Progressive endoplasmic reticulum stress over time due to human insulin gene mutation contributes to pancreatic beta cell dysfunction

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Abstract

Aims: We studied the effects of heterozygous human *INS* gene mutations on insulin secretion, ER stress, and other mechanisms in both MIN6 and hiPSC-derived beta-like cells, as well as the effects of prolonged overexpression of mutant human *INS* in MIN6 cells.

Methods: We modelled the structure of mutant C109Y and G32V proinsulin computationally to examine the *in silico* effects. We then overexpressed either WT, mutant (C109Y or G32V), or both WT and mutant human preproinsulin in MIN6 cells, both transiently and stably over several weeks. We measured the levels of human and rodent insulin secreted, and examined the transcript and protein levels of several ER stress and apoptotic markers. We also reprogrammed patient fibroblasts into hiPSCs and differentiated these into pancreatic beta-like cells, which were subjected to single-cell RNA-Seq, transcript and protein analyses for ER stress and apoptotic markers.

Results: The computational modelling studies, short-term and long-term expression studies in beta cells revealed the presence of ER stress, organelle changes and insulin processing defects, resulting in decreased amount of insulin secreted but not the ability to secrete insulin. By nine weeks of expression of mutant human INS, dominant negative effects of mutant INS were evident and beta cell insulin secretory capacity declined. $INS^{+/C109Y}$ patient-derived beta-like cells and single cell RNA-Sequencing analyses then revealed compensatory upregulation in genes involved in insulin secretion, processing and inflammatory response.

Conclusions: The results provide deeper insights into the mechanisms of beta cell failure during *INS* mutation-mediated diabetes disease progression. Decreasing sXBP1 or inflammatory response could be avenues to restore the function of the remaining WT *INS* allele.

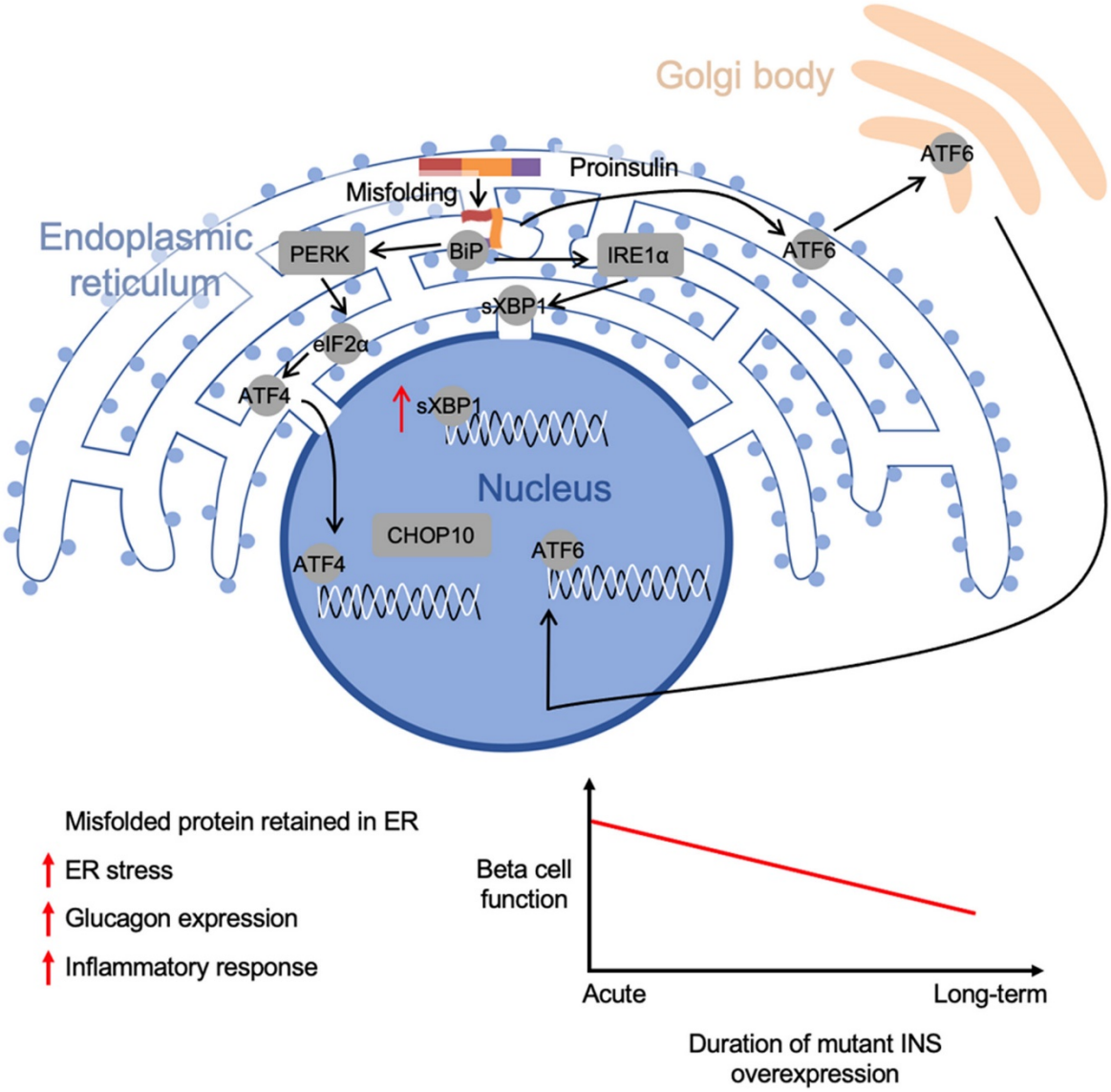

Figure. Model for mechanism of *INS* mutation in beta cell. A schematic which summarises how mutant human preproinsulin results in neonatal diabetes, through upregulated spliced XBP1 as a result of misfolded insulin, as well as increased glucagon expression and inflammatory response.