To the editor:

Do human myeloma cells directly produce basic FGF?

Basic fibroblast growth factor (bFGF) is a growth factor with proangiogenetic properties. Elevated bone marrow (BM) and peripheral serum bFGF levels have been reported in patients with multiple myeloma (MM)[1-3]; however, the source of bFGF in patients with MM is not completely elucidated.

Recently, Bisping et al,[4] in line with others,[1] have reported that human myeloma cell lines (HMCLs) (RPMI-8226, U266, KMS-11, and KMS-18) and sorted CD38^{high}/CD138^{+} cells obtained from 12 of 15 patients with MM produced bFGF, concluding that myeloma cells are the predominant source of bFGF. In contrast, Gupta et al[5] have shown that neither human myeloma cells nor Epstein-Barr virus (EBV)–positive B-cell lines secrete bFGF.

In order to better clarify this issue, we wish to present our evidence. Using reverse transcription–polymerase chain reaction (RT-PCR) (bFGF primer pairs: forward, 5′-GGCTTCTTCCTGCG-CATCCAT-3; reverse: 5′-GGTAACGGTTAGCACACACTCCTTT-3′) we found that XG-6, RPMI-8226, OPM-2, as well as EBV-positive cell line ARH-77 did not express bFGF mRNA, whereas U266 was positive and XG-1 expressed bFGF at low intensity (Figure 1A). Similarly, we failed to detect bFGF either in HMCL lysates by Western blot analysis (antipolyclonal bFGF antibody [Ab]; R&D Systems, Minneapolis, MN) or in HMCL (10^6/mL)–conditioned media by enzyme-linked immunosorbent assay (ELISA; R&D Systems; range of sensitivity, 10 to 640 pg/mL), both in the presence and absence of interleukin-6 (IL-6, 20 ng/mL) with the exception of U266 and XG-1 (Figure 1B-C). Consistently, we previously showed that blocking anti-bFGF Ab failed to inhibit HMCL-induced angiogenesis in an in vitro system, suggesting that any bFGF biologic activity was found in HMCLs.[6]

Purified CD138^{+} MM cells (purity $>$ 95%) isolated by an immunomagnetic method (magnetic-activated cell sorter [MACS]; Miltenyi Biotec, Bergisch-Gladbach, Germany) were positive for bFGF mRNA expression in 11 of 35 patients with newly diagnosed MM in stages I to III (median age, 64 years [range, 33-88 years]; and median plasmacytosis, 35% [range, 12%-95%]) (Table 1). In contrast, BM stromal cells (BMSCs) obtained from all patients were positive for bFGF mRNA (Figure 1A).

bFGF protein has been found in plasma cell lysates in 8 of 30 patients tested. Consistently, bFGF levels were detected by ELISA assay (R&D Systems) in conditioned media of purified MM cells (10^6/mL) in 7 of 28 patients (Table 1). Furthermore, a nuclear bFGF immunostaining with low cytoplasmic positivity has been found in bone marrow myeloma cells of 3 of 21 patients (Table 1; Figure 1D).

No correlation has been found between bFGF expression by MM cells and BM plasmacytosis (Pearson Chi-square, $P = .35$) or the presence of osteolytic lesions. The differences in the microvessel density (MVD) and in the number of microvessels per field, evaluated as previously described,[6] between bFGF mRNA–positive and –negative patients with MM did not reach a statistical significance (MVD $\pm$ SE, 36 $\pm$ 4 vs 24 $\pm$ 3.2; number of microvessels $\pm$ SE, 7.4 $\pm$ 5 vs 3.59 $\pm$ 0.5; Mann-Whitney test, $P = .19$ and $P = .16$, respectively).

In conclusion, our data indicate that bFGF is rarely produced directly by MM cells, suggesting that bFGF is not the major proangiogenetic factor produced by myeloma cells, even if its production could be involved at least in part in the MM-induced angiogenesis.

