Hypoxia and Activated VEGF/Receptor Pathway in Multiple Myeloma

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Abstract. *Background/Aim: Intensified angiogenic pathways are associated with poor prognosis and resistance of multiple myeloma (MM) cells to therapy. The links of the VEGF pathway with the hypoxia inducible factor (HIF) expression in MM are herein investigated. Materials and Methods: The vascular density (VD) and the HIF/VEGF/VEGF-receptor expression in the bone marrows of 106 MM cases were studied using immunohistochemistry. Results: HIF1α and HIF2α were expressed strongly in 33% and 13.2% of the cases, respectively. VEGFR and the phosphorylated (active) form of VEGFR2/KDR receptors were up-regulated in 42.5% and 36.8% of cases, respectively. Both HIF1α and HIF2α were significantly linked with high VD and VEGF expression. Moreover, the expression of the phosphorylated (active) form of VEGFR2/KDR was significantly linked with VEGF and HIF1α expression. The HIF/VEGF/VEGF-receptor pathway is up-regulated in approximately 40% of MM cases and linked with increased angiogenesis. Survival analysis in 37 evaluable patients showed a significantly worse prognosis in cases with high VD. Conclusion: HIFs and VEGF are up-regulated in a significant percentage of MM and are strongly related to each other. Targeting HIFs and the VEGF/receptor autocrine loop may prove of importance in the treatment of the disease.*

The hypoxia inducible factors 1-alpha and 2-alpha (HIF1α and HIF2α) are key transcription factors regulating the expression of a variety of genes involved in glycolysis and angiogenesis (1). These proteins are constantly degraded by the proteasome pathway and, therefore, under normal oxygen tension their concentration remains low. Under hypoxic conditions, however, degradation is inhibited and HIFαs are accumulated in the cytoplasm. Following heterodimerization with the HIF1β protein (aryl-hydrocarbon nuclear receptor translocator), HIFαs bind the DNA to the hypoxia response elements (HREs) of the target genes switching-on the transcription. HIFαs may, however, be constitutively increased in neoplastic cells, regardless of the presence of hypoxic stimuli. Several oncogenes including the *C-ERB* family (2, 3) and the *AKT* gene (4, 5) can induce HIF accumulation in an oxygen-independent manner.

Vascular endothelial growth factor (*VEGF*) is a major target gene of the HIF transcriptional activity (1). This angiogenic factor acts on specific tyrosine kinase receptors, the VEGFR-1 (flt-1), VEGFR-2 (KDR/flk-1) and VEGFR-3 (flt-4), residing on the normal endothelium (6), and in a variety of other cells including monocytes, haematopoietic stem cells (7) and neoplastic cells (8). VEGFRs are glycosylated and undergo phosphorylation in response to VEGF, which is an important step in the signalling of VEGF. It has been suggested that VEGF, produced by neoplastic cells, together with its main signaling receptors which are coexpressed in tumour cells form an autocrine loop contributing to survival and proliferation (9, 10).

Since angiogenesis has been strongly linked with clinical behaviour in myeloma the HIF/VEGF/VEGFR pathway in multiple myeloma (MM) cells was investigated using immunohistochemical techniques and specific antibodies recognizing VEGF, HIF1α and 2α, and the phosphorylated (active) form of VEGFR2/KDR. The vascular density of bone marrow was also calculated in order to compare these findings in the same cases.

Materials and Methods

Formalin-fixed, paraffin-embedded tissues from 106 MM bone marrow biopsies were retrieved from the archives of the Departments of Pathology, Democritus University of Thrace,

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Table I. *Details of the antibodies, dilutions, and antigen retrieval methods used.*

Primary antibody	Dilution/incubation time[a]	Antigen retrieval	Specificity	Source	Ref.
ESEE 122	1:20 (overnight)	MW	HIF-1α	Oxford University	(10)
EP 190b	Neat (overnight)	MW	HIF-2α	Oxford University	(10)
VG1	1:4 (overnight)	MW	VEGF	Oxford University	(10)
34a	1:2 (overnight)	MW	pVEGFR2/KDR	Oxford University	(11)
JC70 (CD31)	1:50 (30 min[a])	Protease XXIV	Endothelium	Dako, Denmark	(12)

[a]At room temperature.

