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Review

Importance of Both Imprinted Genes and Functional Heterogeneity in Pancreatic Beta Cells: Is There a Link?

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Abstract: Diabetes mellitus now affects more than 400 million individuals worldwide, with significant impacts on the lives of those affected and associated socio-economic costs. Although defects in insulin secretion underlie all forms of the disease, the molecular mechanisms which drive them are still poorly understood. Subsets of specialised beta cells have, in recent years, been suggested to play critical roles in "pacing" overall islet activity. The molecular nature of these cells, the means through which their identity is established and the changes which may contribute to their functional demise and "loss of influence" in both type 1 and type 2 diabetes are largely unknown. Genomic imprinting involves the selective silencing of one of the two parental alleles through DNA methylation and modified imprinted gene expression is involved in a number of diseases. Loss of expression, or loss of imprinting, can be shown in mouse models to lead to defects in beta cell function and abnormal insulin secretion. In the present review we survey the evidence that altered expression of imprinted genes contribute to loss of beta cell function, the importance of beta cell heterogeneity in normal and disease states, and hypothesise whether there is a direct link between the two.

Keywords: genomic imprinting; methylation; beta cell function; type 2 diabetes; diet; beta cell heterogeneity; pancreatic islets; single-cell transcriptomics; 'hub' cells; beta cell connectivity

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1. Introduction

Adequate insulin secretion is a sine qua non for the effective control of blood glucose in mammals and defects in this process are involved in all forms of diabetes mellitus. Thus, whilst type 1 diabetes involves the immune-mediated destruction of beta cells [1], residual beta cells often remain, especially in those diagnosed later in life [2] but are unable respond adequately to stimulation with glucose. In type 2 diabetes (T2D), the beta cell mass is thought to remain mostly intact [3] but to become largely refractory to stimulation with glucose [4]. Though certain studies [5] report up to 50% beta cell "loss" in individuals with T2D, it should be emphasised that the histological studies on which these conclusions are based are not prospective (i.e., involving measurements in the same individual). Consistent with the preservation of a substantial number of beta cells, responses to non-glucose stimuli, including certain amino acids, are often largely preserved in subjects with T2D [6].

Healthy beta cells respond to glucose through the uptake of the sugar via glucose transporters (GLUT2/SLC2A2 in rodents, GLUT1-3 in man [7]), phosphorylation of the sugar by a low affinity hexokinase, termed glucokinase (encoded by the *GCK* gene [8]) and enhanced flux through glycolysis [4]. Efficient mitochondrial oxidative metabolism, achieved in part through the absence of alternative metabolic fates for glucose carbon [6,9] then drives increases in ATP/ADP ratio in the cytosol [10] which close ATP-sensitive K^+ channels [11], depolarising the plasma membrane to facilitate Ca^{2+} entry through voltage-gated Ca^{2+} channels [4]. The latter then activate the release of stored insulin from secretory granules through regulated exocytosis. Other, less well-defined, "amplifying" glucose signalling mechanisms potentiate the actions of Ca^{2+} on the granules [12–14].

Compromised beta cell glucose metabolism and the misexpression of critical transcription factors and glucose sensors such as GLUT2/SLC2A2 [15,16] appear to underlie the changes which suppress normal glucose sensing in the islet in T2D. Moreover, re-expression of genes which are usually expressed at low levels (including those which are selectively "disallowed" in the beta cell, but strongly expressed in most other tissues, including *Ldha* and the lactate/pyruvate transporter MCT-1/*Slc16a1* [17]) and markers such as *Aldh1a3* [18] may also be involved in rendering beta cells "blind" to stimulation.

Heterogeneity and connectivity have emerged in recent years as important aspects of the healthy beta cell population [19] and may be compromised in both type 2 [20] and type 1 [21] diabetes. Importantly, subsets of beta cells which appear to be highly connected to other cells within a cellular network [22,23], and to influence overall islet-wide dynamics, appear to be potential targets for dysfunction in both disease settings. The molecular mechanisms leading to the establishment of an apparent beta cell "hierarchy" are poorly defined. Epigenetic changes, including alterations in DNA methylation [24], represent one possibility.

