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Notch signaling in pancreatic endocrine cell and diabetes

Wook Kim¹, Yu-Kyong Shin¹, Byung-Joon Kim², and Josephine M. Egan^{1,*}

¹NIA/NIH, Baltimore, MD 21224, USA

²Department of Internal medicine School of Medicine, Eulji University, Daejeon 302-120, Korea

Abstract

Recent studies have improved our understanding of the physiological function of Notch signaling pathway and now there is compelling evidence demonstrating that Notch is a key regulator of embryonic development and tissue homeostasis. Although further extensive studies are necessary to illustrate the molecular mechanisms, new insights into the role of Notch signaling in pancreas development and diabetes have been achieved. Importantly, the ability to regulate Notch signaling intensity both positively and negatively may have therapeutic relevance for diabetes. Thus, this paper reviews the current knowledge of the roles of Notch signaling in the pancreatic endocrine cell system.

Keywords

Notch signaling; Pancreatic endocrine cell; Diabetes

Overview of pancreatic endocrine cell

The adult pancreas is a heterogeneous organ with three main functions: (1) the exocrine acinar tissue produces digestive enzymes that facilitate nutrient digestion and absorption in the gut, (2) the small ducts, or ductules, collect digestive enzymes produced from exocrine cells, and (3) islets of Langerhans that include α , β , δ , PP and ϵ cells, which produce glucagon, insulin, somatostatin, pancreatic polypeptide (PP) and ghrelin, respectively. The islets of Langerhans are scattered throughout the exocrine tissue and comprise about 1% of the total pancreatic tissue [1]. Endocrine cells are derived from a common pancreatic progenitor cells located within the early gut endoderm (Fig. 1). The earliest endocrine cells detected in the pancreatic bud of the foregut contain glucagon, and a subset of these cells co-expresses insulin and sometimes peptide YY (PYY) [2]. At ~e14.5, there is a secondary transition, and cells divide into distinct lineages that express glucagon and insulin; it seems that this second wave, based on lineage tracing, does not arise from the early primitive endocrine cells of the e9.5 stage [3]. Within the next 24h, the first somatostatin-expressing

*Correspondence should be addressed to: **Josephine M. Egan, MD, NIA/NIH**, 251 Bayview Boulevard, Baltimore, MD 21224 USA, Tel: 410-558-8414, eganj@grc.nia.nih.gov.

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cells arise [4]. Finally, at e18, shortly before birth, PP cells appear while endocrine cells begin to organize into functional islets of Langerhans, embedded within the exocrine matrix [2]. In rodent islets, β cells are most prominent (60-80% of total) and they are concentrated in the islet core, with other cell types arranged closer to the mantle; however, human islets do not show quite the same anatomical subdivisions or stereotypy and α , δ , and PP cells are scattered among the β cells. α cells comprise 15-20% of the islet, with a few percent remaining for the other three cell types [1]. In type 1 diabetic patients, whose β cells are destroyed, α cells comprise approximately 75% of the total cell number [5,6]. However, in type 2 diabetes, α cell hyperplasia occurs and the β cell mass is probably reduced, at least in long-standing type 2 diabetes, as a result of increased apoptosis [5,7].

Notch signaling pathway

Notch signaling is intimately involved in embryonic development and, in adults, in maintenance of homeostasis of multicellular organisms, such as cell differentiation, survival/apoptosis, and the cell cycle under both physiologic and pathologic contexts. In mammals, loss of Notch signaling leads to severe defects in embryonic development and tissue homeostasis and its dysregulation is linked to multiple developmental and physiological disorders [8-11]. Furthermore, mutations that lead to Notch malfunction have been associated with a variety of diseases [11]. The last few years have witnessed the elucidation of new functions for Notch signaling during pancreas organogenesis and adulthood and it is clear that normal, uninterrupted Notch signaling pathways are required for normal pancreatic development. Enforced activation of Notch signaling in pancreatic progenitors impairs their differentiation into the various pancreatic cell lineages, whereas inactivation of Notch signaling leads to premature differentiation of endocrine pancreas [8-10]. Not surprisingly, the Notch pathway has also been implicated in diabetes.