Alexandroupolis, and the University of Ioannina, Ioannina, Greece. The expression of angiogenesis and that of hypoxia-related molecular features were examined in the MM cells of the bone marrow. An additional 10 reactive bone marrow samples from patients without malignancy were also included for immunohistochemical analysis. Survival data with disease specific death events were available for 37 patients.

Table I shows details of the antibodies and methods used for the immunohistchemical detection of VEGF, VEGFR2/KDR and hypoxia inducible factors HIF1α and HIF2α. Briefly, sections were cut at 3 μm and stained as follows. They were dewaxed and rehydrated in graded alcohol solutions. For heat-induced epitope retrieval, the sections were placed in citrate buffer (1:10 dilution, pH 7.2) and heated at 120˚C for 3×5 min. Endogenous peroxidase activity was neutralized using Peroxidase Block for 5 min. The non-specific binding was blocked by preincubation with Protein Block for 5 min at room temperature (Novocastra Laboratories Ltd, Newcastle upon Tyne, UK). Slides were then incubated overnight at 4˚C with primary antibodies (Table I). The slides were washed with PBS (2×5 min) and then incubated with Post Primary Block for 30 minutes at room temperature (Novocastra Laboratories Ltd). Washed with PBS for 2×5 min and incubated with NovoLinkTM polymer for 30 min at room temperature (Novocastra Laboratories Ltd). After extensive washing with PBS (2×5 min), the colour reaction was developed in 3,3'-diaminobenzidine for 5 min. The sections were then counterstained with haematoxylin, dehydrated and mounted. Normal immunoglobulin-G was substituted for the primary antibody as negative control.

The expression of HIFs and of the VEGFR2/KDR is mixed nuclear and cytoplasmic. The percentage of MM cells with nuclear and with strong cytoplasmic expression was separately assessed at ×200 magnification in all available optical fields (2-4 fields per case). The grouping of cases according to the patterns of expression of these proteins was performed using a grading system, as reported previously by this group (11, 12). Briefly, cases with nuclear reactivity in >10% of MM cells and/or strong cytoplasmic expression in >50% of neoplastic cells were considered as bearing high HIF or VEGFR2/KDR reactivity. Lack of any reactivity was considered as negative, while weak cytoplasmic expression (of any extent) or strong cytoplasmic expression in <50% of cells was considered as low expression. The expression of VEGF is purely cytoplasmic and, according to the percentage of cells with strong reactivity, cases were grouped in two categories of low *vs.* high reactivity. Analysis was performed using two different cut-off points: a) reactivity in ≥50% of MM cells and b) reactivity in ≥80% of MM cells.

For the detection of the endothelial cells, the JC70 (anti-CD31) monoclonal antibody was used in conjunction with the alkaline phosphatase anti-alkaline phosphatase immunohistochemical technique (13). Vessel counting was performed at ×200 magnification. Vessels with a clearly defined lumen or well-defined linear vessel shape, but not single endothelial cells, were considered for counting. After examining all available optical fields, the median number of vessels was used to score each case. The 66th percentile of these scores was used to group cases in the low and high vascular density (VD) category.

Statistical analysis was carried out and graphs were produced using the GraphPad Prism® 5.0 and Instat® 3.0 (GraphPad, San Diego CA, USA). A two-tailed Fisher's exact *t*-test was used for testing relationships between categorical tumour variables. Linear regression analysis was used to compare groups of continuous variables. Disease-specific survival curves were plotted using the method of Kaplan and Meier, and the log-rank test was used to determine statistical differences between life tables. For multivariate analysis, a Cox proportional hazard model was used to assess the effect of assessed parameters on death events. A p-value of <0.05 was used for significance.

