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Review

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.

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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-x_L (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-x_L.

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

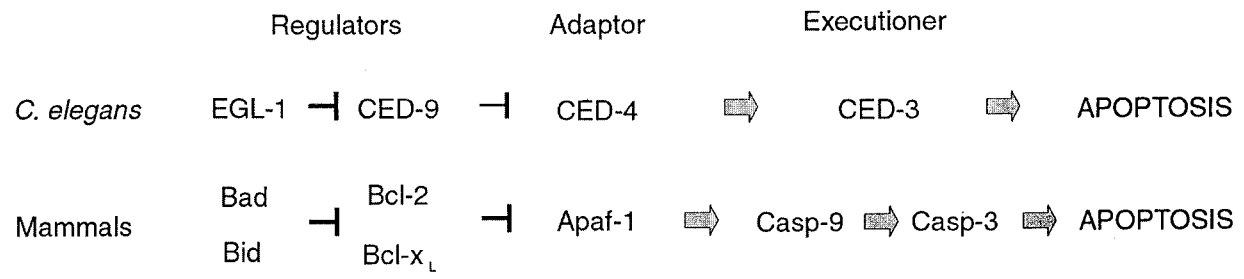

Figure 1. Mechanisms that control cell survival and death in the nematode *C. elegans* and in mammals. The current understanding of genetic pathways of apoptosis is described for *C. elegans* (Top) and mammals (Bottom). Both, the nematode genes and their mammal homologues, are functionally characterized as regulators, adaptors, or executioners of apoptosis. Symbols: inhibition (⊣), activation (→). Modified from Vaux and Korsmeyer [1].

(executioners) [9, 10]. The function of adaptors (also called caspase activators) is necessary to transduce pro-apoptotic signals from regulators to the caspases [25]. For example, Apaf-1 is a human homologue of CED-4 [26] that oligomerizes and activates procaspase-9 when it is not bound to antiapoptotic proteins of the Bcl-2 family [27, 28]. Therefore, antiapoptotic members of the Bcl-2 family may inhibit caspa1se activation by preventing oligomerization of the adapter protein Apaf-1 [29–31].

The key executioners of apoptosis are caspases, a family of cysteine proteases that are normally present in the cell as proenzymes that require proteolytic cleavage to become activated [25, 31]. Based on their sites of action in the apoptotic pathway, these proteases were divided into upstream caspases (initiator caspases) and downstream caspases (effector caspases) [31]. In mammals, Apaf-1 is dissociated from pro-survival Bcl-2 family members and activated by dATP and cytosolic cytochrome c, a protein that is released from the mitochondria during apoptosis [26, 31, 32]. Upon activation, Apaf-1 undergoes a conformational change, which enhances its ability to interact with caspase-9 (an initiator caspase) through its caspase recruitment domain (CARD) [26, 29, 31, 33, 34]. Upon this interaction, caspase-9 is processed by autocatalysis and subsequently activates downstream effector caspases such as caspase-3 [25, 33, 35]. When the downstream caspases are activated, they cleave ICAD (inhibitor of caspase activated DNAse), allowing CAD (caspase activated DNAse) to enter the nucleus and degrade chromosomal DNA [36–38].

A number of cytokines and extracellular proteins initiate apoptosis by signaling through specific cell membrane receptors [39]. The best characterized death receptors are CD95 (also known as Fas) and TNFR1 (also termed p55), that belong to the tumor necrosis factor (TNF) receptor gene superfamily [25, 31, 39]. They have cysteine-rich extracellular domains and a cytoplasmic sequence termed the 'death domain' (DD) [40, 41]. Binding of the CD95 ligand or TNF to their respective receptors activates FADD (adapter or caspase activator) that engages procaspase-8 molecules in a death-inducing signaling complex (DISC) [42]. The aggregation of several procaspase-8 molecules mediates their auto-processing and activation [43, 44]. Active caspase-8 signals through caspase-3 [45] and triggers downstream death-inducing events similar to the ones described above.

Peptides that specifically inhibit caspase activity (e.g. zVAD-FMK, zDEVD-FMK) have been shown to prevent DNA fragmentation and enhance cell survival *in vitro* and *in vivo* [46–49]. The use of caspase inhibitors for treatment of diseases that are caused by excessive apoptosis is already being evaluated in clinical trials. However, there are still several unanswered questions regarding their efficacy and safety for therapeutic use [50]. Therapeutic strategies designed to target the recently discovered caspase-independent pathways of apoptotic death may eventually prove to be a more reliable and safer alternative to the use of caspase inhibitors [51–53].

Vasculogenesis and angiogenesis

During the early stages of embryogenesis a primary vascular plexus develops from endothelial cell precursors or angioblasts by a process termed vasculogenesis [54, 55]. 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

Table 1. Classification of regulators of angiogenesis according to their role in endothelial cell survival.

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 ($\alpha_V\beta_3$)
Transforming Growth Factor-β (TGF-β)	Nitric Oxides (NO)
Lipopolysaccharides (LPS)	Estradiol
Amyloid β-peptide	Adrenomedullin
Cholesterol oxides	Serum albumin
Accutin	
Extracellular ATP	
Alkyllyso-phospholipid ET16-Ome	
2-methoxyestradiol	

regression has been attributed to its cytotoxic effect on endothelial cells that results in disruption of the tumor capillary bed [105]. Several studies have shown that binding of TNF to endothelial cells results in their apoptotic death [105–108]. The ability of TNF to induce endothelial cell apoptosis is enhanced by concomitant exposure to inhibitors of protein synthesis [107–109]. This suggested that TNF might also protect endothelial cells from apoptosis by utilizing a pathway(s) that is dependent on protein synthesis [110]. In fact, TNF was shown to induce expression of the anti-apoptotic protein A1 (a Bcl-2 homologue) and where overexpression of A1 was shown to inhibit apoptosis when endothelial cells were exposed to TNF and actinomycin D [110]. The recent finding that overexpression of Bcl-2 further supports this TNF associated protective mechanism. Bcl-x_L also protects endothelial cells from TNF-mediated apoptosis after sensitization with cyclohexamide [111].

TNF seems to mediate a multitude of intracellular signal transduction pathways that are triggered upon its binding to endothelial cell membrane receptors. These include activation of: (a) NF-κB, which mediates expression of IL-8, VEGF, and E-selectin in endothelial cells [112–115]; (b) Protein kinase C, which leads to the upregulation of A1 expression and subsequent enhancement of cell survival [110]; (c) Sp1, which mediates expression of VEGFR-2 [116]; and (d) Ceramides and *c-jun*, which result in endothelial cell death [110, 117].

Despite extensive research, the effects of TNF in endothelial cells are still unclear. While some reports demonstrate that TNF induces endothelial cell apoptosis, other investigations have shown that TNF is angiogenic [115, 118, 119]. There are a number of possible explanations for these conflicting results. (A) TNF's ability to induce angiogenesis *in vivo* may be due to the synthesis of angiogenic factors such as IL-8, VEGF or bFGF or macrophages [115, 119]. (B) Responses mediated by TNF are dose-dependent. While TNF stimulates angiogenesis at low concentrations [118], it inhibits it in higher concentrations [120]. This suggests that the induction of endothelial cell death or survival depends on the expression levels of TNF that accumulate extracellularly, which in turn determines whether it transmits a death signal or survival signal to endothelial cells.

