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Hypomorphic ERdj4 Expression Induces ER Stress and Compromises Insulin Secretion in Beta Cells

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The DnaJ homologue ERdj4 is essential for protein quality control by facilitating ER associated protein degradation. Approximately one-third of ERdj4 gene trap ($ERdj4^{GT/GT}$) mice that expressed hypomorphic levels of ERdj4 died perinatally associated with growth restriction and aberrant blood glucose levels. Previous studies in our laboratory demonstrated that adult $ERdj4^{GT/GT}$ mice had ER dilation and aberrant secretory granule formation in salivary glands and pancreatic exocrine glands. In this study, we demonstrated that loss of ERdj4 in adult mice resulted in ER stress in beta cells associated with hypoinsulinemia and glucose intolerance. Blood glucose level was effectively reduced by exogenous insulin, suggesting that $ERdj4^{GT/GT}$ mice had defects in insulin secretion rather than insulin resistance.

Murine pancreas sections obtained from 6-8 week old $ERdj4^{GT/GT}$ and ERdj4 wild type ($ERdj4^{WT/WT}$) mice were analyzed for histology. Pancreatic islet cells stained with H&E showed that some beta cells had amorphous inclusions that were not detected in WT beta cells. It was confirmed by electron microscopy that these amorphous inclusions were indeed dilated ER (Figure 1) localized to beta cells, in which degranulation was pronounced. ER dilation also occurred in alpha cells, although it was not prominent.

Confocal microscopy was conducted to determine if ER stress occurred in specific islet cells. Cell profiling by confocal microscopy determined that $ERdj4^{GT/GT}$ mice had more glucagon positive cells in pancreatic islets than WT controls. ER stress monitored by GFP tagged XBP-1 reporter detected elevated ER stress in beta cells, while minimal ER stress was observed in other cells. Colocalization analyses determined that insulin accumulated in ER of beta cells (Figure 2), consistent with hypoinsulinemia and elevated serum proinsulin to insulin ratio in $ERdj4^{GT/GT}$ mice.

To determine whether ERdj4 associated with insulin or insulin processing enzymes, immunoprecipitations were performed using HEK 293 cells transiently coexpressing HA tagged ERdj4 and FLAG tagged insulin, or insulin processing enzymes. Insulin processing enzymes PCSK1, PCSK2, and carboxypeptidase E (CPE) pulled down with HA tagged ERdj4, suggesting that these were substrates of ERdj4. This result was confirmed by DuoLink® proximal ligation assay that FLAG tagged PCSK1 associated with HA tagged ERdj4 in transfected HEK 293 cells. PCSK1 expression assessed by confocal microscopy determined that PCSK1 was significantly lower in beta cells of GT mice compared to WT controls.

Our studies demonstrate that the loss of ERdj4 results in accumulation of proinsulin and subsequent ER stress associated with processing enzyme deficiency. How other putative ERdj4 substrates including PCSK2 and CPE cause ER stress in beta cells will be determined in future studies.