Results

Expression patterns. In reactive bone marrow samples, HIF2α and to a lesser extent HIF1α were expressed in a subset of cells morphologically and immunohistochemically identified as macrophages (positive for CD68 antigen). All other cells were negative. VEGF was expressed weakly and rather focally in some plasma cells, but all other cells were negative. pKDR was expressed in the cytoplasm of myeloid progenitor cells and megakaryocytes, but nuclear expression was randomly noted.

In the bone marrow of patients with myeloma, the mean percentage of MM cells with strong cytoplasmic and nuclear HIF1α expression was 26% (range 0-100%) and 2% (range 0-50%) respectively (Figure 1a). Using the aforementioned scoring system, 35/106 (33%) cases had high HIF1α expression. The mean percentage of MM cells with strong cytoplasmic and nuclear HIF2α expression was 7% (range 0-70%) and 1% (range 0-50%), respectively (Figure 1b). Using the same scoring system, 14/106 (13.2%) cases had high HIF2α expression.

The mean percentage of MM cells with strong cytoplasmic VEGF expression was 31% (range 0-90%). Using the 50% and the 80% cut-off points, 45/106 (42.5%) and 35/106 (33%) cases had high VEGF expression, respectively (Figure 1c). The mean percentage of MM cells

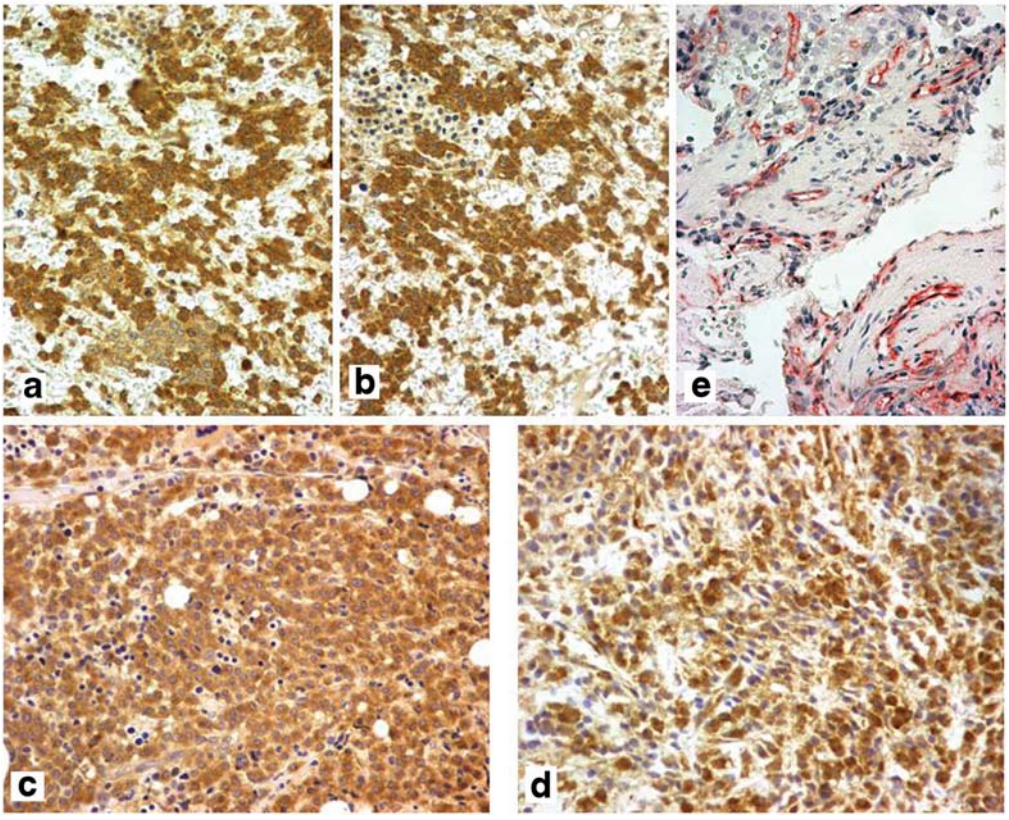

Figure 1. *Immunohistochemical figures of the bone marrow from patients with multiple myeloma (×200): (a) HIF1α, (b) HIF2α), (c), VEGF (d) pVEGFR2/KDR and (e) vascular density using CD31.*

with strong cytoplasmic and nuclear pVEGFR2/KDR expression was 29% (range 0-100%) and 1% (range 0-20%), respectively (Figure 1d). Applying the scoring system, 39/106 (36.8%) cases had high pVEGFR2/KDR expression.

The mean VD was 7 vessels per ×200 optical field (range 1-30). Using the 66th percentile as a cut off point (>7 vessels), 21/106 (19.8%) cases were of high VD (Figure 1e).

Association among variables. In group analysis (Tables II and III) HIF1α expression in MM cells was significantly associated with HIF2α (p=0.01), VEGF (p=0.004), pVEGFR2/KDR (p=0.001) and high VD (p<0.0001). These results were confirmed in linear regression analysis of the percentage of cells expressing the parameters analyzed (Table IV).

