Pancreas development and diabetes

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The past few years have seen an increase in interest about the molecular and genetic events regulating pancreas development. Transcription factors such as Pdx1, p48 and Nkx2.2 have been shown to be essential for the proper differentiation of exocrine and endocrine tissue; however, pancreas development also involves intricate interactions between the pancreatic epithelium and its surrounding mesenchyme. Signalling factors emanating from the notochord have been shown to repress Sonic hedgehog expression in the endoderm whereas signals originating from the pancreatic mesenchyme determine the proportion of exocrine to endocrine tissue. Understanding the molecular and genetic events underlying pancreas development also opens the door for devising new therapeutic strategies against pancreatic diseases such as diabetes and cancer.

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Abbreviations

FGF	fibroblast growth factor
HNF	hepatocyte nuclear factor
Ihh	Indian hedgehog
MODY	maturity-onset diabetes of the young
NIDDM	non-insulin-dependent diabetes mellitus
PP	pancreatic polypeptide
Ptc	Patched
Shh	Sonic hedgehog

Introduction

The pancreas is an essential organ important for digestion and glucose homeostasis in higher organisms. Malfunction of the pancreas results in several debilitating diseases such as diabetes, pancreatitis and pancreatic cancer. Recent advances in pancreas development have allowed the discovery of a certain number of key transcription and signalling factors necessary for the proper differentiation of the various pancreatic cell types. The pancreas possesses both an exocrine function involved in the delivery of enzymes into the digestive tract and an endocrine function by which several hormones are secreted into the bloodstream of an organism to co-ordinate and regulate the use of glucose. The exocrine function is performed by acinar cells that produce various digestive enzymes (amylase, proteases, nuclease, etc.) and duct cells that transport these enzymes to the intestine. The functional unit of the endocrine pancreas are the islets of Langerhans which are dispersed throughout the exocrine portion of the pancreas and are composed of four cell types: α, β, δ and PP cells. The insulin-producing β cells represent the majority of the endocrine cell population and form the core of the islet whereas the sparser α, δ and PP cells secrete glucagon, somatostatin and a pancreatic polypeptide respectively and are found at the periphery of the islets.

Pancreas organogenesis involves a sequential cascade of inductive events in association with the activation of specific transcription factors. During early embryogenesis, the pancreas arises from an evagination of the foregut to form initially a dorsal, and at a later stage, a ventral epithelial bud. Both buds subsequently proliferate to form multiple branches and fuse together to make a functional organ. During this process, it is thought that both exocrine and endocrine cells differentiate from a common pluripotent progenitor derived from the endoderm.

Transcription factors and pancreas development: an increasing list

In the past few years, a growing number of transcription factors have been shown to be necessary for proper pancreas development. These transcription factors are listed in Table 1 and are discussed extensively in two recent reviews [1,2]. Recent additions to the list include the exocrine DNA- binding factor p48 and the NK2 homeobox Nkx2.2. p48 is a pancreas-specific basic helix-loop-helix protein that, along with the ubiquitously expressed p64 and p75 proteins, will form the hetero-oligomeric transcription complex PTF1 [3]. Whereas PTF1 is necessary but not sufficient for regulation of exocrine pancreas-specific gene expression, p48 by itself is also involved in early pancreas development: mutant mice lacking p48 fail to develop any exocrine tissue [4••]. Differentiation of the endocrine cell population does occur in the absence of p48 but these cells are found dispersed throughout the adjoining spleen. The exact circumstances which allow the endocrine cells to populate the spleen are difficult to understand. It is most likely that, in the absence of exocrine tissue, the differentiated endocrine cells migrate freely from the pancreatic epithelium through the surrounding mesoderm and into the spleen. What is less clear is why the spleen is chosen as the final resting place for the cells instead of other tissues, such as the intestine or the liver. It is possible that the spleen may provide a more favourable environment for the survival and function of the endocrine cells in the absence of exocrine tissue.

The transcription factor Nkx2.2 is first expressed in the pancreatic epithelium as the dorsal bud is being formed in day 9.5 mouse embryos [5•]. As differentiation proceeds, Nkx2.2 expression becomes restricted to all β cells and most α and PP cells. Expression is not detected in mature

Table 1

Relevant transcription factors involved in pancreas development.

