Bone marrow angiogenesis in multiple myeloma: closing in on the loop

Angiogenesis refers to the process of new blood vessel formation that occurs during embryonal growth, wound healing, the menstrual cycle, and in certain diseases of the eye. Angiogenesis is also recognized as being critical for tumor growth, invasion and metastasis.[1,2] The various steps of angiogenesis, such as basement membrane disruption, endothelial cell migration and proliferation, and tube formation, occur in response to angiogenic triggers mediated by cytokines such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF).

VEGF (also referred to as VEGF-A) is one of the major inducers of angiogenesis. The protein is structurally related to PlGF (placenta growth factor), VEGF-B, VEGF-C, VEGF-D and Orf virus-derived VEGF (also called VEGF-E).[3] VEGF is a survival and a proliferation factor for human microvascular endothelial cells. There are 5 different isoforms formed by mRNA splicing: VEGF121, VEGF145, VEGF165, VEGF189, and VEGF206. VEGF plays a critical role during embryo development; the loss of just one allele in knockout mouse models results in embryonic death.[4,5] To date, there are two known receptor tyrosine kinases that bind VEGF: VEGFR-1 (also called Fms-like tyrosine kinase, Flt-1) and VEGFR-2 (also called kinase domain region, KDR).[6] VEGFR-3 (also called FLT 4) is the receptor tyrosine kinase that mediates lymphangiogenesis; VEGF-C and VEGF-D serve as ligands for VEGFR-3. VEGF mRNA and protein are upregulated by hypoxia and VEGF mRNA is often elevated near areas of tumor necrosis. Tumor hypoxia and locally increased VEGF concentrations also upregulate VEGFR-1 and VEGFR-2. Stimulation of VEGF receptors in endothelial cells by VEGF leads to activation of the MAP kinase and JAK-STAT signaling pathways.[6]

The role of angiogenesis and VEGF in multiple myeloma has been the subject of intense investigation in recent years. In 1994, Vacca et al. first determined that bone marrow angiogenesis was markedly increased in myeloma compared to in its pre-malignant state, monoclonal gammopathy of undetermined significance (MGUS).[7] Further they showed that the increase in angiogenesis was correlated to plasma cell proliferative rate. Subsequent studies by our group have confirmed these pivotal observations.[8-10] Further confirmation came from more recent work by Vacca et al., who demonstrated that 76% of purified myeloma samples from patients are angiogenic whereas only 20% of MGUS samples are so in the in vitro chick embryo chorioallantoic membrane (CAM) angiogenesis assay.[11] Bone marrow angiogenesis has since been shown to have prognostic value in myeloma,[9,12] and in some studies appears to persist even after conventional dose or high dose chemotherapy.[8,13] In fact, microvessels in the marrow appear to persist even after therapy with thalidomide, an agent with known anti-angiogenic properties;[14] however the lack of resolution of microvessels may not be an accurate way to measure the effect of anti-angiogenic therapy.[15] Overall, these studies suggest that induction of angiogenesis is a feature of, and possibly important in the transformation of MGUS to myeloma, and in the progression of early stage myeloma to advanced, refractory disease.

Increased angiogenesis in myeloma, as in other tumors, is likely mediated by an alteration in balance between pro- and anti-angiogenic cytokines. Several studies show overexpression of VEGF by clonal plasma cells.[16-20] bFGF also appears to be important. Sezer et al. found increased levels of serum bFGF in myeloma and that these levels decreased with effective chemotherapy.[21] Vacca et al. have shown that antibodies to bFGF cause a significant inhibition (>50%) of the angiogenesis induced by myeloma cells in the CAM assay.[11] Besides VEGF and bFGF, aquaporin 1 and matrix metalloproteinase-2 may be important, and their expression appears to correlate with the increased angiogenesis seen in myeloma.[11,22]

Angiogenesis may contribute to the pathogenesis and progression of myeloma in two ways: (i) by ensuring an adequate tumor oxygen and nutrient supply and (ii) by paracrine stimulation of tumor cell migration and proliferation. Likewise, in addition to stimulating angiogenesis, VEGF may also have paracrine or even autocrine effects in myeloma. The paracrine role of VEGF in myeloma was first proposed by Dankbar et al., who found that stimulation of myeloma cell lines with interleukin-6 (IL-6) results in an increase in VEGF secretion.[17] Similarly, stimulation of endothelial cells and bone marrow stromal cells with VEGF induced a significant increase in IL-6 secretion in a dose-dependent manner.

