Endothelial Cell Apoptosis in Angiogenesis and Vessel Regression

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Abstract—The programmed form of cell death (apoptosis) is essential for normal development of multicellular organisms. In the past few years, compelling evidence accumulated that dysregulation of apoptosis can lead to embryonal death and is involved in the pathophysiology of various inflammatory and degenerative diseases. Specifically, the occurrence of endothelial cell apoptosis has deleterious effects on the development of the cardiovascular system leading to embryonal death. Moreover, endothelial cell apoptosis counteracts neovascularization in the adult organism. On the basis of these findings, one may consider the regulation of endothelial cell apoptosis as a potential therapeutic target. The induction of endothelial cell apoptosis may limit unwanted neovascularization of tumors. In contrast, the prevention of endothelial cell apoptosis may improve angiogenesis and vasculogenesis in patients with ischemia. The present work critically reviews the existing data that supports a role of endothelial cell apoptosis for vascular growth and remodeling and provides insights into the mechanisms and the potential therapeutic consequences. (Circ Res. 2000;87:434-439.)

Key Words: cell death ♦ endothelial cells ♦ growth factors ♦ hemodynamic forces

The coordinated regulation of vasculogenesis, angiogenesis, and vessel regression is essential for the development of the vascular system.1,2 The induction of endothelial cell apoptosis dramatically disturbs the establishment of the primordial vascular network termed vasculogenesis in the embryo, causes severe hemorrhage, and leads to embryonal death. Of the various endothelial growth factors, specifically vascular endothelial growth factor (VEGF) seems to play a critical role to protect endothelial cells against apoptotic cell death during embryonal development, whereas angiopoietin-1 (Ang-1) or placenta-derived growth factor seems to exert modulating functions on vessel remodeling.3 Withdrawal of VEGF by targeted inactivation of the VEGF gene results in massive endothelial cell apoptosis, which leads to severe hemorrhage and is lethal in mouse embryo.4,5 Similarly, the VEGF receptor Flk-1, which predominantly mediates the antiapoptotic effects of VEGF,6 is required for definitive vasculogenesis during embryonal development.7 Blockade of antiapoptotic signaling pathways in endothelial cells can lead to vascular defects during embryonic development. Thus, inactivation of the vascular endothelial (VE)-cadherin gene, which mediates adhesion between endothelial cells, was found to induce embryonal death at embryonic day 9.5 caused by vascular insufficiency due to increased endothelial cell apoptosis.8 Importantly, endothelial cell proliferation and differentiation were not affected,8 illustrating the pivotal role of endothelial cell apoptosis suppression for intact embryonal development. Interestingly, the lack of a functional VE-cadherin gene blocked the capability of VEGF-A to stimulate the phosphatidylinositol 3'-kinase (PI3K)/Akt pathway and prevented VEGF-A–induced expression of the apoptosis suppressive pro-

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Angiogenesis

Angiogenesis, the process of postnatal neovascularization, is believed to be mediated by proliferation, migration, and remodeling of fully differentiated endothelial cells from preexisting vessels. Several lines of evidence indicate that endothelial cell apoptosis may play a major regulatory role in adult neovascularization. Counteracting proliferation, excessive apoptosis may limit angiogenesis and may actively lead to vessel regression. Growth factors, which are essential for angiogenesis, not only stimulate endothelial cell proliferation and migration but concomitantly inhibit endothelial cell apoptosis (see Figure). In detail, the vascular endothelial growth factor VEGF and basic fibroblast growth factor potently block endothelial cell apoptosis. Moreover, the ligand of the Tie2 receptor, Ang-1, which exerts critical in vivo angiogenic actions by mediating reciprocal interactions between the endothelium and the surrounding matrix, abrogates endothelial cell apoptosis. Although angiopoietin-1 has a distinct biological activity compared with VEGF, both factors share a common prosurvival activity.

