Vascular Cell Apoptosis in Remodeling, Restenosis, and Plaque Rupture

Kenneth Walsh, Roy C. Smith, Hyo-Soo Kim

Apoptotic death of vascular cells is a prominent feature of blood vessel remodeling that occurs during normal development and fibroproliferative disorders of the vessel wall. This review summarizes a large number of studies that have provided evidence for apoptotic cell death in the vasculature. We also describe reports that shed light on the molecular mechanisms that may control this process. Finally, we highlight the relatively small number of studies that suggest a function for vascular cell apoptosis in controlling the morphology and cellular composition of the blood vessel wall.

Vascular Cell Apoptosis During Development and Flow-Induced Vessel Remodeling

A number of studies have demonstrated apoptotic death of vascular cells in vessels that remodel postnatally. Regionalized apoptosis has been found in vascular smooth muscle cells (VSMCs) during the regression and closure of the human ductus arteriosus before birth and in VSMCs and endothelial cells of the arteries and veins of the umbilical cord, which are subject to dramatic hemodynamic changes at birth. Evidence of VSMC apoptosis in the human neonate has also been found at the branch points of the great arteries arising from the aortic arch when they are exposed to a disturbed blood flow, whereas VSMC apoptosis was not observed in the aorta when a normal flow pattern is maintained. Finally, VSMC apoptosis has been observed during the remodeling of the abdominal aorta in lambs, resulting from the large decrease in blood flow that occurs after the loss of the placenta at birth.

Vascular cell apoptosis during neonatal vascular remodeling appears to be triggered by decreased flow or by perturbation of flow at branch points that results when the organism switches oxygen exchange from the placenta to the lungs. Cell loss occurring upon flow-induced vessel remodeling has been directly demonstrated by inducing changes in flow through the carotid arteries of immature rabbits. In that experimental system, ligation of the left external carotid artery results in a marked reduction in blood flow through the common carotid artery, and this reduction correlates with a large increase in endothelial cell and VSMC apoptosis. Presumably, vascular cell apoptosis contributes to an adaptive process that allows the vessel to permanently constrict to manage the decrease in flow.

Changes in flow affect wall tension and cell matrix interactions, and it may be these factors that alter the survival characteristics of vascular cells. Consistent with this notion, VSMC apoptosis occurs when wall tension is diminished, or enhanced, or when the expression of matrix components or matrix metalloproteinases is altered. Although changes in the proapoptotic proteins Bax and Bcl-X<sub>S</sub> are associated with flow-induced vascular remodeling, relatively little is known about the molecular mechanisms that regulate vascular cell viability under these circumstances. Furthermore, although it makes intuitive sense that cellular elimination would be required for negative remodeling of a vessel, causal data in support of this hypothesis have not yet been provided.
Apoptosis in Chronic Vascular Lesions

Apoptosis also appears to be a feature of the remodeling processes occurring in chronic inflammatory fibroproliferative disorders of the vessel wall. Numerous studies have documented VSMC apoptosis in atherectomy specimens from atherosclerotic and restenotic lesions, and apoptosis has also been observed in macrophages and T cells within atherosclerotic lesions. Consistent with a remodeling function, vascular cell apoptosis has also been reported to be more pronounced in advanced atherosclerotic lesions compared with regions of early intimal thickening and/or fatty streaks. Atherectomy specimens from patients with in-stent restenosis also display abundant apoptotic cells, and a large fraction of VSMCs are positive for cell-cycle protein expression, suggesting high levels of VSMC turnover. Finally, apoptosis within chronic vascular lesions is also observed in animal models including the atherosclerotic plaques of cholesterol-fed rabbits, the advanced vascular lesions of APOE*3-Leiden transgenic mice, and the aortas of hyperlipidemic ApoE- and LDL receptor–deficient mice.

The diminished plaque cellularity of advanced lesions may be attributed to VSMC apoptosis, and it has been proposed that VSMC apoptosis eventually contributes to plaque rupture. This stems from the observation that VSMCs cultured from atherosclerotic coronary atherectomy specimens proliferate more slowly and demonstrate higher frequencies of apoptosis than VSMCs from normal vessels. This process may lead to plaque destabilization since apoptotic and necrotic cells have been detected in atherosclerotic plaques with a recent history of rupture, and VSMC apoptosis can be observed in the fibrous cap and underlying media of nonulcerated lesions obtained from human thoracic aorta and coronary arteries. However, causal data that could shed light on the relative importance of apoptotic cell death in plaque rupture have not been provided. Furthermore, the molecular mechanisms regulating cellular viability in chronic vascular lesions have not been defined in detail.

