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Fbxw7 Deletion Accelerates *Kras*^{G12D}-Driven Pancreatic Tumorigenesis via Yap Accumulation[1,2,3]

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Abstract

Pancreatic cancers driven by *KRAS* mutations require additional mutations for tumor progression. The tumor suppressor FBXW7 is altered in pancreatic cancers, but its contribution to pancreatic tumorigenesis is unknown. To determine potential cooperation between *Kras* mutation and *Fbxw7* inactivation in pancreatic tumorigenesis, we generated P48-Cre;LSL-*Kras*^{G12D};*Fbxw7*$^{fl/fl}$ (KFC$^{fl/fl}$) compound mice. We found that KFC$^{fl/fl}$ mice displayed accelerated tumorigenesis: all mice succumbed to pancreatic ductal adenocarcinoma (PDA) by 40 days of age, with PDA onset occurring by 2 weeks of age. PDA in KFC$^{fl/fl}$ mice was preceded by earlier onset of acinar-to-ductal metaplasia (ADM) and pancreatic intraepithelial neoplasia (PanIN) lesions, and associated with chromosomal instability and the accumulation of Fbxw7 substrates Yes-associated protein (Yap), c-Myc, and Notch. Using KFC$^{fl/fl}$ and FBXW7-deficient human pancreatic cancer cells, we found that Yap silencing attenuated growth promotion by Fbxw7 deletion. Our data demonstrate that Fbxw7 is a potent suppressor of *Kras*^{G12D}-induced pancreatic tumorigenesis due, at least in part, to negative regulation of Yap.

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Introduction

Pancreatic cancer has an overall 5-year survival rate of 8% and is currently the fourth leading cause of cancer-related death in the United States. Nearly all human pancreatic cancers are characterized by mutations in *KRAS* followed in order of mutational frequency by *P16*, *P53*, and *DPC4* [1]. Genetically engineered *Kras* mutant mouse models of pancreatic cancer recapitulate the human disease process beginning with acinar-to-ductal metaplasia (ADM), followed by pancreatic intraepithelial neoplasia (PanIN) formation, and ultimately pancreatic ductal adenocarcinoma (PDA) [2]. The introduction of other mutations such as *p53*, *p16*, or *Dpc4* further accelerates the development of PDA. Using an inducible *Kras* mutant (G12D) mouse model, it was shown that *Kras* mutation is involved not only in initiation but also in the maintenance of PDA, as inactivation of mutant *Kras* in established tumors leads to tumor regression [3]. Furthermore, tumor regrowth following inactivation of mutant *Kras* involves expression of Yap which is also required for initiation of PDA in *Kras* mutant mice [4,5].

FBXW7, a component of the Skp1-Cullin1-Fbox E3 ubiquitin ligase complex, is a characterized tumor suppressor. For instance, conditional *Fbxw7* deletion cooperates with *APC* mutation or *P53*

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deletion to accelerate intestinal tumorigenesis in a haploinsufficient manner [6,7]. Although *FBXW7* is most frequently mutated in colorectal, uterine, and bladder cancers, it is also a significantly mutated gene in pancreatic cancers [8,9]. Furthermore, in a Sleeping Beauty transposon insertional mutagenesis model of $Kras^{G12D}$-induced pancreatic tumorigenesis, *Fbxw7* mutation cooperated with $Kras^{G12D}$ to accelerate PDA formation with a high frequency (24%), suggesting that Fbxw7 may be an important tumor suppressor in Kras-driven pancreatic cancers [10]. In addition to mutation, there is also emerging evidence to suggest that FBXW7 is altered by other mechanisms in pancreatic cancers including by reduced gene expression and heightened protein degradation which correlate with reduced survival [11].

Although the tumor suppressor functions of FBXW7 have largely been attributed to its proteolytic regulation of oncogenic substrates such as Cyclin E, c-MYC, Notch, and c-JUN, FBXW7 regulates over 30 substrates and likely unknown substrates as well [12,13]. Loss of FBXW7 promotes genomic instability in part by increased Cyclin E expression [14] which may contribute to tumorigenesis. We have recently reported that FBXW7 regulates nonhomologous end-joining (NHEJ) via K63-linked polyubiquitination of XRCC4, promoting the interaction of XRCC4 with DNA double-strand breaks and other core NHEJ proteins [15]. This direct involvement of FBXW7 in NHEJ may also contribute to the genomic instability and tumorigenesis in FBXW7-deficient models.

