

Recent advances in pancreas development: from embryonic pathways to programming renewable sources of beta cells

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F1000 Biology Reports 2010, **2**:17 (doi:10.3410/B2-17)

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Abstract

In recent years, there has been significant progress in understanding the detailed mechanisms of pancreas development. These studies have in turn influenced research aimed at producing pancreatic islet cells from stem cells. Here, we review recent progress in both of these areas.

Introduction and context

The pancreas consists of two organs in one: the exocrine compartment, which produces digestive enzymes, and the endocrine compartment (the islets housing insulin-secreting beta cells), which is critical for blood sugar homeostasis. As loss or dysfunction of beta cells results in diabetes, much effort is targeted toward generating renewable sources of islets (e.g., by differentiation of stem cells), and these efforts are in turn influenced by knowledge of pancreas development. Here, we review some recent advances from studies of animal models which are helping us to understand how to build a pancreas.

Major recent advances

The influence of secreted signals on early pancreas development

Over the last decade, there has been an emphasis on understanding how transcription factors establish pancreatic progenitors. For example, pancreatic and duodenal homeobox gene 1 (*Pdx1*), which labels all pancreas progenitors, has been particularly well studied (e.g., [1]). Interestingly, a recent study from Spence and colleagues [2] has shown that *Pdx1*⁺ cells are also the source of biliary progenitors that produce the gall bladder, cystic duct, and common duct. These authors showed that segregation of the extrahepatic biliary system from ventral pancreas is dependent on transcription factor Sox17. Sox17 has a well-known early role in general endoderm

development (e.g., [3]), but the use of sophisticated conditional approaches allowed the critical later role for Sox17 to be uncovered.

In addition to transcription factors, we need to understand the roles of longer-range secreted signals. Wandzioch and Zaret [4] used a 'half-embryo' culture system to manipulate mouse embryos while pancreatic progenitors develop. They found that a highly dynamic series of integrated signals is acting on endodermal progenitors. For example, bone morphogenetic protein (BMP)-Smad4 signaling is required at the 5- to 6-somite stage for expression of pancreas markers, including *Pdx1*, whereas BMP plays an inhibitory role just a few hours earlier. Work in zebrafish is also revealing the complexities of signaling. Chung and Stainier [5] found that while Hedgehog (HH) signaling is required for zebrafish beta-cell development, it does not act directly on beta-cell progenitors. These authors used single-cell lineage tracing to show that the most medial *Pdx1*⁺ endodermal cells form endocrine pancreas, including beta cells, but HH signals received in more lateral endoderm are fated to become exocrine pancreas and intestine. In a related study, Chung *et al.* [6] found that *Bmp2b* signaling from the lateral plate mesoderm influences lateral endoderm progenitors to form liver while cells located further from the *Bmp* source express *Pdx1* and contribute to intestine and exocrine pancreas. While lateral endoderm cells are bipotential, with hepatic versus pancreatic fate

dependent on proximity to Bmp2b signals, the most medial cells, fated to become endocrine pancreas, are refractive to Bmp signaling. Note the apparent discrepancy with the requirement for Bmp signaling to induce Pdx1 expression in mouse endoderm (described above); it remains unclear whether this reflects a difference in timing, species, or the specific Bmp acting. Another novel form of signaling, which has just started to be explored, is between neural crest derivatives and developing pancreas. Nekrep *et al.* [7] revealed a role for crest-derived neurons and glia in controlling beta-cell number. Overall, these examples emphasize that signals often need to act within defined windows of time to elicit specific effects, and they highlight the complexities of interactions between and within tissues during specification of pancreatic progenitors.

Pancreatic progenitors in the embryonic and adult pancreas

How are beta cells replaced in the course of normal cell turnover or during repair following injury? A long-standing but controversial hypothesis is that new beta cells arise from a stem cell-like progenitor via a process that recapitulates embryonic development. A competing second hypothesis is that new beta cells arise from proliferation of existing beta cells and that embryonic signaling pathways are not involved. A major focus has been on the neurogenin-3 (Ngn3) transcription factor, which marks undifferentiated islet cells in the embryo, to determine whether it has a role in adult beta-cell renewal.

