Regulation of Endothelial Cell Survival and Apoptosis During Angiogenesis

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Abstract—The process of angiogenesis plays an important role in many physiological and pathological conditions. Inhibition of endothelial cell (EC) apoptosis providing EC survival is thought to be an essential mechanism during angiogenesis. Many of the angiogenic growth factors inhibit EC apoptosis. In addition, the adhesion of ECs to the extracellular matrix or intercellular adhesion promotes EC survival. In contrast, increasing evidence suggests that the induction of EC apoptosis may counteract angiogenesis. In this review, we focus on the regulation of EC survival and apoptosis during angiogenesis and especially on the effects and intracellular signaling promoted by angiogenic growth factors, endogenous angiogenic inhibitors (such as angiostatin, endostatin, and thrombospondin-1), and the adhesion to the extracellular matrix. Furthermore, we discuss the effects of cross talk between adhesion molecules and growth factors. Understanding the molecular mechanisms involved in the regulation of EC survival and apoptosis may provide new targets for the development of new therapies to enhance angiogenesis in the case of tissue-ischemia (eg, the neovascularization of myocardium) or to inhibit angiogenesis in the case of neovascularization-dependent disease (eg, tumor, diabetic retinopathy). (Arterioscler Thromb Vasc Biol. 2002;22:887-893.)

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Angiogenesis refers to the formation of new capillaries from preexisting vessels. Angiogenesis plays an essential role in physiological processes such as embryonic development, the menstrual cycle, and in pathologic conditions (eg, wound healing, tumor growth and metastasis, rheumatoid arthritis, proliferative diabetic retinopathy, atherosclerosis, and posts ischemic vascularization of the myocardium). The process of angiogenesis consists of several steps, which include the stimulation of endothelial cells (ECs) by growth factors, the subsequent degradation of the extracellular matrix by proteolytic enzymes followed by invasion of the extracellular matrix, migration and proliferation of ECs, and finally the formation of new capillary tubes. Eventually, the recruitment of periendothelial cells (pericytes) stabilizes the newly formed capillary network.

The initiation of angiogenesis, the angiogenic switch, is dependent on a dynamic balance between proangiogenic and antiangiogenic factors in the immediate environment of ECs. A positive balance in favor of angiogenic factors leads to new vessel formation, whereas the prevalence of antiangiogenic factors shifts the equilibrium to vessel quiescence or, under particular circumstances, even to vessel regression. Inhibition of EC apoptosis providing EC survival is thought to be an essential issue during angiogenesis. In contrast, increasing evidence suggests that the induction of EC apoptosis may counteract angiogenesis. In this review, we focus on the regulation of EC survival and apoptosis during angiogenesis and especially on the effects and intracellular signaling promoted by angiogenic growth factors, angiogenic inhibitors, and the adhesion to the extracellular matrix.

Angiogenic Growth Factors and EC Survival

EC survival is maintained by growth factors and by contact to the extracellular matrix. Growth factor deprivation leads in vitro to programmed cell death of ECs. Several endothelial growth factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and angiopoietin-1 are known to provide EC survival by inhibiting apoptosis. Alon et al first demonstrated in a model of hyperoxia-induced retinopathy of neonatal rats that hyperoxia-induced
downregulation of VEGF expression in the neonatal rat retina leads to the regression of retinal capillaries via selective apoptosis of ECs. This apoptotic effect on ECs could be prevented by the intracocular injection of VEGF, establishing a role for VEGF as an in vivo EC survival factor. Moreover, the inhibition of VEGF leads to apoptosis of ECs and vessel regression in several models of tumor angiogenesis. Developmental investigations have indicated that VEGF survival function is only required until the vessel comes in contact with pericytes. Even in an animal model consisting of xenografted tumors with tetracycline-regulated VEGF expression, abrupt VEGF withdrawal leads to selective apoptosis of ECs in immature tumor vessels (newly formed vessels devoid of periendothelial cells) and subsequently to vessel regression, whereas mature tumor vessels with recruited pericytes seem to be resistant to VEGF withdrawal-induced apoptosis and regression. Thus, mature, pericyte–covered vessels are less sensitive to alterations of the VEGF level for both proliferation and regression.

