Available online at www.sciencedirect.com
ScienceDirect
Developmental Biology 314 (2008) 406–417
DEVELOPMENTAL BIOLOGY
www.elsevier.com/developmentalbiology

Genomes & Developmental Control

pdx-1 function is specifically required in embryonic β cells to generate appropriate numbers of endocrine cell types and maintain glucose homeostasis

Maureen Gannon [a,b,c,d,*], Elizabeth Tweedie Ables [b], Laura Crawford [b], David Lowe [a], Martin F. Offield [c], Mark A. Magnuson [b,c,d], Christopher V.E. Wright [c,d]

[a] *Department of Medicine, Vanderbilt University Medical Center, Nashville, TN 37232, USA*
[b] *Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN 37232, USA*
[c] *Department of Cell and Developmental Biology, Vanderbilt University Medical Center, Nashville, TN 37232, USA*
[d] *Program in Developmental Biology, Vanderbilt University Medical Center, Nashville, TN 37232, USA*

Received for publication 27 September 2007; accepted 16 October 2007
Available online 4 November 2007

Abstract

The *pdx1* gene is essential for pancreatic organogenesis in humans and mice; *pdx1* mutations have been identified in human diabetic patients. Specific inactivation of *pdx1* in adult β cells revealed that this gene is required for maintenance of mature β cell function. In the following study, a Cre-lox strategy was used to remove *pdx1* function specifically from embryonic β cells beginning at late-gestation, prior to islet formation. Animals in which *pdx1* is lost in insulin-producing cells during embryogenesis had elevated blood glucose levels at birth and were overtly diabetic by weaning. Neonatal and adult mutant islets showed a dramatic reduction in the number of insulin$^+$ cells and an increase in both glucagon$^+$ and somatostatin$^+$ cells. Lineage tracing revealed that excess glucagon$^+$ and somatostatin$^+$ cells did not arise by interconversion of endocrine cell types. Examination of mutant islets revealed a decrease in proliferation of insulin-producing cells just before birth and a concomitant increase in proliferation of glucagon-producing cells. We propose that *pdx1* is required for proliferation and function of the β cells generated at late gestation, and that one function of normal β cells is to inhibit the proliferation of other islet cell types, resulting in the appropriate numbers of the different endocrine cell types.

Keywords: Pancreas; Islet; Diabetes; Cre-lox; Lineage tracing

Introduction

Mature pancreatic islets are composed of five different endocrine cell types (α: glucagon, β: insulin, δ: somatostatin, ε: ghrelin, and PP: pancreatic polypeptide) arranged in a typical architecture where the β cells, which make up the large majority, are found at the core and the other cell types are located at the periphery or mantle. Pancreatic endocrine cells appear in two waves during mouse pancreatic organogenesis (Pictet et al., 1972; Pang et al., 1994). During the first wave, which begins at embryonic day 9.5 (e9.5), the glucagon- and insulin-expressing cells that arise differ in their gene expression pattern from the mature cells found in adult islets. First wave glucagon-producing cells express pro-hormone convertase 1/3 (PC1/3) rather than PC2, which is expressed by mature α and β cells (Lee et al., 1999; Wilson et al., 2002), and first wave insulin-producing cells lack the glucose transporter, GLUT2, a marker of mature β cells (Pang et al., 1994). Insulin/glucagon double-positive cells have been detected at early developmental stages and have been postulated to represent an intermediate stage of endocrine differentiation (Alpert et al., 1988; Teitelman et al., 1993). Lineage tracing studies, however, suggest that these cells

* Corresponding author. Department of Medicine, Division of Diabetes, Endocrinology, and Metabolism, Vanderbilt University Medical Center, 2213 Garland Avenue 7425C MRBIV, Nashville, TN 37232-0475, USA. Fax: +1 615 936 1667.
E-mail address: maureen.gannon@vanderbilt.edu (M. Gannon).

0012-1606/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2007.10.038

do not in fact give rise to α or β cells of mature islets (Herrera, et al., 1994; Herrera, 2000). Thus, the function of these first wave single and double hormone-positive cells remains unclear.

The second wave of endocrine differentiation occurs between e13.5 and 16.5, and is believed to produce the cells of the mature islets (Pictet et al., 1972; Pang et al., 1994). The mechanism driving the relatively sudden increase in endocrine cell formation at this particular time in development is unknown, although signals including the Notch/Ngn3 pathway that affect precursor proliferation and differentiation are likely to be involved (Apelqvist et al., 1999; Murtaugh et al., 2003; Jensen, 2004). At late gestation and early postnatal stages, the islets begin to adopt their characteristic core/mantle architecture. Little is known about how the different endocrine cell types or their precursors interact with one another to generate the correct proportions of the various cell types. Several studies suggest that early glucagon-producing cells are required specifically for the generation of first wave insulin-producing cells (Dohrmann et al., 2000; Prasadan et al., 2002; Vincent et al., 2003), but it is uncertain whether similar cell–cell interactions are involved in generating endocrine cells at the secondary transition.

