This Review is part of a thematic series on **Apoptosis in the Cardiovascular System**, which includes the following articles:

Apoptosis and Heart Failure: A Critical Review of the Literature

Vascular Cell Apoptosis in Remodeling, Restenosis, and Plaque Rupture

Endothelial Cell Apoptosis in Angiogenesis and Vessel Regression

Apoptosis During Cardiovascular Development

Myocyte Apoptosis in Ischemic Heart Disease

Richard Kitsis, Guest Editor

Apoptosis During Cardiovascular Development

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Abstract—Morphogenesis and developmental remodeling of cardiovascular tissues involve coordinated regulation of cell proliferation and apoptosis. In the heart, clear evidence points toward focal apoptosis as a contributor to development of the embryonic outflow tract, cardiac valves, conducting system, and the developing coronary vasculature. Apoptosis in the heart is likely regulated by survival and death signals that are also present in many other tissues. Cell type–specific regulation may be superimposed on general cell death/survival machinery through tissue-specific transcriptional pathways. In the vasculature, apoptosis almost certainly contributes to developmental vessel regression, and it is of proven importance in remodeling of arterial structure in response to local changes in hemodynamics. Physical forces, growth factors, and extracellular matrix drive vascular cell survival pathways, and considerable evidence points to local nitric oxide production as an important but complex regulator of vascular cell death. In both the heart and vasculature, progress has been impeded by inadequate information concerning the incidence of apoptosis, its relative importance compared with the diverse cell behaviors that remodel developing tissues, and by our primitive knowledge concerning regulation of cell death in these tissues. However, tools are now available to better understand apoptosis in normal and abnormal development of cardiovascular structures, and a framework has been established that should lead to considerable progress in the coming years. **(*Circ Res*. 2000;87:856-864.)**

Key Words: apoptosis ■ development ■ myocardium ■ smooth muscle ■ endothelium

The role of spontaneous cell death in normal embryonic development was appreciated a century ago (for an early review, see Glucksmann[1]). Histological studies by Kerr et al[2] led them to propose in 1972 that the cell death that occurs in some developmental and pathological contexts is distinct from necrotic cell death. They suggested that this form of cell death be termed "apoptosis," for the controlled cell deletion that reminded them of its Greek derivation, "falling off," as of leaves from a tree.[3] The 1990s witnessed an explosion in the study of apoptosis that led to the identification of the components of a programmed cell death (PCD) pathway. Critical discoveries included the caspases, a family of highly conserved proteases that executes the program of cell death[4,5]; receptor (tumor necrosis factor [TNF] superfamily, bone morphogenetic protein [BMP]) and nonreceptor (hypoxia, genotoxic stress, and growth factor withdrawal) means of activation of the caspase cascade[6,7]; the Bcl2 family of proteins, which reside within the mitochondria and regulate cytochrome *c* release and caspase activation[8]; and a family of inhibitor of apoptosis proteins (IAPs) that inhibit caspase activity[9] and thus prevent the fortuitous activation of the pathway.

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This review describes what is known of the role of apoptosis in the development of the cardiovascular system, focusing on three major questions.

1. What cells undergo PCD? Identification of the cell type undergoing PCD in vivo can be problematic since the process of apoptosis, as well as a commonly used detection method, terminal deoxynucleotide transferase–mediated dUTP-biotin nick end-labeling (TUNEL), destroys protein epitopes. This problem may be compounded by the undifferentiated or partially differentiated state of cells in developing organs. Evidence of apoptotic cell death includes chromosomal fragmentation detected by the in situ TUNEL labeling or by DNA laddering on gel electrophoresis, vacuolation and nuclear condensation by electron microscopy, eversion of phosphatidylserine residues of the cell membrane, and activation of caspases. Reliance on a single measure is often misleading.[10]
2. What is the significance of PCD with respect to the formation or the malformation of cardiovascular structures? PCD may have a number of functions in development, including reducing cell numbers, eliminating abnormal or mislocated cells, sculpting tissues, and eliminating vestigial structures.[11] The role of the elimination of a cell or group of cells may be surmised by its coincidence with a morphogenic process, but proof of its role requires targeted perturbations of the PCD process. The significance of the elimination of cells must also be considered in the context of the rates of proliferation of the cells within a tissue.[12]
3. What are the molecular mechanisms? Over the last 5 years, our knowledge of basic genetic pathways that regulate PCD has grown exponentially; however, the role of most apoptotic regulators during cardiovascular development is unclear despite their great importance. Molecular pathways involving such regulators may be the targets for a variety of teratogens, as has been shown in the case of fetal alcohol syndrome.[13]

Apoptosis in the Developing Heart

Early studies of cell death in the cardiovascular system used vital dyes and electron microscopy to identify dead cells in avian, rodent, and human embryos, using what were essentially random sampling methods.[14,15] These studies provided a foundation for future investigations of apoptosis in the developing cardiovascular system.

What Cells Undergo Apoptosis and What Is the Significance?

The ventricular and atrial compartments of the developing heart enlarge throughout development; consequently, it is not surprising that high levels of PCD have not been observed in the cardiomyocytes of these chambers. Some apoptotic myocytes were identified by TUNEL and transmission electron microscopy (TEM) in the compact and trabecular zones of the embryonic day (ED) 11 to 16 mouse ventricle,[16] and TUNEL-positive cells have been identified in the trabeculae and compact zones of the mouse ventricles from ED13 to day 2 after birth.[17] A study of the neonatal rat heart used incorporation of biotinylated dUTP as a marker for DNA strand breaks.[18] Cardiomyocytes of the 1-day-old right ventricle (RV) were positive with a prevalence of 0.1%. The prevalence declined during the first 2 weeks of life and was 4- to 8-fold higher in the RV than the left ventricle (LV), and the authors proposed that this process might contribute to the thinning of the RV after birth. However, the prevalence of apoptosis was well below that of cell division; therefore, it is uncertain whether apoptosis serves a specific morphogenic purpose in these instances.

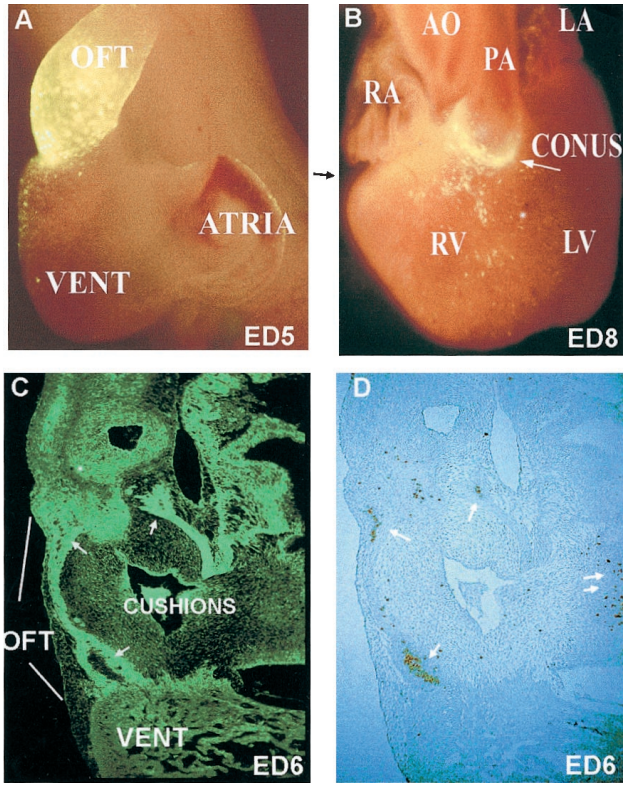

Figure 1. Shortening and rotation of the myocardial portion of the OFT coincident with cardiomyocyte apoptosis. The OFT myocardium labeled with AdCMVGFP undergoes shortening and rotation (compare A and B) in the transition from the single- to dual-circulation heart. At the same time, the atria and ventricles considerably increase in size (A and B are not to scale). Coincident with the OFT remodeling is cardiomyocyte apoptosis. C, Myocardium is delimited by an antimyosin antibody (fluorescein). D, Same section, showing dense foci of TUNEL-positive cardiomyocytes in the OFT (single arrows). There are also uncharacterized TUNEL-positive cells in the OFT and AV cushions (double arrows), which will be sculpted to form the valves of the heart. Approximate embryonic day (ED) is shown. VENT indicates ventricle, AO, aorta; LA, left atria; and RA, right atria. Other abbreviations are defined in the text.