Four Notch receptors (Notch1-Notch4) have been described in mammals and their intracellular portion conveys the signal to the nucleus. The Notch ligands are type I transmembrane proteins and there are two major classes of ligands: Delta or Delta-like (Dll) and Serrate (Jagged in mammals). Mammals possess five ligands (Delta-1, -3 and -4 and Jagged-1 and Jagged-2). In addition to Delta and Jagged, the neural adhesion molecule F3/contactin, the related NB-3 protein, the EGF repeat protein DNER, and a diffusible protein in *C. elegans* have all been identified as possible Notch ligands.

Notch signaling is activated by ligand-receptor interaction between two neighboring cells. The interaction leads to successive proteolytic cleavages by a metalloprotease of the ADAM/TACE/Kuzbanian family and then γ -secretase that ultimately release the Notch intracellular domain (NICD). NICD then translocates to the nucleus, where it assembles into a ternary complex with members of the CSL (for CBF1 in mammals, Suppressor of Hairless in *Drosophila*, Lag-1 in *C. elegans*) family of transcription factors. Several targets of Notch signaling have been identified. One well-known target in *Drosophila* and mammals is the HES (hairy/enhancer of split) family of the basic helix-loop-helix (bHLH)-type transcriptional repressors [12], which negatively regulate the expression of genes by recruiting a set of co-repressors or by sequestering transcriptional activators. In addition, NICD also directly stimulates expression of cell cycle regulators (e.g., p21 and Cyclin D1),

transcription factors (e.g., c-Myc and NF- κ B2), and growth factor receptors (e.g., ErbB2). This complexity of interactions explains why Notch signaling is involved in a variety of cellular events.

Notch signaling during pancreatic endocrine cell development

Notch receptors

Specific Notch pathway elements and intracellular effectors necessary for normal pancreatic development are expressed in the developing pancreas [8,9,13]. Loss-of-function mutation of various Notch signaling pathway genes (*Rbp-Jk*, *Delta1*, and *Hes1*) display up-regulated expression of the basic helix-loop-helix *Neurogenin 3* (*Ngn3*) gene and consequent accelerated and increased pancreatic endocrine development, leading to depletion of precursor cells followed by pancreatic hypoplasia [8,9]. Conversely, expression of constitutively active NICD traps pancreatic precursor cells in an undifferentiated state and prevents both endocrine and exocrine pancreas development [10,14,15]. Mis-expression of activated Notch1 in developing pancreas using *Pdx1* promoter prevents both endocrine and exocrine development and appears to trap both early and late progenitors in an undifferentiated state [10,14], whereas expression in fully differentiated endocrine cells is without effect [10]. These studies suggest that cells undergoing endocrine differentiation lose responsiveness to Notch signaling. More recent study indicates that Notch signaling is active within a committed exocrine progenitor pool in developing mouse pancreas, and that Notch signaling blocks terminal acinar cell differentiation, but not initial commitment to the exocrine lineage [15].

In contrast to NICDs of Notch1, Notch2 and Notch4, NICD of Notch3 represses Notch1-mediated up-regulation of *Hes1* expression through competition with NICD of Notch1 for a common co-activator present in limiting amounts, and for access to *Rbp-Jk* [16]. One recent report assessing knockout and transgenic mice for components of the Notch signaling pathway suggests that NICD expression of Notch3 in the developing pancreas using *Ipf1/Pdx1* promoter impairs pancreatic epithelial proliferation, morphogenesis, and exocrine, but not endocrine, cell differentiation [9]. In this mouse, the pancreatic epithelium is poorly branched and the pancreatic buds are decreased in size. Consistent with the function of Notch3 as a repressor [16], *Hes1* is expressed at a low level, and *Ngn3*-expressing cells are found throughout the epithelium, meaning that differentiated endocrine cells are dispersed throughout the immature pancreatic epithelia. A similar phenotype is observed in mice deficient for *Dll1*, *Rbp-Jk*, and *Hes1* as well as in mouse over-expressing *Ngn3* [8,9].

Notch ligands

Mice deficient for *Dll1* show that epithelial IPF1/PDX1⁺ progenitor cells within the pancreatic bud differentiate prematurely into endocrine cells, causing a lack of expansion of cells within the pancreatic buds [9]. In addition to *Dll1*, the developing pancreatic epithelium expresses Notch ligands of the Serrate/Jagged family, and this ligand family is involved in endocrine development [17]. In knockdown studies in zebrafish embryos, loss of specific Jagged ligands causes ectopic islet-cell differentiation [18]. A similar phenotype has

been observed in mice overexpressing Ngn3 or the intracellular form of Notch3 as discussed above.