Because pancreatic cancer is characterized most frequently by *KRAS* mutation (~100%) [1] as well as mutations or reduced expression of *FBXW7* in a subset of human pancreatic cancers [8,9,11], in this study, we investigated whether FBXW7 acts as a tumor suppressor in $Kras^{G12D}$-driven pancreatic tumorigenesis. Using a pancreas conditional P48-Cre;LSL-$Kras^{G12D}$;$Fbxw7^{fl/fl}$ mouse model, we found that homozygous deletion of *Fbxw7* causes a dramatic acceleration of $Kras^{G12D}$-driven pancreatic tumorigenesis. Based on this finding, we went on to investigate the contribution of Fbxw7 substrates, such as Yap, to the mechanisms of tumorigenesis in both mouse and human pancreatic cancer cells.

Materials and Methods

Mouse Strains

The conditional LSL-$Fbxw7^{flox/flox}$ mouse was a gift from Dr. Iannis Aifantis (New York University) [16]. Conditional LSL-$Fbxw7^{flox/flox}$; LSL-$Kras^{G12D/+}$; and P48-Cre strains were interbred to obtain LSL-$Kras^{G12D/+}$; LSL-$Fbxw7^{flox/flox}$; P48-Cre triple mutant animals (KFC). The mice were housed in specific pathogen-free facilities, and all the studies were conducted in compliance with the Institutional Committee on Use and Care of Animals guidelines. Genotyping was carried out by tail clipping from mice 2 weeks after birth. Tail specimens were incubated for 24 hours at 55°C in tail-lysis buffer containing proteinase K. NaCl was added, and cellular debris were pelleted by centrifugation. DNA was precipitated by the addition of isopropanol and washed with 70% ethanol. DNA pellets dissolved in water were used for polymerase chain reaction (PCR) analysis.

Cell Lines

Panc-1 cells were obtained from the American Type Culture Collection. Cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% FBS, 2 mM glutamine, and antibiotics. The KFC cell line was derived from the tumor of a 3-week-old KFC mouse. Briefly, mouse pancreas was harvested and finely minced and incubated with a Collagenase V/HBSS (with Ca and Mg) solution at 37°C under constant shaking for 1.5 hours. Collagenase digestion of pancreas tissue was neutralized by RPMI-1640 medium containing 10% FBS. Digested tumor cells were filtered through a 40-μm filter and then cultured in RPMI-1640 medium containing 10% FBS, 2 mM glutamine, and antibiotics.

Acinar Cell Culture

The 3D culture of pancreatic acinar cells was prepared by digesting pancreata from 1-week-old mice with Collagenase P followed by culture in Matrigel as previously described [17]. Briefly, pancreata from KFC, KC, and control mice were cut into small pieces and digested with 2 mg/ml of Collagenase P (Roche Diagnostics) in HBSS for 15 minutes at 37°C. Cells were then washed three times with HBSS with 5% FBS and filtered through 100-μm nylon meshes. After centrifugation, the cell suspension was mixed 1:1 with Matrigel and plated onto the collagen layer. The acinar cell/Matrigel mix was allowed to solidify for 1 hour at 37°C before adding medium. The formation of ductlike structures was observed at days 1, 2, and 3.

Histopathologic Analysis

Histopathologic analysis was conducted by a pathologist (Y.Z.) on all deidentified hematoxylin and eosin (H&E)–stained slides. Pancreata sections were evaluated for ADM, PanIN1, PanIN2, PanIN3, and PDA lesions based on a previously reported classification system [2]. Pancreata were diagnosed according to the most severe phenotype observed, and data were expressed as the percentage of animals with each phenotype.

Immunohistochemistry and Immunoblotting

Immunohistochemistry was conducted as previously described [3]. Images were acquired with an Olympus BX-51 microscope, Olympus DP71 digital camera, and DP Controller software. Antibodies used for immunohistochemistry include those recognizing YAP (Cell Signaling, #4912, 1:500), c-MYC (Abcam, ab32072, 1:200), Notch (Cell Signaling, #3608, 1:250), Ki67 (BD Bioscience, Cat. 550609, 1:500), CK19 (Abcam, ab87000, 1:500), and Amylase (Sigma-Aldrich, A8273, 1:500).

Immunoblotting was conducted as previously described [15]. FBXW7 was detected by FBXW7 immunoprecipitation followed by immunoblotting. Antibodies used for immunoblotting in this study include those recognizing FBXW7 (Bethyl, A301-720A, 1:1000, overnight, 4°C), YAP (Cell Signaling, #4912, 1:3000, overnight, 4°C), c-MYC (Abcam, ab32072, 1:2000, overnight, 4°C), Notch (Cell Signaling, #3608, 1:2000, overnight, 4°C), c-JUN (Cell Signaling, #9165, 1:1000, overnight, 4°C), MCL-1 (Santa Cruz, sc-819, 1:1000, overnight, 4°C), Cyclin E (Santa Cruz, sc-198, 1:1000, overnight, 4°C), mTOR (Cell Signaling, #2972, 1:1000, overnight, 4°C), and actin (Sigma Aldrich, Clone AC-40, 1:5000, overnight, 4°C).

