

**P042** A siRNA-based inhibition of STAT1 protects beta-cells against cytokine-induced apoptosis.

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Cytokines are potential mediators of pancreatic  $\beta$ -cell death in T1D. *In vitro* exposure of  $\beta$ -cells to interleukin(IL)-1 $\beta$  + interferon(IFN)- $\gamma$  or to tumor necrosis factor(TNF)- $\alpha$  + IFN- $\gamma$  impairs their function and ultimately induces apoptosis. The transcription factor STAT1 has been implicated in the expression of as yet unidentified pro-apoptotic gene networks in  $\beta$ -cells, and  $\beta$ -cells from STAT1<sup>-/-</sup> mice are protected against diabetogenic stimuli *in vitro* and *in vivo*. In the present study we used small interfering RNA (siRNA) to down regulate STAT1 expression in both rat insulin-producing INS-1E cells and primary  $\beta$ -cells, and to evaluate whether this prevents cytokine-induced cell death. We first tested diverse approaches for siRNA transfection in  $\beta$ -cells, and selected one (DharmaFECT) with the highest transfection efficiency (between 80-90 %) and lowest toxicity (<12%), as evaluated by fluorescent siRNA and nuclear dyes respectively. Transfection of siRNAs targeting STAT1 inhibited IFN- $\gamma$ -induced STAT1 expression in INS-1E cells and primary  $\beta$ -cells by >80% at both mRNA and protein levels. Viability tests using nuclear dyes indicated that siRNA-driven STAT1 inhibition induced a 55-70% protection (p<0.05) against IL-1 $\beta$  + IFN- $\gamma$ - or TNF- $\alpha$  + IFN- $\gamma$ -induced apoptosis in both INS-1E and rat primary  $\beta$ -cells. We hereby conclude that siRNA-based gene modulation is a promising tool for *in vitro* study of the gene networks underlying cytokine-induced  $\beta$ -cell apoptosis.