

P018 Role for forkhead/winged helix box gene, group O-1 (FOXO1) in pancreatic α -cells.
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Whilst FOXO1 has been suggested to be important for pancreatic β -cell development, a role for FOXO1 in the pancreatic α -cell remains unclear. Here we use RNA silencing to determine the role of FOXO1 in the regulation of the preproglucagon gene. An siRNA duplex against mouse FOXO1 and a scrambled RNA duplex were constructed using the Ambion Silencer™ kit and introduced into cells using *TransIT*-TKO transfection reagent (Mirus®). An anti-FOXO1 antibody (Cell Signalling) was used for immunocytochemistry and immunoprecipitation techniques. Fluorescence intensity was quantified by Volocity™ software. Real-time PCR was performed using an Opticon 2 cycler (M J Research). Immunocytochemical analysis of dissociated mouse islets detected the presence of FOXO1 in both β - and α -cells, and derived α TC1-9 cells showed endogenous FOXO1 immunoreactivity largely within the nucleus. Stimulation of cells with insulin revealed a significant translocation of FOXO1 to the cytosol and cell periphery. In the absence of insulin, an anti-FOXO1 siRNA decreased detectable FOXO1 immunoreactivity by $95 \pm 3.5\%$ and Real-time PCR analysis indicated that FOXO1 is a positive regulator of preproglucagon mRNA levels. Correspondingly, chromatin Immunoprecipitation Assay revealed a direct binding of FOXO1 within the preproglucagon promoter. We conclude that FOXO1 plays an important role in the regulation by insulin of preproglucagon gene expression in pancreatic α -cells.