Mechanistically, Ang-1 and VEGF activate the survival promoting PI3K/Akt pathway. Activation of the serine/threonine kinase Akt in turn stimulates the phosphorylation of proapoptotic proteins such as Bad and thereby inhibits apoptosis execution (for review, see Khwaja). Moreover, Akt activates the endothelial NO synthase, leading to enhanced synthesis of NO, which promotes endothelial cell survival by inhibiting the cysteine protease activity of caspasers via S-nitrosylation of the reactive cysteine residue. In addition, recent studies demonstrated a PI3K/Akt-dependent enhanced expression of the antiapoptotic protein survivin being important for the apoptosis protective effects of angiogenic factors. Finally, VEGF mediates upregulation of the antiapoptotic protein Bcl-2.

In addition to the PI3K/Akt pathway, ras-dependent signaling pathways may also play an important role at least for VEGF signaling. Thus, HrasV12G downregulation leads to profound tumor regression, which is initially characterized by massive endothelial cell apoptosis of tumor- and host-derived endothelial cells. Therefore, apoptosis induction is resistant to enforced VEGF expression, suggesting that VEGF requires an intact Ras-dependent signaling pathway to mediate its apoptosis inhibitory effect. Moreover, inhibition of MAPK activation by dominant-negative src kinase abrogates the apoptosis suppressive and the proangiogenic effect of VEGF.

In contrast to the apoptosis inhibitory effects of angiogenic factors, negative regulators of neovascularization were shown to stimulate endothelial cell apoptosis. Endostatin, which inhibits endothelial cell proliferation, angiogenesis and tumor growth, concomitantly stimulates endothelial cell death. The proapoptotic activity appears to be mediated via tyrosine kinase signaling and a reduction of the antiapoptotic proteins Bcl-2 and Bcl-XL. Similarly, the antiangiogenic protein angiostatin directly induces endothelial cell apoptosis. Moreover, endothelial cell apoptosis is also induced by thrombospondin-1, a naturally occurring inhibitor of angiogenesis. Thrombospondin-1 sequentially activates CD36, p59fyn, caspase-3 and the p38 MAPK. Taken together, these data suggest that natural inhibitors of angiogenesis act at least in part by specifically inducing apoptosis of endothelial cells to actively induce vessel regression.

Recent studies demonstrated that postnatal circulating bone marrow–derived endothelial progenitor cells may home to sites of neovascularization and differentiate into endothelial cells in situ, consistent with vasculogenesis. Thus, neovascularization in the adult organism may comprise both embryonic and angiogenic mechanisms. Importantly, VEGF gene transfer significantly augmented circulating endothelial progenitor cells in humans. It is conceivable that the antiapoptotic effect of VEGF might have importantly contributed to the increase in circulating endothelial progenitor cells.
Vessel Regression and Hemodynamic Forces

Vascular remodeling is characterized by a reorganization of blood vessel geometry in response to physiological alterations in blood flow or to pathophysiological stimuli. Physiologically, long-term increases in blood flow will increase lumen diameter and thus accommodate increased blood supply, for example, after surgical anastomosis or arteriovenous fistula, whereas regression of the uterus postpartum is associated with dramatic reductions of arterial diameters up to complete vessel occlusion. Because of its strategic location, positioned at the interface between the flowing blood and the underlying vessel wall, the endothelium should serve as the primary mediator of flow-mediated mechanotransduction to initiate vascular remodeling processes. Indeed, reductions in arterial diameter by long-term decreases in blood flow are strictly endothelium-dependent. A physiological role for endothelial cell apoptosis to contribute to vessel regression was suggested by Langille et al., who demonstrated that long-term reduction of carotid blood flow in rabbits caused loss of endothelial cells within days of flow reduction. Direct evidence for physiological endothelial cell apoptosis was obtained by investigating deletion of endothelial cells during atrophy of the vascular supply to the corpus luteum during luteal regression. In contrast, increases in endothelial cell numbers preceding increases in arterial diameter were observed, when blood flow was greatly increased by arteriovenous anastomosis. Thus, irrespective of circulating survival factors, the hemodynamic forces associated with arterial flow are major determinants of endothelial cell fate.

