Role of endothelial cell survival and death signals in angiogenesis

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Abstract

Angiogenesis, the process of new microvessel development, is encountered in a select number of physiological processes and is central to the pathogenesis of a wide variety of diseases. There is now convincing evidence that regulated patterns of endothelial cell survival and death, a process known as apoptosis, play a central role in the periodic remodeling of the vasculature, and in the timely evolution and regression of angiogenic responses. In this review we discuss the current evidence suggesting a role for inducers and inhibitors of angiogenesis as well as other mediators that modify endothelial cells functions in the survival and death of endothelial cells. We also discuss how dysregulation of apoptosis can lead to aberrant angiogenesis as demonstrated in the pathogenesis of retinopathy of prematurity and cancer.

Introduction

Angiogenesis and apoptosis are biological processes that are indispensable for normal organ and tissue homeostasis. Programmed cell death, also known as apoptosis, maintains normal tissue and organ homeostasis by removing superfluous, damaged or senescent cells. Mounting evidence suggests that apoptosis is also involved in the homeostasis of the vascular system. Recent studies suggest that endothelial cell apoptosis is necessary for repair of damaged blood vessels and for sprouting and branching of capillaries during angiogenesis. Events that govern the survival and death of endothelial cells have emerged as major factors that contribute to angiogenic responses during embryonic development, in the maintenance of organ and tissue homeostasis in adult organisms, and in pathological conditions such as tumor development. In this review we will briefly summarize the literature on apoptosis and angiogenesis, and examine more closely the current knowledge about how the death machinery contributes to vascular remodeling and angiogenesis. Lastly, we will show how disruption of the apoptotic program can contribute to the unrelenting and persistent angiogenesis that is the hallmark of angiogenesis dependent diseases.

Apoptosis

Programmed cell death is necessary for the development and survival of multicellular organisms. Every second, hundreds of thousands of cells are generated in the human body and a similar number of them die [1]. The overwhelming majority of these cells die by apoptosis, a morphologically stereotyped series of cellular events that result in the deletion of cells without inflammation [2, 3]. The process of programmed cell death was first described in 1842 in the neuronal system of the developing toad embryo [4]. However, it was only in 1972 that the term apoptosis was proposed to define this genetically regulated form of cell death [5]. Programmed cell death involves expression of genes and protein synthesis, and is distinguished from necrosis where cell injury and or inflammation results in membrane damage and cell lysis.

Apoptosis is a key component of a number of physiological processes. It accounts for the death of cells necessary for tissue remodeling, for the cell loss that accompanies atrophy of adult tissues following endocrine stimuli, and the removal of cells no longer needed at the late stages of wound healing [1, 2, 6]. Apoptosis is the mechanism by which autoreactive lymphocytes are eliminated during development or after termination of immune responses [7]. It also eliminates excessive neutrophils that are produced continuously in the bone marrow [8, 9].

Apoptosis also maintains organ and tissue homeostasis by eliminating cells that are potentially destructive. Disruption of the function of a cell caused by viral
infection generates a signal that induces apoptosis of infected cells in order to eliminate the virus from the organism [10]. Perhaps one of the best examples of how apoptosis guards against intrusion by potentially harmful cell populations is the protective role that this process plays in the prevention of cancer. Apoptosis is an efficient mechanism designed to recognize and eliminate cells that have acquired genetic lesions that are potentially lethal to the host [7]. In this context, cell death is a physiologically protective event. In summary, apoptosis is a mechanism that regulates the reshaping of tissues and organs, insures the survival of the fittest cells and their optimal adaptation to the environment, and eliminates infected, damaged, or cells at risk for neoplastic conversion [1, 7, 11, 12].

Apoptosis is a genetically programmed event

At the cellular level, apoptosis is characterized by a predictable, well-choreographed series of morphological and biochemical events. Cells undergoing apoptosis shrink in volume, detach their neighbors, lose their microvilli, and detach from one another [2]. The outer membrane bulges (blebs), the chromatin condenses into dense granular caps, and the nuclear membrane eventually breaks down. At this stage, surface convolution takes place and the cell is disassembled into a series of condensed membrane-bound, apoptotic bodies that are phagocytosed by macrophages or by other adjacent cells [2].

The events that initiate the apoptotic program are tightly regulated by an intricate system of controls and checkpoints that are designed to minimize accidental or inappropriate activation of the death cascade. Although it is well recognized that apoptosis plays a central role in numerous in physiological and pathological processes, the genetic and biochemical signals that comprise the cell death machinery are still remain to be defined. We will begin this review with an overview of what is currently known about the molecular regulation of apoptosis and the intracellular and extracellular signals that initiate this cascade.

