Control of pancreatic development by intercellular signals

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Abstract

Understanding pancreatic development is important for at least three reasons: first, from a cognitive point of view, to understand the development of a complex organ, the pancreas; next, because it is now clear that abnormal pancreatic development can give rise to specific forms of diabetes in humans; and finally, because, if we want to define new treatments for diabetes based on cell therapy or regenerative medicine, we will have to understand in detail how β-cells develop. In the present paper, we summarize what we currently know concerning pancreatic development and concentrate on some intercellular and environmental signals controlling pancreatic development.

Introduction

It can easily be postulated that understanding the physiology of β-cell mass development represents a necessary step to better understand the pathophysiology of diabetes and to progress in the definition of new treatments or a cure for diabetes. This point is supported by at least three examples. (i) The demonstration in 1994 that the pancreas did not develop in mice deficient for the transcription factor Pp1 (insulin promoter factor 1)/Pdx1 (pancreatic duodenal homebox-1) [1], leading to the identification of a cause of pancreatic agenesis in humans [2]. (ii) Progress in the definition of the crucial role of ATP-sensitive K+ channels for insulin secretion by pancreatic β-cells [3] which led to the description of ABCC8 (ATP-binding cassette protein C8), which encodes one of the proteins of the ATP-sensitive K+ channel present in β-cells, being mutated in patients with neonatal diabetes [4]. On the basis of these results, treatment with sulfonylureas was initiated in patients with permanent neonatal diabetes [4]. On the basis of these results, treatment with sulfonylureas was initiated in patients with permanent neonatal diabetes [4]. On the basis of these results, treatment with sulfonylureas was initiated in patients with permanent neonatal diabetes [4]. (iii) It now seems certain that production of the way β-cells form during normal development [5].

In the present paper, we review our current knowledge concerning pancreatic development and concentrate on the importance of some intercellular and environmental signals.

Pancreatic development and transcription factors

The mature pancreas contains two types of tissue: endocrine islets composed of cells that produce hormones such as insulin (β-cells), glucagon (α-cells), somatostatin (δ-cells), pancreatic polypeptide (PP cells) and ghrelin (ε-cells), and exocrine tissue composed of acinar cells that produce enzymes (e.g. amylase and carboxypeptidase-A) secreted via the pancreatic ducts into the intestine [6–10]. The pancreas originates from the dorsal and ventral regions of the foregut endoderm directly posterior to the stomach. Over the last few years, important findings have shed light on the processes controlling pancreatic endocrine cell development. Studies of genetically engineered mice identified a hierarchy of transcription factors regulating pancreas organogenesis and islet cell differentiation [11–16]. The endodermal region committed to pancreatic development first expresses the homeodomain-containing transcription factor Pdx1. Pdx1 is detected in mouse embryos at E8.5 (embryonic day 8.5) (E9 in rats, 5 weeks of development in human) in early pancreatic progenitor cells [17–19]. During adulthood, Pdx1 expression becomes largely confined to the β-cells, where it activates insulin gene transcription [17]. Targeted disruption of the Pdx1 gene in mice leads to pancreatic agenesis [20,21]. Interestingly, in 1997, Stoffers et al. [2] reported a case of a young patient with pancreatic agenesis. It was found that this patient was homozygous for a single nucleotide deletion of the human PDX1 gene, whereas both parents were heterozygotes for the same mutation. The deletion was not found in normal individuals, strongly suggesting that PDX1 is necessary for pancreatic development in humans. Taken together, these data indicate that Pdx1 is necessary for the morphogenesis and differentiation of the pancreatic buds both in rodents and humans [2]. Pdx1 is also an efficient marker of early pancreatic progenitor cells.

Differentiation into endocrine and exocrine cells is the next step during this process of differentiation, and cell-tracing experiments have shown that both endocrine and exocrine cells derive from Pdx1-expressing progenitor cells [22,23]. The basic helix–loop–helix transcription factor Ngn3 (Neurogenin3) is expressed in epithelial pancreatic progenitor cells before endocrine differentiation and is subsequently down-regulated during differentiation [24]. Ngn3 is necessary...
for pancreatic endocrine cell development, and Ngn3-deficient mice lack pancreatic endocrine cells [25]. Lineage-tracing experiments have provided direct evidence that Ngn3-expressing cells are islet progenitors [23]. Thus Ngn3 is a marker of choice for detecting the onset of pancreatic endocrine cell differentiation. It was reasonable to speculate that Ngn3 should have the same role in rodents and humans, and thus patients with mutations giving rise to lack of function of Ngn3 should be born diabetic without endocrine cells in their pancreas. However, a recent study showed that people with mutations in Ngn3 have significant congenital, malabsorptive diarrhoea, but are not diabetic at birth. Importantly, this loss-of-function mutation was described as giving rise to an inactive form of Ngn3 [26]. On the basis of such results, it was speculated that, in humans, as is the case in rodents, Ngn3 would have a major role in the development and function of intestinal cells [26,27]. On the other hand, it seemed that Ngn3 was necessary for β-cell development in rodents, but not in humans [25,26]. However, this last hypothesis was not supported by recent data indicating that the described mutation [26] was not null, but hypomorphic, and capable of inducing endocrine development when expressed in the primitive gut endoderm of chicken embryo [28].

