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Trisomy of the *Dscr1* gene suppresses early progression of pancreatic intraepithelial neoplasia driven by oncogenic *Kras*

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ABSTRACT

Individuals with Down syndrome exhibit remarkably reduced incidence of most solid tumors including pancreatic cancer. Multiple mechanisms arising from the genetic complexity underlying Down syndrome has been suggested to contribute to such a broad cancer protection. In this study, utilizing a genetically engineered mouse model of pancreatic cancer, we demonstrate that trisomy of the Down syndrome critical region-1 (*Dscr1*), an endogenous calcineurin inhibitor localized on chromosome 21, suppresses the progression of pancreatic intraepithelial neoplasia-1A (PanIN-1A) to PanIN-1B lesions without affecting the initiation of PanIN lesions mediated by oncogenic $Kras^{G12D}$. In addition, we show that *Dscr1* trisomy attenuates nuclear localization of nuclear factor of activated T-cells (NFAT) accompanied by upregulation of the $p15^{Ink4b}$ tumor suppressor and reduction of cell proliferation in early PanIN lesions. Our data suggest that attenuation of calcineurin–NFAT signaling in neoplastic pancreatic ductal epithelium by a single extra copy of *Dscr1* is sufficient to inhibit the progression of early PanIN lesions driven by oncogenic *Kras*, and thus may be a potential mechanism underlying reduced incidence of pancreatic cancer in Down syndrome individuals.

1. Introduction

Down syndrome is the most common genetic disorder in human arising from the presence of an extra copy of all or part of chromosome 21. Although most effects caused by trisomy 21 are deleterious, including delays in mental and physical development and increased risk of acute megakaryoblastic leukemia (AMKL) in children with Down syndrome, the incidence of most solid tumors, including pancreatic cancer, has been shown to be remarkably reduced in Down syndrome adults [1–6]. Such observations suggest that increased dosage of one or more genes on chromosome 21 is responsible for such a broad cancer protection, and elucidating the underlying mechanisms could potentially reveal new targets for cancer prevention and treatment.

Recent studies have demonstrated that multiple mechanisms derived from the genetic complexity of Down syndrome contribute to reduced cancer incidence in individuals with Down syndrome. The increased dosage of *Ets2* gene has been shown to suppress the incidence of intestinal tumors driven by Apc^{Min}, the multiple intestinal neoplasia mutation in the adenomatous polyposis coli (*Apc*) gene, in Ts65Dn mice, a Down syndrome mouse model [7]. In addition, tumor xenografts studies in Ts65Dn and Tc1 Down syndrome model mice have suggested that cancer protection observed in Down syndrome population is, in part, attributable to angiogenesis suppression by trisomy 21 [8,9]. Furthermore, a more recent study has shown that trisomy for 104 genes orthologous to those located on human chromosome 21 increases survival of NPcis mice spontaneously developing various tumors such as lymphomas, sarcomas and carcinomas, and this cancer protection is attributed to a shift of the tumor spectrum away from rapidly lethal sarcoma and toward adrenal tumors but not either increased *Ets2* dosage or reduced tumor angiogenesis [10]. Taken together, these observations suggest that, depending on types of tumors, distinct mechanisms may contribute to cancer protection in the Down syndrome population.

Sustained calcineurin–NFAT signaling promotes pathological cell growth and proliferation, leading to several disorders such as atherosclerosis and hyperinsulinaemia [11,12]. In addition, constitutively active NFAT expression enhances cell transformation and metastatic potential of cancer cells [13,14]. Furthermore, NFAT upregulation has been observed in several human malignancies,

Abbreviations: Dscr1, Down syndrome critical region-1; NFAT, nuclear factor of activated T-cells; PanIN, pancreatic intraepithelial neoplasia; PDA, pancreatic ductal adenocarcinoma; *Dyrk1a*, dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A; CK-19, cytokeratin-19; CDK, cyclin-dependent kinase; TUNEL, terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labeling.

