Death Receptors, Intimal Disease, and Gene Therapy
Are Therapies That Modify Cell Fate Moving too Fast?

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The pathogenesis of vascular diseases such as atherosclerosis and postangioplasty restenosis is characterized by endothelial cell injury and an abnormal accumulation of vascular smooth muscle cells (VSMCs) within the intimal space. The classic paradigm has emphasized the role of VSMC migration, proliferation, and subsequent elaboration of extracellular matrix as the principal cellular events that mediate neointima formation. In accord with this model, gene therapy strategies directed at inhibiting cell proliferation and migration have been shown to be effective at inhibiting intimal disease in animal models and are currently under study as novel therapies for vascular disease in clinical trials.

It has become increasingly clear that the cellular economy within tissues reflects a balance between cell proliferation and cell death by apoptosis. Studies involving both animal models and human specimens have clearly established that VSMC apoptosis is a prominent feature of the response to injury and the consequent formation of the neointima.

Nevertheless, the studies describing the association between cell death and lesion formation fail to definitively establish the pathogenic role of vascular cell apoptosis in the natural history of intimal vascular disease. Therefore, it remains to be determined whether therapeutic strategies that modulate apoptosis within the vasculature will have efficacy in ameliorating the course of vascular disease. The study by Chan et al in this issue of Circulation Research provides important new insights into the complexities of the intrinsic compensatory mechanisms that VSMCs exhibit to promote cell viability under various conditions. These findings challenge us to develop a deeper, more intricate understanding of apoptosis regulation within the vasculature that is commensurate with our understanding of cell-cycle regulation.

Intimal Disease Progression: Site-Specific Implications of Cell Death

It is well established that loss of endothelial cells from the intimal surface predisposes to vascular lesion formation. Accordingly, it is postulated that the induction of endothelial cell loss by apoptosis may promote vascular lesion formation, whereas interventions that prevent endothelial cell death may inhibit lesion formation. It is noteworthy that factors associated with promoting vascular disease (eg, oxidized LDL cholesterol, angiotensin II, homocysteine, hyperglycemic conditions, and proinflammatory cytokines) induce endothelial cell apoptosis. Conversely, vasculoprotective factors that inhibit lesion formation and promote endothelium regeneration (eg, shear stress, nitric oxide, and vascular endothelial growth factor) inhibit endothelial cell death. The recent observation that acute coronary thrombosis is often associated with areas of endothelial cell loss without plaque rupture raises the intriguing possibility that endothelial cell apoptosis may also participate in the pathogenesis of acute ischemic syndromes. It is speculated that therapeutic interventions directed at preserving the integrity of the intimal lining by preventing endothelial cell apoptosis may have clinical efficacy in the treatment of vascular disease.

Although it is quite conceivable how endothelial cell apoptosis may promote vascular lesion formation, it is less clear whether VSMC death actually stimulates or inhibits intimal lesion formation. Several recent reviews highlight the complex nature of this question and raise the possibility that both outcomes are possible. We speculate that the pathogenic role of VSMC apoptosis is contextual and may vary at different stages in the natural history of the intimal disease. For example, the seminal work of Reidy et al documented that balloon injury induces cell death and the consequent release of fibroblast growth factor, thereby resulting in VSMC replication. More recent studies have shown that the initial pathogenic event after balloon injury involves the induction of acute medial VSMC apoptosis via redox-sensitive signaling pathways. Thus, the initial induction of VSMC apoptosis may promote lesion formation after injury. However, it is postulated that once VSMCs migrate into the intimal space, an ongoing process of apoptosis mitigates the progressive accumulation of intimal VSMCs induced by mitogens. Studies involving atherectomy specimens of human restenosis lesions that document hypercellularity and relatively low VSMC replication rates are consistent with this working hypothesis. Thus, cellular pathways that inhibit VSMC apoptosis may contribute to intimal lesion progression.

In accord with this working model, we have observed that intimal VSMCs exhibit a resistance to apoptosis induced by balloon injury in association with the upregulation of antiapoptotic genes such as Bcl-xL. Furthermore, we have demonstrated that the selective downregulation of Bcl-xL expression in intimal cells using antisense oligonucleotides induces VSMC apoptosis and promotes the regression of the intimal lesion. It is postulated that an additional feature of the phenotypically modified intimal VSMCs involves modulation of the cell-death program such that VSMC survival is enhanced as a mechanism.
for promoting intimal lesion stability and progression. Additional studies are needed to define the spectrum of changes in the cell-death program in medial versus intimal VSMCs. Future studies must definitively demonstrate the functional significance of apoptosis in various stages of intimal lesion formation and progression.

