Gene Ther Mol Biol Vol 9, 41-46, 2005

Vascular endothelial growth factor as an effector of mast cell-induced tumor angiogenesis

Review Article

Domenico Ribatti[1,*], Andrea Marzullo[2], Beatrice Nico[1], Angelo Vacca[4], Enrico Crivellato[4]

[1]Department of Human Anatomy and Histology, University of Bari Medical School, Bari, Italy
[2]Department of Pathological Anatomy and Genetics, University of Bari Medical School, Bari, Italy
[3]Department of Biomedical Sciences and Human Oncology, Section of Internal Medicine and Clinical Oncology, University of Bari Medical School, Bari, Italy
[4]Department of Medical and Morphological Research, Anatomy Section, University of Udine Medical School, Udine, Italy.

***Correspondence**: Prof. Domenico Ribatti, Department of Human Anatomy and Histology, Piazza Giulio Cesare, 11, Policlinico, I-70124 Bari, Italy; Phone/Fax: +39.080.5478240; E-mail ribatti@anatomia.uniba.it

Key words: angiogenesis; mast cells; tumor progression; vascular endothelial growth factor

Abbreviations: chorioallantoic membrane, (CAM); extracellular matrix, (ECM); hepatocyte growth factor, (HGF); interleukin-8, (IL-8); mast cells, (MC); placenta growth factor, (PlGF); platelet-derived growth factor, (PDGF); Prostaglandin E2, (PGE-2); tissue inhibitor of metalloproteinase-1, (TIMP-1); transforming growth factor beta, (TGF-β); tumor necrosis factor alpha, (TNF-α); urokinase-type plasminogen-activator-receptor, (uPAR); vascular endothelial growth factor/vascular permeability factor, (VEGF/VPF)

Received: 11 March 2005; Accepted: 16 March 2005; electronically published: March 2005

Summary

The current wisdom is that tumors are endowed with an angiogenic capability and that their growth, invasion and metastasis are angiogenesis-dependent. Tumor cells are surrounded by an infiltrate of inflammatory cells, namely lymphocytes, neutrophils, macrophages and mast cells (MC), which communicate via a complex network of intercellular signaling pathways, mediated by surface adhesion molecules, cytokines and their receptors. This review article summarizes: i) the MC mediators involved in angiogenesis; ii) the experimental evidence concerning the role played by MC in tumor angiogenesis; iii) the role played by vascular endothelial growth factor contained in MC secretory granules in tumor angiogenesis.

I. Introduction

Angiogenesis

Angiogenesis is the formation of new blood vessel from pre-existing ones and takes place in various physiological and pathological conditions, such as embryonic development, wound healing, the menstrual cycle, chronic inflammation and tumors (Folkman, 1995; Risau, 1997). It is a multistep process that includes different phases: basement membrane degradation, endothelial cell migration and invasion of the surrounding extracellular matrix, endothelial cell proliferation and capillary lumen formation.

It is generally accepted that tumor growth is angiogenesis-dependent and that every increment of tumor growth requires an increment of vascular growth (Ribatti et al, 1999). Tumor angiogenesis is an uncontrolled and unlimited process essential for tumor growth, invasion and metastasis regulated by the interactions of numerous mediators and cytokines with pro-angiogenetic and anti-angiogenetic activity. Tumors lacking angiogenesis remain dormant indefinitely. An expanding endothelial surface also gives tumor cells more opportunities to enter the circulation and metastasize.

New vessels promote growth by conveying oxygen and nutrients and removing catabolities, whereas endothelial cells secrete growth factors for tumor cells and a variety of matrix-degrading proteinases that facilitate invasion. An expanding endothelial surface also gives tumor cells more opportunities to enter the circulation and metastasize, while their release of antiangiogenic factors explains the control exerted by primary tumors over metastasis. These observations suggest that tumor angiogenesis is linked to a switch in the equilibrium between positive and negative regulators. In normal tissues, vascular quiescence is maintained by the dominant influence of endogenous angiogenesis inhibitors over angiogenic stimuli. Tumor angiogenesis, on the other

hand, is induced by increased secretion of angiogenic factors and/or downregulation of angiogenesis inhibitors.

Growth of solid and hematological tumors consists of an avascular and a subsequent vascular phase. Assuming that the latter process is dependent on angiogenesis and depends on the release of angiogenic factors, acquisition of angiogenic capability can be seen as an expression of progression from neoplastic transformation to tumor growth and metastasis.