Transforming growth factor-β (TGF-β)

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].

Lipopolysaccharides

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 Agkistrodon 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 $\alpha_V\beta_3$ 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 cytolysis 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 $VEGF_{165}$ is the predominant molecular species [147, 148]. VEGF has been extensively characterized as a potent permeability factor [150], endothelial cell specific mitogen and chemoattractant [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 $\beta 3$ 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 $\alpha_5\beta_5$ integrin, not $\alpha_5\beta_3$. 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 Bcl-2 and its homologue A1 in endothelial cells *in vitro* [156, 157]. Overexpression of Bcl-2 was sufficient to enhance endothelial cell survival and protect against apoptosis induced by growth factor deprivation [157, 158]. VEGF-mediated Bcl-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 Flk-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 HIFα 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 Bcl-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 $\alpha_V\beta_3$ is preferentially expressed in newly formed microvessels and that monoclonal antibodies to $\alpha_V\beta_3$ 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 $\alpha_V\beta_3$ 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 $\alpha_V\beta_3$ have been extensively studied. Ligation of endothelial cell $\alpha_V\beta_3$ during angiogenesis promotes a specific signal that leads to inhibition of p53 expression and its inducible partner $p21^{WAF1}$ (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 $\alpha_V\beta_3$-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 $\alpha_V\beta_3$-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 β_3 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]. Estradiol, 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 homodymeric 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

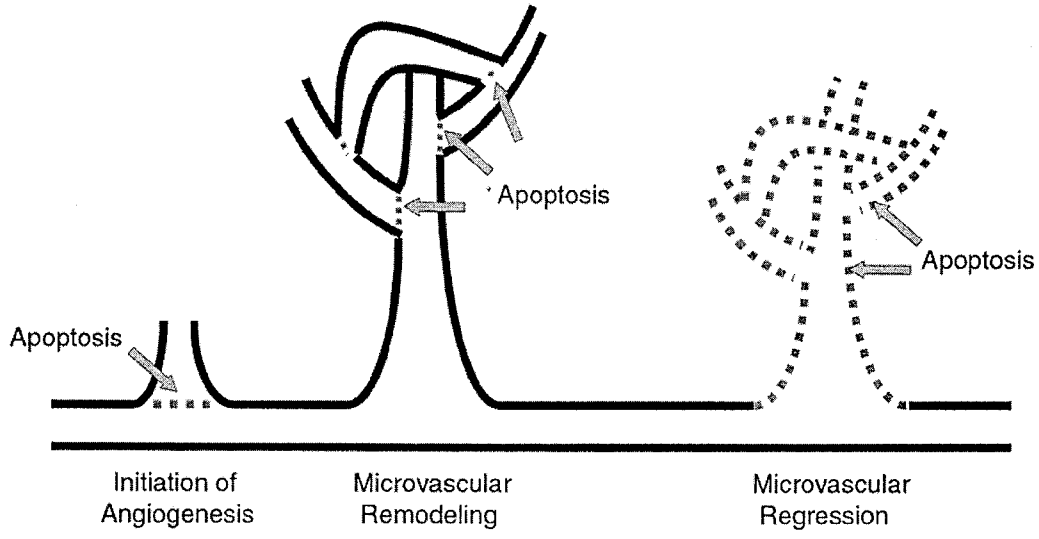

Figure 2. Endothelial cell apoptosis in vessel wall remodeling, angiogenesis and angiosuppression. Localized apoptosis of endothelial cells in the walls of venules is necessary for capillary remodeling. It allows for the communication of lumens of contiguous microvessels during the processes of sprouting and branching. Apoptosis of endothelial cells is also involved in vascular regression. It is the process by which microvessels are eliminated in the absence of inflammation.

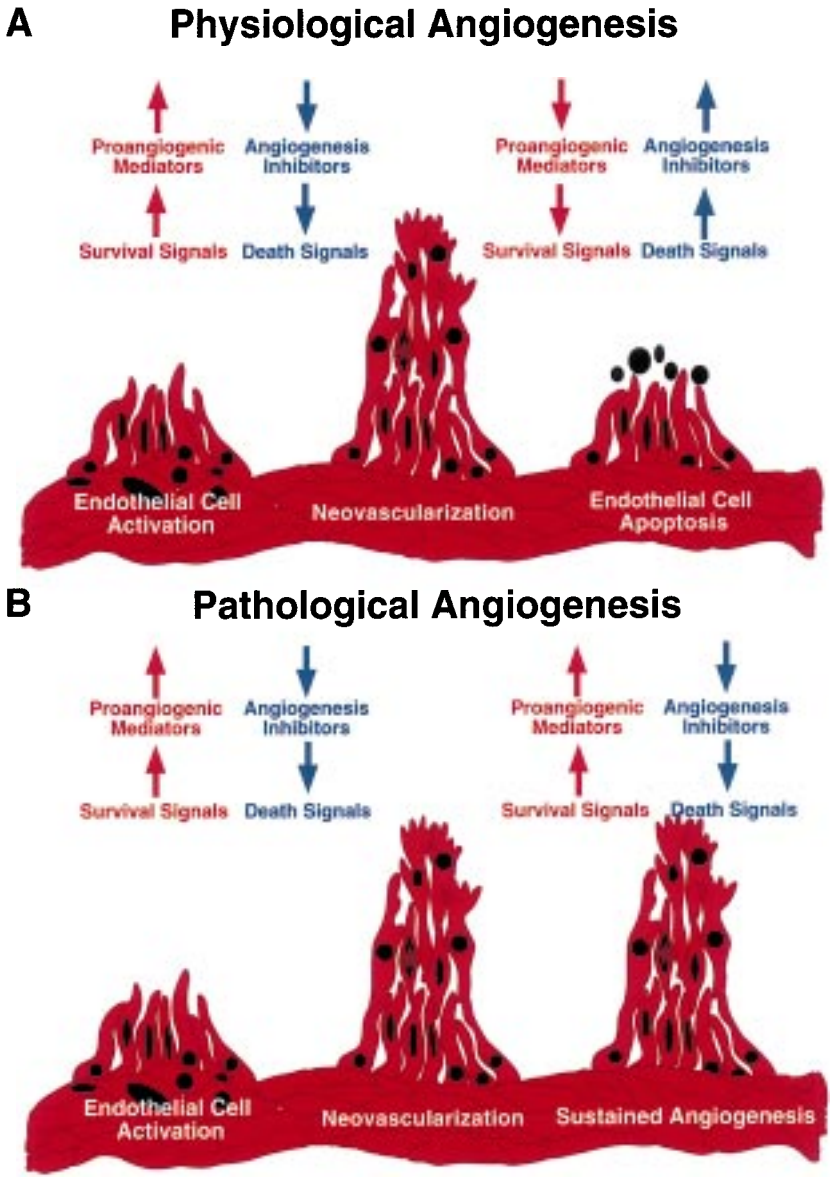

Figure 3. The balance expression of endothelial survival and death signals characterizes physiological angiogenesis. (A) During the initiation phase of a physiological angiogenic response there is an increase in the level of proangiogenic factors coincidental with a reduction in the level of angiogenesis inhibitors. This temporary shift to the angiogenic phenotype is accompanied by a transient upregulation in survival signals that is induced by proangiogenic factors. Also, the number of endothelial cells undergoing apoptosis is reduced due to the diminished level of angiogenesis inhibitors and/or increased resistance of endothelial cells to the pro-apoptotic effects of angiogenesis inhibitors. Once the metabolic demands of the tissue have been met, i.e., during the reparative phase of tissue injury, the level of proangiogenic mediators in the tissue decreases while the level of angiogenesis inhibitors begin to rise. This results in increased endothelial cell apoptosis, and the rapid regression of neovessels. (B) In angiogenesis dependent diseases the angiogenic phenotype is prolonged resulting in a protracted angiogenic response. The reduction in the level of angiogenic inhibitors along with a relative or absolute increase in the level of proangiogenic factors results in prolonged upregulation of endothelial cell survival signals and a marked decrease in endothelial cells undergoing apoptosis. We propose that prolonged upregulation of survival signals and/or a reducing in death signals contributes to sustained neovascularization that characterizes angiogenesis dependent diseases.