Insights into the relation between the hemodynamic forces exerted by the flowing blood (termed shear stress) and endothelial cell fate in situ have been gained by analyzing different regions within the vascular tree. At branches, curvatures, and bifurcations, where separations of the flow streamlines create low or even turbulent flow with reduced shear stress exposure of the endothelial cells, an increased cell cycle activity with proliferating endothelial cells is observed. In contrast, mitotic endothelial cells are rarely found in regions with laminar flow. Because contact inhibition of endothelial cell growth leads to an almost complete suppression of proliferation in the endothelial monolayer in vivo, the presence of proliferating endothelial cells in areas with reduced or absent shear stress indicates preceding endothelial cell loss. Indeed, shear stress seems to be one of the most potent inhibitors of endothelial cell apoptosis induced by a wide variety of stimuli, including withdrawal of serum-derived growth factors. Most importantly, the occurrence of apoptotic endothelial cells overlying atherosclerotic human plaques relates to the prevailing shear stress with dramatically increased apoptosis rates in areas with predictable disturbed flow. Thus, whereas suppression of endothelial cell apoptosis by physiological levels of shear stress contributes to vessel enlargement after increases in blood flow, reduced or even absent shear stress either due to a severe reduction of blood flow or local separation of the flow streamlines is associated with endothelial cell apoptosis leading to vessel regression.

Mechanistically, physiological levels of shear stress interfere with numerous steps of the endothelial cell apoptotic cascade. Sustained physiological levels of shear stress activate the PI3K/Akt pathway in an integrin-dependent fashion, thereby mimicking the signaling pathways used by specific endothelial cell growth factors such as VEGF or Ang-1. Similarly, shear stress increases endothelial NO synthase expression and NO production, which not only inhibits enzyme activity of caspases but is also an absolute requirement for neovascularization of ischemic tissue. In addition, shear stress upregulates a variety of oxygen radical scavenging enzymes in endothelial cells, thus limiting oxidative stress within the vessel wall. Taken together, it is important to note that the signaling cascades activated by hemodynamic forces acting on the endothelial cell layer resemble in many, if not all, respects the effects of endothelial-specific survival factors.

Recent clinical studies suggested that risk factors for coronary artery disease may modify an individual’s capacity for angiogenesis and vascular remodeling. Specifically, hypercholesterolemia or diabetes has been shown to be associated with a significant impairment in adaptive vascular growth of both capillary-like tubes and collateral vessels. Oxidized LDL or oxygen radicals are potent inducers of the caspase cascade in endothelial cells, supporting a potential role for endothelial cell apoptosis to contribute to the impairment in neovascularization. However, it remains to be determined whether the impairment in angiogenesis and vascular remodeling is due to the direct proapoptotic effects of classical risk factors on endothelial cells, or whether risk factors interfere with the signaling cascade used by survival factors. Preliminary data indeed suggest that oxidized LDL or tumor necrosis factor-α is capable to reverse phosphorylation of Akt in endothelial cells thereby shutting off the PI3K/Akt survival pathway. On the other hand, in serum-starved endothelial cells in vitro, tumor necrosis factor-α might induce PI3K activity. Thus, insights into potential mechanisms of interference of risk factors with endothelial cell survival cascades would be of utmost importance for the design of therapeutic angiogenesis by endothelial growth factors in patients with ischemic heart disease.