Finally, the pancreatic phenotype of patients and rodents with mutations in the POU-homeobox TCF2 (transcription factor 2) [also known as vHNF1 (variant hepatocyte nuclear factor 1), HNF1β (hepatocyte nuclear factor 1β)] gene is also interesting. In both rodents and humans, mutations in this gene result in pancreatic atrophy or agenesis [29–33].

In conclusion, during the last few years, a large amount of new information has emerged concerning the hierarchy of transcription factors that are implicated in pancreatic development, and it seems that the same set of transcription factors control pancreatic development in both rodents and humans.

**Pancreatic development: role of intercellular and environmental signals**

Whereas information is accumulating on the transcriptional control of pancreatic development, less is known about the mechanisms by which extracellular signals control pancreatic development. It seems clear, however, that pancreatic development is controlled by permissive signals derived from mesodermal structures that will contact the pancreatic region at specific stages of development [7]. Specifically, at early stages of development, the notochord is in direct contact with the endodermal region that will develop into pancreas. It has been demonstrated that, in the absence of notochord, the expression of markers of dorsal pancreas bud development, including insulin, glucagon and carboxypeptidase A is not induced, whereas when endoderm is grown in vitro with notochord, pancreatic gene expression can be initiated and maintained [34]. On the basis of their pattern of expression and gain of function experiments, activin-βB and FGF (fibroblast growth factor) 2 have been proposed as factors produced by the notochord that mimic notochord activity in inducing pancreatic genes [35]. However, whether activin-βB and FGF2 are the endogenous factors from the notochord that control pancreatic development remains to be demonstrated.

Later on (at approx. E8.5 in mice), signals from the dorsal aorta are crucial for proper pancreatic development [36–38]. It has been shown that pancreatic growth is initiated at three sites on the dorsal and ventral faces of the foregut endoderm, precisely where the endodermal epithelium contacts the dorsal aorta and the vitelline veins. Experiments based on recombination in culture between isolated endoderm and dorsal aorta have demonstrated that the endothelial tissue was necessary to initiate the expression of Pdx1 and insulin [36]. However, the exact signals derived from the endothelium that are necessary for pancreatic cell differentiation remained undefined at this point, but we can expect new information to emerge rapidly on this topic.