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most notably in human pancreatic carcinomas [15]. These observations suggest that calcineurin–NFAT pathway plays a crucial role in development and progression of certain types of human neoplasia including pancreatic cancer. Notably, two genes, Down syndrome critical region-1 (*Dscr1*) and dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A (*Dyrk1a*), on chromosome 21 have been shown to function as negative regulators for calcineurin–NFAT pathway and their expressions are elevated in Down syndrome tissues [16–21]. Hence, reduced pancreatic cancer incidence in Down syndrome individuals may be due, in part, to attenuated calcineurin–NFAT pathway by increased expression of those negative regulators afforded by the extra copy of chromosome 21. However, it has never been examined whether the modest excess of either DSCR1 or DYRK1A afforded by a single extra copy impedes calcineurin–NFAT signaling evoked by oncogenic activation in pancreatic ductal epithelial cells, and thus suppresses development of pancreatic cancer. In addition, the role calcineurin–NFAT signaling pathway in development and progression of pancreatic cancer still remains to be further verified in spontaneous animal tumor models closely recapitulating the characteristics of human pancreatic tumorigenesis

In this study, utilizing the well-characterized *Pdx-1-Cre;LSL-Kras^{G12D}* mouse model of preinvasive human pancreatic ductal adenocarcinoma (PDA) [22], we examined the inhibitory role of *Dscr1* in pancreatic tumorigenesis and evaluated the calcineurin–NFAT signaling pathway in neoplastic pancreatic ductal epithelium. In addition, we specifically addressed the effect of a single extra copy of *Dscr1* on progression of pancreatic intraepithelial neoplasia (PanIN) lesions driven by oncogenic *Kras^{G12D}* using *Dscr1* transgenic mice, a mouse model harboring a third copy of a *Dscr1* transgene targeted into the *Hprt* locus and controlled by the *Dscr1* native promoter [8]. We generated the *Pdx-1-Cre;LSL-Kras^{G12D}* mice trisomic for *Dscr1* and found that trisomy of the *Dscr1* inhibits the progression of early PanIN lesions through attenuating nuclear localization of nuclear factor of activated T-cells (NFAT) accompanied by compromised cell proliferation in neoplastic ductal epithelium.

2. Materials and methods

2.1. Mice

Pdx-1-Cre mice on a C57BL/6 background were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). *LSL-Kras^{G12D}* mice on a 129SvEv background were provided by Dr. Tyler Jacks (MIT Cancer Center) and backcrossed onto a C57BL/6 background for more than 10 generations. *Dscr1* transgenic mice bearing a single extra copy of a *Dscr1* transgene inserted into the *Hprt* locus on a C57BL/6 background were generated as previously described [8]. *LSL-Kras^{G12D}* mice were crossbred with *Pdx-1-Cre* mice and then further crossed to *Dscr1* transgenic mice to generate *Pdx-1-Cre;LSL-Kras^{G12D};Dscr1* transgenic mice. Genotyping of mice was performed on genomic DNA extracted from tails using PCR as previously described [8,22,23]. *Pdx-1-Cre;LSL-Kras^{G12D}* mice littermates on a *Dscr1* wild-type background were used as controls in our studies. All animal studies were performed in accordance with guidelines of the Institutional Animal Care and Use Committee at the Sungkyunkwan University School of Medicine.

2.2. Histology

Mice were euthanized by CO_2 asphyxiation, and pancreatic tissues were removed, fixed with 10% neutral buffered formalin and embedded in paraffin following standard procedures. 4-μm serial sections were prepared and every fifth slide was stained with hematoxylin and eosin (H&E; Sigma, St. Louis, MO, USA) followed by examination under a bright-field microscope (Carl Zeiss, Jena, Germany). The stage of PanIN lesions was determined without knowledge of genetic background on three sections of each pancreas as previously described [18].

2.3. Immunohistochemistry

Paraffin-embedded sections were deparaffinized in xylene, rehydrated by sequential incubations in graded ethanol from 100% to 95% followed by distilled water and antigen retrieved in a pressure cooker containing a sodium citrate buffer (10 mM, pH 6.0). Sections were then incubated in dual endogenous enzyme block reagent (Dako, Glostrup, Denmark) to quench endogenous peroxidase activity, treated with an avidin/biotin blocking solution (Vector Laboratories, Burlingame, CA, USA) and further incubated in serum-free protein blocking solution (Dako). The sections were then incubated with rabbit antibodies to cyclin-dependent kinase 4 (CDK4; Santa Cruz Biotechnology, Santa Cruz, CA, USA), p15^{INK4b} and Ki-67 (Abcam, Cambridge, UK) overnight at 4 °C followed by visualization with Vectastain Elite ABC Universal kit and the DAB Peroxidase Substrate kit (Vector Laboratories) according to the manufacturer's instructions. To stain pancreatic tissues with mouse monoclonal antibodies against cytokeratin-19 (CK-19), NFATc2 (Abcam) and c-Myc (Santa Cruz Biotechnology), the sections processed as described above were further treated with mouse-on-mouse (M.O.M.) blocking reagent (Vector Laboratories) prior to incubation with the antibodies. After incubation with the primary antibodies overnight at 4 °C, sections were incubated with M.O.M. biotinylated anti-mouse IgG followed by visualization as described above. The sections were then counterstained with hematoxylin to detect cell nuclei, mounted and examined under a bright-field microscope.

