RESEARCH COMMUNICATION

DNMT1 represses *p53* to maintain progenitor cell survival during pancreatic organogenesis

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In the developing pancreas, self-renewal of progenitors and patterning of cell fates are coordinated to ensure the correct size and cellular makeup of the organ. How this coordination is achieved, however, is not clear. We report that deletion of DNA methyltransferase 1 (*Dnmt1*) in pancreatic progenitors results in agenesis of the pancreas due to apoptosis of progenitor cells. We show that DNMT1 is bound to the *p53* regulatory region and that loss of *Dnmt1* results in derepression of the *p53* locus. Haploinsufficiency of *p53* rescues progenitor cell survival and cellular makeup of the *Dnmt1*-deleted pancreas.

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The vertebrate pancreas is a complex organ made of functionally different tissue types that arise from a common progenitor cell pool during embryogenesis (Gu et al. 2003; Jensen 2004; Gittes 2009). Lineage tracing analysis indicates that a pool of pancreatic progenitor cells persists throughout organogenesis to give rise to differentiated mature cell types in a spatially and temporally regulated manner (Herrera 2000; Jensen et al. 2000a; Gu et al. 2002). The maintenance of the progenitor cell pool requires that progenitor cell self-renewal is balanced with cell cycle exit to coordinate differentiation of mature cell types of the pancreas (Georgia et al. 2006).

DNA methylation is a potential epigenetic mechanism that may be involved in maintaining the balance between self-renewal and terminal differentiation in the developing pancreas. DNA methylation may serve as a means of repressing pluripotency genes in multipotent progenitor cells or a means of repressing lineage-specific differentiation genes in multipotent progenitor cells (Reik 2007). It is not clear whether pancreatic progenitor DNA methylation patterns must be inherited during self-renewal to maintain a functional progenitor population by repres-

sing differentiation genes or to serve as a basis for establishing lineage-specific methylation patterns during differentiation.

DNA methyltransferase 1 (*Dnmt1*) is the enzyme that propagates DNA methylation patterns during cell division. Previous studies on *Dnmt1* have yielded interesting yet divergent data about its role in maintenance and differentiation of progenitor cell types. *Dnmt1*-null mouse embryonic stem cells (mESCs) undergo limited differentiation and apoptosis and are eliminated in competition assays with wild-type mESCs yet have a proliferative capacity comparable with that of wild-type mESCs when maintained in an undifferentiated state (Lei et al. 1996; Panning and Jaenisch 1996). *Dnmt1*-null embryos expire from growth arrest and severe neural tube distortions by embryonic day 8.5 (E8.5), which indicates that *Dnmt1*-null mESCs are capable of undergoing more extensive differentiation in vivo than in vitro (Li et al. 1992; Lei et al. 1996). Loss of *Dnmt1* in hematopoietic progenitor cells results in defects in self-renewal and niche retention and an inappropriate shift to myeloid progenitor cell cycling and differentiation (Trowbridge et al. 2009). Studies in human adult epidermal stem cells indicate that *Dnmt1* maintains progenitor self-renewal by repressing genes that trigger cell cycle exit and premature differentiation (Sen et al. 2010). During neurogenesis, *Dnmt1* restricts precocious astroglialogenesis of neural precursor cells, but deletion of *Dnmt1* in telencephalic precursors results in cell death and forebrain degeneration (Fan et al. 2005; Hutnick et al. 2009). Taken together, these studies present context-dependent roles for *Dnmt1* that may have differential effects in vitro or in vivo.

To investigate the in vivo role of DNMT1 in progenitor maintenance and concomitant lineage specification during organogenesis, we conditionally deleted *Dnmt1* in pancreatic progenitor cells. Analysis of progenitor cell self-renewal dynamics revealed that *Dnmt1*-null pancreatic progenitor cells undergo G2 arrest. Our studies indicate that DNMT1 functions to repress *p53*. In its absence, *p53* expression is derepressed, resulting in apoptosis of pancreatic progenitor cells. We show that *p53* haploinsufficiency rescues progenitor cell survival and restores pancreatic organogenesis in the *Dnmt1*-null embryos. These data suggest that *Dnmt1* is required to prevent *p53*-dependent apoptosis during lineage specification of pancreatic progenitor cells.

