Impact of islet architecture on beta cell heterogeneity, plasticity and function

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Hypothetical 3D model of a mouse islet of Langerhans illustrating functional beta cell heterogeneity
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Evidence of phenotypical and/or functional heterogeneity among beta cells

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Keypoints:

- Phenotypical and functional beta cell heterogeneity arises during beta cell development but is also acquired postnatally due to cell maturation and plasticity.
- Islet composition, cell polarity, cell-cell and cell-matrix adhesion and endocrine cell interaction with vessels, nerves and microenvironment all contribute to heterogeneity.
- Islet cell plasticity allows islet cells to adapt to physiological changes, e.g. during pregnancy, or may be triggered by pathological insults.
- Heterogeneous beta cell subpopulations can be recognized by the expression of specific markers including insulin, Pdx1, E-Cadherin, Flattop, PSA-NCAM, DKK3, ST8SIA1 and CD9.
- Fltp represent a novel promising Wnt/PCP effector marker able to discriminate proliferative versus more metabolically active beta cells.

  Targeting specific beta cell subpopulations may open the door to therapeutic regeneration of endogenous islet cells in diabetes.

Abstract

Although beta cell heterogeneity has been discovered more than 50 years ago, its underlying principles have only been explored very recently. Islet cell heterogeneity arises during pancreas development and may reflect the existence of distinct progenitor populations and developmental pathways of endocrine cells. Heterogeneity can also be acquired postnatally due to changes in islet architecture or due to beta cell plasticity. Furthermore, beta cell neogenesis, replication and de-differentiation represent alternative sources of beta cell heterogeneity. Beside a role in beta cell physiology, recent studies have shown that beta cell heterogeneity also influences diabetes development and treatment response. Identifying phenotypic and functional markers to discriminate distinct beta cell subpopulations and the underlying mechanisms of their regulation is warranted to unlock the foremost secrets of beta cell function and make beta cell subpopulations optimal targets for regenerative strategies. In this context, we described the Wnt/PCP effector molecule Fltp to be able to distinguish two unique beta cell subpopulations owing specific cellular and functional signatures and specific response to pathophysiological stimuli. *In vivo* targeting of these specific beta cell subpopulations might therefore represent an alternative future strategy for the treatment of diabetes.

1. Beta cell heterogeneity

Although all beta cells are able to sense glucose, produce and release insulin upon metabolic demand to regulate blood glucose levels, many studies have shown remarkable beta cell heterogeneity, both phenotypical and functional, among islets or even within the same islet. The first evidence of phenotypical heterogeneity among beta cells was described in 1960 when Hellerstrom observed differences in nuclear size with regard to the specific regional location in the islet of Langerhans[1]. Few years later Dean and Matthews reported differences in beta cell membrane potentials between central and peripheral beta cells in the same islet[2] proving the concomitant existence of functional beta cell heterogeneity. Several independent studies have later confirmed and expanded these observations by using more advanced technologies and opened a new era of excitement for beta cell scientists (Table 1). Beta cells were found to differ in size but also granularity and insulin abundance[3-5]. Freeze-fracture technique followed by electron microscopy analysis allowed to study the internal organization of beta cell membranes and permitted the visualization of the heterogeneous surface structure of beta cells and their components, in particular gap junctions[6]. In the '80s, autofluorescence-activated cell sorting was used for the first time to analyse and purify beta cells according to their responsiveness to glucose, based on the specific variations in redox state and subsequent changes in the endogenous fluorescence of the nucleotides FAD and NAD(P)H. Such techniques provided evidence of intercellular differences in glucose sensitivity of individual beta cells[7] which in turn lead to cellular diversity in biosynthetic and secretory activities[8]. Furthermore, radioactive labelling of nucleotides and amino acids helped to discover and/or confirm differences in beta cell proliferation[9], metabolic coupling[10] and glucose-dependent stimulation of insulin synthesis and secretion[11]. Glucose infusion techniques were used to explore beta cell functionality *in vivo*[12]. Static and dynamic islet and/or beta cell incubations with insulin secretagogues revealed how beta cells are heterogeneous in terms of insulin release[12,13]. The use of laser confocal scanning microscopy together with three dimensional (3D) imaging software later allowed exploring the role of the islet cytoarchitecture in beta cell heterogeneity both in animals and in humans[14]. Importantly, it was found that human beta cells also display significant variability in terms of insulin production and secretion[15,16].

The development of beta cell-specific transgenic reporter mice further widened our understanding of beta cell heterogeneity allowing a clear discrimination of distinct subpopulations of beta cells based not only on insulin expression but also on the expression of non-beta cell hormones (e.g. glucagon, somatostatin and pancreatic polypeptide)[17]. In the last decades, more sensitive cellular and molecular biological techniques and single cell analysis led to the discovery of important biomarkers of beta cell heterogeneity (Table 1),

which will be described later in this review. A deep understanding of this inter- and intra-islet beta cell heterogeneity is of critical biological importance and of great clinical relevance for diseases like diabetes characterized by the loss or dysfunction of beta cells and consequent dysregulation of glucose metabolism.

2. Establishment of beta cell heterogeneity

Although it is clear that beta cells are different, further questions remain on when this heterogeneity is established and how it is regulated, what its physiological significance is as well as its role in the development of diabetes. Heterogeneity arises during pancreas development, but is also subjected to post-natal mechanisms of regulation due to the ability of beta cells to modify their gene and protein expression in response to specific endogenous and exogenous cues (a phenomenon known as β-cell plasticity). Heterogeneity stems from the fact that not all beta cells face the same environment in the course of their birth, life and death. During development the pancreas originates from the ventral and the dorsal buds, each of them receiving distinct signals from their surrounding tissues[18]. Differences in ontogeny between ventrally and dorsally derived islets (and beta cells) are responsible for differences in innervation, blood supply, and endocrine composition of the islets, all influencing islet cell composition and heterogeneity[19] later in life. Furthermore, even in the same pancreatic region and within an islet, beta cells may originate from different progenitors and therefore display some degree of intrinsic heterogeneity[20]. Indeed, by using aggregation mouse chimeras between non transgenic and transgenic mouse embryos, Deltour et al observed the formation of heterogeneous islets characterized by a mixture of cells originating from progenitors of both embryos[20].

2a. Impact of 3D islet architecture on beta cell heterogeneity

Islet compaction is a pivotal process in determining beta cell function. [212221]. The compaction of the beta and non-beta cells in the islet occurs in a non-random fashion, giving each islet a specific 3D architecture and cellular composition. The islet niche is composed of different cell types (endocrine, neuronal, endothelial, mesenchymal and blood cells) that are interconnected by extracellular matrix, cell-cell and cell-matrix adhesion molecules, and gap junctions, all of which actively support endocrine cell processes. Islet composition, cell polarity, (homotypic and heterotypic) cell-cell contacts, and interaction with the surrounding tissues and environmental cues, provide regional differences in beta cell glucose responsiveness and insulin secretion, indicating that the 3D islet architecture plays a pivotal role in β-cell heterogeneity.

