LEUKEMIA CELLS AND THE BONE MARROW ENDOTHELIUM

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ABSTRACT

Angiogenesis, the recruitment and proliferation of endothelial cells leading to formation of new blood vessels from preexisting ones, plays a critical role in the growth of solid tumors. The biological and clinical implications of bone marrow (BM) endothelium interaction with hematological tumor cells remain controversial. However, there is evidence suggesting the existence of BM niches where endothelial and leukemia cells contribute with mutually beneficial stimuli that promote both an angiogenic phenotype and leukemia expansion. Factors such as SDF1 and VEGF have been implicated in the interplay between endothelium and leukemia, and may constitute targets for therapeutic intervention. Another factor, IL-7, is produced by BM stroma and endothelium, and appears to stimulate endothelial cells. Moreover, we and others have shown that IL-7 is a T-cell leukemia growth factor. IL-7 mediates leukemia proliferation and viability by triggering the activation of PI3K/Akt(PKB) pathway leading to $p27^{kip1}$ downregulation, Bcl-2 upregulation, and consequent cell cycle progression and decreased apoptosis. Given the evidence IL-7 can further stimulate leukemia T-cell motility and directional migration, it is tempting to hypothesize the existence of BM niches where stroma/endothelium-produced IL-7 promotes leukemia expansion.

Keywords: Leukemia; bone marrow endothelial cells; IL-7; angiogenesis

INTRODUCTION

Cancer cells are not isolated entities, multiplying inexorably irrespectively of what surrounds them. On the contrary, they evolve in complex microenvironments and are responsive to exogenous stimuli that include the extracellular matrix, cytokines, chemokines and cell-cell contact. In addition, they are able to modulate their environment in an advantageous manner, for example by releasing defined soluble factors, contributing to extracellular matrix remodeling, and stimulating or inhibiting neighboring cells.

A TUMOR'S NEED FOR ANGIOGENESIS

The recruitment and proliferation of endothelial cells from preexisting vasculature resulting in the formation of new blood vessels defines angiogenesis. Tumors, despite their tendency to expand relentlessly, are nonetheless constituted by cells and thus restricted by two basic needs, which no cell is able to overcome, malignant as it may be: oxygen and nutrients. As the tumor mass grows, how do the cells in the inner portions of the tumor satisfy these essential needs, since passive diffusion is not sufficient to deliver oxygen and nutrients and remove metabolic waste? The answer is known for many years now: they release proangiogenic factors, which promote the formation of new vasculature that penetrates into the tumor mass[1]. More precisely, there is evidence that production of proangiogenic factors by hypoxic cells in the tumor core overcomes the expression of antiangiogenic factors in what is called the 'angiogenic switch', which will ultimately shift the balance towards an angiogenic phenotype[2] (Figure 1). Evidently, the dependency on angiogenesis for tumor growth has stimulated the quest for anti-angiogenic therapeutic agents and strategies involving the use of inhibitors of angiogenesis with the ultimate goal of rendering cancer a controlled, chronic 'disease'[2,3]. However, much is still to be learnt, since tumors often appear to be able to circumvent the effect angiogenesis inhibitors[4].

HOW DO TUMORS PROMOTE ANGIOGENESIS?

Upon the angiogenic switch, the net balance of proangiogenic factors perceived by endothelial cells (ECs) will result in their activation. Activated cells loosen the contacts with adjacent cells and start pro-

