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Nkx6 Transcription Factors and Ptf1a Function as Antagonistic Lineage Determinants in Multipotent Pancreatic Progenitors

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SUMMARY

The molecular mechanisms that underlie cell lineage diversification of multipotent progenitors in the pancreas are virtually unknown. Here we show that the early fate choice of pancreatic progenitors between the endocrine and acinar cell lineage is restricted by cross-repressive interactions between the transcription factors Nkx6.1/Nkx6.2 (Nkx6) and Ptf1a. Using genetic loss- and gain-of-function approaches, we demonstrate that Nkx6 factors and Ptf1a are required and sufficient to repress the alternative lineage program and to specify progenitors toward an endocrine or acinar fate, respectively. The Nkx6/Ptf1a switch only operates during a critical competence window when progenitors are still multipotent and can be uncoupled from cell differentiation. Thus, cross-antagonism between Nkx6 and Ptf1a in multipotent progenitors governs the equilibrium between endocrine and acinar cell neogenesis required for normal pancreas development.

INTRODUCTION

At the earliest stages of pancreas development, most, if not all, pancreatic epithelial cells are thought to be multipotent progenitors which are competent to develop into all pancreatic cell types, namely five different endocrine lineages as well as the exocrine acinar and ductal cells (Gu et al., 2002). During subsequent pancreas morphogenesis, cells residing in the "tips" of the branching epithelium adopt an acinar fate, whereas cells in the core or "trunk" become restricted to a ductal or endocrine fate (Solar et al., 2009; Zhou et al., 2007). Although significant advances have been made in defining the molecular determinants of endocrine cell subtype specification and differentiation (Oliver-Krasinski and Stoffers, 2008), little is known regarding the molecular mechanisms governing the early allocation of multipotent progenitors to either a ductal/endocrine or acinar fate.

In many tissues, the separation of lineage-restricted progenitors from a multipotent progenitor cell pool is mediated by cross-repressive interactions between two transcription factors (Briscoe et al., 2000; Laslo et al., 2006; Olguin et al., 2007). The transcriptional repressors Nkx6.1 and Nkx6.2 (Nkx6) constitute one arm of such a cross-repressive loop during neuronal subtype specification in the spinal cord (Sander et al., 2000a; Vallstedt et al., 2001). In the pancreas, Nkx6.1 and Nkx6.2 are redundantly required for endocrine α and β cell development (Henseleit et al., 2005), but the mechanism by which Nkx6 factors control endocrine development has remained poorly defined. The finding that coexpression of the two Nkx6 factors is only observed in multipotent progenitors, but not in endocrine lineage-committed progenitors marked by expression of the transcription factor Neurogenin 3 (Ngn3) (Henseleit et al., 2005), suggests that Nkx6 proteins play a key role in endocrine cell development prior to the initiation of Ngn3 expression. This raises the possibility that proper endocrine cell development from a multipotent progenitor cell domain first requires the specification of endocrine fate by Nkx6 factors, whereas Ngn3 subsequently promotes cell differentiation by initiating cell cycle exit and by inducing the expression of endocrine lineage-specific genes (Apelqvist et al., 1999; Schwitzgebel et al., 2000). The contention that Nkx6 proteins could be involved in early lineage specification is consistent with the expression domain of Nkx6.1, which becomes progressively compartmentalized to the trunk domain, as the activator of acinar-specific gene transcription, Ptf1a (Krapp et al., 1996; Rose et al., 2001), becomes exclusively restricted to cells residing in the tips (Hald et al., 2008). Interestingly, the resolution of Nkx6.1 and Ptf1a into exclusive compartments appears to coincide with the progressive restriction of trunk and tip cells to the ductal/endocrine or acinar cell lineage, respectively (Solar et al., 2009; Zhou et al., 2007).

In this study, we provide evidence that reciprocal repression between Nkx6 factors and Ptf1a enables a bistable switch in multipotent progenitors that directs progenitors to adopt either a ductal/endocrine or acinar cell fate.