Islet composition and (homotypic and heterotypic) cell-cell contacts

In rodents as well as in other laboratory animals beta cells are the most abundant endocrine cell type and are mainly located in the core of the islets, surrounded by alpha, delta, epsilon and pancreatic polypeptide cells secreting glucagon, somatostatin, ghrelin and pancreatic polypeptide, respectively.[14,16,2324,2524 1423-2514]The islet composition has been shown to be crucial for normal adult islet tuning and functioning. Islet composition is determined during embryonic development[26]. Islets derived from the ventral bud (which gives rise to the pancreas head) contain more cells producing pancreatic polypeptide, whereas islets formed from the dorsal bud (which gives rise to the pancreas tail) contain more alpha cells[27,28]. Importantly, similar differences were found in human pancreata[29]. Evidence indicates that the location of the islet within the pancreas and the location of the beta cell within an islet are important determinants of adult beta cell functional heterogeneity. Different islet composition between pancreas head and tail has been postulated to play a role in the islet regional differences in terms of insulin secretion[27]. Indeed, it has been shown that islets located in the pancreas tail have higher rates of stimulated insulin secretion and biosynthesis than the ones from the pancreas head[27].[30]

The islet composition is crucial for the establishment of heterogeneity between islets but also contributes to intra-islet heterogeneity by determining the amount of homotypic and heterotypic cell-cell contacts. The cellular organization of islets in rodents clearly favours homotypic cell-cell interactions in the core of the islets where the beta cells are harboured, whereas in the periphery alpha, delta, epsilon and pancreatic polypeptide cells and are intermingled and heterotypical cell-cell contacts are more frequently observed[31].

Most information on the role of cell-cell contacts on insulin secretion comes from re-aggregation studies where dissociated beta cells were allowed to re-compact with either beta cells or non-beta cells. The establishment of either homotypic or heterotypic contacts was associated with large differences in insulin secretion[32]. In particular, homologous contacts between beta cells potentiated insulin secretion to a maximum both in animals and humans[15,32]. Contacts between alpha cells and beta cells were also able to increase insulin secretion compared to single beta cells whereas decreased insulin levels were observed in aggregates of beta and delta cells[15,22]. [1215,22]These findings might explain the difference in glucose thresholds and insulin secretion observed between rodent and human islets. In fact, in humans all endocrine cell types are homogenously distributed throughout the islets, meaning that heterotypic cell-cell contacts are more abundant than in rodents. In line with this idea, it was found that in humans the majority of insulin is released by a minority of beta cells[15], these probably being the beta cells that are more closely associated with other beta cells. Although the effect of locally released hormones and peptides on insulin secretion[33]

may partially explain the observed functional differences among homotypic and heterotypic cell-cell contacts, a role for adhesion molecules and/or cell junctions has also been suggested due to the capacity of these surface components to maintain islet integrity and function. In particular, beta cells communicate via gap junctions, which are believed to regulate the coordinated functioning of beta cells by synchronizing their activities[34]. The finding that human islets have a decreased number of homotypic cell-cell contacts[15,25] and lack synchronization of calcium oscillations[16] has suggested a crucial role of gap junctions in mediating functional homotypic beta cell contacts in rodents[16,35,36]. However, this is in contrast with findings showing that gap junctions are more abundant in peripheral beta cells where heterotypic contacts are more frequent[37]. Since beta cells and non-beta cells are also coupled by gap junctions and further interlinked by adhesion molecules, the expression and abundance of specific surface molecules might be the determining factor in the formation of specific homotypic or heterotypic islet cell aggregate. For example, specific expression pattern of the cell adhesion molecules (CAMs) at the surface of islet cells has been postulated to play a role in the ordered distribution of cells within islets in analogy to the sorting out phenomenon described by Townes and Holtfreter in 1955[38].[39]

Beside a role in mediating cell-cell contact that junctional structures such as connexins and cell-cell and cell-matrix adhesion molecules, such as integrins, cadherins and CAMs positively influence intracellular communications[34] and insulin secretion among beta cells[34] even in human[40]. Importantly, the heterogeneous and dynamic distribution of gap junctions on beta cells has been associated with regional differences in beta cell coupling in animals[6,36,41]. To which extent differences in gap junction abundance correlate with beta cell variability in terms of insulin secretion *in vivo* still needs to be elucidated.

On the other hand, the role of adhesion molecules on functional heterogeneity *in vivo* has been consolidated by recent reports showing how distinct patterns of expressions of E-Cadherin and the sialylated form of the Neural CAM (PSA-NCAM) on beta cells correlate with their different functional responses[42,43] (see section 3).

The islet microenvironment

The islet microenvironment is a network of extracellular matrix (ECM), mesenchymal cells, nerves and blood vessels that signal via neighbouring tissue interactions, as well as neuronal and circulatory routes to beta cells and affect their function. Differences in ontogeny between pancreas head and tail sections are responsible *in primis* for differences in innervation and blood supply among native islets. The ventral pancreas is supplied with blood via the mesenteric artery and receives sympathetic innervation from the superior mesenteric ganglion whereas the dorsally derived pancreas is irrigated by the coeliac artery

and is innervated by the celiac ganglion[19]. Heterogeneity within islets is also dependent on vascularization and innervation. Central and peripheral beta cells constantly face a different extracellular environment and differentially interact with the microvasculature and the autonomic innervation. Since blood components (including oxygen) and neurotransmitters participate directly in the regulation of insulin secretion[44,45], these regional differences could raise functional heterogeneity between peripheral and central beta cells.

Distinct patterns of blood perfusion and vascular density among dorsal and ventral islets have been associated with heterogeneity in beta cell function and proliferation[26]. Moreover, static reconstruction techniques[46,47] and live *in vivo* imaging of islet blood-flow within the pancreas[48] have revealed that the vascular anatomy of the islets varies depending on the size of the islet[46] and the location of the islet in relation to the large vessels within the pancreas. Small islets usually have two to three vascular penetration points whereas large islets have more[47]. Islets may receive capillary branches if they are located in the periphery of the pancreas or receive branches directly from large vessels if the islet is more central[47]. It is therefore legitimate to think that some beta cells are bathed directly by the systemic circulation whereas others received blood, which has passed through other pancreatic or islet regions. This implies that some beta cells may be more active due to the higher amount of blood oxygen and blood components they receive, whereas other islets are “dormant” and constitute a reserve pool upon metabolic demand. Indeed, two distinct pools of high and low blood perfused islets[44,49,50] have been described. These islets (and their beta cells) display unique functional features[49,50], indicating that oxygen consumption is a good indicator of islet and beta cell functional heterogeneity. Olsson et al recently proposed a method to monitor oxygen tension and islet function *in vitro* and *in vivo* by using the low oxygen-tension marker pimonidazole. Interestingly, in normally awake rats, pimonidazole accumulated in 20-25% of islets, which are less vascularized and characterized by a lower (insulin) biosynthetic activity[49]. Strikingly, after performing a 60% pancreatectomy, all islets became well-perfused and a dramatic reduction of pimonidazole staining was observed in the remaining islets, indicating that dormant islets can be ‘awakened’ upon metabolic need to maintain normoglycemia[49].