Vascular Cell Apoptosis Induced by Acute Balloon Injury

Apoptotic VSMC death has been documented in numerous animal models of acute vascular injury. Several studies demonstrate that balloon injury of vessels induces two waves of VSMC apoptosis. Generally speaking, the first wave is a rapid burst of apoptosis in the media occurring within hours of the injury, resulting in a marked decrease in vessel wall cellularity. Initial studies in the rat carotid artery model of vascular injury demonstrated that balloon denudation leads to a rapid and relatively synchronous induction of medial VSMC apoptosis. Apoptotic marker expression in this model peaked at ~1 hour after injury but is no longer evident by 4 hours after injury. The frequency of apoptotic cells correlates with the decrease in cellular density (up to 65%) that is observed within hours of injury. This rapid decrease in vessel wall cellularity occurs before the initiation of cell-cycle activity in VSMCs. Although the consequences of early-onset apoptosis in medial VSMCs are unknown, it could exacerbate neointima lesion formation at later time points by provoking a greater wound healing response to overcome the cellular deficit. Consistent with this notion, cells can release cytokines as they undergo apoptosis, and this could enhance the proliferative response after traumatic balloon injury.

The second wave of apoptosis occurs at much later times after injury (days to weeks) and at much lower frequencies. In the rat carotid model, apoptosis at these later time points is confined to the VSMCs of the developing neointima. This second wave of apoptosis may limit lesion growth. It has long been known that VSMC accumulation in the neointima of injured rat carotid arteries reaches a maximal level at 2 weeks after injury, yet cellular proliferation continues for up to 12 weeks. Presumably the rates of neointimal VSMC death and proliferation are in equilibrium from 2 weeks onward, thereby preventing any further increase in lesion size.

Rapid balloon angioplasty–induced apoptosis has also been documented in the rabbit iliac model. In this case, increased balloon-to-artery ratios produce greater frequencies of VSMC apoptosis at early time points, and this correlates with more acute cell loss. The rapid wave of apoptosis resulting from mechanical injury appears to involve a redox-sensitive pathway, because local administration of antioxidants will minimize cell loss. Surprisingly, analyses of diseased vessels have revealed that VSMCs of the neointima are less sensitive to rapid-onset apoptosis than are the VSMCs of the underlying media, suggesting that modulation of the VSMC phenotype influences angioplasty-induced apoptosis. Normocholesterolemic and hypercholesterolemic rabbits display similar profiles of early postinjury apoptosis, but hypercholesterolemia enhances apoptosis in the neointima at 2 weeks after injury. This observation has led to the hypothesis that macrophages present in the vascular lesions of the hypercholesterolemic rabbits may contribute to VSMC turnover at later time points. Macrophage involvement has also been implicated in VSMC apoptosis observed after stent implantation in rabbit vessels.

Finally, VSMC apoptosis has also been described in balloon-injured porcine coronary arteries. In this model, apoptosis is first observed at sites of obvious trauma at 1 hour after injury and, at later times, in the deeper layers of the media. Medial VSMC apoptosis peaks at 18 hours after injury, and lower levels of apoptosis are observed at 3 days and 7 days, but not at 14 days. The time course of apoptosis in cells of the adventitia and loose connective tissue was similar to that of medial cells.

Regulation of Vascular Cell Viability by Bcl-2 Family Proteins

Cell viability is governed at the molecular level by a balance between proapoptotic and antiapoptotic signals mediated by a number of gene families, the most prominent being the Bcl-2 family. Bcl-2 family members that promote cell survival include Bcl-2, A1, and the long form of Bcl-X (Bcl-XL), whereas Bax, Bad, and Bid function to promote apoptosis. Numerous studies have examined the role of Bcl-2 family proteins in controlling vascular cell viability. Bax expression is elevated in VSMCs of human atherosclerotic plaques, where high frequencies of apoptosis are observed. This is reminiscent of the situation in the neonate where vascular remodeling and vessel regression are associated with upregulation of Bax expression in VSMCs. The protective protein Bcl-XL is abundantly expressed in normal medial VSMCs but is downregulated after balloon injury with a time course that
correlates with the early wave of apoptotic cell death. Bcl-XL expression is also elevated in rabbit intimal VSMCs, which are more resistant to angioplasty-induced apoptosis than medial VSMCs.

The functional significance of Bcl-2 family proteins in VSMC survival has been demonstrated by acute ablation experiments. It has been shown that neointimal VSMC apoptosis can be induced in stenotic vessels by Bcl-XL ablation using an antisense strategy, leading to a reduction in intimal thickness. Because Bcl-XL is preferentially expressed in neointimal VSMCs, this factor may contribute to the differential sensitivity of medial and neointimal VSMCs to balloon injury–induced apoptosis. VSMC apoptosis can also be triggered by acute ablation of Bcl-2 using an adenovirus-encoded ribozyme directed against bcl-2 mRNA, which brings about a decrease in vessel wall cellularity and reduced intimal lesion formation after balloon injury. Collectively, these studies show that endogenous levels of Bcl-2 and Bcl-XL are essential for VSMC viability, and thus, stimuli that alter Bcl-XL or Bcl-2 expression could influence VSMC survival in the vessel wall.

