New Insights into Endocrine Pancreatic Development: The Role of Environmental Factors

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Introduction

The pancreas is a mixed gland composed of endocrine and exocrine components. Within the pancreatic islets, β cells produce insulin and control the glycaemia. Their deficiency leads to diabetes and several potential complications. In the last decade, numerous studies have focused on pancreas development. The objective was to characterize the cellular and molecular factors that control the differentiation of endocrine and exocrine cell types. Investigation of the role of transcription factors by using genetic approaches led to the discovery of key molecules that are expressed both in rodents and humans. Some of them are ubiquitous, and some others are specifically involved in endocrine or exocrine specification. In addition to these intrinsic factors, recent studies have focused on the role of environmental factors. In the present review, we describe the roles of nutrients and oxygen in the embryonic pancreas. Interestingly, these extrinsic parameters can interfere with β-cell differentiation and function. Altogether, these data should help to generate β cells in vitro and define strategies for a cell-based therapy of type 1 diabetes.
mutation of a single nucleotide in Pdx1 also resulted in the absence of the pancreas [6], showing that the role of Pdx1 is conserved between species. Other factors, the bHLH factors Ptf1a, Hlx9, and Is1l are involved in the first steps of pancreas development. While p48-Ptf1a and Mist1 control exocrine development, Hlx9 and Is1l are implicated in the initiation of the development of the dorsal pancreas [5, 7–9]. The transcription factor neurogenin 3 (Ngn3) is expressed after Pdx1 during development, in the cells that are committed to the endocrine lineages. The lack of Ngn3 in mice resulted in absence of endocrine differentiation [10]. Mellitzer et al. [11] showed that insulinoma-associated 1 (IA1), a zinc-finger containing factor, is activated by Ngn3 and controls endocrine development through the activation of a bHLH-containing factor, NeuroD1 (BETA2) [10–12]. In contrast to Ngn3-deficient mice, endocrine cells are present in NeuroD1 knockout mice, but they undergo massive apoptosis [10, 12]. Pax4, a paired-box encoding gene, is expressed in the Ngn3-expressing cells and is activated by Ngn3 [13]. Mice lacking Pax4 do not develop β or δ cells [14]. Collombat et al. [15] showed that Arx, a member of the paired-like encoding gene family, and Pax4 are mutually antagonistically regulated. Pax4 favors β- and δ-cell commitment while Arx favors α-cell commitment [15]. MafA and MafB belong to the large Maf family of leucine zipper (bZIP) transcription factors. Nishimura et al. [16] showed that α- and β-cell differentiation occurs from a precursor expressing MafB. Their results suggest that differentiation of β cells proceeds through a MafB+MafA–Ins+ intermediate cell to MafB–MafA+Ins+ cells. The expression pattern of MafB reveals a role of MafB in both α- and β-cell development. Other factors, like the POU homeobox transcription factor 4 brain4/POU3F4, are expressed in glucagon-expressing cells. Some Brn4+ cells co-express Pax6 and Is1l. Later, its expression is restricted to α cells, suggesting that Brn4 is a marker of the α cells’ progenitors [17]. Nkx2.2, Nkx6.1 and Nkx6.2 belong to the NKX family protein. They are involved in endocrine development [18, 19]. Nkx2.2 is expressed in the progenitors during early development and becomes restricted to some Ngn3-positive cells during embryogenesis and next to α, β, and PP cells. In Nkx2.2 mutant mice, β cells are absent and numbers of α and PP cells are decreased [18, 19]. Nkx6.1 is first expressed in the pancreatic epithelium at E10.5. Next its expression is restricted to the Ngn3-expressing cells, and is found later exclusively in the adult β cells [19]. Nkx6.2 is expressed in the undifferentiated epithelium and is restricted to acinar and α cells at E15.5, but is excluded from Ngn3-positive cells [19]. Inactivation of Nkx6.1 leads to reduced β-cell mass [20]. In the absence of Nkx6.2, the development of the pancreas remains normal. However, Nkx6.1−/−Nkx6.2−/− mice display reduced numbers of β and α cells [20], showing the importance of Nkx6.2. In order to compare the role of transcription factors in rodents and human, Lyttle et al. [21] analyzed the transcriptionalome of the human fetal pancreas at different stages. In the human fetal pancreas, Ngn3 is expressed in early development. Q-PCR analyses showed a decrease of Ngn3 expression as development progressed from 8 to 21 weeks. Moreover, microarray experiments revealed that Is1l, NeuroD1, MafB, and Pax6 were significantly increased during human fetal pancreas development whereas Pax4, Nkx2.2, and Nkx6.1 were expressed but remained constant [21]. Recently, microarray analysis has investigated the genes expressed in E15.5 eYFP-Ngn3-positive cells versus Ngn3-negative cells. Interestingly, the Rfx6 factor, which belongs to the Rfx transcription factor family, was enriched in the Ngn3-positive cells [22]. Rfx6 is expressed in Pdx1-
expressing cells within the pancreatic buds and becomes restricted to the endocrine cell types [22]. Expression of Rfx6 was unaffected in Arx-, Pax4-, and NeuroD1-deficient mice, suggesting that Rfx6 acts downstream of Ngn3 and upstream of Arx, Pax4 and NeuroD1 [22]. Interestingly, mice lacking Rfx6 failed to generate any of the normal islet cell types except for the pancreatic polypeptide-producing cells [23]. In human infants with a similar autosomal recessive syndrome of neonatal diabetes, genetic mapping and subsequent sequencing identified mutations in the human Rfx6 gene [23]. Another gene of the same family, Rfx3, is expressed in the Ngn3-positive cells, and is found in the developing and mature islet cells. Rfx3 is involved in the growth and function of cilia. Before birth, Rfx3–/– islets contain reduced numbers of α and β cells whereas the number of PP cells is increased. In adult mice, the lack of Rfx3 leads to small and disorganized islets, with reduced insulin production and impaired glucose tolerance [24]. These data underline the sequential intervention of the transcription factors during the decisions of cell commitment in the pancreas development.

