Assessing the expression of lysosomal proteins in pancreatic islets in type 2 diabetes

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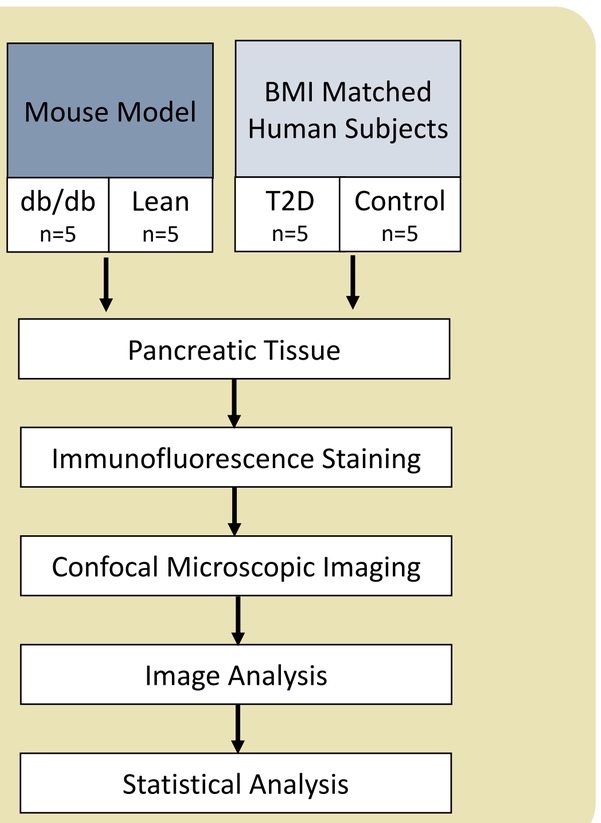
Introduction

- **Type 2 diabetes (T2D)** is a chronic disease characterized by elevation of blood glucose level due to dysfunction in insulin production and action with detrimental impact on patient's quality of life.
- Previous studies suggest that cell recycling pathway known as **autophagy** plays an important role in regulating pancreatic β-cell homeostasis.
- Impairment of this function has been observed in T2D and thought to occur secondary to **lysosomal function impairment which then** drives β-cell loss in T2D.
- Single-cell RNA-sequencing data analysis showed changes in lysosomal gene expression in T2D, however limited investigation has been done at a protein level.

Aim

To explore the expression of lysosomal proteins in pancreatic tissues of control and T2D patients alongside markers of autophagic flux.

Methods



- Pancreatic tissues were embedded in paraffin and cut into 4-5 µm slices.
- Immunofluorescent assay was done on
  - Autophagy markers: p62 & LC3
  - lysosomal proteins: TFEB, LAMP2, Cathepsin B & Cathepsin D
- Image analysis was done using FIJI software.
- Puncta count was measured on insulin positive area.
- Statistical analysis done using unpaired T-test.

Results

Mouse Model

To optimise image quantification methodology, we first performed analysis on existing images captured on tissue from a mouse model of T2D (db/db) and lean control.

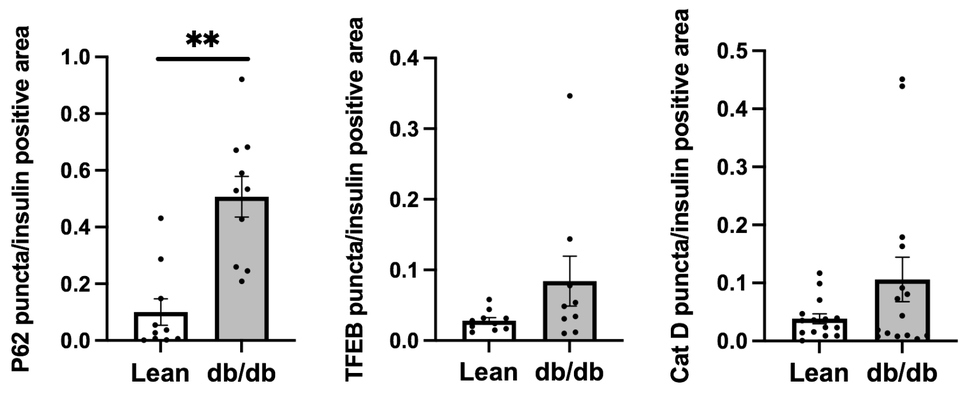


Figure 1. Expression of p62, TEFB and cathepsin D in db/db and lean mouse model. Mean±SEM; **p<0.001

- Significant increase of p62 puncta accumulation in insulin positive cells of db/db mouse pancreatic islet, indicating impaired autophagic flux.
- No significant difference in TFEB and cathepsin D puncta count between subject groups with a trend towards higher expression in db/db mice.

