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Cell type and tissue specific function of islet genes in zebrafish pancreas development

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ABSTRACT

Isl1 is a LIM homeobox transcription factor showing conserved expression in the developing and mature vertebrate pancreas. So far, functions of pancreatic *Isl1* have mainly been studied in the mouse, where *Isl1* has independent functions during formation of exocrine and endocrine tissues. Here, we take advantage of a recently described *isl1* mutation in zebrafish to address pancreatic *isl1* functions in a non-mammalian system. *Isl1* in zebrafish, as in mouse, shows transient expression in mesenchyme flanking the pancreatic endoderm, and continuous expression in all endocrine cells. In *isl1* mutants, endocrine cells are specified in normal numbers but more than half of these cells fail to establish expression of endocrine hormones. By using a lineage tracking approach that highlights cells leaving cell cycle early in development, we show that *isl1* functions are different in first and second wave endocrine cells. In *isl1* mutants, early forming first wave cells show virtually no glucagon expression and a reduced number of cells expressing insulin and somatostatin, while in the later born second wave cells somatostatin expressing cells are strongly reduced and insulin and glucagon positive cells form in normal numbers. *Isl1* mutant zebrafish also display a smaller exocrine pancreas. We find that *isl1* expression in the pancreatic mesenchyme overlaps with that of the related genes *isl2a* and *isl2b* and that pancreatic expression of *isl*-genes is independent of each other. As a combined block of two or three *isl1/2* genes results in a dose-dependent reduction of exocrine tissue, our data suggest that all three genes cooperatively contribute to non-cell autonomous exocrine pancreas extension. The normal expression of the pancreas mesenchyme markers *meis3*, *fgf10* and *fgf24* in *isl1/2* depleted embryos suggests that this activity is independent of *isl*-gene function in pancreatic mesenchyme formation as was found in mouse. This indicates species-specific differences in the requirement for *isl*-genes in pancreatic mesenchyme formation. Overall, our data reveal a novel interaction of *isl1* and *isl2* genes in exocrine pancreas expansion and cell type specific requirements during endocrine cell maturation.

Introduction

The pancreas is a vertebrate-specific endodermal organ with essential functions in food digestion and glucose homeostasis. The mature organ is composed of an exocrine compartment with acinar and duct cells that produce and transport digestive enzymes into the gut, and an endocrine compartment from which metabolism-regulating peptide hormones are secreted into the blood stream. In higher vertebrates, endocrine cells are arranged in small clusters termed islets (islet of Langerhans in mammals) that contain up to five cells types each expressing a specific hormone: α-, β-, δ-, ε- and PP cells expressing *glucagon* (*gcg*), *insulin* (*ins*), *somatostatin* (*sst*), *ghrelin* and *pancreatic polypeptide* (*pp*), respectively. Formation of the pancreas can be formally divided into two phases termed primary and secondary transition. In amniotes, primary transition refers to the outgrowth of epithelial buds of dorsal and ventral foregut endoderm into the surrounding pancreatic mesenchyme demarcating the onset of pancreas morphogenesis. During this process, a population of postmitotic endocrine cells of unknown function, also termed 'first wave' cells, is established. Secondary transition defines the subsequent massive proliferation and expansion of the pancreatic buds and the accompanying differentiation of exocrine and 'second wave' endocrine cells from which the organ is finally formed (Pan and Wright, 2011).

Genetic studies have been extremely successful in defining the importance of specific signaling pathways and transcription factors in regulating pancreas development. While the mouse has been the major model for such studies, more recently the zebrafish had gained attention as a model that is readily amenable to genetic and in vivo imaging approaches (Field et al., 2003;

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Kimmel and Meyer, 2010; Kinkel and Prince, 2009; Tiso et al., 2009). Importantly, pancreas in mouse and fish has a conserved physiological function, a very similar cellular architecture, and conserved expression and function of most developmental genes (Eames et al., 2010; Jurczyk et al., 2011; Yee et al., 2005). Despite these similarities, studies in fish also revealed some evolutionary differences between mammalian and fish pancreas development.

Pancreas development in zebrafish is morphologically visible at 24 h post fertilization (24 hpf) by the formation of an endocrine cluster of 50–60 cells that are located dorsal to the gut endoderm (Biemar et al., 2001; Kimmel and Meyer, 2010). This early islet separates from the endoderm and by 48 hpf becomes encircled by a second endodermal protrusion. Based on their position and by analogy to the buds formed during primary transition in mouse, the early islet and the later forming protrusion in zebrafish have been termed dorsal and ventral bud, respectively (Field et al., 2003). However, the fates of these buds are different in mouse and fish. In mouse, the dorsal and ventral bud both contributes to first and second wave endocrine, as well as to exocrine cells (Pan and Wright, 2011). By contrast, zebrafish dorsal bud consists exclusively of first wave endocrine cells (dorsal bud derived cells, DBCs), while the ventral bud gives rise to second wave endocrine (hereafter termed ventral bud derived cells, VBCs), exocrine and duct cells, and thus to all cells forming the mature pancreas (Field et al., 2003; Hesselson et al., 2009; Wang et al., 2011). Further, first wave cells in mouse mainly express *Gcg*, while in fish, at least 4 early endocrine cell types, expressing *Gcg* (α-cells), *Ins* (β-cells), *Sst* (δ-cells) and *Ghrelin* (ε-cells) can be distinguished (Hesselson et al., 2009; Pan and Wright, 2011; Tiso et al., 2009).