Initiation and execution of the cell death program

Each cell possesses its own death machinery, which can be activated on demand. Our understanding of the molecular events that control apoptosis emerged from studies in the nematode Caenorhabditis elegans [13, 14]. During development of C. elegans, 1090 cells are generated of which 131 undergo apoptosis [15]. Genetic studies in this worm led to the identification and ordering of key components of the cell death machinery. The apoptotic program is a highly conserved mechanism that is mediated by regulators, adaptors, and executioners (effectors) of apoptosis [1, 14]. Figure 1 shows a comparison of intracellular pathways that control apoptosis in nematodes and mammals. In C. elegans, upstream signals promote the binding of EGL-1 (regulator) protein to CED-9 (regulator) that, in its turn, releases CED-4 (adapter). Unbound CED-4 induces proteolytic cleavage of CED-3 (executioner) and a series of downstream events culminating in cell death [16–19].

In humans, the Bcl-2 family of proteins is composed of at least 16 members that are critical regulators of apoptotic pathways and function to either inhibit or to promote cell death [20–22]. These proteins are located within the intracellular membranes of mitochondria, the nucleus, and endoplasmic reticulum and are believed to form ion-channels/pores when these proteins form homo and/or heterodimers [22, 23]. The pro-apoptotic proteins Bad and Bid (human homologues of the nematode EGL-1) regulate the activity of the antiapoptotic Bcl-2 and Bcl-xL (homologues of CED-9). Bad and Bid lack membrane anchoring domains and move from cytosol to the surface of membranous organelles, where they can bind to other members of the Bcl-2 family [24]. For example, the dimerization of Bad with Bcl-2 mediates downstream events that result in apoptosis [22]. Therefore, this step of the apoptotic pathway is dynamically controlled by the ‘regulated’ translocation of mobile proteins such as Bid and Bad, and their binding to anchored proteins such as Bcl-2 and Bcl-xL.

None of the known antiapoptotic Bcl-2 family members (regulators) interacts directly with caspases

![Figure 1. Mechanisms that control cell survival and death in the nematode C. elegans and in mammals. The current understanding of genetic pathways of apoptosis is described for C. elegans (Top) and mammals (Bottom). Both, the nematode genes and their mammal homologues, are functionally characterized as regulators, adaptors, or executioners of apoptosis. Symbols: inhibition (\(\rightarrow\)), activation (\(\rightarrow\)). Modified from Vaux and Korsmeyer [1].](image-url)
and prevent DNA fragmentation and enhance cell survival. zVAD-FMK, zDEVD-FMK) have been shown to describe above.

downstream death-inducing events similar to the ones caspase-8 signals through caspase-3 [45] and triggers their auto-processing and activation [43, 44]. Active aggregation of several procaspase-8 molecules mediates in a death-inducing signaling complex (DISC) [42]. The respective receptors activates FADD (adapter or [40, 41]. Binding of the CD95 ligand or TNF to their cytoplasmic sequence termed the ‘death domain’ (DD) [54–56]. Subsequently, these cells proliferate and organize into primitive blood vessels establishing the initial vascular network. Vascular endothelial growth factor (VEGF) and its receptors VEGFR-1, VEGFR-2 are important molecules in the initial phase of vascular development. Knockout mice lacking any one of these genes die before day 10.5 of embryonic development by absence or delayed endothelial cell differentiation and failure of vasculogenesis [56, 57]. After the primary vascular plexus is formed, endothelial cells start to proliferate and form new capillaries [55]. The emerging vascular plexus is rapidly remodeled to resemble a mature system by a process termed angiogenesis. This term was first used in 1935 to describe the formation of new blood vessels from pre-existing capillaries in the placenta [58]. Recent studies have demonstrated the presence of endothelial cell precursors in peripheral blood as well as the ability of these cells to target sites of active angiogenesis [59]. These findings raise the possibility that vasculogenesis and angiogenesis may not be as distinct from one another as once thought.

Despite their low turnover (measured in years) in adult tissues, endothelial cells still retain the capacity to divide and form new blood vessels in response to specific stimuli [60]. Capillary blood vessels consist of endothelial cells and pericytes that are programmed to form a complete capillary network when stimulated by mediators of angiogenesis [61]. The process of angiogenesis starts with proteolytic degradation of interstitial tissue and the basement membrane of the parent vessel. This is followed by a series of well orchestrated events in which endothelial cells migrate towards the angiogenic stimulus and divide [62]. Endothelial cells elongate and align to form a sprout, and the lumen is formed by a curvature inside each endothelial cell [63]. Individual sprouts elongate and eventually join with each other forming loops through which blood begins to flow. Pericytes originating from migrating and de-differentiation of arterial smooth muscle cells, position themselves along the original sprouts and reposition themselves in the basement membrane that invest newly formed capillaries [64, 65]. The acquisition of a coating with pericytes has been recently described as the end of the ‘plasticity window’ in which the vascular architecture is fine tuned according to the availability of oxygen [66].
Role of endothelial cell survival and death signals in angiogenesis

A tightly controlled balance between cell proliferation and cell death dictates tissue integrity and tissue homeostasis. Traditionally, regression of neovascular responses has been associated with inhibition of endothelial cell proliferation, migration, and adhesion [67]. Only recently has the role of endothelial cell survival and death signals in sustaining and disrupting neovascularization been recognized. It is now well established that key regulators of angiogenesis function, at least in part, by modulating the survival of endothelial cells during the processes of vessel repair and angiogenesis.