Reverse genetic studies in mice are helping to elucidate the complex pathways and gene interactions involved in pancreas development (Wilson et al., 2003). Genetic or cell deletion studies in the absence of lineage tracing techniques can, however, result in difficulties in deciphering cell differentiation pathways. Loss of one cell population in a particular mutant condition may have a profound non-cell autonomous effect on another cell population. For example, selective deletion of PP-producing cells, which results in the loss of both insulin- and somatostatin-producing cells, could be explained either by a PP-expressing precursor cell population giving rise to these two cell types, or by PP-expressing cells producing an inductive signal required for the production of β and δ cells (Herrera et al., 1994). Lineage tracing analyses have helped distinguish between these possibilities, and suggest that mature β cells do indeed arise from cells that previously expressed PP (Herrera, 2000).

The homeobox gene *pdx1* is expressed within the developing pancreatic endoderm in all vertebrates so far examined (Gannon and Wright, 1999). In the mouse, *pdx1* expression begins at e8.0 (Guz et al., 1995; Li et al., 1999), prior to the onset of pancreatic bud formation and islet hormone gene expression, and is initially detected throughout the pancreatic epithelium. By late gestation, *pdx1* expression is selectively maintained at high levels in β cells, with low levels of expression in acinar cells (Guz et al., 1995; Wu et al., 1997). Loss of *pdx1* function results in an early block in pancreatic outgrowth and differentiation in both mice and humans (Jonsson et al., 1994; Offield et al., 1996; Stoffers et al., 1997a,b). The pancreatic rudiment of *pdx1* null mouse embryos does contain transient, first wave insulin$^+$ cells (Ahlgren et al., 1996), and longer-lived glucagon$^+$ cells (Offield et al., 1996), indicating that *pdx1* is not required to generate first wave endocrine cells. In addition to an early role in pancreatic bud outgrowth, studies using tetracycline-inducible *pdx1* inactivation demonstrated that *pdx1* is also specifically required between e11.5 and e13.5 in order for subsequent differentiation of endocrine and exocrine cells (Holland et al., 2002).

Mice heterozygous for a *pdx1* deficiency are glucose-intolerant (Ahlgren et al., 1998; Dutta et al., 1998; Brissova et al., 2002), consistent with the finding that humans carrying dominant *pdx1* mutations are predisposed to a form of Type 2 diabetes called maturity onset diabetes of the young type 4 (MODY4) (Stoffers et al., 1997a,b, 1998; Macfarlane et al., 2000). The continued essential role for *pdx1* in mature β cells (Ahlgren et al., 1998; Holland et al., 2002) fits well with its identification as a direct activator of several β cell-specific genes that control glucose utilization and metabolism, including insulin, IAPP, glucokinase, *Pax4*, and *pdx1* itself (Chakrabarti et al., 2002; Cissell et al., 2002).

Direct evidence that *pdx1* is essential for maintaining mature β cell function comes from studies using tetracycline-inducible *pdx1* inactivation in adult mice (Holland et al., 2002) as well as conditional inactivation studies using an insulin promoter-driven Cre transgene that resulted in a loss of Pdx1 protein between 3 and 5 weeks after birth (Ahlgren et al., 1998). This mature β cell-specific loss of *pdx1* caused a dramatic decrease in insulin, Nkx6.1, and GLUT2 expression, a concomitant increase in the number of glucagon$^+$ cells, and overt diabetes in 3–5 month old mice. The excess glucagon$^+$ cells and large number of insulin/glucagon co-producing cells that were detected in the islets of these mice led to the suggestion that insulin$^+$ cells acquire glucagon expression after removal of the repressive influences of Pdx1. In the absence of lineage tracing, it is impossible to determine the origin of the excess glucagon$^+$ cells. One can envisage several ways in which such cells could arise from β cells or an insulin-expressing precursor cell type: (1) mature β cells de-differentiate to a more immature glucagon/insulin co-expressing cell type; (2) β cells slowly trans-differentiate towards a mature α cell type; or, (3) β cells loss promotes generation of new endocrine cells from an unidentified progenitor cell, which then gives rise to insulin/glucagon double-positive cells.

In summary, therefore, while *pdx1* is critical early in pancreas development for global organ formation and differentiation, as well as later in mature β cells, it is unclear what role it plays at the secondary transition in generating the β cells that will contribute to mature islets. We report here the results of a Cre-lox conditional inactivation study that provides details on *pdx1* function early in the definitive β cell lineage (during the second wave of endocrine differentiation), in which we assessed the consequences of *pdx1* inactivation by including lineage-tracing analysis. In this study, we used an optimized rat insulin promoter-Cre transgenic line (RIP-Cre; Postic et al., 1999; Gannon et al., 2000a,b,c) which shows functional recombination as early as e11.5 in developing pancreas and efficient (>85% of β cells) excision of *loxP*-flanked DNA (Postic et al., 1999; Gannon et al., 2000c). We demonstrate that loss of *pdx1* from early β cells leads to a severe reduction in the number of insulin$^+$ cells beginning at late gestation stages. β cell-specific inactivation of *pdx1* during embryogenesis results in early-onset diabetes, disrupted islet architecture, and increased numbers of glucagon$^+$ and somatostatin$^+$ cells at the expense