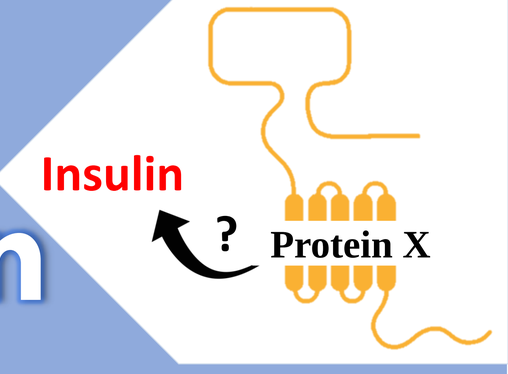
A Novel Strategy to Promote Insulin Secretion



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Introduction:

Under **half-a-billion global population** is affected by Diabetes Mellitus (DM)[1]. Both Type 1 and Type 2 DM are characterised by high blood glucose due to **failure in sufficient insulin secretion** by beta cells within the pancreatic islets[2]. The standard insulin replacement therapy does not provide normal metabolic control. **Islet transplantation** is a potential treatment to provide insulin independence in diabetic patients, however the long-term success is limited by the relatively low efficiency and survival of donor islets post-transplantation[3]. Therapeutic options for diabetes and islet transplantation require glucose metabolism maintenance through enhanced insulin production and release from beta cells. Although much is known about factors limiting insulin secretion, improving beta cell function by targeting the signalling pathways that suppress the production and release of insulin remain largely unexplored as a therapeutic option. **Protein X** – a membrane receptor expressed by all tissues – has multiple roles including inhibition of nitric oxide production and mediation of cellular stress responses such as oxidative damage, cell death and self-renewal, all of which are implicated in DM pathogenesis and islet transplantation failure. The role for Protein X in insulin secretion, however, **remains unexplored**.

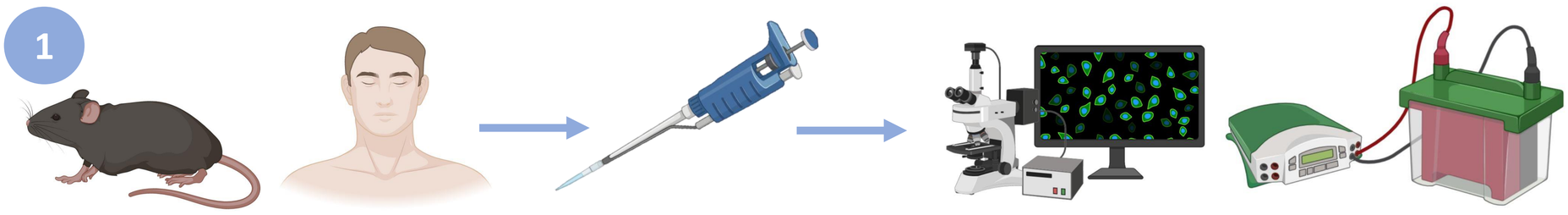
Hypothesis and aim:

Protein X signalling limits insulin secretion.

To determine if Protein X inhibition enhances insulin expression.

Research Plan:

1

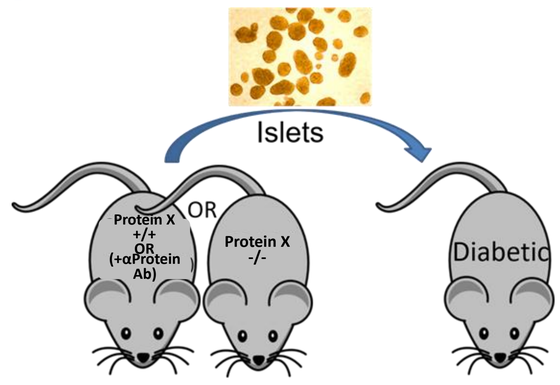


Tissue of interest:
- Wildtype (WT) and Protein $X^{-/-}$ mouse islets
- Human islets

Treatment:
- **Glucose stimulation**
- **Protein X blocking** via antibody in human islets