Hes1

The *Hes1* gene, which is transcriptionally activated by Notch signaling, encodes for bHLH transcriptional repressors. Hes1, although expressed in the pancreatic buds, and later by the pancreatic epithelial precursors together with Notch1 and 2 [8,9,13,19], is present in exocrine cells, but not in endocrine cells, while expression of Ngn3 is restricted to endocrine cells (Fig. 1 and Fig. 2). Hes1 prevents expression of Ngn3 by binding to several silencer sites located near the transcription initiation site, and therefore suppresses endocrine precursor patterning through the Notch signaling pathway [20]. *Hes1*-deficient mice show pancreatic hypoplasia because of depletion of pancreatic epithelial precursors resulting from accelerated differentiation of pancreatic endocrine cells [8], as stated above.

Recently, the Hes1-mediated Notch pathway was shown to control proper regional specification of pancreas in the developing foregut endoderm through Ptf1a regulation [21]. Loss of *Hes1* leads to mis-expression of Ptf1a in localized regions of the primitive stomach and duodenum as well as throughout the common bile duct. Lineage tracing shows that all the ectopic Ptf1a-expressing cells were reprogrammed to multipotent pancreatic progenitors that then differentiated into mature pancreatic exocrine, endocrine, and duct cells [21]. Similar to this study, inactivation of Hes1 induced the conversion of biliary epithelium to pancreatic tissue. Biliary epithelium in Hes1-deficient mice ectopically expressed *Ngn3*, differentiated into endocrine and exocrine cells and formed acini and islet-like structures in the mutant bile duct [22]. In addition, Hes1 likely regulates the binary decision choice of pancreatic progenitor to either exit the cell cycle or self-renew through transcriptional repression of p57, the cyclin kinase inhibitor [23]. Inactivation of *Hes1* caused upregulation of p57 expression in progenitors, leading to cell cycle arrest, early differentiation and depletion of the progenitor pool.

Rbp-Jk

DNA-binding protein Rbp-Jk, which is ubiquitously expressed and associates with all four types of Notch receptors, is a down-stream partner in Notch signaling. NICD interacts with Rbp-Jk to activate expression of *Hes* genes. Rbp-Jk mutant embryos showed accelerated differentiation of pancreatic endocrine cells [9,24]. The loss of Rbp-Jk at the initial stage of pancreatic development leads to accelerated α and PP cell differentiation and a concomitant decrease in the number of Ngn3-positive cells. This mice exhibited insulin-deficient diabetes because of endocrine hypoplasia and exocrine pancreatic hypoplasia was also present [24]. In contrast, the loss of Rbp-Jk specifically in β cells did not affect β cell number and function. Taken together, these studies demonstrate that Notch/Rbp-Jk signaling prevents premature differentiation of pancreatic progenitor cells into endocrine and ductal cells during early development and its presence in mature β cells in young animals is not required. However, no experiments with these mice were carried out over the natural life span of the animals so we do not know if they have an aging phenotype.

Ngn3

As is evident from the above literature, the most important transcription factor for driving pancreatic precursors towards an endocrine cell fate is the Ngn3. Sommer and colleagues were the first group to outline the expression of Ngn3 in the developing pancreas [25]. It is expressed exclusively in endocrine precursor cells before they differentiate and it is thought not to be present in differentiated endocrine cells [9,19,26]. Its expression starts at e9.5, peaks at e15.5 during the major wave of endocrine cell genesis and then decreases at birth, with almost undetectable levels in adult pancreas [20]. Lineage tracing analysis demonstrates that Ngn3-expressing cells are indeed the endocrine cell precursors [27]. Mice lacking Ngn3 function failed to develop any endocrine cells and died postnatally because of elevated blood glucose levels [28]. Conversely, over-expression of Ngn3 in the early pancreas induced massive premature differentiation of the entire pancreas into endocrine cells similar to those in *Hes1* deficient mice [9,26]. Moreover, mis-expression of Ngn3 induced endocrine differentiation throughout the gut epithelium [29] and adenovirus-mediated expression of Ngn3 in adult human pancreatic ductal cells induced an endocrine phenotype [30]. These results suggest that Notch signaling also operates in adult duct cells, driving them into an endocrine phenotype and formation of insulin-expressing cells.

