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FORKHEAD TRANSCRIPTION FACTOR FOXO3A POTENTLY INDUCES SMOOTH MUSCLE CELL APOPTOSIS AND PROMOTES EXTRACELLULAR MATRIX DEGRADATION THROUGH ACTIVATION OF MATRIX METALLOPROTEINASES

A Kennedy, T Littlewood, M Bennett, H Yu *University of Cambridge*

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Background Apoptosis of vascular smooth muscle cells (VSMCs) induces features of plaque vulnerability in atherosclerosis. Overexpression of matrix metalloproteinases (MMPs) leads to extracellular matrix (ECM) degradation, cell migration and plaque rupture. However, it is not clear whether proteins that regulate apoptosis also induce MMP expression and/or activity. We have previously shown that FOXO3a, a downstream target of the serine/threonine kinase Akt, potently promotes VSMC apoptosis. In this study we sought to investigate the role of FOXO3a in MMP regulation.

Methods and Results We generated rat VSMCs expressing a hormone-inducible allele of FOXO3a (FoxO3aA3ER) in which three Akt-phosphorylation sites were mutated to alanine to render

them insensitive to Akt. 4-hydroxytamoxifen activated FOXO3aA3ER, identified through translocation from cytoplasm to the nucleus, and induced apoptosis. In accordance with previous microarray data, selective activation of FOXO3a stimulated MMP13 mRNA (9125-fold) and protein expression (79.9-fold) examined by real-time qPCR, Western blot and immunohistochemistry. MMP2 mRNA and protein expression were also upregulated (11.1-fold and 52.5-fold respectively). Importantly, active cleavage of proMMP13 in conditioned medium was seen to be time- and dose-dependent upon FOXO3a activation on Western blot and gelatin zymography. Reporter gene assay, site-directed mutagenesis and chromatin immunoprecipitation collectively demonstrated that FOXO3a directly regulates MMP13 through binding to the forkhead consensus sequence in its promoter. Finally, MMP2 inhibitors prevented MMP13 activation suggesting MMP2 is required for MMP13 activity.

Conclusion We show that FOXO3a regulates MMPs including MMP13 and MMP2. This provides a potential mechanism by which FOXO3a regulates cell migration and ECM degradation in atherosclerosis.