Angiogenesis and Current Antiangiogenic Strategies for the Treatment of Cancer

Rahmi Oklu, PhD, MD, Thomas G. Walker, MD, Stephan Wicky, MD, and Robin Hesketh, PhD

Angiogenesis is a complex process critical for embryonic development and for survival. It is also a critical player in many pathologic processes, most notably in neoplasia. The cell signaling pathways involved in angiogenesis have become key targets for drug design, with more than 2,500 clinical trials currently under way. This review summarizes the essential features of angiogenesis and discusses therapeutic strategies that have been applied to specific diseases known to be associated with perturbation of normal angiogenic control.

J Vasc Interv Radiol 2010; 21:1791–1805

Abbreviations: bFGF = basic fibroblast growth factor, DLL4 = Notch ligand delta-like 4, ERK = extracellular signal–regulated kinase, FDA = Food and Drug Administration, HIF = hypoxia-inducible factor, MAPK = mitogen-activated protein kinase, MMP = matrix metalloproteinase, mRNA = messenger RNA, mTOR = mammalian target of rapamycin, PDGF = platelet-derived growth factor, PGF = placenta growth factor, PHD = prolyl hydroxylase, PI3K = phosphatidylinositol 3-kinase, RTK = receptor tyrosine kinase, S1P = sphingosine-1-phosphate, TGF = transforming growth factor, TK = tyrosine kinase, VEGF = vascular endothelial growth factor, VEGFR = vascular endothelial growth factor receptor, VHL = von Hippel–Lindau

THE blood vascular system develops from hemangioblasts in the mesoderm that differentiate into angioblasts (also called vasoformative cells). Proliferation of these cells gives rise to dense, syncytial masses that develop into the vascular plexus during the process known as vasculogenesis. Fluorescence imaging of zebrafish reveals that coalescing angioblasts form a single vascular cord that in turn becomes the first embryonic artery (the dorsal aorta). From this primordium a specific subset of angioblasts then sprout to form the first (ie, caudal) vein so that a common precursor has given rise to two unconnected vessels—the first artery and vein. Further extensive development of these primitive networks then forms the arterial and venous systems in the process of angiogenesis—the formation of a continuous network of new blood vessels from an original, established vessel (1). Around the fifth week of embryonic development, lymph sacs form from venous endothelial cells. This is the first step in the process of lymphangiogenesis that involves sprouting of lymphatic endothelial cells from these sacs to form the peripheral lymphatic vascular network.

From this summary of the circulatory system, we proceed to consider the cellular basis of angiogenesis and the molecular pathways that are the regulators thereof. In particular, we focus on the vascular endothelial growth factor (VEGF) family and also on the impact of signaling by Notch and transforming growth factor (TGF)–β. We then consider the importance of hypoxia in regulating VEGF responses. This process establishes the molecular basis on which antiangiogenic agents have been developed, and those currently in clinical use, particularly for the treatment of cancers, are discussed in turn. It was primarily the insight of Judah Folkman (2) approximately 40 years ago that initiated this field, and we conclude by discussing how much more fraught it has become than even the most pessimistic observer might have foreseen but that, nevertheless, there are grounds for therapeutic optimism.

STAGES IN ANGIOGENESIS

Angiogenesis is a complex process that involves the activation, proliferation, and directed migration of endothelial cells to form new capillaries from existing blood vessels. This sprouting of capillaries from preexisting vessels occurs during embryonic development but is almost absent in adult tissues except in wound healing (3). However, normal transient regulated angiogenesis occurs in adult tissues during the female reproductive cycle and during wound healing. Pathologic angiogenesis is characterized by the persistent proliferation of endothelial cells and is a prominent feature of a number of diseases, including rheumatoid arthritis, psoriasis, and proliferative retinopathy. Additionally, many tu-

From the Division of Vascular Imaging and Intervention, Department of Radiology (R.O., T.G.W., S.W.), Massachusetts General Hospital, Harvard Medical School, 55 Fruit St., GRB2, Boston, MA 02114-2696; and Section of Cardiovascular Biology, Department of Biochemistry (R.H.), University of Cambridge, Cambridge, United Kingdom. Received March 3, 2010; final revision received August 2, 2010; accepted August 22, 2010. **Address correspondence to** R.O.; E-mail: r6251@yahoo.com

T.G.W. is a paid consultant for the Scientific Advisory Panel of Medtronic (Minneapolis, Minnesota). None of the other authors have identified a conflict of interest.

DOI: 10.1016/j.jvir.2010.08.009

mors are able to attract blood vessels from neighboring tissues. The induction of new blood vessel growth is necessary if solid tumors are to grow beyond a minimal size, as in the example of relatively thin melanomas residing entirely above the basement membrane that are avascular and therefore rarely metastasize. In addition to promoting tumor growth and metastasis by supplying nutrients and oxygen, and removing waste products, angiogenesis also delivers immune cells, macrophages, and humoral factors to the vicinity of the tumor. The endothelial cells involved in tumor development dissolve their surrounding extracellular matrix, migrate toward the tumor, proliferate, and form a new vascular network (**Fig 1**). Extensive vascularization in early breast tumors appears to correlate with a poor prognosis, and the capacity to quantify angiogenesis and/or angiogenic growth factors may prove to be an important indicator for cancer therapies (4–6).

