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Review

Genomics of Islet (Dys) function and Type 2 Diabetes

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Pancreatic islet dysfunction and beta cell failure are hallmarks of type 2 diabetes mellitus (T2DM) pathogenesis. In this review, we discuss how genome-wide association studies (GWASs) and recent developments in islet (epi)genome and transcriptome profiling (particularly single cell analyses) are providing novel insights into the genetic, environmental, and cellular contributions to islet (dys) function and T2DM pathogenesis. Moving forward, study designs that interrogate and model genetic variation [e.g., allelic profiling and (epi)genome editing] will be critical to dissect the molecular genetics of T2DM pathogenesis, to build next-generation cellular and animal models, and to develop precision medicine approaches to detect, treat, and prevent islet (dys)function and T2DM.

Lay of the Land: (Functional) Genomic Landscape of Islets and T2DM

T2DM is a complex metabolic disorder with both genetic and environmental components. It results from the dysfunction and loss of insulin-secreting beta cells in the endocrine pancreas (Islets of Langerhans) as they work to secrete more insulin to counteract insulin resistance in peripheral tissues (adipose, skeletal muscle, and liver). Ultimately, T2DM manifests as uncontrolled elevations in blood glucose levels. **GWAS** (see Glossary) have systematically identified hundreds of **single nucleotide variants** (SNVs), representing >150 regions of the genome (loci) [1], that are associated with T2DM risk and differences in T2DM-related quantitative metabolic traits, such as insulin, proinsulin, and glucose levels. Most (>90%) of these SNVs reside in noncoding regions of the genome. In parallel, functional (epi)genomics approaches to map open chromatin using **DNase I hypersensitive site sequencing** (DNase-seq), **assay for transposase-accessible chromatin sequencing** (ATAC-seq), and histone modification and transcription factor (TF)-binding patterns using **chromatin immunoprecipitation sequencing** (ChIP-seq) have identified genome-wide location of regulatory elements (REs), such as promoters, enhancers, and insulators, in >150 human cell types and tissues. T2DM SNVs are significantly and specifically enriched in islet-specific REs [2–7], suggesting that changes in islet RE activity and target gene expression are a common mechanism underlying the molecular genetics of islet dysfunction and T2DM [8] (Figure 1A). Indeed, recent studies have identified putative factors binding these REs and have detected allelic effects on their binding and target gene expression [9–11].

In this review, we discuss how recent studies are improving our understanding of how islet REs are perturbed by SNVs contributing to T2DM risk [1,12–19] and are elucidating the transcriptional underpinnings of islet responses to (patho)physiological environmental changes, such as aging, circadian rhythms, Western diet and lifestyle, as well as oxidative, endoplasmic reticulum (ER), and inflammatory stress responses [20–25]. We explore how studies applying next-generation sequencing (NGS) to profile individual cells are improving our comprehension of islet biology and reshaping our view of T2DM pathogenesis. Finally, we examine similarities and differences between mice and humans in the 'omics of islet function and T2DM (summarized in

Trends

T2DM is a multi-tissue metabolic disorder that results when pancreatic islets fail to compensate for insulin resistance in peripheral tissues.

Recent studies reaffirm the common variant origins of T2DM genetic risk. Variants overlap noncoding genomic regions, implicating regulatory defects in T2DM etiology.

Environmental stressors are associated with changes in gene expression programs leading to T2DM progression.

Single cell sequencing technologies permit investigation of islet cell type transcriptomes and epigenomes with single cell resolution and/or precision. Such methods provide greater insight into cell type-specific perturbations and their roles in T2DM.

Recent studies suggest that other cells (alpha, delta, and PP/gamma) in the islet have important roles in islet/and/or beta cell function, resilience, and T2DM pathogenesis.

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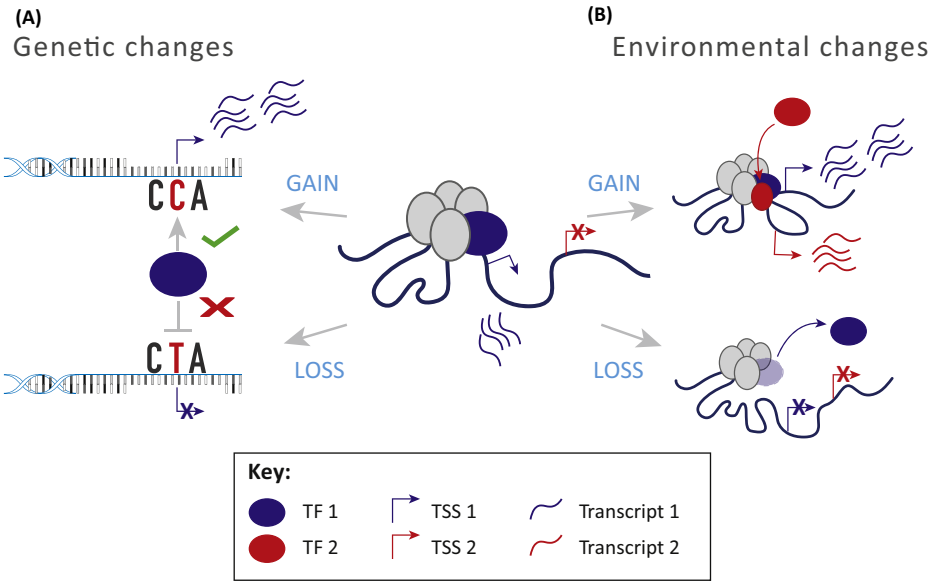