Furthermore, it was discovered that islet capillaries, in addition to supply islets with oxygen and nutrients also enhance insulin secretion and beta cell survival by providing soluble factors and ECM proteins[51-53]. Hence, proximity to capillaries may be an additional cause of beta cell heterogeneity and contribute to their plasticity. [16,242454555656573358]

The autonomic nervous system is responsible for islet innervation[59] and controls islet development, maturation, mass and function[60,61]. Importantly, by employing genetic and chemical ablation of sympathetic neurons in mice, Borden et al showed that sympathetic

innervation is necessary for establishing pancreatic islet shape and cytoarchitecture during development, which in turn is critical for regulation of glucose metabolism[60]. Only a small proportion of beta cells receive axon terminals[62] and recent studies in mice have demonstrated that sympathetic islet innervation is largely concentrated at the islet periphery[63]. [64]Although this can lead to speculations on regional functional heterogeneity within islets, clear evidence in this regard is still lacking. The observed differences in neural adhesion molecules among endocrine cells[38] may however support the role of innervation in beta cell heterogeneity.

Finally within each islet, the endocrine cells communicate with each other by locally secreting their secretory products into the interstitial fluid. This so-called “paracrine hypothesis” has been used to explain the beneficial role of alpha-beta cell aggregates and the detrimental effect of delta-beta cell aggregates, due to the insulin-stimulating effect of glucagon and the insulin-inhibitory effect of somatostatin on beta cells, respectively[33]. Collectively, the organization of the islet and its particular relationship with the vasculature and the nervous system offers a tight spatial compartmentalization of beta cell responses and may subdivide beta cells in distinct functional subpopulations.

Beta cell polarity

Establishment of polarity during development is crucial for terminal differentiation and spatial localization of cells within a tissue. Although beta cells derive from epithelial tissue, they do not show classical [54]columnar epithelial organization where the apical domain is opposite to the basal domain. Instead, evidence suggests that beta cells resemble the polar structure of hepatocytes, with apical regions along the lateral surfaces[65]. The first proves of polarity date back to the late ‘80s. In 1985, Lombardi et al demonstrated polarization of plasma membrane domains in pancreatic endocrine cells by viral budding[66]. Few years later the beta cell glucose transporter Glut2 was found to localize at the lateral side directly adjacent to a neighbouring beta cell[67]. Importantly, Bonner-Weir showed that beta cells in the rat are typically organized in rosette-like structures surrounding blood vessels[54]. In these structures, beta cells segregate the nucleus to the basal side and the insulin granules to the apical side facing the central capillary[54], supporting the concept that insulin secretion into the bloodstream occurs apically. However, based on more recent findings[51], another theory has emerged according to which insulin granule exocytosis in beta cells occurs mainly on the lateral side into the interstitial space[68] from where it reaches the systemic circulation. Nevertheless, it is now clear that beta cells are polarized with respect to the vasculature. By adopting a pancreatic slice preparation that maintains the native structural organization of the islets[69] and by using different 3D imaging methods, Gan et al identified three distinct

polar domains in beta cells: the apical region where cilia are projected into the extracellular lumen, the large lateral domain that express Glut2 and the basal region where beta cells are in contact with the vasculature. Importantly, the same polar organization was recapitulated in humans.

In another recent study it was found that adjacent beta cell (both human and mouse) meet at sharp angles or edges, which form special surface microdomains where important signalling molecules are segregated[70]. Thus, polarization not only plays a role in beta cell positioning in the islets, but likely contributes to define specific signal and process compartmentalization within beta cells.

The first insight into the regulation of beta cell polarity on a molecular level was provided by the functional analysis of the serine-threonine kinase LKB1[65,71]. LKB1 deletion in adult beta cells caused the translocation of the cell cilia to the basal side and of the nucleus to the apical side. Both precise nuclear and ciliary positioning depend on intracellular polarity, strongly pointing at LKB1 as an important regulator of beta cell polarity. The fact that LKB1 also regulated beta cell function and size further suggests a role of polarity acquisition in beta cell morphology and homeostasis.

More recently, planar cell polarity (PCP), defined as the polarity perpendicular to the apical-basal polarity, has been shown to regulate the orientation of cells within the plane of an epithelium, intracellular organelle positioning and ciliary positioning via a conserved set of core PCP genes. These genes translate into membrane bound and cytoplasmic proteins localized symmetrically and asymmetrically which are crucial for PCP establishment and maintenance[72]. The activators of PCP signaling are the secreted non-canonical Wnt proteins (e.g. Wnt4, Wnt5a and Wnt11) that bind to Frizzled receptors and transduce the signal into the cell. Although the role of non-canonical Wnt signaling in pancreas development is established[73], little is known about the role of PCP during islet neogenesis and beta cell maturation. Cortijo et al. were the first to show expression and asymmetric localization of core PCP proteins in the embryonic pancreas[74]. Double knock out mice for two core PCP components, Celsr2 and 3, showed reduced numbers of endocrine cells at E14.5 and as a consequence a diminished glucose clearance capacity[74]. The authors showed that Celsr3 acts on beta cell differentiation probably by activating PCP signaling. Another downstream effector of Wnt/PCP signaling, the actin cytoskeletal regulator RhoA/ROCK, has recently been shown to play a role in adult beta cell function including insulin secretion[75], indicating that beta cell polarity can directly affect beta cell function. Importantly, we recently demonstrated the involvement of Wnt/PCP in islet formation and maturation and the existence of functional beta cell heterogeneity in active PCP signalling, indicated by the expression of the novel marker Flattop (Fltp, see section 4). *In vitro* activation of Wnt/PCP

signalling triggered expression of maturation markers, including Nkx6.1, MafA and Urocortin3 (Ucn3) and improved glucose-stimulated insulin secretion. Importantly, we have evidence, indicating that Wnt/PCP plays a role in the post-natal maturation of beta cells. Collectively, these studies highlight the importance of polarity and (intra)cellular signal positioning for beta cell heterogeneity, maturation and function.

2b. Beta cell plasticity

During the course of life, physiological or pathological conditions can alter islet composition and 3D architecture and further affect beta cell heterogeneity between and within islets. Mostly, this is the result of the highly plastic behaviour of endocrine cells in particular beta cells which adapt their physiology, morphology and function upon exposure to specific endogenous and exogenous cues.

Physiological plasticity

The proliferative activity of beta cells decreases rapidly and dramatically after birth.[976] During pregnancy under the effect of lactogenic hormones, however, beta cells undergo extensive hyperplasia to meet the higher metabolic demand[77]. Strikingly, studies have shown that considerable heterogeneity exists in islet responsiveness to cell proliferation during mid- to late pregnancy in the rat[78] suggesting that variability in hormonal adaptation may be responsible for the development of gestational diabetes.

This boost in beta cell proliferation may furthermore cause changes in islet composition and affect the intra-islet localization of beta cells as well as their function. Indeed, beta cell hyperplasia was accompanied by a significant decrease in the threshold for glucose-stimulated insulin secretion[77,78].

Along the same line, during aging both replication of pre-existing beta cells and neogenesis contribute to maintain an adequate beta cell mass[79]. Different cellular origin may again account for changes in islet composition and may give rise to unique beta cell subpopulations. Studies in rodents have shown that aging regulates the size and granularity of some beta cells and therefore impact their insulin secretion[80,81]. Moreover, Katsuta et al found significant changes in the percentages of beta cells co-expressing multiple hormones between newly born and adult mice[17].

