Brief summary of background, significance and hypothesis

Lipid droplets (LDs) appear to uniquely accumulate in adult primate islets and become elevated in T1D α cell-enriched islets (1). My published results strongly suggest that the levels of LD scaffolding protein, PLIN2, influences adult human islet β cell activity (2). Notably, the PLIN2 levels are higher in human α than β (3,4). Strikingly, α cell dysfunction was detected in single autoantibody positive (GADA+) individuals prior to β cell inactivity and/or loss (5). Single-cell RNA sequencing (scRNA-Seq) of the α cells from GADA+ donors revealed abnormalities in fatty acid (FA) and LD metabolism related signaling pathways (6–8), highlighting a potential role of LDs in maintaining α cell function and health.

In this project, I am using a human pseudo islet culture system to determine how PLIN2 dependent LDs impact adult human islet α cell function. I proposed in Aim 1, to delineate the functional and molecular impact of reducing LDs in adult human islet α cells; and in Aim 2, to determine if improved LD accumulation neutralizes lipotoxicity and proinflammatory induced stress in human α cells. I hypothesized that LDs are crucial in maintaining human α cell function both through storage of metabolically and structurally important hydrophobic molecules and by neutralizing lipotoxic and proinflammatory effectors.

Approach and preliminary results

To examine how LDs specifically affect human islet α cells, I applied an immunomagnetic positive selection strategy to enrich α cells from dissociated non-diabetic adult human islets. Using an anti-CD26 antibody as the selection marker, I was able to produce human pseudo islets greatly