For the purposes of discussion we have divided the regulators of angiogenesis in two broad groups according to their role in endothelial cell survival and death: inducers of endothelial cell apoptosis and enhancers of endothelial cell survival (Table 1). Many but not all of these mediators we will describe below have been shown to function as either antiangiogenic or proangiogenic factors. When viewed in this context perhaps all mediators of angiogenesis will eventually be found to function as either inducers of endothelial cell death or survival factors.

Inducers of endothelial cell apoptosis

*Thrombospondin-1 (TSP1)*

TSP1 is the first member of a family of multifunctional extracellular matrix (ECM) glycoproteins [68–70]. It is intimately associated with matrix surrounding blood vessels where it is able to interact with a variety of growth factors, matrix molecules and cations [71–76]. TSP1 has been implicated in several steps in the angiogenic response [70, 77–79]. It potently inhibits endothelial cell proliferation [80–83], migration [82, 84] and induces vascular disassembly. Endothelial cell sprouting is enhanced by exposure to anti-TSP1 antibodies [76, 85], and endothelial cells transduced with antisense TSP1 [82] exhibit an enhanced ability to form sprout-like structure on artificial matrices *in vitro*.

The potent anti-angiogenic activity of TSP1 has been localized to two domains within the central stock region of the molecule: the procollagen homology region and the properdin-like type I repeats [72, 86–88]. The antiangiogenic effect of TSP1 involves signalling through the endothelial cell membrane receptor CD36 [89]. Recent data suggest that the antiangiogenic effect of TSP1 is mediated in part by its ability to induce endothelial cell apoptosis. TSP1 as well as peptide fragments derived from its antiangiogenic domains have been shown to induce apoptosis of human umbilical vein endothelial cells (HUVECs) *in vitro* [90].

*Angiostatin*

A 38 kDa internal fragment of plasminogen that contains the first four disulfide-linked kringle structures was purified from plasma of mice bearing a Lewis lung carcinoma, sequenced and named angiostatin [91–93]. It was characterized as a specific inhibitor of endothelial cell proliferation *in vitro* and suppressor of tumor growth and metastatic dissemination *in vivo* [91, 92, 94]. Angiostatin can be generated by the hydrolysis of circulating plasminogen by a macrophage-derived metalloelastase [95], matrilysin and gelatinase B [96], or stromelysin [97].

The antiangiogenic function of angiostatin is believed to be mediated, at least in part, by its ability to induce apoptotic death of endothelial cells [98, 99]. Treatment of endothelial cells with angiostatin resulted in decreased cell numbers without significant effects on DNA synthesis, suggesting that the decreased proliferation rates observed in cells exposed to angiostatin were due to enhanced endothelial cell apoptosis [98, 99]. The domains responsible for angiostatin pro-apoptotic function appear to reside in kringle domains 1, 2 and 3 [99].

Tumor necrosis factor

Tumor necrosis factor (TNF) is a multifunctional cytokine secreted by activated macrophages [100–102] and T cells [103]. TNF is an important mediator of inflammatory processes and immune responses, where it was shown to induce tumor regression by hemorrhagic necrosis [101, 104]. TNF’s function in tumor

| Inducers of endothelial cell apoptosis | Enhancers of endothelial cell survival |
|--------------------------------------|---------------------------------------|
| Thrombospondin-1 (TSP1)              | Vascular Endothelial Growth Factor (VEGF) |
| Angiostatin                          | Basic Fibroblast Growth Factor (bFGF)   |
| Tumor Necrosis Factor (TNF)          | Integrins (αVβ3)                       |
| Transforming Growth Factor-β (TGF-β) | Nitric Oxides (NO)                     |
| Lipopolysaccharides (LPS)            | Estradiol                              |
| Amyloid β-peptide                    | Adrenomedullin                         |
| Cholesterol oxides                   | Serum albumin                          |
| Acetatin                             |                                       |
| Extracellular ATP                    |                                       |
| Alkyllyso-phospholipid ET16-Ome      |                                       |
| 2-methoxyestradiol                   |                                       |

Table 1. Classification of regulators of angiogenesis according to their role in endothelial cell survival.
Apoptosis and the regulation of angiogenesis

TGF-β has been shown to induce apoptosis of HUVEC in vitro by downregulating Bcl-2 expression [121]. Choi and Ballerman have demonstrated that TGF-β induces apoptosis of renal glomerular endothelial cells and capillary sprouting in vitro [122]. The authors found that endothelial cell sprouting is inhibited in dominant negative mutants if TGF-β type II receptors are blocked. They concluded that TGF-β-induced endothelial cell apoptosis is necessary for capillary morphogenesis [122].