Pathological plasticity

Studies have shown that in diabetic animals and humans there is a change in islet composition[82,83] accompanied by a displacement of the endocrine cells from their

characteristic location within the islet[82]. Although it is unclear whether these changes are a cause or a consequence of disease, this imbalance likely affects the net hormonal output of the endocrine pancreas and contributes to blood glucose dysregulation.

Experimental models of beta cell dysfunction have shown that under pathological circumstances distinct pools of beta cells can originate from other cell types, including endocrine or exocrine cells[84]. Accordingly, over the last years, several independent studies have demonstrated that single gene manipulation or beta cell depletion is sufficient to drive trans-differentiation of alpha or delta cells towards beta cell lineage[85,86]. Newly formed beta cells may display distinct features such as co-expression of multiple hormones, as shown in a model of islet regeneration[87].

Importantly, beta cell neogenesis from exocrine progenitors (both duct or acinar-derived) also occurs after mild but prolonged hyperglycemia in normal and diabetic rodents[88,89] indicating that progenitor, immature and mature beta cells may co-exist and might contribute to beta cell heterogeneity in diabetic conditions. This idea is supported by the fact that chronic exposure to pathological stressors or artificial gene deletion has been shown to induce de-differentiation of beta cells into an immature state, with the appearance of subpopulations of beta cells expressing progenitor markers (e.g. Ngn3) and lacking expression of maturation markers (e.g. Insulin, Pdx1, Nkx6.1, MafA)[90,91]. Importantly, de-differentiated endocrine cells could be reverted into mature hormone-expressing cells upon insulin therapy[91] suggesting a promising window of therapeutic opportunity. According to the five stages theory of diabetes progression proposed by Weir et al[92], the relentless demise of beta cells is accompanied by sequential changes in beta cell mass, phenotype and function. Hence, research and therapy would benefit enormously from the identification of specific markers able to distinguish discrete beta cell populations during the progressive deterioration that leads to diabetes.

2c. Relevance of beta cell heterogeneity in physiology and disease

The existence of heterogeneous populations of beta cells provides the islets with high functional flexibility in terms of insulin secretion and allows them to properly sense and adapt to physiological changes in the environment.

It was also shown about 30 years ago that central and peripheral beta cells respond to glucose at different kinetics leading to the possibility that one population may be responsible for acute challenge response whereas the other population could contribute to the insulin output after a more sustained challenge[12,29].

Other studies have noted the existence of specialized pancreatic stem cell populations or distinct pools of beta cell precursors both expected to display significant phenotypical and

functional differences from the mature adult beta cells[87,93]. Although this hypothesis remains highly debated[94], islets are believed to contain progenitor-like cells with self-renewal capacity owning a precursor gene expression profile[93]. Similarly, Olsson et al recently described the existence of a low oxygenated and functionally less active or “dormant” subpopulation of islets which may be of functional importance during increased metabolic need and therefore act as a cellular reserve[49].

Lately however, attention has been focused on the role of beta cell heterogeneity in development of disease. A recent study showed that there is a regional heterogeneity in beta cells adaptation to pathological stressors. In this regard, beta cells from the splenic region were more prone to increase proliferation and glucose-induced insulin secretion in response to six weeks of high-fat diet than beta cells from duodenal and gastric regions[26].

Furthermore, beta cell heterogeneity may also determine susceptibility to pathogenic stressors. For example, beta cells with lower glucose responsiveness were found to be markedly more susceptible to oxidative damage than the glucose-responsive beta cells[3,7]. In another study, a pool of highly functional, highly blood-perfused islets in the native pancreas was shown to display higher susceptibility to cellular stress by cytokines and hypoxia[50,95] [30]Cellular and molecular characterization of these metabolically different subpopulations of beta cells is warranted to identify markers and understand novel molecular principles of heterogeneity. Genomic, proteomic and metabolic analysis of functionally distinct populations of beta cells with regard to proliferation, glucose response and insulin secretion but also to pathogenic susceptibility or response to diabetic treatment may help to find a specific signature of markers that can be used to monitor (changes in) heterogeneity in the islets in pathophysiological conditions. A better understanding of the endogenous and exogenous modulation of these markers would lead to new therapeutic strategies aimed to recapitulate a specific beta cell function which is lost or dysfunctional in diabetes.

3. Existing markers of beta cell heterogeneity

Identification and characterization of markers of beta cell heterogeneity still remains the ultimate goal to enable targeting of specific β-cell subpopulations for regenerative therapy in diabetes. Pancreatic insulin-producing beta cells express a unique and complex landscape of transcription factors, membrane transporter, metabolic enzymes, G-protein coupled receptor (GPCR)- and cell adhesion molecules which mirror their phenotypic and functional status.

Insulin represents the oldest and most well described marker of beta cells and insulin expression levels are heterogeneous among beta cells. Morphological analysis of insulin expression revealed that beta cells in the centre of the islet express less insulin compared

with peripheral beta cells[5]. Transgenic mice expressing GFP under the insulin promoter (MIP-GFP) allowed the distinction of three beta cell subpopulations characterized by distinct levels of GFP/insulin expression[80]. Interestingly, this heterogeneity was detected throughout the animal lifespan and correlated with beta cell function. Different islet composition between pancreas head and tail can additionally contribute to insulin heterogeneity[27]. Indeed, islets located in the pancreas tail have higher insulin biosynthesis rates than the ones from the pancreas head[27]. Interestingly, the same result was observed in a mouse model of obesity and diabetes[26]. Furthermore, beta cells structurally coupled to delta cells were reported to have more insulin than those coupled to beta cells[3]. Insulin heterogeneity might also reflect different maturation states of beta cell subpopulations. In this regard, single gene expression analysis of MIP-GFP cells revealed that a small portion of beta cells still co-express multiple hormones[17]. The co-expression of islet hormones that has been reported in endocrine progenitors during development and in adult mice might reflect residual progenitor potential of some beta cell reserve pool under metabolic demand. Finally, the density of pale granules which contain unprocessed (immature) insulin appears to associate with beta cell glucose sensitivity and has been suggested as a marker for highly responsive cells[96].

The transcription factor Pancreatic and duodenal homeobox 1 (Pdx1) is a master regulator of beta cell development and function[97] and has been described in both human and murine pancreas to exhibit cellular heterogeneity, likely reflecting distinct maturation status of beta cell subpopulations[98]. Interestingly 25% of beta cells within human and mouse adult pancreas were found to be $Pdx1^{+}/Ins^{low}$. This population displayed a gene expression profile that resembled the immature developing beta cells with higher levels of the transcription factor MafB and low levels of the glucose transporter Glut2 and Glucokinase , accompanied by increased proliferative capacity and diminished insulin secretory capacity. A part of this population differentiated into mature cells in culture without cell division[98]. Furthermore, chronic mild glucose infusion in rats triggered the formation of small common duct epithelium clusters composed of $Pdx1^{+}/Ins^{-}$ and $Pdx1^{+}/Ins^{+}$ cells suggesting a role for Pdx1 (together with insulin) as a marker for beta cell neogenesis and heterogeneity[88]. In line with this, appearance of precursor $Pdx1^{+}$ (and somatostatin^{+}) cells was also noted in a model of regeneration following beta cell ablation[87].