In addition to the Bcl-2 family proteins, it has been suggested that the transcription factor p53 regulates vascular cell apoptosis because p53 is reported to accumulate in atherosclerotic lesions. This factor promotes apoptosis by functioning, at least in part, as a positive regulator of Bax expression and a negative regulator of Bcl-2 expression. Interestingly, p53 can also promote VSMC apoptosis by increasing cell surface expression of the death ligand receptor Fas. Forced overexpression of p53 induces VSMC apoptosis in vitro and inhibits neointima lesion formation in vivo. In addition to this proapoptotic function, p53 negatively regulates cell growth through its ability to induce the cyclin-dependent kinase inhibitor p21. This observation suggests that endogenous p53 in VSMCs inhibits cell proliferation in response to proapoptotic stimuli. Consistent with this interpretation, VSMCs are sensitized to Fas-mediated apoptosis by interferon γ, which favors receptor clustering by upregulating cell surface Fas expression.

In striking contrast to VSMCs, vascular endothelial cells are normally resistant to Fas-mediated apoptosis and remain resistant even when Fas expression is upregulated by exposure to interferon γ, suggesting that resistance is not due to low levels of receptor expression. The marked differences in the sensitivity of vascular cells to Fas-induced apoptosis may be mediated by the expression of cellular FLIPs (FLICE-like inhibitory proteins) that function as dominant-negative inhibitors of caspase-8 function. FLIP isoforms are abundantly expressed in endothelial cells where they may function to inhibit Fas-mediated cell suicide (or fratricide). Along these lines, it is reported that FLIP expression in rat carotid VSMCs is downregulated after balloon injury and human atherosclerotic plaque VSMCs express relatively low levels of FLIP, suggesting that FLIP may participate in the regulation of VSMC turnover in those lesions.

Many lines of evidence suggest that Fas-mediated cell death is important in the control of vessel wall inflammation. First, it has been shown that a deficiency in Fas-mediated apoptosis will lead to vasculitis, resulting from neutrophilic and mononuclear cell infiltrates, in some strains of gld and lpr mice. Second, FasL-deficient mice display enhanced mononuclear cell infiltration and intimal hyperplasia in a flow-restricted model of vascular injury that induces neointima formation in the presence of an intact endothelium. Third, it has been shown that constitutive overexpression of FasL on the vascular endothelium will inhibit tumor necrosis factor-α–mediated leukocyte extravasation.

Death Receptor/Ligand Interactions in Vascular Cells

Members of the tumor necrosis factor receptor family may also participate in the regulation of vascular cell survival. In the vasculature, most studies to date have focused on cell death mediated by Fas/Fasl signaling. The death receptor Fas is ubiquitously expressed, whereas Fasl is typically expressed on the cell surface of inflammatory cells including T cells and macrophages. Fas-mediated apoptosis has been implicated as functioning mainly to downregulate inflammatory reactions. The gld and lpr strains of mice are null for functional FasL and Fas expression, respectively, and suffer from rheumatic diseases characterized by aberrant inflammation. At that Fas-mediated apoptosis is required for the elimination of autoreactive and peripheral T cells is well established, and spontaneous monocye apoptosis is also controlled by Fas. Consistent with an anti-inflammatory role, Fasl is expressed at sites of “immune privilege,” such as eye and testis where it may inhibit inflammation by killing immune cells as they attempt to infiltrate the tissue. Fasl within the vasculature is expressed at low levels on the surface of endothelial cells where it may serve a similar function by inhibiting adventitious leukocyte extravasation. In marked contrast to the anti-inflammatory function of endogenous Fasl, ectopic expression of this protein in Fas-expressing cells can, in some cases, result in tissue destruction and induce inflammatory responses that are characterized by neutrophil infiltration.

It has been proposed that Fas-mediated apoptosis plays a role in a variety of vascular disorders including atherogenesis, allograft arteriopathy, and acute inflammatory responses. Because VSMCs express Fas and inflammatory cells express FasL, it is possible that Fas-mediated apoptosis contributes to atherosclerotic plaque instability. The susceptibility of VSMCs to Fas-mediated cell death in vitro and in vivo has been documented in numerous studies. Cultured VSMCs undergo apoptosis after infection with a replication-defective adenoviral vector that encodes cell surface Fasl. Consistent with its expression on the cell surface, coculture experiments reveal that Fasl-expressing VSMCs kill cells in a paracrine manner. Local delivery of adenovirus encoding Fasl to balloon-injured rat carotid arteries induces apoptosis in proliferating smooth muscle cells and potently inhibits intimal hyperplasia. Although VSMCs are susceptible to adenovirus-encoded Fasl expressed at the cell surface, they are not efficiently killed by soluble recombinant Fasl or agonist anti–Fas antibody. Presumably these soluble reagents are less efficient than cell surface Fasl at inducing Fas clustering on the membrane of the target cell. Consistent with this interpretation, VSMCs are sensitized to Fas-mediated apoptosis by interferon γ, which favors receptor clustering by upregulating cell surface Fas expression.

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The Fas/FasL system is essential for the inhibition of vessel inflammation. Fas-mediated cell death may also play a role in atherogenesis and plaque rupture, but causal data in support of these hypotheses are lacking.

In conclusion, the data suggesting that apoptosis plays a role in developmentally regulated or pathological vessel remodeling are largely correlative. In this regard, further definition of regulatory pathways and directed gene ablation studies would be helpful in defining the respective roles of these factors in controlling vessel architecture in development and disease.

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