Figure 1. Genomic Effects of Genetic and Environmental Perturbations Contributing to Pancreatic Islet Dysfunction and Type 2 Diabetes Mellitus (T2DM). (A) DNA single nucleotide variants (SNVs) may enhance (gain-of-function) or diminish (loss-of-function) transcription element (e.g., enhancer) activity and islet gene expression. Most T2DM-associated SNVs reside in noncoding regions of the genome and overlap islet regulatory elements (REs) [2,3,12,14,15,32,47], implicating disruptions in gene regulatory network components as a central molecular feature in disease pathogenesis. A subset of SNVs has been linked to changes in basal islet gene expression [11,31]. (B) Environmental factors, such as inflammation, diet, aging, circadian rhythms, and stress, may also influence RE activity, resulting in altered and/or novel transcription of genes essential for islet function [20–25,48–50,57,58]. Abbreviations: TF, transcription factor; TSS, transcription start site

Figure 2, Key Figure). Throughout, we highlight future challenges and opportunities and offer perspectives on how these recent developments set the stage for precision medicine approaches to understand, treat, and prevent T2DM.

Homing in on T2DM Genetic Risk and Architecture

Since initial T2DM GWAS reports in 2007 [26–29], the list of genomic loci in which sequence variation contributes to T2DM risk and variability in quantitative measures of pancreatic islet function has grown to over 150 [1,14,30]. Associated SNVs at each locus contribute modestly to increased T2DM risk [odds ratios (OR) 1.05–1.75]. Together, these loci only explain a fraction of T2DM heritability [13,14]. Genetic consortia continue to dissect the genetic architecture of T2DM using larger cohorts with increasing ethnic diversity and/or representation. Recent efforts have reported [12,14,30,99] fewer 'new' T2DM loci (N=10) than previous studies. Importantly, however, they are refining the genetic signals at known (previously associated) T2DM loci to define **'credible sets' of single nucleotide polymorphisms** (SNPs) that are the most probable causal and/or functional SNPs driving the association and, consequently, the resulting molecular and/or phenotypic consequences.

The GOT2D and T2D-GENES consortia sought to identify less common SNVs (0.1%<MAF<5%) with larger effect size that may underlie common variant associations or may account for some of the T2DM 'missing heritability' using a combined whole-genome sequencing (WGS), exome sequencing, and genotype imputation approach [14]. These efforts identified protein-coding variants and/or mutations that are the most likely causative variant or effector transcripts for 12 out of 78 GWAS loci, confirming five nominated in previous studies (*PPARG, KCNJ11-ABCC8, SLC30A8, GCKR,* and *PAM* loci) and identifying seven

Glossary

Assay for transposase-accessible chromatin sequencing (ATAC-seq): a technique used to profile regions of open chromatin from small cell numbers.
Chromatin immunoprecipitation sequencing (ChIP-seq): a method used to study DNA–protein interactions.
Chromatin interaction analysis by paired-end tag sequencing (ChIA-PET): a method used to study 3D chromatin interactions genome wide.
CpG sites: areas of DNA containing a cytosine nucleotide directly linked to a single phosphate group and guanine nucleotide. These sites are often methylated and influence transcription.
Credible sets of SNPs: lists of sequence variants with 95% posterior probability of containing a/the causal disease-associated SNP (s) [99].
Deconvolution: a statistical framework to resolve a heterogeneous mixture into its constituent elements.
Dedifferentiation: the process in which a mature differentiated cell type reverts to an earlier developmental and/or precursor state.
DNA methylation: molecular process wherein a methyl group is covalently attached to a DNA base without altering the DNA sequence.
DNase I hypersensitive site sequencing (DNase-seq): a method used to characterize regulatory and open chromatin regions of the genome.
Expression quantitative trait loci (eQTL): approach to link sequence variation at a position in the genome to expression of target gene(s).
Genome-wide association study (GWAS): statistical association of sequence variation with disease risk or variability in a measurable phenotypic trait and/or feature.
Glycated hemoglobin (HbA1C): a type of hemoglobin modification that is measured to determine plasma glucose concentration.
RNA-sequencing (RNA-seq): measures the amount of RNA in a sample at a given time.
Single nucleotide polymorphism (SNP): nucleotide variation at a specific location in the genome that exists with >5% frequency in the population.

new ones (*FES, TM6SF2,* and *RREB1* in the *PRC1, CILP2,* and *SSR1* loci, respectively, and *TSPAN8, THADA, HNF1A,* and *HNF4A*). For the remaining loci, noncoding SNVs constitute the putative causal SNVs. Comparison of multiple genetic models with the empirical data generated in this study suggest that a long tail of common variants with lower effect sizes may comprise the missing heritability and reaffirms the importance of common, regulatory variation in the genetic architecture of T2DM (see Outstanding Questions). Perhaps most importantly, this immense effort has narrowed the list of putative causal SNVs to a handful for five loci and by 50% on average for the 78 T2DM-associated autosomal loci investigated [14]. Similar themes and reductions in credible sets were reported for fasting glucose- and insulin-associated loci [30].