References

Results

Human Tissue

Next, we used the optimised quantitative analysis method to assess autophagy and lysosomal markers in tissue from patients with T2D.

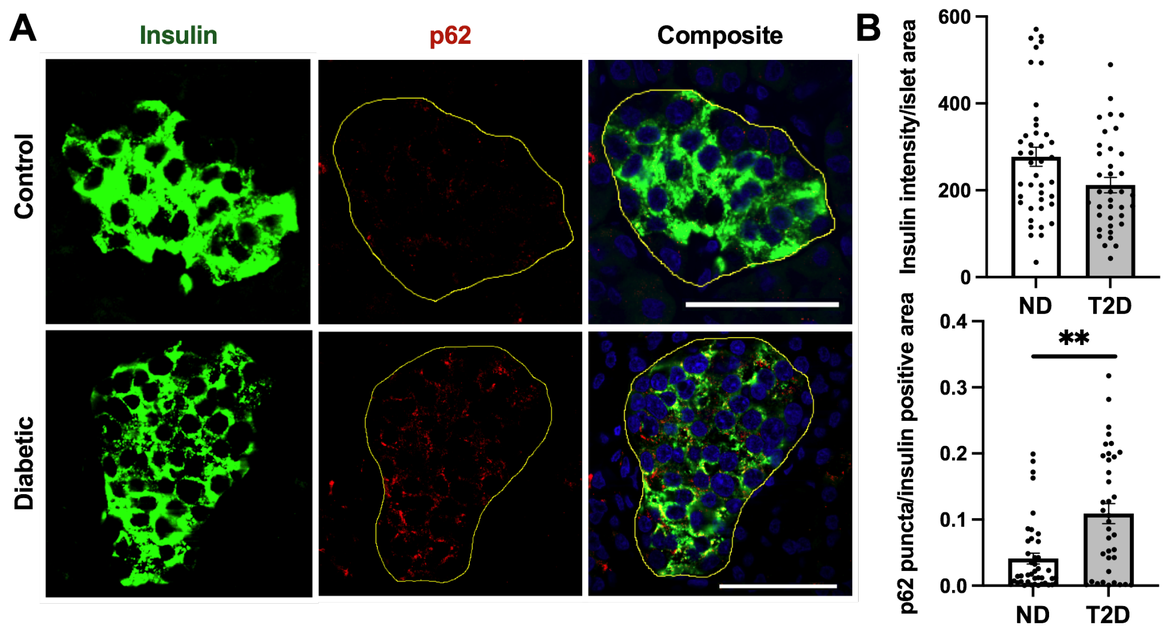


Figure 2. (**A**) Representative immunofluorescence images of p62 puncta on T2D and control human pancreatic islet; Scale bar represents 50 µm. (**B**) Insulin and p62 expression in T2D and Non diabetic (ND) pancreatic islet cell. Mean±SEM; **p<0.001.

