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Short review

Molecular mechanism in β-cell development: the role of Pdx1, Ngn3 and Pax4 proteins

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INTRODUCTION

Insulin-producing pancreatic β-cells are predominant and the most essential cell type of endocrine pancreas. Together with other endocrine cell types (glucagon-producing α-cells, somatostatin-producing δ-cells and pancreatic polypeptide-producing PP cells), beta cells are organized into spherical clusters, each containing about 2000 cells which are dispersed within the exocrine tissue of pancreas. These structures are known as islets of Langerhans. The main protein product of β-cells is insulin, the only hormone in the body with the ability to reduce blood glucose level. Insulin deficiency leads towards *Diabetes mellitus*, a devastating disease that affects approximately 450 million people worldwide. In patients suffering from Type I diabetes all β-cells are destroyed due to strong autoimmune attack. As islet beta cells *per se* have a limited capacity for self renewal after postfetal development, especially in case of active autoimmune response, beta cell mass rapidly decreases and causes diabetes. Most diabetic patients today are committed to lifelong insulin therapy. While insulin therapy offers a means to achieve normoglycemia, only pancreas or islet transplantations represent the actual »cure« for insulin-dependent diabetic patients. Unfortunately, availability of pancreas donors or islets as a source for transplantation is acutely limited.

Nevertheless, these patients with time develop severe (diabetic late complications) life-threatening complications due to the lack of fine tuned mechanism of glucose sensing and physiologically regulated insulin release that would maintain constant level of blood glucose. Therefore diabetes is considered to be an incurable disease, and new approaches in developing therapy are needed to change that fact. One of the most promising approaches is cell replacement therapy based on *in vitro* differentiation of embryonic stem cells toward insulin-producing cells that would display β-cell phenotype. The most common strategy used by numerous research groups is an attempt to recapitulate normal embryonic development of β-cells in the context of pancreatogenesis in human organism. For that reason, it is essential to get insight into complex transcriptional networks governed by numerous cell extrinsic and intrinsic signals during formation of an adult organ from pluripotent cells of an early embryo. Due to strict limitations of work with human embryonic stem cells and difficulties in obtaining experimental material, mouse is the most common model organism for both developmental (*in vivo*) and differentiational (*in vitro*) studies.

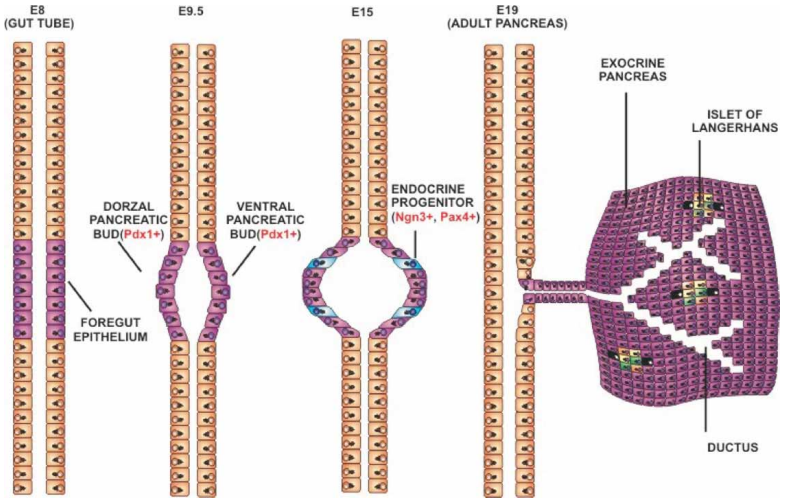

Figure 1. *Schematic diagram of pancreatic development in the mouse. Approximate embryonic age (in days) is designated for each stage of development.*