2.4. TUNEL staining

To assess apoptosis in PanIN lesions, terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining was performed on pancreatic tissue sections using the Dead-End labeling kit (Promega, Madison, WI, USA) following the manufacturer's instructions. In brief, sections were deparaffinized, rehydrated and antigen retrieved as described above. Sections were then treated with avidin/biotin blocking solution, blocked in M.O.M. blocking reagent and incubated with anti-CK-19 antibody followed by serial incubation with M.O.M. biotinylated anti-mouse IgG and Texas Red Avidin DCS (Vector Laboratories). The sections were then incubated in equilibration buffer for 15 min followed by further incubation with fluoresceinated rdNTPs and terminal deoxynucleotidyl transferase for 60 min at 37 °C. Sections were treated with 2× SSC buffer to stop reaction, counterstained with 1 μg/ml Hoechst dye (Sigma) to visualize cell nuclei, mounted and examined under a fluorescence microscope (Carl Zeiss).

2.5. Statistics

Student's two-tailed unpaired *t* test was used to determine the difference between two groups and *p* value less than 0.05 was considered as statistically significant.

3. Results and discussion

3.1. A single extra transgenic copy of Dscr1 inhibits early PanIN progression

The largest epidemiological study to date involving 17,897 individuals with Down syndrome has revealed that the incidence of

pancreatic cancer is remarkably reduced more than 7-fold in Down syndrome population compared to general population controls [4]. Given the emerging role of calcineurin–NFAT signaling as a critical regulatory pathway in development and progression of human pancreatic cancer [15], we hypothesized that the increased dosage of *Dscr1* may contribute to such a protection of Down syndrome individuals from pancreatic cancer. To examine this hypothesis, we generated *Pdx-1-Cre;LSL-Kras^{G12D}* mice, a well-characterized mouse model developing preinvasive PanIN lesions [22], with the targeted third copy of *Dscr1* [8] and monitored the progression of PanIN lesions on a *Dscr1* transgenic background (Fig. 1). As previously shown [22], the proportion of total PanIN lesions in total pancreatic ducts and their grades increased in both *Pdx-1-Cre;LSL-Kras^{G12D};Dscr1* transgenic and littermate control mice with advancing age. However, interestingly, whereas the incidence of total PanIN lesions did not show statistically significant difference between mice on a *Dscr1* transgenic versus *Dscr1* wild-type background at any age examined (13.4 weeks: $p = 0.4546$; 26.7 weeks: $p = 0.6215$; 40 weeks; $p = 0.8699$), the incidence of PanIN-1B lesions at 26.7 weeks of age was remarkably reduced in *Pdx-1-Cre;LSL-Kras^{G12D};Dscr1* transgenic mice compared with that in littermate controls (4.80 ± 1.01% in *Dscr1* transgenic mice versus 13.56 ± 2.66% in *Dscr1* wild-type mice, 95% CI of difference = 2.97 to 16.85; $p = 0.0186$). In addition, at 40 weeks of age, *Pdx-1-Cre;=LSL-Kras^{G12D};Dscr1* transgenic mice exhibited a dramatic accumulation of PanIN-1A lesions accompanied by significantly reduced incidence of PanIN-1B lesions as well as of more advanced PanIN-2 and PanIN-3 lesions compared with littermate control mice (*Dscr1* transgenic versus wild-type mice: PanIN-1A, 56.44 ± 2.96% versus 39.00 ± 1.96%, 95% CI of difference = −25.17 to −9.70, $p = 0.0004$; PanIN-1B, 21.55 ± 2.06% versus 28.57 ± 1.41%, 95% CI of difference = 1.59 to 12.45, $p = 0.0156$; PanIN-2, 4.01 ± 0.53% versus 12.04 ± 1.79%, 95% CI of difference = 3.96 to 12.09, $p = 0.001$; PanIN-3: 1.06 ± 0.22% versus 4.79 ± 1.06%, 95% CI of difference = 0.69 to 6.76, $p = 0.0203$). These observations clearly demonstrate that an increase in *Dscr1* dosage restrains the progression of PanIN-1A to PanIN-1B lesions but not affects the initiation of neoplastic lesions driven by oncogenic *Kras^{G12D}* in pancreas.