The complexity of the genetic network is modulated by the signals provided by the surrounding tissues and also by environmental parameters. The roles of notochord, blood vessels and epithelio-mesenchymal interactions in pancreas development were previously documented [25–28]. Mechanical stresses generated by cell growth also participate to organogenesis [29]. In this review, we focus on the roles of nutrients [30] and oxygen.

**Role of Nutrients**

Low birth weight is an important risk for type 2 diabetes in later life. Both maternal environment and the fetal genome influence the number and function of the β cells in early life and this has lifelong implications for postnatal diabetes [31]. During the past decade, numerous studies have focused on the role of nutrients on pancreas development [32–38]. Indeed, adverse events that include modifications of the diet during early life may affect the growth of different organs and cause metabolic disorders in later life. Recently, Dumortier et al. [35] exposed pregnant rats to low protein or low energy diet during different windows of gestation. The low energy diet decreased the β-cell mass in 21-day-old fetuses by 33 or 56% when administered during the last week or throughout gestation, respectively. Fetal corticosterone was increased. The poor development of β-cell mass was correlated with a decrease of the number of NGN3- and Pdx1-expressing cells, showing an effect of this diet on neogenesis. The low protein diet also induced a reduction of the β-cell mass but this effect was more pronounced when applied during the last week of gestation (~53%) than throughout gestation (~33%). This diet altered β-cell proliferation and islet vascularization but not the expression of NGN3 or PDX1 [35]. Altogether, these data demonstrate that the diet controls β-cell development. One explanation for the effects of the low energy diet could be the increase of corticosterone. Indeed, previous studies showed that overexposure to glucocorticoids in utero mediates the effects of undernutrition on the β-cell mass [39]. Exposure of pregnant rats to low protein diet also causes impaired glucose homeostasis in the young adult offspring [40]. Dietary insult in early life is thus able to affect the development and future function of the endocrine pancreas. Surprisingly, the diet during gestation can also influence the onset of type 1 diabetes. When non-obese diabetic mice were exposed to low protein diet during early life, the onset of diabetes was delayed [41]. Indeed, low protein diet throughout gestation led to decreased levels of cytotoxic cytokines and reduced insulitis. In females, low protein diet was also associated with a reduction of the number and size of the pancreatic islets, lower insulin pancreatic content and lower serum insulin. However, the ability of low protein diet to delay the onset of diabetes was sex-independent. The mechanism by which low protein diet delays the onset of diabetes is likely to involve both immune alterations and changes in β-cell development [41]. A human correlate of such observation could be the lower incidence of type 1 diabetes seen in individuals born of low birth weight or subjected to nutritional deficiencies [42, 43].