**Fas, Fas Ligand, and Cell-Death Signal Transduction**

The regulation of cell fate involves communication links between the extracellular milieu and the intrinsic cell-suicide program. Specialized systems have evolved to convey a death signal from one cell to another. Fas is a death receptor that belongs to the tumor necrosis factor (TNF) receptor family and is abundantly expressed in various tissues including the heart, endothelial cells, and VSMCs. Fas ligand (FasL) is a cytokine in the TNF family that binds to Fas with a high affinity. FasL is synthesized as a membrane protein and undergoes cleavage by a metalloproteinase to generate a soluble form that is less biologically active than the membrane-bound form. Both Fas and FasL gene expression seem to be upregulated by cytokines and stressful stimuli (eg, irradiation) via nuclear factor-κB–dependent mechanisms.26

The death-receptor signaling cascade is outlined in the Figure. FasL engagement promotes the trimerization of Fas and the formation of a signaling complex of molecules linked by protein-protein interactions with the cytoplasmic portion of the receptor. The adapter molecule Fas-associated protein with death domain (FADD) is recruited to Fas by the interaction between the death domains (DDs). Homotypic interactions of proteins that contain DDs, such as RIP, RAIDD, and TRADD, add to the complexity of the signal-transduction regulatory apparatus of Fas. In addition to the DD motif, there is a death effector domain (DED) at the N-terminus of FADD that is responsible for binding caspase 8, promoting its auto-activation, and triggering the cell-execution cascade.

The caspases are a family of cysteine proteases that exist in a zymogen form until activated by proteolytic cleavage. This sequential proteolytic process is reminiscent of the coagulation cascade and begins with proximal caspases (eg, caspase 8 and caspase 9) that are closely coupled to receptor-mediated signal-transduction apparatus. Cells are executed by the consequence of downstream caspase activation (eg, caspase 3). These cysteine proteases act at specific sites downstream of aspartate residues and promote the cleavage of a variety of cellular substrates that result in the biochemical and morphological hallmarks of apoptosis.

The FasL-induced cell-execution pathway seems to be reinforced by the capacity to promote parallel activation of the proapoptotic factor Bid by proteolytic cleavage. This activation of Bid stimulates cytochrome c release from the mitochondria, Apaf-1 activation, and caspase 9 cleavage, and thereby results in caspase 3 stimulation via a caspase 9–dependent pathway.

Several levels of inhibitory control mechanisms modulate the efficiency of the Fas-FasL–mediated cell-execution process. As noted earlier, the shedding of FasL from the cell surface seems to attenuate the signal. In addition, certain cell types exhibit a member of the TNF receptor family that functions as a decoy receptor for FasL and is shed into the extracellular space. This decoy receptor has been described in transformed cells that manifest an antiapoptotic phenotype.27 Similarly, there are proteins with DEDs that lack protease activity, such as c-FLIP, that effectively function as endogenous inhibitors. Moreover, there are inhibitors of apoptosis proteins (IAPs) that inhibit specific caspases such as caspase 3 or caspase 6. Furthermore, the mitochondria-dependent pathway of FasL-induced apoptosis seems to be sensitive to the expression of antiapoptotic genes, such as Bcl-xL, that are capable of antagonizing the release and function of cytochrome c.28 Similarly, antiapoptotic signals generated by integrins and growth factors via extracellular signal–regulated protein kinase or phosphatidylinositol 3′ kinase–Akt are also capable of modulating cell death by this mitochondrial caspase 9–dependent pathway.29–31 Thus, there are several mechanisms by which the cell fate response to FasL can be modulated by intrinsic cellular mechanisms at the receptor level as well as distal to receptor activation.
Fas and VSMC Apoptosis

Whether VSMCs undergo apoptosis in response to FasL activation has been a question of controversy. Some investigators using agonistic anti-Fas antibodies observed human VSMC apoptosis only in the context of priming with cytokine activation. A recent study of cultured human VSMCs by Walsh and colleagues reported that agonistic Fas antibodies and soluble FasL fail to induce VSMC death, but infection with an adenoviral expression vector that upregulates the expression of membrane-associated FasL promotes VSMC suicide. Similarly, we have observed that FasL can induce VSMC apoptosis in certain contexts (unpublished observations, 1998). However, the mechanistic basis for these inconsistent variances in the sensitivity of VSMCs to FasL-induced apoptosis was in need of additional investigation.

