Induction of Vascular Smooth Muscle Cell (VSMC) Gene Expression by Angiotensin II and Platelet Derived Growth Factor (PDGF) via cAMP Response Element Binding Protein (CREB)

Migration and proliferation of VSMCs are important for atherosclerotic lesion formation in the blood vessel wall and involve a complex pathway involving PDGF and Angiotensin II (AII). The Lounsbury lab has shown that CREB is activated by PDGF and that Ca^{2+} signaling induces CREB-dependent transcription of c-fos and MKP-1 (Pulver-Kaste et al., 2006, Rose et al., 2008). This study sought out to test whether the levels of CREB-induced protein gene product MKP-1 in VSMCs would be reduced in the absence of CREB under treatment with PDGF and AII. Low passage VSMCs were initially brought up in culture and transfected with shSCRAM as control and shCREB to knockdown CREB. The VSMCs were also transfected with luciferase constructs pRL-TK (renilla) and pCL100N (mkp-luciferase). Luciferase assays allowed us to see whether CREB reduction had an effect on subsequent MKP-1 induction. Results of these tests on AII induction of CREB gene expression were inconclusive due to poor transfection of shRNA (VSMCs display relative resistance to transfection). As a result, we changed the protocol to siRNA. VSMCs were cultured and transfected with siNon-targeting or siCREB. The same luciferase constructs were transfected as before. Luciferase assays showed that treatment of AII with the siCREB VSMCs may result in the up-regulation of MKP-1. These results would refute our hypothesis and suggest that AII induction of CREB signaling reduces MKP-1. Further tests must be carried out in order to see significant results and these data may lead to a new hypothesis where MKP-1 is downregulated by AII through CREB.