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Molecular mechanism of Intraductal Papillary Mucinous Neoplasm (IPMN) and IPMN-derived Pancreatic Ductal Adenocarcinoma

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Key words

IPMN

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Abstract

Pancreatic ductal adenocarcinoma (PDA) is one of the most lethal human malignancies. Dissecting the mechanisms underlying PDA development is important for developing early detection methods and effective preventions and therapies for the disease. PDA is considered to arise from distinct precursor lesions, including pancreatic intraepithelial neoplasia (PanIN) and intraductal papillary mucinous neoplasia (IPMN). However, little is known about molecular mechanisms of development of IPMN and IPMN-derived PDA. We have recently reported that loss of Brg1, a core subunit of SWI/SNF chromatin remodeling complexes, cooperates with oncogenic Kras to form cystic neoplastic lesions that resemble human IPMN and progress to PDA. Brg1 null IPMN-PDA is less lethal compared to PanIN-derived PDA (PanIN-PDA) driven by mutant Kras and hemizygous p53 deletion, mirroring prognostic trends in PDA patients. Brg1 null IPMN-PDA possesses a distinct molecular signature that supports less malignant potential compared to PanIN-PDA. Furthermore, Brg1 deletion inhibits Kras-dependent PanIN development from adult acinar cells, but promotes Kras-driven preneoplastic transformation in adult duct cells. Therefore, Brg1 is a determinant of context-dependent Kras-driven pancreatic tumorigenesis and chromatin remodeling may underlie the development of distinct PDA subsets.

Understanding molecular mechanism of IPMN and IPMN-derived PDA could provide critical clues for novel diagnostic and therapeutic strategies of the disease.

Introduction

PDA is one of the most devastating and lethal cancers with less than five percent of patients surviving five years after diagnosis, and the incidence of this disease is increasing. Therefore, understanding the pathogenesis of PDA is important for developing early detection methods and effective preventions and therapies for the disease. PDA is assumed to arise from three morphologically different precursor lesions: pancreatic intraepithelial neoplasias (PanINs; the most common precursor lesions in humans), intraductal papillary mucinous neoplasias (IPMNs), or mucinous cystic neoplasias (MCNs). Because IPMN-derived PDA (IPMN-PDA) has a more favorable prognosis for survival compared to PanIN-derived PDA (PanIN-PDA)¹⁻³, it has been considered that IPMN-PDA is biologically distinct⁴. However, to date, very few studies have addressed molecular characteristics and mechanisms underlying the development of IPMN and IPMN-PDA. It has recently been shown that the

mutations of *Kras* and *GNAS* are observed in cystic fluid of IPMNs ⁵ and resected IPMNs ⁶, and the detection of *GNAS* mutation is specific to distinguish IPMNs from other cystic neoplasms ⁵. In addition, *RNF43* has recently been reported to be mutated in both IPMNs and MCNs ⁵, although the functional roles of *GNAS* and *RNF43* in the development of IPMNs are still unknown.

There is growing evidence to support a role for epigenetic regulators in the development and progression of cancer ⁷. Chromatin remodelers are a group of epigenetic regulators that utilize ATP hydrolysis to disrupt DNA-protein contacts in order to regulate gene expression ⁸. Inactivating mutations or loss of expression of several subunits of multi-protein chromatin-remodeling SWI/SNF complexes have recently been identified in various human cancers ⁹⁻¹³, including PDA ¹⁴. For PDA, mutations in *Brg1* (*SMARCA4* in human), a catalytic ATPase subunits of the SWI/SNF complex, and other subunits of the complex are identified in over 30% of human PDA cases ^{15, 16}. Of note, *Brg1* expression was reported to be frequently reduced or lost in human IPMN samples ¹⁷. However, it remained unclear whether *Brg1* plays a functional role in the specification of PDA precursors and PDA development.

Genetically engineered mouse models provide valuable insights into the pathogenesis of diseases and allow the testing of the hypothesis that particular genes are involved in pathogenesis. We have recently generated transgenic mice in which *Brg1* is deleted in the context of oncogenic *Kras* (*Kras*^{G12D}) in the pancreas under the control of endogenous *Ptf1a* promoter (*KP.Brg1* mice)¹⁸. *KP.Brg1* mice results in spontaneous development of cystic neoplasms which resembles human branch-duct IPMNs. Furthermore, the IPMN lesions progress to form IPMN-derived PDA, which carry a better prognosis than PanIN-PDA. In this article, we discuss the molecular mechanism and characteristics of IPMN and IPMN-derived PDA in the aspect of the role of chromatin regulator, Brg1, and their cellular origin.

