Review

Control of early development of the pancreas in rodents and humans: implications of signals from the mesenchyme*

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Abstract

During the last few years, progress has been made in the control of pancreatic development. Many transcription factors have been described in the pancreas and a genetic approach has been used to define their role in pancreatic development. Pancreatic development depends on mesodermic signals, with the initial steps controlled by signals from the notochord that is in close contact with the dorsal endoderm of the gut fated to become pancreas. Later signals from the mesenchyme that surrounds the embryonic pancreat-

ic epithelium regulate the proliferation of immature pancreatic epithelial cells and their differentiation into endocrine or exocrine tissue. This review discusses recent data on the role the signals from the mesenchyme have in the development of the pancreas in rodents and humans. [Diabetologia (2000) 43: 1083–1092]

Keywords Pancreas, development, epithelium, mesenchyme, proliferation, differentiation

Introduction

The pancreas develops from two buds which derive from the epithelium of the digestive tract. The buds increase in size, fuse together and then the epithelial cells differentiate into endocrine and exocrine cells. Finally, the endocrine cells associate into islets of Langerhans and the exocrine cells form the acini. In the last few years, data on different aspects of pancreatic development has increased. First, a large number of key transcriptional factors implicated in pancreatic development have been characterized such as Pdx1 [1–4], Pax 4 and 6 [5–7], islet-1 [8], beta2/NeuroD

[9], Nkx2.2 and Nkx6.1 [10, 11], Hlxb9 [12], neurogenin-3 [13] and p48 [14]. Transgenic mice deficient in such factors have been produced to show the importance of the factors for normal pancreatic development [6, 15–23].

Progress has also been made in understanding the mechanisms that control pancreatic development. It has been shown that the development of the chick dorsal pancreas is tightly controlled by signals derived from the notochord in the early stages [24, 25]. Fibroblast growth factor (FGF) 2 and activin β B have been identified as notochord factors associated with pancreatic development [25].

Later, proliferation and differentiation of the pancreatic epithelial cells, the cells that will give rise to the mature endocrine and exocrine cells, is thought to be controlled by signals from the mesenchyme, which by this time surrounds the epithelium [26, 27]. I will concentrate on the implication of signals from the mesenchyme on the development of the endocrine and exocrine cells of the pancreas reviewing data from animal models and from the development of the human pancreas.

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Abbreviations: FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; BMP, bone morphogenic protein; RTK, receptor tyrosine kinase; EGF, epidermal growth factor; N-CAM, neural cell adhesion molecule; MMP, matrix metalloproteinase.

Control of pancreatic development in animal models

Control of pancreatic epithelial cell proliferation

It was long suspected that the pancreas, like other organs, such as the lung or the kidney, develops by interaction between the epithelium and its surrounding mesenchyme [26, 27]. Experiments in the 1960s and 1970s comparing in vitro pancreatic epithelial cell proliferation in the absence or presence of mesenchyme found that signals from the mesenchyme were necessary for normal proliferation of pancreatic epithelial cells. Although attempts were made to characterize active factors, their exact identity remained elusive [28]. The mesenchyme-derived factors acting on epithelial cell proliferation showed no tissue specificity. Experiments showed that the effects of pancreatic mesenchyme could be mimicked by pancreatic and gut mesoderm, by extracts of young whole rat embryos, or extracts from chicken embryos [28–30]. It was not known whether, as with other organs, ligands of receptor tyrosine kinases could imitate factors produced by the pancreatic mesenchyme and act on epithelial cell proliferation. Specific members of the fibroblast growth factor family were tested. This family is large, containing approximately 20 members which are expressed in various tissues and act on their target cells by binding to specific fibroblast growth factor receptors (FGFRs) [31]. It was found that FGF1, 7 and 10 bind to a specific receptor, FGFR2IIIb which is known to be expressed in the pancreatic epithelium during embryonic/fetal life [32, 33]. First FGF7 [34] and then FGF1 and FGF10 as well were shown to be expressed in the pancreas during development [35]. Two types of experiments which strongly suggest that these FGFs are implicated in pancreatic epithelial cell proliferation have been done in vitro. Firstly, when the embryonic pancreatic epithelium, depleted of its surrounding mesenchyme, was grown in culture in the presence of FGF1, 7 or 10, epithelial cell proliferation was activated. Secondly, when the epithelium surrounded by its mesenchyme was grown in culture in the presence of a recombinant protein corresponding to the extracellular ligand-binding domain of FGFR2b (which acts predominantly as a negative receptor), a statistically significant reduction in cell proliferation was seen [35]. These experiments confirmed earlier ones in various animal models in which FGF7 caused the proliferation of pancreatic ductal epithelial cells in adult rats after 1–2 weeks of treatment [36] and transgenic mice overexpressing *FGF7* under the control of the liver specific apolipoprotein E promoter showed, among other effects, ductal hyperplasia [37]. Finally, transgenic mice expressing a soluble dominant-negative *FGFR* under the control of the metallothionein promoter, (which gave rise to the expression of the transgene relatively early dur-