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

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 administrating 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 enabeling 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

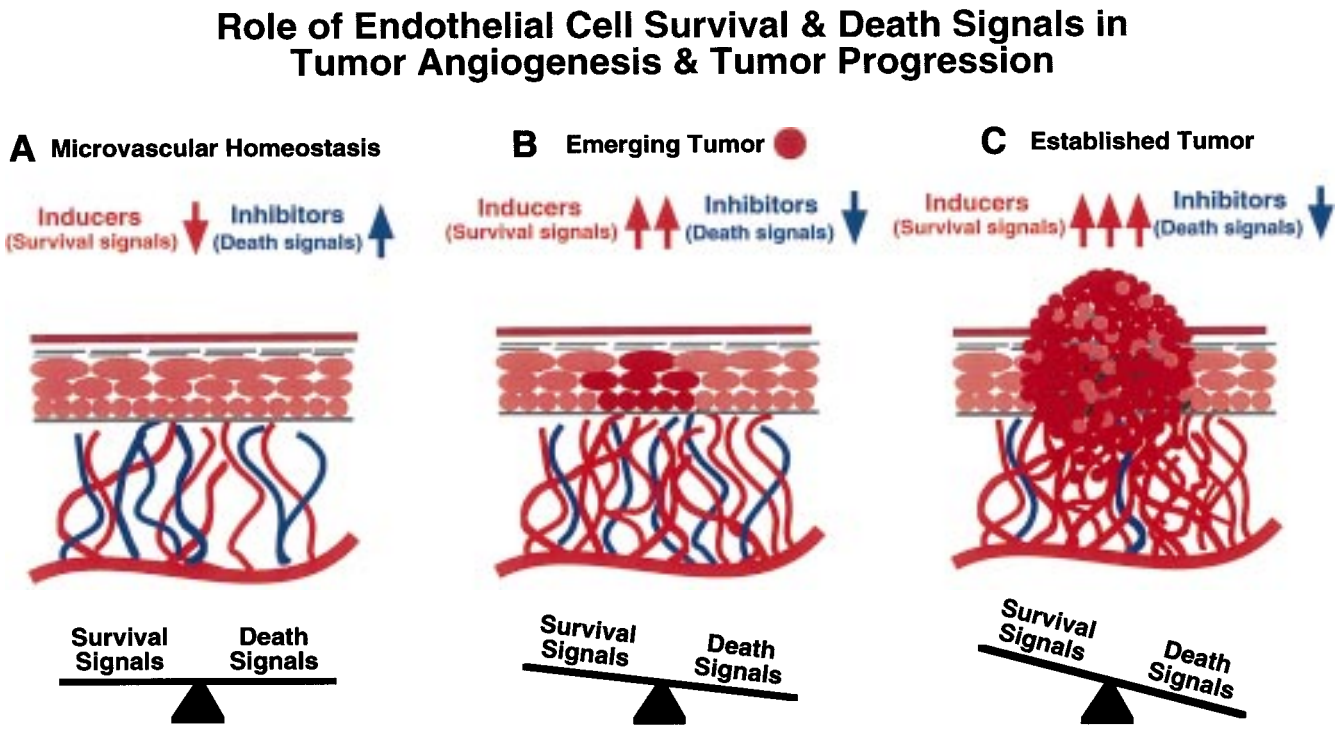

Figure 4. This diagram depicts how aberrant expression of endothelial survival and death signals might contribute to the unrelenting angiogenesis that is a hallmark of tumor development. In (A) vascular remodeling is a feature of normal microvascular homeostasis. In this setting the levels of inducers 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.

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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References

1. Vaux DL, Korsmeyer SJ. Cell death in development. Cell 1999; 96: 245–54.
2. Wyllie AH. Apoptosis and the regulation of cell numbers in normal and neoplastic tissues: An overview. Cancer Metastasis Rev 1992; 11: 95–103.
3. Green DR. Apoptotic pathways: The roads to ruin. Cell 1998; 94: 695–8.
4. Vogt C. Untersuchungen über die Entwicklungsgeschichte der Geburtsshelferkroete (Alytes obstertricians). Solothurn, Switzerland: Jent und Gassman 1842.
5. Kerr JFR, Wyllie AH, Currie AR. Apoptosis: A basic biological phenomenon with wide ranging implications in tissue kinetics. Br J Cancer 1972; 26: 239–57.
6. Greenhalgh DG. The role of apoptosis in wound healing. Int J Biochem Cell Biol 1998; 30: 1019–30.
7. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science 1995; 267: 1456–62.
8. Metcalf D, Linderman GF, Nicola NA. Analysis of hematopoiesis in max 41 transgenic mice that exhibit sustained elevations of blood granulocytes and monocytes. Blood 1995; 85: 2364–70.
9. Raff M. Cell suicide for beginners. Nature 1998; 396: 119–22.
10. Levine B. Conversion of lytic to persistent alphavirus infection by the bcl-2 cellular oncogene. Nature 1993; 361: 739–42.
11. Ameisen JC. The origin of programmed cell death. Science 1996; 272: 1278–9.
12. Steller H. Mechanisms and genes of cellular suicide. Science 1995; 267: 1445–9.
13. Ellis RE, Yuan J, Horvitz HR. Mechanisms and functions of cell death. Annu Rev Cell Biol 1991; 7: 663–98.
14. Metzstein MM, Stanfield GM, Horvitz HR. Genetics of programmed cell death in *C. elegans* past, present and future. Trends Genetics 1998; 14: 410–6.
15. Sulston JE, Horvitz HR. Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. Dev Biol 1977; 56: 110–56.
16. Wu DY, Wallen HD, Nuñez G. Interaction and regulation of subcellular localization of CED-4 by CED-9. Science 1997; 275: 1126–9.

