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Review Article

VEGF-A, cytoskeletal dynamics, and the pathological vascular phenotype

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Abstract

Normal angiogenesis is a complex process involving the organization of proliferating and migrating endothelial cells (ECs) into a well-ordered and highly functional vascular network. In contrast, pathological angiogenesis, which is a conspicuous feature of tumor growth, ischemic diseases, and chronic inflammation, is characterized by vessels with aberrant angioarchitecture and compromised barrier function. Herein we review the subject of pathological angiogenesis, particularly that driven by vascular endothelial growth factor (VEGF-A), from a new perspective. We propose that the serious structural and functional anomalies associated with VEGF-A-elicited neovessels, reflect, at least in part, imbalances in the internal molecular cues that govern the ordered assembly of ECs into three dimensional vascular networks and preserve vessel barrier function. Adopting such a viewpoint widens the focus from solely on specific pro-angiogenic stimuli such as VEGF-A to include a key set of cytoskeletal regulatory molecules, the Rho GTPases, which are known to direct multiple aspects of vascular morphogenesis including EC motility, alignment, multi-cellular organization, as well as intercellular junction integrity. We offer this perspective to draw attention to the importance of endothelial cytoskeletal dynamics for proper neovascularization and to suggest new therapeutic strategies with the potential to improve the pathological vascular phenotype.

Keywords: Pathological angiogenesis; Angioarchitecture; Hyperpermeability; VEGF; Morphogenesis; Endothelial cytoskeletal dynamics; Actin; Rho GTPases; RhoA; Rac1; Cdc42

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Introduction

Pathological angiogenesis, which is characterized by vessels with aberrant angioarchitecture and impaired functionality, is generally associated with cancer and a variety of ischemic and inflammatory diseases. Vascular endothelial growth factor (VEGF-A), one of the most potent pro-angiogenic growth factors identified thus far, has been implicated in orchestrating the generation of neovessels in almost all types of pathological settings (see recent reviews [1–5] and references therein). Whereas the presence of VEGF-A is absolutely essential for the formation of a normal vascular network during both embryonic development [6–8] and neonatal vascularization [9–12], as well as for the maintenance of some specialized capillary beds in the adult [13], the overexpression of VEGF-A associated with various pathologies, e.g., solid and ascites tumor growth, proliferative retinopathy, psoriasis, rheumatoid arthritis [14–17], results in the formation of a neovasculature that is distinguished by serious structural and functional defects. The purpose of this review is three-fold. First, we will briefly summarize the critical defects associated with VEGF-A-driven pathological neovascularization. Next, we will describe recent progress in our understanding of specific cellular and molecular mechanisms and signaling pathways involving the regulation of endothelial cytoskeletal dynamics that may, at least in part, explain the basis for these abnormalities. Finally, we will conclude with a summary of the results of a recent study based on a new strategy designed to manipulate one of the key molecular regulators of vascular morphogenesis. We suggest that this novel approach holds the promise to improve at least some of the deficiencies that characterize the pathological neovasculature.

Pathological angiogenesis: structural and functional defects

A conspicuous, if not pathognomic, feature of tumor growth, retinal pathologies, and chronic inflammation is the appearance of a disordered, often bizarre, neovascular network composed of vessels with increased diameter and tortuous course that stands in stark contrast to the surrounding normal vasculature [18–22]. As outlined in Fig. 1 and discussed in detail below, such pathological vessels possess both structural and functional defects and differ substantially from their normal counterparts.

Aberrant vascular architecture and abnormal blood flow

The blood vessels that supply normal adult tissues are organized into a hierarchy of arteries, arterioles, capillaries, post-capillary venules, and small and large veins that are distributed uniformly and branch at regular intervals [23]. Each of these vessel types has a characteristic size and structure that allows it to perform its specialized function. In contrast, pathological vessels, particularly those that are associated with very high levels of VEGF-A expression, are characteristically heterogeneous and highly abnormal [24–26]. Histological, morphometric, and ultrastructural approaches have been utilized to examine the aberrant vascular topology found in inflammation [27], in syngeneic [28,29] and xenogeneic tumors [30–33] as well as in clinical tumor specimens [34–36]. Vessels observed in these situations do not conform to a clear hierarchical pattern. Rather, they are non-uniformly distributed, branch irregularly, and at atypical branching angles, appear tortuous, and can form arterio-venous (A-V) shunts and malformations [24–38].

Animal model systems have been designed to study more directly the effects of VEGF-A overexpression in vivo, i.e., in the absence of tumor and/or inflammatory cells and the ancillary growth factors that they might contribute [39–43]. In particular, adenoviral vectors expressing high levels of murine VEGF-A164 induce a highly reproducible, dose- and time-dependent angiogenic response when injected into a wide variety of normal mouse and rat tissues [40–43]. Adenoviral mediated delivery of VEGF-A164 leads to the formation of at least four distinct types of new blood vessels: (i) *Mother vessels* (MVs) which are greatly enlarged, thin-walled, serpentine, pericyte-poor, hyperpermeable, strongly VEGFR-positive sinusoids that arise from pre-existing microvessels by a process that involves vascular basement membrane degradation, pericyte detachment, and spreading and thinning of ECs in the absence of EC proliferation. (ii) *Daughter capillaries* that evolve from mother vessels by a process of EC proliferation coupled with transluminal bridging (TLB); (iii) *Glomeruloid bodies* (GBs) that begin as focal collections of primitive, CD-31 positive cells in the endothelial lining of mother vessels, proliferate rapidly, and then project both into the mother vessel lumen and outwardly into the extravascular connective tissue; and (iv) *Vascular malformations* (VMs) that form as mother vessels acquire an irregular coating of smooth muscle cells and/or perivascular fibrosis [41].

The various vascular patterns induced by VEGF-A overexpression that occur in these [40–43] and other experimental models [44–46] can each be found during the pathological angiogenesis that occurs in a variety of clinical settings. Mother vessels are also commonly found in healing skin wounds, myocardial infarcts, tumors, and inflammation [27,47,48]. Originally described in the tumor vasculature [28], bridging also occurs in the mother vessels of healing wounds [47] and in response to chronic vasodilation [28]. Glomeruloid bodies are commonly found in patients with glioblastoma multiforme [49], and also in cases of gastrointestinal carcinoma [50], aggressive malignant melanomas [51], and pulmonary inflammation [52]. Hemangioma-like vessels, similar in appearance to glomeruloid bodies generated by Ad-VEGF-A injection, also form following the intramuscular injection of myoblasts engi-