Up-taking and sensing glucose is one of the most important features of beta cells and it is required to initiate appropriate insulin secretion. The Glucose transporter 2 (Glut2) is the major glucose transporter isoform in murine beta cells and its expression starts early during development and increases during beta cell maturation and acquisition of metabolic competence[99]. Low expression levels of Glut2 have been reported to mark a rare beta cell subpopulation, expressing also low levels of insulin and retaining stem cell/progenitor

properties[93]. Similarly by using a dedifferentiation in vivo model, Beamish et al identified a population of $Ins^{+}/Glut2^{low}$ cells representing 3.5% of all insulin-expressing cells. The majority of these cells localized outside of big islet and they exhibited higher proliferation rate compared to young $Ins^{+}/Glut2^{+}$ beta cells and high plasticity towards endocrine, ductal and neural lineages. The authors postulated that these cells may represent a resident population of cells capable of generating new functional beta cells in vivo[100]. Furthermore, it has been reported that a pool of precursor $Glut2^{+}$ cells appeared during beta cell regeneration following targeted beta cell ablation[87,101], making this marker a very attractive tool to explore plasticity among beta cell subpopulations.

Glucokinase (GK) is a pivotal glucose sensor enzyme among beta cells and its expression correlates with insulin granule abundance and Glut2 expression[102]. Heterogeneous polarized localized patterns of GK immunostaining were detected among beta cells: in beta cells with contact to intra-islet capillaries GK had a polarized localization, with the highest density in the cytoplasmic region close to the pericapillary space[5]. Interestingly, GK expression becomes heterogeneous with changes in metabolic status and precedes insulin heterogeneity[5]. This marker might be therefore of use to discriminate discrete beta cell subpopulations retaining differential metabolic activity.

Beta cells express a variety of cadherins, important calcium-dependent transmembrane protein involved in cell-cell adhesion, islet compaction and formation[103] as well as beta cell activity[104]. In beta cells, E-Cadherin regulates proliferation and glucose responsiveness[105] and its expression is reduced in transgenic animal models of type 2 diabetes[106]. Interestingly, Bosco at al reported heterogeneous expression of E-Cadherin in rodent adult beta cells[42], which was regulated by insulin secretagogues. Beta cells expressing high levels of E-Cadherin showed increased expression of insulin and greater secretory capacity compared to beta cells expressing low levels of E-Cadherin, indicating the importance of tight cell-cell contacts for physiological beta cell function[42]. Similarly to cadherins, other studies have reported beta cell heterogeneity in terms of PSA-NCAM expression[43]. The advantage of PSA-CAM as a marker for beta cell heterogeneity in the pancreas is its restricted expression to beta cells and the regulation of its surface expression by cellular activity, such as insulin exocytosis[107]. PSA-NCAM has been used to sort beta cell subpopulations with distinct glucose responsiveness in normal rats[108]. Karaca et al extended these findings and explored the mechanisms underlying this heterogeneity in both normal and diabetic and hyperglycemic rats. Based on the level of PSA-NCAM expression, rat beta cells were divided in high and low expressing subpopulations, characterized by distinct mRNA profiles, metabolic status, cellular complexity and insulin secretion patterns[43]. PSA-$NCAM^{low}$ beta cells had small raises in calcium after glucose stimulation, reduced ATP

levels, and impaired expression of genes involved in the generation of metabolic signals, such as Glut2 and GK. This subpopulation also showed deficiency in cAMP-dependent pathways and a significant degree of de-differentiation. PSA-NCAM^{high} and PSA-NCAM^{low} cells further differed in F-actin distribution at the apical plasma membrane, and expression of protein, pumps and ion channels needed in insulin exocytosis. Importantly, in two different animal models of diabetes and acute hyperglycemia the proportion of PSA-NCAM^{high} and PSA-NCAM^{low} beta cells varied according to the insulin demand and correlated with disease progression[43], highlighting the pathophysiological relevance of this marker and its potential use in monitoring functional beta cell mass *in vivo*. The validity of this marker in humans, however, still remains to be investigated.

Recent work from Dorrell et al identified two surface markers (ST8SIA1 and CD9) able to distinguish four distinct subpopulations of beta cells in the adult human islets. These subpopulations were named (from most abundant to less abundant) β1 (ST8SIA1$^-$ and CD9$^-$, β2 (ST8SIA1$^-$ and CD9$^+$), β3 (ST8SIA1$^+$ and CD9$^-$) and β4 (ST8SIA1$^+$ and CD9$^+$). They presented clear differences in both basal and stimulated insulin secretion, suggesting that the subpopulations differ in their insulin release kinetics. Interestingly, the ST8SIA1$^-$ population was significantly enriched for Gene Ontology (GO) terms related to protein secretion whereas ST8SIA1$^+$ was enriched for GO terms related to neurogenesis. Indeed, the ST8SIA1$^-$ populations β1 and β2 were able to secrete more insulin than the ST8SIA1$^+$ populations, despite a similar insulin content. Moreover, although the four populations all displayed common beta cell markers like Pdx1, MafA and Nkx6.1, a subset of genes was consistently expressed at different levels in the beta cell subtypes. Most important, the frequencies of beta cell subtypes were altered in individuals with type 2 diabetes, suggesting that the populations may react differently to metabolic stress and pathogenic cues and highlighting the potential medical relevance of these findings[109] (Dorrell et al., 2016, in press; Nat Comm).

In another paper, gene expression profile of isolated human islets revealed high expression of Dickkopf 3 (*DKK3*) and immunohystochemical analysis showed its heterogeneous expression among beta cells[110]. DKK3 is a member of the well-known family of secreted Wnt antagonists, but its function in regulating canonical or non-canonical WNT signaling pathway still remains to be clarified. Studies in Zebrafish reported a specific expression pattern of Dkk3b (the homologous of human DKK3) in islet during pancreas development and its possible involvement in endocrine differentiation[111]. Surprisingly, however, Dkk3 null mutant mice did not display any abnormalities during development or in adulthood[112], suggesting that its function could be redundant.

Furthermore, another marker with heterogeneous expression in human islets is the vesicular monoamine transporter 2 (VMAT2). Saisho et al reported that in human pancreas from diabetic and non-diabetic individuals most beta cells expressed VMAT2 (~80%). The overall pattern of VMAT2 expression was similar in type 1 and type 2 diabetics. Interestingly, the percentage of VMAT2 negative cells increased to 70% in the beta cells scattered in the exocrine tissue far away from islets[113]. However, the role of VMAT2 in beta cells has not been determined yet.

Finally, the advent of single cell-based techniques as the Fluidigm C1 recently allowed the analysis of cell-type specific genes in mouse[114] and human islets (Gromada, personal communication). By using this technique, the group at Regeneron Pharmaceuticals was able to analyze about 20000 human islet cells and found that several genes were differentially expressed between islet cell types and even among beta cells. In particular, they could identify two populations of alpha cells and three populations of beta cells displaying a distinct signature in particular in ECM and markers of cell polarity and adhesion confirming once again the importance of the islet niche in defining beta cell heterogeneity.

Despite the undoubted importance of these discoveries however, the mechanisms underlying the heterogeneous expression of the beta cell markers still need to be elucidated to render these markers ultimately clinically relevant.

