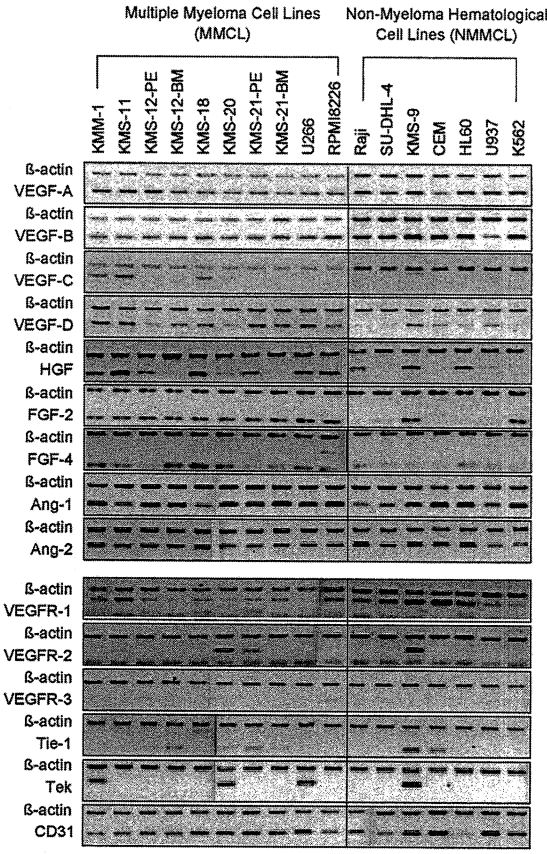


Fig 1. Gel images of MP-RT-PCR products for the angiogenic factors listed in Table 1 in ten MMCLs and seven nMCLs.

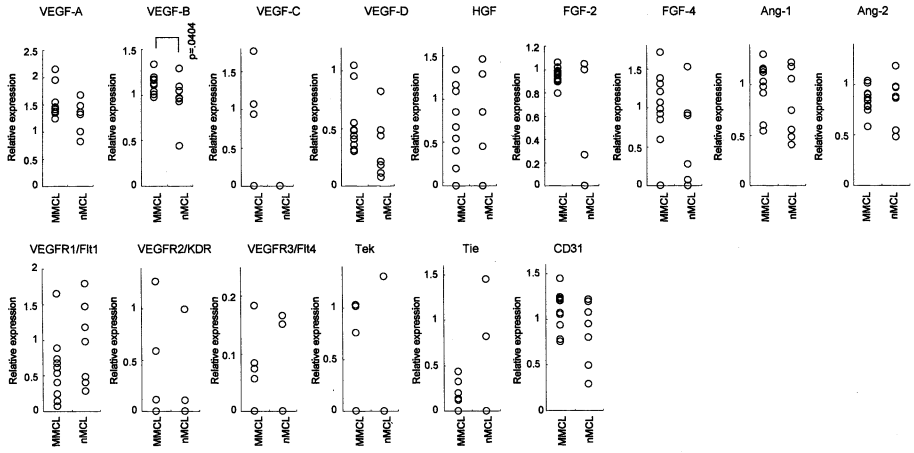


Fig 2. The relative expression levels of the angiogenic factors in MMCLs and nMCLs demonstrated in Fig 1 were calculated as described in "Materials and Methods".

that VEGFs may play an important role in tumor progression not only in solid tumors but also in hematological malignancies, particularly myelomas.

### *Production and release of VEGF-A*

Next, we examined the production and release of VEGF-A from the MMCLs and nMCLs into culture medium. As shown in Fig 3, the MMCLs produced and released significantly higher amounts of VEGF-A into the medium than the nMCLs did.

