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Mouse Pancreatic Tumor Model Independent of Tumor Suppressor Gene Inactivation

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To the Editor

Comprehensive cancer genome analyses identified a number of recurring mutations in PDACs; a prevailing model posits that activating mutations in KRAS are early events, and when combined with inactivation of tumor suppressor genes such as TP53 and CDKN2A, they play crucial roles in initiation and progression of PDACs. This model is well corroborated by findings in genetically engineered mice, where expression of constitutive-active mutant Kras and deletion or point mutations of p53 in the pancreatic epithelia reproduced pre-cancerous PanINs progressing to full-blown metastatic PDACs. Hence, these mouse models are now widely used for both basic and translational studies of PDAC.¹

However, while KRAS mutations are found in more than 90% of PDAC, TP53 mutations are seen only in approximately half of the cases. Evidently, there are a considerable number of PDACs that cannot be faithfully recapitulated by existing models. In order to improve our understanding of PDAC biology and to develop better diagnostic/treatment strategies, additional models are needed.

Although the exact etiology of PDAC has not been fully clarified, risk factors of pancreatic cancer include cigarette smoking, obesity, chronic pancreatitis and family history of pancreatic cancer.² Significance of inflammation in PDAC is repeatedly demonstrated in mouse models; for example, pancreatic tumor formation in mice expressing oncogenic mutant Kras is accelerated upon cerulein-induced pancreatitis; conversely, inhibition of inflammatory pathways protected mutant Kras-expressing mice from pancreatic tumors.^{3–5}

Thus, combining inflammation with Kras mutations provides an alternative platform to investigate pancreatic tumors independent of tumor suppressor gene inactivation. However, a

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Conflict of interest

The authors declare no conflict of interest

relatively long latency of existing models (6–8 months for oncogenic mutant Kras mice with cerulein-induced pancreatitis) limits the utility of these models. Here we report a novel mouse pancreatic tumor model independent of tumor suppressor gene inactivation. Because of its extremely early onset and high penetrance, this could be a useful model to investigate involvement of Kras mutations and pancreatic inflammation in PDACs.

Significance of inflammation on pancreatic cancers is well supported by the chemically-induced pancreatitis model using cerulein. However, in this model, tumor formation requires a long latency after pancreatitis induction. Furthermore, because cerulein is administered systemically, it is conceivable that the disease progression may be influenced by its effect outside of the pancreas.

Therefore, we sought to establish an alternative model of Kras-dependent pancreatic tumors that the inflammation is strictly limited to the pancreas. We reasoned that this is achievable through a genetic approach. Because the NF- κ B pathway activation is the hallmark of inflammation, and IKK β is the critical regulator of the pathway activity, we decided to simulate inflammation by expressing constitutive-active IKK β in the pancreatic epithelia.

To this end, we crossed B6.129S4-Kras^{tm4Tyj/J} mice (Kras G12D) with B6(Cg)-Gt(ROSA)26Sor^{tm4(Ikbbk)Rsky/J} mice (caIKK β) (Fig 1A). The former carries a loxP-flanked STOP cassette followed by the oncogenic G12D point mutant K-RAS in the endogenous Kras locus; the latter carries a loxP-flanked STOP cassette, a cDNA encoding a FLAG-tagged, constitutively active form of murine Ikbbk (caIKK β ; containing two serine to glutamate substitutions (S177E, S181E) in the activation loop of the kinase domain), a frt-flanked internal ribosomal entry site and enhanced green fluorescent protein (IRES-GFP), and a bovine polyA sequence in the ROSA26 locus. The resultant double-mutant mice were further crossed to B6.FVB-Tg(Pdx1-cre)6Tuv/J mice (Pdx1-Cre) that express Cre recombinase in the pancreatic epithelium starting mid-gestation. Consequently, triple mutant mice will express G12D mutant Kras and caIKK β in the pancreatic epithelia.

When the first litter from this cross was born, we noticed one pup, which appeared normal at birth but lagged significantly in growth compared to littermates by postnatal day 8 (Fig 1B). When we dissected this animal after euthanasia, we observed a massive tumor in the abdomen. Histologically, the tumor showed cystic appearance surrounded by non-epithelial cells, with papillary fronds and inflammatory cells filling the lumen of the cysts. Epithelial cells were highly dysplastic and mitotic cells were observed. Genotyping revealed that the affected pup was the only one among the litter that carried the three transgenes (Kras G12D, caIKK β and Pdx1-Cre). We subsequently obtained 7 additional mice of the same genotype, and except for one mouse, they all developed pancreatic tumors and had to be euthanized due to severe growth retardation by postnatal day 10. The reason why one mouse of this genotype failed to develop tumors is not clear at present.