In the present review, we discuss the importance of both imprinted gene expression and functional heterogeneity in beta cells, and hypothesise that changes in imprinted gene expression may be involved in, and contribute to, the loss of beta cell function and heterogeneity in the settings of nutrient excess and T2D.

1.1. Genomic Imprinting

Genomic imprinting is an epigenetic phenomenon resulting in monoallelic and parent-of-origin specific gene expression [25]. The specific requirement for the individual contribution from both the male and female germlines was demonstrated in pioneering studies in the 1980s by showing that pronuclear transfer to create newly-fertilised gynogenetic (two copies of the maternal genome) or androgenetic (two copies of the paternal genome) mouse oocytes did not produce viable embryos [26–30]. Further studies later revealed specific regions of the genome where the presence of two maternal or two paternal chromosomal copies (known as uniparental disomy, UPD) resulted in abnormalities in early growth, development and viability, and thus formed the basis for our understanding of these so-called "imprinted regions" of the genome. The phenomenon of genomic imprinting, as well as its appearance alongside the manifestation of the placenta in mammals, have led to a number of hypotheses regarding the evolutionary advantage of "imprinting" this small subset of mammalian genes. Major examples include "parental conflict" [31] between the two genomes whereby the maternal and paternal genomes are centered around resource conservation and resource extraction, respectively, particularly during early growth and development. Co-adaptation between the mother and her offspring, stipulating that genomic imprinting may benefit their interaction, in terms of the fitness of both individuals, has also been suggested [32].

Allele-specific epigenetic control at specific imprinted *loci* is predominantly mediated by differences in methylation of cytosine residues (at the carbon-5 position) at cytosine-guanine dinucleotides or "CpGs" (reviewed extensively in [33,34]). Silencing of one parental allele is governed by an imprinting control region (ICR), that is a differentially-methylated region (DMR) often controlling multiple imprinted genes within a single genomic cluster [35,36]. The overall result is monoallelic expression of a subset of "imprinted" genes (~150 identified to date) that are defined by expression solely driven from either the paternal or maternal allele. As the epigenetic regulation at imprinted *loci* is re-established in the germline, genomic imprinting is carried through to the next generation in a transgenerational manner.

1.2. Human Imprinting Disorders

Imprinted genes are expressed in several metabolic systems (muscle, adipose, hypothalamic–pituitary–adrenal (HPA) axis and pancreatic beta cells), particularly at early (fetal, neonatal and postnatal) stages where they regulate a diverse range of cellular processes that ulti-

mately mediate key physiological parameters such as growth, development, metabolism and behaviour [37]. It is, therefore, not surprising that a number of disorders exist in humans due to large chromosomal duplications (therefore leading to either paternal or maternal UPD), specific point mutations or mutations to genomic regions critical for epigenetic control of an imprinted locus (e.g., an ICR) [38–42]. Imprinting disorders are typically characterised by perturbed growth and development, particularly in early life, and are associated with "failure to thrive" phenotypes. Furthermore, mutant mouse lines that model human imprinting disorders generally recreate clinical features described in patients and demonstrate that even a two-fold alteration in imprinting gene expression has phenotypic consequences similar to those observed in humans [40,41]. Examples of imprinting disorders include Prader–Willi Syndrome and Angelman Syndrome, two conditions associated with major developmental and metabolic abnormalities caused by genetic disruption at 15q11-13 [43,44]. Angelman syndrome is most likely caused by disruption of the *UBE3A* gene either through direct *UBE3A* point mutations or via paternal UPD, as reviewed in [45]. Inversely, Prader–Willi Syndrome results from the deletion of paternal 15q11-13, maternal 15q11-13 UPD or ICR disruption reviewed in [46], with both disorders highlighting the importance of maintaining correct gene dosage at specific imprinted *loci*. Similar imprinting disorders have been identified with overlapping "failure to thrive" phenotypes (e.g., feeding problems, growth restriction or overgrowth, developmental delays, metabolic syndrome etc) and underlying genetic abnormalities at imprinted *loci*, for example in the cases of Silver–Russell Syndrome and Beckwith–Weidermann Syndrome (11p15.5 or "ICR2" containing *CDKN1C* and *KCNQ1*) [47,48] and Temple Syndrome (14q32.2 containing *DLK1* and *MEG3*) [49]. Importantly in the context of an impact on beta cell function and diabetes, Transient Neonatal Diabetes Mellitus (TNDM) is associated with paternal UPD of chromosome 6q24 [50–54] (containing the *PLAGL1*/*ZAC* gene) or modified methylation at the maternal allele [55,56] with overexpression of *Plagl1* in mice mimicking the impaired glucose homeostasis at the neonatal stage [57].