4. Linking beta cell heterogeneity to 3D architecture and beta cell function

We have recently discovered a Wnt/PCP effector and reporter named Flattop (Fltp) as a novel marker of beta cell heterogeneity[115,116115,116]. Fltp was originally discovered in a screen to identify target genes of the forkhead box transcription factor Foxa2 that regulates polarization and epithelialization in the endoderm germ layer[117,118]. Indeed, Fltp is expressed in definitive endoderm-derived organs, such as the pancreas, lung and gastrointestinal tract, in particular in regions where Wnt/PCP signaling components are expressed and the pathway is active[119,120]. As such Fltp represents a Wnt/PCP reporter, which could be used to study the effects of specific polarity signals on beta cell homeostasis and function. The PCP pathway is known to orient cells and organelles within the plane of tissues and is therefore a prime player in organizing islet architecture and function. Both Foxa2[121] and Wnt/PCP[74] are important for pancreas and islet development and planar polarization and given that tissue organization and cell-cell interaction are critical for beta cell heterogeneity and function, we investigated the expression and the potential role of Fltp in the islets and more specifically on beta cell pathophysiology. To study the establishment of tissue polarity at the molecular level we employed the $Fltp^{ZV}$ knock-in/knock-out mouse model where the entire open-reading frame of Fltp is replaced by a multi-cistronic lacZ-Venus reporter cassette. This so

called Fltp promoter-driven Venus reporter (FVR) allowed us to explore the expression and function of Fltp in all endocrine cells of the islets of Langerhans. FVR expression was undetectable in multipotent pancreatic progenitors at embryonic day (E) 18.5, but its expression rose in beta cells of compacted islets from newborn mice and increased along with the beta cell maturation process. At postnatal day 1 (P1) reporter activity was detectable in about 50% of Nkx6.1^{+}/Ins^{+} beta cells and the number reached 80% in adult islets (Fig. 1). This important finding suggests that heterogeneity could be postnatally driven by Wnt/PCP-mediated planar polarization of beta cells. Importantly, we found FVR^{+} cells often in rosette-like structures (Fig. 1), suggesting that Fltp is important for the polarity and functional tuning of mature insulin-producing beta cells. Contrarily, we observed a higher proliferative capacity of the FVR^{-} beta cells during pregnancy and postnatal beta cell expansion compared to the FVR^{+} population (Fig. 1) . By adopting a genetic lineage tracing strategy we found that Fltp-lineage^{-} and Fltp-lineage^{+} endocrine subpopulations reacted differently in physiological and pathological conditions known to affect glucose metabolism. In particular, Fltp-lineage^{-} beta cells underwent cell expansion during pregnancy whereas Fltp-lineage^{+} beta cells accounted for beta cell growth/hypertrophy after eight weeks of high-fat diet. This suggests that the Fltp-lineage^{+} population of mature beta cells is more prone to cytotoxic stress. Indeed, when we transplanted islets in the anterior chamber of the mouse eye, the Fltp-lineage^{+} population initially decreased in number whereas the Fltp-lineage^{-} population underwent expansion. Strikingly, however, at four weeks after transplantation, the Fltp-lineage^{+} subpopulation recovered, likely due to the maturation of the Fltp-lineage^{-} cells into lineage^{+} cells. This indicates that Fltp-lineage^{-} beta cells can functions as a sort of reservoir pool of immature beta cells that can compensate better for metabolic demand likely due to their higher plasticity, whereas the Fltp-lineage^{+} beta cells are the mature ones with higher metabolic activity. This was further confirmed by genome-wide mRNA expression profile analysis, which indeed revealed distinct gene expression profile, structural and physiological features among the two subpopulations. In particular, Fltp-lineage^{+} beta cells were enriched in gene associated with beta cell maturation markers, receptors and signaling components as well as mitochondrial and metabolic[115]. In line with this, the FVR^{-} beta cells expressed low levels of Slc2a2, a result which has been recently reported for insulin^{+} pancreatic multipotent progenitor cells identified in mouse and human islets[93]. Moreover, the high expression of Proprotein convertase subtilisin/kexin type 1 (Pcsk1) in the FVR^{+} cells and the higher number of mature secretory granules in Fltp-lineage^{+} cells further suggest that Fltp is a marker for mature beta cells. Interestingly, glucose-stimulated insulin secretion was decreased in re-aggregated FVR^{-} beta cells compared to FVR^{+}, indicating differences in metabolic status among Fltp beta cells subpopulations. Moreover, FVR^{+} beta cells displayed

higher expression levels of Wnt4, which in human islets is heterogeneously expressed among beta cells as well as in the other endocrine [115]. Finally, to explore the importance of FLTP in human disease, we genotyped seven SNPs tagging all common variation in the human FLTP ortholog (C1Orf192) and analyzed their association with metabolic traits in a cohort of more than 2000 human pre-diabetic subjects. One SNP was significantly associated with decreased insulin secretion in obese individuals. Together with FLTP also *PDX1* and *SLC2A2* were found to be downregulated in progressive diabetes, suggesting that Wnt/PCP activity could be reduced in human diabetes[115].

In comparison to the previously described markers, the Wnt/PCP effector molecule Fltp represents a unique tool to study the mechanisms by which heterogeneity is established but also the mechanisms that regulate heterogeneity in physiological and pathological conditions. In this context, we described for the first time the role of Wnt/PCP signaling in islet compaction as shown by Fltp increased expression in the time when islets start forming and acquire their unique 3D structure. Our results also support the concept that active Wnt/PCP signaling regulates beta cell polarization, maturation, and function during development but also in the adult. Indeed, by stimulating 2D and 3D *in vitro* culture of beta cells with Wnt/PCP ligands we were able to trigger expression of beta cell maturation markers like Nkx6.1, MafA and Ucn3 and increase glucose-stimulated insulin secretion. The mechanisms of these effects are probably the results of Wnt/PCP (and Fltp)-mediated regulation of actin and microtubule cytoskeleton and impact on gene expression, which we intend to elucidate in future investigations. More open questions remain including the relationship between Wnt/PCP and Fltp with the islet niche. Indeed, when we compared head and tail regions and islets of different size, we observed heterogeneity within and between islets in terms of FVR expression. Moreover, the mechanisms that regulate conversion of FVR^- to FVR^+ beta cells still need to be elucidated in details. Nevertheless, Fltp represent a novel promising Wnt/PCP effector marker able to discriminate proliferative versus more metabolically active beta cells. Hence, exogenous manipulation of Wnt/PCP signaling or endogenous changes mirrored by FVR expression may allow us to explore a new dimension of heterogeneity in conditions known to affect beta cell function. On the other hand, the distinct profile of receptors and signaling pathways that characterize the Fltp subpopulations gives hope for a targeted approach directed to one or the other specific population for *in vivo* beta cell regeneration strategies. Interestingly, Ling et al have recently shown that therapeutic targeting of different subpopulations of beta cells is possible[122]. Chronic treatment with the insulin secretagogue glibenclamide induced heterogeneous degranulation of beta cells. In particular, the heavily degranulated subpopulations showed elevated insulin synthetic activity even in the absence of glucose[122].

In addition to regenerative strategies, Fltp may also allow to recapitulate beta cell maturation *in vitro* (from human stem cells) for beta cell replacement strategies or to create functional islets for tissue engineering approaches. Furthermore, *Fltp* expression is not restricted to beta cells but it is expressed in other endocrine cells, opening alternative avenues to explore intra-islet plasticity and heterogeneity among alpha or delta cells, which may also ultimately affect beta cell function.