Results and Discussion

Loss of Dnmt1 results in a severely atrophic pancreas

We bred mice with a floxed allele of *Dnmt1* (DNMT1^{fl/fl}) with mice transgenic for Cre recombinase under the control of the *Pdx1* promoter (Jackson-Grusby et al. 2001; Gu et al. 2002). This restricted *Dnmt1* excision to the pancreatic epithelium (DNMT1^{PC}). To facilitate lineage tracing studies, we also bred in the stop-floxed-R26R^{YFP} to mark all cells derived from progenitor cells that expressed the *Pdx1*-Cre transgene (Srinivas et al. 2001). To confirm that deletion of *Dnmt1* resulted in hypomethylation of the self-renewing pancreatic progenitor pool, we used immunohistochemistry to detect 5-methyl-cytosine (5mC). Unlike the control epithelium,

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where 5mC uniformly stained the epithelium and surrounding mesenchyme, 5mC staining was grossly diminished in the epithelium of the DNMT1^{PC} pancreas but maintained in the surrounding mesenchyme (Supplemental Fig. 1A,B). This indicated that deletion of *Dnmt1* resulted in hypomethylation of the pancreatic epithelium during organogenesis. Previous reports have shown that loss of *Dnmt1* leads to derepression of intracisternal A particle (IAP), a core retroviral element protein (Fan et al. 2001). Consistent with these previous studies, IAP staining was not detected in the pancreatic epithelium of control E13.5 embryos, but high levels of IAP were detected in the DNMT1^{PC} pancreatic epithelium (Supplemental Fig. 1C,D). Some glucagon-positive cells in the DNMT1^{PC} pancreatic epithelium were negative for IAP expression and were likely derived from cells that had escaped Cre-mediated recombination. These data indicated that *Pdx1*-Cre expression efficiently deleted *Dnmt1* from the large majority of pancreatic progenitor cells early in development and led to hypomethylation of the pancreatic epithelial cells.

We next examined the effects of *Dnmt1* deletion on pancreatic organogenesis by analyzing DNMT1^{PC} litters at birth. DNMT1^{PC} animals were born alive at expected Mendelian ratios. Examination of littermates indicated that the pancreas of pups in which one allele of *Dnmt1* was deleted were grossly normal and comparable with wild-type control littermates (Fig. 1A,B,D,E). YFP expression was absent in the control animals and homogeneously distributed throughout the pancreas in heterozygous pups (Fig. 1G,H). Strikingly, gross examination of the DNMT1^{PC} pancreas revealed a severely atrophic pancreas (Fig. 1C,F). The rudimentary DNMT1^{PC} pancreas displayed little YFP expression, indicating that most of the atrophic pancreatic tissue was derived from cells that had escaped recombination (Fig. 1I). These data

indicated that *Dnmt1* is essential for the formation of the pancreas.

Previous studies in zebrafish indicated that loss of *Dnmt1* resulted in degeneration of the acinar pancreas, but ductal and endocrine lineages were spared, leaving open the possibility that the atrophic pancreas that we observed could consist of primarily endocrine and ductal cells (Anderson et al. 2009). To determine whether the absence of *Dnmt1* resulted in loss of specific cell lineages, we carried out immunohistological analysis for differentiated cell types in the DNMT1^{PC} pancreas. Antibody staining against exocrine cells expressing amylase and endocrine cells expressing insulin showed that scattered clusters of both endocrine and exocrine cells were present in the DNMT1^{PC} pancreas, but the typical rosette architecture of the acinar tissue and islet clusters of insulin cells was disrupted (Supplemental Fig. S2A–D). Cells that stained for the ductal marker mucin were scattered throughout the DNMT1^{PC} pancreas (data not shown). From this analysis, we concluded that deletion of *Dnmt1* from pancreatic epithelial progenitor cells did not impact any particular lineage and instead disrupted the architecture and severely reduced the numbers of differentiated cells of all lineage of the mature pancreas.

