SHORT COMMUNICATION

Oxidative Stress Induced by Inactivation of TP53INP1 Cooperates with KrasG12D to Initiate and Promote Pancreatic Carcinogenesis in the Murine Pancreas

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Accepted for publication
February 27, 2013.

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Tumor protein p53-induced nuclear protein 1 (TP53INP1) is involved in cell stress response. Its expression is lost at the pancreatic intraepithelial neoplasia 1b (PanIN1b)/PanIN2 stage of pancreatic carcinogenesis. Our objective was to determine whether TP53INP1 loss of expression contributes to pancreatic cancer formation in a conditional KrasG12D mouse model. We generated Kras-INP1K0 mice using *LSL-Kras^{G12D/+};Pdx1-Cre^{+/-}* mice (Kras mice) and *TP53INP1^{-/-}* mice. Analysis of pancreases during ageing shows that in the presence of activated Kras, TP53INP1 loss of expression accelerated PanIN formation and increased pancreatic injury and the number of high-grade lesions as compared with what occurs in Kras mice. Moreover, cystic lesions resembling intraductal papillary mucinous neoplasm (IPMN) were observed as early as 2 months of age. Remarkably, TP53INP1 is down-regulated in human IPMN. Activation of the small GTPase Rac1 shows that more oxidative stress is generated in Kras-INP1K0 than in Kras mice pancreas despite elevated levels of the Nrf2 antioxidant regulator. We firmly establish the link between Kras-INP1K0 pancreatic phenotype and oxidative stress with rescue of the phenotype by the antioxidant action of *N*-acetylcysteine. Our data provide *in vivo* functional demonstration that TP53INP1 deficiency accelerates progression of pancreatic cancer, underlining its role in the occurrence of IPMN and highlighting the importance of TP53INP1 in the control of oxidative status during development of pancreatic cancer. (*Am J Pathol* 2013, 182: 1996–2004; <http://dx.doi.org/10.1016/j.ajpath.2013.02.034>)

Pancreatic ductal adenocarcinoma (PDA) displays an overall 5-year-survival rate of less than 5%. It is often not diagnosed before the cancer has metastasized and therefore becomes very challenging to treat. Identification of molecular pathways that cooperate in initiating PDA is essential for diagnosis and prevention. Recent research demonstrated that inflammation plays a contributory role in pancreatic carcinogenesis.^{1,2} Data suggest that oxidative damage links inflammation and pancreatic carcinogenesis, but this link and the role of reactive oxygen species (ROS) are still only partially known.^{3–5}

Tumor protein p53-induced nuclear protein 1 (TP53INP1) is a key stress-response protein highly expressed during pancreatitis.⁶ Recent data^{7–9} demonstrated the role of

TP53INP1 in the control of oxidative status *in vivo*. Indeed, the level of ROS is increased in TP53INP1 knockout mice and their antioxidant defenses reduced, thus showing a chronic oxidative stress in correlation with an increased susceptibility to develop colitis and colitis-associated cancer.^{7–9}

TP53INP1 protein expression is lost during pancreatic cancer progression as early as pancreatic intraepithelial neoplasia 2 (PanIN2).¹⁰ Mice deficient for TP53INP1 did not reveal pancreatic abnormalities,⁹ therefore, we examined in this study the consequences of homozygous deletion of

Supported by INSERM, Agence Nationale pour la Recherche, and Association pour la Recherche contre le Cancer.

TP53INP1 and concomitant activation of the KrasG12D mutation on initiation of PDA. We show that TP53INP1 deficiency accelerates Kras-induced PanIN and cooperates with KrasG12D mutation to induce cystic lesions. We also show that an antioxidant treatment rescues this phenotype.

Materials and Methods

Mouse Strains

The *LSL-Kras^{G12D/+}* mice were from the Mouse Models of Human Cancers Consortium Repository (National Cancer Institute, Frederick, MD) and the *Pdx1-Cre^{+/-}* mice from the DA Melton Laboratory (Cambridge, MA).¹¹ The *TP53INP1^{-/-}* mice were previously described.^{7,9}

LSL-Kras^{G12D/+} and *Pdx1-Cre^{+/-}* mice were bred to generate *LSL-Kras^{G12D/+};Pdx1-Cre^{+/-}* mice (Kras mice) and control wild-type mice.¹² *LSL-Kras^{G12D/+};Pdx1-Cre^{+/-}*; *TP53INP1^{-/-}* mice (Kras-INP1KO mice) were generated by interbreeding Kras mice with *TP53INP1^{-/-}* mice. Control mice were *Pdx1-Cre^{+/-};TP53INP1^{-/-}* or *LSL-Kras^{G12D/+};TP53INP1^{-/-}* littermates (INP1KO mice). All procedures were approved by the animal care committee of the animal facility of INSERM-US006.