ing mice development) had developmental abnormalities in multiple organs. In particular, the pancreas showed a greatly reduced number of morphologically aberrant acinar cells [38].

Although signals mediated by FGFR2b mimic the effects of the mesenchyme from a qualitative point of view, the quantitative effect is different. A soluble dominant-negative FGFR fully represses the development of the lung, the thyroid or the anterior pituitary, in vivo whereas pancreatic development is strongly perturbed but not abolished [38]. In vitro, pancreatic growth was inhibited in the presence of a dominant-negative FGFR2b but it was not completely abolished [35]. One of the various possible explanations for these partial phenotypes is technical: signal transduction mediated by FGFR2b would not be totally abolished in these experiments in the pancreas. Another possibility is that additional soluble factors produced by the mesenchyme are implicated in pancreatic development. Finally, during the last few years, transgenic animals deficient in specific members of the FGF family such as FGF7 or FGF10 have been generated [39–41]. Although no obvious pancreatic abnormalities have been reported in these animals, their pancreatic development should be reevaluated in the light of the studies described above.

In conclusion, signals derived from the mesenchyme play an important part in pancreatic epithelial cell growth. Recent experiments implicate specific ligands of receptor tyrosine kinase (RTK), such as members of the FGF family in this process. But other ligands of RTK seem to be implicated in the process. These factors now have to be characterized.

Pancreatic epithelial cell differentiation

Various studies have shown that signals derived from the mesenchyme are implicated in the control of pancreatic cell differentiation into acinar or endocrine tissue.

Implication of the mesenchyme in the differentiation of the exocrine tissue. It is now clear that the differentiation of the epithelium into acinar tissue is mesenchyme dependent [27, 35, 42, 43]. Amylase or carboxypeptidase A cells develop normally from pancreatic embryonic epithelium grown in vitro in the presence of mesenchyme but in its absence their growth is strongly repressed [42, 43]. In vitro, this effect can be reproduced qualitatively by ligands of FGFR2b, such as FGF1, 7 and 10. In the presence of FGF1, 7 or 10, twenty times more amylase-expressing cells develop from embryonic pancreatic epithelium than in the absence of these factors [35]. It is not fully clear whether FGF1, 7 and 10 induce the differentiation of precursor cells into amylase-expressing cells or act as growth factors for pre-existing amylase-expressing

cells. Experimental design should render possible the separation of effects of specific factors on cell proliferation and cell differentiation. Recently, FGF2 mutants have been designed, in which the differentiation property remains intact but the mitogenic activity is lost [44].

Implication of the mesenchyme in the differentiation of the endocrine tissue. The implication of signals from the mesenchyme in the control endocrine tissue development is more controversial. It was first shown that when adult ductal epithelium is grafted to immuno-incompetent mice in the presence of fetal mesenchyme, islets develop which stain positively for insulin and glucagon, whereas such islets did not develop in the absence of mesenchyme. These experiments strongly suggested that the mesenchyme is necessary for the development of endocrine tissue from epithelial cells, at least during postnatal life [45, 46]. More recently, it was found that when embryonic pancreatic epithelium depleted of its mesenchyme was grafted under the kidney capsule of immuno-incompetent mice for 10 days, a dense aggregate of pure mature islets developed suggesting the mesenchyme is not required for the development of pancreas endocrine tissue during embryonic life [42]. It is therefore possible that the mechanisms controlling endocrine cell differentiation during prenatal and postnatal life are completely different. Alternatively it could be that when the embryonic pancreatic epithelium is grafted under the kidney capsule of mice, the kidney produces factors that induce the proper development of the endocrine tissue. This possibility is supported by the inability to induce embryonic epithelium to develop into endocrine tissue in vitro in the absence of mesenchyme [42].

