Determinants of Notch-3 Receptor Expression and Signaling in Vascular Smooth Muscle Cells

Implications in Cell-Cycle Regulation

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Abstract: The Notch family of receptors and ligands plays an important role in cell fate determination, vasculogenesis, and organogenesis. Mutations of the Notch-3 receptor result in an arteriopathy that predisposes to early-onset stroke. However, the functional role of the Notch signaling pathway in adult vascular smooth muscle cells (VSMCs) is poorly characterized. This study documents that the Notch-3 receptor, the ligand Jagged-l, and the downstream transcription *j* factor, HESR-I, are expressed in the normal adult rat carotid artery, and that this expression is modulated after vascular injury. In cultured VSMCs, both angiotensin II and platelet-derived growth factor (PDGF) markedly downregulated Notch-3 and Jagged-l through ERK-dependent signaling mechanisms and prevented the glycosylation of Jagged-l. The downregulation of Jagged-l and Notch-3 was associated with a decrease in CBF-l-mediated gene transcription; activation and a fall in the mRNA levels of the downstream target transcription factor HESR-l. To test the hypothesis that the Notch pathway was coupled to growth regulation, we generated VSMC lines overexpressing the constitutively active form of Notch-3 (A 7r5-N3IC). These cells exhibited a biphasic growth behavior in which the growth rate was retarded during the subconfluent phase and failed to decelerate at postconfluence, The lack of cell-cycle arrest in postconfluent A 7r5-N3IC was associated with an attenuated upregulation of the cell--cycle inhibitor $p27^{kip}$ relative to control cells. This study documents the regulation of the Jagged-l and Notch-3 genes in VSMCs by growth factor J stimulation as well as a role for Notch-3 as a determinant of VSMC growth.

Key-words: angiotensin II, platelet-derived growth factor, vascular remodelling, neointima, glycosylation.

H11 Kinase Is a Novel Mediator of Myocardial Hypertrophy In Vivo

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Abstract: By subtractive hybridization, we found a significant increase in H11 kinase transcript in large mammalian models of both ischemia/reperfusion (stunning) and chronic pressure overload with hypertrophy. Because this gene has not been characterized in the heart, the goal of the present study was to determine the function of H11 kinase in cardiac tissue, both in vitro and in vivo. In isolated neonatal rat cardiac myocytes, adenoviral-mediated overexpression of H11 kinase resulted in a 37% increase in protein/DNA ratio, reflecting hypertrophy. A cardiac-specific transgene driven by the αMHC-promoter was generated, which resulted in an average 7-fold increase in H11 kinase protein expression. Transgenic hearts were characterized by a 30% increase of the heart weight/body weight ratio, by the reexpression of a fetal gene program, and by concentric hypertrophy with preserved contractile function at echocardiography. This phenotype was accompanied by a dose-dependent activation of Akt/PKB and $p70^{S6}$ kinase, whereas the MAP kinase pathway was unaffected. Thus, H11 kinase represents a novel mediator of cardiac cell growth and hypertrophy.

Key-words: cardiac growth, H11 kinase, hypertrophy, gene expression, ischemia.