Transcription factor	Onset of expression*	Expression in adult pancreas	Pancreas phenotype in null mutant mice	References
Pdx1	8.5	β-cells	Absence of pancreas.	[7,8,39]
Isl1	9.0	β-, α-, δ-, PP-cells Dorsal mesenchyme	Absence of endocrine cells. No differentiation in dorsal bud.	[40]
Pax6	9.0–9.5	β-, α-, δ-, PP-cells	Absence of glucagon cells. Islet malformation.	[41,42]
Pax4	9.5	β-, δ-, PP-cells	Absence of insulin and Somatostatin cells. Increase in glucagon cells.	[28]
Nkx2.2	9.5	β-, α-, PPcells	Islet mass reduced. Absence of insulin cells. Decrease in glucagon cells.	[5•]
Nkx6.1	9.0–9.5	β-cells	NP	[43]
NeuroD/BETA2	9.5	β-, α-, δ-, PP-cells	Reduction of endocrine cells. Islet malformation.	[44]
p48	10	Exocrine cells	Absence of exocrine cells. Endocrine cells found in spleen.	[4••]

*In mouse embryos. NP, not published

δ cells. In *Nkx2.2* homozygous mutant mice, there is a decreased islet cell mass with a complete absence of insulin-positive cells and a severe reduction in the glucagon cell population [5•]. Unsurprisingly, somatostatin-producing cells remain unaffected and a slight reduction in the number of PP-producing cells is observed. Cells in the mutant islet clusters do not express insulin, glucose transporter 2 or glucokinase characteristics of mature hormone-producing β cells but certain early markers such as *Pdx1*, *Pax6*, islet amyloid polypeptide and prohormone convertase 1/3 are detected, suggesting that cells had undergone some level of β-cell differentiation before being arrested in an immature state. Therefore, Nkx2.2 is involved in the terminal differentiation of β cells into functional hormone-producing cells.

Finally, with the exception of p48, almost all transcription factors described to date are involved in endocrine cell differentiation. Although these factors facilitate a better understanding of the genetic mechanisms involved in the generation of exocrine and endocrine cells, much work is still needed before the definitive lineage of each cell type can be determined. In particular, the gene(s) involved in establishing and defining the early multipotent progenitor cell from which all pancreatic cells are putatively derived remains unknown. It is also becoming clear that most transcription factors possess early functions involved in the differentiation of the various cells types and late functions involved in maintenance, function and the optimal expression of hormonal or enzymatic genes in adult mature cells. Tissue- and temporal-specific mouse mutants, as well as *in vitro* organ culture studies, will surely permit us in the future to determine additional functions for each gene.

Early and late inductive events: the role of the notochord and surrounding mesenchyme

In addition to transcription factors, pancreas formation also requires a series of initial and secondary inductive signals emanating from surrounding mesodermic tissues. Early events that define and pattern the region of the endoderm which will give rise to the pancreas are undefined but a pre-patterning of the endoderm seems to occur that will define and specify the area of the embryonic foregut from which the pancreatic buds will form. Spatial expression of members of the hedgehog family in the endoderm strongly suggest such a patterning of the foregut [6]. Although both Sonic hedgehog (Shh) and Indian hedgehog (Ihh) are expressed along the entire endodermic gut, the region that will give rise to the future pancreas does not express either molecules (Figure 1a). The region devoid of hedgehog expression also coincides to the region where Pdx1 will first be expressed [7,8]. It is thought that expression or repression of *Shh* in the endoderm is important in determining the differentiation of the surrounding mesoderm into specialised intestinal or pancreatic mesenchymes. This is supported by the observation that Patched (Ptc), a candidate receptor for Shh, is expressed in the mesoderm surrounding the stomach and duodenum but is absent from pancreatic mesoderm [9••]. Furthermore, ectopic expression of *Shh* in the pancreatic epithelium transforms the pancreatic mesoderm into smooth muscle although it also induces the differentiation of the epithelium into a mixture of pancreatic and duodenal cell types [6].

Experiments performed in chicken have shown that close contact of the notochord to the dorsal endoderm intervenes in *Shh* repression of the pancreatic epithelium and the subsequent activation of pancreatic genes such as

Pdx1, *Pax6*, *Isl1*, insulin and glucagon in the dorsal bud [10]. In the ventral bud, however, transcriptional regulation of these genes does not seem to rely entirely on the notochord. The exact nature of the signals provided by the notochord and its action, whether direct or indirect, on the pancreatic epithelium remains to be clarified. Notochord factors such as activin-βB and fibroblast growth factor 2 (FGF2) appear to participate in the repression of endodermal *Shh* in the pancreatic anlage [9••,11]. Additional factors, possibly from other sources, might also be involved as there is no patterning of either activin-βB, FGF2 or other known signalling factors in the notochord that correspond to the pre-patterning seen in the endoderm. Interestingly, cyclopamine, a steroid alkaloid teratogen produced by the plant species *Veratrum californicum*, can promote pancreatic development [12•]. Cyclopamine acts as an inhibitor of hedgehog signalling possibly by affecting cholesterol biosynthesis and the functional status of Ptc in target tissue [13]. Treatment of endodermic foregut and surrounding mesoderm with cyclopamine increases the region of the endoderm that does not respond to Shh signalling. As a result, there is an extension of *Pdx1* expression in the epithelium and formation of heterotopic pancreatic structures in the distal stomach and duodenum. Cyclopamine effect on the endoderm, however, requires the presence of the surrounding mesenchyme. Nonetheless, stomach and intestinal mesenchymes may also be affected by cyclopamine as *Ptc* expression in these tissues is also reduced.