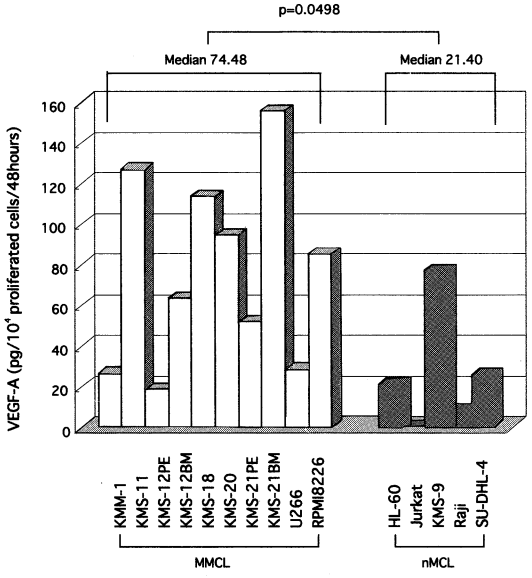


Fig 3. Ten MMCLs and five nMCLs were cultured for 48 hours and a cell count was performed using trypan-blue staining. Then, the cultured medium was applied to measure VEGF-A using the ELISA method. The data are shown as VEGF-A concentrations as "pg/$10^4$ proliferated cells/ 48 hours".

***Effects of anti-VEGF-A MoAb on the growth of the MMCLs***

As shown in Fig 4, three out of the five MMCLs (KMS-21BM, KMS-20 and KMS-21PE) demonstrated significant growth inhibition when they were cultured with anti-VEGF-A MoAb, whereas the growth of the other two lines, KMM-1 and KMS-18, was not altered by anti-VEGF-A MoAb. To explore the differences between lines in which growth was not altered and those in which it was inhibited, the relationship between growth rates (percentage of the control) and the RELs of the VEGFRs was analyzed. Fig 5 shows that a significant inverse correlation existed between the growth rate and the REL of VEGFR-2/KDR in the five MMCLs studied. This finding indicates that there may be an autocrine growth stimulating loop in VEGFR-2 expressing myeloma cells.

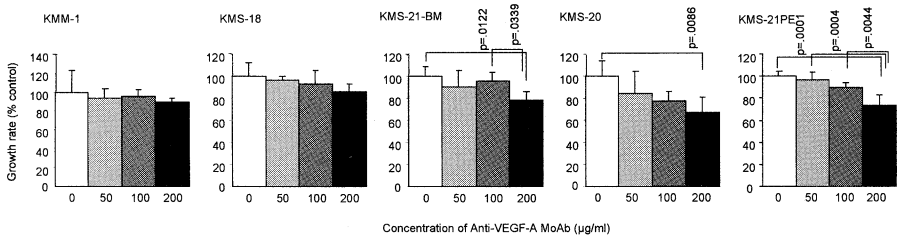


Fig 4. Growth inhibitory effect of anti-VEGF-A MoAb in five human myeloma cell lines. These cells were cultured with 0 (control), 50, 100 and 200 $\mu$g/ml of anti-VEGF-A MoAb for four days and cell numbers were counted. The results were revealed as the percentage of the control. KMS-21BM, KMS-20 and KMS-21PE cells showed a significant reduction in their growth rates as indicated in the figure.

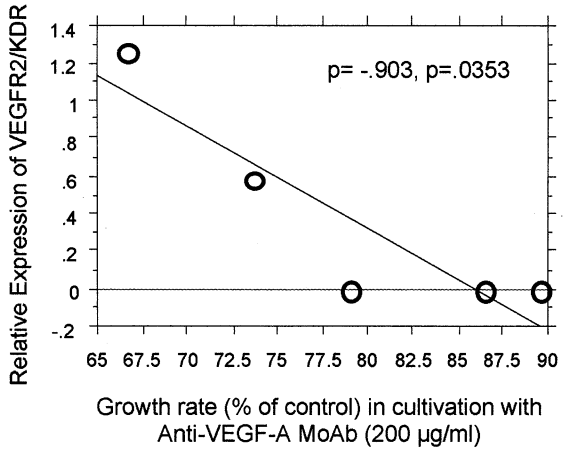


Fig 5. A significant inverse correlation was observed between the growth rate of myeloma cells cultured with 200 $\mu$g/ml of anti-VEGF-A MoAb and the relative expression levels of the VEGFR-2/KDR gene in myeloma cells.

