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MOUSE GENETIC MODELS TO EXPLORE THE FUNCTION OF IGF-I IN SMOOTH MUSCLE

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Unregulated growth of vascular smooth muscle cells (SMC) is a key component in the development of atherosclerosis and restenosis after angioplasty. The proliferation of medial SMC, migration to the intima, and further growth of intimal cells are sequential steps in the formation of a neointima after intraarterial injury. The signals mediating these processes are complex. Significant among them is the paracrine production of growth factors, including basic fibroblast growth factor, platelet-derived growth factor and insulin-like growth factor I (IGF-I).

IGF-I, a small polypeptide with structural homology to IGF-II and proinsulin, is produced by many cell types and acts as an autocrine/paracrine growth factor. It has been postulated to play a role in regulation of growth of SMC of the bladder, uterus, and vasculature. Using embryonic fibroblast cell lines derived from mice with targeted disruption of the type I IGF receptor (IGF-IR), about 60% of the proliferative capacity of serum, or of PDGF, has been found to be dependent on autocrine production of IGF-I. Furthermore, integrity of the IGF-IR is obligatory for the PDGF-dependent increase in proliferating cell nuclear antigen mRNA levels in replicating cells. The critical need for IGF-IR signaling has been further demonstrated by the fact that IGF-I R-null fibroblasts cannot be transformed by the SV40 large T antigen. Until recently, information on the paracrine effects of IGF-I in SMC tissue beds *in vivo* was conjectural, and based primarily on descriptions of the regulation of IGF-I gene expression in association with events that trigger smooth muscle hyperplasia or hypertrophy. For instance, there is a marked induction of IGF-I gene expression in the medial layer of the rat aorta after balloon arterial injury, coincident with the peak time of SMC DNA synthesis. Treatment of rats with a stable antagonist of IGF-I inhibits SMC proliferation and neointimal formation after arterial injury.

Our lab has focused on mouse genetic models to explore the paracrine role of IGF-I in smooth muscle. To this end we first developed SMP8-IGF-I transgenic mice, in which IGF-I is selectively overexpressed in SMC by means of a mouse smooth muscle a actin promoter. These mice exhibit marked overexpression of IGF-I in all smooth muscle-rich tissues (i.e. bladder, stomach, intestine, uterus, aorta), where the growth factor promotes a striking degree of hyperplasia without affecting plasma IGF-I levels or total body weight. To determine the impact of local insulin-like growth factor I (IGF-I) gene expression on vascular injury, the left carotid arteries of SMP8-IGF-I mice and wild type (WT) controls were injured mechanically with an epon resin probe. There was a greater than 4-fold increase in neointimal area in SMP8-IGF-I mice compared to WT. The mitotic index was also markedly elevated, but the effect had subsided by 14 days. In spite of a higher rate of cell division, the relative increase in medial area was less in the SMP8-IGF-I mice than in WT at both 7 and 14 days, consistent with a stimulation of cell migration to the neointima. To further explore the contribution of local IGF-I production to smooth muscle cell biology, we initiated strategies to delete the IGF-I gene exclusively from smooth muscle cells using a cre/lox system. SMP8-Cre mice, in which the P1 bacteriophage cre recombinase is expressed under control of the smooth muscle alpha actin gene promoter, expressed the enzyme only in smooth muscle cell-rich tissues postnatally. However, when SMP8-Cre mice were crossed with the double reporter mouse Z/AP, recombination was seen both in smooth muscle, as well as in cardiac and skeletal myocytes. Further analysis revealed that this is due to transient expression of the SMP8-Cre transgene in somites and in the developing heart during embryogenesis. To overcome this experimental problem, we are presently developing mice in which the Cre recombinase will be conditionally expressed in smooth muscle cells post-natally.

Besides the abundance of IGF-I or of its receptor, IGF action is determined by a family of structurally-related secreted proteins (IGFBPs) that specifically bind IGFs and modulate IGF bioactivity in different tissues. Tissue IGFBP levels are, in turn, subject to regulation by processes that include altered synthesis and degradation. There is no clear understanding of the individual functions that each of the

IGFBPs may serve in vivo, particularly as most cells secrete several IGFBP that share at least some functional properties. In rodent SMC, IGFBP-2 and IGFBP-4 predominate. To explore the role of IGFBP-4 in a paracrine setting, we developed mice overexpressing IGFBP-4 in smooth muscle cells. We found that targeted overexpression of IGFBP-4 in smooth muscle cells of transgenic mice decreases SMC mass. IGFBP-4 is degraded by specific protease/s, which are thought to release free IGF-I for local bioactivity, although the role of IGFBP-4 proteolysis has not been examined *in vivo*. We used our mouse smooth muscle overexpression model to explore this question. IGFBP-4 protease/s recognize basic residues within a 13 aminoacid stretch in the midregion of the molecule (120-132). We engineered a mutant IGFBP-4 in which 7 basic aminoacids within this region were substituted as follows: 119-KHMAKVRDRSKMK-133 to 119-AAMAAVADASAMA-133. Myc-tagged native and IGFBP-4^{7A} expression constructs were transfected into B104 cells, and purified from conditioned media by IGF-I affinity chromatography. Whereas native IGFBP-4 was cleaved by SMC conditioned media, IGFBP-4^{7A} was completely resistant to proteolysis. The mid-region substitutions did not affect IGF-I binding affinity as determined by chemical cross-linking of native and IGFBP-4^{7A} to ^{125}I-IGF-I in the presence of graded concentrations of unlabeled IGF-I. To explore the function of the protease-resistant IGFBP-4 *in vivo*, expression of the mutant and native proteins was targeted to SMC of transgenic mice by means of the SMP8 smooth muscle a actin promoter. Transgene expression was confined to SMC-rich tissues in all lines. Bladder and aortic immunoreactive IGFBP-4/transgene mRNA ratios in SMP8-BP-4^{7A} mice were increased by 2- to 4-fold relative to SMP8-BP4 mice, indicating that the IGFBP-4^{7A} protein was stabilized *in vivo*. SMP8-BP4^{7A} mice had significantly lower aortic, bladder and stomach weight and intestinal length relative to SMP8-BP4 counterparts matched for protein expression by Western blotting. Thus, IGFBP-4^{7A} results in greater growth inhibition than equivalent levels of native IGFBP-4 *in vivo*, supporting a role for IGFBP-4 proteolysis in regulation of IGF-I action. Our findings support the notion that local expression of IGF-I within smooth muscle rich tissues controls myocyte growth. Moreover, locally expressed binding proteins such as IGFBP-4 serve as a local reservoir for the growth factor, which is released by regulated proteolysis.