A significant association was noted between HIF2α and VEGF expression (p=0.03) in group analysis (Table II); interestingly, HIF2α was marginally linked with VD in linear regression analysis (Table IV).

In group analysis, VEGF was directly linked with the expression of phosphorylated VEGFR2/KDR receptors in cancer cells (p=0.004) and with VD (p=0.005); Table III. This was further confirmed in linear regression analysis (Table IV). The expression of VEGFR2/KDR receptors was also linked with high VD (p=0.04).

Overall survival. Table V shows the univariate and multivariate analysis of disease-specific overall survival. High VD was the only variable linked with poor overall survival (p=0.04, hazard ratio 5.8), while a trend for poorer survival was also noted for high VEGF expression (p=0.15, hazard ratio 2.3). Kaplan-Meier overall survival curves for VD and VEGF are shown in Figures 2a and b, respectively. In multivariate analysis, none of the parameters analysed showed an independent prognostic relevance.

Discussion

Increased bone marrow angiogenesis has been documented in patients with MM and this feature has been linked with increased proliferation (14), poorer prognosis and resistance to chemotherapy (15-17). Rajkumar *et al*. showed that bone marrow angiogenesis progressively increases along the spectrum of plasma cell disorders, from the more benign

Table II. *Association of HIF expression with VEGF, pKDR and vascular density (VD).*

	HIF1α			HIF2α		
	L	H	*p*-Value	L	H	*p*-Value
HIF2α						
L	66	26	0.01	--	--	--
H	5	9		--	--	
VEGF (50)						
L	44	17	0.21	56	5	0.03
H	27	18		36	9	
VEGF (80)						
L	65	24	0.004	80	9	0.04
H	6	11		12	5	
pKDR						
L	53	14	0.001	61	6	0.13
H	18	21		31	8	
VD						
L	62	23	<0.0001	74	11	0.94
H	9	12		18	3	

L: Low, H: High.

Table III. *Association of VEGF expression with pKDR and vascular density (VD).*

	VEGF (50)			VEGF (80)			pKDR		
	L	H	*p*-Value	L	H	*p*-Value	L	H	*p*-Value
VD									
L	52	33	0.14	76	9	0.005	58	27	0.04
H	9	12		13	8		9	12	
pKDR									
L	46	21	0.004	59	8	0.17	--	--	--
H	15	24		30	9		--	--	

L: Low, H: High.