There is a great interest in identifying and modulating antiangiogenic pathways and the development of antiangiogenic drugs for therapeutic purpose (Blagosklonny, 2004). Several approaches, inhibit tumor angiogenesis and more than 60 antiangiogenic compounds have been clinically evaluated. Because tumor-associated angiogenesis develops in a physiological context, its inhibition should not induce resistance and should potentiate the oncostatic effect, because each neovessel supplies hundreds of tumor cells. Inhibitors may be synthetic or semi-synthetic agents, endogenous inhibitors, or biological antagonists of the angiogenic cascade. By contrast, vascular targeting focused on specific molecular determinants of neovasculature is used for local delivery of a toxic effect that leads to vascular damage and tumor necrosis.

II. Angiogenic factors

Angiogenic factors can be produced by a number of cells such as embryonic cells, adult resident and inflammatory cells (fibroblasts, macrophages, T cells, plasma cells, neutrophils, eosinophils) and neoplastic cells. Several angiogenic factors have been identified, including vascular endothelial growth factor/vascular permeability factor (VEGF/VPF), placenta growth factor (PlGF), basic fibroblast growth factor/fibroblast growth factor-2 (bFGF/FGF-2), transforming growth factor β (TGF-β), hepatocyte growth factor (HGF), tumor necrosis factor α (TNF-α), interleukin-8 (IL-8) and angiopoietin-1 and –2.

A. VEGF

Vascular endothelial growth factor is a potent, multifunctional cytokine (a dimeric, disulfide-linked glycoprotein) with different actions on the vascular endothelium (Ribatti, 2005). The VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and PlGF. Several VEGF-A isoforms of 121, 145, 165, 189 and 206 amino acids respectively are recognized, generated by alternative splicing that differently encodes exons 6 and 7, where the peptides responsible for the heparin-binding capacity are located. The heparan-sulfate proteoglycans present in the extracellular matrix (ECM) bind the VEGF isoforms with high heparin affinity representing an extracellular VEGF storage and facilitating the interactions with its receptors. The expression of VEGF is regulated by several factors: IL-1β, -6, -10 and -13, FGF-4, platelet-derived growth factor (PDGF), TGF-β, insulin-like growth factor-1, TNF-α, gonadotropins, hypoxia and nitric oxide.

VEGF isoforms can bind to specific receptors, namely VEGFR-1/flt-1, VEGFR-2/ KDR/Flk-1, VEGFR-3/Flt-4, all sharing tyrosine kinase activity, and neuropilin-1, that is a nonkinase co-receptor expressed on the surface of endothelial and tumor cells. VEGFR-1 and VEGFR-2 are expressed on the surface of endothelial cells as well as trophoblast and placenta cells, monocytes, mesangial cells (VEGFR-1), hematopoietic stem cells and retinal progenitor cells (VEGFR-2), while VEGFR-3 is expressed on the surface of lymphatic endothelial cells.

The firstly recognized effect of VEGF was the ability to increase the permeability of the microvasculature to circulating macromolecules, such as plasma fibrinogen, favoring the formation of a fibrin network which serves as a substratum for endothelial cell migration during angiogenesis. Moreover, it has been demonstrated a mitogenic activity of VEGF on endothelial cell proliferation and migration.

VEGF is highly expressed in tissues undergoing to physiological angiogenesis, such as placenta, many fetal tissues, the proliferating endometrium and in the corpus luteum, and in several pathological conditions characterized by an intense angiogenic response, such as healing wounds, asthma, psoriasis, ischemic myocardium and rheumatoid arthritis.

1. VEGF in tumor angiogenesis

VEGF is expressed in most solid tumors such as colon, esophagus, stomach, pancreas, kidney, bladder, breast, head and neck carcinomas and in glioblastomas and also in haematological malignancies (Ribatti, 2005). VEGFRs are predominant in endothelial cells surrounding or penetrating malignant tissue, but are absent from vascular cells in the surrounding normal tissue. This finding suggest that VEGFRs expression is induced in endothelial cells during tumor angiogenesis by VEGF secreted by tumor cells. VEGF overexpression has been correlated with microvascular density in many tumors, which is in turn associated with poor prognosis.