1.3. Imprinted Genes and Pancreatic Beta Cells

Importantly, a significant number of imprinted genes play key functional roles in beta cells, both during their early development and in the adult [58]. These include regulation of insulin secretion (*Nnat* [59,60], *Plagl1* (*ZAC1*) [57,61]), beta cell mass (*Cdkn1c* [62,63], *Dlk1* [64], *Peg3* [65], *Grb10* [66,67], *Rasgrf1* [68]) and epigenetic regulation (*Gtl2* (*MEG3*) [69,70], *H19* [71]) (Table 1). Interestingly, imprinted gene expression is deregulated in subclones of stable mouse-derived MIN6 beta cells that are "poorly responsive" in terms of insulin secretion to glucose and other secretagogues compared with "highly responsive" MIN6 subclones [72], and in pancreatic islets from T2D vs non-diabetic subjects [66,70,73–75]. Furthermore, single nucleotide polymorphisms (SNPs) at imprinted *loci* in multiple human cohorts are associated with T2D, possibly due to altered methylation at these genomic regions [76–79].

Table 1. Effect of modified imprinted gene expression on the function of mouse and human pancreatic beta cells.

Imprinted Gene	Proposed Functional Role in Beta Cells	Effect of Altered Expression in Beta Cells	References
Cdkn1c	Cell cycle control	Increased beta cell replication upon knockdown in human islets	[62,63]
Dlk1	Cellular differentiation	Overexpression resulted in differentiation of human pancreatic ductal cells into beta-like cells and an increase in insulin secretion	[64]
Grb10	Receptor tyrosine kinase adaptor protein	Knockdown in human islets reduced insulin secretion. However, increased beta cell mass, insulin secretion and improved whole body glucose tolerance in knockout mice	[66,67,80]

Table 1. *Cont.*

Imprinted Gene	Proposed Functional Role in Beta Cells	Effect of Altered Expression in Beta Cells	References
Gtl2	Long non-coding RNA	Knockdown in stable mouse beta cells increased sensitivity to cytokine-mediated oxidative stress	[70]
H19	Long non-coding RNA	Knockdown decreased rat beta cell expansion	[71]
Nnat	Mediator of preproinsulin processing	Knockout in mice leads to reduced beta cell insulin content, glucose-stimulated insulin secretion (GSIS) and glucose tolerance	[59,60]
Peg3	Zinc finger protein, regulates apoptosis	Viral-mediated knockdown in vitro activates beta cell cycling	[65]
Plagl1	Zinc finger protein, suppresses cell growth	Transient neonatal diabetes upon overexpression of *Plagl1* in mice	[52,57]
Rasgrf1	Guanine nucleotide exchange factor	Knockout in mice leads to reduced beta cell mass, hypoinsulinaemia and impaired glucose tolerance	[68]