Deletion of Dnmt1 in pancreatic progenitors results in the absence of differentiated cells

We investigated whether a severely reduced pancreas size could result from depletion of the pancreatic progenitor pool during embryogenesis. We examined DNMT1^{PC} and control littermate pancreas by immunostaining for pancreatic progenitor markers *Pdx1* and *Sox9*. At E13.5, the overlapping *Pdx1* and *Sox9* populations were comparable in the control and DNMT1^{PC} pancreatic epithelium ($P = 0.2568$) (Fig. 2A,B,E). In contrast, the number of cells staining for *Sox9* at E15.5 was reduced by >40% in the DNMT1^{PC} pancreas compared with the control littermate pancreas ($P = 0.0137$) (Fig. 2C,D,E). This indicated that the progenitor pool is depleted during pancreatic organogenesis and results in a severely atrophic pancreas. The depletion of pancreatic progenitors could be due to either a defect in self-renewal or precocious differentiation. Precocious differentiation leading to depletion of the pancreatic progenitor pool has been previously described (Jensen et al. 2000b; Bhushan et al. 2001). To assess precocious differentiation, we carried out immunohistochemistry of E12.5 pancreas with antibodies against *Sox9* to mark progenitor cells and glucagon to mark differentiated cells. The size of the pancreatic epithelial bud and the number of glucagon cells from E12.5 embryos immunostained with *Sox9* and glucagon were comparable in DNMT1^{PC} and control littermates (Fig. 2F–G). To further assess differentiation, we compared global gene expression by microarray analysis in control and DNMT1^{PC} pancreas. There was less than a twofold difference between most transcripts in the control and DNMT1^{PC} pancreas, which suggests that the majority of the cells in the DNMT1^{PC} pancreas had a transcriptome similar to that of the control pancreas (Supplemental Fig. 3A). Gene enrichment analysis indicated that a subset of genes involved in pancreatic differentiation was specifically down-regulated. *Neurogenin 3*, the gene required for differentiation of all endocrine cell types, was one of the most down-regulated gene in the array analysis, with a >12-fold decrease in expression in the DNMT1^{PC}

Figure 1. *DNMT1* deletion results in an atrophic pancreas. (A–C) Gastrointestinal tract of control (DNMT1^{fl/fl}) (A), heterozygous (DNMT1^{PC/+}) (B), and conditional knockout (DNMT1^{PC}) (C) pancreas at postnatal day 0 (P0). (D–F) Pancreas dissected from the gastrointestinal tract illustrate the atrophic size of the DNMT1^{PC} pancreas. (G–I) Lineage trace analysis indicates that there is efficient recombination in the heterozygous DNMT1^{PC/+} pancreas (H), but very few recombined cells persist in the DNMT1^{PC} pancreas (I).

Figure 2. DNMT1^{PC} pancreatic atrophy is the result of progenitor cell depletion. (A,B) Immunohistochemistry for Sox9 and Pdx1 at E13.5 indicates no notable difference in the size or morphology in control (A) and DNMT1^{PC} (B) pancreatic epithelium. (C,D) Immunohistochemistry of pancreatic epithelium for Sox9 and Pdx1 at E15.5 reveals a decrease in the number progenitor cells in the DNMT1^{PC} pancreatic epithelium. (E) Quantification of progenitor cells (E13.5) and Sox9 cells (E15.5). (F,G) Immunohistochemical comparison of the progenitor and glucagon populations at E12.5 in the control and DNMT1^{PC} suggests that precocious differentiation is not responsible for progenitor depletion. (H,I). By E15.5, DNMT1^{PC} pancreas have little expression of pre-endocrine cell marker Ngn3 (I) in comparison with control littermates (H). (J) Quantification of Ngn3⁺ cells at E15.5.