17. Chinnaiyan AM, O'Rourke K, Lane BR, Dixit VM. Interaction of CED-4 with CED-3 and CED-9 – a molecular framework for cell death. Science 1997; 275: 1122–6.
18. Spector MS, Desnoyers S, Hoeppner DJ, Hengartner MO. Interaction between the *C. elegans* cell death regulators Ced-9 and Ced-4. Nature 1997; 385: 653–6.
19. Del Peso L, Gonzalez VM, Nuñez G. *Caenorhabditis elegans* EGL-1 disrupts the interaction of CED-9 with CED-4 and promotes CED-3 activation. J Biol Chem 1998; 273: 33495–500.
20. Reed JC. Bcl-2 and the regulation of programmed cell death. J Cell Biol 1994; 124: 1–6.
21. Chao DT, Korsmeyer SJ. BCL-2 family: Regulators of cell death. Ann Rev Imm 1998; 16: 395–419.
22. Reed JC. Bcl-2 family proteins. Oncogene 1998; 17: 3225–36.
23. Reed JC. Double identity for proteins of the Bcl-2 family. Nature 1997; 387: 773–6.
24. Wang K, Yin XM, Chao DT et al. BID: A novel BH3 domain-only death agonist. Genes Dev 1996; 10: 2859–69.
25. Thonberry NA, Lazebnik Y. Caspases: Enemies within. Science 1998; 281: 1312–6.
26. Zou H, Henzel WJ, Liu X et al. Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell 1997; 90: 405–13.
27. Pan G, O'Rourke K, Dixit VM. Caspase-9, Bcl-xL, and Apaf-1 form a ternary complex. J Biol Chem 1998; 273: 5841–5.
28. Hu Y, Benedict MA, Wu D et al. Bcl-x_L interacts with Apaf-1 and inhibits Apaf-1-dependent caspase-9 activation. Proc Natl Acad Sci USA 1998a; 95: 4386–91.
29. Hu Y, Ding L, Spencer DM, Nuñez G. WD-40 repeat region regulates Apaf-1 self-association and procaspase-9 activation. J Biol Chem 1998(b); 273: 33489–94.
30. Srinivassula SM, Ahmad M, Fernandes-Alnemri T, Alnemri ES. Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. Mol Cell 1998; 1: 949–57.
31. Nuñez G, Benedict MA, Hu Y, Inohara N. Caspases: The proteases of the apoptotic pathway. Oncogene 1998; 17: 3237–45.
32. Green DR, Reed JC. Mitochondria and apoptosis. Science 1998; 281: 1309–12.
33. Li P, Nijhawan D, Budihardjo I et al. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 1997; 91: 479–89.
34. Cardone MH, Roy N, Stennicke HR et al. Regulation of cell death protease caspase-9 by phosphorylation. Science 1998; 282: 1318–21.
35. Faleiro L, Kobayashi R, Fearnhead H, Lazebnik Y. Multiple species of CPP32 and Mch2 are the major active caspases present in apoptotic cells. EMBO J 1997; 16: 2271–81.
36. Enari M, Sakahira H, Yokoyama H et al. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. Nature 1998; 391: 43–50.
37. Sakahira H, Enari M, Nagata S. Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. Nature 1998; 391: 96–9.
38. Mukae N, Enari M, Sakahira H et al. Molecular cloning and characterization of human caspase-activated DNase. Proc Natl Acad Sci USA 1998; 95: 9123–8.
39. Ashkenazi A, Dixit VM. Death receptors: Signaling and modulation. Science 1998; 281: 1305–8.
40. Smith CA, Farrah T, Goodwin RG. The TNF receptor superfamily of cellular and viral proteins: Activation, costimulation, and death. Cell 1994; 76: 959–62.
41. Tartaglia LA, Ayres TM, Wong GH, Goeddel DV. A novel domain within the 55 kd TNF receptor signals cell death. Cell 1993; 74: 845–53.
42. Medema JP, Scaffidi C, Kischkel FC et al. FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). EMBO J 1997; 16: 2794–804.
43. Yang X, Chang HY, Baltimore D. Autoproteolytic activation of pro-caspases by oligomerization. Mol Cell 1998; 1: 319–25.
44. MacCorkle RA, Freeman KW, Spencer DM. Synthetic activation of caspases: Artificial death switches. Proc Natl Acad Sci USA 1998; 95: 3655–60.
45. Stennicke HR, Jurgensmeier JM, Shin H et al. Pro-caspase-3 is a major physiologic target of caspase-8. J Biol Chem 1998; 273: 27084–90.
46. Muzio M, Chinnaiyan AM, Kischkel FC et al. FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death–inducing signaling complex. Cell 1996; 85: 817–27.
47. Boise LH, Thompson CB. Bcl-x(L) can inhibit apoptosis in cells that have undergone Fas-induced protease activation. Proc Natl Acad Sci USA 1997; 94: 3759–64.
48. Cursio R, Gugenheim J, Ricci JE et al. A caspase inhibitor fully protects rats against lethal normothermic liver ischemia by inhibition of liver apoptosis. FASEB J 1999; 13: 253–61.
49. Schierle GS, Hansson O, Leist M et al. Caspase inhibition reduces apoptosis and increases survival of nigral transplants. Nat Med 1999; 5: 97–100.
50. Thornberry NA. Caspases: key mediators of apoptosis. Chem Biol 1998; 5: R97–103.
51. Adams JM, Cory S. The Bcl-2 protein family: Arbiters of cell survival. Science 1998; 281: 1322–6.
52. Monney L, Otter I, Olivier R et al. Defects in the ubiquitin pathway induce caspase-independent apoptosis blocked by Bcl-2. J Biol Chem 1998; 273: 6121–31.
53. Gross A, Jockel J, Wei MC, Korsmeyer SJ. Enforced dimerization of BAX results in its translocation, mitochondrial dysfunction and apoptosis. EMBO J 1998; 17: 3878–85.
54. Risau W, Flamme I. Vasculogenesis. Annu. Rev. Cell Dev. Biol. 1997; 11: 73–91.
55. Risau W. Mechanisms of angiogenesis. Nature 1997; 386: 671–4.
56. Ferrara N, Carver-Moore K, Chen H et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. Nature 1996; 380: 439–42.
57. Fong GH, Rossant J, Gertsenstein M, Breitman ML. Role of the flt-1 tyrosine kinase in regulating the assembly of vascular endothelium. Nature 1995; 376: 66–70.
58. Hertig AT. Contrib Embryol 1935; 25: 37.
59. Asahara T, Murohara T, Sullivan A et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997; 275: 964–7.
60. Denekamp J. Progress in applied microcirculation In Hammersen F, Hudlicka O (eds): Perspectives in Methodology for Studies of the Microcirculation. Basel: Karger 1984; 42: 28–38.
61. Folkman J, Shing Y. Angiogenesis. J Biol Chem 1992; 267: 10931–4.
62. Gross JL, Moscatelli D, Rifkin DB. Increased capillary endothelial cell protease activity in response to angiogenic stimuli in vitro. Proc Natl Acad Sci USA 1983; 80: 2623–727.
63. Folkman J, Haudenschild C. Angiogenesis *in vitro*. Nature 1980; 288: 551–6.
64. Nicosia RF, Villaschi S. Rat aortic smooth muscle cells become perycites during angiogenesis in vitro. Lab Invest 1995; 73: 658–66.
65. Folkman J. What is the role of endothelial cells in angiogenesis? Lab Invest 1984; 51: 601–4.
66. Benjamin LE, Hemo I, Keshet E. A plasticity window for blood vessel remodelling is defined by pericyte coverage of the preformed endothelial network and is regulated by PDGF-B and VEGF. Development 1998; 125: 1591–8.
67. Polverini PJ. The pathophysiology of angiogenesis. Crit Rev Oral Biol Med 1995; 6: 230–47.
68. Lawler J. The structural and functional properties of thrombospondin. Blood 1986; 67: 1197–209.
69. Dardik R, Lahav J. The structure of endothelial cell thrombospondin. Characterization of the heparin-binding domains. Eur J Biochem 1987; 168: 347–55.
70. Bornstein P. Diversity of function is inherited in matricellular proteins: An appraisal of thrombospondin-1. J Cell Biol 1995; 130: 503–6.