The study by Chan et al provides important new insights that may reconcile the conflicting findings. These investigators derived human medial VSMCs from healthy coronary arteries and defined 2 subpopulations of Fas-resistant and Fas-sensitive VSMCs. The FasL-resistant VSMCs exhibited normal levels of receptor expression as well as normal receptor engagement mechanisms. However, Fas-resistant VSMCs had deficiencies in the expression of the distal cell-execution apparatus, such as the adapter protein FADD as well as caspase 8 and caspase 3. Using an antisense transfection approach, the authors also demonstrated that the activation of caspase 8 and caspase 3 is essential for FasL-induced VSMC death. However, it is noteworthy that restoration of caspase 8 and caspase 3 in Fas-resistant cells was not sufficient to confer FasL sensitivity. This may reflect the fact that the Fas-resistant VSMCs also exhibited a coordinate up-regulation of antiapoptotic genes in addition to deficiencies in the cell-execution apparatus. Indeed, Chan et al demonstrate that the Fas-resistant cells have a higher level of expression of FLIP (the inhibitor of caspase 8) as well as c-IAP1 (the inhibitor of caspase 3). Finally, as a confirmation that this in vitro model reflects the in vivo context, these investigators documented a similar pattern of heterogeneity in medial VSMCs in human vascular specimens.

Overall, this study extends a growing body of evidence that indicates that the VSMC population within the vessel wall is heterogeneous and that this heterogeneity may be reflected in genetic programs involved in differentiation, cell growth, and cell death. It is also conceivable that the eventual constitution of VSMC phenotypes within the intima during lesion formation and progression may reflect a selection process among a heterogeneous set of different medial VSMCs. This emergence of certain VSMC phenotypes during this process of natural selection is influenced by gene-environment interactions. Intimal diseases such as atherosclerosis are characterized by a chronically activated, proinflammatory state involving genotoxic oxidative stress and cytotoxic cytokines. In this noxious milieu, only the strong will survive. Under these conditions, we speculate that only VSMCs that exhibit adaptive changes in the regulation of intrinsic cell fate determination programs will persist within the intima. Thus, it is postulated that the selection and accumulation of intimal VSMCs in this context involve a coordinate upregulation of antiapoptotic genes and a downregulation of proapoptotic mediators as an essential survival mechanism for maintaining intimal lesion stability and progression over the long-term course of vascular disease. The characterization of heterogeneous subsets of VSMCs with variances in sensitivity to apoptosis stimuli is consistent with this working hypothesis.

Implications for Apoptosis-Mediated Gene Therapy

These insights into the pathobiological mechanisms involved in intimal disease should enable investigators to develop novel therapies for vascular disease. This proof of principle has already been established for antiproliferative therapies involving viral expression vectors as well as antisense oligonucleotide technologies. Similarly, an apoptosis-mediated therapy for the regression of intimal disease has been established using an antisense strategy directed at downregulating the expression of Bcl-xL. This approach has been extended by recent studies of Walsh and colleagues using the rat balloon injury model with a gene therapy strategy involving the local overexpression of FasL. Despite the obvious limitations of current adenoviral vectors, this approach to inducing VSMC apoptosis has efficacy in reducing intimal lesion formation in this context. It is postulated that this approach may ameliorate the course of in-stent restenosis or accelerate the transition to an acellular fibrous cap in stable atherosclerosis.

Thus, apoptosis-modulatory therapies hold promise in the treatment of cardiovascular disease. However, the advancement of these approaches to the clinic requires ongoing diligence in exploring the vascular pathobiology in greater mechanistic depth. The study by Chan et al raises the possibility that intrinsic mechanisms of FasL resistance within certain VSMC subpopulations may render a FasL-based gene therapy strategy relatively impotent in the context of vessels with preexistent vascular disease reminiscent of the clinical context. These concerns are reinforced by the recent observations by Dick et al and colleagues, who used adenoviral expression vectors with FasL in a rabbit model of atheroma formation. In this study, local expression of FasL restricted to the endothelium actually exacerbated the process of intimal lesion formation in hyperlipidemic rabbits. Although the precise mechanism by which FasL seems to promote intimal disease remains to be characterized, it is conceivable that intimal VSMCs were relatively resistant to FasL-induced death and thereby rendered the intervention ineffective. Similarly, it is possible that FasL-induced endothelial cell death may promote lesion formation. Moreover, there is a growing body of evidence to suggest that, similar to TNF receptors, Fas is also capable of activating other cellular processes in addition to the cell-execution pathway. Therefore, it is conceivable that FasL stimulation in Fas-resistant VSMCs could actually stimulate proliferation, migration, or an activated state in these viable VSMCs and thereby potentiate atherogenesis. Thus, in addition to the usual caveats of using first- and second-generation adenoviral vectors, it remains unclear whether a FasL-based gene therapy strategy will be effective in a more complex context that more closely simulates the clinical situation.

As we move to more sophisticated, mechanistically based therapeutics for cardiovascular disease, it is clear that our understanding of the pathobiology of vascular disease must be equally sophisticated. The study by Chan et al provides insight
into the complexities of the intrinsic compensatory mechanisms that VSMCs exhibit to promote cell viability under various conditions. It is hoped that additional elucidation of the cell fate programs and their roles as determinants of vessel function and structure will foster the identification of even better targets for novel strategies to treat vascular disease.

References

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