A previous study has shown that *Pdx-1-Cre;LSL-Kras^{G12D}* mice develop PDA although with a low frequency [22]. Therefore, to further determine the pathological consequence of restrained PanIN progression at the early stage by *Dscr1* trisomy in PDA incidence, we examined the development of PDA in *Pdx-1-Cre;LSL-Kras^{G12D}* mice on *Dscr1* transgenic background (Fig. 2). Of interest, whereas around 13.3% of *Pdx-1-Cre;LSL-Kras^{G12D}* mice developed PDA, none of *Pdx-1-Cre;LSL-Kras^{G12D};Dscr1* transgenic mice showed any evidence of PDA at 40 weeks of age. Although the difference in frequency of PDA incidence between *Pdx-1-Cre;LSL-Kras^{G12D};Dscr1* transgenic and wild-type littermate mice was not statistically significant ($p = 0.1161$) and further studies are necessary to verify the statistical significance of this observation in a larger cohort of mice, it appears that inhibition of the progression from PanIN-1A to PanIN-1B lesions caused by a single extra copy of *Dscr1* resulted in protection of *Pdx-1-Cre;LSL-Kras^{G12D}* mice from developing PDA.

Taken together, these observations suggest that a single extra copy of *Dscr1* suppresses early PanIN progression driven by onco-

