A Transgenic Mouse Model that Enables Inducible Knockout of the cAMP Response Element Binding Protein (CREB) in Vascular Smooth Muscle Cells

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Cardiovascular diseases such as hypertension and atherosclerosis induce vascular remodeling that requires changes in gene transcription. The cAMP response element binding protein (CREB) is a transcription factor that is involved in vascular signaling. The Lounsbury lab has found correlations between CREB activity and gene transcription in both cultured vascular smooth muscle cells (VSMCs) and intact arteries; however, a specific role for CREB in vascular function or disease has not been established. The goal of this study was to characterize a transgenic mouse model in which CREB is selectively reduced in smooth muscle, and then to evaluate the role of CREB in signaling by the vasoactive peptide angiotensin II. Crossing an αSMA-CRE^{-ERT} mouse with a CREB^{loxp} mouse resulted in offspring that exhibit tamoxifen-inducible CREB knockdown specifically in VSMCs. The αSMA-CRE^{-ERT}/CREB^{loxp} animals were injected with tamoxifen to induce knockdown of CREB. Animals injected with vehicle were used as a control. Cultured aortic VSMCs and femoral artery sections were analyzed by immunofluorescence to show the loss of CREB expression only in cells that express α-smooth muscle actin (α-SMA). Cultured VSMCs that were treated with tamoxifen *in vitro* also showed significant knockdown of CREB in α-SMA expressing cells, thus the breeding successfully produced a genetically encoded inducible CREB knockdown. This model was used to test the hypothesis that CREB is necessary for induction of c-fos and microRNA132 (miR132) by angiotensin II (Ang II). Expression of c-fos leads directly to transcription of genes that promote hyperplasia. The presence of miR132 reduces the expression of PTEN, a phosphatase that prevents hyperplasia (cell growth). We examined levels of CREB and c-fos by Western protein analysis and relative amounts of miRNA132 and mRNA (PTEN and c-fos) levels by Taqman analysis. Preliminary experiments suggest that CREB affects miRNA132 and PTEN, but not c-fos. These results could reinforce the hypothesis that CREB is important for cell signaling in VSMCs. Future experiments will use this CREB knockdown model to identify CREB-specific signaling pathways and their effects on the development of cardiovascular diseases.