Table IV. *Linear regression analysis between the hypoxia- and angiogenesis -related variables examined.*

Parameter	HIF1α		HIF2α		VEGF		pKDR	
	p-Value	r-Value	*p*-Value	r-Value	*p*-Value	r-Value	*p*-Value	r-Value
HIF2α	0.01	0.22						
VEGF	0.005	0.26	0.04	0.58				
pKDR	0.002	0.28	0.10	0.15	0.003	0.28		
VD	0.03	0.21	0.05	0.52	0.05	0.17	0.52	0.05

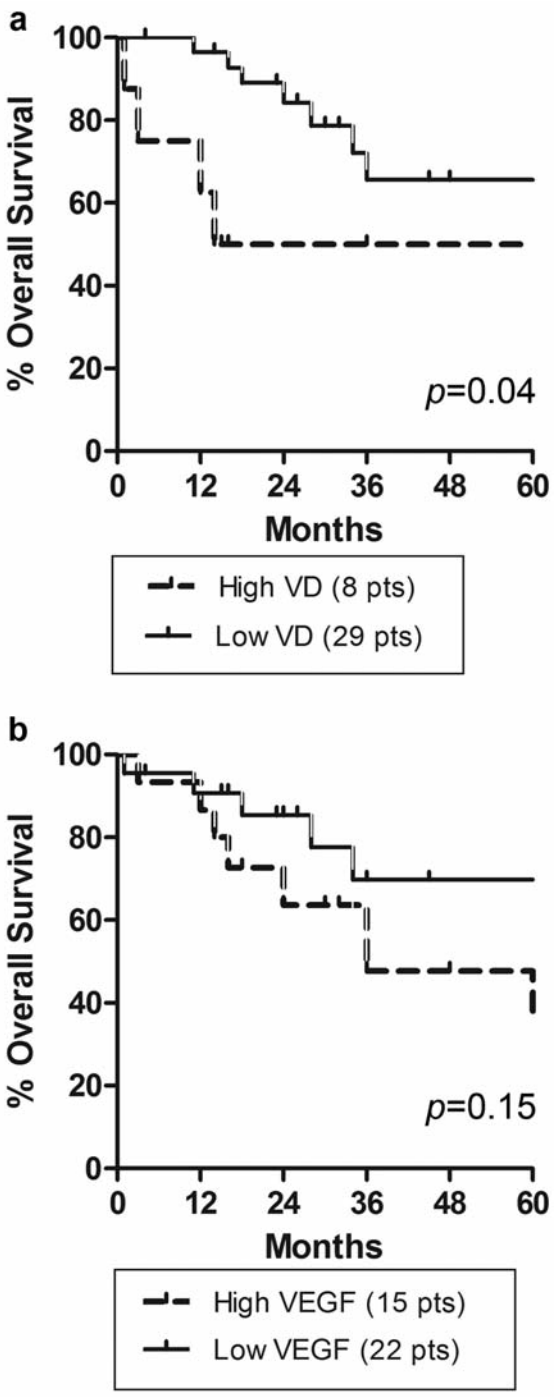

Figure 2. *Disease-specific overall survival Kaplan-Meier curves stratified for VD (a) and VEGF (b).*

stage of monoclonal gammopathy of undetermined significance to advanced myeloma, suggesting an important role of angiogenesis in regulating disease progression pathways (18).

VEGF, a potent angiogenic factor, is produced abundantly by plasma cells in MM, stimulating proliferation and chemotaxis of VEGFRs in bone marrow endothelial cells (19). In addition to the paracrine function of VEGF, an autocrine

Table V. *Disease-specific overall survival analysis of 37 patients with MM.*

Parameter	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*p*-Value	t-Ratio	*p*-Value
VD	5.8 (1.0-31)	0.04	1.2	0.22
VEGF	2.3 (0.7-7.4)	0.15	1.5	0.13
pKDR	1.0 (0.3-3.2)	0.99	0.4	0.66
HIF1α	1.6 (0.4-6.4)	0.45	0.09	0.92
HIF2α	0.9 (0.2-4.5)	0.96	0.42	0.67

mechanism has been documented for this cytokine and its receptors VEGFR1/flt-1 and VEGFR2/KDR in different myeloma cell lines and plasma cells isolated from patients (20). Inhibition of VEGFRs in the bone marrow milieu acts directly on MM cells increasing apoptosis, decreasing angiogenesis and decellerating growth in a mouse xenograft model of human MM (21).

