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The role of islet-1 in the endocrine pancreas

Lessons from pancreas specific islet-1 deficient mice

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Recently, we have reported the LIM-homeodoman (HD) transcriptional regulator, Islet-1 (Isl-1,[1]) as a key regulator for pancreatic islets after the secondary transition and into early postnatal stages in mice. Previously, the role of Isl-1 had only been examined during early pancreas development in vivo[2] and cell lines.[3-5] These early studies concluded that Isl-1 is required for the differentiation of early endocrine cells,[2] and hormone gene expression is regulated by Isl-1 in cell culture.[3-5] However, it was not clear from these studies whether the regulation of hormone gene transcription by Isl-1 was a direct transcriptional event. In addition, the function of Isl-1 during the formation of principle hormone producing endocrine cells had not been investigated since *Isl-1* null animals die prior to the formation of these cells. Using pancreas-specific inactivation of *Isl-1* in mice, we have elucidated the role of Isl-1 during maturation, proliferation and survival of the endocrine pancreas after the secondary transition. We have also identified *MafA*, a potent *Insulin* gene regulator, as the first direct target of Isl-1 in β-cells.

During mouse embryogenesis, the pancreas develops from a pre-patterned domain of the foregut endoderm around embryonic day 9.5 (E9.5). The first visible sign of pancreatic development is the formation of the dorsal and ventral pancreatic buds. At this time, a first wave of endocrine cells expressing glucagon and/or insulin appears as clusters, but these cells do not contribute to the endocrine compartment of the mature pancreas.[6-8] At E13.5, during the secondary transition, coincident with the peak of endocrine and exocrine cell genesis, the generation of the principal population of the endocrine cells that populate the mature pancreas occurs. Over recent years, the analysis of mouse loss-of-function and gain-of-function phenotypes has represented a powerful tool to elucidate pancreas development and has demonstrated the importance of certain transcription factors in the differentiation and function of the endocrine pancreas.[9,10] However, the complete functional repertoire of one of these islet transcription factors, Islet-1 (Isl-1), remained to be explored.

In 1990, Karlsson and colleagues isolated Isl-1 as an *Insulin* enhancer binding protein by screening a rat islet cell (RIN cell) library.[1] They found expression of Isl-1 restricted to all mature pancreatic islet cells (**Fig. 1A**).[11,12] However, during development Isl-1 expression is not only limited to hormone-producing cells (**Fig. 1B–D**) but is also found in the pancreatic mesenchyme. Interestingly, unlike the first wave Isl-1$^+$ endocrine cells that are postmitotic,[2] we found subsets of Isl-1$^+$ cells after the secondary transition possessing proliferating capacity (**Fig. 1E and F**). The function of Isl-1 in the developing pancreas was first examined using *Isl-1* null animals by Ahlgren and coworkers. They observed dorsal pancreatic bud agenesis and complete loss of first wave hormone-expressing cells in *Isl-1* null embryos.[2] In addition, Isl-1 has been shown to regulate the expression of hormone genes including proglucagon, somatostatin and

