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REVIEW ARTICLE

Pancreatic Development and Adult Diabetes

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ABSTRACT

Low birth weight is an important risk factor for type 2 diabetes in later life. Maturity-onset diabetes of the young has been linked to genetic sequence abnormalities in transcription factors known to be involved in endocrine pancreatic development. These observations suggest that both the maternal environment and the fetal genome can influence the number and/or function of pancreatic β cells in early life, and that this has life-long implications for postnatal diabetes. This article reviews the evidence that suggests that β cells derive from a neogenic process within the pancreatic ductal epithelium, controlled by specific transcription factors and locally acting peptide growth factors. In rodents, many of the fetal phenotypes of β cells are destroyed during neonatal life in a developmental apoptosis and are replaced by a second wave of neogenesis. This results in islets with insulin release characteristics suited to postnatal life. The timing and amplitude of these ontological events are altered by nutritional sufficiency, and this may be mediated by changes in pancreatic growth factor expression, particularly of the IGF axis. Because β-cell plasticity after the perinatal period is limited, a dysfunctional programming of β-cell ontogeny may present a long-term risk factor for glucose intolerance and type 2 diabetes. This critical window of pancreatic development is likely to occur in third trimester of human development. **(*Pediatr Res* 48: 269–274, 2000)**

Abbreviations

MODY, maturity-onset diabetes of the young
IUGR, intrauterine growth restriction
Shh, Sonic hedgehog gene
PP, pancreatic peptide
FGF, fibroblast growth factor
DTA, diphtheria toxin
HNF, hepatocyte nuclear factor
PTF1, pancreatic transcription factor 1
VEGF, vascular endothelial growth factor

INTRAUTERINE GROWTH RESTRICTION AND GLUCOSE HOMEOSTASIS IN ADULTS

IUGR is a risk factor for both perinatal disease and diseases of later life. Barker *et al.* showed that a strong inverse correlation exists between mortality from cardiovascular disease below age 65 and birth weight (1). Similarly, the relative risk for prevalence of syndrome X, consisting of type 2 diabetes, hypertension and hyperlipidemia, is 18-fold higher in males born <2.5 kg compared with those >4 kg (1, 2). The at-risk individuals were thin at birth with a low ponderal index. Impaired glucose tolerance can be detected as early as 7 y of age in children who at birth had low weight and were thin (3). This implies that a programming of the metabolic axis can occur in early life, which is modulated by the intrauterine environment. For type 2 diabetes this may result from an altered development and insulin-secreting capacity of the endocrine pancreas, or by altered insulin sensitivity of target tissues. It is possible that perturbations of prenatal growth may lead to inappropriate β-cell ontogeny and result in a population of β cells qualitatively ill suited to subsequently manage metabolic stress. A reduced availability of insulin prenatally is a major contributor to IUGR. This can be experimentally demonstrated in extreme form using insulin gene knockout mice (4) whose birth weight was 25% less than heterozygote littermates at birth; it is also demonstrated by the severe growth retardation of human infants with pancreatic agenesis (5). Insulin deficiency may result from either genetic mutations in transcription factors active during β-cell formation, or from altered expression of transcription factors and peptide growth factors due to environmental influences *in utero*.

Intrauterine growth restriction in rat or man results in a reduced population of pancreatic β cells at birth (6, 7). Maternal calorie restriction by 50% in rat from d 15 of gestation until term showed that β-cell mass was reduced in the newborn due

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to a reduction in the number of islets (8). If normal diet was restored at birth, the β-cell mass returned to that of controls by weaning. However, continuation of energy restriction during neonatal life leads to irreversible changes in β-cell mass. A reduction of dietary protein to 8% throughout gestation caused relative growth restriction at birth with reduced β-cell mass and islet cell size (9, 10). Again, if nutritional restriction was lifted at birth, the islet morphology recovered, but if extended to weaning the changes were irreversible. Such animals are susceptible to glucose intolerance in later life (10). These models show a strong effect of maternal environment on fetal islet development, but also that the neonatal period is a time of islet plasticity that will have life-long consequences for glucose homeostasis.