pancreas (Schwitzgebel et al. 2000). Other transcription factors involved in endocrine and exocrine lineage specification—such as *Nkx6.1*, *Nkx2.2*, *Rfx6*, *Rbjk-1*, and *Ptf1*—were specifically down-regulated (Supplemental Fig. 3B). We sought to confirm the gene expression profiles by immunohistochemistry. Immunostaining of the control pancreas at E15.5 showed Ngn3⁺ cells along the embryonic duct surrounded by pancreatic progenitor cells expressing Pdx1. In striking contrast, the number of Ngn3⁺ cells in the DNMT1^{PC} pancreas was <15% that of the control littermates ($P < 0.005$) (Fig. 2H–J). These

results indicated that in the absence of *Dnmt1*, pancreatic epithelial cells were not depleted due to precocious differentiation, and in fact, very few differentiated cells were observed in the DNMT1^{PC} pancreas.

Loss of *Dnmt1* resulted in accumulation of progenitor cells in the G2 phase

To investigate whether loss of *Dnmt1* resulted in depletion of pancreatic progenitor cells due to defects in self-renewal, we analyzed embryos from pregnant dams injected with a short pulse of BrdU. Control and DNMT1^{PC} pancreas were immunostained with antibodies against Pdx1 to mark pancreatic progenitor cells and BrdU to identify cells actively in S phase of the cell cycle (Fig. 3A,B). The Pdx1⁺BrdU⁺ cells were counted to determine the proliferation index, calculated as (Pdx1⁺BrdU⁺)/Pdx1⁺. There were no differences in the proliferation index between control and DNMT1^{PC} progenitor cells at E12.5 (Fig. 3C). Because a short BrdU pulse only measures the cells that are actively in S phase, we pursued additional proliferation studies to determine whether the progenitor cells were completing the cell cycle. Control and DNMT1^{PC} pancreas were immunostained with antibodies against phosphorylated histone H3, a marker for G2/M phase, and Pdx1 (Fig. 3D,E). Cells with a speckled PHH3 pattern were categorized as G2 phase of the cell cycle (Fig. 3D,E, white arrows), and cells with more robust staining were categorized as M phase (Fig. 3D,E, orange arrows). Ratios of Pdx1⁺PHH3⁺/Pdx1⁺ were used to calculate the proliferation index. Comparing control and DNMT1^{PC} pancreas revealed that the number of M-phase progenitor cells was not significantly different, but the number of progenitor cells in G2 phase in the DNMT1^{PC} pancreas was increased by 40% ($P < 0.005$) (Fig. 3F). The fact that S-phase and M-phase progenitor cells did not change in the DNMT1^{PC} pancreas indicated that cell cycle entry and mitosis of pancreatic epithelial cells were not perturbed by the deletion of *Dnmt1*. The accumulation of a subset of pancreatic

Figure 3. A subset of DNMT1^{PC} progenitor cells accumulates in G2 arrest. (A–C) E12.5 pancreatic sections immunostained for BrdU and Pdx1 to quantify progenitor proliferation. (D–F) Quantification of pancreatic progenitor cell proliferation at E13.5 as a percentage of phosphorylated histone H3⁺ Pdx1⁺ double cells indicates that there is an increase in the number of cells in G2 in the DNMT1^{PC} pancreas. (White arrows) G2-phase cells; (orange arrows) M-phase cells. (***) $P < 0.005$.

epithelial cells in G2 arrest suggested that depletion of these cells could be due to apoptosis.

Deletion of Dnmt1 results in p53-dependent apoptosis of pancreatic progenitors

We next investigated whether apoptosis of pancreatic epithelial cells could result in the atrophic phenotype of the DNMT1^{PC} pancreas at birth. Pancreatic sections from E15.5 DNMT1^{PC} and control littermate embryos were stained for cleaved caspase 3, a marker for apoptosis, and Pdx1. Cleaved caspase 3 was not detected in control pancreas; however, DNMT1^{PC} pancreas showed a number of cells within the pancreatic epithelium that were positive for cleaved caspase 3 (Fig. 4A,B).