Lentivirus-Based shRNA Infection and siRNA Transfection

Cells were infected with lentivirus (control 5′-GCAAGCT GACCCTGAAGTTCAT-3′; FBXW7: 5′-ACAGGACAGTGTTTA CAAA-3′) in the presence of 8 μg/ml of polybrene. Silencing efficiency was detected 72 hours after infection by immunoblot. For Yap RNAi experiments, cells were transfected with Oligofectamine (Invitrogen) transfection reagent. The two independent siYap oligonucleotide sequences used were 5′-CCACCAAGCUAGAUAAAGA-3′ (siYap-2) and 5′-GCACCUAUCACUCUCGAGA-3′ (siYap-4). Nonspecific siRNA was used as a control.

Colony Formation Assay

Cells were infected with shRNA (FBXW7 or control) and then transfected with Yap siRNA. After 3 days, cells were seeded in triplicate in 60-mm dishes and allowed to form colonies for 9 to 14 days. KFC pancreatic tumor cells were transfected with two independent siYap oligonucleotides. Two days posttransfection, KFC cells were seeded to form colonies for 14 days. Colonies were fixed with 100% methanol, stained with methylene blue in methanol, and then scored.

Statistical Analysis

Survival curves were calculated according to the Kaplan-Meier method, and statistical differences were analyzed by the log-rank and Gehan-Breslow-Wilcoxon tests using GraphPad Prism. A two-sided, unpaired Student's *t* test was used for other statistical analyses. *P* values of $< .05$ were considered statistically significant.

Results

Acceleration of Kras^{G12D}-Pancreatic Tumorigenesis by Fbxw7 Deletion

To investigate the involvement of FBXW7 in mutant *KRAS*-driven pancreatic tumorigenesis, we crossed P48-Cre;LSL-*Kras*^{G12D} mice [2] with *Fbxw7*$^{fl/fl}$ mice [16] to generate mice with pancreas specific activation of *Kras*^{G12D} and heterozygous or homozygous deletion of *Fbxw7* (designated KFC$^{fl/+}$ or KFC$^{fl/fl}$, respectively; Figure 1*A*). We first confirmed deletion of Fbxw7 by examining Fbxw7 protein levels in pancreata from 1-week-old mice and found Fbxw7 protein to be substantially reduced in pancreata from KFC$^{fl/fl}$ mice compared with those from KC control mice or KFC$^{fl/+}$ mice (Figure 1*B*). To begin to establish a phenotype for these mice, survival was monitored in KC, KFC$^{fl/+}$, KFC$^{fl/fl}$ as well as in FC$^{fl/fl}$ mice. We observed a profound effect of homozygous Fbxw7 deletion on survival of KFC$^{fl/fl}$ mice (Figure 1*C*) with a median survival time of 28.5 days. Most KFC$^{fl/fl}$ displayed early symptoms of morbidity and were euthanized; 100% of KFC$^{fl/fl}$ mice succumbed to PDA by 40 days compared with only 7% of KFC$^{fl/+}$ and none of the KC or FC mice during the observation period. Gross examination of KFC$^{fl/fl}$ mice revealed smaller body size, enlarged pancreata, ascites, and duodenum adhesions in most mice with pancreas main duct obstruction and liver metastases occurring in a subset of mice (Supplementary Figure 1, *A*–*E*). Histological examination of pancreas sections from 1-month-old KFC$^{fl/fl}$ mice revealed moderately to poorly differentiated PDA accompanied by PanIN3 lesions (Figure 1*D*). In contrast, pancreata from KC or FC$^{fl/fl}$ mice appeared largely normal, although early-stage PanIN lesions were occasionally observed in KFC$^{fl/+}$ pancreata (Supplementary Figure 1*F*). To further explore the effects of heterozygous Fbxw7 deletion, we also examined pancreata from 6-month-old mice. Whereas KC mice showed some PanIN lesions, KFC$^{fl/+}$ contained more PanIN lesions, ranging from PanIN1A through PanIN3 in both genotypes, but no PDA (Figure 1*D*). Pancreata from FC$^{fl/fl}$ mice were overall normal with the exception of occasional enlarged ductal cells, consistent with a prior study [18] (Supplementary Figure 1*G*).