ONTOGENY OF THE ENDOCRINE PANCREAS

The pancreas arises from embryonic mid-gut endoderm. In the mouse, the dorsal pancreatic bud appears at embryonic (E)9.5 closely followed by the ventral bud, and these fuse at embryonic d E16–17 (11). As each bud grows it forms highly branched structures and the acini and ducts become distinguishable at d E14.5. Endocrine cells can be found from the earliest stages of bud development and by d E15.5 make up 10% of the pancreas. Initially, they are seen as individual cells or small clusters close to the pancreatic ducts, and only form as mature islets in the final few days of gestation. This is achieved by early third trimester in the human fetus. The growth and cyto-differentiation of the pancreas depends on mesenchymal-epithelial interaction. Pancreatic mesenchyme accumulates around the dorsal gut epithelium and induces pancreatic bud formation and branching (12–15). The earliest signal may be derived from the notochord, whereby notochordal repression of endodermal *Shh* expression permits pancreas development (16–18). Hebrok *et al.* (17) found in the chick that activin-βB and fibroblast growth factor-2 (FGF-2) are notochord factors that can repress endodermal *Shh* and thereby permit expression of pancreas genes, including *Pdx*-1 and *insulin*. The earliest morphogenetic signaling in pancreas formation therefore depends on interplay between peptide growth factors and tissue transcription factors, an interaction that is apparent throughout islet formation.

Teitelman *et al.* (19) suggested that the precursor cells of the endocrine pancreas coexpress insulin, glucagon, and also the neuronal proteins tyrosine hydroxylase and neuropeptide Y. These proteins were first detectable by immunocytochemistry on d E9.5 in mouse, whereas PP was detected only at birth. However, Herrera *et al.* (20) found PP immunopositive cells as early as d E9.5. The expression of the A chain of the DTA placed under the control of elastase I in transgenic mice, thus killing cells expressing elastase 1, produced animals with a rudimentary pancreas and only a few ductal cells and islets of Langerhans. Elastase I is normally expressed in the exocrine tissue. This suggested that the early disruption of the elastase-expressing cells dramatically affected endocrine pancreatic development. Herrera *et al.* (21) produced transgenic mice expressing the DTA gene placed under the control of the insulin, glucagon, somatostatin, or PP promoters. They demonstrated that only the PP promoter decreased endocrine cell type number within the islets, suggesting that the PP cells might be the endocrine precursor cells.

The differentiation of the endocrine pancreas is ongoing at birth in rodent species. Neogenesis of islets continues through neonatal life but ceases shortly after weaning (22). In the rat fetus the cellular area staining immunopositively for insulin increases twofold over 2 d just before term, deriving both from β-cell replication and ductular neogenesis (23). Conversely, the rate of mitosis in adult pancreatic β cells is normally low (24). This fundamental change in cell phenotype has been linked to a transient wave of apoptosis that occurs in neonatal rat islets 2 wk after birth (25–27), and the replacement of these lost β cells with new islets by ductular neogenesis. This partial replacement of β cells may generate a cell population suited to metabolic control in later adult life. Because the final population of β cells will have only limited potential for regeneration in the adult animal, any aberrant apoptotic deletion of fetal-type cells, or the parallel neogenesis of adult-type islets, is likely to alter the ability of the animal to deal with metabolic stress. The mechanisms leading to such cellular pathologies are likely to involve an altered expression of transcription and/or growth factors.

