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Title: Effect of TGF-β on FoxO activity in airway smooth muscle cells

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Body: Background:TGF-β is a mediator of abnormal airway smooth muscle (ASM) function in asthma and COPD. TGF-β triggers ASM cell (ASMC) hyperplasia and increases intracellular oxidants whilst reducing antioxidant enzyme expression. The O subfamily of forkhead box transcription factors (FoxO1, -3 and -4) activate antioxidant enzyme, cell cycle inhibitor and pro-apoptotic genes. Aims & Objectives:Determine whether TGF-β reduces FoxO activity in ASMCs leading to attenuated antioxidant enzyme expression and resistance to apoptosis. Methods:mRNA and protein expression were determined by qRT-PCR and Western blotting, respectively. FoxO transcriptional activity was determined by a luciferase reporter assay. Smad activity was inhibited by infection with adenoviral vectors expressing dominant-negative Smad3 (DN-Smad3) and Smad7 genes, and histone deacetylase (HDAC) activity by treatment with trichostatin A (TSA). Results:TGF-β (1 ng/ml) reduced the mRNA levels of the FoxO target genes BimEL (~75%; $p<0.01$), PGC-1a (~90%; $p<0.01$), Mn-superoxide dismutase (MnSOD) and catalase (~50%; $p<0.01$) after 24hrs. TGF-β also reduced FoxO transcriptional activity (~25%; $p<0.05$) 24hrs post-treatment. The inhibition of MnSOD, catalase and BimEL by TGF-β was reversed by DN-Smad3, Smad7 and TSA. TGF-β reduced FoxO3 (~40%; $p<0.05$) and FoxO4 (~70%; $p<0.001$) nuclear protein expression after 24hrs. TGF-β strongly increased FoxO1 mRNA and cytoplasmic protein levels (~12-fold; $p<0.05$), however, nuclear levels were only weakly increased (~3-fold; $p<0.05$) whilst DNA binding activity was unaffected, suggesting nuclear exclusion of FoxO1. Conclusion:TGF-β decreases FoxO activity in ASMCs possibly by reducing FoxO nuclear expression.