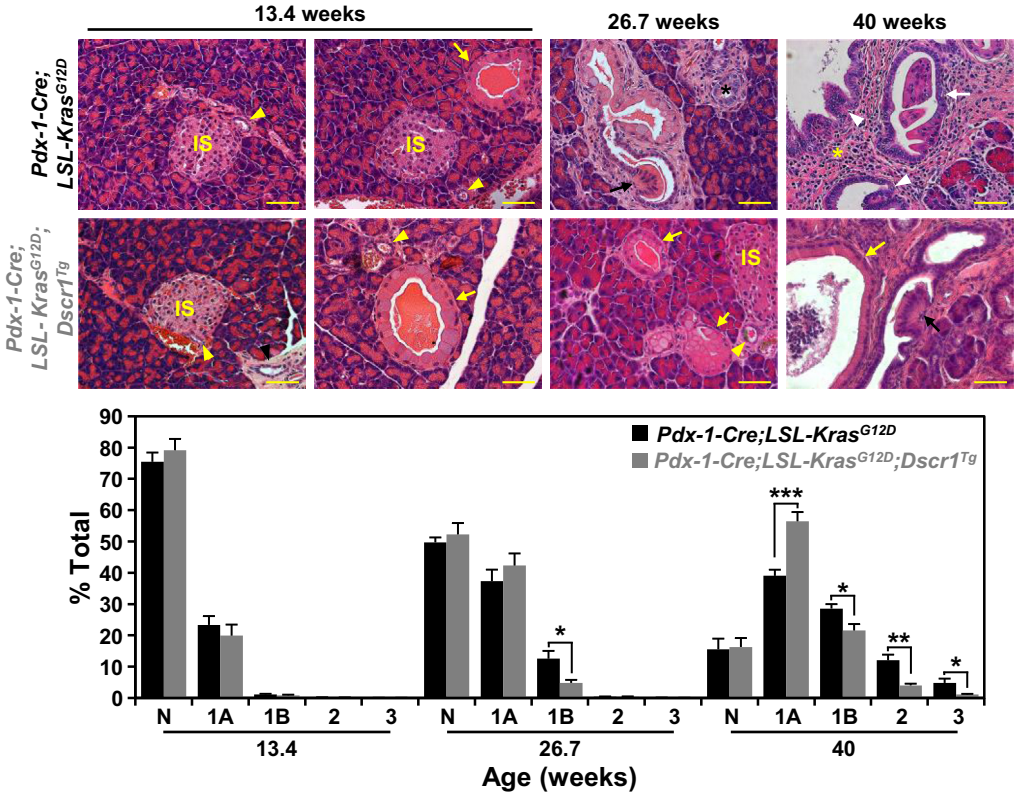

Fig. 1. Trisomy of the *Dscr1* suppresses progression of PanIN-1A to PanIN-1B lesions. Representative H&E-stained images (*upper panels*) and distribution of PanIN stages (*lower panel*) in pancreases isolated from mice of average age 13.4 weeks (*Pdx-1-Cre;LSL-Kras^{G12D}* mice, $n = 8$; *Pdx-1-Cre;LSL-Kras^{G12D};Dscr1* transgenic mice, $n = 7$), 26.7 weeks (*Pdx-1-Cre;LSL-Kras^{G12D}* mice, $n = 11$; *Pdx-1-Cre;LSL-Kras^{G12D};Dscr1* transgenic mice, $n = 7$), and 40 weeks (*Pdx-1-Cre;LSL-Kras^{G12D}* mice, $n = 7$; *Pdx-1-Cre;LSL-Kras^{G12D};Dscr1* transgenic mice, $n = 7$). Yellow and black arrowheads indicate normal intralobular and interlobular ducts, respectively. IS indicates islet. Yellow arrow indicates a PanIN-1A lesion. Black asterisk indicates a reactive duct displaying enlarged nuclei. Black arrow indicates a PanIN-1B lesion showing a papillary structure. White arrowhead indicates a PanIN-2 lesion showing significant loss of cellular polarity and a papillary architecture with higher degree of nuclear atypia. White arrow indicates a PanIN-3 lesion exhibiting complete loss of polarity and dystrophic goblet cells in ductal lumen. Yellow asterisk indicates fibroinflammatory structure. Scale bars: 50 μm. Data are represented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

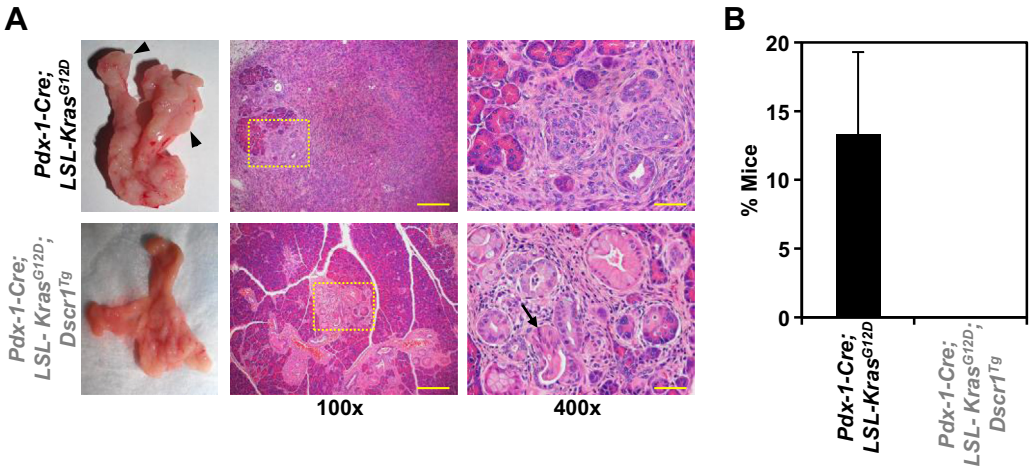

Fig. 2. A single extra copy of *Dscr1* abolishes incidence of pancreatic ductal adenocarcinoma (PDA) in *Pdx-1-Cre;LSL-Kras^{G12D}* mice. (A) Gross photographs and representative H&E-stained images of pancreases. The images in the last column are magnified views of the insets showing the junction area between normal acini and invasive PDA (*upper*) and a PanIN-3 lesion (*lower*). Arrowhead and arrow indicate nodular pancreas and goblet cells, respectively. (B) The percentage of mice developing PDA. Pancreases were harvested from *Pdx-1-Cre;LSL-Kras^{G12D};Dscr1* transgenic ($n = 14$) and littermate control ($n = 15$) mice at 40 weeks after birth. Scale bars: 200 μm; 50 μm (insets). Data are represented as mean ± SEM.

genic *Kras^{G12D}* and support our hypothesis that the increased *Dscr1* dosage contributes to reduced pancreatic cancer incidence in Down syndrome individuals.