Lipoplysaccharides

Escherichia coli endotoxins (LPS) cause acute pulmonary endothelial cell injury in vivo [123] and endothelial cell apoptosis in vitro [124]. Recently, a study has associated LPS with the pathogenesis of endotoxic shock syndrome that is characterized by generalized inflammation, circulatory collapse and death [125]. Co-injection of LPS with its putative effector (TNF-α) induces endothelial cell apoptosis that is associated with enhanced expression of the pro-apoptotic lipid ceramide [125]. The ability of LPS to induce apoptosis of cultured sheep pulmonary artery endothelial cells was attenuated by collagen [124] and by overexpression of the heat shock protein-70 (HSP-70) [126]. Vitamin C and E have also been shown to prevent LPS-mediated apoptosis in HUVEC [127]. The protective effect of these vitamins was associated with enhanced expression of the anti-apoptotic protein Bcl-2 and decreased expression of the pro-apoptotic protein Bax [127].

Other inducers of endothelial cell apoptosis

Several other inducers of endothelial cell apoptosis have been described in the literature. Interferon-γ induces apoptosis of normal endothelial cells through activation of the protein kinase C pathway [128]. Amyloid β-peptide, a molecule involved in neuronal degeneration observed in the brain of patients with Alzheimer’s disease, has been implicated in the induction of endothelial cell apoptosis [129]. It was suggested that amyloid β-peptide-induced endothelial cell apoptosis might be directly involved with the vascular damage frequently observed in these patients [129]. Cholesterol oxides are molecules involved in the initiation and progression of atherosclerosis [130]. Physiological concentrations of cholesterol oxides induce apoptosis of endothelial cells and cause damage to the vessel wall [131]. This observation might explain, at least in part, the role of cholesterol oxides in the etiology of vascular injury in vivo [132].

Accutin, a RGD containing small peptide from the disintegrin family, was recently purified from the viper venom of Akgistrodon acutus [133]. In vitro, accutin induces apoptosis of endothelial cells by inhibiting their adhesion to fibrinogen, fibronectin, or vitronectin [133]. Furthermore, accutin is anti-angiogenic when evaluated in the chick chorioallantoic membrane (CAM) assay. It was suggested that the mechanisms responsible for this anti-angiogenic effect involve selective blockade of the endothelial cell integrin αβ3 and consequent induction of apoptosis [133].

Endothelial cell injury is a component of the ‘increased pulmonary edema’ that manifests in patients with acute respiratory distress syndrome (ARDS) [134]. Dawicki and collaborators showed that extracellular ATP and adenosine induce apoptosis of pulmonary artery endothelial cells [135]. They speculate that ATP released from activated platelets and cells undergoing cytolyis in
ARDS patients causes apoptosis of lung endothelium, and thereby exacerbates pulmonary injury [135].

Pharmacological agents can also induce endothelial cell apoptosis. Alkyllyso-phospholipid ET16-Ome, a putative anti-tumor drug, induces apoptosis when added to the culture medium of human umbilical vein endothelial cells [136]. Moreover, 2-methoxyestradiol, an endogenous estrogen metabolite of the oral contraceptive 17-ethylestradiol, induces apoptosis of bovine pulmonary artery endothelial cells in vitro and inhibits angiogenesis in vivo [137].

Lastly, an important role for accessory cells and changes in blood flow in endothelial cell apoptosis and capillary regression has been proposed in a series of studies by Richard Lang and colleagues [138–142]. During regression of the pupillary membrane, a transient capillary network found in the anterior chamber of the developing rodent eye, macrophages initiate apoptosis of endothelial cells which leads to capillary regression. This selective ablation of endothelial cells results in vessel obstruction and a block in plasma flow within the clogged capillary segment. Endothelial cells then die in a ‘synchronous’ manner because they are deprived of essential survival factors present in plasma. Recently this group has determined that the major survival factor in plasma that is responsible for maintaining the integrity of this embryonic organ is VEGF. The significance of VEGF as a survival factor will be discussed in greater detail below.