Histology and Immunohistochemistry

Pancreases were fixed in 10% neutral buffered formalin and embedded in paraffin. For histopathological analysis, pancreases were serially sectioned (4 μ m) and every 10 sections stained with H&E. Histopathological scoring of pancreatic lesions was performed by two pathologists (J.S. and T.A.S.) using serial H&E-stained sections (50 μ m apart, six sections per pancreas). For quantification of acinoductal metaplasia (ADM)/PanIN/cystic lesions, one representative slide per mouse was imaged using a Hamamatsu NanoZoomer 2 slide scanner (Hamamatsu Photonics, Hamamatsu City, Japan), and lesions were counted and measured on the entire section with the NanoZoomer Digital Pathology view software version 1.2.46.0 (Hamamatsu Photonics). Antibodies used for immunohistochemical studies were the following: anti-cytokeratin19 [rat monoclonal Troma III, developed by Rolf Kemler (Max-Planck Institute, Freiburg, Germany)] obtained from the Developmental Study Hybridoma Bank developed under the auspices of the National Institute of Child Health & Human Development and maintained by the University of Iowa, Department of Biology (Iowa City, IA), anti- α -smooth muscle actin (rabbit polyclonal, 1:100; Thermo Fischer Scientific, Kalamazoo, MI), anti-nuclear factor erythroid 2-related factor 2 (Nrf2) (C-20) (rabbit polyclonal, 1:500; Santa Cruz Biotechnology, Santa Cruz, CA), anti-active Rac1 (mouse monoclonal, 1:1000; New-East Biosciences, King of Prussia, PA), anti-4-hydroxynonenal (HNE) (rabbit polyclonal, 1:250; Alpha Diagnostic International, San Antonio, TX). A25-E12 mouse monoclonal anti-TP53INP1 antibody was previously

described.¹⁰ To ensure specific staining, control slides were stained without primary antibody. For Nrf2, we also pre-incubated the antibody with an excess of immunogenic peptide (sc-722 P; Santa Cruz Biotechnology).

Human tissue samples containing intraductal papillary mucinous neoplastic (IPMN) lesions were provided by the Pathology Department of Beaujon Hospital (Clichy, France). A total of 46 paraffin-embedded IMPN were included in tissue microarray blocks. TP53INP1 expression was scored by combining an estimate of the percentage of immunoreactive cells (0 to 100) with an estimate of the staining intensity (0 = no staining; 1 = low; 2 = moderate; and 3 = high levels of staining) to obtain a score range between 0 and 300.

NAC Treatment

N-acetylcysteine (NAC; Sigma-Aldrich, St. Louis, MO) (40 mmol/L) in drinking water was initiated during embryogenesis via treatment of parent mice and maintained throughout life.¹³

Cells

Primary mouse embryonic fibroblasts from *TP53INP1^{-/-}* embryos were prepared, cultured, and transformed by transduction as previously described.¹⁰

Western Blot Experiments

Mouse embryonic fibroblasts were resuspended in lysis buffer (50 mmol/L Hepes, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 10% glycerol, 1% triton X-100, 25 mmol/L NaF, 10 μ mol/L ZnCl₂). Fifty microgram of proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Antibodies used for immunoblotting were anti-Nrf2 (sc-722; Santa Cruz Biotechnology), anti-TP53INP1 (rat monoclonal antibody generated in our laboratory, clone F8), and anti- β -tubulin (T4026-1; Sigma-Aldrich) as loading control. Proteins were detected using the Immobilon Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA).