3.2. Dscr1 trisomy decreases epithelial proliferation in early PanIN lesions through attenuating nuclear localization of NFAT accompanied by upregulation of *p15^{Ink4b}*

Prior to investigating the molecular mechanism by which *Dscr1* trisomy inhibits early PanIN progression, we first confirmed the epithelial nature of the early PanIN lesions developed in *Pdx-1-Cre;LSL-Kras^{G12D};Dscr1* transgenic and wild-type littermate mice by assessing expression of CK-19, an epithelial cell marker (Fig. 3A). We also verified the expression of transgenic Myc-tagged DSCR1 in ductal epithelial cells in PanIN-1A/B lesions from *Pdx-1-Cre;LSL-Kras^{G12D};Dscr1* transgenic mice using immunohistochemistry (Fig. 3A). Considering that the expression of the *Dscr1* transgene is driven by its native promoter and thus is under the transcriptional control by NFAT [8,17], our data indicates that the calcineurin–NFAT signaling is activated in neoplastic ductal epithelium during PanIN progression mediated by oncogenic *Kras^{G12D}*.

Next, we examined whether the modest increase in *Dscr1* expression afforded by a single extra transgenic copy affects nuclear localization of NFATc2, which is a member of the NFAT family and is upregulated in pancreatic cancers [24], in neoplastic ductal epithelium of early PanIN lesions (Fig. 3A–C). Notably, whereas trisomy of the *Dscr1* showed no effect on overall percentage of ductal cells positive for NFATc2 expression in PanIN-1A/B lesions (*Dscr1* transgenic versus wild-type mice: 46.11 ± 3.07% versus 41.95 ± 3.52%, 95% CI of difference = −0.14 to 0.05, $p = 0.379$) (Fig. 3B), the portion of cells with a predominant nuclear NFATc2 localization among those NFATc2-positive ductal cells was remarkably reduced on a *Dscr1* transgenic background when compared with that on a *Dscr1* wild-type background (*Dscr1* transgenic versus wild-type mice: 3.63 ± 0.84% versus 10.92 ± 1.85%, 95% CI of difference = 0.03 to 0.11, $p = 0.0008$) (Fig. 3C), suggesting that the modestly increased *Dscr1* expression afforded by a single extra copy is sufficient to attenuate nuclear localization of NFATc2 in early PanIN lesions driven by oncogenic *Kras^{G12D}*.

Recently, NFATc2 has been shown to upregulate D-type cyclins and CDKs through transcriptional repression of *p15^{Ink4b}*, a tumor suppressor known to arrest cell cycle progression at G1 phase, leading to promotion of pancreatic cancer cell proliferation [25]. Such observation together with our data showing attenuated NFATc2 activity on a *Dscr1* transgenic background as indicated by its reduced nuclear localization suggests that *Dscr1* trisomy may suppress early PanIN progression via upregulating *p15^{Ink4b}* expression in neoplastic ductal epithelium. Indeed, the population of ductal epithelial cells expressing p15^{INK4b} was dramatically increased up to 1.7-fold in PanIN-1A/B lesions of *Pdx-1-Cre;LSL-Kras^{G12D};Dscr1* transgenic mice compared with that in those of littermate control mice (*Dscr1* transgenic versus wild-type mice: 30.38 ± 2.35% versus 17.77 ± 1.08%, 95% CI of difference = −17.80 to −7.43, $p < 0.0001$) (Fig. 3A and D). To further determine the inhibitory effect of increased p15^{INK4b} expression by *Dscr1* trisomy on cell proliferation in early PanIN lesions, we examined expression of CDK4, a key positive regulator of cell proliferation, and Ki-67, a cell proliferation marker, in PanIN-1A/B lesions (Fig. 3A and D). Notably, both CDK4- and Ki-67-positive cell populations were significantly decreased in those early PanIN lesions in *Pdx-1-Cre;LSL-Kras^{G12D};Dscr1* transgenic mice compared with littermate control mice (*Dscr1* transgenic versus wild-type mice: CDK4-positive ductal cells, 25.62 ± 0.85% versus 32.19 ± 1.49%, 95% CI of difference = 3.16 to 9.99, $p = 0.0003$; Ki-67-positive ductal cells, 14.31 ± 1.30% versus 27.21 ± 2.14%, 95% CI of difference = 7.91 to 17.90, $p < 0.0001$). These data suggest that restrained PanIN progression at an early stage by a single extra copy of *Dscr1* is a consequence of a deficit in oncogenic *Kras^{G12D}*-driven pancreatic ductal epithelial proliferation arising from suppression of calcineurin–NFAT pathway followed by upregulation of *p15^{Ink4b}*.