5. Conclusions

The technological advances of the last decades have allowed not only to confirm previous important principles of beta cell heterogeneity but have also opened a new exciting era marked by novel scientific breakthroughs. Heterogeneity arises during development and may reflect the existence of more than one developmental pathway for beta cells[17,99]. Indeed, beta cells express some degree of intrinsic heterogeneity shown by the fact that when exposed to the same environment at the same time they show different rates of insulin secretion[13]. Moreover, dissociated beta cells display a clear self-organizing ability to form aggregates with appropriate 3D architecture typical of native islets with insulin release patterns comparable to those of intact islets[123]. Postnatally, the islet cytoarchitecture supports the heterogeneous function of beta cells. Thanks to their location within the islet and within the pancreas and the surrounding microenvironment, beta cells are able to provide specific responses. Alternatively, heterogeneity can result from beta cell plasticity or from different routes of beta cell neogenesis during physiological or pathological conditions. A proper visualization and discrimination of heterogeneous beta cell populations with specific markers and a better understanding of the mechanisms that regulate marker expression is warranted to better understand beta cell pathophysiology, and it would open several possibilities for diabetes treatment. Markers like Flattop reporting on the activity of specific signalling pathways of pivotal importance in islet compaction, maturation and function (e.g. Wnt/PCP) will enable a deeper understanding of heterogeneity and the exploration of its clinical relevance. [2416124]Although most studies have been conducted in rodents and using *in vitro* preparations, recent data clearly support the concept that beta cell heterogeneity is present in human islets and may also contribute to diabetes pathogenesis[30]. Understanding how distinct beta cell subpopulations are dynamically regulated upon diabetogenic cues (e.g. high-fat diet) will help to design appropriate strategies to recapitulate physiological heterogeneity and regenerate functional beta cell mass. Technical limitations still need to be overcome and it is indeed possible that isolation of islets and beta cells from their *in vivo* natural environment may alter important beta cells features. For these reasons, research and therapy would definitely benefit from the validation of beta cell-specific surface markers

or the development of more advanced technologies like targeted *in vivo* labelling approaches to monitor *in situ* the extent and the modulation of beta cell heterogeneity as well as the response to pharmacological treatment. This could open the door to a new generation of therapeutic intervention in diabetes where endogenous cellular function could be monitored, reprogrammed and appropriately reinstated.

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Heterogeneous feature	**Consequence/observation[(ref)]**		
Phenotypical			
Nuclear size	**Nuclear DNA content varies among beta cells with the occurrence of diploid, tetraploid and octaploid** The smallest nuclei were found centrally in large islets[1]		
Granularity	Subpopulations of beta cells with distinct granularity have different amount of insulin Beta cells structurally coupled to somatostatin-containing cells are more densely granulated[3]		
Gap junctions	Gap junctions are more frequent in beta cells located at the periphery of the islet. This heterogeneity is preserved during stimulation of insulin secretion		
Homotypic and heterotypic cell contacts	Heterotypic contacts are more abundant at the cell periphery while homotypic beta cell contact are more abundant in the islet core		
Mitochondria	Mitochondria within the beta cell are metabolically heterogeneous. BAD, implicated in mitochondrial recruitment of GK, influences the level of heterogeneity		
Polarity	Beta cells which are positioned around capillaries share the same orientation of granular accumulation[54]		
Hormone content	Beta cells heterogeneously co-express multiple hormonal genes		
Marker expression			
Surface:			
PSA-NCAM	PSA-NCAM^{high} cells are more responsive to secretagogues. The ratio PSA-NCAM$^{high/}$ PSA-NCAM^{low} varies with metabolic conditions. The two populations of beta cells have distinct gene expression profiles		
E-cadherin	Expression of E-cadherin correlates with insulin secretion		
ST8SIA1 and CD9	**Co-staining of human islets with antibodies against these two surface markers revealed clear heterogeneity and distinguished four beta cell subpopulations owing distinct gene expression profile and different insulin secretion kinetics (Dorrell, 2016; Nat Comm, in press)**		[109]
Intracellular:			
Insulin	Cellular fluorescence intensity after insulin immunostaining varies in the same islet Rat beta cells displayed heterogeneous immunoreactivity to insulin which disappears in starving conditions. Central beta cells express less insulin than peripheral ones[5] Beta cells structurally coupled to delta cells have more insulin than those coupled to beta cells[3] Transgenic mice expressing GFP under the insulin promoter show three distinct populations of beta cells based on GFP/insulin expression, with distinct glucose responsiveness[80] **Heterogeneous insulin staining among dispersed**		[5]

	human beta cells[127]		
Pale secretory granules	High glucose responsive beta cells have more pale secretory granules		
Fltp	Fltp distinguishes a mature subpopulation ($Fltp^+$) and a proliferative subpopulation ($Fltp^-$) of beta cells with distinct genetic profiles and differential response to metabolic stressors		[115]
GK	Beta cells display heterogeneous GK immunoreactivity Rat beta cells displayed heterogeneous immunoreactivity to GK which disappears in starving conditions[5]		
Glut2	Low expression levels of Glut2 mark a rare beta cell subpopulation with stem cell properties Glut2 marks a pool of precursor beta cell during regeneration[87,101] In a model of dedifferentiation, $Glut2^{low}$ cells may represent a resident population of cell capable of forming new functional beta cells[100]		
Dkk3	**A subset of beta cells contains Dkk3 in human** sections		
VMAT2	**In pancreata from healthy individuals and patients with diabetes (type 1 and type 2) only 10% of the beta cells were found negative for VMAT2. This percentage increased up to 70% in beta cells scattered in the** exocrine tissue		
Nuclear:			
Pdx1	**Heterogeneous Pdx1 staining among dispersed human beta** cells		
Functional			
Electrical activity/membrane potential	Cells with larger membrane potentials were located near the islet surface Beta cells have different thresholds for glucose-induced electrical activity[130] Some, but not all beta cells are electrically coupled by low-conductance junctional channels[35,131]		

Glucose responsiveness Glucose responsiveness	Differences in redox state and FAD and NAD(P)H fluorescence in autofluorescence-activated cell sorting Different glucose utilization and glucose oxidation attributable to intercellular differences in glucose phosphorylation[129] In rodent dispersed beta cells, different thresholds in glucose-mediated gating of ATP-sensitive K^+ channels, electrical activity and calcium rise were observed[130,132,133] In isolated rat islet beta cells at low glucose (1mM) only 5% of the beta cells were responsive. At higher concentrations (>10mM) 70% of the beta cells were responsive[96] At 1mM, 5mM and 20mM glucose, 18%, 43% and 70% of the beta cells shifted from basal to increased redox state, respectively[128] Beta cells are heterogeneous in glucose-stimulated insulin gene expression[134] Centrally located beta cells degranulated faster than peripheral ones after prolonged glucose exposure[12]		
Insulin secretion	Larger islets secrete significantly more insulin compared to small islets Dissociated beta cells display significant heterogeneity in insulin release[13] Glucose-unresponsive beta cells secrete quantitatively lower insulin compared to glucose-responsive beta cells, despite the presence of comparable insulin stores[8] **A small minority of beta cells is responsible for the majority of insulin being secreted. Pairs of cells secrete more than single cells[15]** 75% of beta cells showed constant secretory patterns under repeated glucose stimulation; 25% shifted from a secretory to a non-secretory state[135] Intraislet and interislet variation in insulin secretion in isolated rat beta cells[136] Subpopulations of beta cells in vivo respond differently to physiological and pharmacological insulin secretagogues[12]		