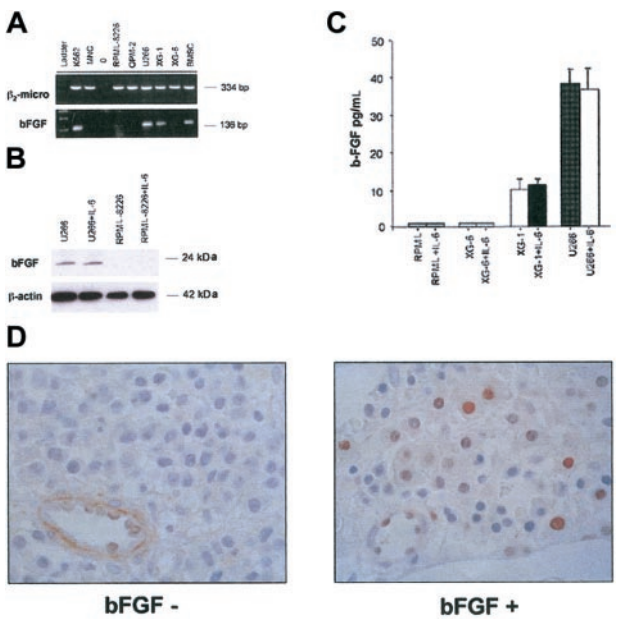

Figure 1. bFGF expression by HMCLs and by MM patients. RT-PCR was performed in order to test bFGF mRNA expression in HMCLs (RPMI-8226, OPM-2, U266, XG-1, and XG-6) and bone marrow stromal cells (BMSCs) obtained from patients with MM. β2-microglobulin was amplified as internal control. K562 and mononuclear cells (MNCs) from healthy subjects were used as positive and negative control, respectively (A). HMCLs (10^6/mL) were incubated in the presence or absence of IL-6 (20 ng/mL). bFGF protein was assessed either in cell lysates by Western blot analysis after 24 hours (B) or in conditioned medium by ELISA after 48 hours (C). (D) bFGF immunostaining in BM biopsies of 2 representative patients with MM with negative (left) and positive (right) myeloma cells performed with anti-bFGF polyclonal Ab (25 μg/mL) using indirect immunoperoxidase detection method.[6,7] Endothelial cells are the internal positive control. Original magnification, × 100.

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Table 1. bFGF expression in MM patients

Patients	Age, y	Type	PC, %	Stage	Osteolysis	bFGF mRNA RT-PCR	bFGF protein		
							ELISA	Western blot	Immunostaining
1	61	Aλ	90	IIIa	+	+	+	+	++
2	68	Gκ	70	IIIa	+	−	−	−	−
3	66	Gκ	30	IIIa	+	−	−	−	−
4	61	Gκ	35	IIIa	+	−	−	−	−
5	88	Gλ	20	IIIa	+	−	−	−	−
6	61	Gλ	70	IIIa	+	+	+	+	+
7	71	Gκ	20	Ia	+< 3	−	−	−	−
8	39	Gκ	30	IIIa	+	+	−	−	−
9	54	G	35	IIIa	+	−	−	−	ND
10	62	G	90	IIa	+< 3	−	−	−	ND
11	60	Aκ	65	IIa	+	+	+	ND	−
12	65	Gκ	40	IIIa	+	−	−	−	−
13	85	κ	50	IIIb	+	+	ND	ND	ND
14	73	Aκ	45	Ia	+< 3	+	ND	+/−	ND
15	71	Aκ	30	IIIa	+	−	−	−	ND
16	82	Gλ	50	IIa	−	−	−	−	ND
17	59	Gκ	20	IIIa	+	−	−	−	ND
18	68	λ	75	IIb	−	−	−	−	−
19	78	Gκ	80	IIIa	+	+	ND	ND	ND
20	48	A	60	IIIa	+	+	+	+	+
21	76	Aκ	25	Ia	−	+	ND	ND	ND
22	61	Aκ	95	IIb	−	+	+	ND	−
23	64	Gκ	60	IIIb	+	−	−	−	−
24	50	Gκ	15	Ia	−	−	−	−	−
25	73	Aλ	70	Ia	+	−	−	−	−
26	41	Gλ	20	IIIa	+	−	−	−	ND
27	77	Gκ	12	Ia	−	−/+	ND	+	ND
28	52	Gκ	15	Ia	−	−/+	ND	+	−
29	66	Aλ	45	IIIa	+	−	−	−	−
30	72	Aλ	28	IIIa	+	+	+	+	ND
31	52	κ	15	IIIa	+	−/+	+	+	ND
32	81	κ	20	Ib	−	−	−	−	−
33	73	Gκ	15	IIa	−	−	−	−	−
34	35	Gλ	80	IIIa	+	−	−	−	−
35	66	Gλ	35	IIIb	+	−/+	ND	+	ND

PC indicates plasmacytosis; +, positive; −, negative; +/−, low expression; and ND, not determined.

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Response:

Source and significance of basic FGF in multiple myeloma

The letter by Colla et al addresses important issues concerning the role of basic FGF (bFGF) as a paracrine mediator and proangiogenic cytokine in multiple myeloma (MM). The authors question whether myeloma cells directly produce bFGF and represent the predominant source of elevated levels in MM marrow.[1,2]

In line with other investigators,[3-7] we have previously shown that several human myeloma cell lines (HMCLs) as well as myeloma cells purified from the marrow of patients with MM express and secrete bFGF. In addition, intracellular bFGF was demonstrable by flow cytometric immunostaining in both HMCLs and patient cells.[2] In our extended series, bFGF expression was detected in 6 of 7 HCMLs (positive: U-266, KMS-11, KMS-18, MM.1S, MM.1R, RPMI-8226; negative: OPM-2) and in sorted myeloma cells from 19 (79%) of 24 patients. Further supporting the notion of bFGF secretion by myeloma cells, Van Riet et al[5] reported a 5-fold increase in bFGF production by U-266 and MM1.S cells upon exposure to conditioned media of cultured bone marrow stromal cells (BMSCs). Likewise, we found significant upregulation in bFGF secretion upon stimulation with interleukin-6 in RPMI-8226, U-266, and myeloma cells from selected patients.[2] Thus, in our view, there is little doubt that a substantial proportion of myeloma cells directly produce bFGF, although their capacity and its regulation may vary considerably between both HCMLs and individual patients. The variability may reflect biologic heterogeneity of the disease, differences in stage and treatment status, and possibly differences in cell processing and culture conditions.