3.3. Trisomy of the Dscr1 does not induce apoptotic cell death in early PanIN lesions

Many studies have demonstrated that calcineurin–NFAT pathway is implicated in control of apoptotic cell death [26]. In addition, a recent study has shown that NFAT exerts anti-apoptotic activity and promotes pancreatic cancer cell survival [27]. Hence, inhibition of early PanIN progression by a single extra copy of *Dscr1* may be due, in part, to increased apoptosis in neoplastic ductal epithelium by attenuated NFAT activity. However, apoptotic cell death was almost undetectable in early PanIN lesions from *Pdx-1-Cre;LSL-Kras^{G12D};Dscr1* transgenic as well as from littermate control mice and did not show any significant difference between those mice

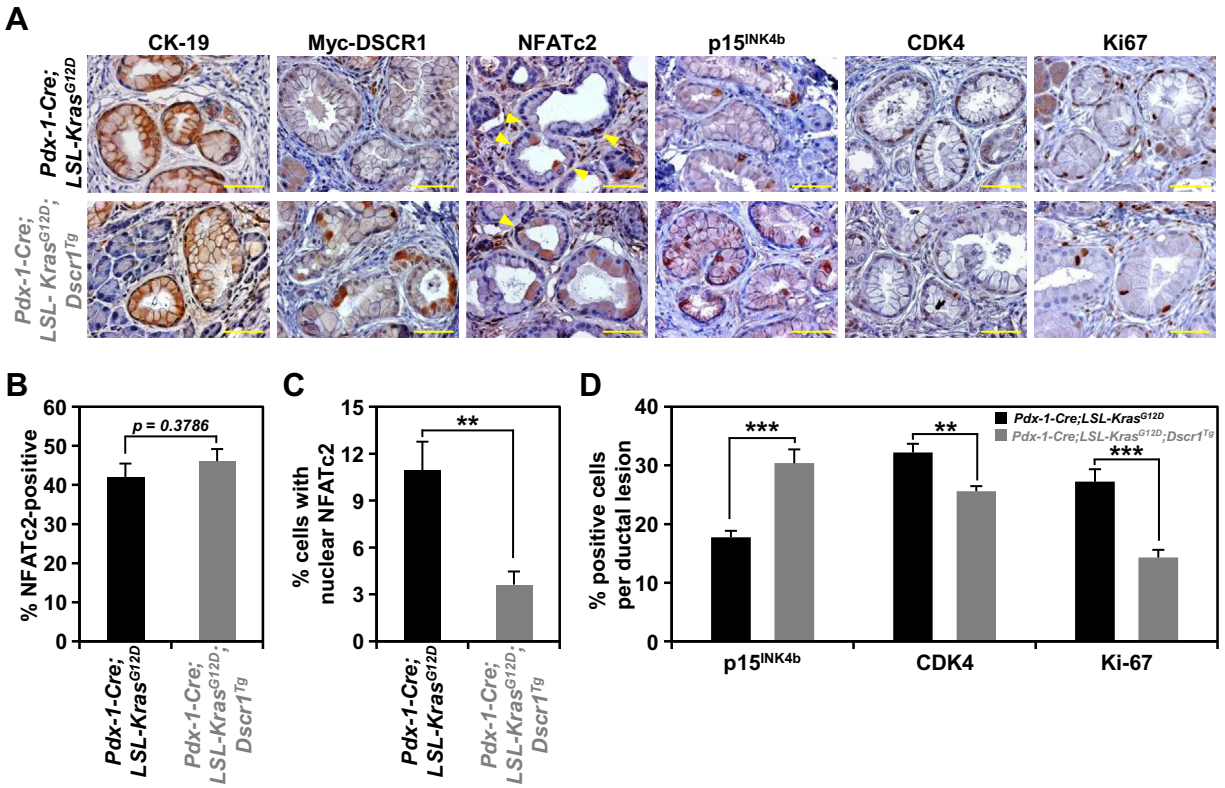

Fig. 3. *Dscr1* trisomy attenuates nuclear localization of NFAT and reduces epithelial proliferation in early PanIN lesions. (A) Representative images of early PanIN lesions stained for CK-19, Myc-epitope tagged DSCR1, NFATc2, $p15^{INK4b}$, CDK4 and Ki-67. Arrowheads indicate cells with nuclear NFATc2. (B) The percentage of NFAT-positive ductal cells in PanIN-1A/B lesions. (C) The percentage of NFATc2-positive ductal cells with predominantly nuclear NFATc2 in PanIN-1A/B lesions. (D) The percentage of $p15^{INK4b}$-, CDK4- and Ki-67-positive ductal cells in PanIN-1A/B lesions. Pancreatic tissues were harvested from *Pdx-1-Cre;LSL-Kras^{G12D};Dscr1* transgenic ($n = 6$) and littermate control ($n = 6$) mice at 26.7 weeks after birth and stained with the indicated antibodies. Scale bars: 50 μm. Data are represented as mean ± SEM. $^{**}p < 0.001$, $^{***}p < 0.0001$.

as assessed by TUNEL assay (Fig. 4). Our data indicate that attenuation of calcineurin–NFAT pathway by *Dscr1* trisomy suppresses oncogenic *Kras^{G12D}*-driven PanIN progression in early stages through impact on cell proliferation but not viability in neoplastic ductal epithelium.

