Diabetes and the pancreas

The two most commonly known forms of diabetes — type 1, also known as juvenile-onset diabetes, and type 2, also known as adult-onset diabetes — are increasing at an alarming pace. Type 1 diabetic patients have partial or complete β-cell deficiency. Type 2 diabetic patients exhibit reduced action of insulin in skeletal muscle, fat and liver cells (insulin resistance), combined with reduced insulin secretion from the pancreatic β-cells. Insulin resistance was previously believed to be the primary cause of the disease. However, it has now become evident that early β-cell defects are a major cause: healthy β-cells can adequately compensate for the increased insulin requirement due to insulin resistance. Type 2 diabetes develops only after β-cell defects arise. Thus, by improving the adaptive β-cell response, diabetes may be treated properly. This paradigm shift makes studies of the generation and physiology of the β-cell of key relevance for type 2 diabetes, enabling the development of mechanistically new drugs that aim to correct the β-cell defects of type 2 diabetic patients.

Type 2 diabetes is a contemporary global epidemic affecting at present over 150 million people and with an expected increase of 5–6 million cases per year. Obesity, western dietary habits, lack of physical activity and aging will continue to drive the dramatic growth in type 2 diabetes for decades to come. Today’s most widely used diabetes drugs were discovered several decades ago when there was little understanding of the disease: they aim only to suppress the characteristically elevated blood glucose levels, without any attempt to correct the underlying causes of the disease. As a result, the disease typically progresses, leading eventually to a loss of drug effectiveness and the onset of serious vascular complications affecting the eyes, kidneys, nervous system, brain and heart. Thus, there is an acute clinical need for novel therapeutic inventions.

Type 1 diabetic patients and patients with severe forms of type 2 diabetes may benefit significantly from transplantation of normal, functional β-cells. The recent development of new protocols to prevent the rejection and improve the viability of transplanted pancreatic islet cells has validated the principle of this approach to restore the number of functional β-cells required to normalize blood glucose levels and thus to cure diabetes [1]. This therapy is, however, not yet practical on a large scale because of a shortage of human islets or β-cells. One attractive approach to creating sufficient numbers of transplantable cells is to generate functional β-cells from stem and/or progenitor cells. To be clinically useful as a replacement for current therapies, these cells should secrete fully processed insulin in response to physiological concentrations of glucose. To attain this goal, in-depth knowledge is required of the processes that operate during normal embryogenesis to control the proliferation, specification, commitment and differentiation of pancreatic progenitor cells. Four specific key steps need to be achieved. These comprise the identification and isolation of: (1) pancreatic stem or progenitor cells that have the capacity to both self-renew and generate differentiated progeny; (2) proliferative signals that can expand pancreatic progenitor cells; (3) instructive signals that can induce the differentiation of stem/progenitor cells into functional β-cells; and (4) signals that maintain viability and the correct physiological state of the β-cells.

Developmental biology studies

Inductive interactions control the specification, proliferation, differentiation and death of cells during organ development in both invertebrate and vertebrate organisms. Information derived from different organisms and from multiple organs can therefore be integrated to generate ideas and models of how individual cells, tissues and organs are generated. The signaling pathways regulating these developmental processes are also involved in controlling the homeostasis of organs, and perturbation of the pathways...
often leads to disease. Thus, information acquired through developmental biology studies can be integrated with the aim of understanding in detail the development of organs, such as the pancreas, and how specific sets of cells, such as the insulin-producing β-cells, are generated. These types of studies will also provide information on how diabetes is linked to developmental perturbations and hopefully identify novel drug targets and strategies for new therapies. This information is also pivotal to our ability to identify, isolate and propagate pancreatic stem and progenitor cells and to differentiate these cells into physiologically active cells, such as insulin-producing cells, that can be used for cell replacement therapy.