In contrast to the growth of the atrial and ventricular compartments, the embryonic outflow tract (OFT) shortens at specific stages of rodent,[19] human,[20] and avian[21–23] development. Early studies identified dying cardiomyocytes by TEM in the conal (myocardial) portion of the chick OFT.[24–26] Subsequent recombinant adenoviral cardiomyocyte tagging delimited the shortening of the chick OFT to ED6 to ED8 of development[23] and demonstrated a coincidence of OFT shortening with OFT cardiomyocyte PCD as evidenced by

TUNEL, annexin V staining, and caspase activity (see Figure 1). The prevalence of apoptosis was stage-dependent and reached a peak of nearly 50% of cardiomyocytes. The authors suggested that the role of the OFT cardiomyocyte PCD is to shorten and rotate the myocardial conus, to form the subpulmonic infundibular connection of the RV to the pulmonary artery (PA) anteriorly, during the transition of the embryonic heart from a single to dual circulation. It is not known whether a similar mechanism is operative in the remodeling of the mammalian OFT, where other cellular mechanisms have been proposed.[19]

During cardiac valve formation, cardiac cushions form as localized expansions of an extracellular matrix, known as the cardiac jelly, at the sites of atrioventricular and ventriculoarterial connections. Endothelial cells invade the cushions and transform into a mesenchymal cell type,[27,28] and the cushions are sculpted to form the fine inflow (mitral, tricuspid) and outflow (aortic and pulmonary) valves and portions of the atrial and ventricular septa. It would appear that this occurs in part by apoptosis. Significant levels of apoptosis have been observed in the mesenchyme of the bulbar and the atrioventricular cushions of birds and mammals and may contribute to the morphogenesis of these structures.[15,17,23]

Cells originating in the neural crest migrate widely throughout the cardiovascular system and are critical to the formation of a number of cardiac structures, including the aorticopulmonary septum and the media of the great arteries.[29] Retroviral labeling of the chick neural crest with lacZ indicated that neural crest cells, in addition to incorporating into these structures, also undergo apoptosis. LacZ-labeled, TUNEL-positive neural crest cells were identified in the developing OFT septum, predominantly beneath the level of the forming semilunar valves and in the endocardial cushions of later-stage (ED5 to ED12) chick embryos.[30] LacZ-positive, TUNEL-positive cells were also observed at the sites of the prospective electrical conduction system of later-stage[30–38] chicken embryos. These regions include the sites of formation of the atrioventricular (AV) node and the bundle of His and right and left bundle branches, ie, the superior aspect of the ventricular septum.[31] Other investigators have also observed a high incidence of cell death at this site[15] (also Watanabe M, unpublished observations, 2000) without identifying the cell type.

Why neural crest cells would migrate these long distances only to die is not clear. Apoptosis of these cells at the site of the mesenchymal septum may facilitate its replacement by myocardium in a process termed "myocardialization."[32] In the region of the prospective conduction system, it has been proposed that cell death may serve as a signal to myocardial cells to differentiate into specialized conduction fibers.[31] Retroviral labeling has demonstrated that Purkinje fibers originate from cardiomyocytes in response to endothelin-1 signaling from adjacent coronary arteries.[33–35] It has also been suggested that apoptosis is involved in the normal postnatal parsing of the human AV node and His bundle.[36]

Apoptosis has also been observed in the developing atrial septum, the blood islands of the forming epicardium, and at the site of formation of the coronary artery orifices.[37] The cell types and morphogenetic roles in these areas are yet to be characterized. Given the rapid clearance of apoptotic cells, it is likely that the incidence of apoptosis is underestimated. More sophisticated techniques for identifying apoptosis, combined with cell fate studies, will likely lead to a greater appreciation of the role of apoptosis in the development of cardiovascular structures.

Role of Apoptosis in Cardiac Malformations

The pathogenesis of most congenital heart defects (CHDs) is unknown; however, CHDs may represent developmental arrest of regional aspects of cardiogenesis, some of which may be due to insufficient numbers of cellular precursors. Studies conducted before the identification of the PCD pathway indicated that teratogens such as cyclophosphamide and glucocorticoids, as well as hemodynamic abnormalities, may cause alterations in the timing or levels of cell death in the embryonic chick OFT cushions.[15] Exposure to such agents was often associated with ventricular septal defects and malalignment of the great vessels. Because these agents may affect many cellular processes, it remains to be determined whether the morphological defects were due to an effect on apoptosis.

Apoptosis has also been suggested to play a role in pathologies of the cardiac conduction system and the RV. Histological examinations of autopsy hearts of two young brothers from a family of five brothers, all of whom had isolated idiopathic AV block and arrhythmias, revealed absent or significantly reduced AV nodes, sinoatrial (SA) nodes, and internodal conduction pathways.[38] TUNEL-positive cells were evident in myocytes and nonmyocytes at the sites of the AV and SA nodes from these specimens, as well as in the heart of a young woman from an unrelated family who had a similar clinical presentation and histological findings.

A primary disorder of the RV, arrhythmogenic right ventricular cardiomyopathy (ARVC), is characterized by the progressive replacement of the RV myocardium with fibrofatty tissue in the young[39] and is commonly associated with heart block.[40,41] Examination of the hearts of patients at autopsy or by endomyocardial biopsy demonstrated abnormal numbers of TUNEL-positive myocytes selectively in the RV of affected patients.[38,42,43] In a series of 20 patients with ARVC who were biopsied, the presence of TUNEL-positive cells in the RV biopsy material was more common in those with an acute presentation (5 of 6 patients) than those with a more insidious onset (2 of 12 patients).[43]

Demonstration of TUNEL-positive cells, if indicative of apoptosis, does not mean that direct activation of the PCD pathway is the primary cause of these diseases. As discussed below, there may be considerable interplay between the molecular pathways of cell differentiation and cell death so that the latter in some instances could reflect a failure of the former, rather than a primary activation of the PCD pathway. In this regard, several families have been identified with an autosomal-dominant form of congenital heart block, frequently associated with atrial septal defects and occasionally with other congenital heart defects.[44,44] The patients have mutations in the gene encoding the transcription factor NKX2.5, a homeodomain protein that regulates the differen-

tiation of cardiomyocytes in mice and flies.[45–47] The molecular mechanisms by which NKX2.5 mutations cause disease is not known, but there is no evidence that NKX2.5 directly regulates the PCD pathway.

