

P003 Biphasic induction of PDX-1 in mouse embryonic stem cells can mimic development of pancreatic beta-cells
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Pdx1 is a homeodomain transcription factor that plays a crucial role in the developing pancreas. The aim of this study was to develop a model system which mimicked β -cell development in vitro. An inducible DNA construct was generated in the pTP6 plasmid by fusing Pdx1VP16 to the mutated ligand binding domain of the estrogen receptor (ER^{T2}), which is dependent on 4' OH Tamoxifen (4OHT) for its activity. This was introduced into the CGR8 mouse ES cell line and a stably transfected clonal line generated. Cells were induced to differentiate as Embryoid Bodies (EBs) to generate a population of cells enriched in markers of definitive endoderm (DE). These cells were then plated as monolayers in the presence or absence of 4OHT, to activate the exogenous Pdx1VP16ER^{T2} protein. The optimum conditions for inducing a β -cell like phenotype mimicked the pattern of expression of Pdx1 in the developing mouse pancreas, i.e. activation in two waves involving day 0-10 and day 15-21. The differentiated β -like cells formed clusters reminiscent of islets that expressed C-peptide as measured by immunocytochemistry, flow cytometry and radioimmunoassay. The cells exhibited stimulated C-peptide secretion in response to KCl and IBMX but not to high glucose. This model system, in which the temporal expression of transcription factors can be externally modulated, may provide important insights into the developmental biology of the pancreas, particularly when applied to human ES cells.