71. Good DJ, Polverini PJ, Rastinejad F et al. A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin. Proc Natl Acad Sci USA 1990; 87: 6624–8.
72. Tolsma SS, Volpert VO, Good DJ et al. Peptides derived from two separate domains of the matrix protein thrombospondin-1 have anti-angiogenic activity. J Cell Biol 1993; 122: 497–511.
73. DiPietro LA, Nissen NN, Gamelli RL et al. Thrombospondin 1 synthesis and function in wound repair. Am J Pathol 1996; 148: 1851–60.
74. O'Shea KS, Dixit VM. Unique distribution of the extracellular matrix component thrombospondin in the developing mouse embryo. J Cell Biol 1988; 107: 2737–48.
75. Iruela-Arispe ML, Porter P, Bornstein P, Sage EH. Thrombospondin-1, an inhibitor of angiogenesis, is regulated by progesterone in the human endometrium. J Clin Invest 1996; 97: 403–12.
76. Iruela-Arispe ML, Bornstein P, Sage H. Thrombospondin exerts an antiangiogenic effect on cord formation by endothelial cells *in vitro*. Proc Natl Acad Sci USA 1991; 88: 5026–30.
77. Lahav J. The functions of thrombospondin and its involvement in physiology and pathophysiology. Biochim Biophys Acta 1993; 1182: 1–14.
78. Bornstein P, Sage EH. Thrombospondins. Methods Enzymol 1994; 245: 62–85.
79. Adams JC. Thrombospondin-1. Int J Biochem Cell Biol. 1997; 29: 861–5.
80. Bagavandoss P, Wilks JW. Specific inhibition of endothelial cell proliferation by thrombospondin. Biochem Biophys Res Commun 1990; 170: 867–72.
81. Taraboletti G, Roberts D, Liotta LA, Giavazzi R. Platelet thrombospondin modulates endothelial cell adhesion, motility, and growth: A potential angiogenesis regulatory factor. J Cell Biol 1990; 111: 765–72.
82. DiPietro LA, Nebgen DR, Polverini PJ. Downregulation of endothelial cell thrombospondin 1 enhances *in vitro* angiogenesis. J Vasc Res 1994; 31: 178–85.
83. Panetti TS, Chen H, Misenheimer TM et al. Endothelial cell mitogenesis induced by LPA: Inhibition by thrombospondin-1 and thrombospondin-2. J Lab Clin Med 1997; 129: 208–16.
84. Bagavandoss P, Kaytes P, Vogeli G et al. Recombinant truncated thrombospondin-1 monomer modulates endothelial cell plasminogen activator inhibitor 1 accumulation and proliferation *in vitro*. Biochem Biophys Res Commun 1993; 192: 325–32.
85. Canfield AE, Schor AM. Evidence that tenascin and thrombospondin-1 modulate sprouting of endothelial cells. J Cell Sci 1995; 108: 797–809.
86. Vogel T, Guo NH, Krutzsch HC et al. Modulation of endothelial cell proliferation, adhesion, and motility by recombinant heparin-binding domain and synthetic peptides from the type I repeats of thrombospondin. J Cell Biochem 1993; 53: 74–84.
87. Prochownik EV, O'Rourke K, Dixit VM. Expression and analysis of COOH-terminal deletions of the human thrombospondin molecule. J Cell Biol 1989; 109: 843–52.
88. Castle VP, Dixit VM, Polverini PJ. Thrombospondin-1 suppresses tumorigenesis and angiogenesis in serum- and anchorage-independent NIH 3T3 cells. Lab Invest 1997; 77: 51–61.
89. Dawson DW, Pearce SF, Zhong R et al. CD36 mediates the *In vitro* inhibitory effects of thrombospondin-1 on endothelial cells. J Cell Biol 1997; 138: 707–17.
90. Guo N, Krutzsch HC, Inman JK, Roberts DD. Thrombospondin 1 and type I repeat peptides of thrombospondin 1 specifically induce apoptosis of endothelial cells. Cancer Res 1997; 57: 1735–42.
91. O'Reilly MS, Holmgren L, Shing Y et al. Angiostatin: A circulating endothelial cell inhibitor that suppresses angiogenesis and tumor growth. Cold Spring Harb Symp Quant Biol 1994a; 59: 471–82.
92. O'Reilly MS, Holmgren L, Shing Y et al. Angiostatin: A novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell 1994b; 79: 315–28.
93. O'Reilly MS. Angiostatin: An endogenous inhibitor of angiogenesis and of tumor growth. EXS 1997; 79: 273–94.
94. Cao Y, Ji RW, Davidson D et al. Kringle domains of human angiostatin. Characterization of the anti-proliferative activity on endothelial cells. J Biol Chem 1996; 271: 29461–7.
95. Dong Z, Kumar R, Yang X, Fidler IJ. Macrophage-derived metalloelastase is responsible for the generation of angiostatin in Lewis lung carcinoma. Cell 1997; 88: 801–10.
96. Patterson BC, Sang QA. Angiostatin-converting enzyme activities of human matrilysin (MMP-7) and gelatinase B/type IV collagenase (MMP-9). J Biol Chem 1997; 272: 28823–5.
97. Lijnen HR, Ugwu F, Bini A, Collen D. Generation of an angiostatin-like fragment from plasminogen by stromelysin-1 (MMP-3). Biochemistry 1998; 37: 4699–702.
98. Claesson-Welsh L, Welsh M, Ito N et al. Angiostatin induces endothelial cell apoptosis and activation of focal adhesion kinase independently of the integrin-binding motif RGD. Proc Natl Acad Sci USA 1998; 95: 5579–83.
99. Lucas R, Holmgren L, Garcia I et al. Multiple forms of angiostatin induce apoptosis in endothelial cells. Blood 1998; 92: 4730–41.
100. Matthews N. Tumour-necrosis factor from the rabbit. II. Production by monocytes. Br J Cancer 1978; 38: 310–5.
101. Tracey KJ, Cerami A. Tumor necrosis factor, other cytokines and disease. Annu Rev Cell Biol 1993a; 9: 317–43.
102. Diez-Roux G, Lang RA. Macrophages induce apoptosis in normal cells *in vivo*. Development 1997; 124: 3633–8.
103. Steffen M, Ottmann OG, Moore MA. Simultaneous production of tumor necrosis factor-alpha and lymphotoxin by normal T cells after induction with IL-2 and anti-T3. J Immunol 1988; 140: 2621–4.
104. Tracey KJ, Cerami A. Tumor necrosis factor: An updated review of its biology. Crit Care Med 1993b; 10: S415–22.
105. Robaye B, Mosselmans R, Fiers W et al. Tumor necrosis factor induces apoptosis (programmed cell death) in normal endothelial cells *in vitro*. Am J Pathol 1991; 138: 447–53.
106. Wendt CH, Polunovsky VA, Peterson MS et al. Alveolar epithelial cells regulate the induction of endothelial cell apoptosis. Am J Physiol 1994; 267: C893–900.
107. Polunovsky VA, Wendt CH, Ingbar DH et al. Induction of endothelial cell apoptosis by TNF alpha: Modulation by inhibitors of protein synthesis. Exp Cell Res 1994; 214: 584–94.
108. Pober JS. Activation and injury of endothelial cells by cytokines. Pathol Biol 1998; 46:159–63.
109. Marino MW, Dunbar JD, Wu LW et al. Inhibition of tumor necrosis factor signal transduction in endothelial cells by dimethylaminopurine. J Biol Chem 1996; 271: 28624–9.
110. Karsan A, Yee E, Harlan JM. Endothelial cell death induced by tumor necrosis factor-alpha is inhibited by the Bcl-2 family member, A1. J Biol Chem 1996; 271: 27201–4.
111. Badrichani AZ, Stroka DM, Bilbao G et al. Bcl-2 and Bcl-XL serve an anti-inflammatory function in endothelial cells through inhibition of NF-kappaB. J Clin Invest 1999; 103: 543–53.
112. Read MA, Whitley MZ, Williams AJ, Collins T. NF-kappa B and I kappa B alpha: An inducible regulatory system in endothelial activation. J Exp Med 1994; 179: 503–12.
113. Collins T, Read MA, Neish AS et al. Transcriptional regulation of endothelial cell adhesion molecules: NF-kappa B and cytokine-inducible enhancers. FASEB J 1995; 9: 899–909.
114. Lakshminarayanan V, Drab-Weiss EA, Roebuck KA. H_2O_2 and tumor necrosis factor-alpha induce differential binding of the redox-responsive transcription factors AP-1 and NF-kappaB to the interleukin-8 promoter in endothelial and epithelial cells. J Biol Chem 1998; 273: 32670–8.
115. Yoshida S, Ono M, Shono T et al. Involvement of interleukin-8, vascular endothelial growth factor, and basic fibroblast growth factor in tumor necrosis factor alpha-dependent angiogenesis. Mol Cell Biol 1997; 17: 4015–23.
116. Giraudo E, Primo L, Audero E et al. Tumor necrosis factor-alpha regulates expression of vascular endothelial growth factor