In support of the second hypothesis, two groups performed extensive lineage-tracing studies and found that in control animals as well as in regeneration models, new beta cells are derived from replication of existing beta cells, with no evidence of a stem cell or progenitor contribution [8,9]. Consistent with this, Lee *et al.* [10] showed that Ngn3 was not expressed during regeneration of the mouse islet. From these and other studies, it seemed likely that in the adult pancreas, neither normal beta-cell turnover nor regeneration of the islet involves a progenitor population or recapitulates an embryonic differentiation program.

However, in the last two years, both conclusions have had to be reassessed in light of new evidence that provides strong support for the existence of a pancreatic progenitor or precursor pool in adults. Xu *et al.* [11] found Ngn3⁺ progenitors residing in the ductal epithelium of the adult mouse pancreas. Furthermore, these Ngn3⁺ cells gave rise to differentiated endocrine cells during regeneration. The major difference with this study and the studies mentioned above is in the regeneration

models. The earlier studies [8-10] used partial pancreatectomy whereas Xu *et al.* used partial duct ligation, a more severe injury that triggered a strong immune response.

Consistent with this work, Wang *et al.* [12] used three Ngn3-GFP mouse reporter lines to show that Ngn3 is expressed in adult pancreatic duct cells. Significantly, they showed that Ngn3 is necessary not only for islet cell differentiation in embryos but for maintaining cell function in adults as well. Desgraz and Herrera [13] used the MADM (mosaic analysis with double markers) technique to genetically label Ngn3⁺ cells during development and found that individual cells are unipotent; that is, one Ngn3⁺ cell gives rise to only one differentiated islet cell type. They suggest that, at least during embryonic development, the Ngn3⁺ pool should be considered a precursor pool rather than a progenitor pool.

Several important advances were also made using the zebrafish model. Parsons *et al.* [14] found evidence that Notch-responsive islet progenitors reside in the pancreatic ductal epithelium. Hesselson *et al.* [15] developed a new Cre-based technique, termed HOTcre, for labeling beta cells in a heat-inducible, temporal manner. Their labeling revealed two populations of beta cells in the embryo: an early, dorsally derived population that quickly becomes quiescent and a later, ventrally derived population that is more proliferative. Further studies of the two populations may reveal what factors are critical for maintaining beta cells in a proliferative state. Finally, in studies of endoderm patterning, Kinkel *et al.* [16,17] showed that the Cdx4 transcription factor prevents beta-cell differentiation in posterior endoderm and that the Cyp26 enzymes limit insulin expression in anterior endoderm. Loss of Cdx4 or Cyp26 enzymes resulted in a dramatically expanded beta-cell population.

Renewable sources of islets

The knowledge gained from the embryo in understanding the signaling pathways and transcription factors involved in islet development has had a significant impact on efforts to differentiate beta cells from stem cells. Kroon *et al.* [18] made an important methodological advance over their earlier protocol for differentiating stem cells [19]. As in their earlier work, they progressively introduced combinations of signaling molecules to step cells through a program that recapitulated *in vitro* the embryonic beta-cell differentiation program. In the new protocol, human embryonic stem (ES) cells were differentiated to endocrine precursors *in vitro* and then implanted into mice, where they further differentiated to become glucose-responsive and insulin-secreting cells.

Using another promising approach, Zhou *et al.* [20] demonstrated that adult pancreatic exocrine cells can be reprogrammed into beta cells *in vivo*. They focused on several transcription factors critical during embryonic development and found that three of them (Ngn3, Pdx1, and Mafa) were sufficient to induce transdifferentiation.

Future directions

While exploiting information from developmental studies to differentiate ES cells is clearly powerful, this is not the only route to produce beta cells. Recently, small molecules that can efficiently direct human or mouse ES cells to an early endoderm fate [21] and that can efficiently direct definitive endoderm to Pdx1⁺ progenitors have been identified [22]. Future screens will likely identify more small molecules that can assist in the efficient production of beta cells *in vitro*. Other potential sources of beta cells are adult pancreatic stem cells and liver cells, the latter of which share a developmental origin with pancreatic cell types and can be transdifferentiated to a beta-cell phenotype (e.g., [23]). A particularly exciting recent advance is the ability to induce pluripotential stem cells from diabetic patients [24], providing a potentially powerful source of patient-specific stem cells for differentiation and eventual cell replacement therapy.

Abbreviations

BMP, bone morphogenetic protein; ES, embryonic stem; HH, Hedgehog; Ngn3, neurogenin-3; Pdx1, pancreatic and duodenal homeobox gene 1.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

The authors are supported by funds from the Juvenile Diabetes Research Foundation and the National Institutes of Health.

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