RESULTS

Loss of Nkx6 Activity Results in an Endocrine-to-Acinar Fate Switch

To confirm that Nkx6.1 and Ptf1a resolve into exclusive compartments (Hald et al., 2008) and to more carefully examine the extent of coexpression between both Nkx6 factors and Ptf1a, we performed coimmunofluorescence analysis for Nkx6.1 and Nkx6.2 together with Ptf1a. At embryonic day (e) 10.5, a large percentage of pancreatic epithelial cells coexpressed Nkx6.1

and Ptf1a, but cells that exclusively expressed one of the two transcription factors, although scarce, were also present (Figure 1A). At the onset of branching morphogenesis at e12.5, Nkx6.1 became progressively confined to the trunk of the developing organ, while Ptf1a marked the tips of the branches (Figure 1B). At this stage, tip cells were additionally also largely devoid of Nkx6.2 (Figure 1D). By e14.5, when tip cells have fully committed to an acinar fate (Zhou et al., 2007), Nkx6.1 was almost completely excluded from the tips and formed a sharp boundary with Ptf1a (Figure 1C). As previously noted (Burlison et al., 2008), from even the earliest stages of development, Ngn3$^+$ endocrine progenitors failed to express Ptf1a (Figure 1G).

To assess whether loss of *Nkx6* gene function affects exclusion of Ptf1a from the core of the developing pancreas, we analyzed organ patterning in *Nkx6.1;Nkx6.2* (*Nkx6*) compound mutant mice, which exhibit normal organ size (see Figures S1M and S1N available online). In Nkx6.1- and Nkx6-deficient embryos, we detected significant ectopic expression of Ptf1a in the trunk region (Figures 1K–1M and 1O), which was accompanied by a 90.7% reduction in the number of Ngn3$^+$ cells at e12.5 (Figures 1K–1N). At e10.5, when the majority of epithelial cells express both Nkx6 factors (Henseleit et al., 2005), *Nkx6.1* single mutants exhibited a smaller, 38.2% reduction in Ngn3$^+$ cells, whereas *Nkx6* compound mutants displayed a much more extensive, 87.5% decrease compared to wild type embryos (Figures 1G–1J). This suggests that compensation by Nkx6.2 partially restores formation of Ngn3$^+$ cells in *Nkx6.1* single mutants at e10.5.

Because Ptf1a is a critical regulator of acinar cell differentiation (Esni et al., 2004), we next examined whether expansion of the Ptf1a$^+$ domain in *Nkx6* mutants manifests in increased numbers of acinar cells. Transcriptional profiling and qRT-PCR analysis of pancreatic anlagen indeed confirmed an upregulation of acinar genes in Nkx6.1-deficient pancreata at e15.5 (Figures 1E and 1F), which was associated with increased numbers of amylase$^+$ acinar cells in *Nkx6* mutants (Figures 1P–1R and 1T). However, acinar cell markers did not appear prematurely (Figures S1A–S1C), indicating that the absence of Nkx6 activity does not affect the timing of acinar cell differentiation.

Significantly, in contrast to earlier developmental stages, Nkx6-deficient embryos no longer displayed a reduction in Ngn3$^+$ progenitors at e14.5 (Figures 1P–1S). We speculate that these Ngn3$^+$ cells arise from a residual Ptf1a$^-$/Sox9$^+$/Pdx1$^+$ trunk-like progenitor cell domain that was still discernable in *Nkx6* mutants (Figures S1D–S1L) and might seed a partial recovery of endocrine cell differentiation later during development. The absence of the early but presence of the later wave of Ngn3 cell genesis in *Nkx6* mutants might also explain why formation of α and β cells, but not of the later-arising δ and pancreatic polypeptide cells, is selectively affected in *Nkx6* mutants (Henseleit et al., 2005; Johansson et al., 2007).