Ongoing islet epigenomic and transcriptomic analyses are progressively defining the regulatory potential of variant loci, identifying SNV-RE overlaps, and nominating potential target genes, whose dysfunction is likely to contribute to T2DM [2,3,11,12,14,15,30–32]. Open chromatin (DNase-seq, ATAC-seq) and histone modification and/or TF-binding profiling (ChIP-seq) indicate that T2DM and related trait-associated SNVs are especially prominent in islet distal REs and **stretch/super enhancers** [2,3,5,33,34]. Due to the long distances over which REs might act, additional work to elucidate the target genes of T2DM SNV-containing REs is needed. Chromosome conformation capture techniques, such as 3C, 4C, 5C [35], Hi-C [36], **chromatin interaction analysis by paired-end tag sequencing** (ChIA-PET) [37], and HiCHIP [38] will be important components to effectively map interactions between REs and their target genes (see Outstanding Questions). In two separate studies, **RNA-sequencing** (RNA-seq) of 89 [31] and 118 [11] human islet samples identified 616 and 2341 **expression quantitative trait loci** (eQTLs), respectively. These analyses were the first studies linking SNVs to gene expression changes in islets to define the putative genetic control of islet function and failure. However, of the 216 eQTLs common to both studies, only 14 overlapped with T2DM-associated loci [11]. This may be due to power limitations and an inability to detect eQTLs beyond their primary signal. Alternatively, this relatively low overlap could suggest that T2DM SNVs affect islet physiological or pathophysiological responses, not just basal expression, as has been measured to date. Indeed, a recent study suggested that several putative T2DM GWAS genes are regulated by NFAT, a TF involved in calcineurin signaling responses [39]. Alternatively, the detection of eQTLs overlapping T2DM-associated SNVs in peripheral tissues, such as skeletal muscle [40] and adipose [41] tissue, reminds us that these other metabolic tissues should not be ignored in the T2DM molecular genetics and pathogenesis, and warrant further investigation of genomic variation in these tissues.

Recent islet studies suggest that regulatory noncoding RNAs (ncRNAs) contribute to diabetes progression and beta cell (dys)function [31,42,43]. Aberrant expression of 17 long noncoding (lncRNAs) has been associated with **glycated hemoglobin** (HbA1c) levels [31]. This study identified eQTLs for two of these transcripts (*LOC283177* and *SNHG5*), but the eQTL SNVs did not overlap with T2DM SNVs [31]. Similarly, a study by Morán and colleagues identified nine out of 55 T2DM-associated loci that contained lncRNAs located within 150 kb of, but not directly overlapping, the reported lead SNVs [42]. In the *KCNQ1* locus, T2DM risk SNVs overlap both *KCNQ1* and *KCNQ1OT1* [43,44], a long intergenic noncoding RNA (lincRNA) also found to be significantly induced in T2DM islets [42]. We anticipate that additional links will emerge in the coming years. Other studies suggest that islet lncRNA alterations could also contribute to type 1 diabetes mellitus (T1DM), because a T1DM GWAS SNV (rs941576) was identified in the *MEG3* lincRNA locus [43,45]. Functional analyses in human islets and rodent models will clarify the roles of these ncRNAs in islet development, (dys)function, and diabetes.

DNA methylation studies of nondiabetic (ND) and T2DM islets have suggested that epigenetic dysregulation promotes T2DM development [46,47]. DNA methylation profiling of 15 T2DM and

Single nucleotide variant (SNV): changes in a given nucleotide sequence in the genome.
Stretch/super enhancers: extended (>3 kb) regions of the genome marked by enhancer chromatin states; enriched near genes that are important for cell type identity and cell type-specific functions.
Subpopulation: a subset of cells within a tissue distinguished by the expression of specific marker genes and/or proteins.
***Trans*-differentiation:** the process in which a mature cell type converts into another mature cell type.