The identification of the molecular components of the PCD pathway (discussed below) facilitates a targeted analysis of the role of this pathway in cardiac malformations. Experiments have recently been described in which chick OFT cardiomyocyte PCD is specifically affected via recombinant adenoviral-mediated expression of activators or inhibitors of the pathway. The results suggest that changes in the levels of OFT cardiomyocyte apoptosis may lead to malalignment of the great vessels, ie, cardiac OFT defects, with associated ventricular septal defects (S.A.F., unpublished data, 2000). In mice with deletions of genes in the PCD pathway, FADD (Fas-associated death domain protein, also known as Mort-1)[48] and caspase-8[49] die before ED11.5 and display a dilated cardiac phenotype that results in hemodynamic insufficiency. Whether this is due to an effect on the incidence of apoptosis, where in the cardiovascular system this effect occurs, and whether the dilated cardiac phenotype is primary or secondary to hemodynamic or other alterations remains to be determined.

Molecular Mechanisms

The first molecular evidence of cell death/survival machinery came in the form of the *Bcl2* gene. Human Bcl2 could prevent PCD in the worm, *Caenorhabditis elegans*, indicating a high degree of conservation in apoptotic pathways throughout evolution. More recent studies have revealed a remarkable conservation of most members of the apoptotic pathway (reviewed by Vaux and Korsmeyer[50]). Briefly, numerous death signals and death receptors, including TNF, its receptor, and many growth factors, culminate on a pathway regulated by proapoptotic (eg, Bax, Bad) and antiapoptotic members of the Bcl2 family (eg, Bcl2, BclX).[51] Relative balances of the two classes of Bcl2 proteins affect interaction of adaptor proteins, such as APAF-1 and FADD, with caspases,[52] which exist as inactive zymogens but become activated on interaction with proteins such as APAF-1. Caspase activation often leads to a feedback loop resulting in amplification of cell death signals.[53] A class of IAPs is thought to function in part by inhibiting caspases.[54] Caspase-mediated cleavage of numerous essential proteins ultimately results in cellular demise. Regulation of cell death is also mediated through overlapping pathways involving the tumor suppressor p53.[55]

Within the heart, little is known regarding the molecular basis for PCD. As with most organs, appropriate apoptosis is necessary for tissue remodeling, particularly within the inflow and outflow tracts of the heart, as described above. Surprisingly, most components of the apoptotic pathways described above are expressed in the developing heart, although their expression has not been examined in detail and their role remains unclear. Which factors play important roles in development is unknown, but it is likely that numerous divergent apoptotic pathways converge on a final execution pathway.

Numerous components of the apoptotic pathway have been mutated in mice but, as indicated above, only null mutation of caspase-8 or FADD affects cardiac development.[48,49] The trabeculae in both mouse models were disorganized and hypoplastic. Oddly, the phenotype was opposite what one might expect after disruption of proapoptotic pathways, with fewer rather than more cells present. These findings may suggest that the function of proteins involved in apoptosis depends in part on the cellular environment and interactions with other death and survival pathways.

It is possible that the spatiotemporal specificity of cell death during cardiac development is achieved through cell-specific regulatory pathways. Disruption of the retinoic acid pathway by gene targeting of RXRα and RARβ in combination results in increased apoptosis of the OFT mesenchyme and subsequent conotruncal defects.[56,57] Similarly, deletion of the signaling peptide, endothelin-1, or its receptor, ETA, causes a variety of OFT and aortic arch defects.[58,59] The neural crest–derived pharyngeal arches display higher than normal levels of apoptosis in endothelin-1/ETA mutants, suggesting that the endothelin pathway in part regulates survival of cardiac neural crest cells. *dHAND*, a tissue-specific basic helix-loop-helix transcription factor, is downstream of the endothelin-1 signaling cascade and is necessary for survival of neural crest–derived mesenchyme, possibly through regulation of the homeobox gene *Msx1*.[60] Mice lacking *dHAND* also display hypoplasia of the right ventricular segment.[61] Our recent studies indicate that *dHAND* is necessary for survival of cells after they have been specified to the right ventricular lineage. The proapoptotic Bcl2 binding factor Nip3 was found to be upregulated in the *dHAND* mutant heart in a screen for mediators of *dHAND* function (A. Aiyer and D. Srivastava, unpublished observations, 2000). These examples of tissue-specific signaling and transcriptional pathways that regulate cell survival suggest that general and specific pathways converge to regulate decisions of cell death and cell survival.

Vascular Cell Apoptosis During Development

The vascular system, like the heart, continuously remodels throughout development, first as primitive vessels form and reorganize, then as the circulation accommodates the demands of organ development and adaptation to ex utero life after parturition. Although tissue growth is the most obvious feature of vascular development, many embryonic and later vessels regress or are totally deleted. Examples of the latter include the paired first, second, and fifth aortic arches and one of the fourth arches (right in mammals, left in birds), as well as vessels in the microvasculature, especially at sites of bone formation.[62,63] These processes may be dominated by cell death, although cell migration or transdifferentiation to mesenchyme,[62] matrix degradation, and internal division of vessels to form multiple smaller channels (intussusception)[64,65] may also occur. In addition to vessel deletion, the vasculature undergoes continuous reorganization during development that occasionally is very dramatic. For example, the aortic origin of the embryonic vessel destined to become the left subclavian artery migrates from the dorsal aorta to the left aortic arch and bypasses other aortic branches, including the ductus arteriosus, before achieving its final position proximal to the latter vessel.[64] The mechanisms underlying such reor-

ganization of vessels have received little study but likely involve highly coordinated regulation of cell migration, cell proliferation, and/or cell death. Apoptotic cell death even contributes to developmental vascular remodeling that is dominated by tissue growth.[66,67] Apoptosis in these vessels probably reflects the demands for independent adjustment of vessel diameter, wall thickness, and length in accord with the demands of hemodynamics and growth of contiguous tissues. Even in quiescent mature vessels, a low rate of cell death prevails.[68]

A role for cell death in the development of the vasculature was first recognized decades ago. In 1918, Clark[69] had argued that all endothelial cells of regressing embryonic vessels retracted into neighboring vessels; however, he later described degeneration and "disappearance" of smooth muscle in these vessels.[70] Early descriptions of ultrastructurally identifiable apoptosis of endothelial cells were based on TEM showing vascular changes in the regressing corpus luteum[71]; however, there has subsequently been surprisingly few studies of the role of cell death in vascular development.

Hemodynamics and Apoptosis

The mechanical forces imposed on arterial tissue are important stimuli for developmental vascular remodeling.[72,73] Chronic changes in blood flow rates cause corresponding changes in arterial diameters, whereas alterations in blood pressure affect wall thickness. By these means, vascular structures continually adapt to changes in hemodynamic loads. Fluid shear stress in the case of flow and circumferential tensile stress in the case of pressure elicit this remodeling. Modulation of these hemodynamic loads in developing arteries affects extracellular matrix accumulation and remodeling, and it influences accumulation of vascular cells in the vessel wall.[72] Recent work indicates that sensitivity of apoptosis of vascular cells to mechanical forces is important in vascular growth regulation.

Cho et al[66] focused on the immediate perinatal period because of the profound arterial remodeling that accompanies physiological adjustments to parturition. Examination of vascular cell kinetics in this period, using a Monte Carlo analysis, demonstrated that arterial cell proliferation rates in the lower aorta much overestimated cell accumulation in this vessel. Additional experiments demonstrated that this disparity was due to high rates of apoptosis postpartum. Apoptosis in this vessel contributes to profound narrowing and tissue growth arrest in the lower aorta after birth, in concert with a 95% reduction in blood flow rate that is largely due to closure of the downstream umbilical arteries. Subsequent studies confirmed that experimental changes in arterial blood flow rates could both initiate apoptosis and suppress cell proliferation rates in developing arteries.[67] Indeed, application of techniques that permitted assessments of daily cell death rates indicated that apoptosis rates exceeded cell proliferation rates in the first days after 70% flow reduction in young rabbit carotid arteries.

