

BIOMECHANICAL MODULATION OF GENE EXPRESSION IN THE DEVELOPMENT OF ARTERIOSCLEROSIS

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Arteriosclerosis is a major contributor to morbidity and mortality in Europe and the USA. The arteriosclerotic lesion is characterized by smooth muscle cell (SMC) hyperplasia or hypertrophy and matrix protein accumulations in the intima and/or media resulting in thickening and stiffness of the arterial wall. *In vivo*, vessel walls are exposed to two main hemodynamic forces or biomechanical stresses: shear stress, the dragging frictional force created by blood flow and mechanical stretch, or tension, a cyclic strain stress created by blood pressure. Since biomechanical stress uniquely exerts its effects on the vessel wall, it could play an important role in the development of arteriosclerosis.

Physical stimuli must be sensed by cells and transmitted through intracellular signal transduction pathways to the nucleus resulting in quantitative and qualitative changes in gene expression in the vessel wall. Recent evidence indicates that mechanical force initiates intracellular signal pathways, especially mitogen-activated protein kinase (MAPK) cascades. MAPKs are thought to play a pivotal role in transmitting transmembrane signals required for cell proliferation, differentiation and apoptosis. MAPKs comprise a ubiquitous family of tyrosine/threonine kinases, and include extracellular signal-regulated kinases (ERKs), stress-activated protein kinases (SAPKs) or c-Jun NH₂-terminal kinases (JNKs), and p38 MAPKs. They are highly activated or expressed in arterioaclerotic lesions and vessel wall stimulated by acute hypertension.

Biomechanical stress-induced cell death. Although the vein vessel does not develop spontaneous arteriosclerosis, accelerated arteriosclerosis develops rapidly in venous bypass grafts, which bear increased biomechanical forces due to alterations in blood pressure, i.e. vein (0-30 mm Hg) vs. artery (120 mm Hg). Therefore, vein bypass grafts could be an optimal model for studying the role of biomechanical stress in the pathogenesis of arteriosclerosis. Recently, we have established the first mouse model of vein graft arteriosclerosis by grafting autologous jugular vein or vena cava to carotid arteries. In many respects, the morphological features of this murine vascular graft model resemble those of human venous bypass graft disease. Using this model, we studied the role of biomechanical stress-induced cell apoptotic death in the development of vein graft arteriosclerosis.

Apoptosis in vessel walls of mouse vein grafts was confirmed by morphological changes and by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL). TUNEL⁺ cells in vein grafts 1, 4, and 8 weeks postoperatively was 13%, 29%, and 21%, respectively, and apoptosis occurred mainly in veins grafted to arteries, remaining unchanged in vein-to-vein grafts. When mouse, rat and human arterial SMCs cultured on a flexible membrane were subjected to cyclic strain stress, apoptosis was observed in time- and strength-dependent manners. All three types of SMCs showed apoptotic death as confirmed by TUNEL, propidium iodide and annexin V staining. To study the signal pathways leading to apoptosis, activities of p38 MAPKs were determined. Mechanical stretch results in p38 MAPK activation. SMC lines stably transfected with a dominant negative rac, an upstream signal transducer, or overexpressing MAPK phosphatase-1, a negative regulator for MAPKs, completely inhibited mechanical stress stimulated p38 activation, and abolished mechanical stress-induced apoptosis. Obviously, the grafted veins are subjected to increased biomechanical forces in the form of stretch stress due to blood pressure. The sudden elevation in mechanical forces could be a strong stimulus to the grafted vessel wall and may result in activation of intracellular signal pathways leading to gene expression and cell death. Thus, one of the earliest events in venous bypass grafts is apoptosis, in which mechanical stress-induced p38-MAPK activation is, at least in part, responsible for transducing signals leading to apoptosis.

Biomechanical stress-induced gene expression related to inflammation. Vein bypass graft arteriosclerosis has an inflammatory nature characterized by mononuclear cell infiltration followed by SMC proliferation. The molecular mechanism by which monocytes/macrophages are

continuously recruited to the neointima of vein bypass grafts is currently unknown. We posit that biomechanical stress plays a role in adhesion molecule expression via MAPK signal transduction pathways, leading to NF- κ B activation. Supporting this concept is the fact that neointimal lesions of vein grafts in ICAM-1 $-/-$ mice were reduced 30% to 50% compared to wildtype controls. Immunofluorescent analysis revealed that increased ICAM-1 expression was observed on the endothelium and SMCs of the grafted veins in wildtype, but not ICAM-1 $-/-$ mice. The number of Mac-1 (CD11b/18)-positive cells adhering to the surface of vein grafts in ICAM-1 $-/-$ mice were significantly less as identified by *en face* immunofluorescence, and these positive cells were more abundant in intimal lesions of vein grafts in wildtype mice. Thus, ICAM-1 is critical in the development of venous bypass graft arteriosclerosis.