Pancreas development also requires signals originating from the pancreatic mesenchyme (Figure 1b). These signals will determine the exocrine–endocrine tissue ratio in the pancreas. The mesenchyme is particularly important in stimulating exocrine development while restricting endocrine differentiation. Studies have shown that the removal of large portions of the mesenchyme in pancreas organ culture will abolish development of the exocrine cells and promote exclusive differentiation of the epithelium into endocrine tissue [14]. One of the secreted mesodermic factors that could participate in the induction of exocrine development is follistatin, an inhibitor of factors important for cellular differentiation such as activin and bone morphogenetic protein 7. Indeed, follistatin can replace the effect of mesenchyme on exocrine differentiation in organ culture assays [15••]. Follistatin is also expressed in the pancreatic mesenchyme during embryogenesis. As activin is known to stimulate endocrine differentiation, it is conceivable that follistatin restricts the effect of activin on the epithelium and therefore favours exocrine differentiation.

Pancreatic transcription factors and diabetes

In general, it is assumed that most cases of non-insulin-dependent diabetes mellitus (NIDDM) are the results of polygenic disorders; however, several monogenetic forms have been identified. Maturity-onset diabetes of the young (MODY) is characterised by autosomal dominant inheritance and defects in insulin secretion causing an early onset of NIDDM, usually before the age of 25. Members of the hepatocyte nuclear factors (HNF) family turned out to be responsible for different types of monogenetic MODY. Recent findings [16,17] have shown that mutations in the homeobox-containing gene *HNF-1α* and in the orphan nuclear receptor HNF-4α can be associated with MODY1 and MODY3 respectively. These mutations interfere particularly with the transactivation, dimerization and DNA-binding activities of the HNF-proteins, implying that the diabetic phenotype is caused by loss in the regulation of HNF target genes. Transgenic mouse models and analysis of the mutated proteins showed that HNF-1α and HNF-4α participate in the control of glucose transport, glycolysis and insulin-secretion processes [18,19•]. In addition, HNF-3α, -β, -γ, HNF-4γ and possibly HNF-6 are also expressed in pancreatic islets and are therefore probable candidates for new MODY genes [20]. This is supported by the finding that HNF-1α, HNF-4α and their respective target genes are regulated by HNF-3α and -β in

Figure 1

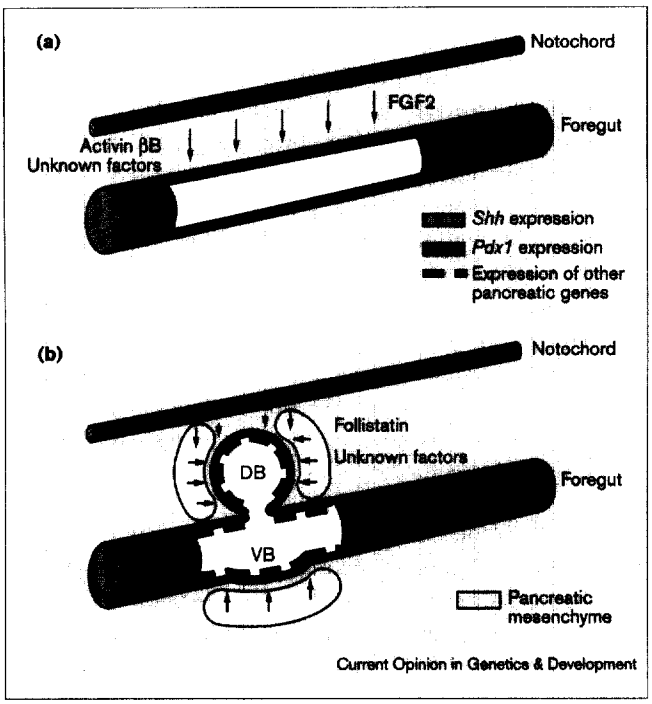

Illustration representing the mesenchymal–epithelial signalling occurring during pancreas development. **(a)** Before the formation of the pancreatic buds, a pre-patterning of the gut endoderm renders the dorsal endoderm competent to respond to specific signals coming from the notochord. Notochord factors such as activin-βB, FGF2 and probably other unidentified factors will repress endodermal Shh expression thereby permitting the expression of Pdx1 and other pancreatic developmental genes. **(b)** As the pancreatic buds form and proliferate, factors produced by the mesenchyme surrounding the pancreatic epithelium will favour the development of the exocrine tissue while restricting endocrine differentiation. Follistatin and additional unknown factors probably participate in this process. The gut endoderm and possibly the notochord may also be involved in specifying the pancreatic mesenchyme from intestinal mesenchyme. DB, dorsal bud; VB, emerging ventral bud.

embryoid bodies [21•]. HNF-3β is also involved in the β-cell-specific regulation of the *Pdx1* gene which is essential for proper β-cell function [22].