Enhancers of endothelial cell survival

Vascular endothelial growth factor (VEGF)

In 1983, vascular permeability factor (VPF) was identified in tumors and characterized as an endothelial factor that enhances the permeability of microvessels to circulating molecules [138]. In 1989, two independent groups reported the cloning and sequencing of an endothelial cell specific mitogen with heparin binding properties that was called vascular endothelial growth factor (VEGF) [143–145]. Further sequencing and characterization led to the conclusion that VPF and VEGF shared the same biological functions and were encoded by the same gene. Alternative splicing of VEGF mRNA originates five human VEGF isoforms, of which VEGF165 is the predominant molecular species [147, 148]. VEGF has been extensively characterized as a potent permeability factor [150], endothelial cell specific mitogen and chemotaxant [143, 151] an angiogenic factor [148, 149], and a mediator of adhesion of natural killer cells to tumor endothelium by inducing expression of VCAM-1 and ICAM-1 [152].

More recently, the role of VEGF as an enhancer of endothelial cell survival has been investigated. VEGF was shown to protect endothelial cells from apoptosis induced by TNF by inducing upregulation of β3 integrin and fibronectin [153]. It was proposed that the sustained endothelial cell survival observed in cells exposed to VEGF was mediated by their enhanced adhesion to matrix [153, 154]. At approximately the same time, another group of investigators determined that VEGF enhanced the survival of microvascular endothelial cells cultured in hydrophobic polystyrene [155]. Interestingly, the mechanism suggested for VEGF-induced endothelial survival in this experimental design was dependent on vitronectin and α5β1 integrin, not α5β2. The ability to enhance endothelial cell survival was specific to VEGF, since other angiogenic factors such as bFGF were tested and did not exhibit the same effect [155].

The mechanisms underlying VEGF’s survival function are starting to be unveiled. VEGF was shown to upregulate expression of the antiapoptotic protein Bel-2 and its homologue A1 in endothelial cells in vitro [156, 157]. Overexpression of Bel-2 was sufficient to enhance endothelial cell survival and protect against apoptosis induced by growth factor deprivation [157, 158]. VEGF-mediated Bel-2 upregulation in endothelial cells unequivocally potentiates angiogenic responses in vitro and in vivo [157]. VEGF’s survival signal was shown to be mediated by the Fk-1/KDR receptor and engagement of the phosphatidylinositol 3’-kinase/Akt transduction pathway [159]. Another group has characterized VEGF’s survival function for endothelial cells cultured in collagen as dependent on the activation of the mitogen activated protein kinase [MAPK], rather than the Akt/PKB, signaling pathway [160].

One of the most potent stimuli for VEGF secretion and angiogenesis is oxygen deprivation. Hypoxia is associated with a variety of responses at the cellular and tissue level. Reduced levels of oxygen induce glycolysis to enhance energy production. It also enhances erythropoietin synthesis to increase the oxygen carrying capacity of the blood, and VEGF secretion which enhances tissue oxygenation by increasing vessel permeability and promoting neovascularization. An important component of the transcriptional response to hypoxia is the transcription factor HIF-1. This hypoxia-inducible transcription factor functions by controlling the expression of a series of target genes that regulate tissue oxygenation in a number of physiological and pathological settings. Recent work from several laboratories has established a key role for HIF-1 in the hypoxic response during embryonic development and angiogenesis [161–163]. For example teratocarcinomas derived from embryonic stem cells null for HIF-1α exhibited a dramatic reduction in growth and vascularization [161–163]. This finding correlated with a reduced capacity of these cells to release VEGF. Furthermore it was shown that HIF-1α null mutant embryos exhibited a complete lack of cephalic vascularization, reduced numbers of somites and abnormal neural fold formation. Carmeliet and collaborators have reported that embryonic stem cells null for HIF-1α showed reduced hypoxia-induced expression of VEGF. This was associated with a reduction in the formation of large, mature vessels and impaired vascular function [163].
The consequences of aberrant expression of HIF-α have been revealed in several recent reports that examined the molecular basis of unregulated angiogenesis in the hereditary cancer syndrome, the von Hippel-Lindau (VHL) disease [164–166]. Individuals affected by this disorder develop hemangioblastomas in the retina, cerebellum and spine as well as the adrenals and kidney. Interestingly, individuals with this disease exhibit a molecular and biochemical phenotype reminiscent of oxygen deprivation. It was recently reported [164] that the VHL gene binds to the transcription factors HIF-1α and HIF-2α where it targets them for destruction. Cells lacking the VHL gene cannot degrade these two transcription factors. This in turn drives excessive VEGF synthesis and angiogenesis. Although a number of questions remain, these recent observations suggest that VEGF may utilize a number of different molecular pathways to enhance the survival of endothelial cells.