The development of ductal epithelial cells into an endocrine lineage, and ultimately into β cells, is likely to involve a specific sequence of expression of transcription factors, interlinked with locally acting growth factor signals. A fundamental branch point is the progression of a pluripotential epithelial cell to either the endocrine or exocrine lineage (Fig. 1). One of the most important transcription factors identified so far for the endocrine lineage is Pdx-1, also known as STF-1, IDX-1, or IPF-1 (28). In animals with a targeted deletion of *Pdx*-1, pancreatic buds form but no further differentiation or morphogenesis occurs (29). Expression of *Pdx*-1 in undifferentiated ductal epithelium is associated with the glucose transporter GLUT 2, which by fetal d 15 in the rat has been lost from acinar cells, but is retained by developing β cells. Pdx-1 therefore appears to have a dual role of an inducer of an early endocrine cell lineage from ductal epithelial cells, and in the maturation of β cells and the control of insulin gene expression. A single deletion of a nucleotide in man leads to the complete agenesis of the pancreas (5). Pdx-1 expression is directed by at least two other nuclear transcription factors, HNF-3β and BETA-2/NeuroD (28). Conversely, PTF1 is able to transactivate the 5′ regions of genes that are specifically expressed in the exocrine tissue. Mice carrying null mutations of the p48 sub-unit of *PTF*1 did not have any pancreas, although groups of endocrine cells were detected, notably in the spleen (30). This result shows that PTF1 is a key factor for exocrine development.

What then are the trophic signals that determine the choice of endocrine or exocrine lineage, and the expression of transcription factors such as Pdx-1 or PTF1? They are likely to include growth factors expressed within the pancreatic stroma adjacent to the ductal epithelium, such as FGF-7 and VEGF (Table 1). Systemic injection of FGF-7 into adult rats for up to 2 wk caused a rapid increase in DNA synthesis within the ductal epithelium, which was seen within 24 h (31). Pancreatic

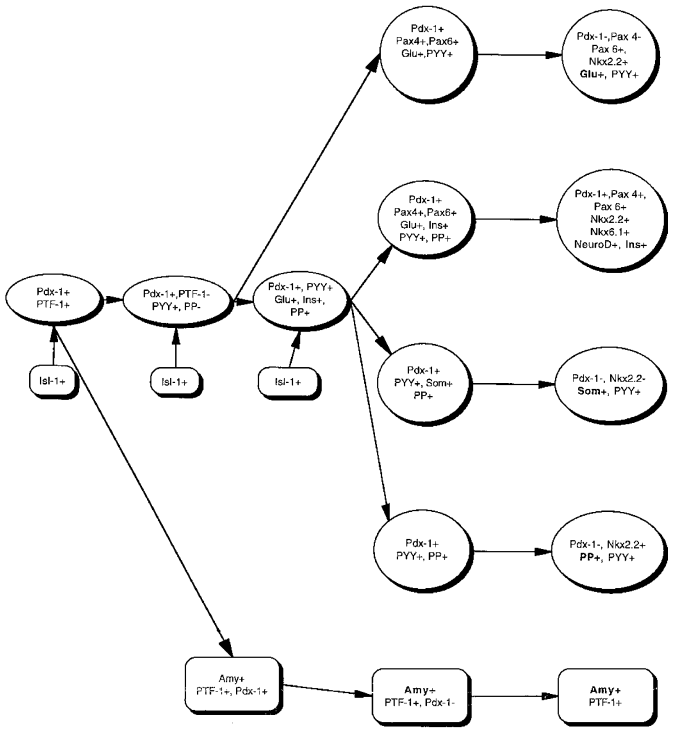

Figure 1. Schematic plan of the likely roles of transcription factors in the generation of pancreatic endocrine and exocrine lineages from a common stem cell within the developing pancreas. Early in development, precursor cells express transcription factors that commit them either to an endocrine (*Pdx*-1) or exocrine (*PTF*-1) lineage. The discrimination depends in part on growth factor signals from surrounding mesenchyme under the control of transcription factors such as *Isl*-1. The early endocrine lineage expresses peptide YY (PYY+) and later, pancreatic polypeptide (PP+). Before the activation of the PP gene (PP−) a subpopulation of cells develops into glucagon-secreting α cells (Glu+). This involves a loss of *Pdx*-1 expression (*Pdx*-1-) and the expression of the transcription factors *Pax*-6 and *Nkx*2.2. Precursor cells that continue to express PP and PYY develop into insulin-expressing β cells with the transcription factor profile *Pdx*-1+, *Pax* 4+ and *Pax* 6+, and *Nkx*2.2+, *Nkx*6.1+ and *NeuroD*+; somatostatin-expressing δ cells with the profile *Pdx*-1− and *Nkx*2.2−; and PP cells with a profile of *Pdx*-1− and *Nkx*2.2+. Insulin secretion from β cells depends on the expression of the additional transcription factors *HNF*-1α and -4α, together with *Pdx*-1, mutations in each of which are associated with MODY. Exocrine cells remain *PTF*-1+, lose *Pdx*-1 expression and synthesize amylase (Amy).