receptor-2 and of its co-receptor neuropilin-1 in human vascular endothelial cells. J Biol Chem 1998; 273: 22128–35.
117. Obeid LM, Linardic CM, Karolak LA, Hannun YA. Programmed cell death induced by ceramide. Science 1993; 259: 1769–71.
118. Leibovich SJ, Polverini PJ, Shepard HM et al. Macrophage-induced angiogenesis is mediated by tumour necrosis factor-alpha. Nature 1987; 329: 630–2.
119. Frater-Schroder M, Risau W, Hallmann R et al. Tumor necrosis factor type alpha, a potent inhibitor of endothelial cell growth in vitro, is angiogenic *in vivo*. Proc Natl Acad Sci USA 1987; 84: 5277–81.
120. Ryuto M, Ono M, Izumi H et al. Induction of vascular endothelial growth factor by tumor necrosis factor alpha in human glioma cells. Possible roles of SP-1. J Biol Chem 1996; 271: 28220–8.
121. Tsukada T, Eguchi K, Migita K et al. Transforming growth factor beta 1 induces apoptotic cell death in cultured human umbilical vein endothelial cells with down-regulated expression of bcl-2. Biochem Biophys Res Commun 1995; 210: 1076–82.
122. Choi ME, Ballerman BJ. Inhibition of capillary morphogenesis and associated apoptosis by dominant negative mutant transforming growth factor-β receptors. J Biol Chem 1995; 270: 21144–50.
123. Brigham KL, Meyrick B. Endotoxin and lung injury. Am Rev Respir Dis 1986; 133: 913–27.
124. Hoyt DG, Mannix RJ, Rusnak JM et al. Collagen is a survival factor against LPS-induced apoptosis in cultured sheep pulmonary artery endothelial cells. Am J Physiol 1995; 269: L171–7.
125. Haimovitz-Friedman A, Cordon-Cardo C, Bayoumy S et al. Lipopolysaccharide induces disseminated endothelial apoptosis requiring ceramide generation. J Exp Med 1997; 186: 1831–41.
126. Wong HR, Mannix RJ, Rusnak JM et al. The heat-shock response attenuates lipopolysaccharide-mediated apoptosis in cultured sheep pulmonary artery endothelial cells. Am J Respir Cell Mol Biol 1996; 15: 745–51.
127. Haendeler J, Zeiher AM, Dimmeler S. Vitamin C and E prevent lipopolysaccharide-induced apoptosis in human endothelial cells by modulation of Bcl-2 and Bax. Eur J Pharmacol 1996; 317: 407–11.
128. Maier JA, Morelli D, Balsari A. The differential response to interferon gamma by normal and transformed endothelial cells. Biochem Biophys Res Commun 1995; 214: 582–8.
129. Blanc EM, Toborek M, Mark RJ et al. Amyloid beta-peptide induces cell monolayer albumin permeability, impairs glucose transport, and induces apoptosis in vascular endothelial cells. J Neurochem 1997; 68: 1870–81.
130. Imai H, Werthessen NT, Taylor CB, Lee KT. Angiotoxicity and arteriosclerosis due to contaminants of USP-grade cholesterol. Arch Pathol Lab Med 1976; 100: 565–72.
131. Lizard G, Deckert V, Dubrez L et al. Induction of apoptosis in endothelial cells treated with cholesterol oxides. Am J Pathol 1996; 148: 1625–38.
132. Geng YJ, Libby P. Evidence for apoptosis in advanced human atheroma. Colocalization with interleukin-1 beta-converting enzyme. Am J Pathol 1995; 147: 251–66.
133. Yeh CH, Peng HC, Huang TF. Accutin, a new disintegrin, inhibits angiogenesis *in vitro* and *in vivo* by acting as an integrin $\alpha_V\beta_3$ antagonist and inducing apoptosis. Blood 1998; 92: 3268–76.
134. Polunovsky VA, Chen B, Henke C et al. Role of mesenchymal cell death in lung remodeling after injury. J Clin Invest 1993; 92: 388–97.
135. Dawicki DD, Chatterjee D, Wyche J, Rounds S. Extracellular ATP and adenosine cause apoptosis of pulmonary artery endothelial cells. Am J Physiol 1997; 273: L485–94.
136. Araki S, Tsuna I, Kaji K, Hayashi H. Programmed cell death in response to alkyllysophospholipids in endothelial cells. J Biochem 1994; 115: 245–7.
137. Yue TL, Wang X, Louden CS et al. 2-Methoxyestradiol, an endogenous estrogen metabolite, induces apoptosis in endothelial cells and inhibits angiogenesis: Possible role for stress-activated protein kinase signaling pathway and Fas expression. Mol Pharmacol 1997; 51: 951–62.
138. Lang R, Bishop MJ. Macrophages are required for cell death and tissue remodeling in the developing mouse eye. Cell 1993; 74: 453–62.
139. Lang RA, Lustig M, Francois F et al. Apoptosis during macrophage- dependent tissue remodelling. Development 1994; 120: 3395–403.
140. Meeson A, Palmer M, Calfon M, Lang RA. A relationship between flow and apoptosis during programmed capillary regression is revealed by vital analysis. Development 1996; 122: 3929–38.
141. Meeson AP, Argilla M, Witte L, Lang RA. VEGF deprivation-induced apoptosis is a component of programmed capillary regression. Development 1999; 126: 1407–15.
142. Senger DR, Galli SJ, Dvorak AM et al. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 1983; 219: 983–5.
143. Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem Biophys Res Commun 1989; 161: 851–8.
144. Plöuet J, Schilling J, Gospodarowicz D. Isolation and characterization of a newly identified endothelial cell mitogen produced by AtT-20 cells. EMBO J 1989; 8: 3801–6.
145. Gospodarowicz D, Abraham JA, Schilling J. Isolation and characterization of a vascular endothelial cell mitogen produced by pituitary-derived folliculo stellate cells. Proc Natl Acad Sci USA 1989; 86: 7311–5.
146. Ferrara N. Vascular endothelial growth factor. Eur J Cancer 1996; 32A: 2413–22.
147. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. FASEB J 1999; 13: 9–22.
148. Nicosia RF, Nicosia SV, Smith M. Vascular endothelial growth factor, platelet-derived growth factor, and insulin-like growth factor-1 promote rat aortic angiogenesis *in vitro*. Am J Pathol 1994; 145: 1023–9.
149. Leung DW, Cachianes G, Kuang WJ et al. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 1989; 246: 1306–9.
150. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 1986; 315: 1650–9.
151. Yoshida A, Anand-Apte B, Zetter BR. Differential endothelial migration and proliferation to basic fibroblast growth factor and vascular endothelial growth factor. Growth Factors 1996; 13: 57–64.
152. Melder RJ, Koenig GC, Witwer BP et al. During angiogenesis, vascular endothelial growth factor and basic fibroblast growth factor regulate natural killer cell adhesion to tumor endothelium. Nat Med 1996; 2: 992–7.
153. Spyridopoulos I, Brogi E, Kearney M et al. Vascular endothelial growth factor inhibits endothelial cell apoptosis induced by tumor necrosis factor-alpha: Balance between growth and death signals. J Mol Cell Cardiol 1997a; 29: 1321–30.
154. Meredith JE, Fazeli B, Schwartz MA. The extracellular matrix as a cell survival factor. Mol Cell Biol 1993; 4: 953–61.
155. Watanabe Y, Dvorak HF. Vascular permeability factor/vascular endothelial growth factor inhibits anchorage-disruption-induced apoptosis in microvessel endothelial cells by inducing scaffold formation. Exp Cell Res 1997; 233: 340–9.
156. Gerber HP, Dixit V, Ferrara N. Vascular endothelial growth factor induces expression of the antiapoptotic proteins Bcl-2 and A1 in vascular endothelial cells. J Biol Chem 1998a; 273: 13313–6.
157. Nör JE, Christensen J, Mooney DJ, Polverini PJ. Vascular Endothelial Growth Factor (VEGF)-mediated angiogenesis is associated with enhanced endothelial cell survival and induction of Bcl-2 expression. Am J Pathol 1999; 154: 375–84.