OVERVIEW OF PANCREATOGENESIS

Pancreas is a derivative of the endoderm germ layer that is formed during the process of gastrulation on embryonic days (E) 6.5 – 7.5 of the mouse. Definitive endoderm forms the primitive gut tube along anterior-posterior axes of an embryo on E 8.5. In the anterior region of the tube (foregut), two cell thickenings, about a hundred of cells each, arise both ventrally and dorsally (E 9). These regions are considered to be pancreatic primordium because endodermal cells of the region acquire unique developmental program directing the differentiation towards pancreatic fate. During the subsequent process of budding, primordial epithelial monolayers proliferate and form dorsal (E 9.5) and ventral (E 10.5) evaginations, referred to as dorsal and ventral stratified epithelial buds *(1)* (Figure 1.). It is interesting that ventral and dorsal programs vary considerably, and the development is slightly asynchronous because the programs are initiated by different signals from the surrounding tissues *(2)*. Dorsal pancreatic bud first gets in close proximity of the notochord and thereafter dorsal aorta takes up the major role in patterning of the prepancreatic tissue *(2)*. During ventral budding, the endoderm epithelium is first intimately connected with liver and bile duct epithelium, which is afterwards displaced by cardiac mesoderm. Pancreatic mesenchyme influences both ventral and dorsal bud, mainly through proliferative signaling *(2)*. It is noteworthy that pancreatic program in ventral pancreatic region is engaged when inhibitory effect of cardiac mesoderm that operates through FGF and BMP signaling is avoided by spatial distancing *(3, 4),* indicating that pancreatogenesis is a default pathway in ventral bud. In contrast, signals from notochord (repression of Shh transduction) and dorsal aorta (VEGF signaling) promote dorsal pancreas development *(5, 6, 7, 8)*. After definition of the two buds they continue to grow due to strong proliferation of pancreatic mesenchyme that underlies multilayered stratified ephitelium. Almost all epithelial cells display characteristics of common endocrine and exocrine cell precursors *(9, 10, 11)*. At the same time the process of branching is initiated by intraepithelial tubulogenesis resulting in the formation of pancreatic ducts that drain exocrine products in the adult organism (E 10.5-11). On E 13.5 two pancreatic buds fuse and form a single organ with fully developed exocrine and endocrine cells that afterwards assemble and form organized structures: exocrine acini on E 15.5 and islets of Langerhans on E16 (1) (Figure 1).

During the process of differentiation, the cascade of numerous signaling molecules is engaged, and each member of the signaling network is activated at a particular time point in a particular cell type *(1)*. Therefore it is essential to identify spatial and temporal requirements for transcription factors that could serve as high specificity molecular markers during specification, proliferation and directed differentiation of ES cells *in vitro*. Importance of molecular stage identification is even more pronounced since the anatomy and morphology of cell structures grown in cultures are often completely incomparable with the structures existing *in vivo*. This review presents current knowledge on three transcription factors that are apparently the most suitable markers for the most significant stages in β-cell development (Figure 1).

Pdx1 is a transcription factor – regulatory circuit in differentiated pancreatic cells

Pancreatic and duodenal homeobox gene 1 (*pdx1*) encoding homeodomain transcription factor was classified as a member of the Hox-like homeobox containing group (ParaHox gene cluster) *(12)*. It operates at different time points during pancreatic development. At early stages (E 8.5) *pdx1* is activated in the dorsal endoderm of the gut, and this activation is the first sign of specification toward pancreatic fate *(13)*. On E 9.5 *pdx1* expression is detectable within dorsal and ventral pancreatic buds as well as

duodenal endoderm *(13)*. At that point, Pdx1$^+$ cells make pluripotent population that gives rise to all cell types of the neonatal pancreas (endocrine, exocrine and duct) (Figure 3) and epithelium of the duodenum and posterior stomach *(13, 14)*. Later in fetal development, *pdx1* is strongly expressed in beta cell lineage progenitors and plays a major role in specific differentiation toward beta cells *(13, 15)*. Finally, in the adult pancreas, Pdx1 is essential in maintaining beta cell function. It activates transcription of several genes involved in glucose homeostasis including insulin, glucokinase and glucose transporter *GLUT2 (16, 17, 18, 19, 20)*.