***Effects of INF-$\alpha$ on the growth and production of VEGF-A in KMS-21BM cells***

IFN-$\alpha$ has been used in maintenance therapy for myeloma.[17] In addition, growth inhibition of myeloma cells induced by IFN-$\alpha$ has been reported to have been caused by suppression of the interleukin (IL)-6 autocrine loop,

downregulation of IL-6-R and induction of apoptosis.[18] Recently, IFN-$\alpha$ was found to inhibit angiogenesis in human umbilical vein endothelial cells.[19] Therefore, the production of VEGF-A for KMS-21BM cells, the growth of which was inhibited by IFN-$\alpha$, was studied. When the KMS-21BM cells were cultured with 1,000 U/ml of IFN-$\alpha$, their growth was reduced significantly, as shown in Fig 6A, and as reported previously.[20] However, the production and release of VEGF-A (pg/$10^4$ proliferated cells/48 hours) into culture medium was enhanced (Fig 6B).

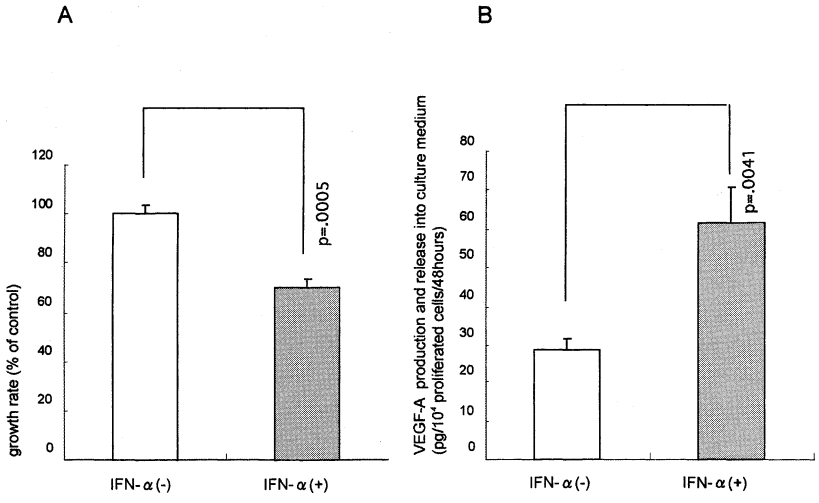


Fig 6. The effect of IFN-$\alpha$ on growth and production of VEGF-A in KMS-21BM myeloma cells. KMS-21BM cells were cultured with or without 1,000 U/ml of IFN-$\alpha$ for two days. Cell numbers were counted by trypan-blue staining and expressed as the percentage of the control (Panel A). The culture medium was applied for measurement of VEGF-A by ELISA and was expressed as "pg/$10^4$ proliferated cells/48 hours". (Panel B).

## DISCUSSION

Recently, it has been investigated whether neoangiogenesis in bone marrow is related to tumor progression and a poor prognosis in myeloma patients in a similar manner to other solid tumors.[7-10] In addition, recent targeting in myeloma therapy has been focusing on the microenvironment and angiogenesis based on the lack of improvement resulting from usage of multi-drug and/or high-dose chemotherapeutic agents.[21-22] However, the roles of various angiogenic factors and their receptors in myeloma cells themselves have not been studied in detail. Therefore, we examined the gene expression of these factors and receptors in myeloma cell lines and studied the effects of anti-VEGF-A MoAb and IFN-$\alpha$ on myeloma cells.