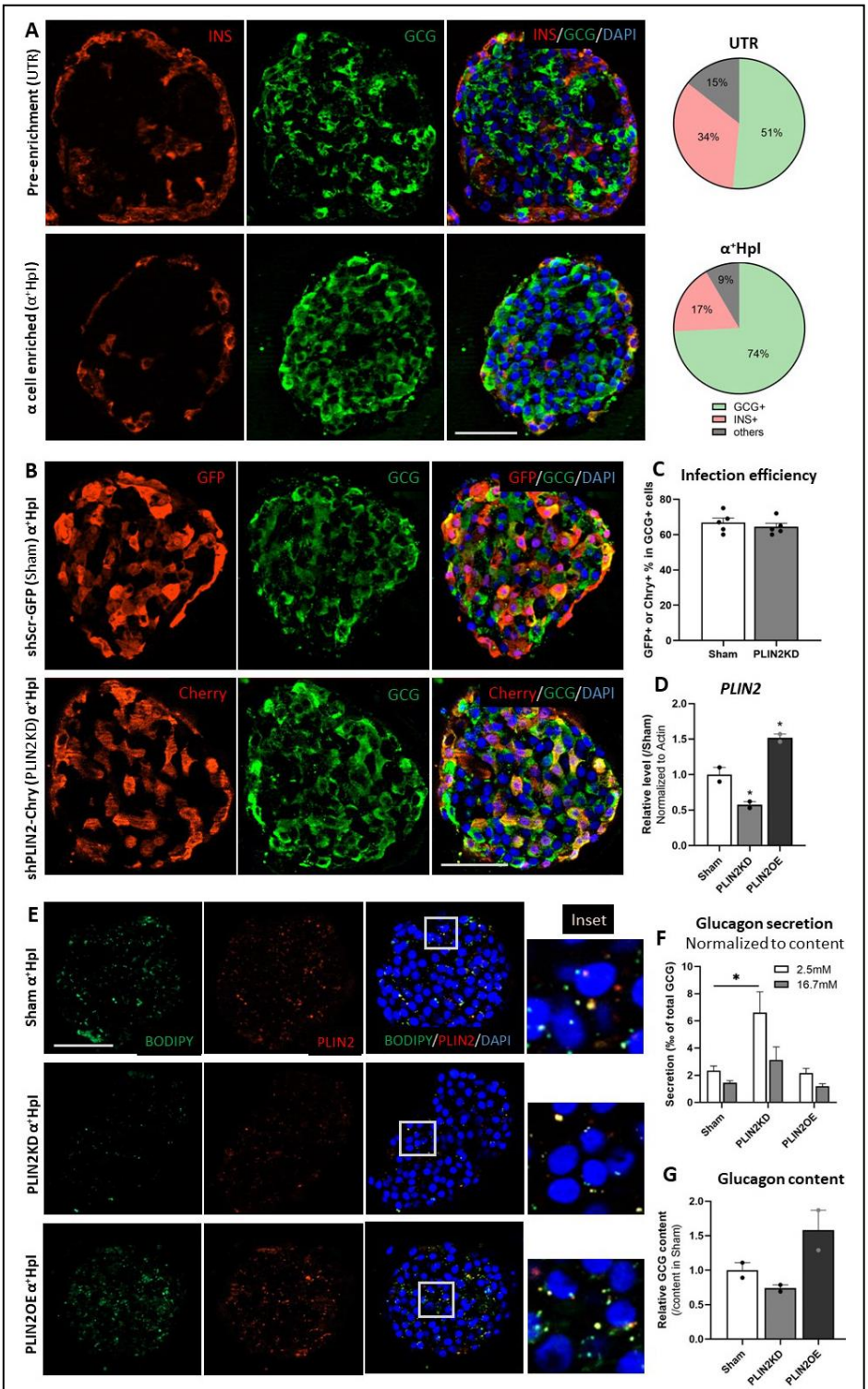

Fig. 1. PLIN2 regulates lipid droplets (LDs) and glucagon (GCG) secretion in human α cell enriched pseudo islets (α⁺HpI). (A-C) Immunofluoresent staining of insulin (INS), GCG, GFP and Cherry (Chry) were performed in pre-enriched (UTR), CD26 selected α⁺HpI, shScramble-GFP or shPLIN2-Cherry infected α⁺HpI. The quantified cell type composition shown in pie charts demonstrate significant α cell enrichment in α⁺HpI (A), and the infection efficiency was around 70% (B-C). **(D-E)** Effectiveness of PLIN2KD and PLIN2OE on PLIN2 mRNA, protein and LD levels in α⁺HpI compared to Sham. **(F-G)** Glucagon secretion at 2.5mM and 16.7mM glucose (*summary of 2 donors was shown*). PLIN2KD α⁺HpI showed significantly elevated glucagon secretion under low glucose and a trend towards higher secretion under high glucose, whereas PLIN2OE α⁺HpI resembled the Sham. No overt impact of PLIN2 on glucagon content was observed **(G)**.

enriched for α cells (Fig. 1A, termed α⁺HpI or α plus Human pseudo islets). Lentiviruses carrying a universal promoter driving shPLIN2 or PLIN2 were used to knockdown (KD) or over-express (OE) the protein. In addition, a GFP or Cherry reporter was used to mark cells infected in α⁺HpI. Around 70% of α cells (i.e., GCG+) expressed GFP or Cherry (Fig. 1B-C), and both PLIN2 mRNA and protein expression was significantly impacted in KD and OE pseudo islets (Fig. 1D-E). Consistently, LD status evaluated by BODIPY staining revealed that PLIN2 KD compromised, while PLIN2 OE elevated LD levels, as expected (Fig. 1E). No overt cell death was observed in any of the experimental groups.

To evaluate how PLIN2 levels regulate glucagon secretion, I performed static incubation with a low stimulating (2.5mM) and high inhibiting (16.7mM) [glucose]. Interestingly, PLIN2 KD α⁺HpI elevated glucagon secretion at 2.5mM glucose and a trend towards higher secretion at 16.7mM. The secretion profile of PLIN2 OE α⁺HpI resembled Sham pseudo islets (Fig. 1F), and no overt changes in glucagon content were observed in PLIN2KD or PLIN2OE (Fig. 1G). This same pattern was observed in the two donors analyzed. In summary, my preliminary data highlight that limiting LD formation directly impacts human α cell glucagon secretion.

Discussion and Specific plans for the next 6 months

Increased LD levels were observed in T1D α cells, in which the glucagon secretion showed a blunted response to the inhibitory effect of high glucose. This contrasts from the elevated glucagon secretion in the low-LD PLIN2 KD α⁺HpI. I hypothesize that despite relatively high LDs in T1D α cells, LDs is still inadequate to neutralize toxicity from circulating cytokines and fatty acids. Consequently, and as proposed in Aim 2, I will evaluate if increasing LD accumulation capacity, i.e., PLIN2 OE, protects α cells from the negative impact of such stressors on glucagon secretion. I will perform similar static incubation of glucose stimulated glucagon secretion assay in Sham and PLIN2 OE α⁺HpI, with or without preincubation of palmitic acid or proinflammatory cytokine mix. I expect that LD levels will be influential on α⁺HpI activity under these conditions. Significantly, I have found that PLIN2 OE protects human β cells from lipotoxicity induced dysfunction (2,9,10).

Additional plans: Human islets are composed of (at least) two functionally and molecular distinct α cell subpopulations (11). I will perform scRNA-Seq on dispersed Sham and PLIN2KD α⁺HpI as part of Aim 1 to examine how PLIN2 levels impact these subpopulations. I hypothesize the effect of PLIN2 on FA metabolism (3) will influence the levels of key α cell regulators, like the *MAFB* and *ARX* transcription factors (3), genes/proteins important to mitochondrial function (12), and glucagon secretion itself (e.g., through regulation of vesicle trafficking components *SYNT13* and *VAMP4* and/or the potassium *ABCC8* and *KCNJ8* channels) (3). Thus, I expect that lowering PLIN2 levels will shift the α cell composition towards compromised functional cell integrity (13).

Overall, the results of my study will provide evidence in how LD formation (i.e., lipid handling) alters α cell-enriched T1D-like islet function.

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