Results

Loss of TP53INP1 Accelerates mPanIN Formation

Histopathological analysis of pancreas of mice euthanized at different time points showed that progression of more advanced mPanIN was significantly accelerated in the pancreas of Kras-INP1KO mice compared with *Kras^{G12D/+};Pdx1-Cre^{+/-}* (Kras mice). We found mPanIN1a in 61%, mPanIN1b in 54%, and mPanIN2 in 7.7% of examined pancreases of young Kras-INP1KO mice. Interestingly, mPanIN2 were not observed in age-matched Kras animals (not shown and¹²). Remarkably, before 1 month of age,

50% of examined Kras-INP1KO displayed mPanIN1a ($n = 4$), which was only occasionally found in age-matched Kras animals (not shown and¹⁴). We detected mPanIN2 lesions in one animal age 17 days (Figure 1B). As

Figure 1 Histological progression of mPanIN in Kras-INP1KO mice. **A:** Kras-INP1KO mice show early mPanIN development before 3 months of age ($n = 13$). mPanIN1 were present in 100% of older mice (3 to 6 months, $n = 11$; 6 to 10 months, $n = 11$). mPanIN2 were found in 27.3% of mice age 3 to 6 months, and 91% of mice age 6 to 10 months. mPanIN3 lesions were present in 27.3% of Kras-INP1KO age 3 to 6 months, and 63.7% of mice aged 6 to 10 months. **B:** mPanIN2 in one Kras-INP1KO mouse age 17 days. **C:** Percentages $\pm$ SEM of normal and preneoplastic ducts by grade (1a, 1b, 2, and 3) in a cohort of nine Kras-INP1KO (black bars) and nine Kras (white bars) mice 6 to 10 months of age. We counted 22 ± 3 and 34 ± 7 normal ducts per section in Kras-INP1KO and Kras pancreas, respectively. * $P > 0.01$ and < 0.05 , ** $P > 0.001$ and < 0.01 .

the animals aged, there was an increase in the level of dysplasia and the frequency of mPanIN3 (Figure 1A).

The total number of ductal lesions and their grades were scored in representative pancreas sections of cohorts of mice of 6 to 10 months of age (Figure 1C). The incidence of normal ducts relative to the total number of ducts (including ductal lesions) significantly decreased by twofold in Kras-INP1KO mice compared to Kras mice ($P = 0.0011$). Although approximately 50% of ducts were mPanIN1a in both strains, the incidence of mPanIN1b, mPanIN2, and mPanIN3 significantly increased in Kras-INP1KO mice compared to Kras mice (threefold, $P = 0.004$, 10-fold, $P = 0.005$, and sevenfold, $P = 0.01$, respectively) and represented 18.6%, 3%, and 2% of ducts, respectively. Taken together, these data suggest that TP53INP1 deficiency accelerates PanIN initiation and progression.

Loss of TP53INP1 Cooperates with KrasG12D to Induce Cystic Lesions of the Pancreas

Cystic lesions resembling IPMN occurred in the pancreas of 45.7% of analyzed Kras-INP1KO mice ($n = 35$). Cysts were multilocular (Figure 2A) and positive for cytokeratin19 (Figure 2B), and the presence of abundant quantities of apical mucins distinguished them from retention cysts (Figure 2C). They were observed as early as 2 months of age (23% of mice), suggesting that they probably develop during the early postnatal period; by contrast, age-matched Kras mice had no cystic lesions (Figure 2D). Examination of older mice (ages 3 to 6 months and 6 to 10 months) revealed a gradual increase in the frequency of these cystic lesions to 45% and 73% of examined pancreas, respectively, whereas the frequencies were 7% and 13% at these ages in Kras mice. The size of the cystic lesions was variable but unrelated to the strain or age. Very large cysts (>1 mm) were already observed at 2 months of age in Kras-INP1KO mice. However, the number of lesions was significantly increased in Kras-INP1KO mice, resulting in replacement of larger pancreatic areas than in Kras mice (Figure 2E).

TP53INP1 Expression Is Down-Regulated in Human IPMN

Positive expression of TP53INP1 was previously reported in IPMN with low dysplasia but was never explored in samples with higher dysplasia.¹⁰ We found TP53INP1 in the cytoplasm of acinar and ductal cells of human pancreas (Figure 2F). In IPMN, TP53INP1 staining was significantly lower in samples of moderate- and high-grade dysplasia than in samples of lower-grade dysplasia (Figure 2, G–I). Although the TP53INP1 staining score was high in 14% and 31% of IPMN of moderate and high grade, respectively, the proportion of individuals that did not express TP53INP1 increased with the degree of dysplasia (Figure 2J).