The VEGF family gene expression was higher in the MMCLs than in the nMCLs. VEGF-C expression was detected only in the MMCLs. Based on recent reports indicating an important role for VEGF-C and -D in lymphagenic metastasis in solid tumors[14,23-25] and higher expression of VEGF-C in the cells of myeloma, which are tumors of terminally differentiated B-lymphocytes, it is suggested that these VEGFs may play an important role in the progression of

myeloma. Regarding the VEGFs, it has been suggested that a paracrine growth loop exists between bone marrow stromal cells and myeloma cells since IL-6 and VEGF and known as proliferation factors for myeloma cells.[26] The higher production of VEGF-A in the MMCLs than the nMCLs also supports the conclusion that myeloma cells have cellular characteristics closer to those of solid tumor cells than other hematological malignant cells, and indicates the importance of angiogenic factors in myeloma.

It was also demonstrated that an autocrine loop exists between VEGF-A and VEGFR2. Podar *et al* recently reported that VEGF triggers tumor cell proliferation via a protein kinase C (PKC)-independent Raf-1-MEK-extracellular signal-regulated protein kinase pathway, and migration via a PKC-dependent pathway.[27] Although analyses of the protein expressions of VEGFRs and the involvement of signaling pathways related to the VEGF-mediated autocrine loop in myeloma cells are necessary, the inhibition of angiogenic factors and or their receptors may be a good tool in myeloma therapy.

Finaly, as has been reported previously,[18,20] IFN-$\alpha$ inhibited myeloma cell growth. However, production and release of VEGF-A into culture medium was enhanced. These results indicate the existence of an independent cellular mechanism between the IFN-$\alpha$ induced growth inhibition and VEGF-A, production in myeloma cells. In fact, it has been reported that IFN-$\alpha$ transcriptionally stimulated VEGF in human fibrosarcoma cell lines[28] and that long-term treatment of Hela cells with IFN-$\alpha$ resulted in upregulation of the VEGF-A expression.[29] Further study is needed to clarify the *in vivo* effects of IFN-$\alpha$ on interaction between myeloma and stromal cells.

Recent advances in myeloma therapy, such as the use of thalidomide and its analogues (immunomodulatory drugs; ImiDs), proteosome inhibitors, VEGF inhibitors, and tumor necrosis factor (TNF) inhibitors,[30–33] have shown a tendency to target the bone marrow microenvironment and angiogenesis surrounding myeloma cells. The results of this study support the importance of anti-angiogenic therapy for myeloma. Further studies are necessary to clarify the molecular mechanisms involved in angiogenic properties and to discover the molecular targets of angiogenesis in myeloma cells.

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## REFERENCES

1) Folkman J: Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med **1**: 27-31, 1995
2) Holash J, Wiegand SJ, Yancopoulos GD: New model of tumor angiogenesis: dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF. Oncogene **18**: 5356-5362, 1999
3) Carmeliet P, Jain RK: Angiogenesis in cancer and other diseases. Nature **407**: 249-257, 2000