The phenotype of tumor endothelial cells seems to be induced, at least in part, by VEGF. VEGF acts as a vascular permeability factor increasing fenestrations in endothelial cells. When endothelial cells invade a neoformed tumor, such as glioma or glioblastoma, they come into contact with tumor cells that produce growth factors, in particular VEGF, which may be responsible not only for vascular proliferation but also for the altered permeability properties of the neoformed vessels.

Blocking anti-VEGF antibodies, antisense VEGF cDNA and dominant negative VEGFR mutant inhibit tumor growth in different experimental models. There is strong evidence that antibodies that neutralize VEGF and antibodies that actually block VEGFR retard tumor growth and may reduce tumor size in mice, effects that are closely mediated through inhibition of angiogenesis. Antibodies that selectively recognize the complex that VEGF forms with VEGFR-2 on vascular endothelium have also been developed.

B. Mast cells and angiogenesis

MC are multi-functional long-lived cells characterized by the presence of numerous large cytoplasmic granules. MC develop, like other leukocytes,

from hematopoietic stem cells; in particular, in humans, they derive from CD 34^+, CD 13^+, FcεRI-, c-kit^+ multipotential precursors. As mononuclear agranular cells (undifferentiated committed cells) traverse the vascular space and complete their maturation in the peripheral tissues where they acquire concomitant phenotypic diversity under the control of micro-environmental factors, such as the c-kit ligand (Stem Cell Factor, SCF), secreted by fibroblasts, stromal cells and endothelial cells. MC survival and the differentiation is regulated also by other cytokines (IL-4, IL-6 and IL-10).

MC are found in almost all of the major organs and tissues of the body, particularly in association with connective tissue structures such as blood vessels, lymphatic vessels and nerves, and in proximity to surfaces that interface the external environment such as those of the respiratory and gastrointestinal system and the skin. This selective accumulation at tissue sites where foreign material attempts to invade the host suggests that MC are among the first cells to initiate defence mechanisms. MC are not found in avascular tissues such as mineralized bone, cartilage and the cornea

Most studies on neoplastic transformation have focused on events that occur within transformed cells. Recent works have addressed the microenvironment of tumor cells and documented its importance in supporting tumor progression. The pathogenesis of most cancers include complex and mutual interactions that affect the number and phenotype of the tumor cells and various normal stromal cells. The intricate tumor-microenvironmental interactions are increasingly recognized as critical features of several neoplasias. Tumor cells are surrounded by an infiltrate of inflammatory cells, namely lymphocytes, neutrophils, macrophages and MC, which communicate via a complex network of intercellular signaling pathways, mediated by surface adhesion molecules, cytokines and their receptors

There is an overwhelming evidence that the density of MC is strictly correlated with the extent of both normal and pathological angiogenesis, occurring in chronic inflammation and tumors (Meininger and Zetter 1992; Norrby and Woolley 1993). In experimentally induced tumors, an increasing number of MC has been demonstrated before the onset of angiogenesis in proximity of tumor cells (Kessler et al, 1976) and, in tumors induced in MC-deficient mice, a reduced angiogenesis and ability to produce metastasis have been reported (Starkey et al, 1988; Dethlefsen et al, 1994).

MC accumulation has been associated with enhanced growth and invasion of human mammary carcinoma, cervical carcinoma, gastric cancer, rectal cancer, haemangioma, Kaposi's sarcoma, lung cancer, laryngeal squamous cell carcinoma, a variety of skin tumors and haematological malignancies, such as multiple myeloma, B-cell non Hodgkin's lymphoma, B-cell chronic lymphocytic leukemia amd myelodysplastic syndrome (Ribatti et al, 2004). The functional sugnificance of tumor infiltrating MC is not enterely clear. They are thought to act as a host response to neoplasia and display tumoricidal activity in some *in vitro* assays.

MC contain several angiogenic factors including tryptase, chymase, heparin and histamine (Sorbo et al. 1994; Blair et al 1997), TGF-β, TNF-α (Moller et al, 1988) IL-8 (Grutzkau et al, 1997), FGF-2 (Qu et al. 1995, 1998) and VEGF (Grutzkau et al, 1998). Isolated MC and their secretory granules, but not degranulated MC, induced an angiogenic response *in vivo* in the chick embryo chorioallantoic membrane (CAM) assay (Ribatti et al, 2001). The addition of anti-FGF-2 or anti-VEGF antibodies reduced the angiogenic response of both MC and their secretory granules by 50% and 30%, respectively. These data support the evidence that the angiogenic properties of MC depend on the angiogenic molecules contained in their secretory granules and indicate that FGF-2 and VEGF are the angiogenic cytokines primarily and synergistically responsible for this vasoproliferative activity.