Several studies in fibroblast and cancer cell lines indicated that loss of *Dnmt1* resulted in p53-dependent apoptosis, although the mechanism remains unclear (Jackson-Grusby et al. 2001; Chen et al. 2007; Anderson et al. 2009). To determine whether deletion of *Dnmt1* in pancreatic progenitors resulted in changes in p53, we first assessed whether levels of p53 were altered in DNMT1^{PC} pancreas. Immunohistochemical analysis using a p53 antibody revealed that p53 accumulated in the DNMT1^{PC} epithelium, while no p53 staining was

observed in control littermate pancreas (Fig. 4C,D, white arrows). To confirm this accumulation of p53 in DNMT1^{PC} pancreas, we measured p53 using Western blot analysis at E12.5, prior to detectable differences in the pancreatic epithelium, and at E15.5, after the onset of p53 expression. Western blot analysis indicated that there was no difference in the accumulation of p53 at E12.5, but there was a significant sixfold increase in p53 in the DNMT1^{PC} pancreas compared with control pancreas from littermates at E15.5 ($P = 0.0098$) (Fig. 4E,F). Western blot analysis indicated that the p53 downstream transcriptional target p21 accumulated in the DNMT1^{PC} pancreas (Fig. 4E). To assess whether the increase in p53 protein correlated with an increase in mRNA levels of *p53*, we used quantitative PCR to quantify any changes in *p53* gene expression. Our analysis revealed a 20-fold increase in *p53* mRNA in DNMT1^{PC} pancreas compared with control pancreas from littermates (data not shown). The increase in *p53* mRNA indicated that *Dnmt1* could potentially regulate p53 at a transcriptional level.

We next assessed how loss of *Dnmt1* resulted in transcriptional up-regulation of *p53*. We hypothesized that DNMT1 could play a role in directly repressing *p53* transcription and that loss of *Dnmt1* would result in derepression of *p53*. Supporting our hypothesis, a previous study showed that DNMT1 was bound to the *p53* promoter 2 kb upstream of the *p53* transcription start site. Interestingly, this upstream regulatory region also contained a binding sequence for the transcriptional repressor CTCF. Binding of CTCF to this region suppressed reporter activity (Su et al. 2009). We reasoned that DNMT1 and CTCF associated at the *p53* regulatory region, and binding of CTCF to the *p53* regulatory region may be disrupted in the absence of *Dnmt1*. To test this, E14.5 pancreas from DNMT1^{PC} and control littermates were subjected to chromatin immunoprecipitation (ChIP) analysis (Fig. 4G). In control pancreas, both DNMT1 and CTCF were bound to the *p53* regulatory region, confirming that DNMT1 and CTCF binding was associated with *p53* repression ($P < 0.005$) (Fig. 4H). In contrast, there was no enrichment of CTCF in DNMT1^{PC} embryos ($P = 0.0268$) (Fig. 4H). Moreover, enrichment of H3K27ac within the *p53* promoter region was observed in the DNMT1^{PC} embryos, which is consistent with transcriptional activation ($P = 0.0423$) (Fig. 4H). These results suggested that DNMT1 directly repressed *p53* transcription, and loss of *Dnmt1* resulted in derepression of *p53*.

Haploinsufficiency of p53 rescues pancreatic organogenesis

To investigate whether inactivation of *Dnmt1* resulted in p53-mediated apoptosis of pancreatic progenitors, we crossed the *p53*-null animals into the DNMT1^{PC} background. *p53*-null animals are phenotypically indistinguishable from wild-type littermates at birth, and pancreas formation was not perturbed (Jacks et al. 1994).