Recent analysis of a human patient with pancreatic agenis led to the identification of a single-base deletion in the homeobox gene *PDX1* (also known as *Ipf1*, *Stf1* and *Idx1* in mouse). This mutation results in the premature termination in translation, creating a PDX1 protein without its DNA-binding properties [23,24••,25]. In this case, the patient was homozygous for this point mutation whereas both parents were heterozygous for the same mutation. Subsequent analysis of the family pedigree showed a high prevalence of diabetes mellitus with a heterogeneous phenotype ranging from normal glucose tolerance to overt NIDDM. These data imply that *PDX1* may represent a new genetic locus for MODY4.

New information has been obtained concerning the function of the *Pdx1* gene in the differentiation and function of β cells. Mutant mice in which *Pdx1* has been specifically deleted just in insulin-producing cells, become overtly diabetic as they age [26••]. Analysis of the pancreas of mutant mice demonstrated a progressive loss of Glut2 and insulin expression in the absence of Pdx1 and an upregulation of glucagon occurs in some cells. Hence, Pdx1 is required for functional expression of the Glut2 and insulin gene in mature β cells. Furthermore, the homeodomain transcription factor *Nkx6.1* is also lost in the absence of Pdx1. As it is thought that Nkx6.1 may act as a transcriptional repressor, it is possible that Pdx1 may activate expression of Nkx6.1, which in turn downregulates the expression of the glucagon gene in β cells. The phenotype observed in the mutant mice is reminiscent of symptoms observed in MODY4 individuals possessing a mutation in the human *PDX1* gene. Interestingly, mice lacking only one copy of *Pdx1* also develop impaired glucose tolerance, a step leading to overt NIDDM [27]. Therefore, both mutant mice may prove to be feasible animal models to study some aspects of the human disease.

The Pax4 transcription factor has been shown to play an essential role in β-cell differentiation. Pax4-deficient mice do not develop β cells and die shortly after birth from impaired insulin production [28]. The *Pax4* gene could therefore represent a possible susceptibility gene for diabetes. Wolcott–Rallison syndrome is a rare disease characterised by skeletal malformations and neonatal diabetes caused by an absence of β-cell development. Sequence analysis of two unrelated patients suffering from WRS did not show any mutation in the *Pax4* cDNA [29]. The fact that no mutation has been found and that Pax4 is not involved in skeletal development makes it unlikely that Pax4 is associated with WRS but it may also be possible that the unknown gene responsible for WRS acts either directly or indirectly on *Pax4* expression. Nonetheless, transcription factors involved in β-cell differentiation and function should be studied in greater detail to determine their relevance in monogenetic and polygenetic forms of diabetes.

Conclusions and future prospects

As we begin to understand the different genes and signals involved in the differentiation of β cells during embryogenesis, it may be possible to use this knowledge to promote the generation of β cells *in vitro* for transplantation or even *in vivo* regeneration of missing or malfunctioning β-cells in diabetic patients. Several lines of evidence suggest that some cells forming the exocrine duct system of the pancreas may possess characteristics similar to the multipotent progenitor cells found in the pancreatic epithelium of embryos. In different models of experimental diabetes, but also in adult human pancreas, duct cells seem to retain the capacity for endocrine differentiation [30,31•]. Activating the appropriate combination of transcription factors in duct cells by using a gene-delivery system to introduce ectopic copies of these genes or applying signalling factors involved in pancreas development to induce expression of the endogenous genes could offer new therapeutic strategies against diabetes.

Along this line of thinking, several DNA delivery systems are being studied for possible use in the pancreas. Adenovirus-mediated gene transfer into islets has been achieved successfully *in vitro* and *in vivo* [32–34] but existing adenovirus vectors also produce a potent immune response and, in most cases, show only transient expression [35,36]. Lentiviral vectors offer the advantage of the stable introduction of foreign DNA into the host genome allowing long-term gene expression. They are also capable of infecting post-mitotic cells and were used recently to introduce the β-galactosidase reporter gene and the Interleukin 4 gene into whole islets [37,38]. Although these results open exciting new prospects for gene therapy, the development of absolutely biosafe vectors will be required considering the pathogenicity of the parental virus.

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