The induction and patterning of tissues and organs in multicellular organisms are regulated by extracellular signals derived from neighboring cells and tissues that control the specification, proliferation, differentiation and death of the cells. The signals, either soluble or membrane-bound, interact with receptors or receptor complexes that stimulate intracellular signaling pathways, which in most cases lead to the nucleus, resulting in changes in gene expression. A limited number of different families of extracellular signals appears to be sufficient to induce and pattern most tissues and organs. Each family of signals and receptors consists, however, of multiple members that interact with different specificity. Most of these signals and pathways were initially identified and analysed genetically in Drosophila and Caenorhabditis elegans but have subsequently been shown to perform the same or similar functions in vertebrate organisms. The concerted actions of signals such as Hedgehog (HH), bone morphogenic protein (BMP)/transforming growth factor-β (TGFβ), fibroblast growth factor (FGF), epidermal growth factor (EGF), retinoic acid, Wingless (Wnt/Wg), Notch, and ligands to G-protein-coupled receptors, etc., appear to control many of the processes that lead to the formation of most organs. The evolutionary conservation of these pathways and their functions allows information derived from different organisms and from multiple organs to be integrated to generate ideas, and models how individual cells, tissue and organs are generated and how perturbation of these pathways leads to disease.

Thus, the challenge now is not so much to identify novel signals but to understand the mechanisms by which a limited number of signaling pathways interact to control the development and physiology of cells and organs. One needs to understand ligand receptor and signal transduction specificity and how at different developmental stages signals can act in either a convergent, opponent or hierarchical manner, and how cells in different organs change with time their competence to respond to these signals. Information on basic mechanisms of organogenesis is crucial for our understanding of the development and homeostasis of the pancreas and of mechanisms that cause diabetes. This information is also pivotal to any attempt to generate functional insulin-producing β-cells suitable for transplantation.

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The morphogenesis and structural development of the pancreas are well characterized [2, 3]; studies of pancreatic gene expression have identified a large number of markers, mainly transcription factors [4–8], that can be used to define pancreatic cells at different stages of development. The pancreas is also suitable for the genetic study of organ homeostasis, because blood glucose levels can be monitored easily in the living animal and they reflect the physiological state of the insulin-producing β-cell, and relevant animal models of diabetes have been generated. The genetic dissection of pancreas development and β-cell function in the mouse has thus provided valuable information on the basic mechanisms of organogenesis, mature β-cell function and maintenance of normal blood sugar levels.

Early pancreatic development

The embryonic pancreas is an endodermally derived organ that forms via a dorsal and a ventral protrusion of the primitive gut epithelium. The majority of cells of the primitive pancreatic bud that forms from the evaginating gut epithelium between embryonic day (e) 8 and e9.5 represent the pancreatic stem, or progenitor cells, that will proliferate, self-renew and differentiate in response to instructive cues, to finally give rise to the definitive pancreas consisting of a mixture of exocrine and endocrine cells. The endocrine pancreas makes up only a small percentage of the entire pancreas and consists of four cell types, α-, β-, δ- and PP-cells, which respectively produce the hormones glucagon,
insulin, somatostatin and pancreatic polypeptide. The homeodomain protein IPF-1/PDX-1 and the basic Helix-Loop-Helix (bHLH) class transcription factor Ptf1a/p48 are both expressed in the early pancreatic progenitor cell (Fig. 1), and genetic analyses in mice have shown that these genes are crucial for pancreatic development [8–12]. Mice mutant for either of these genes fail to develop a pancreas, although a few endocrine cells still form, in particular in Ptf1a/p48 mutant mice [9–12]. Specification of the pancreatic anlage still occurs in both Ipf1/Pdx1 and Ptf1a/p48 mutant mice and hence the function of these genes is required downstream of the initial specification of the gut endoderm to a pancreatic fate [9–12]. In contrast, the homeobox gene Hlx9, encoding HB9, which is transiently expressed in the dorsal and ventral pancreatic anlage, is required for the specification of the dorsal but not the ventral pancreatic anlage [13, 14]. In mice lacking Hlx9 function, dorsal pancreatic development is blocked, whereas the ventral pancreas develops virtually normally into both endocrine and exocrine cells [13, 14]. Recent data from analyses of mice lacking the homeobox gene Hex further emphasize the differences in dorsal and ventral pancreatic development. Hex is expressed in the ventral foregut endoderm of early embryos, including the prospective ventral pancreatic region, but not the dorsal endoderm [15]. In Hex−/− mice the ventral endoderm is truncated and hence no ventral endoderm protrudes beyond the juxtaposed cardiac mesoderm [15]. Cardiac mesoderm promotes liver development but blocks pancreatic development [16, 17], consequently the ventral, but not the dorsal, pancreatic program is impaired in Hex−/− mice [15].