Figure 2 Loss of TP53INP1 induces cystic lesions in the pancreas of Kras mice. **A:** Representative pancreas section showing cysts in a 10-month-old mouse. **B:** Cytokeratin19 expression indicating the ductal phenotype of cysts. **C:** Alcian blue reveals acidic mucin secretion (arrowheads, upper panel). Periodic acid Schiff coloration shows neutral mucins (arrowheads, lower panel). **D:** Kras-INP1KO mice develop cystic lesions before 3 months of age ($n = 13$ mice). These lesions are more frequent in Kras-INP1KO mice than in Kras mice (age 3 to 6 months, $n = 11$; 6 to 10 months, $n = 11$). **E:** Cystic lesions replaced a significantly larger area of pancreas in Kras-INP1KO than in Kras mice at 6 to 10 months of age ($n = 9$). $*P > 0.01$ and < 0.05 . **F:** In human pancreas, acinar (a) and ductal cells (d) express TP53INP1. **G** and **H:** TP53INP1 is expressed in low-grade dysplasia areas of human IPMN (arrows); cells are negative in higher-grade dysplasia (arrowheads). **I:** TP53INP1 staining score in low dysplasia (LD), moderate dysplasia (MD), and high dysplasia (HD). $*P > 0.05$ and < 0.01 , $***P < 0.001$, Student's t -test. **J:** Distribution of TP53INP1 score according to IPMN dysplasia grade. Significant differences between scores in pairs of tissues are indicated by asterisks. $***P < 0.0001$, χ^2 test.

Metaplastic/Dysplastic Changes Are Frequent in Kras-INP1KO Mice

Histological changes reflecting pancreatic injury were frequent in Kras-INP1KO mice with development of extensive fibrosis, infiltration of inflammatory cells, and acinar atrophy (Figure 3A and Figure 4G). Expansion of a reactive stroma strongly positive for α -smooth muscle actin was associated with several distinct metaplastic ductal lesions mirroring human chronic pancreatitis (Figure 3, B–D). ADM was detected at 2 weeks, and partial or complete ADM of several pancreatic lobules was observed in the majority of Kras-INP1KO mice (Figure 3E). Numerous ADM/mPanIN1a hybrid structures containing cells that coexpress amylase and CK19 were found (Figure 3F). Foci of cellular dysplasia suggestive of an *in situ* malignant behavior were also frequent in the pancreas of Kras-INP1KO mice (Figure 3, G and H). The area occupied by the abnormal pancreas represented 5% of the pancreas by the age of 4 to 5 months in Kras-INP1KO mice and reached 30% to 75% by 6 to 10 months, indicating that TP53INP1 deficiency is associated with an

increased proliferation of both the preneoplastic epithelium and the stromal tissue.

Oxidative Stress Promotes Preneoplastic Lesions Formation in Kras-INP1KO Mice

On the basis of the known antioxidant function of TP53INP1, we reasoned that oxidative stress could play a major role in the pancreatic phenotype of Kras-INP1KO mice and investigated the activation state of Rac1, a known mediator of ROS production via NADPH oxidase, by performing immunostaining with an antibody against active Rac1. We observed a more intense staining in Kras-INP1KO pancreas than in Kras mice (Figure 4, A and B). In both strains, staining of acinar cells was cytoplasmic and apical, ducts were negative. Activity of Rac1 was heterogeneous in mPanIN and IPMN cells. ADM and hybrid ADM/mPanIN1a were positive. Similar staining patterns were observed with an antibody against HNE, an aldehydic lipid peroxidation product that is a good marker for oxidative stress. HNE was not detected in the exocrine pancreas of *LSL-Kras*^{G12D/+} and INP1KO