Finally, signals from the mesenchyme, which will condense around the underlying committed endoderm, have been proposed to control the subsequent steps of pancreatic development [6]. Around mid-gestation in rodents, the pancreas is composed of an epithelium that contains early Pdx1-positive progenitor cells, surrounded by mesenchyme. Classic culture explant experiments highlighted the importance of the mesenchyme for exocrine pancreas growth and differentiation [39,40]. However, both the way mesenchymal signals control pancreatic development and the exact nature of the mesenchymal signals implicated in this process remained undefined for a long period. More recent experiments using E12–E15 rat embryonic pancreases have shown that more acinar cells developed in vitro from epithelium grown in the presence of mesenchyme than when the epithelium is removed from its surrounding mesenchyme [41]. It was also shown that the FGF receptor FGFR-2 IIIb and its ligands FGF1, FGF7 and FGF10 were expressed throughout pancreatic development. Moreover, in mesenchyme-free cultures of embryonic pancreatic epithelium, FGF1, FGF7 and FGF10 stimulate the growth, morphogenesis and cytodifferentiation of the exocrine cells of the pancreas. The role of FGFs signalling through FGFR-2 IIIb was investigated further by inhibiting FGFR-2 IIIb signalling in organocultures of pancreatic explants (epithelium and mesenchyme) using either antisense FGFR-2 IIIb oligonucleotides or a soluble recombinant FGFR-2 IIIb protein. Abrogation of FGFR-2 IIIb signalling resulted in a considerable reduction in the size of the explants and in reduction of the development of the exocrine cells. These results demonstrate that FGFs signalling through FGFR-2 IIIb play an important role in the development of the exocrine pancreas [42]. The next step was to determine which FGF produced by the mesenchyme controlled pancreatic development. It was demonstrated that FGF10 plays an essential role in this process. Using in situ hybridization, it was found that FGF10 is expressed in the mesenchyme directly adjacent to the early dorsal and ventral pancreatic epithelial buds. In Fgf10−/− embryos, the evagination of the epithelium and the initial formation of the dorsal and ventral buds appeared to be normal. However, the subsequent growth, differentiation and branching morphogenesis of the pancreatic epithelium were...
arrested; this was primarily due to a dramatic reduction in the proliferation of the epithelial progenitor cells marked by homeobox protein PDX1. Furthermore, FGF10 restored the population of PDX1-positive cells in organ cultures derived from Fgf10−/− embryos. Taken together, these results indicated that FGF10 signalling is required for the normal development of the pancreas [43]. However, the role of FGF10 produced by the mesenchyme on the regulation of β-cell mass remains controversial. Although, as described above, results based on loss-of-function of FGF10 suggested that signals from the mesenchyme directly and positively control the final number of β-cells [43], when FGF10 was over-expressed in the pancreas, β-cell development was inhibited [44,45]. Thus the exact effect of FGF10 on β-cell development remained to be defined, as studies of loss and gain of FGF10 function suggest opposite conclusions. However, recently, by using an in vitro culture model on rat embryonic pancreas, some progress was made on this topic. In vitro, the number of β-cells that developed from rat embryonic pancreatic epithelia was larger in cultures with than without FGF10. The effect of FGF10 was due, not to an increase in β-cell proliferation, but to increased proliferation of early-PDX1-positive progenitor cells, as confirmed by BrdU (bromodeoxyuridine) incorporation [46]. A similar effect of FGF10 early-PDX1-positive progenitor cells was observed in cultures of human fetal pancreas [47]. Consequently, the window during which early PDX1-positive pancreatic progenitor cells differentiated into endocrine progenitor cells expressing Ngn3 was extended. Taken together, such results indicate that expansion of early PDX1-positive pancreatic progenitor cells with FGF10 represents a way to increase the final number of β-cells developing from early embryonic pancreas [46].

Although some information is available on mesodermal signals controlling pancreatic development, not much is known about environmental factors, such as the levels of nutrients, including amino acids or glucose, that may control this process. A long time ago, it was shown that, when embryonic pancreases were grown with large amounts of amino acids, pancreatic development was enhanced [48]. A more recent study analysed the effects of glucose on pancreatic cells development. For this purpose, an in vitro model where both endocrine and acinar cells develop from early PDX1-positive embryonic pancreatic progenitors was used. It was shown that glucose does not have a major effect on global pancreatic cell proliferation, survival and acinar cell development. On the other hand, glucose controlled both α- and β-cell development. Specifically, 20-fold more insulin-positive cells developed in the presence than in the absence of glucose and this effect was dose-dependent over the range 0.5–10 mM. Glucose did not appear to control β-cell development by activating the proliferation of early progenitors or β-cells themselves, but instead tightly regulated cell differentiation. Thus glucose did not modify the pattern of expression of Ngn3, the earliest marker of endocrine progenitor cells, but was necessary for the expression of the transcription factor NeuroD, a direct target of Ngn3 known to be important for proper pancreatic endocrine cell development. It was concluded that glucose interferes with pancreatic endocrine cell development by regulating the transition between Ngn3 and upstream NeuroD.

Conclusion
During the last few years, we have learned a lot concerning the way β-cells develop during prenatal life. We have also learned that the best way to produce in vitro β-cells from embryonic stem cells is to replicate the physiological development of β-cells [5]. In a recent study, a multistep protocol was designed that tries to reproduce in a culture dish the major steps of β-cell development: formation of definitive endoderm, followed by gut tube formation, posterior foregut formation, pancreatic endoderm and endocrine precursor development and finally by hormone-expressing endocrine cells [49]. The cells produced at the end of the protocol had similarities to and differences from human β-cells. For example, their insulin content was not so different from that found in normal β-cells, and the cells secreted insulin upon depolarization. However, a large number of insulin-expressing cells produced with this protocol also expressed glucagon, which is not the case for mature β-cells found in the pancreas. Moreover, the cells did not secrete insulin upon glucose stimulation. In conclusion, although this latest work that tried to mimic in vivo the way β-cells develop in vivo, represents a major step forward in progress when compared with what had been previously done, it can be postulated that key information is still lacking to generate fully mature human β-cells. We thus begin to have a clear picture of transcription factors and intercellular signals important for proper pancreatic development, but additional work is needed before being able to generate functional β-cells from embryonic stem cells.

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References


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