Another important component of the early specification of the pancreatic program involves the exclusion of the expression of the Hedgehog genes Sonic hedgehog (Shh) and Indian hedgehog (Ihh) in the regions of the gut endoderm from which the pancreas forms. Genetic analyses in mice have revealed that Hedgehog promotes an intestinal differentiation program at the expense of pancreatic development (Fig. 1) [18]. Factors emanating from notochord have been suggested to ensure that the expression of the Hedgehog genes is excluded from the presumptive dorsal pancreatic region [19–21], but the exact mechanisms by which Hedgehog gene expression is excluded from the domains of the prospective pancreatic anlage remain unknown. Data from chick in vitro cultures point towards a role for lateral plate mesoderm in ensuring the expression of early pancreatic markers such as Ipf1/Pdx1, Ptf1a/p48 and Nkx6.1 [22]. Moreover, lateral plate mesoderm from the prospective pancreatic region could induce the expression of these pancreatic markers in more anterior endoderm, i.e. endoderm that later will generate the stomach [22]. This mesodermal activity is partly mimicked by members of the TGFβ/BMP family of signaling factors and retinoic acid [22]. Yet another mesodermally derived group of cells, endothelial cells, has been shown to promote pancreatic development by ensuring robust expression of key pancreatic genes such as Ipf1/Pdx1 and p48/Ptf1a [23, 24]. Thus, a number of mesodermally derived cells and tissues (for summary see Table I) control important aspects of early pancreatic development.

Pancreatic growth

Although the pancreatic stem cells that reside in the early pancreatic buds will generate all the different pancreatic cell types, they critically rely on

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<th>Table 1: Mesenchymal tissues and cells that influence pancreatic development.</th>
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<td><strong>Type of mesodermal tissue</strong></td>
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<td>Later plate mesoderm</td>
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external signals for proper proliferation and differentiation. Experiments performed in the 1960s described the mesenchyme to epithelium signaling during pancreas development, an event that is crucial for pancreatic growth, morphogenesis and differentiation [27]. Mesenchyme of non-pancreatic origin could, to a large extent, support the development of the pancreatic epithelium, suggesting that, at least at this stage, the cues to pancreatic identity are intrinsic to the pancreatic epithelium. The true nature of the mesenchymal factor(s) remained, however, elusive.

Today, accumulating data suggest that two families of signaling factors, EGF and FGF, stimulate growth and morphogenesis of the pancreas. Mice with perturbed EGF signaling due to deletion of the EGF receptor genes $\text{ErbB1}$ and $\text{ErbB3}$ show perturbations in pancreatic growth [28, 29]. Moreover, EGF stimulates growth of embryonic pancreatic epithelium when cultured in vitro [30]. The FGF family of growth factors exerts key roles in stimulation of cellular growth during embryogenesis and has been implicated in the development of many organs, in particular those that, like the pancreas, depend on epithelio-mesenchymal interactions [31, 32]. Several independent studies suggest a role for FGF signaling during pancreatic development and for $\beta$-cell function [25, 26, 33–37]. In vitro cultures of embryonic pancreatic rudiments have shown a stimulatory effect of various FGFs on pancreatic epithelial cell proliferation and exocrine cell differentiation [33, 34]. In vivo gain- and loss-of-function analyses also provide evidence for a stimulatory role of FGFs on pancreatic growth. Mice that express a dominant negative form of FGFR2b under the control of the inducible, ubiquitously active metallothionein promoter [34], and mice in which the gene encoding the FGFR2b high-affinity ligand FGF10 has been inactivated by targeted disruption [25, 36] have underdeveloped pancreata. Gain-of-function analyses of Fgf10 in vivo in transgenic mice show that Fgf10 stimulates pancreatic progenitor cell proliferation while inhibiting pancreatic cell differentiation (Fig. 1) [26]. In these mice the persistent expression of Fgf10 in the developing pancreatic epithelium leads to a greatly enhanced proliferation of pancreatic progenitor cells at late stages of development. Consequently, these mice show pancreatic hyperplasia. Not surprisingly, their cell differentiation is drastically impaired. Thus, under these conditions FGF10 appears to promote not only the proliferation of pancreatic progenitor cells but also the maintenance of a progenitor-like state [26]. The latter seems to involve crosstalk between FGF and Notch signaling (described below). Although both genetic and in vitro analyses imply a role for EGF and in particular FGF10-FGFR2b signaling in the stimulation of pancreatic growth and morphogenesis, additional factors are likely to influence the growth of the embryonic pancreas.