Another relevant issue is whether myeloma cells rather than BMSCs are the prevailing source of bFGF in MM marrow. In our series, bFGF transcripts were present in BMSC monocultures from 7 of 8 patients with MM, whereas bFGF concentrations in culture supernatants (10^5 cells/mL) were below the detection limit of the enzyme-linked immunosorbent assay in all cases (Quantikine; R&D Systems, Minneapolis, MN). Moreover, our previously published experiments demonstrated that bFGF secretion in sorted ex vivo samples of MM marrows was almost quantitatively accounted for by myeloma cells rather than BMSCs.[2] In addition, Van Riet et al[5] showed that myeloma cells (U-266, MM1.S) had no effect on stromal production of bFGF. Taken together, the data strongly suggest that myeloma cells are the major source of elevated bFGF concentrations in MM marrow. However, to our knowledge, it has not been studied whether BMSCs contribute to a membrane-bound fraction of bFGF in the bone marrow of patients with MM.

Despite some heterogeneity in disease biology, we conclude from our published data and those cited[1,3-7] that myeloma-derived bFGF is a significant mediator supporting myeloma cell expansion and survival. To what extent myeloma-derived bFGF contributes to the increased microvessel density in MM marrow is beyond the scope of our studies.

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To the editor:

Hypereosinophilic syndrome with elevated serum tryptase versus systemic mast cell disease associated with eosinophilia: 2 distinct entities?

We read with interest the report by Klion et al[1] that describes a myeloproliferative variant of hypereosinophilic syndrome (HES) that is associated with elevated serum tryptase and hyperplasia of dysplastic mast cells (MCs) in the bone marrow (HES-tryptase). It was argued that HES-tryptase is distinct from systemic mast cell disease associated with eosinophilia (SMCD-eos). In support of this notion, the authors considered several features that were absent in HES-tryptase but expected in SMCD-eos: focal mast cell aggregates, MC coexpression of CD2 and CD25, and the presence of the D816V *c-kit* mutation. However, we question the accuracy of such a classification.

Clinical presentation in adults with SMCD is markedly heterogeneous,[2] and making or refuting the diagnosis requires a careful morphologic analysis of the bone marrow. In general, SMCD is characterized by focal, dense aggregates of dysplastic MCs. However, the bone marrow MC infiltration pattern in aggressive SMCD, including SMCD-eos, can be diffuse, and whether one appreciates a "dense" or "loose" scattering of MCs in this setting is open to subjective bias.[3] In our experience, either dense or loose aggregates of dysplastic MCs are seen in both *FIP1L1-PDGFRA*$^+$ and *c-kit* D816V$^+$ SMCD-eos. This was illustrated in a recent report of 5 patients with SMCD-eos in which all 3 patients who carried the *FIP1L1-PDGFRA* fusion had pathognomonic MC aggregates in the bone marrow in a pattern that was not different from 1 of the patients with the *c-kit D816V* mutation.[4]

In regard to immunophenotypic characteristics of neoplastic MCs, we have recently reported that aberrant MC expression of CD2 is not a uniform disease feature in SMCD.[5] While CD25 was aberrantly expressed in all 22 patients studied in that report, the prevalence of CD2 coexpression was much lower (41%), and CD2 expression was only occasionally seen in SMCD that was associated with another clonal hematologic disorder. In our recent report of the 5 patients with SMCD-eos, MC CD25$^+$CD2$^-$ expression profile was seen in all 5 patients, including the 3 with the *FIP1L1-PDGFRA* fusion and the 2 with the *c-kit* D816V mutation.[4]

Not considering a diagnosis of SMCD on the basis of absence of *c-kit* D816V mutations is not accurate given the wide variation in the reported prevalence of such mutations in sporadic SMCD, which may be as low as 20%, depending upon the source of patient sample analyzed and the patient population being studied.[6] The occurrence of the *FIP1L1-PDGFRA* fusion gene has only recently been described for patients with HES[7] and has also been demonstrated in clonal eosinophilia, including chronic eosinophilic leukemia and chronic myeloproliferative disorder associated with eosinophilia.[8] Therefore, the suggestion that patients carrying this fusion gene have HES as opposed to SMCD-eos may not be accurate. In fact, we had reported on the efficacy of imatinib in SMCD-eos well before the discovery of the drug target, the *FIP1L1-PDGFRA* fusion protein.[9] We have subsequently shown that SMCD-eos patients who responded to imatinib carried the *FIP1L1-PDGFRA* fusion.[4]

These observations suggest that the cases described by Klion et al may actually represent SMCD-eos rather than HES associated with reactive mast cell proliferation. Further clarification awaits the performance of either interphase cytogenetics or other molecular approaches for *FIP1L1-PDGFRA* detection in purified primary mast cells. Until then, our data indicate, as has been demonstrated for gastrointestinal stromal tumors,[10] that activating mutations in