It has been established that exposure of endothelial cells to shear (mechanical) stress results in increased expression of ICAM-1 and monocyte chemoattractant protein-1 (MCP-1) via activation of transcription factor NF- κ B and AP-1. These molecules are essential for leukocyte-endothelial cell interaction and subsequently cell infiltration, which is characteristic of the early lesions of vein grafts that undergo elevated blood pressure. Interestingly, mechanical stress also leads to SMCs expressing ICAM-1 via activation of NF- κ B. In animal models, SMCs express ICAM-1 associated with monocyte/macrophage accumulation in vein grafts, and SMCs of ICAM-1 $-/-$ mice do not express ICAM-1 correlated with reduced neointimal lesions. We postulate the role of ICAM-1 expression on SMCs in the development of intimal hyperplasia via three ways: First, the interaction of MAC-1 and ICAM-1 expressed on SMCs may initiate intracellular signaling necessary for cytokine secretion by monocytes/macrophages. Support for this notion comes from the fact that macrophage inflammatory protein-1 production was induced in monocytes cultured on ICAM-1-coated plates. Second, the binding of MAC-1 to ICAM-1 expressed on SMCs may be responsible for monocyte retardation in the vessel wall. Third, it has been reported that expression of ICAM-1 on SMCs may be relevant to the phenotypical change of SMCs, which is considered to be essential to the migration and proliferation of SMCs in the pathogenesis of atherosclerosis. The binding of MAC-1 to ICAM-1 on SMCs might result in intracellular signaling within SMCs, which initiates the gene expression needed for phenotypical change. Thus, our observations, together with others, suggest that mechanical stress is one of the most important factors in initiating ICAM-1 expression in vein grafts. Mechanical stress-induced adhesion molecule and chemokine expression in the vessel wall could be important for the inflammatory response.

Biomechanical stress-activated PDGF-MAPK pathways leading to SMC proliferation. It has been established that mechanical stress stimulates DNA synthesis and proliferation of *in vitro* cultured SMCs. Hypertension increases mechanical force on the arterial wall up to 30%, resulting in marked alterations in signal transductions and gene expression in SMCs, which contribute to matrix protein synthesis, cell proliferation and differentiation. Recently, several reports demonstrated that angioplasty results in stretching of the arterial wall leading to rapid activation of the MAPKs in the regenerating carotids. The magnitude of ERK2 activation positively correlated with the degree of balloon injury to the arterial wall. *Ex vivo* stretching of the vessel wall also induces significant activation of ERK2 kinases. These findings suggest that the kinase activation in the early phase following injury may be due to mechanical stimulation of the vessel wall.

In cultured SMCs, mechanical forces evoked ERK activation followed by enhanced DNA-binding activity of transcription factor AP-1. Interestingly, physical forces rapidly result in phosphorylation of PDGF receptor, an activated state. When GRB2, an adapter protein, was immunoprecipitated from treated SMCs followed by Western blot analysis with anti-phosphotyrosine, -PDGF receptor and -GRB2 antibodies, respectively, PDGF receptor phosphorylation was observed in stretch-stressed SMCs, further supporting the mechanical stress-induced activation of PDGF receptors. Conditioned medium from stretch-stressed SMCs did not result in PDGF receptor phosphorylation; antibodies binding to all forms of PDGFs did not block stress-induced PDGF receptor activation. Thus, mechanical stresses may directly perturb the cell surface or alter receptor conformation, thereby initiating signaling pathways normally used by

growth factors.

Suramin has been shown to be a growth factor receptor antagonist that inhibits cell proliferation. When vein isografts in mice were treated *ex vivo* and *in vivo* with suramin, intimal lesions were reduced up to 70% compared to untreated controls. The mechanism of suramin-inhibited neointima hyperplasia mainly involves inhibition of SMC migration and proliferation via blocking PDGF receptor-MAPK-AP-1 signal pathways. Since suramin is a smaller molecule that should easily penetrate human vessel walls, locally applied suramin might be effective for treatment of bypass patients.

Summary. According to our hypothesis, one of the earliest cellular events in neointima formation in arteriosclerosis could be cell death, in which biomechanical stress is a critical initiator of SMC apoptosis. Following cell death, massive mononuclear cell infiltration into the vessel wall occurs. The mechanism by which monocytes/macrophages are continuously recruited to the neointima of the vessel wall may involve two factors: Biomechanical stress directly stimulating endothelial cell and SMC expressing adhesion molecules and chemokines; dead cells may be an additional force for the induction of inflammatory responses in the vessel wall. Eventually, SMC migration, proliferation and accumulation in the intima occur. In this process, biomechanical stress activates PDGF receptor-MAPK pathways, leading to SMC migration and proliferation. Additional factors stimulating SMC growth could be growth factors and cytokines released by inflammatory cells. Finally, arteriosclerotic lesions comprised of mononuclear cells, SMCs and matrix proteins forms. Thus, research into biomechanical stress-regulated gene expression in arteriosclerosis using these models could lead to a new therapeutic strategy in the treatment of vascular diseases in humans.

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