Table 1. *Peptide growth factors involved in pancreatic β cell development*

Growth factor	Origin	Biological actions
FGF-7	Pancreatic stroma	Endocrine cell neogenesis from ductal epithelium
VEGF	Pancreatic stroma	Endocrine cell neogenesis and angiogenesis
IGF-II	Ductal epithelium and islet endocrine cells	Islet cell proliferation and survival in the fetus and neonate
IGF-I	Islet endocrine cells	Islet cell proliferation and survival in childhood

duct hyperplasia followed, but not a progression to increased numbers of endocrine cells. However, when FGF-7 was expressed within the embryonic liver of transgenic mice, pancreatic duct hyperplasia was seen with increased numbers of ductal cells containing immunoreactive insulin (32). VEGF is a potent mitogen for endothelial cells using two high-affinity tyrosine kinase-type receptors, Flt-1 and Flk-1. *Flk*-1 mRNA is expressed within RINm2F islet cells, as well as in fetal rat islets where VEGF is able to increase insulin content (33). In intact fetal rat pancreas, immunoreactive Flk-1 was localized to pancreatic ductal cells and vascular endothelium, suggesting that ductal cells may also be a target for VEGF action. VEGF was found to enhance ductal cell replication and insulin content, implying that a β-cell neogenesis was also occurring (34). In adult human islets and insulinomas, VEGF was localized to β cells, whereas in the human fetus it was found within immature islets (35, 36). Although VEGF must be a prime candidate for initiating an angiogenic growth response in support of an increasing islet cell mass, it may also act directly as a mitogen and morphogen for ductal epithelial cells.

Once ductal epithelial cells have entered the endocrine lineage they become further differentiated into the separate endocrine cell types, a process controlled by the Pax transcription factors, of which Pax-4 and Pax-6 are expressed in the developing pancreas (Fig. 1). In the early mouse embryo Pax-4 was detected in insulin-containing cells, and after birth remains restricted to the β cells (37). Conversely, Pax-6 coexpressed with glucagon-containing cells, but after d E15.5 was found in all the endocrine cells (38). Deletion of *Pax*-4 caused a complete loss of pancreatic β cells and δ cells, but an increased number of α cells. Conversely, functional deletion of the *Pax*-6 gene in mouse decreases the presence of all four endocrine cell types in the pancreas, with the presence of α cells being totally abolished (39, 40). Pax-6 has been shown to transactivate both the glucagon and insulin genes promoters. Mice lacking both Pax-4 and Pax-6 failed to develop any mature endocrine cells in the pancreas (39). Pax-4 and Pax-6 appear to be important in distinguishing the α cell lineage from β and δ cells, but act in parallel, not in series with Pdx-1 expression. Other transcription factors such as Nkx 2.2, Nkx 6.1, and Isl-1 also play defined roles, as outlined in Figure 1.

The ongoing proliferation and developmental differentiation of β cells, once formed, is highly dependent on the expression of the IGF within the islets (Table 1). Multiple studies indicate that IGF potentiate β-cell growth, maturation, and function, and are expressed by β cells throughout life. IGF-II mRNA is greatest in the fetal pancreas, and declines during the neonatal period (41, 42). Using *in situ* hybridization, IGF-II mRNA was shown to be expressed within islet cells in the fetus and neonate, and also in focal clusters of ductal epithelial cells. Levels of IGF-II mRNA in the human fetal pancreas are 100-fold greater than those for IGF-I (43). Exogenous IGF-I or -II promote increased DNA synthesis by isolated fetal or neonatal rat islets (44, 45), and isolated α and β cells from rat islets contain the high-affinity type 1 IGF signaling receptor (46). Using a transgenic mouse model we showed that an overexpression of IGF-II caused a four- to fivefold increase in the mean islet size at birth, affecting all endocrine cell types, but that the total number of mature islets was not altered (47). This implies that, *in vivo*, IGF-II functions as a growth factor for existing islet cells but does not promote islet neogenesis.