Recently, in vitro conditions were, however described in which rat embryonic epithelium was induced to develop into endocrine tissue in the absence of mesenchyme [43]. Not only was the mesenchyme not necessary for the development of the endocrine tissue but it was even found to act quantitatively as a repressor of endocrine tissue development with five times more insulin-expressing cells developing during a 7-day period in the absence of rather than in the presence of mesenchyme. It was thus proposed that signals from the mesenchyme act as a repressor of endocrine cell differentiation. Based on these data [42, 43], it can be proposed that the default pathway for the epithelial primordium is islet formation. Extrinsic signals would exist to exert a negative control on the proportion of endocrine tissue to be generated. A search for specific factors expressed in the mesenchyme and repressing endocrine cell differentiation has been made. The working hypothesis was that the epithelium would produce factors such as activins or bone morphogenic proteins (BMPs) which would act on pancreatic epithelial cells in an autocrine manner to favour differentiation of pancreatic precursor cells

into endocrine cells. The basis of this hypothesis is that: (1) activin and *BMP-7* have been found to be expressed in the pancreatic anlagen [47–49], (2), AR42J cells, which are pluripotent like the precursor cells of the pancreas, can be converted by activin, in combination with either betacellulin or with hepatocyte growth factor into insulin-producing cells [50, 51]; (3) hypoplasia of pancreatic islets occurs in transgenic mice expressing activin receptor mutants under the control of the insulin promoter [52] or under the control of the β actin promoter [53]. This hypoplasia was found to occur in transgenic mice expressing a dominant-negative form of the activin receptor and in those expressing a constitutively active form of the activin receptor which led the authors to postulate that precise regulation of activin signalling intensity is necessary for the normal development of islets [52].

The possible implication of activin in the control of the differentiation of precursor cells into endocrine cells was tested indirectly by investigating the role of follistatin, a known inhibitor of activin [54] in regulating the relative proportions of endocrine and exocrine tissue during pancreatic development. It was shown that exogenous follistatin increases the development of immature pancreatic epithelial cells into amylase-expressing cells and represses the development of insulin-expressing cells [43]. Exogenous follistatin thus mimics the effects of the mesenchyme. Because mice deficient in follistatin seem, however, to have a normally developed pancreas [55], other factors with a function identical to that of follistatin could be implicated in the control of endocrine pancreas development.

Not only do signals derived from the mesenchyme repress endocrine development from a quantitatively but also qualitatively. Insulin-expressing cells developed in vitro from embryonic pancreatic epithelium in the presence of mesenchyme are immature, whereas those developed without mesenchyme become mature [43, 56]. The cells developed in the presence of mesenchyme resemble the insulin-expressing cells developed in vivo before E15 in rat embryos but those developed without mesenchyme resemble cells developed in vivo at a later stage [27]. Thus it seems that different factors control the development of these two cell populations.

At this point, it is important to redefine whether a relationship exists between insulin-expressing cells developed before E15 and those that appear after E15. Various studies have suggested that insulin-expressing cells at an early developmental stage are precursors of those that are developed later. This is strongly suggested from data from which it seems that beta cell precursors co-express insulin and glucagon [57–60]. The authors assumed that pancreatic cells co-expressing more than one hormone during development would divide and differentiate into mature cells expressing a single hormone but did not

show this directly [57, 61]. Up to now, there is no direct evidence that cells co-expressing insulin and glucagon are precursors for the insulin-expressing cells found later during development. Alternative hypotheses are that these double positive cells, whose development is Pdx1 independent [16, 23] could either die during development or be diluted in the beta-cell mass developed later on. Cell tracing experiments or finding ways to specifically destroy cells co-expressing insulin and glucagon are crucial to answer this type of question.

Pancreatic epithelial cell proliferation: link with cell differentiation

It is important to establish whether there is a link between pancreatic epithelial cell proliferation and differentiation into endocrine or exocrine cells. In Figure 1 a simple diagram summarizes the development of the endocrine and exocrine pancreatic tissue. In brief, precursor cells would have the capacity either to proliferate, or to differentiate into endocrine or exocrine cells.