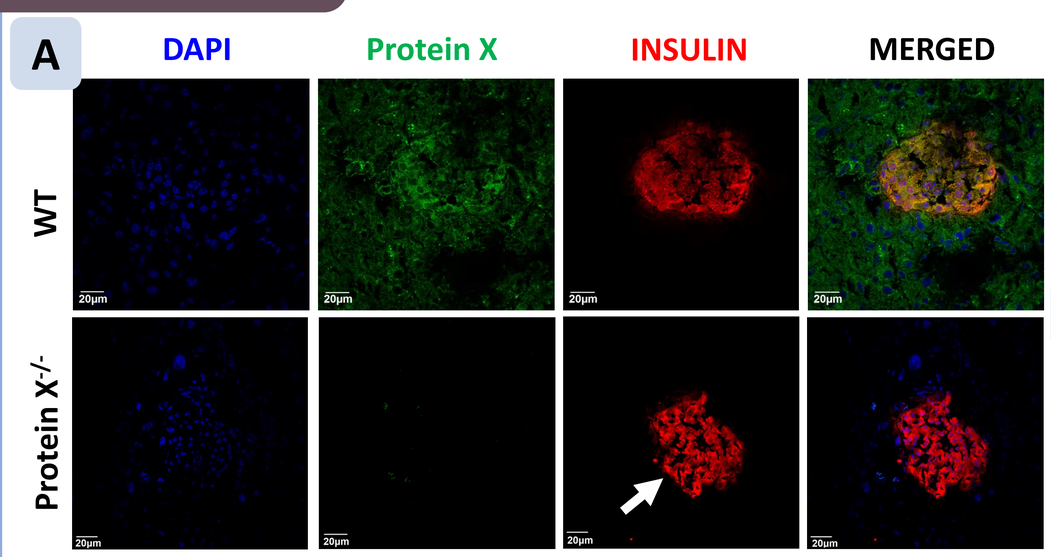
Assess protein expression:
- Immunofluorescence staining
- SDS-PAGE (Western Blot)
- Electron microscopy

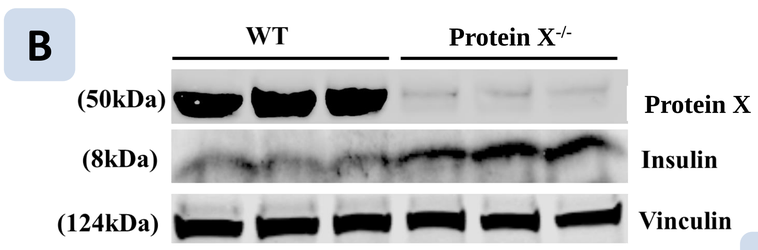
2 **Islet transplantation:**

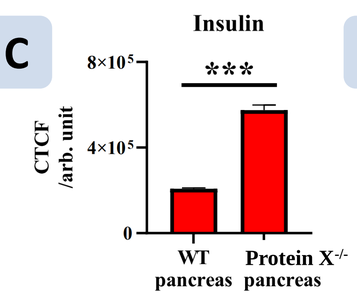


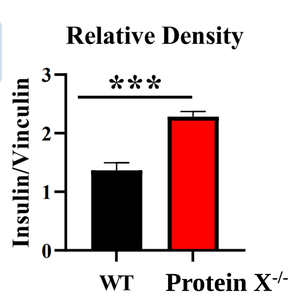
Results:

1 **Deletion of Protein X increases insulin expression and secretion**









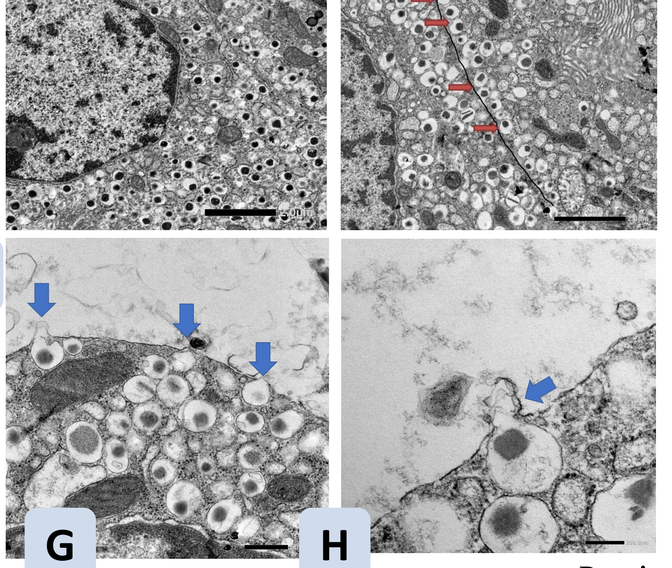


Figure 1. A) Confocal imaging and **B)** SDS-PAGE of WT and Protein $X^{-/-}$ mouse pancreas under high glucose conditions. Increased insulin expression indicated with white arrow. Scale bar = 20µm. **C)** Corrected Total Cell Fluorescence (CTCF) and **D)** Relative band density with error bars indicating SEM. ***p < 0.01. **E)** Insulin granules of WT and Protein $X^{-/-}$ beta cells from mouse islets, under 20mM glucose conditions. Docked insulin granules in Protein $X^{-/-}$ beta cells indicated by red arrows. Scale bar = 2µm. **F)** Insulin granule exocytosis (indicated by blue arrows) in Protein $X^{-/-}$ beta cells under 20mM glucose conditions. Right image: scale bar = 200nm is magnified from left image: scale bar = 500nm. **G)** Number of docked insulin granules in WT and Protein $X^{-/-}$ beta cells under 2mM and 20mM glucose conditions. Significantly greater number of docked insulin granules detected in Protein $X^{-/-}$ cells of 20mM than WT of the same. **H)** Number of exocyting insulin granules in WT and Protein $X^{-/-}$ beta cells under 2mM and 20mM glucose conditions. Significantly greater number of exocyting insulin granules detected in Protein $X^{-/-}$ cells of 20mM than WT of the same. **p < 0.01, *** p < 0.05.