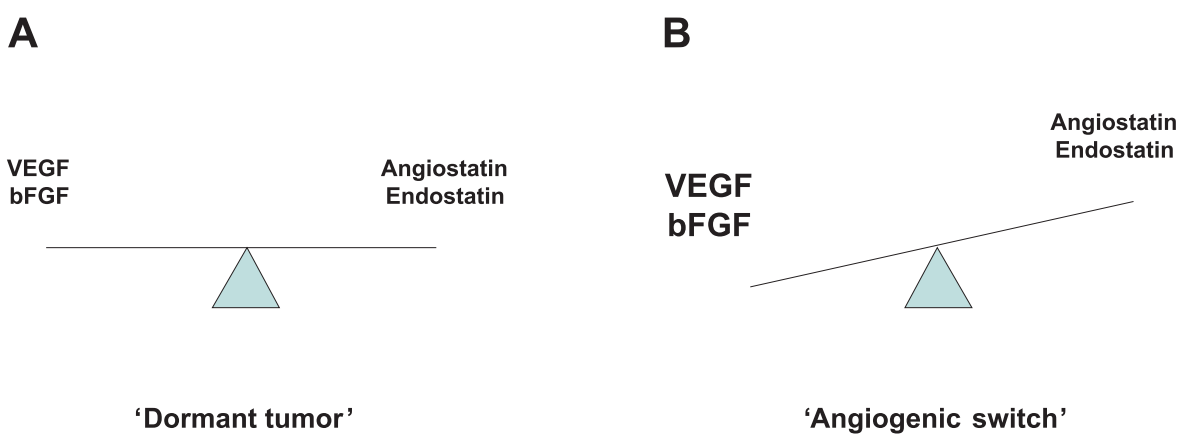

Fig. 1 – Schematic simplified representation of the "angiogenic switch". **(A)** Dormant tumors present a balance between the levels of proangiogenic factors (represented here by VEGF and bFGF) and antiangiogenic factors (represented here by angiostatin and endostatin). **(B)** Upon increase in hypoxic conditions tumors tend to increase the production of proangiogenic factors and/or block the synthesis of antiangiogenic molecules. The "angiogenic switch" occurs when the net signal received by nearby endothelium is angiogenic.

ducing proteases (including matrix metalloproteases – MMPs) that locally degrade the basement membrane. Subsequently, ECs are able to move through the gap in the basement membrane and into the extracellular matrix (ECM). Neighboring ECs may subsequently follow the leading cells into the ECM[5]. After extravasation, ECs continue to secrete proteases, which also degrade the ECM, allowing the ECs to move away from the parent vessel and towards the tumor, forming sprouts that eventually originate capillary structures with a lumen, form anastomoses, and allow for actual blood flow. To do so, ECs respond to the proangiogenic factors first by migrating in a chemotactic-like fashion and second by proliferating.

ANGIOGENESIS IN HEMATOLOGICAL TUMORS?

It is rather intuitive that solid tumors should rely on promoting angiogenesis to grow. However, why would hematological tumors develop such an approach, since in general they do not assemble into masses of malignant cells? The surprising answer is that most of them do. For example, the leukemic bone marrow (BM) is commonly characterized not only by a significant infiltration with malignant blasts that disrupt the normal BM architecture but also by increased numbers of endothelial cells and blood vessels. This is true for both myeloid and lymphoid leukemia[3,6]. Interestingly, there appears to be a clear correlation between the number of leukemic blasts and the number of vessels in the BM[7,8], hinting on the existence of a crosstalk and interdependency between BM endothelial cells (BMECs) and leukemia cells.

INTERPLAY BETWEEN LEUKEMIA AND ENDOTHELIAL CELLS IN THE BONE MARROW

What are the factors involved in the interplay between leukemia cells and BMECs? It is known that hematological tumors produce VEGF, bFGF and other proangiogenic factors[3,9], and their BM and plasma levels are usually elevated in patients as compared to normal controls[9,10]. Similarly to what happens in solid tumors, these factors are implicated in angiogenesis in the BM of leukemia patients[11]. In turn, BMECs were shown to produce leukemia-stimulatory agents. One of these factors, SDF-1/CXCL12 is a potent chemoattractant for CXCR4-expressing leukemia cells[12], and exemplifies the capacity that BMECs also have to modulate the function of tumor cells. Evidently, VEGF and SDF-1 are not the only players in the leukemia-BMEC crosstalk and identification of other molecules with an impact on these processes should have great therapeutic potential.

INTERLEUKIN-7

The BM stroma produces interleukin 7 (IL-7), which promotes T-cell acute lymphoblastic leukemia (T-ALL) cell viability and proliferation[13]. The oncogenic potential of IL-7 was shown several years ago by experiments with IL-7 transgenic mice that demonstrated that these animals developed B and T-cell neoplasms[14]. Subsequently, we demonstrated that IL-7 promotes both T-ALL cell viability and cell cycle progression by downregulating the cyclin-dependent kinase inhibitor $p27^{kip1}$. Decreased expression of $p27^{kip1}$ contributes to cyclin-dependent kinase activity, Rb hyperphosphorylation and progression towards S and G2/M phases of the cell cycle. In addition, $p27^{kip1}$ downregulation contributes to Bcl-2 upregulation, which is mandatory for IL-7-mediated survival of T-ALL cells[15]. These downstream effects are dependent on activation of PI3K/Akt(PKB) signaling pathway[16] (Figure 2).