4) Veikkola T, Karkkainen M, Claesson-Welsh L, Alitalo K: Regulation of angiogenesis via vascular endothelial growth factor receptors. Cancer Res **60**: 203-212, 2000
5) Bellamy WT, Richter L, Frutiger Y, Grogan TM: Expression of vascular endothelial growth factor and its receptors in hematopoietic malignancies. Cancer Res **59**: 728-733, 1999
6) Luo JC, Yamaguchi S, Shinkai A, Shitara K, Shibuya M: Significant expression of vascular endothelial growth factor/vascular permeability factor in mouse ascites tumors. Cancer Res **58**: 2652-2660, 1998
7) Schreiber S, Ackermann J, Obermair A, Kaufmann H, Urbauer E, Aletaha K, Gisslinger H, Chott A, Huber H, Drach J: Multiple myeloma with deletion of chromosome 13q is characterized by increased bone marrow neovascularization. Br J Haematol **110**: 605-609, 2000
8) Rajkumar SV, Leong T, Roche PC, Fonseca R, Dispenzieri A, Lacy MQ, Lust JA, Witzig TE, Kyle RA, Gertz MA, Greipp PR: Prognostic value of bone marrow angiogenesis in multiple myeloma. Clin Cancer Res **6**: 3111-3116, 2000
9) Vacca A, Ribatti D, Presta M, Minischetti M, Iurlaro M, Ria R, Albini A, Bussolino F, Dammacco F: Bone marrow neovascularization, plasma cell angiogenic potential, and matrix metalloproteinase-2 secretion parallel progression of human multiple myeloma. Blood **93**: 3064-3073, 1999
10) Ribatti D, Vacca A, Nico B, Quondamatteo F, Ria R, Minischetti M, Marzullo A, Herken R, Roncali L, Dammacco F: Bone marrow angiogenesis and mast cell density increase simultaneously with progression of human multiple myeloma. Br J Cancer **79**: 451-455, 1999
11) Otsuki T, Yamada O, Yata K, Nakazawa N, Taniwaki M, Sakaguchi H, Yawata Y, Ueki A: Genetic and biological characterization of human myeloma cell lines: an overview of the lines established at Kawasaki Medical School. Gene Funct Dis **1**: 48-56, 2000
12) Otsuki T, Yamada O, Kurebayashi J, Moriya T, Sakaguchi H, Kunisue H, Yata K, Uno M, Yawata Y, Ueki A: Estrogen receptors in human myeloma cells. Cancer Res **60**: 1434-1441, 2000
13) Tanaka K, Otsuki T, Sonoo H, Yamamoto Y, Udagawa K, Kunisue H, Arime I, Yamamoto S, Kurebayashi J, Shimozuma K: Semi-quantitative comparison of the differentiation markers and sodium iodide symporter messenger ribonucleic acids in papillary thyroid carcinomas using RT-PCR. Eur J Endocrinol **142**: 340-346, 2000
14) Kurebayashi J, Otsuki T, Kunisue H, Mikami Y, Tanaka K, Yamamoto S, Sonoo H: Expression of vascular endothelial growth factor (VEGF) family members in breast cancer. Jpn J Cancer Res **90**: 977-981, 1999
15) Asano M, Yukita A, Matsumoto T, Hanatani M, Suzuki H: An anti-human VEGF monoclonal antibody, MV833, that exhibits potent anti-tumor activity in vivo. Hybridoma **17**: 1851-1890, 1998
16) Asano M, Yukita A, Suzuki H: Wide spectrum of antitumor activity of a neutralizing monoclonal antibody to human vascular endothelial growth factor. Jpn J Cancer Res **90**: 93-100, 1999
17) The Myeloma Trialists' Collaborative Group: Interferon as therapy for multiple myeloma: an individual patient data overview of 24 randomized trials and 4012 patients. Br J Haematol **113**: 1020-1034, 2001
18) Otsuki T, Yamada O, Sakaguchi H, Tomokuni A, Wada H, Yawata Y, Ueki A: Human myeloma cell apoptosis induced by interferon-alpha. Br J Haematol **103**: 518-529, 1998
19) Minischetti M, Vacca A, Ribatti D, Iurlaro M, Ria R, Pellegrino A, Gasparini G, Dammacco AF: TNP-470 and recombinant human interferon-alpha2a inhibit angiogenesis synergistically. Br J Haematol **109**: 829-837, 2000
20) Otsuki T, Sakaguchi H, Yata K, Yamada O, Sugihara T, Ueki A: Paradoxical growth enhancement of myeloma cells induced by interferon-$\alpha$. Kawasaki Med J **27(1)**: 15-21, 2001
21) Tricot G, Jagannath S, Vesole D, Nelson J, Tindle S, Miller L, Cheson B, Crowley J, Barlogie B: Peripheral blood stem cell transplants for multiple myeloma: identification of favorable variables for rapid engraftment in 225 patients. Blood **85**: 588-596, 1995
22) Desikan R, Barlogie B, Sawyer J, Ayers D, Tricot G, Badros A, Zangari M, Munshi NC, Anaissie E, Spoon D, Siegel D, Jagannath S, Vesole D, Epstein J, Shaughnessy J, Fassas A, Lim S, Roberson P, Crowley J: Results of high-dose therapy for 1000 patients with multiple myeloma: durable complete remissions and superior survival in the absence of chromosome 13 abnormalities. Blood **95**: 4008-4010, 2000