Starting with their pioneering observations in 1994, Vacca et al. have contributed immensely to our understanding of the role of angiogenesis and angiogenic cytokines in multiple myeloma. In this issue of Haematologica, they add to their vital contributions in an important paper that presents further evidence for the role of VEGF in myeloma.[23] In a series of well conducted experiments, Vacca et al. show that VEGF is expressed and secreted by myeloma cells and that it stimulates proliferation and chemotaxis in both endothelial cells (VEGFR-2 signaling) and stromal cells (VEGFR-1 signaling). Their data provide further support for the presence of a paracrine loop for tumor growth and angiogenesis in multiple myeloma. The study adds to prior data about the role of VEGF during myeloma progression and provides further rationale for considering the

VEGF pathway as a target for myeloma therapy. Although the authors found interesting results with VEGF-C and VEGF-D secreted by stromal cells on plasma cell DNA synthesis rate, the role of these cytokines needs to be further studied and confirmed.

Is VEGF overexpressed in myeloma compared to in MGUS? Vacca *et al.* report higher VEGF expression in myeloma than in MGUS. Using immunohisto-chemistry methods, Bellamy previously made the same observation.[16] However, in a recent study using immunohistochemistry we were unable to find a significant increase in expression of VEGF by plasma cells in myeloma compared to in MGUS.[20] In our experience, quantifying the level of VEGF expression using bone marrow immunohistochemistry is difficult because of several factors, including intense VEGF staining by erythroid precursors[24] and diffi-culties in identifying plasma cells accurately in MGUS. After adjusting for the difference in plasma cell numbers between MGUS and myeloma, we found no significant differences in proportion of plasma cells that express VEGF between the two groups. We also found no significant difference in mRNA expression between MGUS and myeloma using quantitative real time reverse transcriptase polymerase chain reaction (RT-PCR) studies. Thus, in our opinion, the question of whether overexpres-sion and secretion of VEGF in myeloma is due to increased expression by each myeloma cell or mere-ly a function of the increase in plasma cells in myeloma compared to MGUS remains to be deter-mined.

What about expression of VEGF receptors by clon-al plasma cells? Bellamy has reported expression of VEGFR-1 by myeloma cells.[16] We have found expres-sion of both VEGFR-1 and VEGFR-2 by both myelo-ma cell lines and primary myeloma cells using immunohistochemical and RT-PCR studies.[19] In this issue of Haematologica, Vacca *et al.* describe intense expression of VEGFR-3 by myeloma cells. The expression of VEGF receptors by myeloma cells rais-es the intriguing possibility of autocrine effects, especially in the light of recent data showing that VEGF can enhance myeloma cell migration and pro-liferation.[17,25] This is also particularly interesting because there is evidence suggesting that VEGF has autocrine effects in other malignancies.[26,27]

Vacca *et al.* show that the increased proliferation and chemotaxis exhibited by endothelial and stro-mal cells in response to plasma cell conditioned media is not abolished fully by addition of VEGF antibody, suggesting the presence of other secret-ed angiogenic cytokines. This is important because future translational therapeutic efforts must take into account the redundancies built into the angio-genesis pathways, and the fact that blocking one cytokine (such as VEGF) with a small molecule inhibitor or humanized antibody will result in sub-optimal results in the clinic. There is significant interest in anti-angiogenic therapy for myeloma based on results with thalidomide, and given the variety of novel anti-angiogenic compounds avail-able. But clinical trials will need to be carefully designed and guided by findings from the laborato-ry to maximize chances of success. Research such as that presented in this issue of Haematologica cer-tainly gives us a better understanding of myeloma and provides attractive targets to pursue in our fight against this currently incurable malignancy.