Repression of cell differentiation by growth factors. In other cell types, proliferation and differentiation seem to be exclusive events. For example, in vitro, when CNS stem cells from striata of embryonic or adult mice are grown in culture, epidermal growth factor (EGF) [62, 63] or FGF2 [64] activate cell proliferation leading to the development of clonal spheres, which contain undifferentiated cells. When EGF or FGF are removed, cell proliferation stops and differentiation into neurons, astrocytes and oligodendrocytes takes place. These experiments indicate that in the brain, specific growth factors can be found that induce stem cells to proliferate and repress differentiation. In the absence of such growth factors differentiation occurs [65].

To find whether such a link exists in the the pancreas, a search for factors inducing the proliferation of embryonic pancreatic epithelial cells while repressing their differentiation into endocrine or exocrine cells is currently under way. Ligands of receptor tyrosine kinases were first tested because they have a role in the proliferation/differentiation of stem cells derived from the brain [62, 64–68]. Under the conditions tested, different FGF family members (FGF1, FGF7, FGF10) were strong inducers of pancreatic epithelial cell proliferation [35, 69] but none of them showed a repressive effect on epithelial cell differentiation into endocrine or exocrine tissue. Other ligands of RTK have now to be tested. The current challenge in this area is to characterize the factors which lead to pancreatic precursor cell proliferation. Large quantities of putative precursor cells could then be generated to enable

Fig. 1. Model of development of the pancreas

their specific properties to be investigated. Furthermore, it would also be possible to generate specific antibodies against the membrane proteins present in such cells, which would be useful for many purposes.

Control of endocrine/exocrine differentiation by growth factors. A second hypothesis is that RTK ligands activate cell proliferation and control the balance between endocrine and exocrine cell differentiation. Such growth factors could act at different levels. As described above, a working hypothesis is that signalling through the activin/BMP signalling pathway could be implicated in endocrine cell development [43]. Specific ligands of receptor tyrosine kinases have been shown to interact with the BMP signalling pathway. The BMP-2 and BMP-4 signalling requires phosphorylation of the tumour suppressor Smad 1 proteins leading to their nuclear accumulation. Both EGF and HGF induce, however, the phosphorylation of Smad1 in a way which inhibits its nuclear accumulation. Opposite effects between BMP family members and tyrosine kinase receptor ligands have previously been observed in tooth development [70], limb bud outgrowth [71], digit formation [72] and anterior pituitary development [73].

Another possibility is that growth factors control the Notch pathway known to be implicated in pancreatic cell type specification [74–76]. A growth factor could control this pathway in the pancreas in devel-

Fig. 2. In vitro development of the endocrine tissue from rat embryonic epithelium. At the beginning of the culture (day 1), the embryonic epithelium acquires a spherical shape when cultured in a collagen gel. Such an epithelium mainly stains negatively for insulin and glucagon. At day 4, the epithelium remains round, while insulin- and glucagon-expressing cells differentiate (green and red staining respectively). Between days 4 and 7 differentiated endocrine cells migrate away from the central epithelium. At day 7 they are found associated into islets of Langerhans with a core of insulin-expressing cells surrounded by glucagon-expressing cells

opment at different levels as for example in the dental epithelium [77].

In conclusion, signals from the mesenchyme could control the development of the embryonic pancreatic epithelial cells at different levels. Understanding this type of regulation should permit to generate large amounts of endocrine cells from precursor cells.

Control of islet morphogenesis

Although, epithelial cell proliferation and acinar cell differentiation seem to be closely dependent on mesenchyme-derived signals, islet morphogenesis, i.e. the association of endocrine cells into islets of Langerhans, seems to be mesenchyme-independent. When embryonic pancreatic epithelium depleted of its surrounding mesenchyme was grown in culture on a collagen gel, epithelial cell differentiation into endocrine cells occurred first, followed by the association of such cells into islets [43]. This pattern of development in vitro is presented in Figure 2. It mimics what is thought to occur in vivo, where putative precursor cells present in the immature epithelium first differentiate into endocrine cells expressing either insulin or glucagon. Once differentiated, such cells have to migrate into the extracellular matrix, recognize each other, and associate to form islets. An islet is not clonal and does not derive from one precursor cell, which would proliferate and differentiate, as seen using a model of aggregation mouse chimaeras where islet cell origin can be identified using histolog-

ical methods. This model has shown that islets are formed by cell aggregation [78].