Significant increase of p62 puncta in insulin positive cells in T2D vs controls

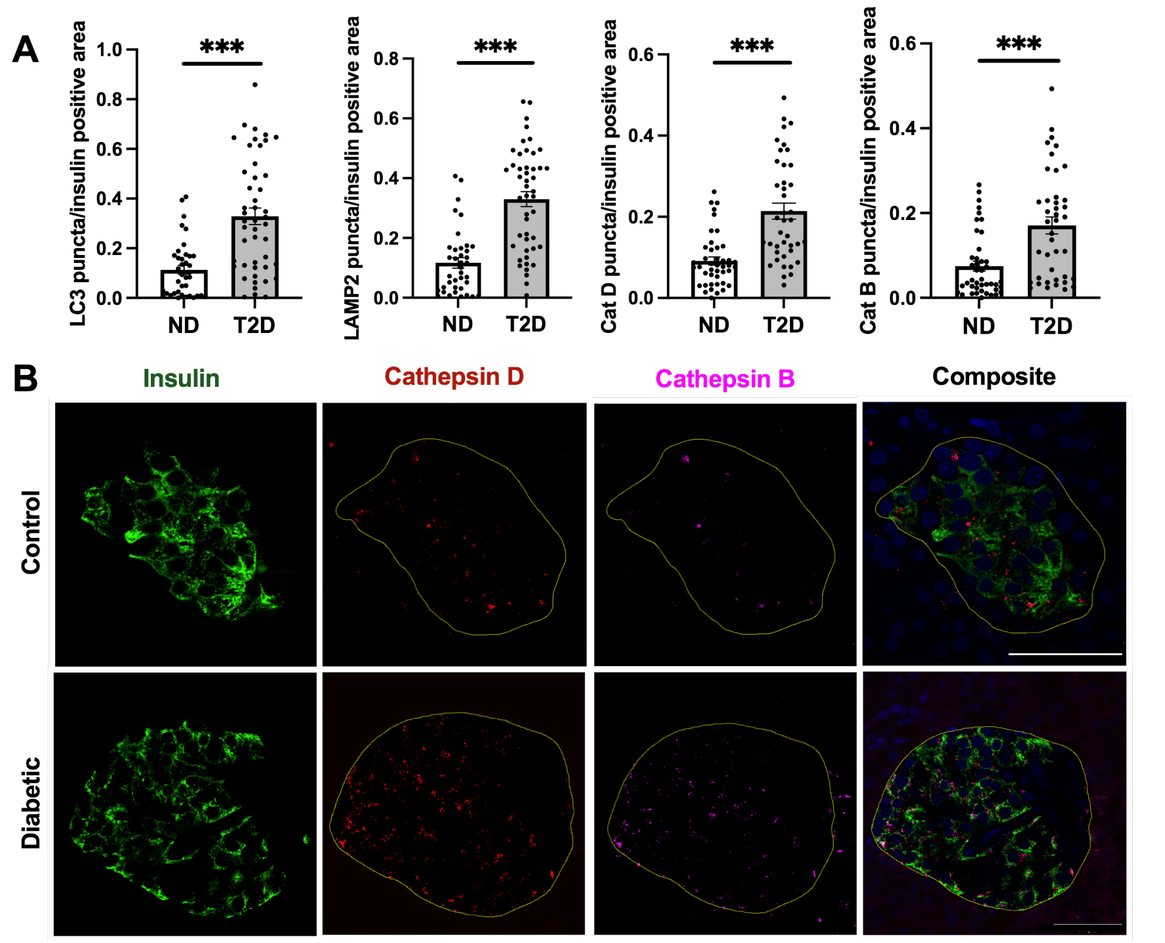


Figure 3. (**A**) LC3, LAMP2, cathepsin D, and cathepsin B on T2D and ND pancreatic islet. Mean±SEM; ***p<0.0001. (**B**) Representative immunofluorescence images of cathepsin D and cathepsin B expression on T2D and control pancreatic islet; Scale bar represents 50 µm.

Significant increase in LC3, LAMP2, cathepsin B and D puncta in insulin positive cells in T2D vs controls

Conclusion and Future Work

- Accumulation of p62 and LC3 indicates autophagic flux dysregulation in db/db mouse and T2D patients.
- Increased cathepsin D, cathepsin B, and LAMP2 puncta in T2D patient is not consistent with decreased mRNA expression detected by previous RNA-sequencing analysis.
- Increasing staining pattern of lysosomal markers suggests a dysfunction in lysosomal clearance due to blocked autophagic flux
- Future work exploring the underlying cause of lysosomal dysfunction in T2D and further analysis on its impact towards autophagic activity may bring further understanding of autophagy and lysosomal roles in T2D pathophysiology and development of future treatment.