Pancreatic loss of Brg1 in the context of oncogenic *Kras* results in formation of cystic neoplasms that resemble human IPMNs.

Brg1 was expressed in all pancreatic cell types in mice¹⁸. Mutant *Kras* (*Kras*^{G12D}) is sufficient to drive the development of PanIN and PDA when targeted to the developing mouse pancreas¹⁹. To investigate whether *Brg1* loss alters *Kras*-driven pancreatic transformation, we generated *Ptf1a-Cre; Kras*^{G12D};

Brg1^{ff} (*KP.Brg1*) mice. *KP.Brg1* mice developed grape-like cystic structures throughout the pancreas (Fig. 1a,b)¹⁸. The epithelial-lined cystic lesions were *Brg1*-negative, CK19-positive, and alcian blue-positive indicative of mucin-production. Further examination revealed that (i) the pancreatic duct system had a direct functional connection to the multiple branch-duct dilated cystic structures without main-duct dilatation (Fig. 1a), (ii) the cystic lesions lacked ovarian-like stroma characterized by expression of ER α and PR. (iii) The cystic epithelium was Muc1+, Muc2-, Muc5+. Collectively, the neoplastic cystic lesions most closely resembled human branch-duct IPMNs of pancreatobiliary type.

IPMN-like lesions progress to form IPMN-derived PDA

IPMN lesions in *KP.Brg1* mice contained varying grades of dysplasia. Furthermore, IPMN-derived PDA was formed in a subset of *KP.Brg1* mice (hereafter referred to as IPMN-PDA mice) (Fig. 1c,d). Morphologically, IPMN-PDAs were indistinguishable from PanIN-derived PDAs in *Ptf1a-Cre; Kras^{G12D}; p53^{ff/+}* mice (hereafter referred to as PanIN-PDA mice)²⁰.

Brg1 null IPMN-PDA is less lethal than PanIN-PDA and carries a distinct molecular signature

Similar to the human situation^{2, 3, 21}, IPMN-PDA mice carried a more favorable prognosis for survival compared to PanIN-PDA mice. Cell proliferation of IPMN-PDA was significantly lower than that of PanIN-PDA. After establishing cancer cell lines derived from PDA that formed in IPMN- and PanIN-PDA mice, subcutaneous transplantation experiments showed that IPMN-PDA cells are intrinsically less proliferative compared to PanIN-PDA cells.

Deep sequencing analysis of RNA isolated from IPMN- and PanIN-PDA cells revealed that several genes previously shown to support malignancy (including *Mmp7*, *Gabrp*, *Hmga2*, *Clic3*, and *Adamts1*) in PDA were among the most down-regulated genes in IPMN-PDA compared to PanIN-PDA²²⁻²⁸. Additional pathway analysis revealed that IPMN-PDAs displayed a decreased gene expression signature for those signaling cascades regulating invasion and metastasis, supporting for the notion that IPMN-PDA possesses less malignant potential compared to PanIN-PDA. Neither significant changes in expression of *GNAS* and *RNF43* nor SNPs/mutations in *GNAS* were detected when comparing IPMN- vs. PanIN-PDA.

Brg1 blocks IPMN formation from adult pancreatic duct cells but is critical for acinar-derived PanIN formation

The cellular origins of PDA have been extensively debated. Considerable functional evidence from mouse models suggests that cellular origin of PanINs is acinar cell²⁹⁻³². Because IPMNs are located within and are continuous with the main and/or branch duct, they are thought to arise from duct cell compartments. However, cellular origin of IPMNs remained unclear. We next asked the question of whether the striking biological differences between PanIN-PDA and IPMN-PDA might reflect a difference in the cellular origin of the cancer precursor lesions. We generated transgenic mice permitting expression of mutant *Kras* and *Brg1* deletion specifically in adult acinar cells upon tamoxifen treatment^{30, 33}, and could not detect any IPMN lesions. Moreover, the number of PanINs was significantly reduced in the absence of *Brg1* in adult acinar cells. Of note, the majority of the few PanINs developing in the mice retained *Brg1* expression, indicating that *Brg1* plays a critical role in acinar-derived PanIN formation. Next we examined whether IPMN lesions can originate from adult pancreatic duct cells by using the tamoxifen inducible *Hnf1b-Cre^{ERT2}* line that permits Cre