Potential Therapeutic Options

Inhibition of endothelial cell apoptosis may facilitate angiogenesis. Therefore, endothelial cell apoptosis could be inhibited by blockade of caspases, the apoptosis-executing enzymes, or by upregulation of antiapoptotic molecules such as Bcl-2 or survivin. However, an improvement of angiogenesis by inhibiting endothelial cell apoptosis has not been demonstrated so far. A more attractive concept might be the stimulation of pathways, which improve endothelial cell survival but further stimulate endothelial migration and proliferation, which also are required for angiogenesis. Experimental and initial clinical studies established VEGF as a therapeutic strategy for postnatal neovascularization of ischemic tissue (for review, see Isner and Asahara). However, one should keep in mind that high-risk patients may have defects in the signaling pathways mediating the effects of VEGF. For example, patients with elevated cholesterol levels are char-
characterized by increased oxidative stress, which might limit the bioavailability of NO, an essential downstream signal for adult neovascularization of ischemic tissue. In addition, one may consider the PI3K/Akt pathway as a potential therapeutic target. Overexpression of the protein kinase Akt potently blocks apoptosis by interfering with various apoptosis signaling pathways,18 stimulates endothelial cell migration,55,56 and enhances the expression of the hypoxia-inducible factor, which is known to stimulate VEGF expression,57 suggesting a potent proangiogenic effect.17,18 Alternatively, Akt inactivation could be prevented by inhibiting phosphatases such as PTEN, which dephosphorylates and thus deactivates Akt. Indeed, loss of PTEN was shown to facilitate angiogenesis.59,60

Finally, endothelial progenitor cells, which also contribute to the formation of new blood vessels (vasculogenesis), may be a target for antiangiogenic therapy. The VEGF receptor KDR/Flik-1, which predominantly mediates the antiangiogenic activity of VEGF, has been shown to be required for the generation of progenitor cells of the endothelial lineage.7 In addition, compared with differentiated endothelial cells, endothelial progenitor cells were shown to be much more sensitive for apoptosis induction, for example, by angiotatin.61 Therefore, one may speculate that supporting endothelial progenitor cell survival by the above-mentioned strategies may improve vasculogenesis.

In contrast, stimulation of endothelial cell apoptosis may prevent tumor blood supply and result in tumor regression. Indeed, withdrawal of the survival signals mediated by VEGF not only leads to endothelial cell apoptosis during embryonal development but also in tumors. Shutting off VEGF in tumors leads to endothelial cell detachment and apoptosis, followed by vascular collapse, hemorrhage, and tumor necrosis.62 Furthermore, antitumor activity was achieved by ribozymes directed against the VEGF receptors either by conditional switching or administration of ribozymes against the VEGF receptor.63 Alternatively, one could use antiangiogenic factors with known proapoptotic activity in combination with conventional therapy. Thus, a recent study combining radiation therapy with angiotatin to additionally target tumor vasculature demonstrated pronounced endothelial cytotoxicity and tumor regression.64 Another possibility to induce endothelial cell apoptosis might be the blockade of antiapoptotic signals, thereby inhibiting the survival-promoting effects of growth factors. Indeed, kinase inhibitors stimulate endothelial cell apoptosis and decrease growth and metastasis of pancreatic carcinoma.65 The ability to block angiogenesis by activation of the classical apoptotic APO-1/Fas pathway using Fas ligand is discussed controversially.66,67 This discrepancy might be explained by different cell type- and tissue-specific effects of Fas activation. Indeed, Fas activation in smooth muscle cells results in the synthesis and release of monocyte chemoattractant protein-1 and interleukin-8,68 which, by virtue of inducing an inflammatory response, are potent mediators of arteriogenesis, the process of collateral vessel growth.

In summary, endothelial cell apoptosis is intimately involved in vascular growth and vessel regression. Understanding the signaling pathways leading to inhibition of endothelial cell apoptosis undoubtedly will provide important tools to develop novel therapeutic strategies not only to enhance neovascularization of ischemic tissue but also to interfere with focal, dysregulated vascular remodeling, the key mechanism for atherosclerotic disease progression. On the contrary, selective stimulation of endothelial cell apoptosis is a promising prospect as a candidate for tumor therapy.

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