Finally, the issue of whether angiogenesis is truly important for the pathogenesis of hematologic malignancies such as myeloma is a point of debate. Given the growing evidence, there is no reason to believe that angiogenesis is any less important in myeloma than it is in solid tumors. Moreover, angio-genesis appears to have important prognostic value in solitary bone plasmacytoma, which is arguably the solid tumor equivalent of myeloma.[28] Two types of studies may settle the debate.[10] One is a serial follow-up study of MGUS patients over time to demonstrate that increased angiogenesis occurs just prior to progression. The second type is a study showing that myeloma responds to a drug whose sole mechanism of action is anti-angiogenesis such as endostatin or angiostatin. Preliminary data from Fujii *et al.* show that endostatin does indeed induce regression of myeloma in a mouse model of myelo-ma, supporting a pathogenetic role for angiogene-sis.[29]

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References

1. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971;285:1182-6.
2. Folkman J. Seminars in Medicine of the Beth Israel Hospital, Boston. Clinical applications of research on angiogenesis. N Engl J Med 1995;333:1757-63.
3. Ferrara N, Gerber HP. The role of vascular endothelial growth factor in angiogenesis. Acta Haematol 2001;106:148-56.
4. Gerber HP, Hillan KJ, Ryan AM, Kowalski J, Keller GA, Rangell L, et al. VEGF is required for growth and survival in neona-tal mice. Development 1999;126:1149-59.
5. Haigh JJ, Gerber HP, Ferrara N, Wagner EF. Conditional inac-tivation of VEGF-A in areas of collagen2a1 expression results in embryonic lethality in the heterozygous state. Develop-ment 2000;127:1445-53.
6. Veikkola T, Karkkainen M, Claesson-Welsh L, Alitalo K. Reg-ulation of angiogenesis via vascular endothelial growth fac-tor receptors. Cancer Res 2000;60:203-12.

7. Vacca A, Ribatti D, Roncali L, Ranieri G, Serio G, Silvestris F, et al. Bone marrow angiogenesis and progression in multiple myeloma. Br J Haematol 1994;87:503-8.
8. Rajkumar SV, Fonseca R, Witzig TE, Gertz MA, Greipp PR. Bone marrow angiogenesis in patients achieving complete response after stem cell transplantation for multiple myeloma. Leukemia 1999;13:469-72.
9. Rajkumar SV, Leong T, Roche PC, Fonseca R, Dispenzieri A, Lacy MQ, et al. Prognostic value of bone marrow angiogenesis in multiple myeloma. Clin Cancer Res 2000;6:3111-16.
10. Rajkumar SV, Mesa RA, Fonseca R, Schroeder G, Plevak MF, Dispenzieri A, et al. Bone marrow angiogenesis in 400 patients with monoclonal gammopathy of undetermined significance, multiple myeloma, and primary amyloidosis. Clin Cancer Res 2002;8:2210-6.
11. Vacca A, Ribatti D, Presta M, Minischetti M, Iurlaro M, Ria R, et al. Bone marrow neovascularization, plasma cell angiogenic potential, and matrix metalloproteinase-2 secretion parallel progression of human multiple myeloma. Blood 1999;93:3064-73.
12. Sezer O. Bone marrow microvessel density is a prognostic factor for survival in patients with multiple myeloma. Ann Hematol 2000;79:574-7.
13. Kumar S, Fonseca R, Dispenzieri A, Lacy MQ, Lust JA, Witzig TE, et al. Bone marrow angiogenesis in multiple myeloma: effect of therapy. Br J Haematol 2002;119:665-71.
14. Singhal S, Mehta J, Desikan R, Ayers D, Roberson P, Eddlemon P, et al. Antitumor activity of thalidomide in refractory multiple myeloma. N Engl J Med 1999;341:1565-71.
15. Folkman J. Angiogenesis-dependent diseases. Semin Oncol 2001;28:536-42.
16. Bellamy WT, Richter L, Frutiger Y, Grogan TM. Expression of vascular endothelial growth factor and its receptors in hematopoietic malignancies. Cancer Res 1999;59:728-33.
17. Dankbar B, Padro T, Leo R, Feldmann B, Kropff M, Mesters RM, et al. Vascular endothelial growth factor and interleukin-6 in paracrine tumor-stromal cell interactions in multiple myeloma. Blood 2000;95:2630-6.
18. Rajkumar SV, Yoon SY, Li CY, Roche PC, Fonseca R, Dispenzieri A, et al. Angiogenesis in myeloma: expression of basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and their receptors by neoplastic plasma cells. Blood 1999;94:303b[abstract].
19. Kumar S, Timm T, Haug J, Wellik L, Witzig TE, Greipp PR, et al. Expression of VEGF and its receptors by myeloma cells. Blood 2001;98:373a[abstract].
20. Kumar S, Witzig TE, Thompson MA, Haug J, Timm T, Wellik L, et al. Expression of angiogenic cytokines by plasma cells: A comparison of MGUS, smoldering myeloma and newly diagnosed symptomatic myeloma. Blood 2002;100:807a [abstract].
21. Sezer O. Serum levels of the angiogenic cytokines basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) in multiple myeloma. Eur J Haematol 2001;66:83-8.
22. Vacca A, Ribatti D, Roccaro AM, Figeri A, Dammaco F. Bone marrow angiogenesis in patients with active multiple myeloma. Semin Oncol 2001;28:543-50.
23. Vacca A, Ria R, Ribatti D, Semeraro F, Djonov V, Raimondo F, et al. A paracrine loop in the vascular endothelial growth factor pathway triggers tumor angiogenesis and growth in multiple myeloma. Haematologica 2003;88:176-85.
24. Tordjman R, Delaire S, Plouet J, Ting S, Gaulard P, Fichelson S, et al. Erythroblasts are a source of angiogenic factors. Blood 2001;97:1968-74.
25. Podar K, Tai YT, Davies FE, Lentzsch S, Sattler M, Hideshima T, et al. Vascular endothelial growth factor triggers signalling cascades mediating multiple myeloma cell growth and migration. Blood 2001;98:428-35.
26. Bellamy WT, Richter L, Sirjani D, Roxas C, Glinsmann-Gibson B, Frutiger Y, et al. Vascular endothelial cell growth factor is an autocrine promoter of abnormal localized immature myeloid precursors and leukemia progenitor formation in myelodysplastic syndromes. Blood 2001;97:1427-34.
27. Bachelder RE, Crago A, Chung J, Wendt MA, Shaw LM, Robinson G, et al. Vascular endothelial growth factor is an autocrine survival factor for neuropilin-expressing breast carcinoma cells. Cancer Res 2001;61:5736-40.
28. Kumar S, Fonseca R, Dispenzieri A, Lacy MQ, Lust JA, Wellik L, et al. Prognostic value of angiogenesis in solitary bone plasmacytoma. Blood 2003;in press.
29. Fujii R, Yaccoby S, Epstein J. Control of myeloma with the anti-angiogenic agent endostatin. Blood 2002;96:360a [abstract].