Key Figure

Converging and Diverging Genetic, Environmental, and Cellular Aspects of Islet (Dys)function and Type 2 Diabetes Mellitus (T2DM) in Mice and Humans

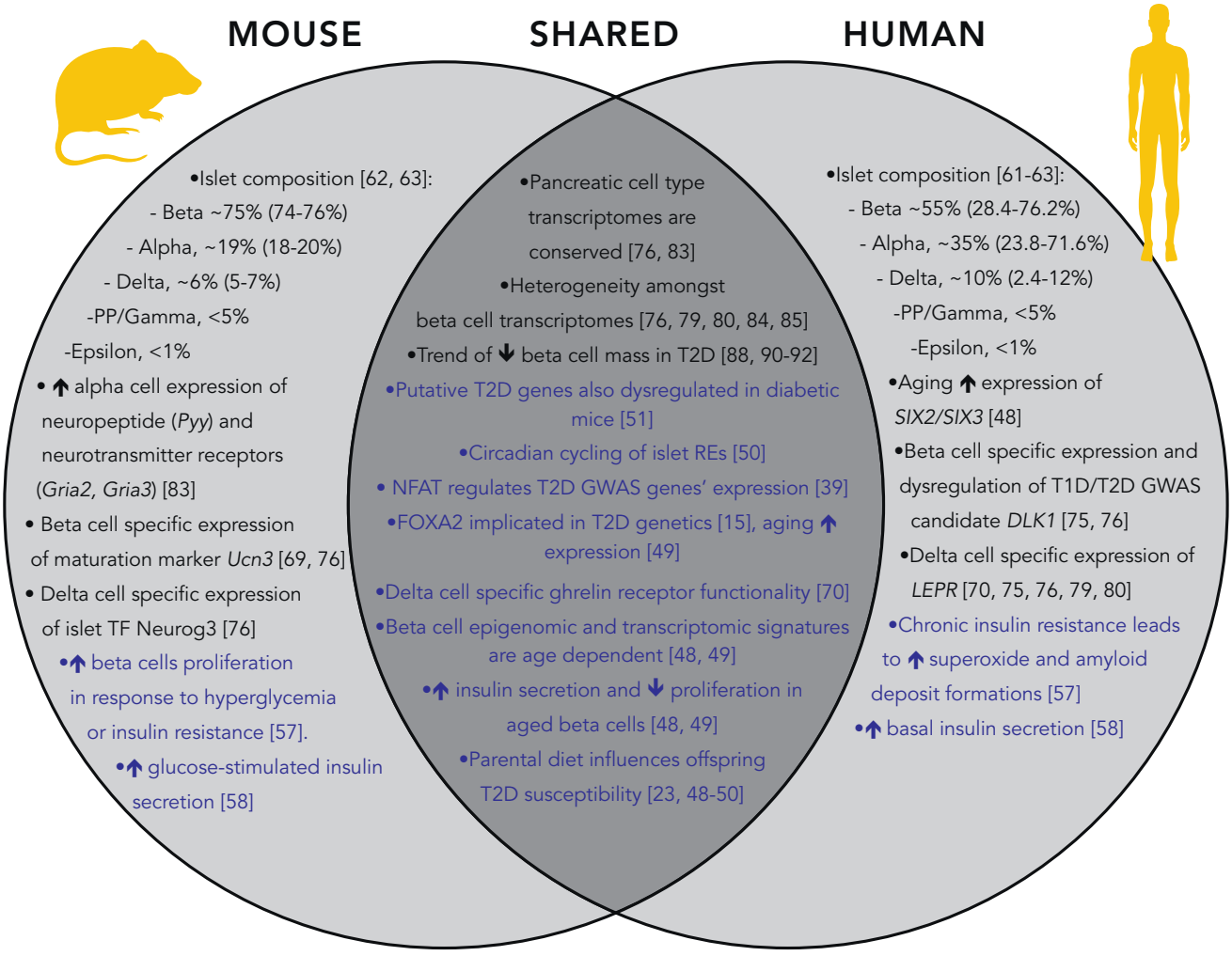

Figure 2. Parallel analyses of human and mouse islets are revealing important similarities [15,23,39,48–51,70,76,79,80,83–85] (A) and differences [48,57–58,61–63,69-70,75-76,79–80,83] (B,C) between molecular features of islet identity and (dys)function in mice and humans. Black text highlights significant findings regarding islet cellular composition and identity. Blue text highlights longitudinal and/or comparative analyses of genome-wide molecular data sets and environmental effects on islet (dys)function. These features reaffirm the value of modeling T2DM in mice to delineate important species-specific differences in islet biology that may reflect distinct T2DM causative mechanisms. Abbreviations: ↑, increase; ↓, decrease; GWAS, genome-wide association study RE, regulatory element; TF, transcription factor; T1DM, type 1 diabetes mellitus

34 ND islets using the Illumina 450BeadChip identified 1649 differentially methylated **CpG sites** for 853 genes, 17 of which reside in T2DM-associated loci [46]. Surprisingly, most (97%) of these CpG sites were hypomethylated in T2DM islets, suggesting that they suffer from decreased methyl donor levels or decreased activity of DNA methyltransferases.