An elegant series of experiments by Dimmeler et al[74,75] demonstrated that both production of nitric oxide (NO) and activation of the phosphatidyl inositol (PI) 3′-kinase pathway by shear stress are antiapoptotic for endothelium (Figure 2).

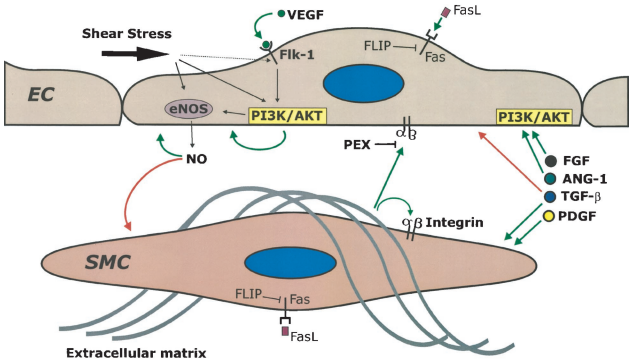

Figure 2. Regulation of apoptosis in the developing vascular system. Red arrows indicate proapoptotic, green arrows prosurvival, pathways. Hemodynamic shear stress inhibits apoptosis through activation of PI 3′-kinase, phosphorylation of eNOS by AKT, upregulation of eNOS gene expression, and possibly by activation of the VEGF receptor Flk-1. NO suppresses apoptosis in ECs but may induce death of smooth muscle cells, probably via peroxynitrite production (see text). Shear stress also upregulates endothelial expression of PDGF and TGF-β, which are high in developing arteries. These regulators of cell proliferation and matrix elaboration also control vascular cell apoptosis rates. IGF-1 (not shown) is also a potent survival factor for SMCs. ANG-1 is developmentally expressed and inhibits endothelial cell apoptosis, at least in part, by activating the PI 3′-kinase pathway. Extracellular matrix, or matrix degradation products, also can provide survival signals to vascular cells via integrin activation that can be suppressed by a noncatalytic breakdown product of MMP-2 and PEX. EC indicates endothelial cell; SMC, smooth muscle cell. Other abbreviations are defined in the text.

A link between these two signals was provided by demonstrations that one of the downstream targets of PI 3-kinase–dependent kinase signaling, AKT, phosphorylates and activates endothelial nitric oxide synthase (eNOS),[76] although chronic shear stress also upregulates expression of eNOS.[77–80] We recently found that in vivo inhibition of the PI 3-kinase pathway suppresses flow sensitivity of apoptosis in developing arteries (Yazer M, Cho A, and Langille BL, unpublished data, 1999). Subsequent in vivo[81,82] and in vitro[83] studies have shown that reductions in blood pressure/wall tension also upregulate arterial cell apoptosis; however, the developmental implications of these observations have not been elucidated. The dramatic decline in pulmonary arterial pressures at parturition, in concert with much increased pulmonary blood flows, could provide an intriguing model for the study of hemodynamic influences on vascular cell death.

Not surprisingly, extensive postnatal apoptosis occurs in vessels that regress after birth, ie, the umbilical arteries[66,84] and ductus arteriosus.[84] The extent to which death of these highly specialized cells is linked to hemodynamics is unknown. Coincident upregulation of proapoptotic members of the Bcl2 family (Bax and the short form of BclX) was observed in umbilical arteries.[84] Importantly, Kim et al[84] also observed apoptosis near large arterial branch sites postpartum, a finding consistent with a role for cell death in remodeling of arterial bifurcations during perinatal development.

Studies of the influence of hemodynamics on vascular cell apoptosis have focused on large arteries in later development. Their roles in early embryology and in the developing

microcirculation have received less study, despite observations that hemodynamic perturbations dramatically affect remodeling of these vessels.[85]

Apoptosis in the Developing Microvasculature

Meeson et al[86,87] have examined the transient vasculature of the pupillary membrane of the eye to study apoptosis during developmental vascular regression. They propose a model of macrophage-dependent initiating apoptosis that induces flow stasis, followed by further, stasis-dependent secondary apoptosis. Vascular endothelial growth factor (VEGF) inhibited apoptosis in this system via its Flk-1 receptor, a finding consistent with previous inferences that VEGF is a survival factor for endothelium.[88,89] VEGF, like shear stress, promotes endothelial cell survival activation of the PI 3-kinase pathway.[88] Interestingly, Chen et al[90] report that shear stress can activate Flk-1 signaling, so there may be overlap between these survival pathways. VEGF also upregulates expression of the caspase inhibitor survivin.[91] Drake et al and Brooks et al[92,93] have found that matrix interactions with endothelial $\alpha_v\beta_3$ integrin, which is upregulated during angiogenesis, promote cell survival. This finding is consistent with other reports of matrix-mediated survival of endothelium[94,95] as well as smooth muscle.[83,96] There also appears to be a role for matrix degradation in endothelial cell survival. Cryptic RGD sequences in native collagen are made accessible to $\alpha_v\beta_3$ integrin after cleavage by matrix metalloproteinase (MMP).[97] Thus, the matrix degradation that facilitates endothelial cell migration during angiogenesis may promote survival of these vessels. Under other circumstances, it is possible that extensive matrix degradation, which often accompanies apoptosis, may ultimately deprive vascular cells of matrix-related survival signals and thereby promote transition to an apoptotic pathway. Also, there is now evidence that a noncatalytic fragment of MMP-2 (hemopexin-like domain, PEX) can block matrix-integrin interactions during angiogenesis[98] and thereby inhibit cell survival signals. Given that matrix remodeling and cell death often are coordinately regulated during development, it is likely that further matrix-receptor interactions will prove important in regulation of apoptosis in the developing vasculature.

Regulation of Vascular Cell Apoptosis During Development

Control of apoptosis in the vasculature has been extensively reviewed[99–102]; therefore, we focus on those aspects that are potentially most important in vascular development.

Interestingly, much of the progress that has been made in understanding apoptosis in vascular (and other) tissues has focused on its inhibition through production of survival factors, including both soluble factors and extracellular matrix, probably because many cells appear poised for apoptosis that must be suppressed for their survival. For example, both endothelial cells and vascular smooth muscle normally express the proapoptotic receptor Fas, and endothelial cells express the Fas ligand (FasL),[103] but autocrine induction of apoptosis appears to be suppressed by the inhibitor of downstream signaling, FLICE-inhibitory protein (FLIP).[104] FLIP is highly regulated in smooth muscle in response to vascular injury,[105] which elicits partial reversion of these cells to a developmental phenotype,[106] so a role in normal vascular development is an attractive, but unproven, hypothesis. Fas-mediated vascular smooth muscle cell death appears particularly interesting given that different subpopulations of these cells display different susceptibilities.[107]

Survival of vascular and other cells during development appears to be controlled by mitogens that are also important in regulating proliferation (Figure 1). Such a role was cited above for VEGF and this inference is consistent with the underdevelopment of the aorta and reduced vascular density reported for mice that are heterozygous for null mutation of VEGF.[108] VEGF homozygotes display extreme defects in vasculogenesis that probably have multiple origins. It is noteworthy that modest VEGF overexpression in embryos results in selective enlargement of epicardial coronary vessels,[109] where significant apoptosis normally occurs,[37] so a role for control of local cell death may be particularly important to the growth of this vascular system. The sensitivity of vascular development to both heterozygous mutation and modest overexpression of VEGF underscore the importance of tight control of VEGF expression to normal vascular development.