Stem cell transplantation for patients with solid tumors

In this issue, Nieto[1] analyzes the relative efficacy of high-dose chemotherapy with stem cell support compared to standard treatment for high-risk primary or metastatic breast-cancer. He concludes that high dose chemotherapy remains too important an option to be prematurely discarded after preliminary analyses of a portion of the data. Ongoing studies must mature and then the data will speak for itself.

Hematopoietic stem cells are used for other purposes in medical oncology. As underlined by Bregni,[2] the observation that the benefits of allogeneic bone marrow transplantation in hematology depend, to a large extent, on an immunologic effect, has opened the way to exploitation of the same effect in oncology. The transfer of allografting to the solid tumor area has opened a new field of clinical research, focused on the alloreactive T-cell, and more generally, on adoptive immunotherapy as a treatment modality for selected malignancies. Several solid tumors are susceptible to the graft-versus-tumor effect. We also know that T-cells can eradicate tumor cells of host origin, but are also responsible for graft-versus-host disease, which still represents a major problem in allogeneic transplant. Many efforts are being devoted to understanding the graft-versus-tumor effect, and more specific strategies are being developed to increase selectivity of the allogeneic transplant.

These issues were addressed at the meeting *Allogeneic Hematopoietic Cell Transplantation for Solid Tumors* held in Milan, Italy, on June 28, 2002. These papers[3-11] are available online on the journal's web site.

References

1. Nieto Y. The verdict is not in yet. Analysis of the randomized trials of high-dose chemotherapy for breast cancer. Haematologica 2003;88:201-11.
2. Bregni M. Foreword. Haematologica 2002;87 Suppl 1:1.
3. Igarashi T, Mena O, Re F, Srinivasam R, Childs R. Exploring the role of allogeneic immunotherapy for non-hematologic malignancies: proof of concept and potential immune mechanisms of graft-vs-tumor effects in solid tumors. Haematologica 2002;87 Suppl 1:2-5.
4. Chung CY, Ueno NT. Non-myeloablative allogeneic peripheral blood progenitor cell transplantation for metastatic breast cancer and metastatic renal cell carcinoma: the M.D. Anderson Cancer Center experience. Haematologica 2002;87 Suppl 1:6-9.