Genomics of Islet Responses to Environmental Changes and T2DM Pathogenesis

Intrinsic and extrinsic environmental changes, such as aging, and Western diet and/or lifestyle, respectively, are linked to islet dysfunction and T2DM risk [23,48–50] (Figure 1B). Multiple groups have begun to characterize the genomic effects of these environmental inputs and insults on islets. Transcriptome profiling of adult and juvenile islet beta cells identified 565 (209 up, 356 down) and 6123 (2083 up, 4040 down) differentially expressed genes in humans and mice, respectively [48,49]. Signatures of decreased proliferative capacity in aged islets and/or beta cells were apparent in both species, perhaps best illustrated by increased *CDKN2A/B* expression, a gene cluster with established cellular senescence functions and implicated as 'Type 2 Diabetogenes' for a T2DM GWAS signal on 9p21 [48,49,51]. Unexpectedly, transcriptome and epigenome signatures suggested superior insulin secretory capacity of adult islets, which was confirmed functionally by glucose-stimulated insulin secretion (GSIS) assays [48,49]. DNA methylation and histone profiling indicated that these expression differences were largely mediated by chromatin remodeling and epigenetic modification of distal Res, such as enhancers. Using whole-genome bisulfite sequencing (WGBS), Avrahami and colleagues identified approximately 14 368 aging-related differentially methylated regions (DMRs) between the beta cells of juvenile and adult mice. DMRs overlapping distal REs outnumbered those overlapping promoters 3:1, and exhibited larger changes in magnitude of methylation. Distal DMRs that lost methylation with aging were enriched for binding sites of important islet TFs, such as Foxa2, Neurod1, and Pdx1, suggesting these factors mediate the expression differences and improved functionality in adult islets. Finally, genes showing differential expression in adult islets were accompanied by differential methylation at nearby distal REs more often than at their promoters. These data suggest that, in addition to their importance in T2DM genetic risk, enhancers also govern important transcriptional regulatory changes accompanying or mediated by aging.

Circadian rhythm links behavior and metabolism to day–night cycles. Notably, insulin secretion oscillates with a circadian periodicity. Analysis of mouse islet transcriptomes revealed that approximately 27% of the beta cell transcriptome (*N*=3905 genes) demonstrated circadian oscillation, including genes responsible for insulin synthesis, transport, and stimulated exocytosis [50]. The human orthologs of 481 of these genes exhibited circadian oscillations in human islets. ChIP-seq identified 742 out of 3905 of these oscillatory genes as direct targets of the circadian clock TFs CLOCK and BMAL1. As with aging, most differential sites were at distal REs. Beta cell-specific deletion of *Bmal1* resulted in islet failure and diabetes in mice. This study demonstrates the importance of circadian rhythms in islet function and suggests that genetic or environmental perturbation of this program contribute to T2DM risk and pathophysiology. GWAS results suggest that this could be the case, because SNVs in the *CRY2* locus, a component of the circadian machinery, and *MTNR1B,* a gene encoding a melatonin receptor, are associated with altered islet function and T2DM [1,52]. It will be interesting to see whether genetic perturbations in circadian clock TFs or their binding sites emerge as one of the molecular mechanisms underlying T2DM GWAS.

Maternal nutrition and *in utero* stresses have been linked to T2DM risk for offspring in humans and rodents [23,53–55]. Although changes in fetal nutrition are suggested to influence offspring metabolism via epigenetic modifications [23,56], the genome-wide effects on the islet (epi) genome have not been determined. Similarly, stress responses to elevated oxidative and/or ER stress lead to islet failure, impaired insulin secretion, and T2DM susceptibility [57–59]. Ultimately, these responses converge on the nucleus and involve the redistribution or covalent modifications of master TFs (MAFB, NKX6-1, and PDX1) or stress response factors (FOXO1, ATF4, and HIF1alpha) [20,22,53,57,58]. (Epi)genomic analyses of these stress responses are warranted and may reveal important connections between T2DM SNVs and altered islet stress

responses. Moving forward, it will be crucial to understand the extent to which genetic and epigenetic changes interact in T2DM pathogenesis (see Outstanding Questions). Response QTL (reQTL) and epigenome-wide association studies (EWAS) [56] should provide these important links (see Outstanding Questions). Indeed, studies of SNV effects on immune cell responses identified 121 reQTLs, 38 of which overlapped autoimmune disease-associated SNVs [60]. Specific factor(s) and pathway(s) activated by insulin resistance appear to differ between mouse and human islets [57,58] (Figure 2); thus, we emphasize that caution must be taken in study design and interpretation to interrogate this and possibly other islet responses.

Deconstructing Pancreatic Islet Cellular and/or Functional Heterogeneity

Islets comprise 1–5% of the pancreas and consist of at least five endocrine cell types performing coordinated but distinct functions and each producing a unique hormone in the islet: beta (insulin), alpha (glucagon), delta (somatostatin), gamma (pancreatic polypeptide), and epsilon (ghrelin) cells [61–64]. Precise understanding of islet molecular changes during T2DM development is likely complicated by variability in islet cell type composition. On average, islets comprise 55% beta cells, 35% alpha, 10% delta, and less than 5% and 1% gamma/PP and epsilon cells, respectively [61–63]. However, this can vary considerably between donors, with ranges of 28.4–76.2%, 23.8–71.6%, and 2.4–12% for beta, alpha, and delta cell compositions, respectively [61] (Figure 2). This cellular heterogeneity, combined with donor-to-donor variability, masks the molecular repertoire of each cell type and impedes clear understanding of the molecular programs perturbed in each cell type by T2DM pathogenesis.

