**MYOCARDIN ACTIVITY IS REGULATED BY PROTEASOMAL DEGRADATION**

**X-L. Zheng**

Dept. of Biochemistry and Molecular Biology, Libin Cardiovascular Institute of Alberta Smooth Muscle & Gastrointestinal Research Groups, The University of Calgary, Alberta, Canada

Several vascular diseases result from dysfunction of smooth muscle cells (SMCs). Vascular SMCs, unlike skeletal and cardiac muscle cells, exhibit phenotypic plasticity and undergo phenotypic transition from contractile to synthetic and proliferative, as occurs in atherosclerosis. The underlying mechanism was not clear until the discovery of myocardin (MyoCD) by Wang et al. in 2001 (Cell 105: 851-862, 2001). It has been well established that MyoCD activity determines the phenotype of SMCs. As a transcriptional co-activator of serum response factor (SRF), MyoCD controls the transcription of most smooth muscle (SM) and cardiac muscle differentiation marker genes. Therefore, its transcriptional activity determines the phenotype of SMCs, and any factor that regulates MyoCD activity has the potential to induce phenotypic transition. Notably, MyoCD can be degraded through ubiquitin–proteasome system. Our preliminary studies unexpectedly revealed that accumulation of MyoCD in response to proteasome inhibition by MG132 or lactacystin resulted in decrease of transcriptional activity of MyoCD as indicated by reduced expression of SMC contractile marker genes (SM α-actin, SM22, and calponin) and muscle-enriched microRNAs (miR-143/145 and miR-1/133a), and reduced contractility of human vascular SMCs embedded in collagen gel lattices, suggesting that MyoCD degradation is required for its transcriptional activity. Further studies using chromatin immunoprecipitation assay revealed that proteasome inhibition, although increased the occupancy of MyoCD and SRF on the promoter of SM α-actin gene, abolished MyoCD-dependent recruitment of RNA polymerase II. We further examined the degradation of MyoCD in epithelioid and spindle-shaped SMCs and revealed that MyoCD in more differentiated spindle-shaped SMCs was more quickly degraded and had shorter half-life than in epithelioid SMCs. In neointimal lesions, we found that stabilization of MyoCD protein was companied by downregulation of transcripts of ubiquitin and proteasome subunits, further illustrating the mechanism underlying reduction of MyoCD transcriptional activity. In summary, our results have suggested that proteasomal degradation of MyoCD is required for its transcriptional activity.