Targeted disruption of *pdx1* in mice results in arrested pancreatic development. Soon after initial pancreatic bud formation, these rudimentary outgrowths regress. Nullizygous *pdx1* mice are therefore apancreatic and die within a few days after birth *(21, 22)*. Intestingly, *pdx1* mutant mice during early embryogenesis generate some insulin and glucagon coexpressing cells *(23),* which raises the possibility of pdx1 independent differentiation of alpha and beta cells. Using *Cre-LoxP* tagging system, Herrera *(10)* clearly demonstrated that double hormone positive cells do not give rise to mature islet cells. The answer to the next obvious question, i.e. whether any other Pdx1 negative cell type contributes to adult pancreatic tissue was given by Gu *et al.* who also used *Cre-LoxP* system to show that pancreatic tissue is derived exclusively from Pdx1 positive progenitors *(9)*.

Since *pdx1* null mutants exhibit a complete arrest in pancreatic development, another model of conditional inactivation helped to elucidate the necessity of continuous *pdx1* expression throughout the process of pancreatogenesis. Tetracycline-induced inactivation of *pdx1* on E 11.5 (a time point which precedes strong proliferation and terminal differentiation of immature endocrine and exocrine cells) resulted in animals with undeveloped pancreas consisted only of large ducts and primitive epithelium, lacking both acinar and islet tissue *(24)*. On the other hand, combined forced expression of Pdx1 and Ptf1a (basic helix–loop–helix transcription factor involved in exocrine differentiation) is sufficient for conversion of extrapancreatic endoderm into endocrine and exocrine pancreatic progenitors (25). Besides continuous expression of Pdx1, it has been demonstrated recently that the tight regulation of the gene activity is also essential for pancreas development *(26)*.

The best of the Ngn3

Neurogenin 3 (Ngn3) is a member of the basic helix–loop–helix transcription factor family that includes important transcriptional regulators of the cell fate determination in a number of cell types. In the mammalian species, Ngn3 is involved in determination of neural precursor cells and common precursors of all pancreatic endocrine cell types *(27, 28)* (Figure 3). Ngn3 positive cells are scattered within the pancreatic epithelium and their number peaks on E 15.5 during genesis of all four endocrine cell types. Afterwards, *ngn3* expression gradually diminishes and ceases completely in the adult pancreas *(29, 30, 31)*. *Ngn3* deficient mice (homozygous null-mutants) lack Langerhans islets and die shortly after birth although exocrine tissue and pancreatic ducts are nearly normal. Moreover, not one islet cell type or endocrine precursor is detectable at any stage of pancreas development. These observations indicate that Ngn3 is required for endocrine fate determination and can therefore be characterized as a functional marker of islet precursor cells in the developing mouse pancreas *(28)*. The role of Ngn3 as an early endocrine marker was further demonstrated by its ectopic expression under the regulatory control of the *pdx1* promoter in transgenic mice *(29)*. Actually, forced *ngn3* expression causes premature differentiation of common pancreatic precursors (exocrine and endocrine) into endocrine cells. The resulting »organ« has poorly branched pancreatic buds with a few endocrine-like cells and without the capacity to differentiate into exocrine tissue. Recently, Johansson and coworkers *(32)* generated *ngn3* (–/–) mice expressing only tamoxifen-inducible *ngn3* under the control of the *pdx1* promoter. Early activation of *ngn3* almost exclusively induced glucagon cells, while depleting the pool of pancreas progenitors. There is a number of studies that identify *neuroD1* (gene for another basic helix–loop–helix transcription factor) *(28, 33, 34, 35)* and a recent study identifying IA1 (gene for a zinc-finger transcription factor *(36)* as a direct genomic targets of Ngn3. Three gene products share very similar expression pattern: NeuroD1 maintains the pro-endocrine differentiation program initiated by Ngn3 and is required for the survival and terminal differentiation of beta cells; IA1 is required for normal differentiation of all endocrine cell types.

Ngn3 is presumably involved in the process of lateral specification by forming a negative cross-regulatory feedback loop between neighboring cells. At certain time point during differentiation of common pancreatic precursors, endocrine and exocrine precursors segregate. At first, there are a few Ngn3+ cells scattered within the prepancreatic tissue. After reaching a certain threshold, Ngn3 turns on Notch ligand genes such as *delta-like 1*, which is thought to suppress differentiation in the surrounding cells. This mechanism is mediated by activation of Hes1 which is a direct repressor of the *ngn3* gene *(37)* and hence the major negative regulator of endocrine cell commitment *(38)*. Lateral inhibition could therefore be responsible for control and modulation of the number of endocrine cells that are generated *(29, 30, 39)* (Figure 2).