Basic fibroblast growth factor (bFGF)

The FGF family consists of nine structurally related polypeptides. bFGF was originally purified from the bovine pituitary gland [167], sequenced, and characterized as an angiogenic factor [168]. Araki and colleagues in 1990 [169] were the first to implicate FGF as a survival factor for endothelial cells. Enhanced activity of protein kinase C was associated with the ability of bFGF to protect endothelial cells against apoptosis induced by growth factor deprivation [170] or ionizing radiation in vitro and in vivo [170–172]. In contrast, tyrosine phosphorylation, but not protein kinase C activation, was shown to mediate bFGF’s protective effect [173]. The role of bFGF in endothelial cell survival was further characterized by the finding that its removal from culture medium of murine aortic endothelial cells was sufficient to activate the pro-apoptotic cysteine protease interleukin-1β-converting enzyme (ICE) and mediate DNA fragmentation [174]. More, recently, the anti-apoptotic function of bFGF was associated with its ability to enhance expression of Bel-2 [175].

Integrins

Endothelial cells rapidly undergo apoptosis when their interactions with the extracellular matrix are inhibited, in a process called ‘anoikis’ [154]. Integrins were identified as key transducers of extracellular matrix signals that were required for maintaining cell survival [154]. There is increasing evidence that integrins also play a critical role in the regulation of angiogenesis by modulating endothelial cell survival [176–180]. The finding that αvβ3 is preferentially expressed in newly formed microvessels and that monoclonal antibodies to αvβ3 induce endothelial cell apoptosis restricted to these vessels was immediately considered a major breakthrough as a potential anti-angiogenic tumor therapy [177–180]. The authors hypothesized that if αvβ3 ligation is prevented; the endothelial cells will no longer receive necessary survival signals from the ECM and undergo apoptosis by default.

The signaling pathways that mediate endothelial cell survival upon activation of αvβ3 have been extensively studied. Ligation of endothelial cell αvβ3 during angiogenesis promotes a specific signal that leads to inhibition of p53 expression and its inducible partner p21WAF1 (mediator of cell cycle arrest), and suppression of the pro-apoptotic Bax pathway [181]. The activation of the transcription factor NF-κB was shown to be dependent on the GTP-binding protein Ras and the tyrosine kinase Src, and is necessary for αvβ3-mediated endothelial cell survival [182]. Another study has identified the activation of the phosphatidylcholine-specific phospholipase C and production of diacylglycerol (DAG) as essential components of integrin mediated survival signals in endothelial cells [183].

Exposure of human endothelial cells to gamma interferon (IFN-gamma) was shown to inhibit the αvβ3-mediated survival pathway and to induce endothelial cell apoptosis in vitro [184]. Treatment of patients with IFN-gamma also resulted in enhanced endothelial cell apoptosis in metastatic melanoma [184]. Inactivation of the integrin pathway during endothelial cell apoptosis may be due to cleavage of its cytoplasmic domain. Calpain-mediated proteolysis of the β3 cytoplasmic domain was observed during apoptosis of human umbilical vein endothelial cells, and its inhibition with sodium orthovanadate (a phosphatase inhibitor) rescued these cells from apoptosis [180].

Other enhancers of endothelial cell survival

Nitric oxide is a multifunctional molecule that is synthesized by endothelial cells in low doses through the activity of the enzyme nitric oxide synthase (eNOS) [185, 186]. Inhibition of NO was associated with enhanced endothelial cell apoptosis in confluent cultures of bovine aortic endothelial cells [187]. The authors suggested that NO has an important role in maintaining vascular homeostasis and architecture [186].

Enhanced endothelial cell apoptosis has been associated with the pathogenesis of atherosclerosis [188, 189]. The incidence of coronary disease in post-menopausal women increases concomitantly with a decrease in the synthesis of estrogens, and the administration of this hormone is being considered one of the most effective anti-atherogenic therapies available for women [190]. Estriol, a key estrogen metabolite, has been now characterized as a potent anti-apoptotic mediator for endothelial cells. Estradiol protected endothelial cells against apoptosis induced by TNF-α [192]. It was also shown that estradiol’s anti-apoptotic function was mediated by increased tyrosine phosphorylation of a focal adhesion kinase that stabilized focal adhesion contacts [189].

Adrenomedullin, a potent vasorelaxant/hypotensive peptide, was shown to suppress serum deprivation-induced apoptosis of rat endothelial cells via a cAMP-
independent mechanism [192]. Human umbilical vein and microvascular endothelial cell apoptosis were inhibited by physiological concentrations of serum albumin [193]. The authors suggested that the removal of excessive blood vessels in remodeling tissues might be mediated by a reduced supply of serum albumin to the endothelial cells.

