cAMP Divergently Regulates Nuclear Actin And Myocardin related Transcription Factor (Mkl1) In VSMCs and ECs

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Aim: Proliferation and migration of VSMC and EC contribute positively and negatively, respectively, to intimal hyperplasia. Moreover, current generation anti-mitotics increase thrombotic risk by impairing endothelial regrowth. Hence, targets for VSMC-specific anti-proliferative/migratory agents are required. Here we investigated the effect of cAMP-induced nuclear actin remodelling on the serum response factor (SRF) co-factor Megakaryoblastic Leukemia-1 (MKL1) in VSMC and EC.

Methods: Rat aortic VSMC (RaVSMCs) and human coronary artery ECs (HCAECs) were treated with physiological cAMP activators BAY60-6583 and Cicaprost. G-actin was detected by Dnase1. Migration analysis was performed using IncuCyteZOOM live-cell imaging and proliferation measured by bromodeoxyuridine (BrdU).

Results: cAMP elevation increased nuclear G-actin in RaVSMCs (1.54±0.16 fold,n=6,$p<0.05$), which inhibited proliferation and migration. By contrast, cAMP did not affect nuclear G-actin levels or inhibit proliferation and migration in HCAECs. Elevated cAMP inhibited mitogen-induced nuclear translocation of MKL1 in RaVSMC (from70.4±2.92 to 4.27±2.55%,n=4,$p<0.001$) but not HCAECs, which was replicated by expression of an unpolymerisable nuclear actin mutant (NLS-ACTIN-R62D). cAMP elevation or expression of NLS-ACTIN-R62D significantly inhibited SRF-dependent reporter activity and mRNA expression of pro-proliferative/migratory MKL1 genes in VSMCs but not HCAECs. MKL1 silencing or NLS-ACTIN-R62D significantly inhibited proliferation and migration of RaVSMC. In the human saphenous vein organ culture model, MKL1 inhibition significantly reduced intimal thickness (to 0.134±0.088μm of control,n=4,$p<0.01$) and intimal proliferation (to 0.272±0.044% of control,n=4,$p<0.001$).

Conclusions: These data demonstrate cAMP-dependent increases in nuclear G-actin inactivate MKL1 and inhibit proliferation/migration of VSMC but not EC. Further elucidation of this pathway promises to identify targets for specific inhibition of VSMC proliferation/migration.