We first assessed whether haploinsufficiency of *p53* restored pancreatic progenitor popula-

Figure 4. Progenitor depletion is a result of apoptosis in the DNMT1^{PC} pancreatic epithelium. (A,B) Immunohistochemistry for cleaved caspase 3 did not reveal any apoptosis in the control pancreas (A), but apoptosis was widespread in the DNMT1^{PC} pancreas (B). (C,D) The presence of apoptosis markers coincides with the appearance of p53 accumulation in the DNMT1^{PC} pancreatic epithelium (D, white arrows) and is absent in control littermates at E15.5 (C). (E) Western blot analysis for p53 protein and its downstream target, p21, at E12.5 and E15. (F) Densitometric quantification of Western blots indicates a sixfold increase in p53 protein at E15.5. (G) Schematic of the ChIP assay binding in the *p53* locus. (H) ChIP analysis indicates that DNMT1 binds to the *p53* locus in control pancreas but is lost in DNMT1^{PC} pancreas. Chromatin insulator protein CTCF is also bound to the *p53* locus in control pancreas but is lost in the DNMT1^{PC} pancreas. The activating histone mark H3K27ac is enriched in the DNMT1^{PC} pancreas.

Figure 5. Haploinsufficiency of *p53* rescues apoptosis and allows for cell survival during differentiation in *DNMT1^{PC}p53^{+/-}* pancreas. (A,B) Immunohistochemical staining for Sox9 and Pdx1 indicates no significant difference in the control (A) and *DNMT1^{PC}p53^{+/-}* (B) cell populations at E15.5. (C,D) Immunohistochemical analysis of control (C) and *DNMT1^{PC}p53^{+/-}yfp⁺* (D) pancreas revealed a restoration of Ngn3⁺ expression in the absence of *Dnmt1*. Overlap with YFP indicates that these cells have arisen from cells that underwent recombination. (E,F) Gross analysis of control and *DNMT1^{PC}p53^{+/-}yfp⁺* indicates that mutant pancreas are comparable with control littermates at P0. (G,H) Lineage tracing by YFP expression indicates that most cells in the *DNMT1^{PC}p53^{+/-}yfp⁺* pancreas have undergone recombination.

tions at E15.5. Immunohistochemical assessment of Sox9 and Pdx1 revealed no significant differences in the pancreatic epithelium between the control and *DNMT1^{PC}p53^{+/-}* pancreas (Fig. 5A,B). To evaluate whether *p53* haploinsufficiency would restore the expression of *ngn3*, we examined *DNMT1^{PC}p53^{+/-}* pancreas for expression of Ngn3. Immunohistochemistry at E16.5 indicated that Ngn3⁺ and Pdx1⁺ cell populations persisted through organogenesis with a staining pattern similar to that of control littermates. Lineage tracing indicated that *DNMT1^{PC}p53^{+/-}* cells expressing Ngn3 had undergone recombination, thus leading us to conclude that the expression of Ngn3 was restored by *p53* haploinsufficiency (Fig. 5C,D). At birth, *DNMT1^{PC}* and *DNMT1^{PC}p53^{+/-}* animals were present at the expected Mendelian ratios. Gross histological examination of the neonatal pancreas revealed that while the pancreas of *DNMT1^{PC}* animals were severely atrophic, *DNMT1^{PC}p53^{+/-}* littermates had a restoration of a full, albeit smaller, pancreas (Fig. 5E,F). Lineage tracing suggested

that recombination was extensive, although not complete, in the *DNMT1^{PC}p53^{+/-}* background (Fig. 5G,H). Insulin and YFP immunostaining indicated normal morphology of the endocrine tissue compartment in the control and *DNMT1^{PC}p53^{+/-}* littermate pancreas (Supplemental Fig. 4A,B). Immunostaining for amylase and YFP indicated that exocrine cells formed morphologically normal rosette structures (Supplemental Fig. 4C,D). Mucin⁺/YFP⁺ ductal structures were present and morphologically normal in both the control and *DNMT1^{PC}p53^{+/-}* pancreas (Supplemental Fig. 4E,F). Taken together, these data indicated that *p53* haploinsufficiency rescued the ability of *Dnmt1*-null pancreatic progenitor cells to differentiate into a normal mature organ during embryogenesis. The area of *DNMT1^{PC}p53^{+/-}* islets decreased by postnatal day 15, and blood glucose levels were elevated into the diabetic range. This suggests that while haploinsufficiency of *p53* may permit organogenesis in the *DNMT1^{PC}* pancreas, these cells are not able to support glucose homeostasis in adolescent mice (Supplemental Fig. 5A–E).