Figure 3 Inflammatory and dysplastic lesions in Kras-INP1KO mouse pancreas. **A:** H&E-stained representative pancreas section showing inflammatory infiltrate and fibrosis in the pancreas of a 10-month-old Kras-INP1KO mouse. **B:** α -Smooth muscle actin expression. **C:** H&E-stained pancreas section showing small tubular complexes (TC) formed by a monolayer of 4 to 10 very flat cells (dashed line) and large TC lined by numerous cells (solid line). **D:** Tubular complexes express cyto-keratin19. **E:** The majority of Kras-INP1KO mice displays ADM. **F:** Amylase-containing cells (arrow-heads) are found in ADM next to mPanIN (asterisks) in Kras-INP1KO mouse pancreas. **Inset:** Double immunofluorescence for amylase (red) and CK19 (green) reveals double-positive cells (arrow-heads) in ADM. **G** and **H:** Higher dysplastic changes in Kras-INP1KO mouse pancreas. Acini are replaced by ductal structures exhibiting loss of architectural orientation, cellular atypia, increased nuclear to cytoplasmic ratio, nuclear atypia, anisokaryosis, and presence of necrotic debris in the lumen.

control littermates (Supplemental Figure S1, A and B). Staining was heterogeneous in mPanIN in both Kras and Kras-INP1KO pancreas (Supplemental Figure S1, C and D). Interestingly HNE was detected in acinar cells, ADM, and hybrid ADM/mPanIN1a of Kras-INP1KO mice (Supplemental Figure S1, D–F).

Because the Nrf2 antioxidant program was shown to promote KrasG12D pancreatic carcinogenesis, we examined whether expression of Nrf2 was modulated by TP53INP1 deficiency. Normal ducts (not shown) and islets did not express detectable levels of Nrf2 in Kras mice pancreas, whereas Nrf2 expression was cytoplasmic, basolaterally found in acini, and nuclear in mPanIN cells; most stromal

cells were negative (Figure 4C). Intense Nrf2 signal was found in Kras-INP1KO mice pancreas in the nucleus of most cells, including acini (Figure 4D). Western blot analysis further demonstrated that Nrf2 levels are increased in TP53INP1-deficient cells (Figure 4E). Finally, we examined the impact of prolonged treatment with NAC, a well-known antioxidant. Because loss of TP53INP1 accelerates Kras-induced development of mPanIN, NAC treatment was initiated early during Kras-INP1KO mouse embryogenesis. After 10 months and consistent with the data reported above, histological analysis of untreated Kras-INP1KO ($n = 6$) shows large areas of pancreas replaced by ADM, mPanIN, and cystic lesions with abundant surrounding stroma

Figure 4 Oxidative stress increases in the pancreas of Kras-INP1KO mice. **A:** Immunostaining of Kras mice pancreas with anti-active Rac1 antibody. **Boxed area** shows activity of Rac1 in the cytoplasm of acini (**arrowhead**) and of hybrid ADM/mPanIN1a (**arrows**), specificity of active Rac1 immunostaining was verified as indicated in *Materials and Methods* (not shown). **B:** Activity of Rac1 in the pancreas of Kras-INP1KO mice. **Boxed area** shows intense labeling of acini (**arrowhead**), tubular complexes (**asterisks**), and heterogeneous activity in mPanIN (**arrows**). **C:** Nrf2 levels in Kras mice pancreas. **Boxed area** shows basolateral expression of Nrf2 in acinar cells and nuclear immunostaining of mPanIN (**arrows**). Islets (i) are negative, and specificity of Nrf2 immunostaining was verified as indicated in *Materials and Methods* (not shown). **D:** Nrf2 levels in Kras-INP1KO mice pancreas. **Boxed area** shows high levels of Nrf2 in the nuclei of most of the cells. **E:** *TP53INP1*^{-/-}-transformed mouse embryonic fibroblasts were transduced with empty murine stem cell virus (MSCV-neo), MSCV-TP53INP1 α , or MSCV-TP53INP1 β retroviral vectors. Nrf2 protein level was analyzed by Western blot in total cell lysates from *TP53INP1*^{-/-}-transformed mouse embryonic fibroblasts, re-expressing one or the other isoform of TP53INP1 (**upper panel**). Re-expression of TP53INP1 is shown by immunoblotting using anti-TP53INP1 antibodies (**lower panel**). **F:** Representative histology of Kras-INP1KO pancreas without NAC treatment (10 months of age). **G:** NAC-treated Kras-INP1KO mice pancreas at 10 months of age. **H:** Proportion of untreated (NAC-) ($n = 6$) and treated (NAC+) ($n = 6$) 10-month-old Kras-INP1KO mice with pancreatic mPanIN, cystic lesions (CL), and ADM. **I:** Quantitative analysis of mPanIN lesions. Percentage of pancreatic ducts with no pathology (normal), mPanIN1a, mPanIN1b, mPanIN2, and mPanIN3 in NAC- ($n = 6$) and NAC+ Kras-INP1KO mice ($n = 6$) at 10 months of age. $*P > 0.01$ and < 0.05 , $**P > 0.001$ and < 0.01 . **J:** Nrf2 levels in NAC-treated Kras-INP1KO mice pancreas. **Boxed area** shows basolateral expression of Nrf2 in acinar cells and nuclear immunostaining of mPanIN (**arrows**).