1.4. Beta Cell Heterogeneity

All beta cells are not equal, and within the same islet, individual beta cells display functional heterogeneity. Early evidence for beta cell heterogeneity was provided as long ago as 1986, using a haemolytic plaque assay developed to visualise insulin release from dispersed rat islet cells. This approach showed that beta cells are heterogeneous in terms of their ability to secrete insulin [81]. These experiments also provided evidence that cell-to-cell adhesion and/or junctional communication regulate hormone secretion from individual beta cells [81]. Other studies demonstrated intercellular differences in the secretory activity of glucose-stimulated beta cells, both in terms of glucose sensitivity and amplitude of insulin secretion. Furthermore, these highly sensitive beta cells were shown to release insulin in greater quantities at the same glucose concentration when compared to less glucose-sensitive cells [82–84]. Moreover, repeated stimulation with high glucose showed that individual rat beta cells from dispersed rat islets demonstrate/retain distinct and lasting secretion patterns, indicating that their excitability level remains unchanged, at least acutely [85]. Accordingly, insulin secretion from human beta cells is also heterogeneous and appears to be dependent on cell-to-cell contact [86]. Interestingly, a subset of beta cells that were poorly responsive to glucose still secreted insulin in response to other stimuli such as tolbutamide or glucagon-like peptide 1 (GLP-1) [87,88].

The molecular mechanisms that lie behind beta cell functional heterogeneity involve regulation of cellular glucose sensing and differential activity or expression of factors and pathways contributing to insulin secretion in response to glucose. For instance, variation in the expression of glucokinase (*Gck*), the flux-determining enzyme for beta cell glycolysis [4], is observed between individual beta cells from rat islets [89]. Accordingly, highly responsive beta cells have a 60% increase in glucokinase activity versus weaker responders [90]. In mouse islets expressing GFP under the control of the insulin promoter, GFP-"bright" cells (i.e., signifying a highly active insulin promoter) accounted for ~20% in comparison to GFP-"medium" cells that represented ~70% of the beta cell population [91]. Indeed, both GFP-"bright" and GFP-"medium" beta cells contained higher insulin mRNA levels and a higher secretion index when compared to GFP-"low" beta cells [91].

To further characterise beta cell heterogeneity in the human context, a study using dissociated human islets showed that four antigenically-distinct subtypes of beta cells could be identified [92]. Beta cells subpopulations designated "β1-4" display differential expression of ST8SIA1 and CD9, as well as different transcriptional signatures in general, with some of the differentially-expressed genes associated with beta cell maturation, glucose

metabolism and insulin secretion [92]. These subpopulations are always present in normal adult human islets, and interestingly, the authors also described that the distribution of these beta cell subtypes is altered in T2D islets, demonstrating that beta cell heterogeneity is functionally relevant [92]. Of note, other antigenic markers have been described, such as polysialylated-neural cell adhesion molecule (PSA-NCAM) in rat beta cells, separating two populations that differ notably in their insulin secretion as well as *Gck* and *Glut2*/*Slc2a2* expression levels [93].

Another criterion of beta cell heterogeneity is whether a given subgroup has the capacity to proliferate. A study in 2016 showed that Flattop (*Fltp)*, a Wnt/planar cell polarity effector, acts as a marker gene to distinguish a subpopulation of proliferating beta cells from more mature (quiescent) beta cells, and that these two populations had distinct molecular and physiological signatures [94]. Though *Fltp* in itself is not necessary for beta cell development, proliferation or maturation, *Fltp*-positive cells showed higher insulin secretion, a lower number of immature insulin granules and higher mitochondrial function, as well as higher expression of genes involved in glucose metabolism and lower proliferation rates [94]. As shown by the several studies above, heterogeneity between beta cells can be observed at the transcriptomic level, and the impressive and rapid development of single-cell RNA sequencing technologies in recent years has allowed us to explore whole genome mRNA expression levels at cellular resolution. Despite some obvious technical limitations due to factors such as sample size, dropout effects and the requirement for efficient computational analysis methods [95–97], studies using single cell mRNA sequencing to explore the beta cell transcriptome have no doubt played a major role in bringing new insights for beta cells identity and heterogeneity.

The first studies that performed single cell sequencing from human pancreatic islets of healthy donors and T2D patients were stepping-stones in terms of assessing beta cell heterogeneity in the context of diabetic states and also validated previously described marker genes for endocrine cell types within the islet [98,99]. However, a limitation of these studies was the number of sequenced cells, notably beta cells. Although Li et al. explored cellular heterogeneity using a separate principal component analysis (PCA) for each cell type, the number of cells was ultimately too low to distinguish clear cellular subpopulations amongst cell types [98].