In vitro experiments have demonstrated that VEGF inhibits EC apoptosis that is induced by growth factor deprivation and tumor necrosis factor-α (TNF-α) stimulation. Recent investigations have provided more insight into the antiapoptotic signaling that is mediated by VEGF (Figure 1). VEGF was shown to induce the expression of antiapoptotic proteins such as Bcl-2, A1, survivin, and XIAP. The targeting of survivin by antisense oligonucleotides abolished the antiapoptotic function of VEGF against TNF-α- or ceramide-induced cell death and induced regression of capillary tubes in a 3-dimensional angiogenic assay. In contrast, the inhibition of survivin had no effect on the stimulatory effect of VEGF on EC migration.

Furthermore, VEGF was shown to promote EC survival by activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. Pharmacological inhibition of PI3K or transfection with a dominant-negative Akt mutant abolished the antiapoptotic effect of VEGF on ECs. Interestingly, the survival effect of VEGF was dependent on the binding of VEGF on the VEGFR2 (KDR/flk-1), whereas VEGFR1-specific ligands (such as PlGF) did not promote survival of ECs. These findings have identified the VEGFR2 and the PI3K/Akt signal transduction pathway as crucial elements in the promotion of EC survival induced by VEGF. The downstream effector pathways mediating the antiapoptotic VEGF effect include Akt-dependent activation of the endothelial nitric oxide synthase (NOS), resulting in an enhanced endothelial NO synthesis. Endothelial NO in turn promotes EC survival, as demonstrated by in vitro studies as well as in studies with ECs from endothelial NOS (eNOS)−/− mice. Alternatively, the PI3K/Akt pathway also upregulates the transcription of survivin and can inhibit the p38 mitogen-activated protein kinase (MAPK). Finally, Gupta et al demonstrated that the VEGF-induced activation of the MAPK/extracellular signal–regulated kinase (ERK) pathway and inhibition of the stress-activated protein kinase/c-Jun amino-terminal kinase pathway is also implicated in the antiapoptotic effect mediated by VEGF. Interestingly, the activation of the PI3K/Akt pathway mediates not only the survival effect but also the migratory effect of VEGF on ECs via Akt-dependent phosphorylation and activation of eNOS. Beyond this, Akt is capable of promoting EC chemotaxis via phosphorylation of the G-protein–coupled receptor EDG-1.

**Angiopoietin-1**

Another endothelial-specific growth factor/growth factor receptor system involved in angiogenesis is the angiopoietin/Tie2 system. Angiopoietin-1 and -2 are the ligands for the Tie2 receptor tyrosine kinase. Angiopoietin-1 stimulation has in contrast to VEGF no mitogenic effect on ECs. Unlike VEGFR2- and VEGF-deficient mice, which fail to develop a primary vascular system, angiopoietin-1− or Tie2-deficient mice reveal a primary vascular plexus, which fails to recruit periendothelial cells (pericytes) and to remodel into a more mature and differentiated vascular system with small and large vessels adapted to the metabolic demands of the tissues. These results suggest that angiopoietin-1 and Tie2 receptor are important for the later steps of the angiogenic process, the remodeling, and the maturation of the newly formed vascular system and have a stabilizing effect on the capillaries. In vitro experiments have demonstrated that angiopoietin-1 activation of the Tie2 receptor inhibits EC apoptosis and induces EC migration and capillary tube formation. The angiopoietin-1–induced survival effect on ECs is dependent on the activation of PI3K and Akt. Papapetropoulos et al demonstrated that angiopoietin-1 induces PI3K-dependent activation of Akt and upregulates the antiapoptotic protein survivin, whereas it has no effect on the transcription of Bcl-2. Expression of a dominant-negative Akt mutant or a dominant-negative survivin mutant abolished the antiapoptotic effect of angiopoietin-1 in ECs. Another possible mechanism by which angiopoietin-1 may affect EC survival is the recruitment of pericytes. The constitutive pattern of expression of angiopoietin-1 suggests that angiopoietin-1 has a permissive stabilizing effect on the vascular system. In contrast to angiopoietin-1, angiopoietin-2 does not lead to activation of the Tie2 receptor and it is believed to be a naturally occurring antagonist of the Tie2 receptor. This assertion is supported by the fact that...
transgenic mice overexpressing angiopoietin-2 demonstrate a phenotype reminiscent of the angiopoietin-1– and Tie2-deficient mice. Angiopoietin-2 is believed to promote a destabilizing effect on capillary vessels by inhibition of the angiopoietin-1 signaling and disruption of the endothelial cell–pericyte interactions and to lead to vessel regression in the absence of VEGF. Angiopoietin-2 blocks the angiopoietin-1–induced phosphorylation and activation of Akt. Yu and Stamenkovic demonstrated that angiopoietin-2 overexpression in tumors leads to increased aberrant angiogenic vessels with increased EC apoptosis. Nevertheless, angiopoietin-2 in high concentrations was also demonstrated to promote EC survival in vitro by the activation of the PI3K/Akt pathway.

bFGF

Another important angiogenic factor is bFGF, which inhibits EC apoptosis induced by radiation or growth factor deprivation. As shown for VEGF, bFGF also upregulates the expression of the antiapoptotic proteins Bcl-2 and survivin. The overexpression of Bcl-2 in ECs prevents apoptosis induced by serum and growth factor deprivation, whereas it has no effect on the bFGF-induced EC proliferation. Furthermore, bFGF also activates the protein kinase Akt in ECs.