recombination in adult duct cells ³⁴. Notably, a subset of *Hnf1b-Cre^{ERT2}*; *Kras^{G12D}*; *Brg1^{fl/fl}* mice showed the formation of a mucinous dilated duct structure reminiscent of the IPMN lesions. Therefore, Brg1 modulates cell-type specific responses to oncogenic Kras, representing a critical node for acinar to PanIN transformation, but an inhibitor of duct cell -derived neoplasia.

Conclusion and future direction

In summary, the chromatin regulator Brg1 inhibits formation of IPMN and IPMN-derived PDA in the context of oncogenic Kras. Brg1 blocks IPMN formation from adult pancreatic duct cells but is critical for acinar-derived PanIN formation. Although IPMN-PDA per se is histologically indistinguishable from PanIN-PDA, IPMN-PDA is biologically and molecularly distinct subtype with different cellular origin. *KP.Brg1* mice are a valid mouse model for investigating mechanisms underlying development of IPMN and IPMN-derived PDA. Future studies are needed to clarify which Brg1-regulated epigenetic and genetic changes contribute to the cell-type specific effects, and how such differences determine the development and character of PDA arising from different precursor lesions with different biological behavior. An improved understanding

of molecular mechanism of IPMN and IPMN-derived PDA and their cellular origin could reveal new avenues for novel diagnostic and therapeutic strategies of the disease.

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Figure Legends

Figure 1

Pancreatic loss of Brg1 in the context of oncogenic Kras results in formation of IPMNs and IPMN-derived pancreatic duct adenocarcinoma

(a) Macroscopic view of a *Ptf1a-Cre; Kras^{G12D}; Brg1^{ff}* pancreas at 9 weeks of age. The pancreas showed extensive grape-like cystic structures throughout the pancreas that resembles human branch-duct IPMNs. To prove a possible connection of the cystic neoplastic lesions to the pancreatic duct system blue dye (bromophenolblue) was injected in the common bile duct to allow retrograde filling of the duct system. (b) H&E staining of the IPMN-like lesions in *Ptf1a-Cre; Kras^{G12D}; Brg1^{ff}* pancreas. (c,d) PDA was formed in *Ptf1a-Cre; Kras^{G12D}; Brg1^{ff}* mice at 18 weeks of age. (c) Microscopic (H&E) view (arrowheads; PDA, arrow; adjacent IPMN cystic lesion). (d) Ki67/CK19/DAPI costaining of the IPMN-derived PDA in *Ptf1a-Cre; Kras^{G12D}; Brg1^{ff}* mice.

Figure 2.

A suggested mechanistic model of IPMN and IPMN-derived PDA

The chromatin regulator Brg1 blocks IPMN formation from adult pancreatic duct cells in the context of oncogenic Kras, while Brg1 is critical for acinar cell-derived PanIN formation.

