Regulating angiogenesis

Vessels Under Control

Professor Czesław Cierniewski researches cell adhesion and migration

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Angiogenesis (the growth of new blood vessels) is an important body mechanism that can nevertheless be "hijacked" by certain types of cancer

A large number of different diseases are associated with the formation of new vasculature, i.e. blood vessel structure. Such pathologies include tissue damage after the reperfusion of ischemic tissue or cardiac failure, where such angiogenesis is low and should be enhanced in order to improve disease conditions. In several diseases, on the other hand, for example in cardiovascular diseases (atherosclerosis), chronic inflammation (rheumatoid arthritis, Crohn's disease), diabetes (diabetic retinopathy), endometriosis, and adiposity, excessive angiogenesis in fact constitutes part of the pathology itself.

Yet the most spectacular dependence upon the formation of new blood vessels is observable during the growth of solid cancers. While still less than 1-2 mm in size, such tumors begin to recruit new capillary blood vessels and tumor cells switch into the so-called angiogenic phenotype. This means that they start producing VEGF (vascular endothelial growth factor) and other biologically active molecules which increase tumor angiogenesis. That is why the current interest in angiogenesis stems mainly from oncology researchers, and antiangiogenesis therapy is considered a promising approach thought to hold a potential key for making a breakthrough in cancer therapy and the treatment of other proangiogenic diseases.