158. Kondo S, Yin D, Aoki T et al. bcl-2 gene prevents apoptosis of basic fibroblast growth factor-deprived murine aortic endothelial cells. Exp Cell Res 1994; 213: 428–32.
159. Gerber HP, McMurtrey A, Kowalski J et al. Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3′-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. J Biol Chem 1998; 273: 30336–43.
160. Ilan N, Mahooti S, Madri JA. Distinct signal transduction pathways are utilized during the tube formation and survival phases of in vitro angiogenesis. J Cell Sci 1998; 111: 3621–31.
161. Maxwell PH, Dachs GU, Gleadle JM et al. Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. Proc Natl Acad Sci USA 1997; 94: 8104–9.
162. Ryan HE, Lo J, Johnson RS. HIF-1α is required for solid tumor formation and embryonic vascularization. EMBO J 1998; 17: 3005–15.
163. Carmeliet P, Dor Y, Herbert J-M et al. Role of HIF-α in hypoxia-mediated apoptosis, cell proliferation, and tumor angiogenesis. Nature 1998; 394: 485–90.
164. Maxwell PH, Weisener MS, Chang G-W et al. The tumor suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 1999; 399: 271–5.
165. Stebbins CE, Kaelin WG, Pavletich NP. Structure of the VHL-elonginC-elonginB complex: implications for VHL tumor suppressor function, Science 1999; 284: 455–61.
166. Kumura T, Koepp DM, Conrad MN et al. Rbx1, a conponent of the VHL tumor suppressor complex and SCF ubiquitin ligase. Science 1999; 284: 657–61.
167. Esch F, Baird A, Ling N et al. Primary structure of bovine pituitary basic fibroblast growth factor (FGF) and comparison with the amino-terminal sequence of bovine brain acidic FGF. Proc Natl Acad Sci USA 1985; 82: 6507–11.
168. Abraham JA, Mergia A, Whang JL et al. Nucleotide sequence of a bovine clone encoding the angiogenic protein, basic fibroblast growth factor. Science 1986; 233: 545–8.
169. Araki S, Shimada Y, Kaji K, Hayashi H. Apoptosis of vascular endothelial cells by fibroblast growth factor deprivation. Biochem Biophys Res Commun 1990a; 168: 1194–200.
170. Araki S, Simada Y, Kaji K, Hayashi H. Role of protein kinase C in the inhibition by fibroblast growth factor of apoptosis in serum-depleted endothelial cells. Biochem Biophys Res Commun 1990b; 172: 1081–5.
171. Fuks Z, Persaud RS, Alfieri A et al. Basic fibroblast growth factor protects endothelial cells against radiation-induced programmed cell death *in vitro* and *in vivo*. Cancer Res 1994; 54: 2582–90.
172. Langley RE, Bump EA, Quartuccio SG et al. Radiation-induced apoptosis in microvascular endothelial cells. Br J Cancer 1997; 75: 666–72.
173. Satake S, Kuzuya M, Ramos MA et al. Angiogenic stimuli are essential for survival of vascular endothelial cells in three-dimensional collagen lattice. Biochem Biophys Res Commun 1998; 244: 642–6.
174. Kondo S, Kondo Y, Yin D et al. Involvement of interleukin-1 beta-converting enzyme in apoptosis of bFGF-deprived murine aortic endothelial cells. FASEB J 1996; 10: 1192–7.
175. Karsan A, Yee E, Poirier GG et al. Fibroblast growth factor-2 inhibits endothelial cell apoptosis by Bcl-2-dependent and independent mechanisms. Am J Pathol 1997; 151: 1775–84.
176. Brooks PC. Role of integrins in angiogenesis. Eur J Cancer 1996; 32A: 2423–9.
177. Brooks PC, Montgomery AM, Rosenfeld M et al. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. Cell 1994; 79: 1157–64.
178. Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science 1994; 264: 569–71.
179. Stromblad S, Cheresh DA. Integrins, angiogenesis and vascular cell survival. Chem Biol 1996a; 3: 881–5.
180. Meredith J Jr, Mu Z, Saido T, Du X. Cleavage of the cytoplasmic domain of the integrin beta3 subunit during endothelial cell apoptosis. J Biol Chem 1998; 273: 19525–31.
181. Stromblad S, Becker JC, Yebra M et al. Suppression of p53 activity and p21WAF1/CIP1 expression by vascular cell integrin $\alpha_V\beta_3$ during angiogenesis. J Clin Invest 1996b; 98: 426–33.
182. Scatena M, Almeida M, Chaisson ML et al. NF-kappaB mediates alphavbeta3 integrin-induced endothelial cell survival. J Cell Biol 1998; 141: 1083–93.
183. Miao JY, Araki S, Hayashi H. Relationships between phosphatidylcholine-specific phospholipase C and integrins in cell-substratum adhesion and apoptosis in vascular endothelial cells. Endothelium 1997; 5: 297–305.
184. Ruegg C, Yilmaz A, Bieler G et al. Evidence for the involvement of endothelial cell integrin alphaVbeta3 in the disruption of the tumor vasculature induced by TNF and IFN-gamma. Nat Med 1998; 4: 408–14.
185. Alonso J, Sanchez de Miguel L, Monton M et al. Endothelial cytosolic proteins bind to the 3′ untranslated region of endothelial nitric oxide synthase mRNA: Regulation by tumor necrosis factor alpha. Mol Cell Biol 1997; 17: 5719–26.
186. Lopez-Farre A, Rodriguez-Feo JA, Sanchez de Miguel L et al. Role of nitric oxide in the control of apoptosis in the microvasculature. Int J Biochem Cell Biol 1998; 30: 1095–106.
187. Lopez-Farre A, Sanchez de Miguel L, Caramelo C et al. Role of nitric oxide in autocrine control of growth and apoptosis of endothelial cells. Am J Physiol 1997; 272: H760–8.
188. Shimokawa H, Flavahan NA, Vanhoutte PM. Natural course of the impairment of endothelium-dependent relaxations after balloon endothelium removal in porcine coronary arteries. Possible dysfunction of a pertussis toxin-sensitive G protein. Circ Res 1989; 65: 740–53.
189. Alvarez RJ, Gips SJ, Moldovan N et al. 17β-estradiol inhibits apoptosis of endothelial cells. Biochem Biophys Res Commun 1997; 237: 372–81.
190. Barrett-Connor E, Bush TL. Estrogen and coronary heart disease in women. JAMA 1991; 265: 1861–7.
191. Spyridopoulos I, Sullivan AB, Kearney M et al. Estrogen-receptor-mediated inhibition of human endothelial cell apoptosis. Estradiol as a survival factor. Circulation 1997b; 95: 1505–14.
192. Kato H, Shichiri M, Marumo F, Hirata Y. Adrenomedullin as an autocrine/paracrine apoptosis survival factor for rat endothelial cells. Endocrinology 1997; 138: 2615–20.
193. Zoellner H, Hofler M, Beckmann R et al. Serum albumin is a specific inhibitor of apoptosis in human endothelial cells. J Cell Sci 1996; 109: 2571–80.
194. Deveraux QL, Reed JC. IAP family of proteins-suppressors of apoptoisis. Genes Dev 1999; 13: 239–52.
195. Crook NE, Clem RJ, Miller LK. An apoptosis inhibiting baculovirus gene with a zinc finger-like motif. J Virol 1993; 67: 2168–74.
196. Birnbaum MJ, Clem RJ, Miller LK. An apoptosis inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motifs. J Virol 1994; 68: 2521–8.
197. Deveraux QL, Roy N, Stennicke HR et al. IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. EMBO J 1998; 17: 2215–23.
198. Sandberg M, Vuorio T, Hirvonen H et al. Enhanced expression of TGF-beta and c-fos mRNAs in the growth plates of developing human long bones. Development 1988; 102: 461–70.
199. Roberts AB, Sporn MB, Assoian RK et al. Transforming growth factor type beta: Rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. Proc Natl Acad Sci USA 1986; 83: 4167–71.
200. Madri JA, Pratt BM, Tucker AM. Phenotypic modulation of endothelial cells by transforming growth factor-beta depends upon the composition and organization of the extracellular matrix. J Cell Biol 1988; 106: 1375–84.
201. Frisch SM, Francis H. Disruption of epithelial cell–matrix interactions induces apoptosis. J Cell Biol 1994; 124: 619–26.