Taken together, various growth factors are involved in the initiation and promotion of angiogenesis and in the maintenance of the vascular network. A common property of these growth factors is the induction of EC survival. The inhibition of EC apoptosis by the distinct growth factors is dependent on PI3K/Akt signaling but may also include the upregulation of apoptosis inhibiting proteins such as survivin and Bcl-2 (see Figure 1).

**Adhesion and EC Survival**

Adhesion of ECs to extracellular matrix proteins and intercellular adhesion are essential for EC survival and angiogenesis.

**Cell Matrix Interactions**

In the absence of any extracellular matrix interactions, ECs rapidly undergo apoptosis, a phenomenon called anoikis. Integrins mediate the adhesion of ECs to extracellular matrix proteins and the EC migration. The interaction of cells via integrins with the extracellular matrix also provides a potent survival signal. In a previous study, programmed cell death was blocked by plating cells on an immobilized integrin beta-1 antibody but not by antibodies to vascular cell adhesion molecule-1, suggesting that integrin-mediated signals were required for maintaining cell viability. Moreover, the attachment of ECs on extracellular matrix proteins as vitronectin or fibronectin reduced the susceptibility of ECs to apoptosis. The vitronectin receptors (α₃β₁ and α₅β₁ integrin) are expressed during in vivo angiogenesis and are markers of the angiogenic phenotype of ECs. Blocking antibodies or antagonistic peptides to α₅β₁-integrin, which interrupt the α₅β₁-mediated adhesion to extracellular matrix proteins leads to the inhibition of tumor- and growth factor–induced angiogenesis in vivo by selectively inducing apoptosis of ECs in newly formed vessels. Interestingly, the inhibition of α₃β₁-integrin ligation did not affect quiescent vessels that are not involved in the angiogenic process. These results suggest that ligation of the α₅β₁-integrin is required for the survival of ECs of the angiogenic phenotype. In addition, the angiogenic effect exerted by TNF-α and interferon-γ results in a reduced activation of α₅β₁-integrin, leading to a decreased α₅β₁-integrin–dependent EC adhesion and survival. Surprisingly, genetically engineered mice with an ablation of the α₅-subunit gene lacking all five α₅-integrins display extensive vasculogenesis and angiogenesis during embryonic development. Likewise, genetic ablation of β₁- and β₁-integrin subunits results in enhanced hypoxia- and tumor-induced angiogenesis. A possible explanation for this discrepancy could be that the physiological developmental angiogenesis and the postnatal or the pathological angiogenesis are dependent at least in part on distinct molecular mechanisms. Another conceivable explanation is the functional redundancy between different integrins. Indeed, recent knockout studies indicate that other integrins such as β₁, α₁, and α₁-integrin also are involved in angiogenesis. Furthermore, in vitro studies demonstrated that α₁β₁- and α₅β₁-integrins mediate the VEGF-induced angiogenesis and that α₅β₁-integrin is involved in the shear stress–induced EC migration and survival.

Various signaling cascades have been considered to mediate the antiapoptotic effect of integrins (Figure 2). Regarding the intracellular signaling mediated by the α₅β₁-integrin, Stromblad et al demonstrated that the ligation state of α₅β₁-integrin influences p53 activity and the Bax cell death pathway. Ligation of the α₅β₁-integrin on ECs suppressed p53 activity, decreased the expression of the cell cycle inhibitor p21 WAF1/CIP1, and increased the Bcl2/Bax ratio, thereby promoting EC survival. Moreover, Scatena et al demonstrated that the attachment of ECs specifically on vitronectin or osteopontin (extracellular matrix proteins that are known α₅β₁-integrin ligands) induces nuclear factor kappa B (NFκB) activity. Inhibition of the adhesion by a specific anti–α₅β₁-integrin antibody or inhibition of NFκB by overexpression of a nonphosphorylatable IκB blocked the survival effect of α₅β₁-integrin ligation. The α₅β₁-integrin–induced activation of NFκB was shown to be mediated by the small GTP-binding protein Ras and the tyrosine kinase Src but not by the MAPK or PI3K. Recently, Malynankar et al demonstrated that the α₅β₁-integrin–mediated EC survival depends on osteoprotegerin induction by NFκB. The angiogenic and apoptotic effect on ECs mediated by inhibition of the α₅β₁-integrin was shown to be associated with an increase in the intracellular ceramide level, which may induce apoptosis.