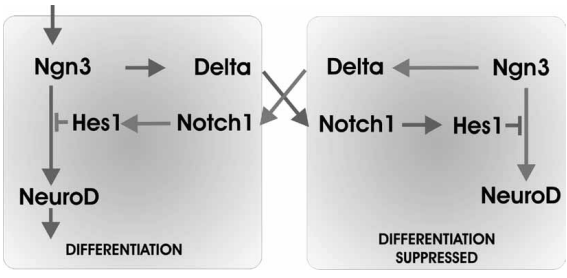

Figure 2. *Lateral inhibition.*

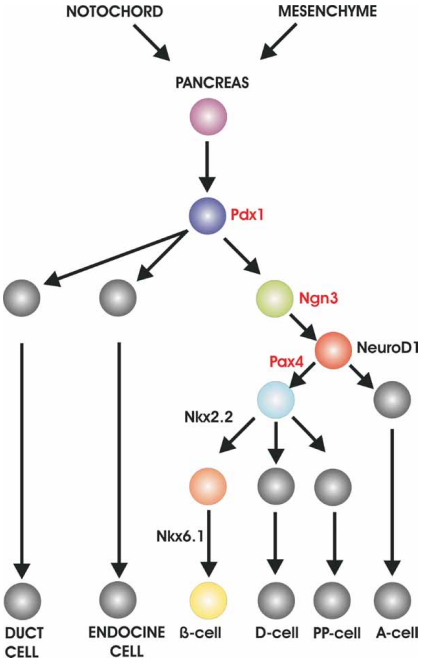

Figure 3. *Model for the role of transcription factors during cellular differentiation of beta cells.*

Role of Pax4 in pancreatic β/D-cell development

Pared box gene 4 (*pax4*) is a paired domain homeobox gene (containing both a paired domain and a homeodomain), a member of a common subclass of the Pax-gene family *(40, 41, 42)*. Expression of *pax4* begins early in the development of embryonic pancreas (E 9.5), peaks on E 13.5 and becomes undetectable in adult islets *(43)*. It appears that *pax4* is a direct target of Ngn3 *(39, 44)* since Ngn3 was found to bind and transactivate *pax4* promoter *(45, 46)*. Moreover, kinetics of *pax4* expression closely follows that of *ngn3*. Homozygous *pax4* deficient mice completely lack beta and delta cells and die within three days of birth *(47)*. The indicates that Pax4 plays an essential role in sub-specification of beta and delta cell types within the endocrine progenitor pool during the development of islet cells *(47, 48, 49)* (Figure 3).

Interestingly, loss of *pax4* does not affect differentiation of endocrine progenitors toward alpha cell lineage since knock out animals even have an increased number of alpha *(43)* and epsilon *(50)* cells. It appears that Pax4 can function as a transcriptional repressor that may suppress alpha and permit beta cell differentiation *(43)*. This action is possibly accomplished through direct or indirect suppression of another transcription factor (Arx) known to promote alpha cell fate of endocrine progenitors *(51)*. Moreover, it was shown that Pax4 competes with Pax6 (transcription factor essential for alpha-cell development) for target sites of the glucagon gene promoter where it acts as a transcriptional inhibitor *(51)*. It is therefore likely that Pax4 plays a role in restricting glucagon gene expression to alpha cells during pancreas development *(52)*.

CONCLUSION

For identification of three major events in acquiring beta cell identity, three intermediate cell types are now possible to distinguish according to highly specific, transiently expressed transcription factors: Pdx 1 as a marker of common pancreatic progenitors, Ngn 3 as a marker of common endocrine progenitors, and Pax 4 as a marker of committed beta cell precursors. These findings are of paramount importance not only for studying pancreas development, but also in an attempt to obtain insulin-producing cells *in vitro* with a final goal to improve diabetes mellitus treatment.

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