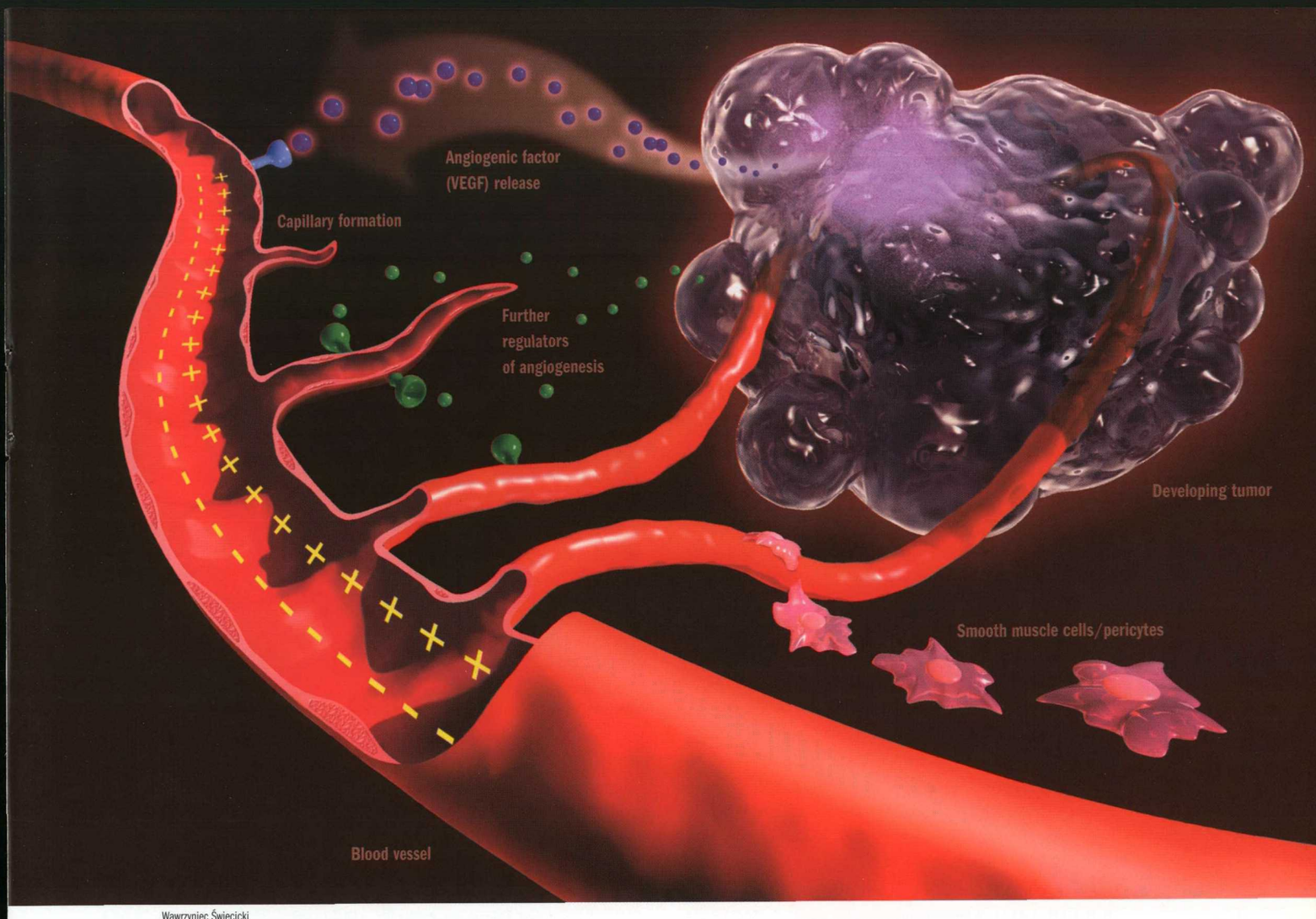
Phases of vessel formation

The growth of normal tissue depends upon new vessel formation to ensure the proper supply of oxygen and nutrients as well as the removal of waste products. Blood vessels themselves consist of various components: endothelial cells that are in direct contact with the blood, subendothelially located pericytes, smooth muscle cells, fibroblasts, basement membrane, and the extracellular matrix (ECM). Generally, the process of angiogenesis may be divided into two steps - a phase of activation and a phase of maturation. The formation of new blood vessels from preexisting ones requires endothelial cells to migrate, proliferate, and ultimately assemble into tubes that regulate the selective transport of blood components from their lumen (the blood vessel interior) to the interstitium (the space between tissues), and vice versa.

Like most other biological processes, angiogenesis depends on carefully controlled interactions between cells and the ECM. This process is mediated by specific proteins in the cell membrane, principally by proteins called integrins, which act as sensors (receptors) providing a link between the extracellular matrix and the cell interior. Integrins are involved in transmitting signals, hold a cell at a specific location, have an impact on its shape, and facilitate its migration. During angiogenesis, the production of integrin receptors is modulated by different factors and plays an important role in endothelial migration and in the formation of new vessels.

Research to uncover the secrets of angiogenesis could give us another strategy for halting tumor growth

Armed with this knowledge, we have recently developed a novel approach to inhibiting cancer-related angiogenesis, employing DNAzymes (DNA particles demonstrating enzymatic activity) to control the production of 2 kinds of integrins in endothelial cells. Our DNAzymes, in the presence of magnesium ions, specifically cleaved their substrates - synthetic integrin mRNA fragments. In the mouse model used in our research, this activity led to abolished capillary tube formation



Wawrzyniec Święcicki

Substances released by a developing tumor stimulate endothelial cells (the wall surface marked here with plusses), causing them to divide, migrate towards the tumor, and form new capillary tubes. The formation of mature blood vessels requires the presence of soft muscle cells and pericytes

and significantly reduced tumor growth. Thus it seems that DNAzymes are potentially useful as gene-inactivating agents and may ultimately provide a therapeutic means to inhibit angiogenesis in vivo.

Molecular balance

Angiogenesis is controlled by an intricate balance between molecules that increase new vessel formation and ones that impede this process. Endothelial cells may switch into a proangiogenic phenotype due to the induction of a positive regulator and/or the loss of a negative regulator. Positive regulators of angiogenesis include the vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) families of molecules and their receptors. A number of endogenous negative regulators have likewise been identified and described in the literature, including inhibitors of extracellular proteinases, thrombospondins 1 and 2, and bioactive fragments of the ECM and other molecules. In addition, extracellular proteolysis (degradation of proteins) appears to be an absolute requirement implicated in most phases of these processes, particularly during basement membrane degradation, cell migration/ECM invasion, and capillary lumen formation. Most of the relevant extracellular proteolytic enzymes belong to one of two families: the serine proteases and the matrix metalloproteinases (MMPs).

There is a carefully controlled proteolytic balance resulting from the precise protease-antiprotease equilibrium during all steps of angiogenesis. This means that even when increased proteolysis is consistently observed during the migration and invasion of endothelial cells, protease inhibitors still play an important role. These inhibitors have quite often been localized in cells surrounding proliferating vessels, where they may serve as guards preventing uncontrolled matrix destruction. This mechanism is crucial since the matrix scaffold required for invasion could be destroyed by excessive proteolysis, whereas insufficient proteolysis could in turn lead to the inhibition of invasion and lumen formation.

Angiogenic molecules

Interestingly, a number of fragments of the ECM and other molecules generated by extracellular proteolysis have been reported to influence vessel formation, either positively or negatively. The greatest attention has

been focused on angiostatin and endostatin, which are negative regulators derived from plasminogen and collagen XVIII, respectively. Fragments of some other ECM components likewise show either stimulatory or inhibitory activity towards angiogenesis. Non-ECM components from which fragments exhibiting antiangiogenic activity have been produced include prolactin, platelet factor 4, MMP-2, calreticulin, high-molecular-weight kininogen, and antithrombin.