Until recently, most studies had focused on epigenetic and transcriptional analyses of whole islets or, at the expense of other cell types, beta cells. However, recent studies demonstrating roles for alpha [65–67] and delta cells [68–71] in modulating beta cell function and/or resilience and in T2DM pathogenesis are fueling renewed interest in these cell types. First attempts to overcome these obstacles and understand the molecular repertoire of each islet cell type focused on transcriptomic analyses of sorted and enriched cell type populations [61,72–74]. However, such methods were unable to effectively isolate and enrich the less abundant nonbeta cells [75], leaving much of the functional genomic landscape of islets imprecisely assigned and/or classified or, in the case of rarer islet cell types, undefined.

Within the past year, multiple groups have applied single cell transcriptome profiling to islets to begin to address questions about islet physiology [75–83] (see Outstanding Questions) with single cell resolution, such as: (i) what is the gene repertoire of each islet cell type? (ii) Does the gene repertoire reveal any new and/or unexpected roles for each cell type in islet (patho) physiology? (iii) Are there novel cell types or unappreciated **subpopulations** in islets? These studies are providing new appreciation of the repertoire of both islet beta and nonbeta cells. Given that much of the beta cell transcriptional repertoire has been extensively studied [61,72–74], several features have been validated, including genes involved in cell survival and/or maturation (*PDX1*), regulation of insulin secretion (*RGS16, SYT13,* and *ENTPD3*), and diabetes-associated genes (*DLK1, MEG3,* and *SLC2A2*) [75,76,78–81,83]. Unique expression of genes encoding TFs (*IRX2*), membrane glycoproteins (*DPP4*), and hormone transporters (*TTR*) were also validated in alpha cells. Analysis of single alpha cell transcriptomes uncovered signatures involved in wound healing (*FAP*), blood clotting (*F10*), and tissue biogenesis (*LOXL4*) [75,76,78–81,83], suggesting that they share functions akin to pancreatic fibroblast and/or mesenchymal cells.

Single cell profiling has provided new views of the roles of delta and PP/gamma cells in islet physiology and the molecular genetics of islet failure and diabetes. For example, these studies revealed that delta cells uniquely express appetite-suppressing leptin (*LEPR*) and appetite-stimulating ghrelin (*GHSR*) hormone receptors [75,79,80], implicating them as the integrators

and regulators of these pathways in the islet. *GHSR* functionality has been demonstrated in both human and mouse delta cells [70]. *LEPR* expression is unique to human delta cells, suggesting that these cells uniquely mediate the leptin response in human islets [70,75,76,79,80] (Figure 2). Expression of genes associated with congenital hyperinsulinemia (CHI) (*UCP2* and *HADH*) in delta cells further implicates this cell type in the molecular genetics of CHI [75]. PP/gamma cell transcriptomes exhibited enrichment of genes involved in neuronal development (*MEIS2* and *FEV*) [75,78–80] and serotonin catalysis and reuptake (*TPH1* and *SLC6A4*) [75,79,80,83]. Together, these findings suggest that delta and PP/gamma cells act as the 'brains' of the pancreatic islets, capable of receiving and integrating various neuronal signals to coordinate islet function. Due to their scarcity in human pancreatic islets (<1% of islet volume), our knowledge of the epsilon cell repertoire and its putative function(s) remain speculative. Nonetheless, the insights gleaned from these initial studies undoubtedly motivate follow-up studies that continue transitioning from whole-islet to functional constituent cell studies. Identification of genes encoding cell type-specific surface markers (beta, *LRRTM3* and *CASR*; alpha, *DPP4* and *PLCE1*; delta, *LEPR*, *GHSR*, and *ERBB4*; PP/gamma, *SLC6A4* and *PTGFR*; and epsilon, *ANXA13*) [75,79] provide new targets that may be exploited for more accurate purification of each islet cell type and analysis of its specific responses to genetic and environmental stressors.

Islet Subpopulations and Cell Type Heterogeneity

Detection of heterogeneous beta cell subpopulations was reported for enriched cell and single cell studies. These include four subpopulations with differing expression of *ST8SIA1* and *CD9* [84], five subpopulations defined by *RBP4, FFAR4/GPR120, ID1, ID2*, and *ID3* expression [80], and subpopulations characterized by ER stress-associated [76] and oxidative stress-associated genes [79]. *Fltp/CFAP126* expression has been reported to distinguish proliferating and mature beta cell subpopulations in mice [85], but single cell transcriptome analyses failed to detect this distinction in human beta cells [75,83]. However, proliferative and mature human beta cells were identified by single cell mass cytometry analysis [86], suggesting that mice and humans make use of distinct cell growth pathways. Given that each study detected distinct beta cell subpopulations with different gene signatures, it remains difficult to distinguish whether these subpopulations are functionally distinct cells or the result of technical confounders, such as the time to sort and enrich in a harsh cell sorting environment, time for cell capture, or cell and transcript capture efficiency [87].