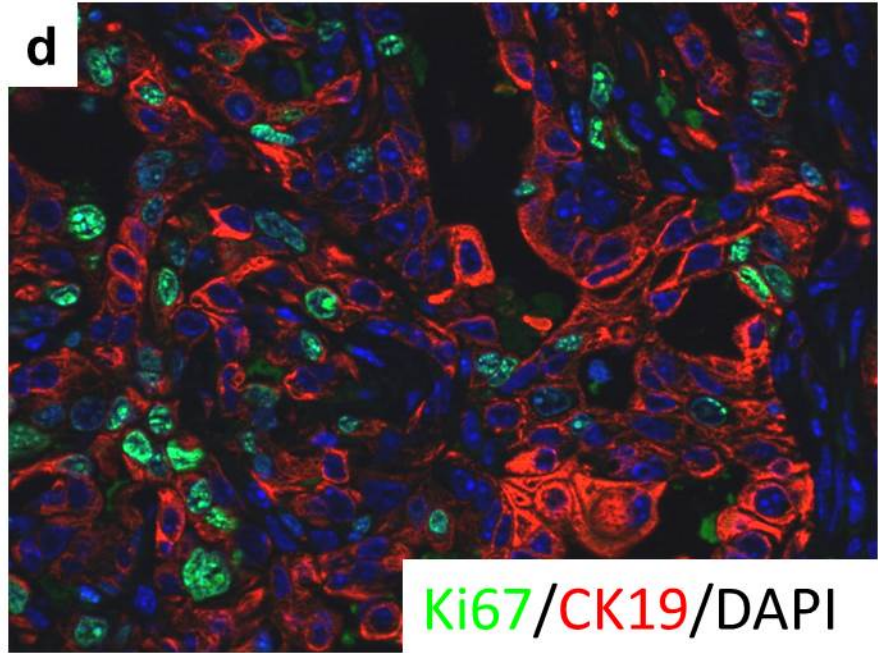
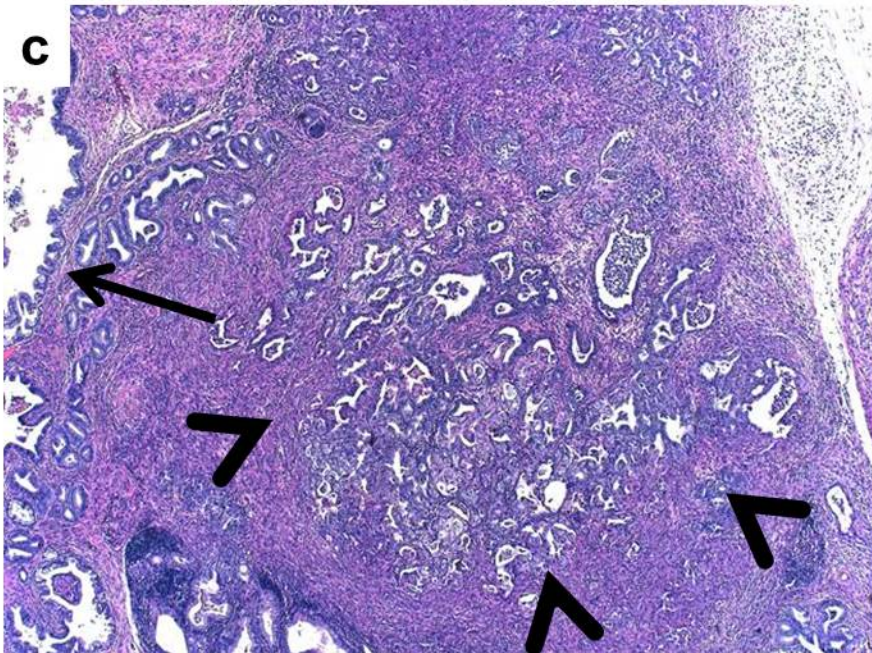
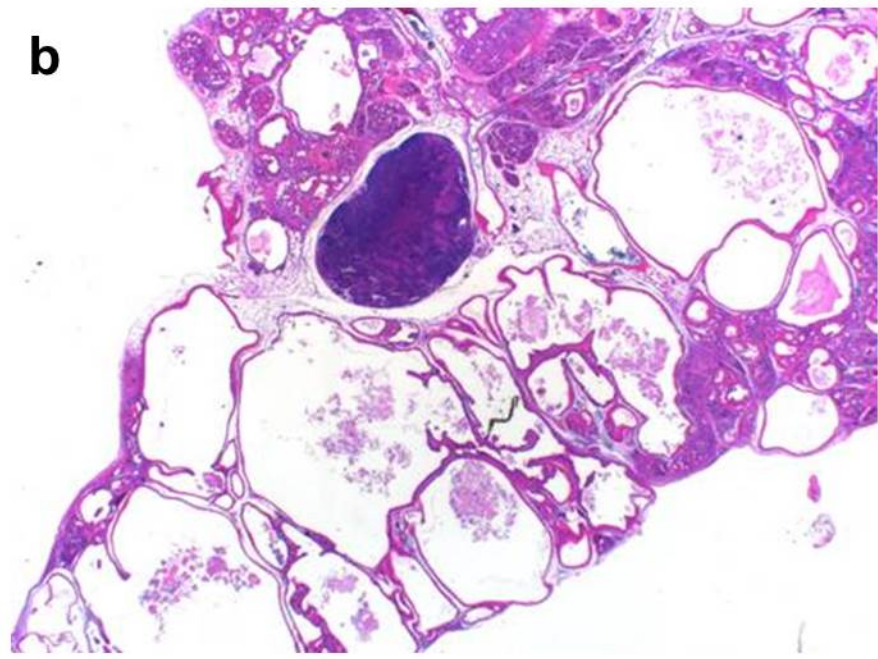
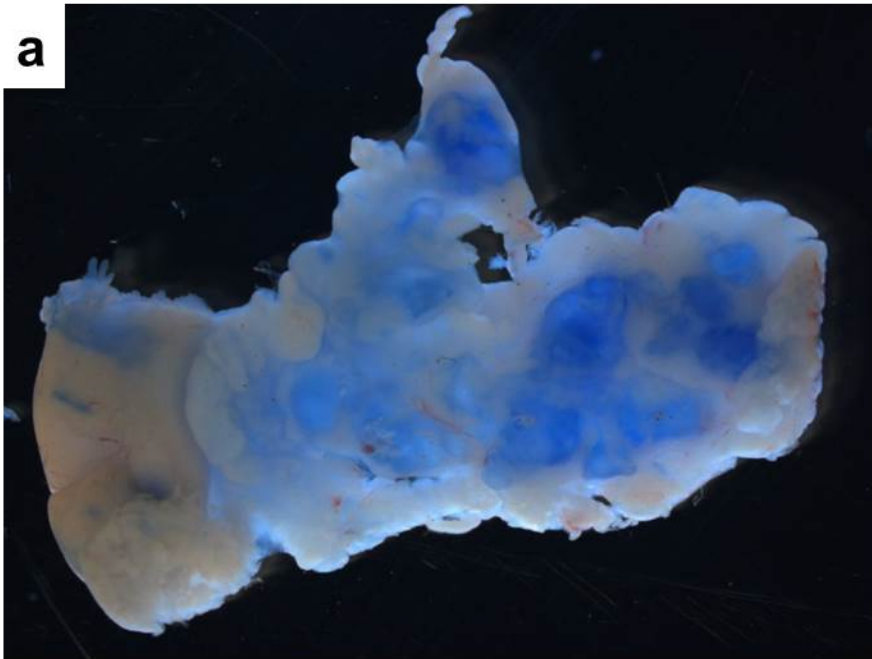
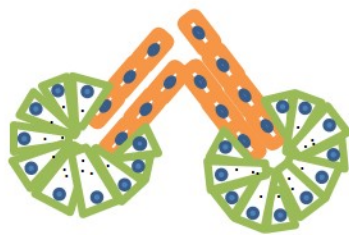
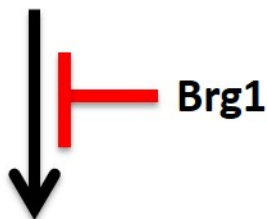
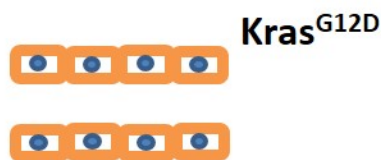


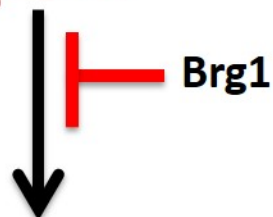
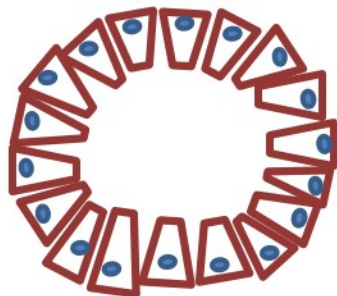
Figure 2



Pancreatic duct cell

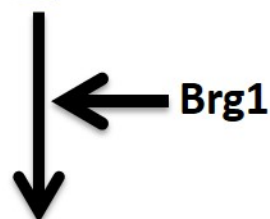
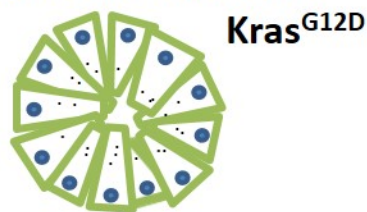


IPMN



IPMN-derived PDA

Pancreatic acinar cell



PanIN



PanIN-derived PDA