(Figure 4F), whereas antioxidant treatment strongly reduced the incidence of mPanIN and cystic lesions (Figure 4, G and H). mPanIN1a represented $22.4 \pm 10.1\%$ of ducts (2.6-fold reduction compared to untreated, $P = 0.007$), mPanIN1b represented $1.7 \pm 1.6\%$ of ducts (11.2-fold reduction, $P = 0.004$); no mPanIN2 and mPanIN3 were found (Figure 4I). One cystic lesion <1 mm in size was found in one treated mouse (Figure 4H). Focal ADM represented $0.3 \pm 0.2\%$ of total area in 50% of treated mice, whereas it was $7.5 \pm 2.7\%$ of the pancreas in all untreated mice ($P = 0.013$). The area occupied by the abnormal pancreas represented $<1\%$ of the pancreas in treated mice ($n = 6$). Interestingly, Nrf2 immunostaining performed in treated Kras-INP1KO mice (Figure 4J) showed decreased Nrf2 levels compared to untreated Kras-INP1KO mice (Figure 4D). The immunostaining pattern was similar to the one in untreated Kras mice, with Nrf2 expressed in the cytoplasm of acini and in the nuclei of mPanIN cells (Figure 4C). Taken together, these results demonstrate that oxidative stress mediates pancreatic damage in Kras-INP1KO mice.

Discussion

Results of our *in vivo* study provide clear genetic evidence for a role of TP53INP1 in initiation steps and progression of pancreatic cancer. We show that the deficiency of TP53INP1 in the context of an activated KrasG12D promotes the formation of PanIN in young mice, indicating that TP53INP1 may impede transformation of embryonic pancreatic cells by oncogenic Kras. Moreover, both accelerated development of PanIN and increased incidence of high-grade lesions demonstrate the functional impact of TP53INP1 deletion on pancreatic cancer evolution, with a higher susceptibility to develop preneoplastic lesions. Our results also indicate that TP53INP1 can restrain the initiation of cystic lesions in the context of KrasG12D heterozygosity in mice, thus establishing a novel genotype–phenotype relationship underlying the formation of these neoplasms.¹⁵ IPMN do not develop in young Kras mice with or without loss of a tumor suppressor such as tumor protein 53 (Tp53), p16Ink4a, or p19Arf,^{12,14,16,17} but are induced by loss of SMAD4 or transcriptional intermediary factor 1 gamma (TIF1 γ) or overexpression of transforming growth factor alpha (TGF α).^{18–21} Interestingly, IPMN that develop in Kras-INP1KO mice show similarities to those observed in the absence of SMAD4 or of TIF1 γ that could support a role of TP53INP1 in the modulation of the TGF β pathway.²²

Our results underline for the first time the role of TP53INP1 in the progression of IPMN in human pancreas. Indeed, we show that TP53INP1 expression decreases with increasing dysplasia in these lesions. Our data also substantiate IPMN and PanIN having different genetic progression, because in contrast with our findings in IPMN, high-grade PanIN never express TP53INP1.¹⁰ Although the relationship between PanIN and IPMN is still unclear, both lesions

frequently exist in the same patients synchronously.^{23,24} Therefore, mice models such as Kras-INP1KO mice might thus reflect more accurately the human pancreatic premalignant conditions.