Single Cell Dissection of Islet Dysfunction and T2DM

Single cell transcriptome analyses provide a fresh and agnostic opportunity to investigate the putative mechanisms underlying islet dysfunction in T2DM. To date, single cell transcriptome profiling has been completed for a total of 1831 and 1970 islet cells from 26 ND and 15 T2DM donors, respectively [75,80,81,83]. Comparison of T2DM and ND single cell transcriptomes suggest that specific alterations in islet cell type transcriptomes underlie T2DM pathogenesis (Figure 3A). However, changes in cell proportions (Figure 3B), identity, and plasticity (Figure 3C, D) have also been regarded as potential contributors to T2DM [72,88–92]. Specifically, decreases in diabetic beta cell mass were suggested to be caused by reversion to endocrine progenitor (hormone-negative) cells (Figure 3C) or different islet cell types (Figure 3D) rather than to apoptosis. The model of transformed beta cell identity remains controversial. A recent study concluded that the observed magnitude of decline in beta cell numbers in T2DM islets is not accompanied by proportionate increases in cells exhibiting ***trans*-differentiation** markers or increases in other islet cell types [93]. Rather, the presence of endocrine progenitor-like cells in T2DM islets may represent newly forming endocrine cells [93]. Single cell profiling also did not identify transcriptomic evidence of **dedifferentiated** or *trans*-differentiated cells in T2DM islets (Figure 3C,D) [75,80,83]. Similar trends were observed in whole-islet RNA-seq data upon **deconvolution**, where cell type proportions did not significantly vary between hypoglycemic

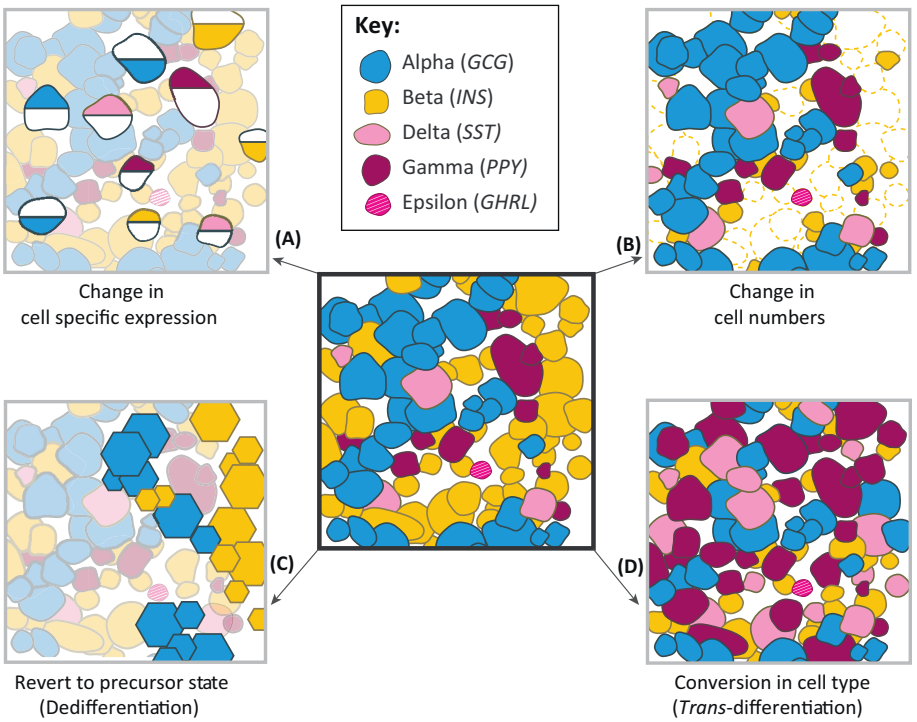

Figure 3. Proposed Cellular Mechanisms Contributing to Type 2 Diabetes Mellitus (T2DM) Development. (Center) Cartoon representation of human islet cellular composition. Studies have described the following phenomena: (A) Islet single cell transcriptomic studies [75,80,83] suggest that cell type-specific changes in gene expression (depicted as half-shaded cells) contribute to T2DM pathogenesis. These studies suggest that potential pathogenic expression changes occur in each islet cell type, not just beta cells. (B) Decreases in beta cell (in yellow) numbers [25,92,100,101], thought to precede islet dysfunction and development of insulin resistance. (C) Alterations in islet cellular identity may also account for islet failure. Dedifferentiation of islet cell types to precursor cell types and/or states (hexagons) has been proposed to underlie the loss of beta cell mass and function in T2DM [88,90–92]. (D) Similarly, *trans*-differentiation of islet cell types may lead to imbalances in islet cell proportions and improper function [72,88,89]

and hyperglycemic islets [76]. Thus, the transcriptome data to date do not provide supporting evidence of dedifferentiation in T2DM islets.