Shortly afterwards, two back-to-back studies [100,101] sequenced 2209 and 1492 single cells, respectively, from human islets of healthy and T2D donors. In addition to the large degree of overlap of gene expression for both alpha and beta cells when compared between the two studies, the number of cells sequenced also allowed Segerstolpe et al. [100] (but not Xin et al. [101]) to identify subpopulations within endocrine cell types, including beta cells. Sub-clustering of beta cells revealed five clusters with combinational expression of *RBP4*, *FFAR4*/*GPR120*, *ID1*, *ID2* and *ID3* [100] and, of note, cells of all five clusters expressed insulin (*INS*) at similar level. A later study also showed heterogeneity amongst human beta cells, in terms of the regulation of genes relating to functional maturation (*UCN3*) and ER stress (*HERPUD1*, *HSPA5* and *DDIT3*) [102]. A similar study assessed beta cell heterogeneity using an algorithm to detect outliers within the beta cell population to show that the most significant genes differentially expressed between beta cell subtypes were *SRXN1*, *SQSTM1* and three ferritin subunits, genes notably highly expressed in one of the clusters and implicated in the response to the ER and oxidative stress [103].

Very recently, Camunas-Soler et al. implemented a technique to collect both transcriptomic and electrophysiological ("Patch-seq") data from the same endocrine cell [104]. Using human islets, the authors patch clamped 1,369 individual cells before RNA-seq analysis. This allowed them to determine how heterogeneity in gene expression correlates with functional heterogeneity recorded by patch-clamp, including exocytosis, Ca^{2+} and Na^{2+} currents. Thus, they identified genes positively or negatively associated with beta cell exocytotic capacity such as beta cell transcription factors *MAFA* and *ETV1*, insulin granule-associated *SLC30A8*, *VAMP2*, *SCG2* and *INS* as well as several metabolic enzymes

and ion channels. Impressively, they also identified a gene set associated with functional heterogeneity in beta cells that can be used to predict electrophysiologal capacity [104].

Functional, metabolic, and transcriptomic heterogeneity between beta cells is now widely described in the literature (for reviews see [105–108]). A potential advantage of a heterogeneous beta cell population would be to achieve a more precise regulation of global insulin secretion while responding to different physiological conditions and, therefore, fine-tuning the control of blood glycaemia. Different excitability levels of multiple cells belonging to the same network might also contribute to the organisation of the network. For instance, one of the roles attributed to beta cell heterogeneity within the islet is to determine spatiotemporal Ca^{2+} wave dynamics in order to coordinate insulin release across the islet, where waves appear to originate in regions of the islet with elevated excitability [109]. Indeed, cells are not isolated within the islet, and islet multidimensional structure, cell-cell communication and beta cell connectivity are crucial to coordinate adequate insulin secretion in response to glucose [108]. Cell-to-cell communication is achieved by neural regulation, paracrine signalling, and possibly through primary cilia and gap junctions [20]. In mouse and human islets, beta cells are electrically coupled by Connexin 36 (*Cx36*) [110]. *Cx36/Gjd2* charge- and size-selective channels that notably permit intercellular passage of ions such as Ca^{2+} are important for calcium waves/oscillations, coordination and insulin secretion in the intact islet under elevated glucose [19,111,112]. Interestingly, fluorescence recovery after photobleaching (FRAP) experiments showed that cell coupling was heterogenous, with cells having either high or low coupling [113].