Lastly, the inhibitor of apoptosis protein family (IAPs) has received considerable attention over the past 5 years [194]. Initially discovered in baculoviruses they appear to be highly conserved across several species including humans [195, 196]. The IAPs appear to suppress apoptosis through direct caspase inhibition (primarily caspase 3 and 7) and by modulation of the transcription factor NF-κB [197]. Although a role for the IAPs in endothelial survival and angiogenesis has yet to be established, given the wide spread distribution of these proteins, it would not be entirely surprising to find that they play a role in endothelial cell survival and angiogenesis.

Endothelial cell survival and death signals in physiological angiogenesis

In response to angiogenic stimuli, endothelial cells undergo a series of tightly controlled events that result in sprouting and development of a capillary network and the remodeling of established vessels. When newly formed blood vessels are no longer necessary, they undergo regression. Figure 2 depicts the process of programmed cell in both angiogenesis and angiosuppression. Traditionally, the initiation of angiogenesis has been described as involving degradation of basement membrane followed by proliferation and migration of endothelial cells to form a capillary loop. In 1995, Choy and Ballermann [122] raised the intriguing possibility that capillary morphogenesis depended on the selective apoptosis of endothelial cells mediated by transforming growth factor-β1 (TGF-β1). This homodimeric polypeptide is strongly expressed in sites of tissue morphogenesis [198]. It has been shown to induce angiogenesis in vivo [199] and endothelial cell sprouting in vitro [200]. During the process of capillary morphogenesis, TGF-β1 mediates secretion of plasminogen activator that cleaves the proenzyme plasminogen and activates plasmin, which in turn degrades ECM proteins [122]. This results in detachment and apoptosis of endothelial cells from selective areas of the developing capillary, and allows for the initiation of a new capillary loop [122, 201]. Targeted apoptosis of endothelial cells seem to have an important physiological role in allowing for the communication between the newly formed capillary and their ‘parent’ venules.

Aberrant expression of endothelial cell survival and death signals characterizes pathological angiogenesis

The role of endothelial cell proliferation and migration in the pathogenesis of angiogenesis-dependent diseases and the mediators responsible for aberrant angiogenic responses have been extensively characterized. More recently researchers have turned their attention to characterizing the impact of endothelial survival and death signals in pathological angiogenesis. The accumulated evidence to date suggests that disruption in the cell death machinery is central to the pathogenesis of a number of angiogenesis-dependent diseases (Figure 3). Two excellent examples of this phenomenon are retinopathy of prematurity, that is caused by excessive endothelial cell apoptosis in its initial stages [202]; and (b) cancer, that is associated with an unrelenting...
angiogenic response [203]. These two diseases were selected for discussion here because there is a considerable body of literature about their pathogenesis. However, it is anticipated that in time new data will emerge linking changes in the survival profile of endothelial cells to other angiogenesis dependent diseases.

Retinopathy of prematurity

With increasing survival of premature infants weighing less than 1000 g, the incidence of retinopathy of prematurity-induced blindness has also increased steadily [204]. The pathogenesis of retinopathy of prematurity has been directly associated to the fact that premature infants are placed in oxygen chambers and exposed to a hyperoxic environment to provide enough oxygen for their immature lungs [205]. The exposure of the immature retina to hyperoxia results in excessive apoptosis of endothelial cells [67, 206, 208]. When the lungs of the infant mature, the transition to room air causes excessive retinal neovascularization that is mediated by the relative ischemia in the retina resulting from the initial

![Diagram of Physiological and Pathological Angiogenesis](image-url)
disruption of blood supply [206, 208–210]. The resulting aberrant neovascularization is the cause of the severe ocular disease observed frequently in these infants, including blindness [211].

The pathogenesis of retinopathy of prematurity has been extensively studied at the molecular level, and VEGF is believed to play a major role in this process. It is known that VEGF is very responsive to subtle changes in oxygen tension. For example, its expression levels are increased in hypoxic areas and result in local induction of neovascularization that re-establishes normal oxygen supply [145, 212, 213]. Recent reports demonstrated that VEGF expression is downregulated soon after the level of oxygen rises in the retina [206, 207, 210, 212], and precedes blood vessel regression caused by endothelial cell apoptosis [193]. When the child starts breathing room air, the retina becomes hypoxic because most blood vessels have been previously disrupted. In attempt to re-establish normal oxygen tension to the area, VEGF is upregulated above physiological levels and causes excessive neovascularization that is responsible for the ocular pathology [206, 207, 210, 212]. This knowledge provides a better rationale for administering exogenous VEGF in infants prior to exposing them to the hyperoxic environment of oxygen chambers in an attempt to prevent endothelial cell apoptosis and maintain the retinal vasculature [66, 206, 210]. This clinical setting underlines the important function of VEGF as an angiogenic mediator and endothelial cell survival factor. It also demonstrates that this growth factor provides a signal that is necessary to sustain endothelial cell survival in vivo and that its premature downregulation results in the disruption of established as well as newly formed microvessels.