**Fig. 1:** Schematic summary of pancreatic cell differentiation. Gastrulation results in formation of the endoderm from which the pancreas is later specified. Sonic hedgehog (SHH) inhibits pancreatic development and promotes an intestinal program. $\beta$-Cells activate the Notch receptor in neighboring cells. In cells where Notch is not activated, neurogenin3 (Ngn3) expression is allowed and these cells subsequently differentiate into mature endocrine cells. Cells in which Notch is activated turn on Hes1 expression, which in turn represses Ngn3 expression. These cells remain as proliferating progenitors in response to FGF10 and serve as a source for the generation of differentiated pancreatic cells, both endocrine and exocrine, throughout pancreatic development. A subset of endogenous markers is indicated at the different stages.

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**Control of differentiation**

Lateral specification mediated by the Notch signaling pathway is a classical way of specifying a particular cell fate within a field of initially
equivalent cells. Notch signaling involves cells expressing high quantities of the ligands (Delta or Serrate) that signal activation of Notch receptors on neighboring cells, in which the activated intracellular Notch receptor (NIC) suppresses the primary cell fate, i.e. the cell fate adopted by the signaling cell [5, 38]. Activated NIC subsequently interacts with the DNA-binding protein RBP-Jκ to activate expression of bHLH repressor genes, such as the Hes genes, which in turn repress expression of downstream target genes that if expressed would promote the primary cell fate [5, 38]. Analyses of mice in which the expression of Notch signaling has been genetically perturbed show a direct role for Notch signaling during pancreatic development. Ectopic expression of neurogenin3 (Ngn3) (also known as Math-4b) or intracellular Notch1 in early pancreas progenitors [39], and the inactivation of the genes encoding the ligand Delta-like 1 (Dll1) [39], the intracellular mediators RBP-Jκ [39], Hes1 [40] or Ngn3 [41], perturb pancreatic cell differentiation. Taken together these studies collectively demonstrate that Notch signaling controls the choice between differentiated endocrine and progenitor cell fates in the developing pancreas (Fig. 1). Moreover, these studies show that not only is Ngn3 expressed in pancreatic endocrine progenitors but it is also critically required for endocrine cell specification [39, 41]. Blocking Notch receptor activation in early pancreatic progenitors results in high Ngn3 expression which promotes early endocrine cell differentiation at the expense of pancreatic cell proliferation [39, 40]. Accordingly, maintenance of NIC in pancreatic progenitor cells, as observed in mice with persistent expression of Fgf10 [26], impairs pancreatic cell differentiation and instead enhances proliferation. Thus, activation of Notch signaling is essential to prevent premature pancreatic progenitor cell differentiation, thereby allowing subsequent proliferation and morphogenesis of the pancreatic progenitor cells.