Correlating with these differences, the molecular control underlying formation of the two pancreatic buds appears slightly different in mouse and fish. Differences were found for example for the role of the homeobox transcription factors *Pdx1* and *Mnx1*/*Hb9*, that both show a conserved expression in pre-pancreatic endoderm and later in differentiating β-cells. In mouse knock-out studies the two phases of expression were shown to be required for early pancreas morphogenesis and for β-cell maturation (Harrison et al., 1999; Larsson et al., 1996; Li and Edlund, 2001; Offield et al., 1996; Pan and Wright, 2011). By contrast, morpholino-based (MO) knockdown studies of these genes in fish revealed no major defects in early pancreas morphology but did provide evidence for a conserved function in specifying second wave endocrine cells (*pdx1*) and β-cell maturation (*hb9*) (Kimmel et al., 2011). These data revealed differences mainly in the regulation of early pancreas morphogenesis and in first wave endocrine specification in fish and mouse. Formation of second wave endocrine cells appears to be more similar in fish and mouse, even though not that much is known about these processes in zebrafish.

In this study, we focus on the LIM-Homeodomain (LIM-HD) transcription factor *isl1* (Insulin gene enhancer protein 1) which in mouse has been shown to be required for early pancreas morphogenesis and endocrine differentiation (Ahlgren et al., 1997; Du et al., 2009; Liu et al., 2011, 2012; May, 2010). While initially identified as a direct regulator of insulin expression in RIN 14B endocrine cells (Karlsson et al., 1990), *isl1* in zebrafish shows evolutionary conserved expression in pancreatic mesenchyme during embryogenesis and in endocrine cells of the embryonic and adult pancreas, including first wave endocrine cells (Manfroid et al., 2007). Mesenchymal *isl1* expression is limited to the initial stages of pancreas development. In mouse, this expression is restricted to the cells flanking the dorsal pancreatic endoderm (Ahlgren et al., 1997), while in fish expression is found in the vicinity of the late forming ventral bud. Knock-out analyses in the mouse revealed that the distinct expression domains in mesenchyme and endoderm are independently required to induce formation of the dorsal pancreatic bud and pancreatic mesenchyme and to initiate endocrine hormone expression, respectively. Due to the lethality of the *Isl1* mutant shortly after onset of pancreas morphogenesis, analyses of later pancreatic fates could only be done in organ culture. It was found that wild type but not *Isl1* mutant pancreatic mesenchyme is able to induce differentiation of exocrine tissue in co-cultured mutant dorsal endoderm, demonstrating a non-cell autonomous function of mesenchymal *Isl1* in inducing exocrine pancreas (Ahlgren et al., 1997).

More recently, a conditional knock-out approach has been used to address *Isl1* functions in second wave endocrine cells. Based on a *Pdx1*-Cre driven knock-out strategy, it was possible to efficiently remove endodermal *Isl1* expression shortly after onset of secondary transition (Du et al., 2009; Liu et al., 2011). In these mutants, expression of the endocrine progenitor marker Pax6 was initiated in normal numbers but unlike in the control embryos, only a few of these Pax6 positive cells established expression of the hormones Gcg, Ins, Sst and PP. During later development, the mutants displayed a reduced number of Pax6 expressing endocrine cells and this reduction was accompanied by a decreased rate of β-cell proliferation and an increased rate of apoptosis within the pancreatic islet (Du et al., 2009). Furthermore, a transgenic increase of *Isl1* expression was shown to enhance glucose-induced insulin secretion (Liu et al., 2012). The mouse data reveal requirements for *Isl1* during induction of exocrine tissue and for the expansion, maturation and physiological responses in endocrine cells. Moreover, molecular approaches provided first hints for the molecular targets and interaction partners of *Isl1*. *MafA* and *Arx*, two genes playing fundamental roles in the process of β- and α-cell differentiation, have been found as direct transcriptional targets of mouse *Isl1* (Du et al., 2009; Liu et al., 2011). In vitro studies also identified *Isl1* as a direct regulator of *c-Myc* and *Cyclin D1*, providing a possible link between *Isl1* function and regulation of islet-cell proliferation (Guo et al., 2011). Most recently, the Lim-domain-binding coregulator Ldb1 was shown to be a critical cofactor for Isl1-dependent expression of *MafA*, *Arx*, *ins* and *Glp1r*, which encodes an important regulator of Ins synthesis and release (Hunter et al., 2013).

Though pancreatic expression of *Isl1* is highly conserved, its functions have not been analyzed in any model system besides the mouse. In order to study pancreatic *isl1* functions in a non-mammalian system, we make use of a recently described *isl1* TILLING mutant in zebrafish. In these fish, an A to T transition creates a premature stop codon at amino acid 88 leading to a complete loss of the homeodomain (de Pater et al., 2009). Mutants are viable until late embryogenesis and therefore enable studies on *isl1* function during both early and later stages of pancreas development. In contrast to mouse, we find that *isl1* in zebrafish is not essential for pancreatic mesenchyme formation or for the formation of hormone-expressing first wave endocrine cells. We show that *isl1* has overlapping function with *isl2a* and *isl2b* in pancreatic mesenchyme in promoting expansion of the exocrine pancreas. We also show that *isl1* alone has cell type specific functions in endocrine cell maturation, and that these functions are different in first and second wave endocrine cells. The data reveal *isl1* functions that are unexpected when considering the mouse data, and show a major difference in the molecular regulation of first and second wave cells in zebrafish.

Materials and methods

Zebrafish maintenance and fish lines

Zebrafish (*danio rerio*) were maintained according to standard protocols. Developing embryos were staged by hours post fertilization