**Islet biology in 2021**

**New insights into β-cell failure, regeneration and replacement**

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**Standfirst:** In 2021, several discoveries shed light on the pathomechanism of β-cell failure during diabetes mellitus initiation and progression and validated novel molecular targets for intervention. Moreover, the field of stem cell-derived replacements for β-cells is rapidly advancing. These advances bring us closer to therapies to protect and/or regenerate β-cell mass in patients with diabetes mellitus

**Key advances**

* Single-cell epigenomics and genome-wide association studies (GWAS) of type 1 diabetes mellitus (T1DM) detected enrichment of risk variants in *cis*-regulatory elements across blood and pancreatic cell types, indicating the contribution of pancreatic exocrine dysfunction in T1DM development2.
* Combining single-cell epigenomics and GWAS of type 2 diabetes mellitus (T2DM) provided endocrine lineage-specific and state-specific accessible chromatin profiles, identifying a risk variant at the *KCNQ1* locus, which impacts insulin synthesis3.
* Multi-omics analysis of fresh biopsy samples from deeply metabolically profiled individuals uncovered molecular changes associated with the history of β-cell pathophysiology in T2DM and identified novel lipid biomarkers4.
* The unexpected discovery of an insulin inhibitory receptor (inceptor) provided insights into how insulin signalling and its receptor re regulated at the source of the ligand in β-cells7.
* An innovative strategy using ready-made microvessels improved the survival and function of transplanted stem cell-derived islets and primary human islets into mice, enhancing diabetes T1DM remission9.

A century after the discovery of insulin as a treatment for diabetes mellitus, no therapy has yet been developed to stop or reverse disease progression and its secondary complications1. The two major forms of autoimmune type 1 diabetes mellitus (T1DM) and glucotoxic and lipotoxic type 2 diabetes mellitus (T2DM) cause either loss or dysfunction of insulin-producing pancreatic β-cells, respectively. Thus, it is crucial to uncover the molecular mechanisms of β-cell pathophysiology to design therapies for protecting or restoring functional β-cell mass in diabetes mellitus. In 2021, several studies provided novel and important insights into pathological changes to islets in T1DM and T2DM. These together with identification and validation of novel drug targets, and establishment of a novel transplantation strategy, open new avenues for β-cell protection, regeneration and replacement.

The cause and mechanisms of T1DM are poorly understood. Several genome-wide association studies (GWAS) have identified genetic risk variations linked with T1DM susceptibility, largely at non-coding genomic regions. Nevertheless, the causality of these variants to T1DM onset and progression remains unclear. In an elegant study by Chiou et al. to validate disease genes, GWAS data was combined with single-cell epigenomics to map the genetic basis of T1DM2. The investigators used single-nucleus ATAC-Seq to generate an epigenomic map of candidate cis-regulatory elements (cCREs) in peripheral blood and pancreas samples from individuals without diabetes mellitus By performing a large single nucleotide polymorphism (SNP)-based GWAS of T1DM and integrating it with the epigenomic landscape, they discovered enrichment of many T1DM risk variants in cell type-specific cCREs (Fig. 1). These risk variants included reference SNP (rs)7795896,which they mapped to a ductal cell-specific cCRE. Functional analysis revealed that rs7795896 suppresses cystic fibrosis transmembrane conductance regulator expression, uncovering a possible causal role of pancreatic exocrine dysfunction in T1DM pathogenesis2.

Chiou and colleagues then took a similar approach to investigate the genetic basis of T2DM risk3. scATAC-seq analysis of healthy human islets provided lineage-specific and state-specific accessible chromatin profiles, indicating the existence of epigenomic heterogeneity in endocrine cells3. The mapping of *cis*-regulatory programmes onto SNP-based GWAS of T2DM identified enrichment of many T2DM risk variants, including rs231361 at the *KCNQ1* locus (Fig. 1). Data analysis predicted state-specific effects of rs231361 on β-cell chromatin accessibility. Gene-edited hESC-derived β-like cells revealed that rs231361 regulates insulin synthesis, further highlighting the functional impact of non-coding genetic risk variants in T2DM3. For further human translation, it would be interesting to stratify a cohort of rs231361 homozygous carriers for the minor and major alleles to confirm the phenotype in glucose metabolism and identify disease gene candidates for personalized medicine. Future studies should functionally validate more of the identified risk variants to detect more T1DM and T2DM-associated disease genes and potential therapeutic targets.