In order to analyse beta cell connectivity, functional high-speed Ca^{2+} imaging experiments have been performed on intact islets with acquisitions subjected to computational methods to identify the cells with correlated activity; thus, a connectivity map can be constructed based on the location of significantly correlated cell pairs [20,114,115]. A subpopulation of highly-connected cells was identified in this way ex vivo, in both mouse and human islets, and these cells exerted a tight control over islet response to glucose [22]. The identified "hub" cells, which also appeared to be the first cells to show an increase in cytosolic Ca^{2+} during oscillations in this parameter and have also been termed "leaders" [116], exhibited lower PDX1 and higher GCK expression levels, as assessed by immunocytochemistry, indicative of a less mature but highly metabolic state. Hub cells accounted for ~10% of the beta cell population and, remarkably, their inactivation via an optogenetic approach in which the Cl^- pump halorhodopsin was activated in selected cells to achieve their reversible electrical silencing cells greatly impaired calcium dynamics across the plane of the islet interrogated. Thus, hub cells may act as "pacemaker" cells in response to elevated glucose and appear to be more sensitive than "follower" cells to pro-inflammatory factors [22] (Figure 1).

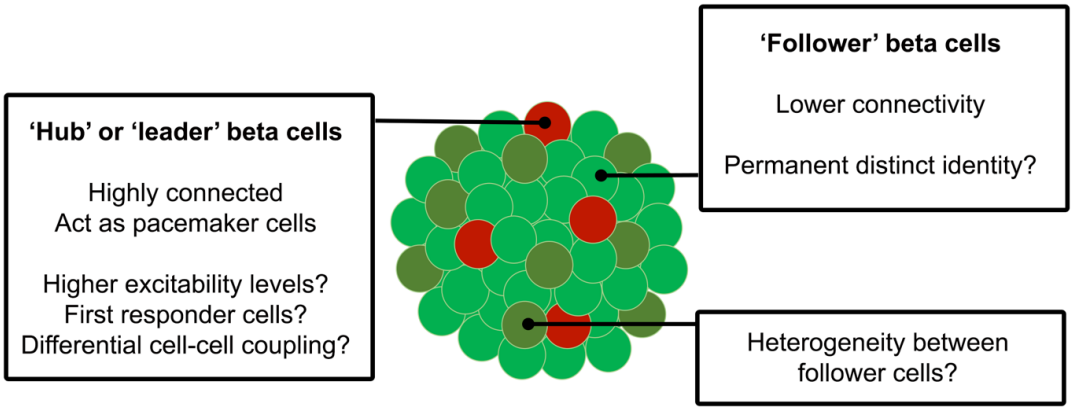

Figure 1. Beta cells display a heterogenous response to glucose including differences in excitability levels. A subpopulation of "hub" cells has been described that account for up to 10% of total beta cells. These "hub" cells, as opposed to "follower" beta cells, exert an acute control over islet response to high glucose and may therefore determine a key component of beta cell connectivity by coordinating the calcium response across the islet, acting as "pacemakers".

Hub cells were also observed in mouse islets as well as in the living fish embryo, with the former becoming revascularized and innervated when engrafted into the anterior chamber of the eye [116]. Similar to what was observed in mouse islets in vitro, photoablation of "leader" cells in the zebrafish led to loss of a coordinated calcium response [116], confirming their possible role as pacemakers. Moreover, these new findings suggest that beta cell "hubbiness" is an intrinsic property of this population and not simply reflective of the localisation of these cells within the islet (e.g., their proximity to blood vessels, nerve termini, etc.). Others [117] have demonstrated that optogenetic activation of subpopulations leads to the activation of Ca^{2+} waves, consistent with the above model, though the degree to which different sub-groups of "hubs", "leaders" and "first responders" overlap is a matter of contention. Importantly, the means through which "hubs" transmit Ca^{2+} waves across the islet remains unclear, with both a direct mechanism involving cell-cell contacts and gap junctions [118] and the involvement of other cell types such as delta cells [119] both possible. Indeed, whilst theoretical considerations have prompted some authors to query the role of gap junctions [120], the grounds for these concerns can be questioned [121], and modelling by others [122] is consistent with these cells playing a coordinating role though gap junctions. Certain characteristics of this "hub/leader" subpopulation are nevertheless still unclear, including their complete transcriptomic (and proteomic) signature. Nevertheless, analysis of published RNA-seq data has shown higher *Gck* and lower *Pdx1* and insulin expression, and revealed a notable enrichment in genes involved in glucose oxidation [116]. We attempt, in Figure 2, to ascribe known differences in gene expression in the mouse to different beta cell subclusters [22,94,123]. Whether imprinted gene expression differs between beta cell populations (e.g., hubs and followers) is yet to be established and is an active area of research.