Fibroblast growth factors (FGFs) also promote survival of endothelium and smooth muscle,[110,111] whereas the suppressor of endothelial cell growth, transforming growth factor-β (TGF-β), promotes endothelial apoptosis while providing a survival stimulus for smooth muscle.[112] Similarly, platelet-derived growth factor (PDGF) and insulin-like growth factor-1 are potent survival factors for smooth muscle.[113] The angiopoietins appear to play a primary role in assembly of the blood vessel wall and in regulating angiogenesis,[114,115] but angiopoietin-1 (ANG-1) also promotes endothelial cell survival,[116] apparently through activation of the PI 3′-kinase pathway.[117] This finding is consistent with observations that mice with null mutation of the receptor for ANG-1, Tek (Tie-2), display subnormal populations of endothelium.[118] It is unclear whether apoptosis is also related to the abnormal vascular branching patterns seen in these mice,[119] which die at mid-gestation.

NO is a bifunctional regulator of apoptosis (for review, see Kim et al[120]) that very often induces cell death, including death of smooth muscle cells,[121] most often through formation of peroxynitrite that may induce DNA damage and increase p53 activity. However, NO inhibits endothelial apoptosis[74,120] through inhibition of caspases, particularly caspase-3, and this mechanism likely participates in shear stress–related endothelial survival that was described above. Given the highly modulated expression of nitric oxide synthase isoforms during development of blood vessels,[122] NO production is potentially an important regulator of developmental vascular apoptosis.

Cell-matrix interactions are potent modulators of vascular cell proliferation,[123] migration,[124,125] and survival.[126] The $\alpha_v\beta_3$ integrins were cited above as being particularly important in vascular cell survival during development, although other integrins are also important. The promiscuous $\alpha_v\beta_3$ integrins interact with multiple matrix constituents, but tenascin-C is of proven importance in developmental control of vascular

smooth muscle cell apoptosis as well as epidermal growth factor–mediated proliferation.[127] Interaction of vitronectin with $\alpha_v\beta_3$ and/or $\alpha_v\beta_3$ integrins regulates endothelial cell survival,[95] and survival is also promoted by interaction with antibodies that recognize β_1 integrins.[126]

Conclusions

During morphogenesis and subsequent development, cells of the cardiovascular system differentiate, proliferate, migrate over large distances, and they elaborate, degrade, and remodel extracellular matrix. It is now clear that tightly controlled apoptosis is an important addition to this repertoire of remodeling modalities. Apoptosis appears to be particularly important in reshaping of cardiac and vascular structures in early morphogenesis, and new links between aberrant control of apoptosis and congenital defects point to some exciting avenues for future work. During later development, apoptosis contributes to regulation of growth of established cardiovascular tissues in accord with changing hemodynamic demands imposed on them and with changing demands of the tissues they perfuse. Our current knowledge concerning regulation of apoptosis in the developing cardiovascular system is primitive. Intriguing recent findings indicate important roles in the heart for the retinoic acid pathway and for *dHAND*-mediated transcriptional control under the regulation of endothelin. In the vasculature, attention has focused on hemodynamic loads, mitogenic survival factors, and extracellular matrix as regulators of apoptosis, and some aspects of downstream signaling have been elucidated. Much more work is needed, especially to determine whether specific cells of the cardiovascular system have a genetically determined susceptibility to apoptosis that is regulated by the expression of pro- and antiapoptotic factors. It is likely that continuous variations in cardiovascular cell phenotype during development will be associated with different modes of control of cell death and with variable susceptibility to apoptosis during normal and pathological development of the cardiovascular structures.

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References

1. Glucksmann A. Cell deaths in normal vertebrate ontogeny. *Biol Rev*. 1951;26:59–86.
2. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer*. 1972;26:239–257.
3. Kerr JFR, Harmon BV. Definition and incidence of apoptosis: an historical perspective. In: Tomei LD, Cope FO, eds: *Apoptosis: The Molecular Basis of Cell Death*. Plainview, NY: Cold Spring Harbor Laboratory Press; 1991:5–29.
4. Ellis RE, Yuan JY, Horvitz HR. Mechanisms and functions of cell death. *Annu Rev Cell Biol*. 1991;7:663–698.
5. Cohen GM. Caspases: the executioners of apoptosis. *Biochem J*. 1997; 326:1–16.
6. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. *Science*. 1998;281:1305–1308.
7. White E. Life, death, and the pursuit of apoptosis. *Genes Dev*. 1996; 10:1–15.
8. Gross A, McDonnell JM, Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. *Genes Dev*. 1999;13:1899–1911.
9. Deveraux QL, Reed JC. IAP family proteins–suppressors of apoptosis. *Genes Dev*. 1999;13:239–252.
10. McCarthy NJ, Evan GI. Methods for detecting and quantifying apoptosis. *Curr Top Dev Biol*. 1998;36:259–278.
11. Jacobson MD, Weil M, Raff MC. Programmed cell death in animal development. *Cell*. 1997;88:347–354.
12. Thompson RP, Lindroth JR, Wong Y-MM. Regional differences in DNA synthetic activity in the pre-septation myocardium of the chick. In: Clark EB, Takao A, eds. *Developmental Cardiology: Morphogenesis and Function*. Mt Kisco, NY: Futura Publishing Co; 1990:219–234.
13. Ikonomidou C, Bittigau P, Ishimaru MJ, Wozniak DF, Koch C, Genz K, Price MT, Stefovska V, Horster F, Tenkova T, Dikranian K, Olney JW. Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. *Science*. 2000;287:1056–1060.
14. Okamoto N, Akimoto N, Satow Y, Hidaka N, Miyabara S. Role of cell death in conal ridges of developing human heart. *Perspect Cardiovasc Res*. 1980;5:127–137.
15. Pexieder T. Cell death in the morphogenesis of the heart. *Adv Anat Embryol Cell Biol*. 1975;51:6–100.
16. Abdelwahid E, Pelliniemi LJ, Niinikoski H, Simell O, Tuominen J, Rahkonen O, Jokinen E. Apoptosis in the pattern formation of the ventricular wall during mouse heart organogenesis. *Anat Rec*. 1999;256:208–217.
17. Zhao Z, Rivkees SA. Programmed cell death in the developing heart: regulation by BMP4 and FGF2. *Dev Dyn*. 2000;217:388–400.
18. Kajstura J, Mansukhani M, Cheng W, Reiss K, Krajewski S, Reed JC, Quaini F, Sonnenblick EH, Anversa P. Programmed cell death and expression of the protooncogene bcl-2 in myocytes during postnatal maturation of the heart. *Exp Cell Res*. 1995;219:110–121.
19. Ya J, van den Hoff MJ, de Boer PA, Tesink-Taekema S, Franco D, Moorman AF, Lamers WH. Normal development of the outflow tract in the rat. *Circ Res*. 1998;82:464–472.
20. Goor DA, Dische R, Lillehei CW. The conotruncus, I: its normal inversion and conus absorption. *Circulation*. 1972;46:375–384.
21. De La Cruz MV, Sanchez GC, Arteaga MM, Arguello C. Experimental study of the development of the truncus and the conus in the chick embryo. *J Anat*. 1977;123:661–686.
22. Thompson RP, Abercrombie V, Wong M. Morphogenesis of the truncus arteriosus of the chick embryo heart: Movements of autoradiographic tattoos during septation. *Anat Rec*. 1987;218:434–440.
23. Watanabe M, Choudhry A, Berlan M, Singal A, Siwik E, Mohr S, Fisher SA. Developmental remodeling and shortening of the cardiac outflow tract involves myocyte programmed cell death. *Development*. 1998;125: 3809–3820.
24. Hurle JM, Ojeda JL. Cell death during the development of the truncus and conus of the chick embryo heart. *J Anat*. 1979;129:427–439.
25. Icardo J. Development of the outflow tract: a study in hearts with *situs solitus* and *situs inversus*. *Ann N Y Acad Sci*. 1990;588:26–40.
26. Manasek FJ. Myocardial cell death in the embryonic chick ventricle. *J Embryol Exp Morphol*. 1969;21:271–284.
27. Eisenberg LM, Markwald RR. Molecular regulation of atrioventricular valvuloseptal morphogenesis. *Circ Res*. 1995;77:1–6.
28. Huang JX, Potts JD, Vincent EB, Weeks DL, Runyan RB. Mechanisms of cell transformation in the embryonic heart. *Ann N Y Acad Sci*. 1995; 752:317–330.
29. Kirby ML, Waldo KL. Neural crest and cardiovascular patterning. *Circ Res*. 1995;77:211–215.
30. Poelmann RE, Mikawa T, Gittenberger-de Groot AC. Neural crest cells in outflow tract septation of the embryonic chicken heart: differentiation and apoptosis. *Dev Dyn*. 1998;212:373–384.
31. Poelmann RE, Gittenberger-de Groot AC. A subpopulation of apoptosis-prone cardiac neural crest cells targets to the venous pole: multiple functions in heart development? *Dev Biol*. 1999;207:271–286.
32. van den Hoff MJ, Moorman AF, Ruijter JM, Lamers WH, Bennington RR, Markwald RR, Wessels A. Myocardialization of the cardiac outflow tract. *Dev Biol*. 1999;212:477–490.
33. Gourdie RG, Mima T, Thompson RP, Mikawa T. Terminal diversification of the myocyte lineage generates Purkinje fibers of the cardiac conduction system. *Development*. 1995;121:1423–1431.
34. Cheng G, Litchenberg WH, Cole GJ, Mikawa T, Thompson RP, Gourdie RG. Development of the cardiac conduction system involves recruitment within a multipotent cardiomyogenic lineage. *Development*. 1999;126: 5041–5049.