23) Skobe M, Hawighorst T, Jackson DG, Prevo R, Janes L, Velasco P, Riccardi L, Alialo K, Claffey K, Detmar M: Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. Nat Med **7**: 151-152, 2001
24) Mandriota SJ, Jussila L, Jeltsch M, Compagni A, Baetens D, Prevo R, Banerji S, Huarte J, Montesano R, Jackson DG, Orci L, Alitalo K, Christofori G, Pepper MS: Vascularendothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis. EMBO J **20**: 672-682, 2001
25) Stacker SA, Caesar C, Baldwin ME, Thornton GE, Williams RA, Prevo R, Jackson DG, Nishikawa S, Kubo H, Achen MG: VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. Nat Med **7**: 186-191, 2001
26) Dankbar B, Padro T, Leo R, Feldmann B, Kropff M, Mesters RM, Serve H, Berdel WE, Kienast J: Vascular endothelial growth factor and interleukin-6 in paracrine tumor-stromal cell interactions in multiple myeloma. Blood **95**: 2630-2636, 2000
27) Podar K, Tai YT, Davies FE, Lentzsch S, Sattler M, Hideshima T, Lin BK, Gupta D, Shima Y, Chauhan D, Mitsiades C, Raje N, Richardson P, Anderson KC: Vascular endothelial growth factor triggers signaling cascades mediating multiple myeloma cell growth and migration. Blood **98**: 428-435, 2001
28) Der SD, Zhou A, Williams BR, Silverman RH: Identification of genes differentially regulated by interferon alpha, beta, or gamma using oligonucleotide arrays. Proc Natl Acad Sci USA **95**: 15623-15628, 1998
29) Lopez-Ocejo O, Perea SE, Bequet-Romero M, Arana MJ, Lopez Saura P: Impaired angiogenic balance and suppression of tumorigenicity in Hela cells chronically exposed to interferon-alpha. Biochem Biophys Res Commun **277**: 410-416, 2000
30) Hideshima T, Chauhan D, Shima Y, Raje N, Davies FE, Tai YT, Treon SP, Lin B, Schlossman RL, Richardson P, Muller G, Stirling DI, Anderson KC: Thalidomide and its analogs overcome drug resistance of human multiple myeloma cells to conventional therapy. Blood **96**: 2943-2950, 2000
31) Davies FE, Raje N, Hideshima T, Lentzsch S, Young G, Tai YT, Lin B, Podar K, Gupta D, Chauhan D, Treon SP, Richardson PG, Schlossman RL, Morgan GJ, Muller GW, Stirling DI, Anderson KC: Thalidomide and immunomodulatory derivatives augment natural killer cell cytotoxicity in multiple myeloma. Blood **98**: 210-216, 2001
32) Hideshima T, Richardson P, Chauhan D, Palombella VJ, Elliott PJ, Adams J, Anderson KC: The proteasome inhibitor PS-341, inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. Cancer Res **61**: 3071-3076, 2001
33) Mitsiades CS, Treon SP, Mitsiades N, Shima Y, Richardson P, Schlossman R, Hideshima T, Anderson KC: TRAIL/Apo2L ligand selectively induces apoptosis and overcomes drug resistance in multiple myeloma: therapeutic applications. Blood **98**: 795-804, 2001