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Understanding the biology of angiogenesis: Review of the most important molecular mechanisms

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Abstract

Angiogenesis is an important process for forming new blood vessels. It is fundamental in many biological processes including development, reproduction and wound repair. Under these conditions, angiogenesis is a highly regulated process. Numerous inducers of angiogenesis have been identified, including the members of the vascular endothelial growth factor family, angiopoietins, transforming growth factors, platelet-derived growth factor, tumor necrosis factor-α, interleukins and members of the fibroblast growth factor family. Vascular endothelial growth factor-A is the most potent pro-angiogenic protein described to date. It induces proliferation, sprouting and tube formation of endothelial cells. Angiogenesis is therefore a putative target for therapy. The potential application of different angiogenesis inhibitors is currently under intense clinical investigation. A better understanding of the biology of angiogenesis may reveal new targets for treating many diseases that are associated with this complex process. In this review, we summarize the most important molecular mechanisms mediating angiogenesis.

Contents

Abbreviations: Ang, angiopoietin; ECs, endothelial cells; ECM, extracellular matrix; FGF, fibroblast growth factor; HSCs, hematopoietic stem cells; HIF, hypoxia-inducible factor; MMP, matrix metalloproteinases; PA, plasminogen activator; PDGF, platelet-derived growth factor; PKC, protein kinase C; TGF-β, transforming growth factor-β; tPA, tissue plasminogen activator; VEGFR, vascular endothelial growth factor; VEGF, vascular endothelial growth factor-receptor; uPA, urokinase-type plasminogen activator; uPAR, uPA receptor.

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Introduction

Angiogenesis is an important biological process, not only under physiological conditions, but in a variety of diseases including cancer, diabetic retinopathy and rheumatoid arthritis [1]. It is an essential process for forming new blood vessels [2]. It is fundamental in development, reproduction and wound repair. Under these conditions, angiogenesis is a highly regulated process, i.e. turned on for brief periods and then completely inhibited [3].

Tumor growth and metastasis are dependent on angiogenesis [4]. The first experiments to test the hypothesis that tumors produce angiogenic factors were performed by Greenblatt and Shubi [5] and Ehrmann and Knoth [6] demonstrating that transplantation of melanoma or choriocarcinoma cells promoted blood vessel proliferation even when a filter is interposed between the host and the tumor; this provided evidence that tumor angiogenesis was mediated by diffusible factor(s) produced by the tumor cells.

After the primary vascular plexus is formed, more endothelial cells (ECs) are generated, which can form new capillaries by sprouting or by splitting from their vessel of origin in a process termed angiogenesis [1]. Angiogenesis depends on the balance between different molecules released by the host and tumor cells and consists of a series of steps, including separation of endothelial cells from pericytes and the basement membrane, invasion and migration across basement membranes, and eventually resulting in the extension into the tumor body [7,8]. Specific angiogenic molecules can initiate this process and specific inhibitory molecules can stop it. Angiogenic factors and inhibitors have been discovered only in the past decade, the elucidation of their interactions with each other is only beginning to be uncovered [3].

Numerous inducers of angiogenesis have been identified, including the members of the vascular endothelial growth factor (VEGF) family, angiopoietins, transforming growth factors (TGF), platelet-derived growth factor, tumor necrosis factor-α, interleukins and the members of the fibroblast growth factor (FGF) family [9,10]. In addition, many factors control and influence angiogenesis including soluble growth factors, membrane-bound proteins, cell–matrix and cell–cell interactions, and many interacting systems [9].

Soluble factors in regulating angiogenesis

Vascular endothelial growth factor

Vascular endothelial growth factor-A (VEGF-A; also referred to as VEGF) is the best characterized and the most studied of the VEGF family members. It is a tumor-secreted cytokine with grave importance in both normal and tumor-associated angiogenesis [11]. The VEGF gene which is located on the short arm of chromosome 6 is composed of eight exons and is differentially spliced to yield four mature isoforms (VEGF121, VEGF165, VEGF189 and VEGF206) [12]. In addition, some less commonly expressed isoforms were identified (VEGF145 and VEGF183) [13]. VEGF165 is the predominant isoform and is over-expressed in a variety of solid tumors. VEGF189 is thought to be most potent for vascularization in various cancers [14].

VEGF-A exerts its biologic effect through interaction with cell surface receptors. These receptors are transmembrane tyrosine kinase receptors and they include VEGF receptor-1 (VEGFR-1) and VEGFR-2, selectively expressed on vascular ECs, and the neuropilin receptors (NP-1 and NP-2), expressed on neurons and vascular endothelium [15]. Upon binding of VEGF-A to the extracellular domain of the receptor, a cascade of downstream proteins are activated after the dimerization and autophosphorylation of the intracellular receptor tyrosine kinases. VEGFR-2 appears to be the major receptor responsible for mediating the pro-angiogenic effects of VEGF-A [16,17]. VEGF-A is the most potent pro-angiogenic protein described to date. It induces proliferation, sprouting and tube formation of endothelial cells (ECs) [16]. In addition, it causes vasodilatation by inducing the endothelial nitric oxide synthase and so increasing nitric oxide production [18]. VEGF-A binds many receptors on hematopoietic stem cells (HSCs), monocytes, osteoblasts and neurons [16]. It induces HSC mobilization from the bone marrow, monocyte chemoattraction and osteoblast-mediated bone formation [16,19].

In vivo, VEGF-A expression has been shown to be associated with significant steps in angiogenesis and physiologic vasculogenesis [20,21]. In mice, deletion of the VEGF-A gene is lethal, resulting in vascular defects and cardiovascular abnormalities [22]. VEGF-A affects an important number of angiogenic processes including wound healing, ovulation, maintenance of blood pressure, menstruation and pregnancy [23]. In humans, VEGF-A is expressed in practically all solid tumors studied as well as in some hematological malignancies [16].

VEGF-B yields two polypeptide forms, VEGF-B_{167} and VEGF-B_{186} by alternative splicing [24]. VEGF-B binds to VEGFR-1 but not VEGFR-2 or VEGFR-3 [25]. The precise role of VEGF-B *in vivo* is not known. VEGF-B might have a role also in inflammatory angiogenesis, and this was deduced in knockout mice which displayed reduced angiogenic responses in collagen-induced arthritis [26]. Silvestre and co-workers clearly demonstrated for the first time that VEGF-B promotes angiogenesis in association with activation of Akt and eNOS-related pathways [27].