35. Gourdie RG, Kubalak S, Mikawa T. Conducting the embryonic heart: orchestrating development of specialized cardiac tissues. *Trends Cardiovasc Med*. 1999;9:18–26.
36. James TN. Normal and abnormal consequences of apoptosis in the human heart: from postnatal morphogenesis to paroxysmal arrhythmias. *Circulation*. 1994;90:556–573.
37. Aikawa E, Kawano J. Formation of coronary arteries sprouting from the primitive aortic sinus wall of the chick embryo. *Experientia*. 1982;38: 816–818.
38. James TN, Nichols MM, Sapire DW, DiPatre PL, Lopez SM. Complete heart block and fatal right ventricular failure in an infant. *Circulation*. 1996;93:1588–1600.
39. Uhl HSM. A previously undescribed congenital malformation of the heart: almost total absence of the myocardium of the right ventricle. *Bull Johns Hopkins Hosp*. 1952;91:107–209.
40. Kullo IJ, Edwards WD, Seward JB. Right ventricular dysplasia: the Mayo Clinic experience. *Mayo Clin Proc*. 1995;70:541–548.
41. Higuchi S, Caglar NM, Shimada R, Yamada A, Takeshita A, Nakamura M. 16-year follow-up of arrhythmogenic right ventricular dysplasia. *Am Heart J*. 1984;108:1363–1365.
42. Mallat Z, Tedgui A, Fontaliran F, Frank R, Durigon M, Fontaine G. Evidence of apoptosis in arrhythmogenic right ventricular dysplasia. *N Engl J Med*. 1996;335:1190–1196.
43. Valente M, Calabrese F, Thiene G, Angelini A, Basso C, Nava A, Rossi L. In vivo evidence of apoptosis in arrhythmogenic right ventricular cardiomyopathy. *Am J Pathol*. 1998;152:479–484.
44. Schott JJ, Benson DW, Basson CT, Pease W, Silberbach GM, Moak JP, Maron BJ, Seidman CE, Seidman JG. Congenital heart disease caused by mutations in the transcription factor NKX2.5. *Science*. 1998;281: 108–111.
45. Lints TJ, Parsons LM, Hartley L, Lyons I, Harvey RP. Nkx-2.5: a novel murine homeobox gene expressed in early heart progenitor cells and their myogenic descendants. *Development*. 1993;119:969.
46. Bodmer R. The gene *tinman* is required for specification of the heart and visceral muscles in *Drosophila* [published erratum appears in *Development* 1994;119:969.]. *Development*. 1993;118:719–729.
47. Komuro I, Izumo S. Csx, a murine homeobox-containing gene specifically expressed in the developing heart. *Proc Natl Acad Sci U S A*. 1993;90:8145–8149.
48. Yeh WC, Pompa JL, McCurrach ME, Shu HB, Elia AJ, Shahinian A, Ng M, Wakeham A, Khoo W, Mitchell K, El Deiry WS, Lowe SW, Goeddel DV, Mak TW. FADD: essential for embryo development and signaling from some, but not all, inducers of apoptosis. *Science*. 1998;279: 1954–1958.
49. Varfolomeev EE, Schuchmann M, Luria V, Chiannilkulchai N, Beckmann JS, Mett IL, Rebrikov D, Brodianski VM, Kemper OC, Kollet O, Lapidot T, Soffer D, Sobe T, Avraham KB, Goncharov T, Holtmann H, Lonai P, Wallach D. Targeted disruption of the mouse Caspase 8 gene ablates cell death induction by the TNF receptors, Fas/Apo1, and DR3 and is lethal prenatally. *Immunity*. 1998;9:267–276.
50. Vaux DL, Korsmeyer SJ. Cell death in development. *Cell*. 1999;96: 245–254.
51. Sattler M, Liang H, Nettesheim D, Meadows RP, Harlan JE, Eberstadt M, Yoon HS, Shuker SB, Chang BS, Minn AJ, Thompson CB, Fesik SW. Structure of Bcl-xL-Bak peptide complex: recognition between regulators of apoptosis. *Science*. 1997;275:983–986.
52. Chou JJ, Matsuo H, Duan H, Wagner G. Solution structure of the RAIDD CARD and model for CARD/CARD interaction in caspase-2 and caspase-9 recruitment. *Cell*. 1998;94:171–180.
53. Nicholson DW, Thornberry NA. Caspases, killer proteases. *Trends Biochem Sci*. 1997;22:299–306.
54. Deveraux QL, Takahashi R, Salvesen GS, Reed JC. X-linked IAP is a direct inhibitor of cell-death proteases. *Nat Lond*. 1997;388:300–304.
55. Clarke AR, Purdie CA, Harrison DJ, Morris RG, Bird CC, Hooper ML, Wyllie AH. Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nat Lond*. 1993;362:849–852.
56. Kastner P, Grondona JM, Mark M, Gansmuller A, LeMeur M, Decimo D, Vonesch JL, Dolle P, Chambon P. Genetic analysis of RXR α developmental function: convergence of RXR and RAR signaling pathways in heart and eye morphogenesis. *Cell*. 1994;78:987–1003.
57. Gruber PJ, Kubalak SW, Pexieder T, Sucov HM, Evans RM, Chien KR. RXR α αeficiency confers genetic susceptibility for aortic sac, conotruncal, atrioventricular cushion, and ventricular muscle defects in mice. *J Clin Invest*. 1996;98:1332–1343.