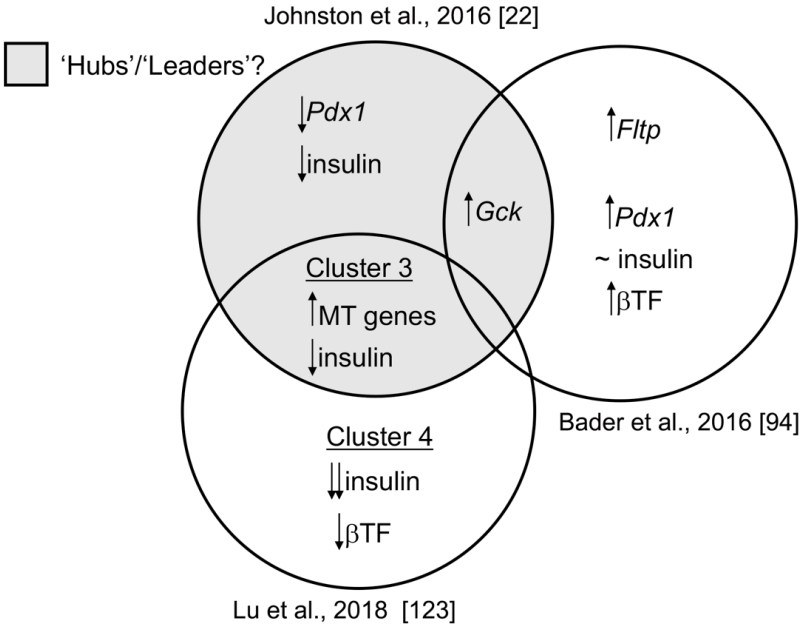

Figure 2. Evaluating the overlap of defined transcriptomic heterogeneity between beta cell subpopulations in mice. "Hub" cells [22] exhibit lower *Pdx1*, *Ins1* and higher *Gck* expression levels, as assessed by immunocytochemistry, indicative of a less mature but highly metabolic cell state. According to Bader et al. [94], FACS-sorting of islet beta cells into *Fltp*-positive and *Fltp*-negative populations revealed that the former was marked by expression of *Pdx1* and several key beta cell transcription factors (βTF) and, similar to "hub" cells, by increased expression of *Gck*. Expression of insulin was similar between *Fltp*-positive and *Fltp*-negative beta cells. scRNA-sequencing of primary mouse beta cells by Lu et al. [123] revealed a population of beta cells (termed "cluster 3") with increased mitochondrial (MT) gene expression and reduced expression of insulin, but with comparable levels of beta cell transcription factors (βTF) as mature beta cells (termed "clusters 1 and 2"), consistent with "hub" cells. "Cluster 4", in this analysis, represents immature beta cells with significantly reduced insulin levels and key beta cell transcription factors (βTF). Whether the expression of imprinted genes is enriched in any of the above subpopulations remains to be determined. Black arrows represent up- or down regulation of gene expression.

1.5. Transcriptomic Diversity between Beta Cell Subpopulations

Early work [81,83,84] and recent single cell transcriptomic profiling [92,99] and imaging studies [22,94,116] have all demonstrated functional heterogeneity amongst individual beta cells within the islet in terms of metabolism, Ca^{2+} influx and insulin secretion. These studies have also revealed diverse transcriptional signatures and secretory profiles amongst beta cell subpopulations, and alterations in subtype distribution in T2D that are associated with partial dedifferentiation and loss of beta cell "identity" [92,99]. Cells from the 'hub' beta cell subpopulation described above [22,116], appear to be transcriptionally immature and highly metabolic. Targeting of these hubs by "glucolipotoxic" insults may thus contribute to the development of T2D [22,116].