The differentiating activity of Ngn3 is under control of Notch signaling, because studies with the *Ngn3* promoter have identified that it contains multiple-binding sites for the Hes-1 and reduced Notch signaling leads to increased expression of Ngn3. Indeed, null mutant mice for Dll-1 and Rbp-jk suffered from accelerated differentiation of pancreatic epithelial cells expressing Ngn3 and *Hes1*-deficient mice also showed premature endocrine differentiation and exocrine cell defects similar to those broadly mis-expressing Ngn3 [8,9]. In addition, the expression of NICD in Ngn3-positive endocrine progenitors was shown to inhibit their differentiation [10], but the ultimate fate of these cells could not be analyzed due to embryonal death, presumably because of Notch mis-expression in the *Ngn3*⁺ domain outside of the pancreas. In summary, in the developing pancreas, Notch signaling controls the choice between differentiated endocrine and progenitor cell fates through the regulation of Ngn3 expression such that when Notch pathway signaling is defective, the resultant high Ngn3 levels drives the epithelial cells to an endocrine fate. In contrast, cells with over-active Notch signaling result in an up-regulation of Hes1 expression and down-regulation of Ngn3 and the epithelial cells remain in an undifferentiated progenitor state (Fig. 2a).

Lateral inhibition by Notch signaling

Several studies have shown that, analogous to the generation of neurons during neurogenesis, the endocrine and exocrine cells of the pancreas via Notch signaling are constructed by lateral specification within ductal epithelium [20,31]. Ngn3 induces expression of Notch ligands such as Delta and Jagged that then activate Notch receptors on neighboring cells [30]. In concert with Rbp-Jk, NICD promotes expression of Hes1 in the adjacent cells. Hes1 or/and *Hes1*-modified genes repress expression of Ngn3 and other target genes, thereby preventing premature endocrine differentiation in the adjacent cells or at a stage that would not allow sufficient proliferation of endocrine precursor cells. Additionally, *Hes1* activation in pancreatic progenitors suppresses the expression of p57 to prevent exiting from the cell cycle and subsequent premature differentiation [23]. Therefore,

cells with active Notch signaling resulting in up-regulation of Hes1 expression maintain their proliferative capacity, while cells lacking Notch signaling express Ngn3, exit the cell cycle, and differentiate into endocrine cells [23]. As a consequence, Notch signaling helps to specify endocrine-cell differentiation and the lateral specification model provides a mechanism by which cells destined to progress along the endocrine lineage (*ngn3* positive) inhibit endocrine differentiation of their neighboring cells, forcing them to retain a non-endocrine fate (Fig. 2a). This lateral specification model in developing pancreas has been supported by several transgenic studies. Loss of various Notch signaling pathway genes (*Hes1*, *Dll1*, *Rbp-Jk*) during pancreatic development led to up-regulation of Ngn3 and consequently accelerated differentiation of pancreatic endocrine cells paralleled by a depletion of the pool of pancreatic precursor cells [8,9], and a similar phenotype was observed in mice overexpressing Ngn3 and overexpressing the intracellular form of Notch3 [9]. Similarly, the increased expression of Dll-1 and Dll-3 in Hes1 mutants [8] and the activation of Dll-1 and Dll-4 in pancreatic duct cells transfected with adenovirus-*Ngn3* [30] also support the lateral specification model in pancreas development.

Suppressive maintenance by Notch signaling; Mesenchyme, FGF10, and Notch signaling

The earlier and highly sophisticated studies on pancreas development have shown that signals originating in the mesenchyme play an essential role in the proliferation of pancreatic epithelial cells, precursor pool maintenance and the ratio of endocrine- to exocrine-cell differentiation [31]. In the absence of mesenchyme, embryonal pancreatic epithelium gives rise to endocrine cells, but not acinar structures, and the same experiments provided the first evidence that islets derive directly from early pancreatic precursor cells and not from ducts or early ductular structures [31]. A member of the fibroblast growth factor (FGF) family, FGF10, which is produced and secreted by pancreatic mesenchyme at stages that coincide with the rapid growth of epithelial buds, is also an important player for normal, fully developed pancreas. Loss of FGF10 results in pancreatic hypoplasia and absence of endocrine cells because there is a dramatic reduction in the proliferation of the epithelial progenitor cells marked by the production of Pdx1 [32]. In contrast, two recent studies have shown that persistent expression of FGF10 in the embryonic pancreas resulted in a large pancreas due to enhanced and prolonged proliferation of pancreatic precursor cells and a block in exocrine, ductal, and endocrine cell differentiation [17,33]. In these mice and in contrast to the wild-type situation [8,9], the pancreatic precursor cells remain strongly positive for Notch1 and Notch2 as well as Hes1, whereas Ngn3 expression is repressed [17,33]. Thus, both studies provide evidence that ectopic FGF10 signaling maintains Notch signaling in an active state throughout the developing pancreatic epithelium, which results in impaired expression of Ngn3 within the pancreatic epithelium and prevention of its differentiation [17,31,33]. Furthermore, Notch ligands Jagged-1 and Jagged-2 are expressed in the normal pancreatic epithelial cells, thereby overlapping with Notch1 and Notch2 [9,13,17], and this pattern was maintained in the presence of ectopic FGF10 [17]. These observations have led to the suggestion that “suppressive maintenance”, another mechanism different from lateral specification, is defined by Notch-mediated Hes1 activation throughout the precursor cell population with the outcome that cell differentiation cues are suppressed and the progenitor state is maintained [17,31] (Fig. 2b).