Our results suggest that *p53* repression mediated by DNMT1 is not required for self-renewal of pancreatic progenitor cells per se but is critical for progenitor cell survival during differentiation. Furthermore, *p53* haploinsufficiency prevented apoptosis and rescued the survival of *Dnmt1*-null progenitor cells; this suggests that *p53* is a primary target of DNMT1 action in pancreatic progenitor cells. Recently, the role of *Dnmt1* in epidermal progenitor cells suggested that *Dnmt1* was required to retain proliferative stamina and suppress differentiation (Sen et al. 2010). Deletion of *Dnmt1* in epidermal progenitors resulted in precocious differentiation and eventual tissue loss. Although the phenotype in the pancreatic deletions of *Dnmt1* also was the eventual loss of tissue, no evidence of premature differentiation or defects in proliferation were observed. The mechanism by which *Dnmt1* maintains progenitor populations may vary in different tissue depending on the cellular context. This suggests that at a molecular level, DNMT1 may associate with different effectors to carry out repressive functions depending on the cellular context.

We attribute the inability of the *DNMT1^{PC}* pancreatic progenitor population to undergo differentiation to the accumulation of *p53*. Part of *p53* accumulation may be due to the derepression of the *p53* locus when DNMT1 is not present on the promoter. It is to be noted that there is no CpG methylation around the DNMT1-CTCF-binding area (Su et al. 2009; our unpublished results), suggesting that DNMT1 is working as part of a repressive complex independent of its DNA methylation activity. Methylation-independent functions of DNMT1 have been shown to repress target genes as part of complexes with E2F1 and histone deacetylases (Valdez et al. 2011; Clements et al. 2012). The inability of *DNMT1^{PC}* cells to differentiate because of derepression of *p53* is similar to the *mdm2^{-/-}* mouse phenotype (Jones et al. 1995; Montes de Oca Luna et al. 1995). While *mdm2^{-/-}* are embryonic-lethal at E6.5, *mdm2^{-/-}p53^{+/-}* compound mutants are normal and viable. Analysis of compound *mdm2^{-/-}p53^{515c-515c}*, which express a mutated *p53* that can mediate growth arrest but not apoptosis, revealed hematopoietic and lymphoid deficiencies because of progenitor cell cycle and differentiation defects (Liu et al. 2007). Thus, it is possible that repression of *p53* serves

to facilitate pancreatic progenitor cell differentiation during normal organogenesis and that the absence of *Dnmt1* results in transcriptional derepression and subsequent accumulation of p53 that initiates cell cycle arrest and apoptosis during differentiation. The ability of DNMT1^{PC}p53^{+/-} pancreatic progenitor cells to persist through differentiation and form a pancreas at birth suggests that *Dnmt1* is not required to maintain progenitor cell self-renewal or differentiation but is required to attenuate the role of p53 as a checkpoint during differentiation.

Materials and methods

Information on mouse strains is in the Supplemental Material. All animal experiments were performed in accordance with NIH policies on the use of laboratory animals and approved by the Animal Research Committee of the Office for the Protection of Research Subjects at University of California at Los Angeles. For immunohistochemical experiments, tissues were prepared, oriented, and stained, and images were captured as previously described (Georgia et al. 2006). Calculations of the proliferative index were done as previously described using at least three embryos from each genotype (Zhong et al. 2007). ChIP experiments with the E14.5 dorsal pancreas cells were carried out using the micro-ChIP protocol (Dahl and Collas 2008). Statistical significance was determined using Student's *t* test. Information on mouse strains, antibodies, and primers is in the Supplemental Material.

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