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Characterization of Model Systems for the Study of Smooth Muscle Cell Phenotype

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SUMMARY

Introduction: Modulation of the expression of Smooth Muscle Cell (SMCs)-specific genes is a key feature of major human pathologies, including hypertension, atherosclerosis, kidney or hepatic fibrosis, airway obstructive diseases and cancer metastasis.

The aim of this work was to characterize two *in vitro* models for the study of the molecular control of SMC phenotype.

Methodology: Mesenchymal Stem Cells (MSCs) were isolated from Wharton's Jelly of neonate umbilical cords, characterized for MSC markers and used at passages 2-3. Human Umbilical Vein Endothelial Cells (HUVECs) were used at passages 2-3.

Results and Discussion: By Western blotting analysis, naive MSCs expressed extremely low levels of the specific SMC proteins Smooth Muscle-α-Actin (SM-α-A), SM-Calponin (SM-CNN) and SM-Myosin Heavy Chain (SM-MHC), compared to differentiated SMCs. In addition, minimal promoters of the above genes and of SM-22α, another SMC marker, driving a luciferase reporter, also showed similar low activity in MSCs. Transforming Growth Factor-β1 (TGF-β1) increased, while Platelet-Derived Growth Factor-BB (PDGF-BB) decreased, SM-α-A protein levels and promoter activity.

SMC phenotype and specific gene expression depend on two crucial transcription regulators: Serum Response Factor (SRF) and Myocardin. Over-expression of Myocardin via an adenoviral vector induced both the activity of the SMC gene minimal promoters as well as the expression of SM-α-Actin and SM-Calponin protein in the MSCs. However, Myocardin was unable to induce minimal promoters of SM-Calponin and of SM-22α bearing mutated SRF-binding elements, indicating that cooperation between SRF and Myocardin is essential in inducing the expression of genes that establish SMC identity in Wharton's Jelly MSCs.

Moreover, in preliminary studies using HUVECs, over-expression of Myocardin resulted in striking cell shape changes and *de novo* expression of SMC markers such as SM-α-Actin. These results are compatible with a phenotypical change referred to as Epithelial-to-Mesenchymal Transition (EMT), a process that plays a crucial role in organ growth, physiological and pathological tissue remodeling and cancer.

Both cell systems are therefore useful in understanding how Myocardin can induce undifferentiated cells (MSCs) or epithelial-type cells (HUVECs) to acquire a Smooth Muscle Cell-like phenotype, and ultimately how its expression may modulate the onset and course of human disease.