Adventitial Fibroblasts
Backstage Journeymen

Francis J. Miller, Jr

Although it has been 20 years since the acceptance of the endothelial layer as more than a hemostatic barrier in the blood vessel, the adventitia continues to be primarily considered a supporting structure, and its role in vascular disease has been easily dismissed. However, there is increasing support for the adventitia as a mediator of vascular dysfunction and a potential therapeutic target. In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Shi et al report elevated superoxide levels in coronary adventitial fibroblasts within 1 day of balloon injury. The source of superoxide appears to be NAD(P)H oxidase.

The observation that adventitial fibroblasts generate reactive oxygen species (ROS) in response to vessel injury is not necessarily surprising. After injury, growth factors and cytokines are released from platelets and cell debris. NAD(P)H oxidase expression and superoxide production in fibroblasts increase within hours after exposure to angiotensin II. If vessel injury is severe and there is medial disruption, adventitial cells are activated, whereas when injury is mild, without rupture of the internal elastic membrane, adventitial activation is modest. These observations suggest that in response to endoluminal injury, locally released substances activate fibroblasts.

How can cells in the adventitia, which are relatively distant from the endothelium and subendothelial space, contribute to vascular dysfunction and neointimal formation? The findings of Shi et al suggest that increased adventitial superoxide levels after balloon injury may modulate fibroblast growth. Redox-mediated events in activated fibroblasts, which may include the release of a variety of paracrine substances and the stimulation of cell migration and proliferation, have the potential to markedly influence vascular function and structure.

Paracrine Effects
Superoxide levels rapidly increase in fibroblasts after vessel injury. Adventitia-derived superoxide can inactivate endothelium-derived NO and form the oxidant peroxynitrite. Perhaps more importantly, vascular cells, when activated, appear to secrete substances that can react with adjacent vascular cells, causing a “wave” of cell activation. Within hours after vascular injury, transforming growth factor-β is secreted in the adventitia and may stimulate cell proliferation.

After injury, the release of several paracrine substances by vascular cells may be modulated by increased cellular ROS. For example, in response to oxidative stress, smooth muscle cells secrete cyclophilin A, which causes extracellular signal–regulated kinase activation and cell growth. After 24 hours after balloon injury, cyclophilin A is also detected in the adventitia. Secretion of cyclophilin A is inhibited by catalase, 4,5-dihydroxy-1,3-benzene disulfonic acid (Tiron), and diphenylene iodonium, all of which also inhibit fibroblast growth in serum. Several other proteins, including heat shock protein 90-α, are secreted by smooth muscle cells in response to oxidative stress and have been referred to as secreted oxidative stress–induced factors. Secretion of oxidative stress–induced factors may be a general response of vascular cells to injury, resulting in the recruitment of adjacent cells in the repair response.

Migration of Fibroblasts
After balloon injury, translocation of bromodeoxyuridine–labeled cells suggests that proliferating adventitial cells migrate to the neointima. This interesting observation was confirmed by implanting LacZ–positive fibroblasts into the adventitia of carotid arteries and tracking their migration from the adventitia, through the medial layer, and into the neointima after endoluminal injury. The relative proportion of smooth muscle cells and fibroblasts participating in neointimal formation after vascular injury remains unclear.

The mechanism by which adventitial fibroblasts migrate to the neointima after injury is not well characterized. Fibroblasts may migrate to the neointima across a chemotactic gradient. Selective injury to the adventitia, however, without endothelial disruption, is also associated with the formation of a neointima. Matrix metalloproteinases are necessary for the migration of cells into the neointima after vascular balloon injury. Adventitial expression of matrix metalloproteinases is increased after vascular injury and may facilitate the migration of fibroblasts to the neointima.

Proliferation of Fibroblasts
Proliferating cells are evident in the adventitia on the day of vascular injury. The findings by Shi et al suggest that the proliferation of fibroblasts is dependent on ROS, especially H2O2. The observation that diphenylene iodonium inhibited fibroblast proliferation suggests a possible role for NAD(P)H oxidase in mediating cell growth. These observations are similar to the finding that growth of smooth muscle cells, in response to angiotensin II, is mediated by intracellular H2O2 derived from NAD(P)H oxidase.

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The role of ROS in vascular cell growth is only beginning to be understood. An increase in vascular cell ROS is not a general phenomenon resulting in unregulated activation of indiscriminate redox-mediated events. For example, although transfection of fibroblasts with either nox-1 or nox-4, which are different homologs of the NAD(P)H oxidase subunit gp91phox, increases NAD(P)H oxidase activity and superoxide generation, the consequences of overexpression of nox-1, compared with nox-4, are quite different. Fibroblasts proliferate after the overexpression of nox-1, but they undergo senescence with the overexpression of nox-4. It is not known whether compartmentalization of cellular ROS imparts specificity to this response.

Vascular Remodeling

Activation of myofibroblasts induces the expression of α-actin and phenotypic modulation to myofibroblasts. Expression of contractile proteins in myofibroblasts may contribute to vascular remodeling by constricting vessels and contributing to late lumen loss. In addition, myofibroblasts are involved in tissue repair by deposition of extracellular collagen, which also contributes to vascular remodeling.

Do the findings of Shi et al indicate that antioxidant therapy would prevent the fibroblast activation and proliferation in response to injury? Although oxidative stress is clearly increased in vascular injury, it has not been conclusively shown that antioxidants can prevent lesion formation. Antioxidants significantly decreased superoxide levels in balloon-injured vessels and promoted vessel remodeling but did not clearly affect neointimal size. The challenge is to identify the source of ROS within vascular cells, specifically, its compartmentalization or site of production, and the ability of injury to activate specific cell-signaling pathways. In the same way that an increase in ROS does not necessarily result in cell proliferation, a generalized reduction in tissue ROS may not “normalize” cell function.

The study by Shi et al reemphasizes the potential role of the adventitia in vascular disease. These data also add to the growing evidence that ROS contribute to the pathophysiology of blood vessel injury. Furthermore, because cells throughout the vessel wall appear to be involved in the response to injury, the adventitia is a novel potential therapeutic target.

References

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