Transcriptomes of each cell type from ND and T2DM donors exhibited remarkable correlation overall. However, specific changes in gene expression were reported in T2DM beta cells, including reduced expression of *INS* [75,80], genes important for insulin secretion (*STX1A*) [75] and beta cell proliferation (*FXYD2*) [80,83], as well as elevated expression of genes implicated in T2DM GWAS (*DLK* and , *DGKB*) [75]. Transcriptional differences were also identified in T2DM alpha cells, including expression of *CD36* [75,80], a crucial activator of the NLRP3 inflammasome [94], and *RGS4,* a negative regulator of GSIS [80]. Several genes were dysregulated in T2DM delta cell transcriptomes [75,83]. However, the underlying biology of these candidates remains undefined, with no association with islet growth or function [83]. Aside from these encouraging examples, these single cell studies have not reached consensus regarding differentially expressed genes between T2DM and ND cell types. Differences in islet donor variability, islet isolation and/or transport, and single cell dissociation and/or sequencing protocols may explain these inconsistencies across studies. We expect that sampling thousands of single cells each from hundreds of individuals for large-scale meta-analyses will provide a more convergent list of cell type-specific genes and pathways disrupted in T2DM islets. It will also be important for future studies to profile cells from individuals at different points

along the T2DM pathogenesis spectrum, such as prediabetic individuals (5.5<$HbA1_c$<6.0) to identify and distinguish primary from secondary genomic changes that may be the cause or consequence of progression to T2DM.

Concluding Remarks and Future Directions

The past few years have marked exciting developments in our understanding of the underlying genomic, environmental, and cellular components driving T2DM pathogenesis. Numerous common (and only few rare) genetic SNVs have been implicated in T2DM progression [13,14]. It is unclear whether the 'missing T2DM heritability' is explained by a larger distribution of common SNVs with minimal effect sizes, whether current methods have missed critical rare SNVs, or whether it will be captured by gene–gene and gene–environment interactions (such as detected by reQTL). Thus far, most catalogued T2DM-SNVs occur in, and disrupt, islet RE function; however, the causal connections between the two remain challenging to decipher. eQTL and chromatin accessibility QTL (caQTL) [95,96] studies have been, and will continue to be, essential for linking genetic variants to molecular phenotypes. A subsequent challenge will be to link these molecular effects to pathways [39] and (patho)physiological phenotypes [97].

Functional genomic studies have identified minimal overlap between islet eQTLs and T2DM-SNVs [11,31], suggesting that responses to environmental stress factors are key mediators of T2DM pathogenesis. Mouse models have been instrumental in elucidating the genetic and molecular regulation of these responses and how environmental stressors influence islet (dys) function. However, observed differences between mice and humans in islet morphology, composition, expression, and function remind us to exercise caution when extrapolating findings in mice to human T2DM. Studies comparing the genomic features of human islets and models are essential to define conserved features and those that require modification to determine what aspects of islet dysfunction and T2DM we can model effectively and to decide how and/or where we should manipulate or humanize the mouse (epi)genome to better model human T2DM. (Epi)genome editing technologies, such as CRISPR/Cas9, can then be applied to develop new cellular and animal models to more effectively study islet phenotypic changes resulting from genetic and environmental variation. We anticipate that these integrative genomic studies and techniques will also serve as valuable resources to determine the underlying genetic changes and mechanisms of beta cell dysfunction that lead to T1DM [98].

Rapid developments in single cell NGS technologies have renewed interest in the less-studied islet cell types. Deconstructing the major molecular changes that occur in each cell type during T2DM progression has proven challenging, yielding inconsistent results between studies due to patient donor variability and technical sequencing artifacts. This is also likely the result of limited statistical power. In the future, it will be interesting to perform meta-analyses of available transcriptomic data to maximize our confidence of changes in cell specific expression programs. Together, the innovative new genomic technologies of the past few years will allow us to more precisely define, model, and manipulate the genes and pathways that have gone awry in T2DM, with the ultimate goal of designing novel therapeutic approaches.

Outstanding Questions

T2DM-associated GWAS variants explain only a small portion of T2DM heritability, with rare variants showing minimal contribution. Does a long tail of common variants with small effect sizes explain this missing heritability? Or are we simply 'underpowered' to detect rare variants and their contribution to T2DM heritability?

What are the genes targeted by T2DM GWAS sequence variant (SV)-containing regulatory elements? Are these links context specific? Does the risk allele enhance (gain-of-function) or repress (loss-of-function) RE function?

How do the transcriptomes and/or epigenomes of islets and islet cell types change when subjected to variable environmental stressors (oxidative stress, inflammation, diet, etc.)? How are they changed by intrinsic (aging, circadian rhythms, etc.) environmental factors? Which SVs regulate and alter these islet responses?

What are the precise cellular and molecular pathophysiological changes in each cell type that lead to T2DM? Are the major pathological changes beta cell specific or do they involve other islet cell types and/or non-islet cell types?

How many islet and single cell samples must be obtained to effectively capture combined cell type heterogeneity while controlling for technical and experimental confounders? How many samples are needed to observe genetic and/or epigenetic differences between T2DM and ND states? Would stratification of islets by T2DM risk genotype improve cell type-specific T2DM signatures?

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