Imprinted genes play key functional roles in beta cells [57,59–67,69–71] and a disproportionate number display deregulated expression in a model of diminished glucose-stimulated insulin secretion (GSIS) [72] and in pancreatic islets from T2D patients [66,70,73–75]. Interestingly, overnutrition (high fat or high sugar diets) has been linked to long-term, programmed epigenetic changes in gene expression at imprinted *loci* in humans and rodents [124–126]. It will be interesting to investigate whether imprinted genes are preferentially expressed across beta cell subtypes and whether or not differences between expression of these genes in different subsets impact beta cell heterogeneity and islet function in both normal and diabetic states (Figure 3). In this scenario, the targeting of imprinted genes, in *loci* with well understood epigenetic control and functional importance in beta cells, would enable us to understand the type and genomic distribution of epigenetic and transcriptional control that mediates stable gene expression between beta cell subtypes and their modification by environmental factors [127] (e.g., diet).

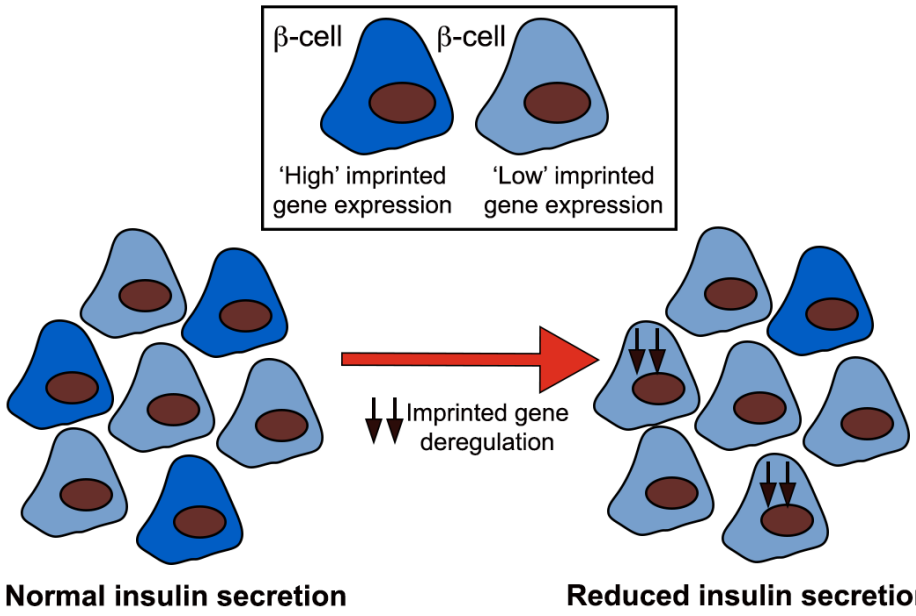

Figure 3. A proposed model for beta cell heterogeneity in terms of imprinted gene expression. Deregulation of imprinted gene expression by environmental factors (i.e., diet) would, in this scenario, reduce beta cell heterogeneity and therefore insulin secretion. Black arrows represent deregulation of imprinted gene expression.

Conclusions: a role for altered imprinted gene expression in reducing beta cell heterogeneity and function?

As discussed above, alterations in the islet transcriptome are likely to be a key driver of beta cell dysfunction in diabetes. Important questions for the future are whether imprinted genes are mis-expressed in beta cells in models of type 1 and type 2 diabetes and whether such altered expression is driven by epigenetic pathways that are key to controlling imprinted gene expression (DNA methylation, modifications to histone proteins). New tools,

including those in which imprinted *loci* can be examined in mice through the "knock-in" of reporter genes such as firefly luciferase [128,129], may provide an exciting means to determine the extent to which gene dysregulation occurs over time in the beta cell in the living animal and whether these changes are reversible. Imprinted genes, with their transgenerational epigenetic maintenance and functional importance in pancreatic beta cells, therefore provide an excellent opportunity to assess epigenetic change in the context of overnutrition and in other settings such as gestational diabetes, with previous studies also linking the possibility of altered imprinted gene expression and perturbed beta cell function being "passed on" from diabetic parents to the next generation [125,126,130–132].

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