Kras-INP1KO mice have a less aggressive pancreatic phenotype compared with KrasG12D animals with homozygous deletions in Tp53 or p16Ink4A, because we observed no invasive cancer. Interestingly, this was also the case for KrasG12D mice with loss of SMAD4 or TIF1 γ that also developed IPMN.^{19,21} This observation is consistent with epidemiological data in humans showing that IPMNs only rarely give rise to aggressive tumors.²⁵ It is also possible that absence of TP53INP1 is not a sufficient oncogenic hit. Therefore, the role of TP53INP1 is currently investigated in *Kras*^{G12D}/*p16Ink4Arf*^{−/−} mice that are a more appropriate model for tumor progression. Another explanation is that we had to euthanize most of the mice because of signs of illness, including weight loss. Therefore, we cannot rule out the possibility that these mice die before the development of invasive cancer because of pancreatic failure due to growing cystic lesions.

Mouse models also revealed the involvement of different pancreatic cell types in PDA initiation. In particular, recent studies showed that activation of oncogenic Kras in acinar cells results in spontaneous induction of mPanIN, via reprogramming of acinar cells into ductal cells, thus suggesting that PDA may originate from acinar cells.^{26–29} One important feature of the Kras-INP1KO mice phenotype is a significant increase in the development of metaplastic ductal changes that are characteristic of chronic pancreatitis. These changes are observed in human pancreas, associated with an increased risk of neoplasia, and proposed to represent precursor lesions of pancreatic cancer.^{29–32} In Kras-INP1KO mice, ADM occurs early in the postnatal development in the majority of mice and exhibits highly dysplastic changes consistent with *in situ* premalignant lesions in some mice. Such dysplastic changes were not previously reported in the KrasG12D mice model. Given that TP53INP1 is an important element of cellular stress response identified in rodent acinar cells, we suggest that a metaplasia–dysplasia sequence may specifically result from the combination of mutated Kras and the absence of TP53INP1 in acinar cells in Kras-INP1KO mice, representing an alternative pathway to PanIN.

Endogenous levels of the Kras oncogene were recently found to reduce ROS levels and to induce the Nrf2 antioxidant program that serves as a mediator of pancreatic oncogenesis.⁵ We show that TP53INP1-deficient cells have increased levels of Nrf2 in accordance with the known role of TP53INP1 as a negative oxidative stress regulator.⁸ Nrf2 levels are also increased in Kras-INP1KO pancreatic cells together with elevated activity of Rac1, a known mediator of ROS production, thus demonstrating that ROS overwhelm the cellular antioxidant defense system, induced by mutated Kras, and therefore generate oxidative stress. Antioxidant treatment of Kras-INP1KO mice clearly demonstrates that

oxidative stress in the absence of TP53INP1 facilitates development of preneoplastic lesions, including cystic lesions. Interestingly, incomplete phenotype rescue was associated with NAC-resistant nuclear expression of Nrf2 in early PanIN, consistent with their previously reported insensitivity to NAC in Nrf2-expressing Kras mice.⁵

TP53INP1 was recently described as a molecule associated with autophagy that interacts with ATG8 family proteins and induce autophagy-dependent cell death.³³ Other tumor suppressors, such as Tp53, PTEN, TSC1, TSC2, and death-associated protein kinase (DAPK), were shown to be involved in autophagy, suggesting that autophagy could be involved in their antitumoral action.^{34–37} In fact, autophagy has been proposed to play a dual role in cancer, functioning to suppress cancer progression at the earliest steps, but allowing advanced cancers to meet their metabolic demands. Because TP53INP1 is lost very early in pancreatic carcinogenesis, we hypothesize that the absence of TP53INP1 favors cancer progression. TP53INP1-mediated autophagy could be part of the cellular response against oxidative stress that protects against tumor formation by inducing death of cells with excessive oxidative damage.

This study highlights the context-dependent roles of ROS in pancreatic carcinogenesis. Indeed, it demonstrates that although both TP53INP1 and Nrf2 are essential upstream regulators of antioxidant cellular defense, they act through different pathways and have opposite roles in pancreatic carcinogenesis. Therefore, cellular stress generated by homozygous deletion of TP53INP1 significantly accelerates the PanIN progression, leads to the development of cystic lesions, and induces numerous metaplastic ductular changes in the Kras mice.

Acknowledgments

We thank Yvan Nicaise (CMEAB, IFR-BMT, University of Toulouse III) for technological assistance and Dr. Romina D'Angelo for assistance with imaging (Cellular Imaging Facility Rangueil-I2MC/TRI Plateform).

Supplemental Data

Supplemental material for this article can be found at <http://dx.doi.org/10.1016/j.ajpath.2013.02.034>.

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