A number of different transcription factors are implied in the differentiation of islet cells. Pax4, which is predominantly expressed in the embryonic pancreas but has no or a very low level of expression in the adult pancreas, is critically required for the generation of β- and δ-cells and is thought to act at the level of cell specification [42]. The NK-related homeobox genes Nkx2.2 and Nkx6.1 appear to act sequentially to assure β-cell differentiation. In mice mutant for these genes, β-cells are specified but fail to terminally differentiate [43, 44]. In contrast to Nkx6.1, the expression of which is restricted to β-cells at late stages of pancreatic development, Nkx2.2 is expressed also in α- and PP-cells and the differentiation of these cells is also perturbed in Nkx2.2 mutants [43]. The genes Iis1, Pax6 and NeuroD are expressed in all pancreatic endocrine cells, and in mice mutant for any of these genes pancreatic endocrine cell differentiation is perturbed [45–48]. In contrast to Ngn3, these genes are not expressed at the pro-endocrine stage and hence act at a later stage of endocrine cell differentiation.

β-Cell function

In fasting states the β-cell stores insulin and C-peptide within storage granules in readiness for the release of insulin following glucose stimulation. Glucose is taken up by the cell via the low-affinity glucose transporter type 2 (GLUT2), and in the cell glucose becomes phosphorylated by the key glycolytic enzyme glucokinase. Oxidative metabolism of glucose then subsequently leads to an increase in the cytosolic ATP/ADP ratio, which in turn results in a closure of the ATP-sensitive K+ ATP transmembrane channel. The resulting membrane depolarization and subsequent activation of the voltage gated Ca2+ channel then leads to an influx of Ca2+, which stimulates exocytic release of insulin and C-peptide into the bloodstream (Fig. 2) [49]. Thus, efficient glucose uptake and subsequent metabolism ensure the generation of the secondary signals that are critical for glucose-stimulated insulin secretion.

The homeobox gene Ipfl/Pdx1 exerts a dual role during pancreatic ontogeny: it is required at early stages for pancreatic development but has a later, distinct role in adult β-cells. These dual roles for Ipfl/Pdx1 are conserved between mice and humans [9, 10, 50]. Thus, genetic inactivation of the gene in mice [9, 10] or homozygosity for a nonsense mutation in the human IPF1 gene [50] results in complete agenesis of the pancreas. Inactivation of Ipfl/Pdx1 specifically in β-cells of adult mice leads to development of diabetes in the animal [51], and heterozygosity for a nonsense mutation in the human IPF1 gene [52] has been linked to maturity-onset diabetes of the young (MODY) [4]. These studies demonstrate that Ipfl/Pdx1 gene function is crucial for β-cell function and maintenance of glucose homeostasis. Additional genes that have been shown to be required in humans, and in mice, to control β-cell function include other MODY genes: glucokinase (MODY2), Hnf1α (MODY3), Hnf1β (MODY5), Hnf4α (MODY1), and NeuroD/Beta2 (MODY6) [53, 54].
Like Ipf1/Pdx1, FGF signaling appears to operate at more than one stage during pancreatic development. In the adult pancreas, FGFR1 and 2, along with several ligands, are expressed in β-cells and the attenuation of FGFR1c signaling in mouse β-cells leads to diabetes [26]. Mice with impaired FGFR1c signaling develop diabetes as a result of impaired glucose sensing, due to loss of Glut2 expression, and increased proinsulin content in β-cells, due to impaired expression of prohormone convertases 1/3 (Fig. 2) [26]. In addition, intact FGFR1c signaling appears to be required for the postnatal expansion of β-cells. In mice Ipf1/Pdx1 seems to act upstream of FGFR1 signaling in β-cells to maintain proper glucose sensing, insulin processing and glucose homeostasis. These data demonstrate that FGF signaling is required not only during pancreatic organogenesis to assure pancreatic growth but is also required in adult β-cells to ascertain their mature function.

The use of stem or progenitor cells as a source for β-cell replacement therapy will critically depend on two important aspects: (1) the use of appropriate markers [8, 55, 56] that allow the classification of distinct stages of cell differentiation; and (2) information regarding key signaling factors that operate at the different stages of differentiation to ultimately guide the cell towards a mature β-cell (Figs 1 and 2). Studies of pancreatic development have and will continue to generate information that is of relevance to both these aspects. The information thus obtained needs to be integrated with our current knowledge of pancreatic development, β-cell differentiation and function, to ascertain that stringent criteria regarding marker gene expression and functionality are used in the evaluation of stem and progenitor cell-derived β-cells.

References

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