Irrespectively of the associated molecular mechanisms, IL-7 is a clear *in vitro* growth factor for T-ALL cells and our most recent studies indicate that IL-7 may significantly contribute to human leukemia progression *in vivo* (Silva et al, unpublished data). Thus, it is interesting to speculate whether IL-7 might also play a role on the crosstalk between leukemia cells and BMECs. The answer is not yet known. How-

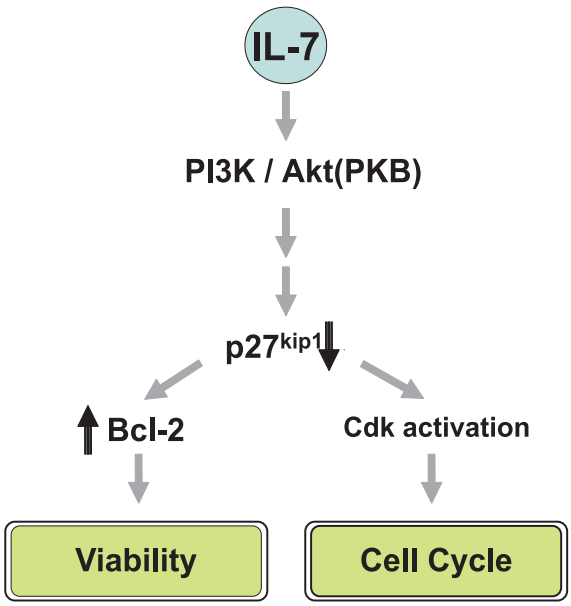

Fig. 2 – Summarized molecular and functional effects of IL-7 on leukemia T-cells.

ever, BMECs appear to express IL-7Rα and secrete IL-7 (Andres Yunes et al, unpublished data), suggesting the possibility of an autocrine/paracrine stimulatory loop (Figure 3). Stimulation with IL-7 prevents BMEC cell death in medium without growth factors, and potentiates the proliferative effect of proangiogenic factors. Finally, IL-7 contributes to the formation of capillary-like structures in matrigel-cultured BMECs (Andres Yunes et al, unpublished data).

As stated above, tumor cells including leukemias, produce angiogenic factors that stimulate BMEC migration. Likewise, it is currently known that BMECs are also able to attract leukemia cells in vitro[10] and in vivo[12]. Evidently, there are several candidates as mediators of this effect, the most prominent of which is SDF-1[17]. However, it is possible that IL-7 might also contribute to this effect in particular niches. Our preliminary data indicate that IL-7 promotes T-ALL cell motility and directional migration in vitro (Henriques et al, unpublished data). Hence, IL-7 produced by BMECs could not only participate in the stimulation of BMECs themselves but also in the recruitment of leukemia cells, with consequent stimulation of their viability and proliferation. In turn, it is known that IL-7 stimulates VEGF production at least in some cells, including normal thymocytes and breast cancer cells[18]. This raises the possibility that IL-7 stimulation of leukemia cells will further contribute to the production of VEGF that will serve as a growth factor for both leukemia cells and BMECs[11]. The model formulated from the studies and hypotheses described here is presented in Figure 3.

CONCLUSIONS

In summary, evidence arising from different studies indicates that there is a clear interplay between leukemia cells and BMECs that eventually leads to a positive loop, which contributes to increased angiogenesis and tumor expansion. Whether such a loop includes a relevant role for the BM microenvironmental cytokine IL-7 remains to be elucidated, but it is tempting to hypothesize the existence of BM niches where

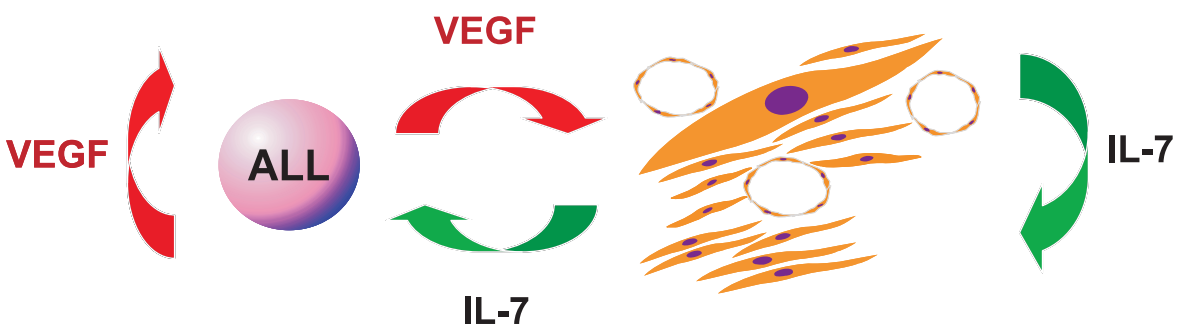

Fig. 3 – Hypothetical model of the contribution of IL-7 to endothelium – leukemia interactions in the BM.

stroma and/or endothelium-produced IL-7 participates in leukemia growth. Future studies should address this question and explore the possibility of targeting it for therapeutic purposes.