It can be speculated that endocrine cells would have to degrade the extracellular matrix before migrating into it. In other tissues, matrix metalloproteinases (MMPs) are known to be able to degrade the extracellular matrix and are implicated in morphogenesis [79]. It has been proposed that activation of MMPs is necessary for proper islet morphogenesis. The model proposed (Fig.3) is that endocrine cells degrade the extracellular matrix to migrate and form islets with a core of insulin-expressing cells surrounded by glucagon-expressing cells. This migration would depend on the activation of MMPs [80], the control of which needs to be precise. In vitro when MMP is overactivated by TGF-beta treatment, islet morphogenesis occurs but the normal localization of

Fig. 3. Model of islet morphogenesis. Precursor cells (yellow) are present in the epithelium. They differentiate here either into insulin-expressing cells (green) or into glucagon-expressing cells (red). To form an islet of Langerhans, with a core of insulin-expressing cells surrounded by glucagon-expressing cells, such mature cells have to migrate and degrade the extracellular matrix. These events would require the activation by TGFβ of the inactive enzyme Pro-MMP-2 into its active form MMP-2

glucagon-expressing cells at the periphery of the islets is lost [80]. Recently, neural cell adhesion molecule (N-CAM) has been shown to be required for cell type segregation in pancreatic islets. One hypothesis is that an excess of TGF- β perturbs the normal expression of N-CAM. This is supported by the following arguments: (1) it is known that TGF β controls N-CAM expression in different experimental systems [81]; (2) perturbation of N-CAM expressing affect islet cell segregation in vitro but also in vivo [82, 83].

In conclusion, islet cells are organized in a specific fashion, with a core of insulin-expressing cells surrounded by the three other polypeptide-expressing endocrine cell types, i.e. glucagon, somatostatin and pancreatic cells [84]. This normal islet architecture is perturbed in animal models of diabetes [85–87] and is thought to be required for normal secretion of insulin after glucose stimulation [88, 89]. Understanding the control of islet morphogenesis is therefore important.

Control of human pancreatic development

The question now is whether the progress made over the last few years in rodents is of use in the understanding of pancreatic development in humans.

Transcription factors and human pancreatic development

It seems that the same transcription factors are implicated in pancreatic development in rodents and in humans. For example, it has been shown that homozygosity for a mutation in the *IPF-1/Pdx1* gene results in pancreatic agenesis in rodents [15, 23] and in humans [90]. Moreover, mutations in the *IPF-1/Pdx1* gene predispose to Type II (non-insulin-dependent) diabetes mellitus [91, 92]. It has also recently been shown that a mutation in *beta2/NeuroD1*, a helix-loop-helix protein implicated in islet morphogenesis and insulin gene transcription in rodents [9, 22], leads to the development of Type II diabetes in humans [93].

In conclusion, recent progress in rodents led to our first understanding of the importance of transcription factors in human pancreatic development and in specific forms of diabetes.

Control of human pancreatic development

To test whether signals from the mesenchyme that are important for pancreatic development in rodents, also control pancreatic development in humans experimental systems need to be designed to mimic normal human pancreatic development so as to produce a clear description of this process. Experiments

would then have to be carried out to define factors that control endocrine cell development.

Description of human pancreatic development. Over the last 20 years light and electronic microscopy [94] and immunohistochemistry, have been used in studies to define the time of the appearance of the endocrine cells [95–98]. The first pancreatic endocrine cells were detected around 7–8 weeks of development. Moreover, these embryonic cells seem to proliferate poorly [97–99], indicating that the increase in endocrine cell mass is mainly due to the differentiation of precursor cells and not to the proliferation of pre-existing endocrine cells. The implication of differentiation in the control of human beta-cell mass is also important during adult life. During adult life, mature beta cells are mitotically inactive [100].

In conclusion, cell differentiation is crucial for the control of beta-cell mass in the humans. Based on anatomical data, it can be proposed that this differentiation process is negatively controlled by signals from the mesenchyme. In vivo during embryonic life, the epithelial cells in close contact with the mesenchyme seem to have a higher rate of proliferation and a lower rate of differentiation into endocrine tissue than those found at a distance from the mesenchyme [98]. This hypothesis has still, however, to be experimentally tested in an adapted experimental system.