Implication for Notch signaling and diabetes

Pancreatic development follows three well-defined steps: endoderm formation, pancreatic morphogenesis, and, finally, differentiation of exocrine and endocrine cells. In this process, nowhere is it obvious why β cells only, as happens in type 1 diabetes, should be subject to later autoimmune destruction. In islets of type 1 diabetic patients, β cells only are destroyed by the immune system and so far no one has figured out a method in human of differentiating or trans-differentiating existing pancreatic cells, in vivo, to become β cells in order to replenish an effective pool of cells. Initially it had been thought that *Sel1L*, a Notch repressor, might be involved in type 1 diabetes, but on subsequent genetic studies, it was found not to be linked to type I diabetes in a panel of 20 Danish patients [34,35]. Isolating islets from cadavers and using them for transplantation is simply not feasible on any large scale. First, there is a paucity of donors. Second, islets from more than one donor are required for exogenous insulin-free living, and, third, the transplanted islets eventually become non-functioning [36]. The non-functionality may be due to lack of cell turnover within the transplanted islets, in addition to the use of immunosuppressors. Lack of cell turnover within islets may be overcome with use of specific Notch receptor agonists, once greater knowledge is gained on the transplantation and embedding process that occur in the liver. It is possible that as the islets become vascularized in their new site in the liver, that Notch would become activated, and in a window-of-opportunity, be capable of being activated and allow cell proliferation. However, in the long run, alternate strategies of cell-based transplanted material besides islets are required. One possibility that has been frequently touted is the use of embryonic stem (ES) cells that might be guided to a β -cell-like phenotype. So far, this has not been accomplished in any meaningful or useful way [37,38]. Continuously sustained Notch activation with exogenous ligands might lead to continued proliferation of ES cells that could then be differentiated as needed. One possibility is the use of γ -secretase inhibitors that may be able to rapidly prevent or suppress Notch activation and allow differentiation to occur by favoring *Ngn3* expression. It has already been shown that Notch signaling (using Delta-4 and Jagged-1 analogs) markedly reduced fetal neural stem cell death [39]. Additionally, the cells retained the capacity to become neurons, astrocytes and oligodendrocytes. Pancreatic precursors from mouse *e13.5* were also maintained in an undifferentiated state by *DI-4* treatment and continued to proliferate.

It is intriguing that in type 2 diabetes, β -cell apoptosis appears to be a consistent feature [7]. Normalization of blood glucose may prevent the apoptosis, though this cannot really be tested in individual humans. Because Notch ligands can prevent apoptosis of ES cells the development of Notch agonists may also find a use in treating type 2 diabetes.

Conclusion and perspective

We have come a long way to understanding the routes taken by the embryo during development from formation of primitive gut through to a mature pancreas, complete with all its diverse functions. So far, we have not been successful in turning this knowledge into useful treatments for type 1 and type 2 diabetes. As we study Notch pathways beyond the developmental stage and into the mature pancreas, we may uncover ways of activating it

within existing β cells and be able to modify the rate on cell turnover. Additionally, the knowledge may be useful in tissue engineering for replenishing β cells in type 1 diabetes.

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Fig. 1.
Schematic model for pancreatic cell differentiation.

Fig. 2.
The bimodal pathway of Notch signaling during pancreas development. (a) Lateral inhibition. (b) Suppressive maintenance.