Cancer

Judah Folkman and colleagues first proposed the hypothesis that ‘solid tumors are angiogenesis-dependent’ in 1971 [214]. Since then the validity of this statement has been widely confirmed [214]. The observation that tumors grow as ‘cylinders’ and enter into dormancy unless they acquire a new blood supply strengthened the concept that tumors were angiogenesis dependent [215, 216]. A recent study confirmed this hypothesis by demonstrating the existence of an inverse relation between spontaneous apoptosis of tumor cells and intratumoral microvessel density [217]. The intense competition for limited oxygen and nutrient supplies, as well as for physical space inside the mass of a solid tumor, generates a hostile microenvironment for normal and neoplastic cells. However, it is clear that tumor cells have developed mechanisms for enhancing their survival and enabling them to grow in nutrient deprived environments. A question arises with regards to normal endothelial cells that populate tumor-associated blood vessels. How do they survive in this hostile environment and sustain tumor neovascularization?

Tumor-associated endothelial cells receive a continuous input of survival signals from the ECM and depend on these signals to remain viable and functional. These conclusions are supported by the findings obtained in two elegant experiments from Eli Keshet’s laboratory. VEGF expression was ‘shut down’ with an inducible VEGF expression system (‘Tet-off’) in xenografted glioma tumors in nude mice. The authors observed that upon VEGF withdrawal endothelial cells became apoptotic, tumor neovascularization decreased, and extensive tumor necrosis took place [202]. In a second model system, these investigators demonstrated that castration of SCID mice bearing an androgen-dependent tumor resulted in decreased intratumoral expression of VEGF and tumor regression [218]. The observation that endothelial cells began to undergo apoptosis before neoplastic cells confirmed the hypothesis that tumor-associated endothelial cells require specific survival signals mediated by VEGF to remain viable.

These findings reported above suggest that tumor regression mediated by withdrawal of VEGF is not due to inhibition of endothelial cell proliferation. The turnover of tumor-associated endothelial cells is thought to occur over several days or weeks. However, in this study the tumors started to regress after 24 h. Therefore, the lack of endothelial cell proliferation and migration cannot be the only mechanism responsible for the vascular regression observed in these tumors. An alternative hypothesis is that VEGF is required for maintaining endothelial cells survival and to sustain tumor angiogenesis. When this positive ‘survival’ signal is eliminated endothelial cells become more responsive to inhibitors of angiogenesis leading to endothelial cell apoptosis, vessel disassembly, and tumor regression (Figure 4).

Conclusions

Angiogenesis is absolutely necessary for embryonic morphogenesis and for maintaining tissue and organ homeostasis in adult organisms. Disruption of this biological process has been unequivocally associated with several diseases that are now described as being angiogenesis-dependent. Traditionally, angiogenic mediators have been categorized as promoting endothelial cell proliferation, migration, and adhesion. There is now compelling evidence that angiogenesis is modulated by a tightly controlled series of cellular and biochemical events that determine whether endothelial cells survive or die. The events that govern the survival and death of endothelial cells significantly influence the stability and duration of an angiogenic response. After all, angiogenesis would be of little benefit to developing organisms or adult tissues undergoing repair if endothelial cells did not survive and eventually die in a predetermined manner. Similarly, in pathological settings such as in neoplasia, tumor cells would be unable to withstand the
onslaught of nutrient deprivation and physiological signals designed to cause their demise if endothelial cells were not able to organize into a microvascular network. Thus as we look to develop novel strategies designed to retard pathological angiogenic responses or enhance physiological angiogenesis, we will have to consider factors that influence endothelial cell survival as an integral component of any therapeutic strategy.

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Figure 4. This diagram depicts how aberrant expression of endothelial survival and death signals might contribute to the unrelenting angiogenesis that is a hallmark of tumor development. In (A) vascular remodeling is a feature of normal microvascular homeostasis. In this setting the levels of inducers and inhibitors of angiogenesis are in balance. The proportion of long-lived endothelial cells populating established vessels is balanced by those endothelial cells undergoing periodic, selective apoptosis. With the emergence of neoplastic cell populations (B) the level of inducers begin to rise in association with a reduction in the level of inhibitors of angiogenesis. This is reflected in a greater number of long-lived endothelial cells and fewer endothelial cells undergoing apoptosis. Lastly in (C) as tumor neovascularization is accelerated coincidental with an increase in the tumor mass, the level of stimulators of angiogenesis that enhance endothelial cell survival greatly exceed the level of inhibitors and thus death signals. As a consequence death signals are minimized and long-lived endothelial cells accumulate in even greater numbers. We predict this would result in a more sustained and stable vascular supply to the tumor and contribute to tumor growth and progression.

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