202. Benjamin LE, Keshet E. Conditional switching of vascular endothelial growth factor (VEGF) expression in tumors: Induction of endothelial cell shedding and regression of hemangioblastoma-like vessels by VEGF withdrawal. Proc Natl Acad Sci USA 1997; 94: 8761–6.
203. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1995; 1: 27–31.
204. Gibson DL, Sheps SB, Uh SH et al. Retinopathy of prematurity-induced blindness: Birth weight-specific survival and the new epidemic. Pediatrics 1990; 86: 405–12.
205. Ticho BH, Dreger V. Retinopathy of prematurity. Insight 1997; 22: 57–61.
206. Alon T, Hemo I, Itin A et al. Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. Nat Med 1995; 1: 1024–8.
207. Stone J, Chan-Ling T, Pe'er J et al. Roles of vascular endothelial growth factor and astrocyte degeneration in the genesis of retinopathy of prematurity. Invest Ophthalmol Vis Sci 1996; 37: 290–9.
208. Penn JS, Tolman BL, Henry MM. Oxygen-induced retinopathy in the rat: relationship of retinal nonperfusion to subsequent neovascularization. Invest Ophthalmol Vis Sci 1994; 35: 3429–35.
209. Aiello LP, Northrup JM, Keyt BA et al. Hypoxic regulation of vascular endothelial growth factor in retinal cells. Arch Ophthalmol 1995; 113: 1538–44.
210. Pierce EA, Foley ED, Smith LE. Regulation of vascular endothelial growth factor by oxygen in a model of retinopathy of prematurity. Arch Ophthalmol 1996; 114: 1219–28.
211. Hebbandi SB, Bowen JR, Hipwell GC et al. Ocular sequelae in extremely premature infants at 5 years of age. J Paediatr Child Health 1997; 33: 339–42.
212. Donahue ML, Phelps DL, Watkins RH et al. Retinal vascular endothelial growth factor (VEGF) mRNA expression is altered in relation to neovascularization in oxygen induced retinopathy. Curr Eye Res 1996; 15: 175–84.
213. Claffey KP, Shih SC, Mullen A et al. Identification of a human VPF/VEGF 3′ untranslated region mediating hypoxia-induced mRNA stability. Mol Biol Cell 1998; 9: 469–81.
214. Folkman J. Tumor angiogenesis: Therapeutic implications. N Engl J Med 1971; 285: 1182–6.
215. Tannock IF. The relation between cell proliferation and the vascular system in a transplanted mouse mammary tumour. Br J Cancer 1968; 22: 258–73.
216. Folkman J, Klagsburn M. Angiogenic factors. Science 1987; 235: 442–7.
217. Lu C, Tanigawa N. Spontaneous apoptosis is inversely related to intratumoral microvessel density in gastric carcinoma. Cancer Res 1997; 57: 221–4.
218. Jain RK, Safabakhsh N, Sckell A et al. Endothelial cell death, angiogenesis, and microvascular function after castration in an androgen-dependent tumor: Role of vascular endothelial growth factor. Proc Natl Acad Sci USA 1998; 95: 10820–5.