In the current study, an intense VEGF expression was documented in 42.5% of the bone marrow MM cells, while phosphorylated (activated) VEGFR2/KDR was present in 36.8% of the cases examined. VEGF was significantly co-expressed with pVEGFR2/KDR in MM cells, a finding that supports the experimental evidence for a VEGFR/receptor autocrine loop in haematological (21) and other malignancies (9, 10). The VD was significantly increased in the bone marrow of cases with VEGF and/or pVEGFR2/KDR expression. Of interest, intense angiogenic activity in the bone marrow was a significant factor linked with increased disease-specific death rates, but this finding must be confirmed in larger series of patients. Nevertheless, this is in accordance with previously published studies (16, 17, 22, 23).

The role of HIF expression on MM cells was further examined. HIF1α and 2α are important transcription factors directly regulating the expression of the *VEGF* gene (1). As yet, their expression status in MM has not been thoroughly investigated. In a recent report by Shin *et al*. (24), treatment of MM cells with bortezomib, a proteasome inhibitor under study for the treatment of MM and several solid tumours, resulted in the abrogation of the HIF1α function. Also inhibition of growth family member 4 (*ING4*), a recently discovered tumour-suppressor gene, seems to be involved in the suppression of HIF1α expression in MM cells (25).

In this study, HIF1α and, to a lesser extent, HIF2α were strongly expressed in the cytoplasm and the nuclei of MM cells, and these factors were often co-expressed. Of the 106 cases studied, 33% had high HIF1α and 13.2% high HIF2α expression. Both factors were significantly linked with high VEGF and VD expression, confirming their role as transcriptional regulators of VEGF. Although this finding strongly supports the role of hypoxia-regulated genes in controlling VEGF and angiogenesis in MM cells, there is a subset of myelomas in which the mechanism of HIF up-regulation remains obscure. Constitutive HIF1α/HIF2α expression as a result either of activation of genes, such as *AKT* (26, 27) and *HER2* (28), or even repression of tumour suppressor genes, such as *ING4* (25), may be part of the HIF up-regulation mechanism in MM.

It is concluded that the HIF/VEGF/VEGFR pathway is up-regulated in approximately 40% of MM cases and linked with increased angiogenesis, a feature previously shown to be of high prognostic relevance. These findings indicate the need for pre-clinical studies and clinical trials targeting HIFs and the VEGF/receptor autocrine loop for the treatment of multiple myeloma.

References

1 Semenza GL, Shimoda LA and Prabhakar NR: Regulation of gene expression by HIF-1. Novartis Found Symp *272*: 2-8, 2006.

2 Pore N, Jiang Z, Gupta A, Cerniglia G, Kao GD and Maity A: EGFR tyrosine kinase inhibitors decrease VEGF expression by both hypoxia-inducible factor (HIF)-1-independent and HIF-1-dependent mechanisms. Cancer Res *66*: 3197-3204, 2006.

3 Peng XH, Karna P, Cao Z, Jiang BH, Zhou M and Yang L: Cross-talk between epidermal growth factor receptor and hypoxia-inducible factor-1alpha signal pathways increases resistance to apoptosis by up-regulating survivin gene expression. J Biol Chem *281*: 25903-25914, 2006.

4 Toschi A, Lee E, Gadir N, Ohh M and Foster DA: Differential dependence of hypoxia-inducible factors 1 alpha and 2 alpha on mTORC1 and mTORC2. J Biol Chem *283*: 34495-34499, 2008.