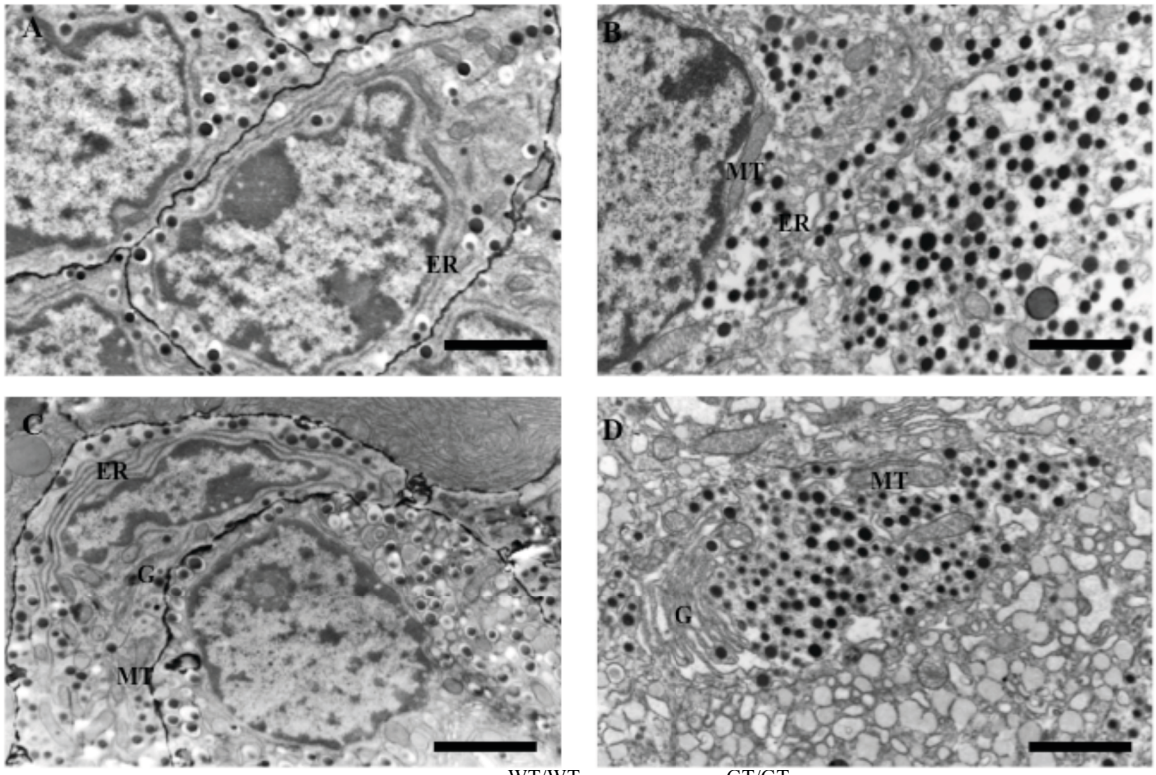

Figure 1. Islet of Langerhans of ERdj4$^{WT/WT}$ and ERdj4$^{GT/GT}$ mice. Alpha cells of (A) ERdj4$^{WT/WT}$ and (B) ERdj4$^{GT/GT}$ mice. Beta cells of (C) ERdj4$^{WT/WT}$ and (D) ERdj4$^{GT/GT}$ mice. ER: endoplasmic reticulum; G: Golgi complex; MT: mitochondria. Scale Bar is 2 μm.

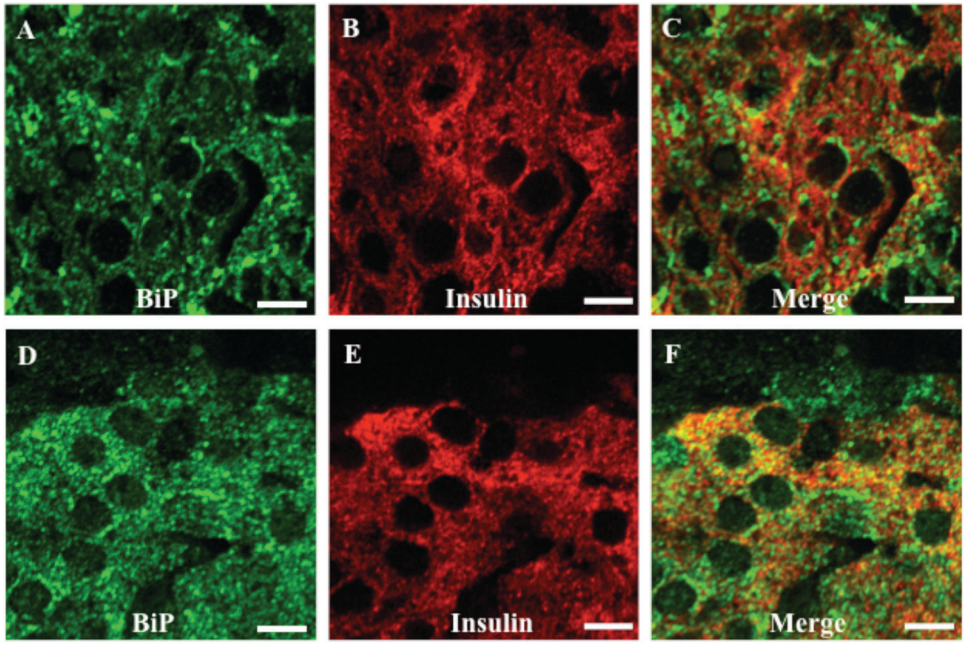

Figure 2. Localization of insulin to the ER of beta cells. (A-C) Beta cells of ERdj4$^{WT/WT}$ mice. (D-F) Beta cells of ERdj4$^{GT/GT}$ mice. Colocalization was demonstrated using antibodies directed against ER marker BiP and insulin. Noted that insulin accumulated in the ER of beta cells of ERdj4$^{GT/GT}$ Scale Bar is 10 μm.

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