58. Kurihara Y, Kurihara H, Oda H, Maemura K, Nagai R, Ishikawa T, Yazaki Y. Aortic arch malformations and ventricular septal defect in mice deficient in endothelin-1. *J Clin Invest*. 1995;96:293–300.
59. Clouthier DE, Hosoda K, Richardson JA, Williams SC, Yanagisawa H, Kuwaki T, Kumada M, Hammer RE, Yanagisawa M. Cranial and cardiac neural crest defects in endothelin-A receptor- deficient mice. *Development*. 1998;125:813–824.
60. Thomas T, Kurihara H, Yamagishi H, Kurihara Y, Yazaki Y, Olson EN, Srivastava D. A signaling cascade involving endothelin-1, dHAND and Msx1 regulates development of neural-crest-derived branchial arch mesenchyme. *Development*. 1998;125:3005–3014.
61. Srivastava D, Thomas T, Lin Q, Kirby ML, Brown D, Olson EN. Regulation of cardiac mesodermal and neural crest development by the bHLH transcription factor, dHAND [published erratum appears in *Nat Genet* 1997;16:410.]. *Nat Genet*. 1997;16:154–160.
62. Latker CH, Feinberg RN, Beebe DC. Localized vascular regression during limb morphogenesis in the chick embryo, II: morphological changes in the vasculature. *Anat Rec*. 1986;214:410–417.
63. Feinberg RN, Latker CH, Beebe DC. Localized vascular regression during limb morphogenesis in the chicken embryo, I: spatial and temporal changes in the vascular pattern. *Anat Rec*. 1986;214:405–409.
64. Heuser CH. The branchial vessels and their derivatives in the pig. *Contrib Embryol*. 1923;15:121–139.
65. Djonov V, Schmid M, Tschanz SA, Burri PH. Intussusceptive angiogenesis: its role in embryonic vascular network formation. *Circ Res*. 2000;86:286–292.
66. Cho A, Courtman DW, Langille BL. Apoptosis (programmed cell death) in arteries of the neonatal lamb. *Circ Res*. 1995;76:168–175.
67. Cho A, Mitchell L, Koopmans D, Langille BL. Effects of changes in blood flow rate on cell death and cell proliferation in carotid arteries of immature rabbits. *Circ Res*. 1997;81:328–337.
68. Hansson GK, Schwartz SM. Evidence for cell death in the vascular endothelium in vivo and in vitro. *Am J Pathol*. 1983;112:278–286.
69. Clark ER. Studies on the growth of blood-vessels in the tail of the frog larva: by observation and experiment on the living animal. *Am J Anat*. 1918;23:37–88.
70. Clark ER, Clark EL. Microscopic observations on the extra-endothelial cells of living mammalian blood vessels. *Am J Anat*. 1940;66:1–49.
71. Azmi TI, O'Shea JD. Mechanism of deletion of endothelial cells during regression of the corpus luteum. *Lab Invest*. 1984;51:206–217.
72. Langille BL. Blood flow-induced remodeling of the artery wall. In: Bevan JA, Kaley G, Rubanyi G, eds: *Flow-Dependent Regulation of Vascular Function*. New York, NY: Oxford; 1995:277–299.
73. Langille BL. Mechanical forces in vascular growth and development. In: Weir E, Archer SL, Reeves JT, eds. *The Fetal and Neonatal Pulmonary Circulations*. Armonk, NY: Futura; 2000:201–212.
74. Dimmeler S, Hermann C, Galle J, Zeiher AM. Upregulation of superoxide dismutase and nitric oxide synthase mediates the apoptosis-suppressive effects of shear stress on endothelial cells. *Arterioscler Thromb Vasc Biol*. 1999;19:656–664.
75. Dimmeler S, Assmus B, Hermann C, Haendeler J, Zeiher AM. Fluid shear stress stimulates phosphorylation of Akt in human endothelial cells: involvement in suppression of apoptosis. *Circ Res*. 1998;83:334–341.
76. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nat Lond*. 1999;399:601–605.
77. Topper JN, Cai JX, Falb D, Gimbrone MA Jr. Identification of vascular endothelial genes differentially responsive to fluid mechanical stimuli: cyclooxygenase-2, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively up-regulated by steady laminar shear stress. *Proc Natl Acad Sci U S A*. 1996;93:10417–10422.
78. Nadaud S, Philippe M, Arnal J-F, Michel J-B, Soubrier F. Sustained increase in aortic endothelial nitric oxide synthase expression in vivo in a model of chronic high blood flow. *Circ Res*. 1996;79:857–863.
79. Ranjan V, Xiao ZS, Diamond SL. Constitutive NOS expression in cultured endothelial cells is elevated by fluid shear stress. *Am J Physiol*. 1995;269:H550–H555.
80. Fulton D, Gratton JP, McCabe TJ, Fontana J, Fujio Y, Walsh K, Franke TF, Papapetropoulos A, Sessa WC. Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. *Nat Lond*. 1999;399: 597–601.
81. Bayer IM, Adamson SL, Langille BL. Atrophic remodeling of the artery-cuffed artery. *Arterioscler Thromb Vasc Biol*. 1999;19: 1499–1505.