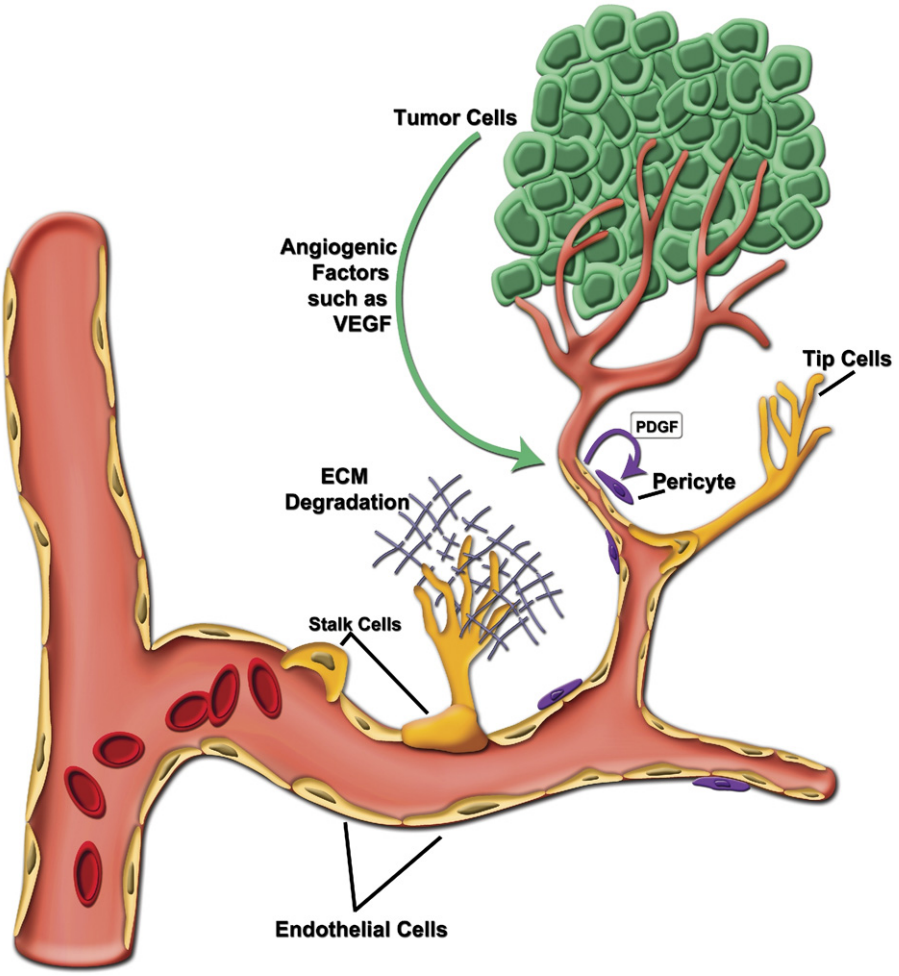

Figure 1. Angiogenic sprouting. In response to vascular endothelial growth factor A (VEGFA), endothelial cells release MMPs that degrade the extracellular matrix into which proliferating endothelial tip cells migrate. VEGFA also activates Notch signaling to inhibit proliferation of stalk endothelial cells. PDGF released from endothelial cells recruits smooth muscle cells (pericytes) that stabilize the neovasculature. MMP = matrix metalloproteinase, PDGF = platelet-derived growth factor. (Available in color online at *www.jvir.org*.)

The relatively detailed picture of these events that we now have has, of course, been built up gradually, but there have arguably been two major landmarks in the story of angiogenesis. The first was contributed in 1971 by Judah Folkman (2) when he speculated, in view of the evidence that angiogenesis was essential for tumor development, if ways could be found to inhibit it, they might form "a powerful adjunct to other cancer therapies." This notion was based on Folkman's observation that, in the absence of neovascularization, most solid tumors become dormant—that is, they fail to grow beyond a diameter of approximately 2 mm—together with the isolation of a diffusible entity released by malignant tumor cells that he called tumor angiogenesis factor because it promoted the formation of new vasculature in solid tumors (2). Several earlier reports had also noted that tumors elicit the growth of capillary endothelium (7–9), and Tannock et al (10,11) determined that the probability of a tumor cell undergoing mitosis was inversely proportional to its distance from the nearest capillary. Even more remarkably, Warren and Shubik (7) used a transparent chamber to observe transplantable tumors in the hamster cheek pouch and recorded that, as new blood vessels grew, they formed extremely tortuous patterns with many anastomoses and cross-linkages. Nevertheless, despite this apparent disorganization, it was possible to identify tumor types (eg, melanoma or mammary carcinoma) just from the vascular patterns generated by the tumor transplants (7,12,13). Indeed, it was Greenblatt and Shubik (14) in 1968 who first coined the term "tumor angiogenesis" to describe the vascularization associated with growing tumors.

Notwithstanding Folkman's prescience, the avalanche of effort that was to be brought to the subject moved almost undetectably until the second major angiogenesis event—the specific identification of proangiogenic agents. The first to be isolated in this quest were basic fibroblast growth factor (bFGF) (15) and VEGF, which was originally called "vascular permeability factor" (16,17). The significance of these discoveries is perhaps illustrated by the fact that, of nearly 70,000 published articles dealing with angiogenesis (68,703 to be precise), more than 96% have appeared in the 16 years since 1993.

For detailed summaries of the molecular biology of angiogenesis and lymphangiogenesis, readers are referred to numerous reviews, in particular those by Jain, Kerbel, Alitalo, Car-