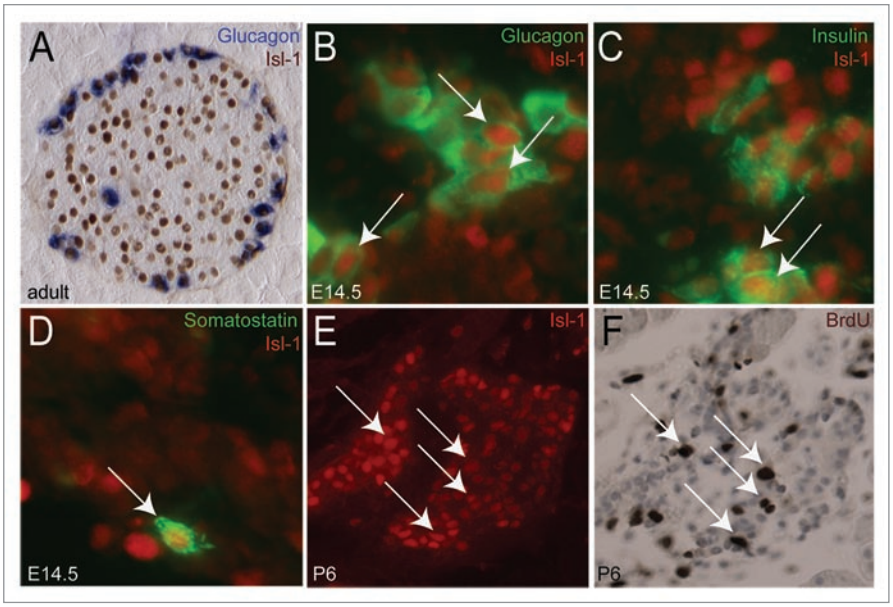

Figure 1. Immunostaining of Isl-1 in the pancreas of E14.5, Postnatal day 6 and adult animals. (A) Isl-1 expression (brown) and glucagon expression (blue) in adult islet. (B–D) Immunostaining of Isl-1 (red) with glucagon (green; arrowheads in B), insulin (green; arrowheads in C) and somatostatin (green; arrowhead in D) in E14.5 pancreas. (E and F) Immunostaining of Isl-1 (red; E) and BrdU (brown; F) on P6 pancreatic section. Arrowheads denote Isl-1^{+}/BrdU^{+} cells.

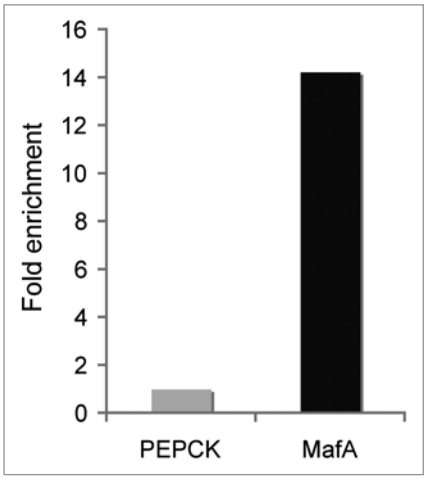

Figure 2. Real-time PCR analysis of ChIP DNA from mouse islets shows a 14-fold enrichment for *MafA* as compared with the inactive *phosphoenolpyruvate carboxykinase PEPCK* promoter set as onefold.

insulin in vitro.[4,5,13] However, due to the lack of better tools at that time to examine direct regulation, these conclusions were based on the use of electrophretic mobility shift assays (EMSA) and reporter assays in cell lines.

To investigate the role of Isl-1 after the secondary transition, our group derived pancreas-specific *Isl-1* deficient (*Pdx1-Cre;Isl-1*$^{L/L}$) mice.[14] *Pdx1-Cre;Isl-1*$^{L/L}$ mice showed progressive loss of islet mass perinatally, characterized by reduced cell proliferation and survival. During development, Isl-1 broadly affects endocrine hormone production without affecting endocrine cell specification. This is supported by the lack of hormone expression in most of the Pax6^{+} endocrine cells in the *Pdx1-Cre;Isl-1*$^{L/L}$ mice.[14] Our observation of downregulation of MafA expression, a potent *Insulin* gene regulator, further confirmed the involvement of Isl-1 during final stages of beta cell differentiation. Although hormone gene production was impacted in the *Isl-1* deficient pancreas, we could not detect direct binding of *Insulin* gene by Isl-1 in our chromatin immunoprecipiation assays (ChIP).[14] However, through a series of biochemical studies including EMSA, luciferase reporter experiments and ChIP, we identified *MafA* as a direct transcriptional target of Islet-1 in βTC3 cells and mouse islets (**Fig. 2**).[14] Our study suggests that the downregulation of *Insulin* expression in the remaining β-cells of the *Pdx1-Cre;Isl-1*$^{L/L}$ mice was partly contributed by the loss of MafA expression.[14] Our work clearly demonstrates the importance of Isl-1 in maturation, proliferation and survival of the endocrine pancreas during early phases of life. The next question is whether Isl-1 is also required for maintaining proper beta mass and function in adult animals. To address this, beta-cell specific *and* inducible ablation of Isl-1 mice will be necessary since *Pdx1-Cre;Isl-1*$^{L/L}$ mice die from severe hyperglycemia before reaching adulthood.

In summary, our work furthered our understanding of the function of Isl-1 beyond early pancreatic development and uncovered the importance of Isl-1 in both proliferating and postmitotic cells after the secondary transition. Interestingly, a human genetic study of a morbidly obese population suggests the association of *Isl-1* with type 2 diabetes.[15] Furthermore, a nonsense mutation of *Isl-1* gene has been found in a type 2 diabetes Japanese family.[16] These reports along with our work conducted in mice strongly suggest a strong link of Isl-1 to diabetes; however, the precise role of Isl-1 during normal physiology as well as stressed conditions and its direct connection to diabetes awaits further investigation.

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