In this study, we demonstrated that the modestly increased *Dscr1* expression afforded by a single extra copy is sufficient to suppress pancreatic tumorigenesis mediated by oncogenic *Kras^{G12D}* in mice through attenuation of calcineurin–NFAT signaling, which subsequently leads to reduced epithelial proliferation in early PanIN lesions. These results not only strongly suggest that reduced pancreatic cancer incidence in Down syndrome individuals may be, in part, attributable to increased *Dscr1* dosage but also further reinforce the previous notion that calcineurin–NFAT pathway is a key regulatory pathway governing pancreatic tumorigenesis. Of note, we previously have shown that upregulation of *Dyrk1a*, another negative regulator of NFAT signaling localized on human chromosome 21 [18,19,21], enhances the impairment of calcineurin–NFAT signaling in *Dscr1* transgenic endothelial cells [8]. Hence, increased dosage of *Dyrk1a* could act together with *Dscr1* to suppress PanIN progression by further attenuating calcineurin–NFAT signaling in neoplastic ductal epithelium and thus could also contribute to reduced incidence of pancreatic cancer in Down syndrome individuals. Further studies will be required to explore whether progression of PanIN lesions would also be suppressed in animals trisomic for *Dyrk1a*.

Collectively, our study reveals a novel role of *Dscr1* as a tumor suppressor in oncogenic *Kras*-mediated pancreatic tumorigenesis

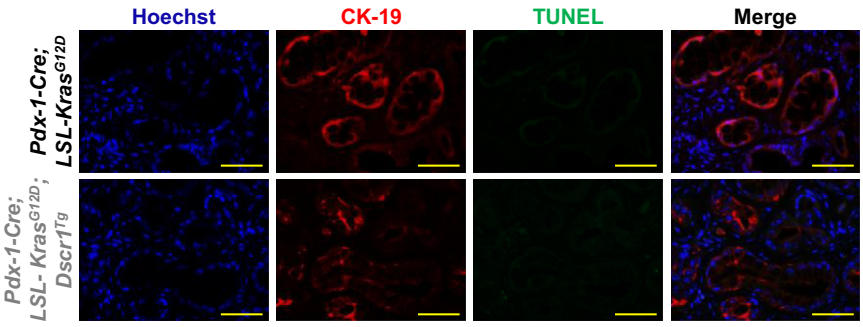

Fig. 4. A single extra copy of *Dscr1* does not elicit apoptosis in early PanIN lesions. Representative images of early PanIN lesions coimmunostained for CK-19 and TUNEL reactivity (*left panels*) and the percentage of TUNEL-positive cells in the lesions (*right panel*). Pancreatic tissues were isolated from *Pdx-1-Cre;LSL-Kras^{G12D};Dscr1* transgenic ($n = 6$) and littermate control ($n = 6$) mice at 26.7 weeks. Scale bars: 50 μm. Data are represented as mean ± SEM. $^{\#}p = 0.8763$.

as well as the potential of calcineurin–NFAT signaling pathway as an attractive target for prevention and treatment of pancreatic cancer.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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