Profiling of islets from people with diabetes mellitus is hampered by possible molecular changes during isolation of the samples from deceased donors and lack of natural history of the disease development. To overcome this hurdle, Wigger et al. snap-froze pancreatic tissue from metabolically profiled living individuals undergoing pancreatectomies and isolated islets using laser-capture microdissection. These individuals represented a wide range of glycaemia conditions, including normoglycaemia, impaired glucose tolerance and overt T2DM4. The researchers performed bulk mRNA sequencing and proteomics of isolated islets along with plasma lipidomics from similar patients (Fig. 1). The transcriptomic analysis revealed progressive alterations in gene expression patterns in impaired glucose tolerance and islets from people with T2DM, including in genes associated with insulin secretion and oxidative phosphorylation. Furthermore, based on the disharmonic changes in β-cell mRNA expression profiles and lack of alterations of maturation and identity genes, the authors suggested that β-cells were not transdifferentiating or de-differentiating in T2DM. Previous studies of pancreatic β-cell dedifferentiation in patients with T2DM have revealed contradictory results5,6, possibly due to different patient characteristics, medication or clinically-determined T2DM subtypesThe integration of transcriptomic and lipidomic data uncovered associations between plasma levels of ceramide, dihydroceramides and ether-linked phosphatidylcholines with HbA1c levels, suggesting their potential use as biomarkers4. Further analysis should apply this multiple level data integration to different islet cell types and distinct β-cell sub-populations derived from different T2DM subtypes characterized by clinical parameters (i.e. insulin resistant versus insulin deficient) to identify the disease proteins and pathways.

Coming from a completely different angle as the above introduced studies, a screen for novel endocrine regulators during pancreas development identified a novel regulator of insulin/IGF signaling7Whether insulin-producing β-cells experience autocrine insulin signalling at the source of the ligand, and how they are shielded from constitutive pathway activation is a point of controversy in the field of β-cell biology. The identification and characterization of a novel regulator of insulin and insulin-like growth factor (IGF) signalling, called the insulin inhibitory receptor (inceptor) (Fig. 1), shed some new lights onto these old questions7. Whole-body knockout of the inceptor resulted in increased β-cell insulin signalling and β-cell mass, causing hyperinsulinaemia and hypoglycaemia in mice. Inceptor physically interacts with insulin and IGF1 receptors and facilitates clathrin-mediated endocytosis of the activated insulin and IGF receptor complexes to desensitize insulin signalling in β-cells. Importantly, mice lacking inceptor specifically in β-cells exhibited increased β-cell proliferation and improved glucose tolerance7. As insulin and/or IGF resistance in β-cells leads to diabetes mellitus8, ways of sensitizing β-cells to insulin and/or IGF signalling might prevent β-cell loss and dysfunction in diabetes mellitus.

Replacing lost β-cells is another possible strategy to restore β-cell mass. Great efforts have been dedicated to transplant cadaveric human islets or *in vitro* differentiated pluripotentstem cell-derived islet-like clusters (SC-islets). However, this promising approach requires further optimization on graft delivery, graft–host interactions and graft survival, safety and function (Fig. 1). In a study by Aghazadeh et al. , adipose tissue-derived ready-made microvessels were used to circumvent some of these hurdles9. The co-transplantation of human SC-islets with microvessels butincreased graft survival, expansion and β-cell maturation (Fig. 1). These results were attributed to the efficient integration of the microvessels with the host vasculature system, which improved function of the grafted SC-islets to restore normoglycaemia in various diabetic mouse models. Importantly, microvessels also enhanced the survival of grafted primary human islets and boosted their function *in vivo*9. Despite these exciting findings, more refinements are still required. For example, combining an encapsulation device10 with microvessels could be a more efficient way of delivering and sustaining functional SC-islets and primary human islets through reducing graft rejection by the host immune system.

These studies have important implications for deciphering the pathomechanism of β-cell failure and developing new approaches to regenerate or replace them. Continuing advancements in single-cell multi-omics approaches,coupled with functional validations, will provide deeper insights into β-cell dysfunction in T1DM and T2DM. These advancements will find novel intervention targets for β-cell protection and/or regeneration, which together with improvements in regenerative medicine will improve quality of life in people with diabetes mellitus.

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**Competing interests**

The authors declare no competing interests.

**Figure 1:** **Novel approaches and targets for -cell protection, regeneration and replacement.**

Combining single-cell epigenomics of human pancreatic cells with GWAS data and multi-omics analyses of living human pancreatic biopsy samples revealed novel genes linked with β-cell failure in T1DM and T2DM. Identifying inceptor provides a new target for enhancing insulin signalling to restore β-cell mass. Co-transplanting SC-islets or primary human islets with ready-made microvessels enhances engraftment.