5 Lee BL, Kim WH, Jung J, Cho SJ, Park JW, Kim J, Chung HY, Chang MS and Nam SY: A hypoxia-independent up-regulation of hypoxia-inducible factor-1 by AKT contributes to angiogenesis in human gastric cancer. Carcinogenesis *29*: 44-51, 2008.

6 Klagsbrun M and D'Amore PA: Vascular endothelial growth factor and its receptors. Cytokine Growth Factor Rev *7*: 259-270, 1996.

7 Katoh O, Tauchi H, Kawaishi K, Kimura A and Satow Y: Expression of the vascular endothelial growth factor (VEGF) receptor gene, *KDR*, in hematopoietic cells and inhibitory effect of VEGF on apoptotic cell death caused by ionizing radiation. Cancer Res *55*: 5687-5692, 1995.

8 Fox SB, Turley H, Cheale M, Blazquez C, Roberts H, James N, Cook N, Harris A and Gatter K: Phosphorylated KDR is expressed in the neoplastic and stromal elements of human renal tumours and shuttles from cell membrane to nucleus. J Pathol *202*: 313-320, 2004.
9 Sher I, Adham SA, Petrik J and Coomber BL: Autocrine VEGF-A/KDR loop protects epithelial ovarian carcinoma cells from anoikis. Int J Cancer *124*: 553-561, 2009.
10 Santos SC, Dias S. Internal and external autocrine VEGF/KDR loops regulate survival of subsets of acute leukemia through distinct signaling pathways. Blood *103*: 3883-3889, 2004.
11 Giatromanolaki A, Koukourakis MI, Turley H, Sivridis E, Harris AL and Gatter KC; Tumour and Angiogenesis Research Group: Phosphorylated KDR expression in endometrial cancer cells relates to HIF1alpha/VEGF pathway and unfavourable prognosis. Mod Pathol *19*: 701-707, 2006.
12 Koukourakis MI, Giatromanolaki A, Sivridis E, Gatter KC and Harris AL; Tumour Angiogenesis Research Group: Lactate dehydrogenase 5 expression in operable colorectal cancer: strong association with survival and activated vascular endothelial growth factor pathway – a report of the Tumour Angiogenesis Research Group. J Clin Oncol *24*: 4301-4308, 2006.
13 Giatromanolaki A, Koukourakis M, O'Byrne K, Fox S, Whitehouse R, Talbot DC *et al*: Prognostic value of angiogenesis in operable non-small cell lung cancer. J Pathol *179*: 80-88, 1996.
14 Alexandrakis MG, Passam FH, Dambaki C, Pappa CA and Stathopoulos EN: The relation between bone marrow angiogenesis and the proliferation index Ki-67 in multiple myeloma. J Clin Pathol *57*: 856-60, 2004.
15 Bhatti SS, Kumar L, Dinda AK and Dawar R: Prognostic value of bone marrow angiogenesis in multiple myeloma: use of light microscopy as well as computerized image analyzer in the assessment of microvessel density and total vascular area in multiple myeloma and its correlation with various clinical, histological, and laboratory parameters. Am J Hematol *81*: 649-56, 2006.
16 Kumar S, Gertz MA, Dispenzieri A, Lacy MQ, Wellik LA, Fonseca R, Lust JA, Witzig TE, Kyle RA, Greipp PR and Rajkumar SV: Prognostic value of bone marrow angiogenesis in patients with multiple myeloma undergoing high-dose therapy. Bone Marrow Transplant *34*: 235-239, 2004.
17 Pruneri G, Ponzoni M, Ferreri AJ, Decarli N, Tresoldi M, Raggi F, Baldessari C, Freschi M, Baldini L, Goldaniga M, Neri A, Carboni N, Bertolini F and Viale G: Microvessel density, a surrogate marker of angiogenesis, is significantly related to survival in multiple myeloma patients. Br J Haematol *118*: 817-820, 2002.