ACKNOWLEDGEMENTS

The author wishes to thank the contribution of all the members of Unidade de Biologia do Cancro, Instituto de Medicina Molecular: Ana Silva, Catarina Henriques, Bruno Cardoso, Leila Martins and Cristina Santos. Special acknowledgements to Dr. José Andrés Yunes for his ongoing collaboration, and to Dr. Angelo Cardoso for his past mentorship and for generously providing cartoon elements used in Figure 3. Part of the work mentioned or described herein was financially supported by grants from Fundação para a Ciência e a Tecnologia (POCI/SAU-OBS/58913) and from Associação Portuguesa contra a Leucemia.

REFERENCES

1. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med. 1971; 285:1182-1186.
2. Bergers G, Benjamin Le. Tumorigenesis and the angiogenic switch. Nat Rev Cancer. 2003; 3:401-410.
3. Moehler Tm, Neben K, Ho Ad, Goldschmidt H. Angiogenesis in hematologic malignancies. Ann Hematol. 2001; 80:695-705.
4. Yu Jl, Rak Jw, Coomber Bl, Hicklin Dj, Kerbel Rs. Effect of p53 status on tumor response to antiangiogenic therapy. Science. 2002; 295:1526-1528.
5. Paweletz N, Knierim M. Tumor-related angiogenesis. Crit Rev Oncol Hematol. 1989; 9:197-242.
6. Dong X, Han Zc, Yang R. Angiogenesis and antiangiogenic therapy in hematologic malignancies. Crit Rev Oncol Hematol. 2007; 62:105-118.
7. De Bont Es, Rosati S, Jacobs S, Kamps Wa, Vellenga E. Increased bone marrow vascularization in patients with acute myeloid leukaemia: a possible role for vascular endothelial growth factor. Br J Haematol. 2001; 113:296-304.
8. Hussong Jw, Rodgers Gm, Shami Pj. Evidence of increased angiogenesis in patients with acute myeloid leukemia. Blood. 2000; 95:309-313.
9. Bellamy Wt, Richter L, Frutiger Y, Grogan Tm. Expression of vascular endothelial growth factor and its receptors in hematopoietic malignancies. Cancer Res. 1999; 59:728-733.
10. Veiga Jp, Costa Lf, Sallan Se, Nadler Lm, Cardoso Aa. Leukemia-stimulated bone marrow endothelium promotes leukemia cell survival. Exp Hematol. 2006; 34:610-621.
11. Dias S, Hattori K, Heissig B, et al. Inhibition of both paracrine and autocrine VEGF/ VEGFR-2 signaling pathways is essential to induce long-term remission of xenotransplanted human leukemias. Proc Natl Acad Sci USA. 2001; 98:10857-10862.
12. Sipkins Da, Wei X, Wu Jw, et al. In vivo imaging of specialized bone marrow endothelial microdomains for tumour engraftment. Nature. 2005; 435:969-973.
13. Barata Jt, Cardoso Aa, Boussiotis Va. Interleukin-7 in T cell acute lymphoblastic leukemia: An extrinsic factor supporting leukemogenesis? Leukemia & Lymphoma. 2005; 46:483-495.
14. Rich Be, Campos-Torres J, Tepper Ri, Moreadith Rw, Leder P. Cutaneous lymphoproliferation and lymphomas in interleukin 7 transgenic mice. J Exp Med. 1993; 177:305-316.
15. Barata Jt, Cardoso Aa, Nadler Lm, Boussiotis Va. Interleukin-7 promotes survival and cell cycle progression of T-cell acute lymphoblastic leukemia cells by down-regulating the cyclin-dependent kinase inhibitor p27(kip1). Blood. 2001; 98:1524-1531.
16. Barata Jt, Silva A, Brandao Jg, Nadler Lm, Cardoso Aa, BOUSSIOTIS VA. Activation of PI3K Is Indispensable for Interleukin 7-mediated Viability, Proliferation, Glucose Use, and Growth of T Cell Acute Lymphoblastic Leukemia Cells. J Exp Med. 2004; 200:659-669.
17. Netelenbos T, Van Den Born J, Kessler Fl, et al. Proteoglycans on bone marrow endothelial cells bind and present SDF-1 towards hematopoietic progenitor cells. Leukemia. 2003; 17:175-184.
18. Al-Rawi Ma, Watkins G, Mansel Re, Jiang Wg. Interleukin 7 upregulates vascular endothelial growth factor D in breast cancer cells and induces lymphangiogenesis in vivo. Br J Surg. 2005; 92:305-310.