Aims

Pro-inflammatory cytokines are known inducers of beta cell death in the pathogenesis of type 1 diabetes and mediate their effects through the perturbation of multiple regulatory gene networks. Growth arrest-specific transcript 5 (GAS5), a long non-coding RNA whose expression negatively correlates with cell survival in cancer cell lines, was previously identified by DNA microarray analysis as overexpressed in INS1 cells in response to inflammatory cytokines. This study aimed to confirm GAS5 expression by RT-PCR and its functional effects on beta cell survival in BRIN-BD11 cells.

Methods

Expression of GAS5 was determined 24hours post cytokine exposure by semi-quantitative PCR, relative to 18S rRNA and effects of cytokine toxicity confirmed by MTT and vital dye exclusion assays. Full length rat GAS5 transcript was subcloned into the pcDNA3.3 expression vector and transfected into cells using lipofectamine reagent to determine GAS5 effects on cell viability.

Results

Treatment of BRIN-BD11 cells with 5:25ng/ml (IL-1β:IFNγ) for 24hours increased GAS5 expression compared to untreated cells. Overexpression of GAS5 decreased viability to 72.5% (+/-2.8) and 56.7%(+/- 1.5) compared to control after 24 and 48hours as determined MTT reduction and reduced viable cell numbers from 8.8x105 to 4.9x105 cells/ml as determined by vital dye exclusion (P<0.05).

Conclusion

This data indicates a potential role for GAS5 in cytokine-induced beta cell death, demonstrating for the first time that this long non-coding RNA is overexpressed under conditions of cytokine toxicity and that GAS5 overexpression decreases cell viability. Further studies are on-going using siRNA to determine effects of endogenous GAS5 in models of cytokine toxicity.