Furthermore, various studies suggest an essential role for the PI3K/Akt pathway in the antiapoptotic signaling promoted by integrin–cell matrix interactions. Khwaja et al provided evidence that adhesion to the extracellular matrix induces the PI3K-dependent activation of Akt and that overexpression of a constitutively active PI3K or Akt mutant inhibited detachment-induced apoptosis of epithelial cells (anoikis). The cell adhesion–dependent phosphorylation of Akt on Ser 473 and its activation is mediated by the
integrin-linked kinase, a serine/threonine kinase capable of interacting with the cytoplasmic domains of integrin β₁, β₂, and β₃ subunits. Moreover, Wary et al demonstrated that the association of specific integrins such as the α₁β₁-, the α₁β₂-, and the α₂β₃-integrin with the adaptor protein Shc can regulate cell survival and cell cycle progression via the Ras/MAPK/ERK pathway.

An interesting issue is that integrin signaling may affect and influence growth factor signaling. Angiopoietin-1, bFGF, or VEGF failed to prevent EC apoptosis (anoikis) in suspension culture. However, another study demonstrated an inhibitory effect for angiopoietin-1 on EC apoptosis induced by anchorage disruption. Soldi et al demonstrated that during EC stimulation with VEGF, the α₁β₁-integrin co-immunoprecipitates with the VEGFR2. Furthermore, EC adhesion to the α₁β₁-integrin ligand vitronectin increases the tyrosine phosphorylation and the biological function mediated by VEGFR2 and that anti–α₁- and anti–β₁-antibodies inhibit the VEGF-induced phosphorylation of VEGFR2 and the subsequent activation of PI3K, suggesting that α₁β₁-integrin ligation may enhance antiapoptotic signaling mediated by VEGF. Inversely, VEGF has been shown to mediate its angiogenic functions through the VEGFR2 by activating various integrins involved in angiogenesis in a PI3K/Akt-dependent manner. EC adhesion mediated by the β₁-integrin or α₁-integrin also has been shown to induce tyrosine phosphorylation and activation of the EGF receptor even in the absence of EGF receptor ligands and leads to MAPK/ERK activation and EC survival. In conclusion, there is a functional cross talk between integrin- and growth factor–mediated signaling, which may act synergistically to promote EC survival (Figure 2).

Cell-Cell Adhesion
Recent evidence suggests that not only cell matrix contacts but also cell-cell contacts between ECs may support cell survival. For example, platelet EC adhesion molecule-1 (PECAM-1, CD-31) homophilic adhesion rescues ECs from serum deprivation–induced apoptosis, whereas it has no effect on EC migration and proliferation. Furthermore, the VE-cadherin, an adhesive protein contained at endothelial adherens junctions that mediates interendothelial cell adhesion, has been demonstrated to be essential for the VEGF-induced antiapoptotic effect. In detail, targeted inactivation of the VE-cadherin gene or truncation of the β-catenin–binding cytosolic domain of the VE-cadherin in mice caused embryonic lethality by impairing the maturation and remodeling of the initially formatted vascular plexus via apoptosis induction. The VEGF-induced activation of the PI3K/Akt pathway, upregulation of Bcl2, reduction of p53 and p21 expression, and prevention of serum deprivation–induced apoptosis of ECs was abolished by the inactivation of the VE-cadherin gene or by the truncation of the cytosolic β-catenin–binding domain of VE-cadherin, suggesting that VE-cadherin signaling via the β-catenin is essential for the survival signaling mediated by VEGF. Taken together, cell matrix and cell-cell interactions provide EC survival by inhibiting EC apoptosis that acts synergistically to growth factors. This survival signaling is essential for the promotion of angiogenesis.

Angiogenic Inhibitors and EC Apoptosis
Because angiogenesis is the result of a dynamic balance between angiogenic inducers and angiogenic inhibitors, we
will focus in the following part of this review article on the regulation of EC apoptosis by endogenous angiogenic inhibitors (Figure 3).