Nkx6 Misexpression Prevents Acinar Cell Differentiation

Next, we investigated whether continuous expression of Nkx6 factors throughout the entire pancreatic progenitor cell field is sufficient to repress their differentiation into acinar cells. We utilized the *Pdx1* promoter to misexpress *Nkx6.1* or *Nkx6.2* (Figures 2A and 2H). As predicted, *Pdx1-Nkx6.1* or *Pdx1-Nkx6.2* embryos expressed Nkx6.1 or Nkx6.2, respectively, throughout the entire pancreatic epithelium, including the presumptive tip domain (Figures 2C, 2E, 2G, 2J, 2L, and 2N). Organ size as well as Ngn3$^+$ and endocrine cell numbers were unaffected in *Pdx1-Nkx6.1* and *Pdx1-Nkx6.2* embryos (Figure S2; data not shown), demonstrating that Nkx6 misexpression does not induce premature endocrine cell differentiation. However, sustained expression of Nkx6.1 or Nkx6.2 significantly repressed Ptf1a expression (Figures 2B–2E and 2I–2L) and consequently blocked acinar cell differentiation (Figures 2F, 2G, 2M, and 2N). Thus, the misexpression of either Nkx6.1 or Nkx6.2 in multipotent progenitors is sufficient to prevent the initiation of an acinar program.

Nkx6.1 Specifies Endocrine Fate in Multipotent Pancreatic Progenitors

To determine whether expression of Nkx6.1 predisposes multipotent pancreatic progenitors to adopt an endocrine fate, we generated *CAG-Bgeo,-Nkx6.1,-eGFP* transgenic mice (hereafter abbreviated to *Nkx6.1^{OE}*), in which concomitant expression of Nkx6.1 and enhanced green fluorescent protein (eGFP) can be stably and heritably induced by expression of Cre recombinase (Figure 3B). The transgene design is analogous to that used in *CAG-Bgeo,-eGFP* (*Z/EG*) mice, in which *lacZ* is ubiquitously expressed in all cells unless Cre recombinase-mediated excision removes the *lacZ* gene and allows for expression of *eGFP* (Figure 3A). *Z/EG* mice therefore serve as a control for the *Nkx6.1^{OE}* model. To validate the *Nkx6.1^{OE}* transgenic model, we intercrossed *Nkx6.1^{OE}* with *Pdx1-CreER^{TM}* mice and induced Cre-mediated recombination by administration of tamoxifen (TM) to pregnant dams. As expected, the majority of unrecombined cells expressed β-galactosidase (β-gal) but not GFP, whereas recombined cells coexpressed Nkx6.1 and GFP but were negative for β-gal (Figure S3A). No GFP expression was observed in *Nkx6.1^{OE}* mice without the *Pdx1-CreER^{TM}* transgene (Figure S3B), thus demonstrating no leakiness in our system. These results were confirmed for mouse lines established from two independent founders.

To analyze whether stable, heritable expression of Nkx6.1 biases the cell fate choice of multipotent progenitors, we injected mice with TM at e8.5, which results in nuclear translocation of CreER^{TM} and mosaic induction of Nkx6.1 and GFP in Pdx1$^+$ progenitors for a period of ~12–36 hr after TM administration (Zhou et al., 2007). To assess subsequent progenitor cell fate, we quantified the percentages of recombined cells that had initiated expression of specific lineage markers at different time points following induction of Nkx6.1 expression. At e10.5, when Nkx6.1 and Ptf1a are still coexpressed in most progenitors (Figure 1A), Nkx6.1-misexpressing cells showed the same propensity to activate the pre-endocrine markers Ngn3 and Pax6, hormones, or Ptf1a as normal progenitors (Figures 3C–3K). This suggests that progenitors are either not competent to repress Ptf1a in response to Nkx6.1 prior to e10.5 or that the time window between TM-mediated Nkx6.1 induction and analysis is too short to observe a response. By e12.5, however, we observed preferential activation of pre-endocrine markers in cells that heritably expressed Nkx6.1 (Figures 3L–3N, 3R–3T, and 3X). Crucially, we found that this increase in pre-endocrine cells was not the result of increased cell proliferation