The wave of developmental β-cell apoptosis in the neonatal rat seen 2 wk after birth (27) coincides temporally with a diminished pancreatic expression of IGF-II within islets. A

nadir in total IGF availability may therefore exist in pancreas when apoptosis is transiently high. IGF inhibit apoptosis in a wide range of cell types *in vitro* (48, 49), and it has been demonstrated that endogenous IGF-II within isolated neonatal rat islets was able to protect them from cytokine-induced apoptosis (27). This protection was lost by weaning when islets no longer expressed IGF-II, but could be replaced with exogenous IGF-II. Further functional proof that changes in IGF-II availability provoke developmental β-cell apoptosis in the rat was obtained from a transgenic mouse model overexpressing IGF-II in skin, leading to increased circulating levels that did not fall postnatally. In these animals the neonatal wave of β-cell apoptosis is suppressed. There is therefore substantive data to show that IGF-II has a role in the homeostasis of β-cell mass in early life, but is predominantly a growth and survival factor for existing islet cells.

ABNORMAL EXPRESSION OF TRANSCRIPTION AND GROWTH FACTORS LEADS TO ALTERED B-CELL MASS AND FUNCTION, AND DIABETES

The consequences of a functional absence of a transcription factor can be dramatic as a single deletion in *Pdx-1* led to the complete agenesis of the human fetal pancreas (5). Several other cases of pancreatic agenesis have been described in the human infant, but no genetic disorder was established. The phenotype is characterized by severe growth retardation with term birth weights of 1.7–1.9 kg, poor muscle mass, and an absence of adiposity, which can be directly related to the lack of insulin (50–53).

MODY, a form of type 2 diabetes, is a monogenic disease with autosomal dominant inheritance, and is characterized by an early onset (childhood, adolescence ,or young adulthood) of failure of insulin secretion (54). The clinical phenotype is very variable, perhaps attributed to genetic heterogeneity. The known genes that are involved in MODY are hepatocyte nuclear factor 4α (*HNF*-4α) (MODY 1) (55), glucokinase (MODY 2) (56, 57), *HNF*-1α (MODY 3) (58), and *Pdx*-1 (MODY 4) (59). MODY 4 was observed in patients carrying a heterozygous mutation in *Pdx*-1. The resulting phenotype differed very much between individuals, some being normal, others being glucose intolerant, suggesting that the penetrance of this mutation was not complete. Targeted disruption of the mouse *Pdx*-1 gene in β cells alone resulted in those animals progressively becoming diabetic with age (60), showing that Pdx-1 is required to maintain the β-cell identity by positively regulating insulin and islet amyloid polypeptide expression, and by repressing glucagon expression. The expression of the HNF factors were first found in the liver, but a role in the pancreas now also seems likely, as in MODY 3 there is an impaired insulin secretion (61). Mice lacking the *HNF*-1α gene showed inadequate insulin secretion and β-cell intracellular calcium responses after stimulation with nutrient secretagogues such as glucose (62). A family of patients with MODY 1 has been described with mutations in *HNF*-4α who developed diabetes requiring insulin therapy in 30% of cases (54). Such patients demonstrate primary defects in β-cell insulin-release mechanisms (63, 64).

An abnormal β-cell mass may also result from altered expression of structurally intact transcription factors because of environmental pressures. *Pdx*-1 gene expression within islet-like cell clusters was down regulated by hyperglycaemia (65), as was *HNF*-3β but less so *BETA*-2 (66), and by fatty acids (66). Low glucose levels will also limit *Pdx*-1 expression in β cells (68). Partial pancreatectomy in the juvenile rat leads to a reduction in the expression of Pdx-1, GLUT 2, and insulin mRNA (69), most likely due to the associated hyperglycemia.