To confirm that the tumor in our model originated from cells that underwent Cre-mediated DNA recombination, we stained the tissue sections with antibodies against FLAG and GFP (Fig 1C). caIKK β is tagged with FLAG, and GFP is an additional marker for Cre-mediated DNA recombination at the ROSA26 locus in B6(Cg)-Gt(ROSA)26Sor^{tm4(Ikbbk)Rsky/J} mice.

We found that cells with epithelial appearance stained positive for FLAG and GFP while cells with mesenchymal appearance did not.

In previously reported genetic models of mouse pancreatic tumors, affected animals survived between 2 months to over 12 months after birth. Thus, the extremely early onset of pancreatic tumors in the Pdx1-Cre; Kras G12D; caIKK β model was rather unexpected (Fig 1D). To exclude the unlikely possibilities that the Kras G12D and/or Pdx1-Cre strains we employed carried unidentified mutations that accelerated tumor growth, we crossed these mice from our colony to those with the conditional Trp53 allele (B6.129P2-Trp53^{tm1Bm/J}; Trp53 flox). As previously reported, Pdx1-Cre; Kras G12D; Trp53 flox mice developed pancreatic tumors and became moribund around 5~6 months of age, suggesting that there were no additional unidentified mutations in Kras G12D or Pdx1-Cre mice in our colony.

Using the tumors from Pdx1-Cre; Kras G12D; Trp53 flox mice as reference, we characterized Kras G12D; caIKK β tumors by immunohistochemistry. Both tumors stained positive for cytokeratin 19, confirming their epithelial origin (Fig 1E). However, former expressed MUC5AC, the latter did not stain for this marker. In conclusion, forced expression of constitutive active IKK beta in the pancreatic epithelium accelerates Kras G12D driven tumor formation.

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References

1. Tuveson DA, Hingorani SR. Ductal pancreatic cancer in humans and mice. Cold Spring Harb Symp Quant Biol. 2005; 70:65–72. [PubMed: 16869739]
2. Midha S, Chawla S, Garg PK. Modifiable and non-modifiable risk factors for pancreatic cancer: A review. Cancer Lett. 2016; 38:269–277.
3. Daniluk J, Liu Y, Deng D, et al. An NF- κ B pathway-mediated positive feedback loop amplifies Ras activity to pathological levels in mice. J Clin Invest. 2012; 122:1519–15128. [PubMed: 22406536]
4. Ling J, Kang Y, Zhao R, et al. KrasG12D-induced IKK2/beta/NF- κ B activation by IL-1 α and p62 feedforward loops is required for development of pancreatic ductal adenocarcinoma. Cancer Cell. 2012; 21:105–120. [PubMed: 22264792]
5. Maniati E, Bossard M, Cook N, et al. Crosstalk between the canonical NF- κ B and Notch signaling pathways inhibits Ppargamma expression and promotes pancreatic cancer progression in mice. J Clin Invest. 2011; 121:4865–4899.

FIGURE 1.

A, Schematic representation of mutant alleles used in this study. B, Macroscopic appearance of the abdominal tumor in the nine day old Pdx1-Cre; *Kras* G12D; *calKKβ* mouse. The arrow indicates the tumor (demarcated with the dotted white line). H&E stain of the tumor. C, Anti-FLAG antibody stain (brown) of the tumor. Anti-GFP antibody stain (brown) of the tumor. D, Kaplan-Meier survival analysis of Pdx1-Cre; *Kras* G12D (blue), Pdx1-Cre; *Kras* G12D; Trp53 flox (gold), Pdx1-Cre; *Kras* G12D; *calKKβ* (red), and their littermates (black). E, Pancreatic tumors from Pdx1-Cre; *Kras* G12D; Trp53 flox (top) and Pdx1-Cre; *Kras* G12D; *calKKβ* (bottom) were stained with antibodies against cytokeratin 19 (left) and MUC5AC (right).