82. DeBlois D, Tea B-S, Dam T-V, Tremblay J, Hamet P. Smooth muscle apoptosis during vascular regression in spontaneously hypertensive rats. *Hypertension*. 1997;29:340–349.
83. Cowan KN, Jones PL, Rabinovitch M. Regression of hypertrophied rat pulmonary arteries in organ culture is associated with suppression of proteolytic activity, inhibition of tenascin-C, and smooth muscle cell apoptosis. *Circ Res*. 1999;84:1223–1233.
84. Kim H-S, Hwang K-K, Seo J-W, Kim S-Y, Oh B-H, Lee M-M, Park Y-P. Apoptosis and regulation of Bax and Bcl-2 proteins during human neonatal vascular remodeling. *Arterioscler Thromb Vasc Biol*. 2000;20: 957–963.
85. Stephan F. Les suppleances obtenues experimentalement dans le systeme des arcs aortiques de l'embryon d'oiseau. *Comptes Rendu*. 1949;36:647–651.
86. Meeson AP, Argilla M, Ko K, Witte L, Lang RA. VEGF deprivation-induced apoptosis is a component of programmed capillary regression. *Development*. 1999;126:1407–1415.
87. Meeson A, Palmer M, Calfon M, Lang R. A relationship between apoptosis and flow during programmed capillary regression is revealed by vital analysis. *Development*. 1996;122:3929–3938.
88. Gerber HP, McMurtrey A, Kowalski J, Yan MH, Keyt BA, Dixit V, Ferrara N. 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;273:30336–30343.
89. 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;154:375–384.
90. Chen K-D, Li Y-S, Kim M, Li S, Yuan S, Chien S, Shyy JYJ. Mechanotransduction in response to shear stress: roles of receptor tyrosine kinases, integrins and Shc. *J Biol Chem*. 1999;274:18393–18400.
91. O'Connor DS, Schechner JS, Adida C, Mesri M, Rothermel AL, Li F, Nath AK, Pober JS, Altieri DC. Control of apoptosis during angiogenesis by survivin expression in endothelial cells. *Am J Pathol*. 2000; 156:393–398.
92. Drake CJ, Cheresh DA, Little CD. An antagonist of integrin $\alpha_v\beta_3$ prevents maturation of blood vessels during embryonic neovascularization. *J Cell Sci*. 1995;108:2655–2661.
93. Brooks PC, Montgomery AMP, Rosenfeld M, Reisfeld RA, Hu T, Klier G, Cheresh DA. Integrin $\alpha_v\beta_3$ antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell*. 1994;79:1157–1164.
94. Meredith JEJ, Fazeli B, Schwartz MA. The extracellular matrix as a cell survival factor. *Mol Biol Cell*. 1993;4:953–961.
95. Isik FF, Gibran NS, Jang YC, Sandell L, Schwartz SM. Vitronectin decreases microvascular endothelial cell apoptosis. *J Cell Physiol*. 1998; 175:149–155.
96. Cowan KN, Jones PL, Rabinovitch M. Elastase and matrix metalloproteinase inhibitors induce regression, and tenascin-C antisense prevents progression, of vascular disease. *J Clin Invest*. 2000;105:21–34.
97. Brooks PC, Strömblad S, Klemke R, Visscher D, Sarkar FH, Cheresh DA. Anti-integrin $\alpha_v\beta_3$ blocks human breast cancer growth and angiogenesis in human skin. *J Clin Invest*. 1995;96:1815–1822.
98. Brooks PC, Silletti S, Von Schalscha TL, Friedlander M, Cheresh DA. Disruption of angiogenesis by PEX, a noncatalytic metalloproteinase fragment with integrin binding activity. *Cell*. 1998;92:391–400.
99. Köhler R, Distler A, Hoyer J. Increased mechanosensitive currents in aortic endothelial cells from genetically hypertensive rats. *J Hypertens*. 1999;17:365–371.
100. Kockx MM, Herman AG. Apoptosis in atherosclerosis: beneficial or detrimental? *Cardiovas Res*. 2000;45:736–746.
101. McCarthy NJ, Bennett MR. The regulation of vascular smooth muscle cell apoptosis. *Cardiovas Res*. 2000;45:747–755.
102. Romeo F, Li DY, Shi M, Mehta JL. Carvedilol prevents epinephrine-induced apoptosis in human coronary artery endothelial cells: modulation of Fas/Fas ligand and caspase-3 pathway. *Cardiovas Res*. 2000;45:788–794.
103. Sata M, Suhara T, Walsh K. Vascular endothelial cells and smooth muscle cells differ in expression of Fas and Fas ligand and in sensitivity to Fas ligand-induced cell death: implications for vascular disease and therapy. *Arterioscler Thromb Vasc Biol*. 2000;20:309–316.
104. Sata M, Walsh K. Endothelial cell apoptosis induced by oxidized LDL is associated with the down-regulation of the cellular caspase inhibitor FLIP. *J Biol Chem*. 1998;273:33103–33106.
105. Imanishi T, McBride J, Ho Q, O'Brien KD, Schwartz SM, Han DKM. Expression of cellular FLICE-inhibitory protein in human coronary arteries and in a rat vascular injury model. *Am J Pathol*. 2000;156:125–137.
106. Schwartz SM, Heimark RL, Majesky MW. Developmental mechanisms underlying pathology of arteries. *Physiol Rev*. 1990;70:1177–1209.
107. Chan SW, Hegyi L, Scott S, Cary NR, Weissberg PL, Bennett MR. Sensitivity to Fas-mediated apoptosis is determined below receptor level in human vascular smooth muscle cells. *Circ Res*. 2000;86:1038–1046.
108. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C, Declercq C, Pawling J, Moons L, Collen D, Risau W, Nagy A. Abnormal vessel development and lethality in embryos lacking a single VEGF allele. *Nat Lond*. 2000;380:435–439.
109. Miquerol L, Langille BL, Nagy A. Embryonic development is disrupted by modest increases in vascular endothelial growth factor gene expression. *Development*. 2000;127:3941–3946.
110. Fox JC, Shanley JR. Antisense inhibition of basic fibroblast growth factor induces apoptosis in vascular smooth muscle cells. *J Biol Chem*. 1996;271:12578–12584.
111. Fuks Z, Persaud RS, Alfieri A, McLoughlin M, Ehleiter D, Schwartz JL, Seddon AP, Cordon-Cardo C, Haimovitz-Friedman A. Basic fibroblast growth factor protects endothelial cells against radiation-induced programmed cell death in vitro and in vivo. *Cancer Res*. 1994;54: 2582–2590.
112. Pollman MJ, Naumovski L, Gibbons GH. Vascular cell apoptosis. Cell type-specific modulation by transforming growth factor-β_1 in endothelial cells versus smooth muscle cells. *Circulation*. 1999;99:2019–2026.
113. Evan G, Harrington E, Fanidi A, Land H, Amati B, Bennett M. Integrated control of cell proliferation and cell death by the c-*myc* oncogene. *Phil Trans R Soc Lond B*. 1994;345:269–275.
114. Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, Sato TN, Yancopoulos GD. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell*. 1996;87:1171–1180.
115. Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand S, Radziejewski C, Compton D, McClain J, Aldrich TH, Papadopoulos N, Daly TJ, Davis S, Sato TN, Yancopoulos GD. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science*. 1997;277:55–60.
116. Kwak HJ, So JN, Lee SJ, Kim I, Koh GY. Angiopoietin-1 is an apoptosis survival factor for endothelial cells. *FEBS Lett*. 1999;448:249–253.
117. Kim I, Kim HG, So JN, Kim JH, Kwak HJ, Koh GY. Angiopoietin-1 regulates endothelial cell survival through the phosphatidylinositol 3′-kinase/Akt signal transduction pathway. *Circ Res*. 2000;86:24–29.
118. Dumont DJ, Gradwohl G, Fong G-H, Puri MC, Gertsenstein M, Auerbach A, Breitman ML. Dominant-negative and targeted null mutations in the endothelial receptor tyrosine kinase, *tek*, reveal a critical role in vasculogenesis of the embryo. *Genes Dev*. 1994;8: 1897–1909.
119. Sato TN, Tozawa Y, Deutsch U, Wolburg-Buchholz K, Fujiwara Y, Gendron-Maguire M, Gridley T, Wolburg H, Risau W, Qin Y. Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation. *Nat Lond*. 1995;376:70–74.
120. Kim YM, Bombeck CA, Billiar TR. Nitric oxide as a bifunctional regulator of apoptosis. *Circ Res*. 1999;84:253–256.
121. Fukuo K, Hata S, Suhara T, Nakahashi T, Shinto Y, Tsujimoto Y, Morimoto S, Ogihara T. Nitric oxide induces upregulation of Fas and apoptosis in vascular smooth muscle. *Hypertension*. 1996;27:823–826.
122. Mason CAE, Chang P, Fallery C, Rabinovitch M. Nitric oxide mediates LC-3-dependent regulation of fibronectin in ductus arteriosus intimal cushion formation. *FASEB J*. 1999;13:1423–1434.
123. Schwartz EA, Bizios R, Medow MS, Gerritsen ME. Exposure of human vascular endothelial cells to sustained hydrostatic pressure stimulates proliferation: involvement of the α_v integrins. *Circ Res*. 1999;84:315–322.
124. Hou G, Mulholland D, Gronska MA, Bendeck MP. Type VIII collagen stimulates smooth muscle cell migration and matrix metalloproteinase synthesis after arterial injury. *Am J Pathol*. 2000;156:467–476.
125. Bendeck MP, Regenass S, Tom WD, Giachelli CM, Schwartz SM, Hart C, Reidy MA. Differential expression of α_1 type VIII collagen in injured platelet-derived growth factor-BB-stimulated rat carotid arteries. *Circ Res*. 1996;79:524–531.
126. Meredith JE, Fazeli B, Schwartz MA. The extracellular matrix as a cell survival factor. *Mol Biol Cell*. 1993;4:953–961.
127. Jones PL, Crack J, Rabinovitch M. Regulation of tenascin-C, a vascular smooth muscle cell survival factor that interacts with the $\alpha_V\beta_3$ integrin to promote epidermal growth factor receptor phosphorylation and growth. *J Cell Biol*. 1997;139:279–293.