An altered expression of IGF or IGF binding protein (BP) associated with IUGR due to neonatal undernutrition may severely alter β-cell ontogeny after birth, leading to a population of β cells less than optimally equipped to handle metabolic challenge in later life. IUGR in rats, induced either by maternal fasting (70), maternal diabetes (71), or restricted uteroplacental blood flow (72), is associated with reduced circulating IGF levels, pancreatic weights, and pancreatic insulin contents. Parallel findings exist in human infants following IUGR (73). A low-protein diet causes an altered β-cell ontogeny in the fetal and neonatal rat in which the rate of β-cell replication is decreased, but the incidence of apoptosis is increased (74). Analysis of the cell cycle kinetics of β cells *in situ* using immunologic detection of cell cycle-specific proteins suggests that the cell cycle length of β cells is prolonged or arrested in low-protein-fed rats, with an extended G1 phase. This is associated with a reduced expression of IGF-II within the pancreatic islets. Thus, IGF-II may not only determine β-cell mass, but also the phenotype of adult β cells. What could depress pancreatic IGF-II expression during IUGR? Perhaps a direct effect of selected amino acids, inasmuch as we have recently shown that supplementation of the pregnant rat given a low-protein diet with taurine can reverse the deficit in pancreatic IGF-II mRNA (75). Another possibility is that a reduced expression of IGF-II mRNA could be due to increased levels of circulating corticosteroid in IUGR.

Glucocorticoids have been shown to decrease the expression of IGF-II and the IGF receptor in late fetal life (76, 77), and a neonatal increase in corticosterone may precipitate a decrease in pancreatic IGF-II expression, and a wave of β-cell apoptosis within islets. In addition, the expression of several IGFBP is under glucocorticoid control (78). Glucocorticoids are also capable of down-regulating *Pdx*-1 expression, mediated by a blockade of an enhancer region in the *Pdx*-1 promoter that recognizes HNF-3β and BETA-2 (79).

ABNORMAL PANCREATIC DEVELOPMENT *IN UTERO* AND ADULT DIABETES

Plasticity exists in the fetal and neonatal pancreas allowing changes in β-cell number through both β-cell replication and neogenesis, but later becomes restricted. Consequently, any deficiency in β-cell mass occurring *in utero* as a result of either genetic mutations within transcription factors, or maternal malnutrition or placental dysfunction leading to inappropriate expression of transcription or growth factors, will have only a limited opportunity for correction postnatally. In the rat and mouse at least two developmental windows exist in pancreatic ontogeny. The first covers the initial embryogenic process of

endocrine cell formation, and the subsequent cellular expansion of those islets. Insulin release from the β cells within these fetal-type islets is poorly responsive to glucose, with no acute release pharmacokinetics, but is very responsive to amino acids. Shortly after birth a developmental apoptosis appears to delete many of these β cells and they are simultaneously replaced with new islets derived from a second wave of neogenesis. The β-cell population is now sensitive to glucose with acute, first phase insulin release. This developmental change may prepare the animal for postnatal metabolism. Parallel changes may occur in the exocrine pancreas concerning the ontogeny of expression of lipases (80). Early environmental insults may alter the timing or amplitude of developmental changes leaving the individual with a β-cell population poorly suited both quantitatively and qualitatively for postnatal life, and ultimately leading to glucose intolerance. The finding of a similar developmental apoptosis of β cells in the third trimester human fetus (81) would coincide with the acquisition of glucose sensitivity by human fetal β cells, the maturation and functional activation of adipocytes, and the laying down of white adipose tissue. Thus, the establishment of postnatal insulin release kinetics and end organ sensitivity may be coordinated events in the third trimester as the human fetus prepares itself for extrauterine life. The precise ontogeny is likely to be controlled by the integrated effects of pancreatic transcription and growth factors whose expression can be modulated by nutritional metabolites or glucocorticoid availability. Abnormal programming of the fetal pancreas could be a major risk factor for adult diabetes.

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