In neoplastic diseases MC are recruited via several mediators produced by tumor cells, such as c-kit receptor or SCF (Poole and Zetter 1983; Norrby and Woolley 1993), FGF-2, VEGF and PDGF, which are effective to picomolar concentrations and this finding suggests that MC would express surface receptors for these pro-angiogenic cytokines (Gruber et al 1995).

Heparin induces endothelial cell proliferation and migration *in vitro* (Thorton et al 1983; Alessandri et al 1984), whereas controversial results have been found *in vivo*, probably depending by its molecular size and degree of sulphation (Ribatti et al, 1987; Taylor and Folkman, 1982; Jakobson and Hahnenberg, 1991).

Histamine has angiogenic effect through both H1 and H2 receptors (Sorbo et al, 1994) and also contribute to the hyperpermeability of new formed microvessels during tumor angiogenesis, increasing leakage of plasma proteins and hence deposition of fibrin. Degradation products of fibrin are angiogenic *in vivo* (Thompson et al, 1995). Moreover, *in vitro* experiments revealed that histamine induces VEGF production in the granulation tissue (Ghosh et al, 2001).

SCF may induce urokinase-type plasminogen-activator-receptor (uPAR) expression in MC which, in turn, can chemotactically respond to uPA released by endothelial cells (Sillaber et al, 1997).

MC containing both chymase and tryptase are predominantly present in skin and other connective tissues, namely gut submucosa, while MC containing only tryptase are found in lung alveolar tissue and gut mucosa. Tryptase is a protease MC specific that is a potent mitogen for fibroblasts, smooth muscle cells, and epithelial cells (Brown et al 1995; Cairns and Walls, 1996) and could play an important role in neovascularization favoring the formation of capillary structures via a direct action on endothelial cells (Blair et al, 1997) or by activating latent metalloproteinases and plasminogen activator (Stack and Johnson, 1994). It has been demonstrated that in B-cell non Hodgkin's lymphoma, myelodysplastic syndrome, B-cell chronic lymphocytic leukemia, and cutaneous melanoma angiogenesis is highly correlated with the total and MC tryptase-positive counts (Ribatti et al, 2000, 2002, 2003a, b).

Chymase is angiogenic by activating matrix metalloproteinases and inactivating tissue inhibitor of metalloproteinase-1 (TIMP-1). Muramatsu et al, (2000a, b) showed, by using the hamster sponge-implant assay, that angiogenesis is mediated by angiotensin II. Moreover, the angiogenic response is inhibited by treatment with chymase inhibitors.

III. Mast cells and VEGF

Human MC produces and secretes VEGF by the activation of the Fcε I receptor, which binds the Fc region of IgE antibody molecules. This activity is strongly increased by the exposition to high concentrations of IgE, thus explaining the role of VEGF in inflammatory diseases with an allergic pathogenesis and parasitic infections (Boesiger et al, 1998). Prostaglandin E2 (PGE-2), another essential mediator of inflammation, via the activation of the PGE-2 receptor, represents the most important inducer of the VEGF-A isoform by MC (Abdel-Majid and Marshall, 2003). MC syntesize and release VEGF also by a degranulation-independent mechanism (Nguyen et al, 2002).

MC are the main site of VEGF production in laryngeal squamous carcinomas (Sawatsubashi et al, 2000), in small lung carcinoma, where most intratumoral MC express VEGF (Imada et al, 2000; Takanami et al, 2000; Tomita et al, 2000), and in melanoma, where MC express both VEGF (Toth et al, 2001) and FGF-2 (Ribatti et al, 2003). Aoki et al, (2003) examined the expression of VEGF in basal cell carcinoma and demonstrated that the number of VEGF-positive cells was significantly higher than in controls. Wimazal et al, (2002) assessed the microvascular density in the bone marrow of patients with systemic mastocytosis and demonstrated by immunohistochemistry the VEGF was expressed in MC infiltrates.

Acknowledgments

Supported in part by Associazione Italiana per la Ricerca sul Cancro (AIRC), Milan, Fondazione Italiana per la Lotta al Neuroblastoma , Genoa, Ministero per la Salute – Regione Puglia (grant BS2) and Ministero dell'Istruzione, dell'Università e della Ricerca (Consorzio Carso Grant N. 72/2), Rome, Italy.

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