More recently, Guillemain et al. [44, 45] also examined the effects of glucose levels in endocrine differentiation. They found that glucose interferes with the pancreatic endocrine cells development by regulating the transition between Ngn3 and NeuroD [45]. Moreover, glucose activates β-cell development in vitro in a dose-dependent manner [45]. Altogether, these data should increase our control on the regimens during pregnancy that could impact on the occurrence of type 1 and type 2 diabetes.

**Oxygen and β-Cell Development**

At low oxygen tension (pO₂), cells undergo adaptive changes, including increased angiogenesis and erythropoiesis, and a switch to glycolytic metabolism. The cellular response to hypoxia is tightly controlled by the hypoxia-inducible factor (HIF) complex. HIF is regulated in an O₂-
HIF and the β-Cell Function

The HIF complex is an α/β heterodimer. Although HIF1α is sensitive to the level of oxygen, HIF1β, also called ARNT, is constitutively expressed. The possible role of ARNT in the β cells was examined. β-Cell specific knockout of ARNT in mice led to abnormal glucose tolerance and impaired insulin secretion [56]. Interestingly, microarrays analysis in pancreatic islets from type 2 diabetic patients showed a 90% reduction of ARNT when compared to human glucose tolerant islets, showing that in addition to its physiological role, ARNT may be involved in the pathogenesis of type 2 diabetes [56].

The VHL protein controls the degradation of HIF1α. In mice lacking VHL in β cells, HIF1α was stabilized in the islets, and a defect of glucose-stimulated insulin se-
cretion was observed [47–49]. The deletion of HIF1α in β cells restored a normal insulin secretion in response to glucose in these mice, indicating that the abnormal glu-

cose homeostasis was dependent on HIF1α upregulation [47]. HIF1α induces the expression of the high-affinity glucose transporter GLUT1 and glycolytic enzymes, and decreases mitochondrial oxygen consumption [57]. Since glucose uptake, glycolysis and mitochondrial respiration have a major role in glucose sensing, these effects of the HIF pathway might be involved in the impaired stimulated insulin secretion that occurs in the VHL knockout mice. Interestingly, Cantley et al. [47] found that islets lacking VHL in the β cells have impaired glucose uptake, altered mitochondrial metabolism, and the glucose-stimu-
lated flux of Ca2+ was attenuated as compared to con-
trols, supporting this hypothesis. Altogether, these data demonstrate that the oxygen (HIF) pathway plays an im-
portant role in β cells, and can impact on their function when hypoxia occurs.

In conclusion, recent data shed light on the roles of en-
vironmental factors, as nutrients and oxygen, on the de-
velopment and the function of β cells. We think that un-
derstanding the cellular and molecular mechanisms that control endocrine differentiation should help generate β cells in vitro and to improve the protocols for a cell-
based therapy of type 1 diabetes. Moreover, an associa-
tion of HIF1α gene polymorphism with type 1 and type 2 diabetes was discovered recently [58]. The characterization of the role of oxygen/HIF in diabetes should render possible the use of molecules that interfere with this path-
way and facilitates the function of β cells.

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