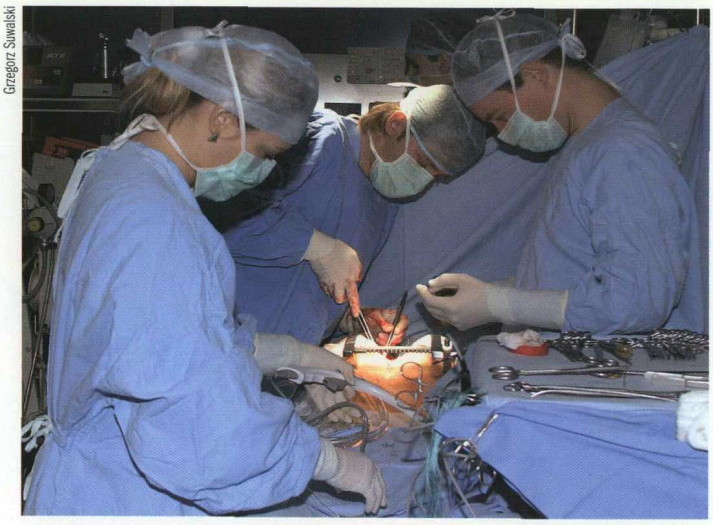
The overall significance of such naturally generated fragments in controlling angiogenesis seems to be supported by the failure of clinical attempts to apply synthetic inhibitors of MMPs. Most of these protein fragments are believed to be produced by MMPs. Since MMPs play an important role in extracellular proteolysis during new vessel formation as evidenced by preclinical data, they have long been considered to be potential targets in inhibiting angiogenesis. Nevertheless, the clinical application of MMP inhibitors has been disappointing and a number of phase III trials have been discontinued. One possible explanation may be that because MMPs have been implicated in the generation of numerous protein fragments with antiangiogenic activity, their inhibition may result in the stimulation rather than inhibition of angiogenesis. Another explanation could be that the synthetic MMP inhibitors are not specific to a single MMP.

Modulated angiogenesis

Researchers worldwide are currently analyzing a broad spectrum of angiogenesis modulation strategies, leading to the inhibition of different functions of endothelial cells during blood vessel formation. The most successful approach to date involves the use of agents that specifically inhibit growth of endothelial cells. Because the process of angiogenesis also depends on endothelial cell adhesion to the extracellular matrix and the migration of cells through it, efforts are being made to search for modulators of these interactions. Another mechanism related to the inhibition of endothelial cell adhesion and migration involves the use of specific inhibitors of enzymes (proteinases) that dissolve the connective tissue, thereby facilitating endothelial cell migration and subsequent vessel formation.

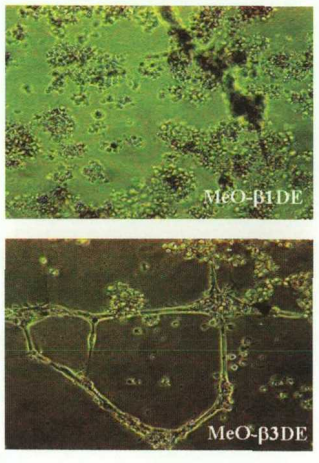
Overall, increased knowledge about the complex roles of the MMP and plasmin systems in the regulation of angiogenesis has opened up novel therapeutic opportunities. Significant benefits can also be anticipated in different clinical settings, from the regulation of extracellular proteolysis during vessel formation. ■

Grzegorz Suwalski



Current research interest in angiogenesis comes from oncologists, who might be able to "tame" a tumor by halting this process. Might such knowledge replace cardiac surgery in the future? Attempts are indeed already being made at developing the reverse application: using genetic therapy to stimulate the growth of new blood vessels – to treat coronary heart disease, for example

Czesław Cierniewski



In our experiments, DNAzymes (DNA molecules demonstrating enzymatic activity) efficiently abolished capillary tube formation (above photo) when compared to the control (below)

Further reading:

Griffioen A.W., Molema G. (2000). Angiogenesis: Potentials for Pharmacologic Intervention in the Treatment of Cancer, Cardiovascular Diseases, and Chronic Inflammation. *Pharmacol. Rev.* 52:237-268,

Cieslak M., Niewiarowska J., Nawrot M., Koziolkiewicz M., Stec W.J., Cierniewski C.S. (2002). DNA zymes to b1 and b3 mRNA downregulate expression of the targeted integrins and inhibit cell capillary tube formation in fibrin and Matrigel. *J. Biol. Chem.* 277: 6779-87,

De S., Razorenova O., McCabe N.P., O'Toole T., Qin J., Byzova T.V. (2005). VEGF-integrin interplay controls tumor growth and vascularization. *PNAS* 102: 7589-7594