18 Rajkumar SV, Mesa RA, Fonseca R, Schroeder G, Plevak MF, Dispenzieri A, Lacy MQ, Lust JA, Witzig TE, Gertz MA, Kyle RA, Russell SJ and Greipp PR: Bone marrow angiogenesis in 400 patients with monoclonal gammopathy of undetermined significance, multiple myeloma, and primary amyloidosis. Clin Cancer Res *8*: 2210-2216, 2002.
19 Vacca A, Ria R, Ribatti D, Semeraro F, Djonov V, Di Raimondo F and Dammacco F: A paracrine loop in the vascular endothelial growth factor pathway triggers tumor angiogenesis and growth in multiple myeloma. Haematologica *88*: 176-185, 2003.
20 Kumar S, Witzig TE, Timm M, Haug J, Wellik L, Fonseca R, Greipp PR and Rajkumar SV: Expression of VEGF and its receptors by myeloma cells. Leukemia *17*: 2025-2031, 2003.
21 Podar K, Tonon G, Sattler M, Tai YT, Legouill S, Yasui H, Ishitsuka K, Kumar S, Kumar R, Pandite LN, Hideshima T, Chauhan D and Anderson KC: The small-molecule VEGF receptor inhibitor pazopanib (GW786034B) targets both tumor and endothelial cells in multiple myeloma. Proc Natl Acad Sci USA *103*: 19478-19483, 2006.
22 Marković O, Marisavljević D, Cemerikić V, Vidović A, Perunicić M, Todorović M, Elezović I and Colović M: Expression of VEGF and microvessel density in patients with multiple myeloma: clinical and prognostic significance. Med Oncol *25*: 451-457, 2008.
23 Ribas C, Colleoni GW, Silva MR, Carregoza MJ and Bordin JO: Prognostic significance of vascular endothelial growth factor immunoexpression in the context of adverse standard prognostic factors in multiple myeloma. Eur J Haematol *73*: 311-317, 2004.
24 Shin DH, Chun YS, Lee DS, Huang LE and Park JW: Bortezomib inhibits tumor adaptation to hypoxia by stimulating the FIH-mediated repression of hypoxia-inducible factor-1. Blood *111*: 5258-5259, 2008.
25 Colla S, Tagliaferri S, Morandi F, Lunghi P, Donofrio G, Martorana D, Mancini C, Lazzaretti M, Mazzera L, Ravanetti L, Bonomini S, Ferrari L, Miranda C, Ladetto M, Neri TM, Neri A, Greco A, Mangoni M, Bonati A, Rizzoli V and Giuliani N: The new tumor-suppressor gene inhibitor of growth family member 4 (*ING4*) regulates the production of proangiogenic molecules by myeloma cells and suppresses hypoxia-inducible factor-1 alpha (HIF-1alpha) activity: involvement in myeloma-induced angiogenesis. Blood *110*: 4464-4475, 2007.
26 Lentzsch S, Chatterjee M, Gries M, Bommert K, Gollasch H, Dörken B and Bargou RC: PI3-K/AKT/FKHR and MAPK signaling cascades are redundantly stimulated by a variety of cytokines and contribute independently to proliferation and survival of multiple myeloma cells. Leukemia *18*: 1883-1890, 2004.
27 Frost P, Shi Y, Hoang B and Lichtenstein A: AKT activity regulates the ability of mTOR inhibitors to prevent angiogenesis and VEGF expression in multiple myeloma cells. Oncogene *26*: 2255-2262, 2007.
28 Otsuki T, Kurebayashi J, Ohkubo S, Uno M, Fujii T, Sakaguchi H, Hatayama T, Takata A, Tsujioka T, Sugihara T and Hyodoh F: Expression of HER family receptors and effects of anti-HER2-antibody on human myeloma cell lines. Int J Oncol *23*: 1135-1141, 2003.

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