2 **Protein X blockade increases glucose-stimulated insulin expression in human islets**

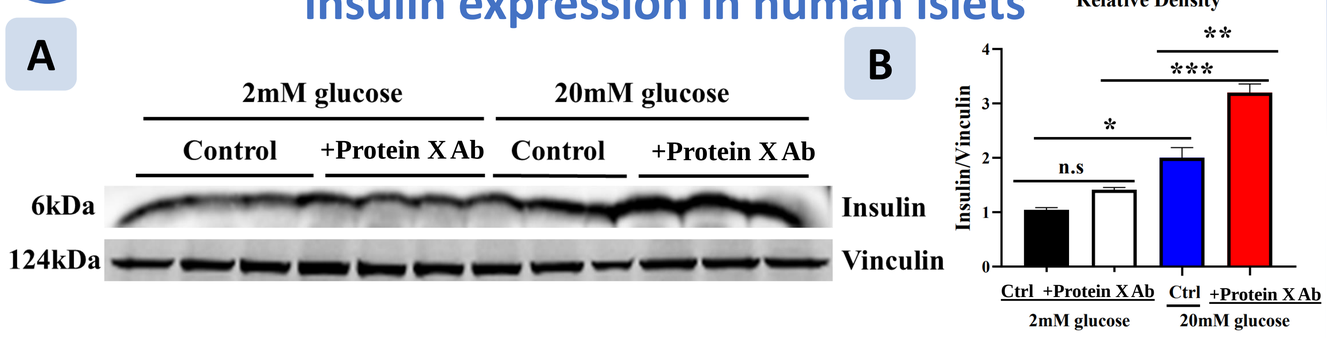


Figure 2. A) SDS-PAGE of control and Protein X-blocked (+Protein X Ab) human islets under 2mM and 20mM glucose conditions. Significantly greater insulin expression was detected in Protein X-blocked islets of 20mM than control islets of the same. **B)** Relative band density with error bars indicating SEM.

3 **Transplantation of Protein $X^{-/-}$ or Protein X-blocked islets improves glucose control in diabetic mice**

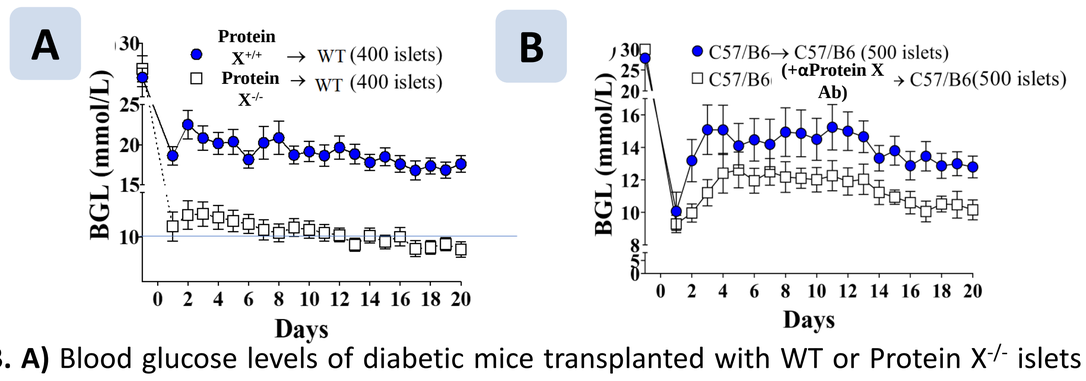


Figure 3. A) Blood glucose levels of diabetic mice transplanted with WT or Protein $X^{-/-}$ islets (400 islets, each). **B)** Blood glucose levels of diabetic mice transplanted with WT or Protein X-blocked islets (500 islets, each). Glucose control improved in mice transplanted with Protein X-inhibited islets compared to mice transplanted with WT islets. Error bars indicate SEM.

Conclusions and future directions:

- **Downregulating Protein X increases insulin expression and secretion.**
- **Transplanting Protein $X^{-/-}$ or Protein X-blocked islets in diabetic mice improves glucose control and islet transplantation outcomes.**
- **Future directions:** To check if Protein X – an inhibitor of angiogenesis – can be inhibited to improve islet revascularisation post-transplantation.
- **Health outcome:** Protein X can be a useful **therapeutic target** to improve beta cell function and islet transplantation outcomes in diabetic patients.

References:

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