Metabolic coupling	Nucleotide exchange was observed only in 50% of the recipient beta cells contacting donor beta cells in monolayer culture		
Dye coupling	Dye exchange among beta cells is restricted to specific directions		
Calcium oscillations	Intracellular calcium rise in response to glucose is heterogeneous within islets		
Biosynthetic activity	High glucose-responsive, larger beta cells exhibited higher rates of insulin synthesis after glucose incubation		
Replicative activity	In the adult islet only 3% of the beta cells have proliferative capacity		
Adaptation to physiological and pathological stressors	Beta cells in the splenic region of the pancreas (compared to beta cells from gastric and duodenal regions) increased proliferation and glucose-induced insulin release in response to high fat diet		
Response to treatment	Tolbutamide induced heterogeneous intracellular calcium increase in rat beta cells		
Susceptibility to damage	The subpopulation of glucose unresponsive beta cells was markedly more susceptible to oxidative damage[7] Intercellular differences in oxidative state can explain differences in sensitivity to beta cell cytotoxic agents[3]		
Blood supply	Most but not all beta cells are aligned along capillaries **Most beta cells are in close proximity to vascular cells and aligned along blood vessels in random order[16]**		
Innervation	Only a small proportion of beta cells receive an axon terminal		

Table 1. Evidence of phenotypical and/or functional heterogeneity among beta cells.

In bold, human studies. GK = Glucokinase; Glut2 = Glucose transporter2; Fltp = Flattop, Pdx1 = Pancreatic and duodenal homeobox 1; PSA-NCAM = Sialylated form of the neural cell adhesion molecule; Dkk3 = Dickkopf3. VMAT2= vesicular monoamine transporter 2.

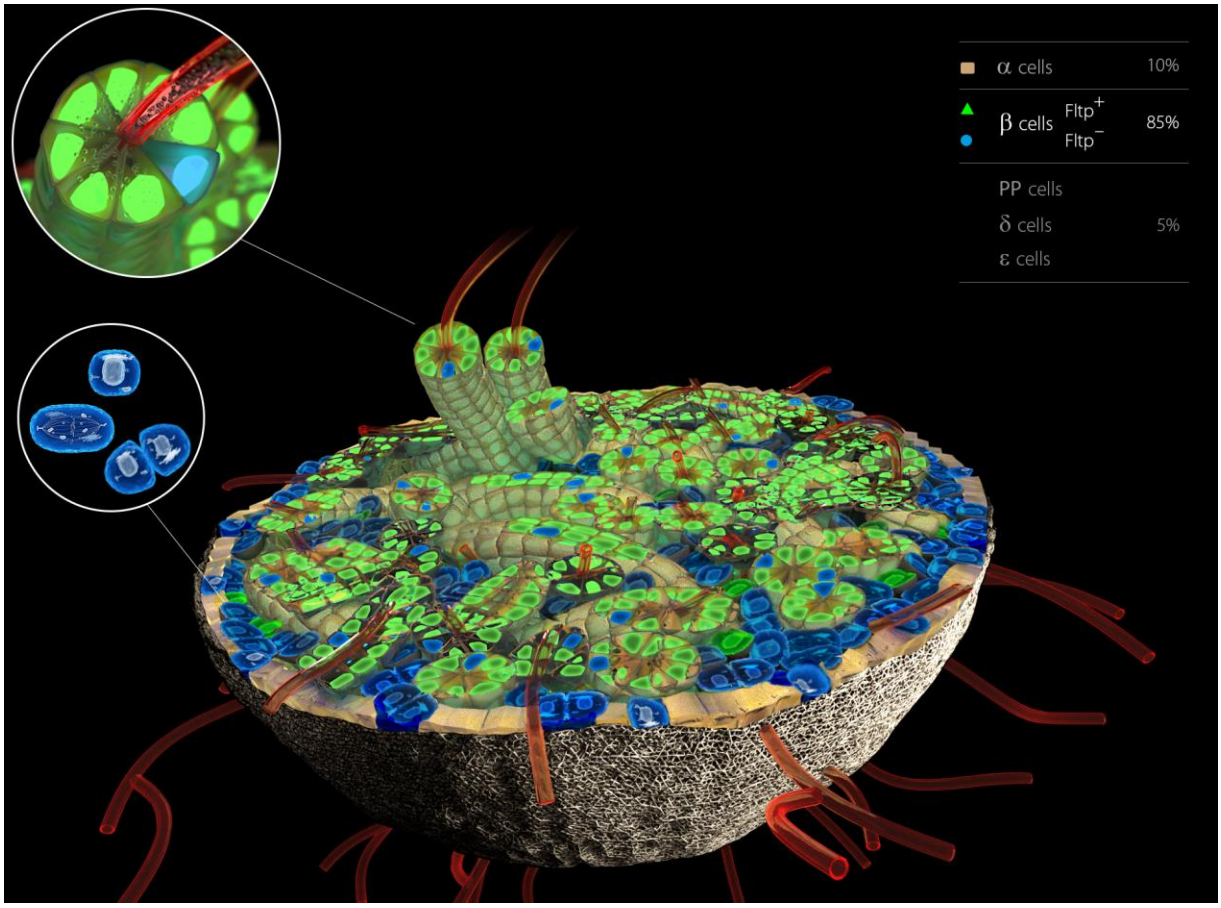
α cells 10%
β cells Fltp+ Fltp− 85%
PP cells
δ cells 5%
ε cells

Figure 1. Hypothetical 3D model of a mouse islet of Langerhans illustrating functional beta cell heterogeneity.

Beta cells (blue and green) are the most abundant cell type in the islet (85%) and are located in the core of the mini-organ. Non-beta cells, including ca. 10 % alpha cells (shown in yellow) and remaining 5% delta, pancreatic polypeptide and epsilon cells (not shown for clarity) are normally located at the islet periphery. The blood vessels penetrate the islet at different levels and heterogeneously interact with the endocrine cells. The novel Wnt/PCP effector and reporter molecule Flattop (Fltp) distinguishes two unique beta cell subpopulations with distinct molecular, physiological and ultrastructural features. The Fltp-Venus reporter $(FVR)^{+}$ beta cells (green) comprise 80% of the total beta cell population. They are terminally differentiated, highly polarized and more mature, likely due to active Wnt/PCP signalling that allows them to preferentially localize in rosette-like structures around blood vessels for proper tuning of glucose sensing and insulin secretion. Contrarily, FVR^{-} beta cells (blue) constitute only 20% of the total beta cell mass, they are less polarized and less mature, but more responsive to environmental cues. This beta cell subpopulation has a higher proliferative capacity and can mature into FVR^{+} beta cells, suggesting that these cells constitute a reserve pool of immature cells that can compensate for metabolic demand or pathological insult.

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