**Angiostatin**

Angiostatin is a 38-kDa fragment of plasminogen containing kringle 1 to 3, which was purified from mice bearing a Lewis lung carcinoma. Angiostatin was shown to inhibit in vivo tumor angiogenesis and to induce dormancy of tumors in mice by inhibition of the EC proliferation. Angiostatin can be generated by proteolysis of plasminogen by a macrophage-derived metalloelastase or by the reduction of plasmin. Angiostatin exerts its antiangiogenic function at least in part by induction of EC apoptosis.

Intriguingly, the proapoptotic effect of angiostatin was not restricted to mature ECs. Thus, Ito et al demonstrated an inhibitory effect of angiostatin on the growth of endothelial progenitor cells, which are believed to play a key role in postnatal neovascularization. With respect to the mechanism, Claesson-Welsh et al demonstrated that angiostatin has no effect on growth factor–induced signal transduction but leads to an arginine-glycine-aspartic acid (RGD)-independent activation of focal adhesion kinase. Furthermore, Gupta et al have shown that angiostatin-induced EC apoptosis is associated with ceramide generation and RhoA activation. Interestingly, the antioxidant N-acetylcysteine inhibited the cytotoxic effects of angiostatin. Finally, angiostatin can bind and block an α/β ATP-synthase on the surface of ECs, thereby inhibiting proliferation. It is not known at this time whether this inhibitory effect on the ATP-synthase activity of angiostatin is involved in the induction of EC apoptosis.

**Endostatin**

Endostatin is a 20-kDa C-terminal fragment of collagen XVIII, which is produced by hemangiendothelioma. Endostatin is, like angiostatin, a potent inhibitor of in vivo tumor angiogenesis. Endostatin can be generated by proteolytic processing of collagen XVIII performed by cathepsin L or by a protease with elastase-like activity. The major antiangiogenic effect of endostatin seems to be mediated by inhibition of EC migration. Endostatin inhibits VEGF-induced EC migration by inducing eNOS dephosphorylation on Ser 1177 without affecting Akt activity. Aside from the antimigratory effect, endostatin has been demonstrated to induce EC apoptosis. Indeed, endostatin stimulation of ECs leads to marked reduction of Bcl-2 and Bcl-XL antiapoptotic proteins without affecting the level of the proapoptotic Bax protein. Furthermore, the Shb adaptor protein has been suggested to be involved in the mediation of the apoptotic signaling of endostatin. Rehn et al demonstrated that soluble endostatin is capable of binding to α5- and αv-integrins, thereby inhibiting the integrin functions, such as EC migration. It is conceivable that such an interaction with integrins may affect EC survival.

**Thrombospondin-1**

Thrombospondin-1 (TSP-1) is a large multi-domain glycoprotein that has been shown to inhibit angiogenesis. TSP-1 exerts its antiangiogenic activity via binding to the CD36 receptor by triggering an apoptotic signaling pathway. Binding of TSP-1 to CD36 receptor leads to the recruitment of the Src-related kinase, p59-fyn, and to activation of p38 MAPK. The activation of the p38 MAPK was shown to be p59-fyn–dependent and to require a caspase-3–like proteolytic activity. Furthermore, activated p38 MAPK led to the activation of caspase-3 and to apoptosis. Interestingly, the apoptotic effect of TSP-1 was restricted to ECs activated to take part in the angiogenic process and not in quiescent vessels.

**Conclusion**

Angiogenesis is dependent strongly on the suppression of EC apoptosis. Many of the proangiogenic growth factors promote the survival of ECs. Both angiogenesis and EC survival also are dependent on the attachment of ECs to the extracellular matrix and to cell-cell contacts. Inhibition of growth factor signaling or adhesion-dependent signaling can induce apoptosis directly and concomitant angiogenesis inhibition. Moreover, a common property of many angiogenic inhibitors is the induction of EC apoptosis. Therefore, the events that induce survival or apoptosis of ECs affect angiogenesis. It is conceivable that ECs integrate exogenous angiogenic and antiangiogenic stimuli and transform them intracellularly into conflicting survival and apoptotic signals. The prevailing signals may determine the fate of the ECs and, subsequently, the fate of the growing vessel. Elucidation of the molecular mechanisms that are involved in EC apoptosis and survival may lead to the development of new therapeutic approaches to enhance angiogenesis in the case of tissue ischemia (eg, revascularization of ischemic tissue) or to inhibit angiogenesis in the case of neovascularization-dependent disease (eg, tumor, diabetic retinopathy).

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**References**


Regulation of Endothelial Cell Survival, Apoptosis


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