Definition of experimental systems which mimic human pancreatic development. Different laboratories have generated and used specific in vitro or in vivo experimental systems but have mainly used fetal pancreases at 18–24 gestational weeks, which is a late stage of pancreatic development [101–104]. At this stage, the human pancreas is already rich in endocrine cells, the concentrations of mRNAs coding for insulin, glucagon or somatostatin being identical to or higher than those found in the adult pancreas [105]. It is thus difficult to define whether the increase in the relative endocrine cell mass detected after treatment with specific factors is due to the differentiation of precursors, to the proliferation of pre-existing endocrine cells or to an enrichment in relation to the death of non-endocrine cells. The acinar tissue is already well developed [94, 106] and the acinar tissue is known to degenerate very rapidly in vitro [107], inducing a relative increase in the concentration of endocrine tissue. It is also important to note that the increase in beta-cell mass has always been poor in this type of experimental system.

In vitro, it was shown that nicotinamide is a potent inducer of endocrine differentiation in cultured human fetal pancreatic cells [103]. After nicotinamide treatment, cell proliferation occurs and endocrine cells develop. These data confirm and extend that obtained in a previous study [108], where it was also shown that insulin secretion after glucose stimulation

was activated in beta cells developed in the presence of nicotinamide [103]. These functional data are, however, different from that obtained in a previous study where no difference was found in insulin secretion [108]. It has been shown in this experimental model that transforming growth factor alpha, platelet-derived growth factor, nerve growth factor, epidermal growth factor and insulin-like growth factor II have no effect on cell proliferation, DNA or insulin content. Fibroblast growth factor 2 induced, however, an increase in cell proliferation and insulin content and fibroblast growth factor 7 induced cell proliferation [109]. In this study, hepatocyte growth factor increased the DNA content of the pancreatic fragments compared with the starting material but had no statistically significant effect on cell proliferation. It also increased insulin content by a factor of three [109]. Finally, the mitogenic effect of matrices from a variety of different cell lines was tested. It was clearly found that specific matrices were strong inducers of pancreatic fetal cell growth [110, 111].

In conclusion, although this experimental system is currently one of the best to study the control of human pancreatic development and some progress has been made, it has limitations [112].

In vivo models have also been used. It was shown that when human embryonic pancreatic tissue was grafted to immuno-incompetent mice, pancreatic growth occurs. In addition endocrine cell mass and insulin content increased, suggesting that endocrine cell differentiation took place [104, 113]. Because the grafted tissues came from fetuses late in development (14–20 weeks gestation) and already rich in islets [104], final conclusions were difficult to draw.

In another series of experiments human islet cell clusters were used. Human islet cell clusters are spherical cell aggregates formed in vitro from fetal pancreases and contain a minority of endocrine cells. The exact nature of the other cell types in the clusters is not known [101, 102] but when they were grafted to immuno-incompetent mice, they developed into mature functional islet-like structures [110, 114]. This in vivo model should be useful to study the control of human pancreatic development and we can expect the rapid arrival of new data.

In my laboratory, an alternative model has recently been designed. Human embryonic pancreases were grafted between 7–8 weeks of development under the kidney capsule of immuno-incompetent mice. At this early stage, the tissue stained negatively for insulin, and the completely immature human pancreatic tissue grafts which were initially 1 mm³ grew to 1 cm³ after 4–6 months. Moreover, after 4 months in the mouse approximately 10% of the cells stained positively for insulin. Various criteria show the newly formed human beta cells to be mature (Castaing et al, manuscript in preparation). Because the starting tissue stained negatively for insulin, it can be concluded

that endocrine differentiation occurred. This model which seems to recapitulate human pancreatic development in vivo, will now help to understand the mechanisms controlling the development of this complex human tissue.

Conclusion

Over the last few years, a large number of groups have studied pancreatic development for at least three reasons. Firstly, understanding the development of a complex organ represents an interesting challenge. Secondly, it is becoming clear that certain forms of diabetes might be linked to abnormal pancreatic development. Thirdly, strategies to treat diabetes by cell therapy have been proposed. In addition to the use of adult islets, the use of precursor cells differentiating into mature beta cells has been proposed. To reach that goal, the mechanisms controlling pancreatic development have, however, to be understood. Although huge progress has been made with transcription factors necessary for pancreatic development, data now appear for soluble factors controlling pancreatic development. Additional work is necessary to make this new information useful for medical purposes.

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