Tumorigenesis in Kras^{G12D};Fbxw7-Deleted Pancreata Is Preceded by ADM and PanIN and Associated with Chromosomal Instability

We then sought to characterize the progression of precursor lesions leading to PDA in KFC$^{fl/fl}$ mice. To this end, pancreata from 1-, 2-, and 3-week-old KC, KFC$^{fl/+}$, and KFC$^{fl/fl}$ mice were examined histologically and scored for the presence of ADM, PanINs, and PDA. In KC and KFC$^{fl/+}$ mice at 1 to 2 weeks of age, pancreata were composed mainly of acinar cells with no observable ADM or PanIN lesions (Figure 2, *A* and *B*). By 3 weeks of age, pancreata from KC and KFC$^{fl/+}$ mice began to develop ADM with progression toward PanIN2 in the KFC$^{fl/+}$ mice. In striking contrast, KFC$^{fl/fl}$ pancreata contained PanIN1 and PanIN2 lesions as well as stroma accumulation at 1 week, which advanced to a combination of PanIN3 and PDA at 2 weeks. By 3 weeks of age, 100% of KFC$^{fl/fl}$ pancreata contained PDA. To confirm the acceleration of ADM in KFC$^{fl/fl}$ pancreata, we used 3D cultures of acinar clusters and observed the formation of ductlike structures by as early as 1 day in KFC$^{fl/fl}$ acini (Figure 2, *C* and *D*). Further characterization of KFC$^{fl/fl}$ pancreata showed increased CK19, decreased amylase, and increased Alcian Blue staining, confirming ADM and progression toward PanIN lesions, respectively (Supplementary Figure 2).

Genomic deletion or mutation of *FBXW7* has been associated with genomic instability in various cancers [6,14,19,20]. Given the high degree of chromosomal instability (CIN) in human PDA, we examined KFC$^{fl/fl}$ pancreatic tumors for signs of CIN and found abnormal mitotic figures (Supplementary Figure 3*A*). Furthermore, using a cell line derived from KFC$^{fl/fl}$ pancreatic tumors (Supplementary Figure 3*B*), we found increased numbers of micronuclei, which are commonly observed in cells with CIN (Supplementary Figure 3*C*). Consistent with this finding, flow cytometry also revealed that the KFC tumor cell line, as well as the established KPC cell line (with *p53* deletion), is aneuploid (Supplementary Figure 3*D*). These data suggest that CIN is associated with the accelerated pancreatic tumorigenesis observed in KFC$^{fl/fl}$ mice.

Accumulation of Fbxw7 Substrates in Fbxw7-Deleted Pancreata

To begin to determine the mechanisms leading to accelerated pancreatic tumorigenesis in the KFC$^{fl/fl}$ mice, pancreata from 1-week-old mice were analyzed for expression of oncogenic substrates of Fbxw7 [12,13]. We hypothesized that Fbxw7 substrates involved in *Kras*^{G12D}-driven tumorigenesis would be accumulated in response to homozygous *Fbxw7* deletion. Of the substrates investigated, we found that the levels of c-Myc, Yap, and Notch, but not c-Jun, Cyclin E, and Mcl-1, were elevated in KFC$^{fl/fl}$ pancreata as well as in the KFC$^{fl/+}$ pancreata, although to a lesser extent (Figure 3*A*). Evaluation of these proteins immunohistochemically confirmed their increased expression within aberrant ductal structures in KFC$^{fl/fl}$ pancreata (Figure 3*B*). In addition, we also assessed the proliferative marker Ki67 and found significantly higher levels of Ki67 in KFC$^{fl/fl}$ pancreata (Figure 3*B*, Supplementary Figure 4*A*). These changes in KFC$^{fl/fl}$ pancreata appeared to be independent of Kras activity, as downstream effectors of Kras (pErk1/2 and pAkt) were not elevated in KFC$^{fl/fl}$ pancreata (Supplementary Figure 4*B*). Taken together, these data demonstrate an association between increased c-Myc, Yap, and Notch expression and *Kras*^{G12D}-induced tumorigenesis in *Fbxw7* homozygous deleted mice.

Rescue of Growth Promotion Caused by Fbxw7 Deletion by Depletion of Yap

Based on recent studies implicating Yap in *Kras*^{G12D}-induced pancreatic tumorigenesis [4,5], we hypothesized that Fbxw7 may promote tumorigenesis through a Yap-dependent mechanism. To test this hypothesis, we first evaluated the ability of Fbxw7 to regulate Yap protein levels in a cell line established from KFC$^{fl/fl}$ pancreatic

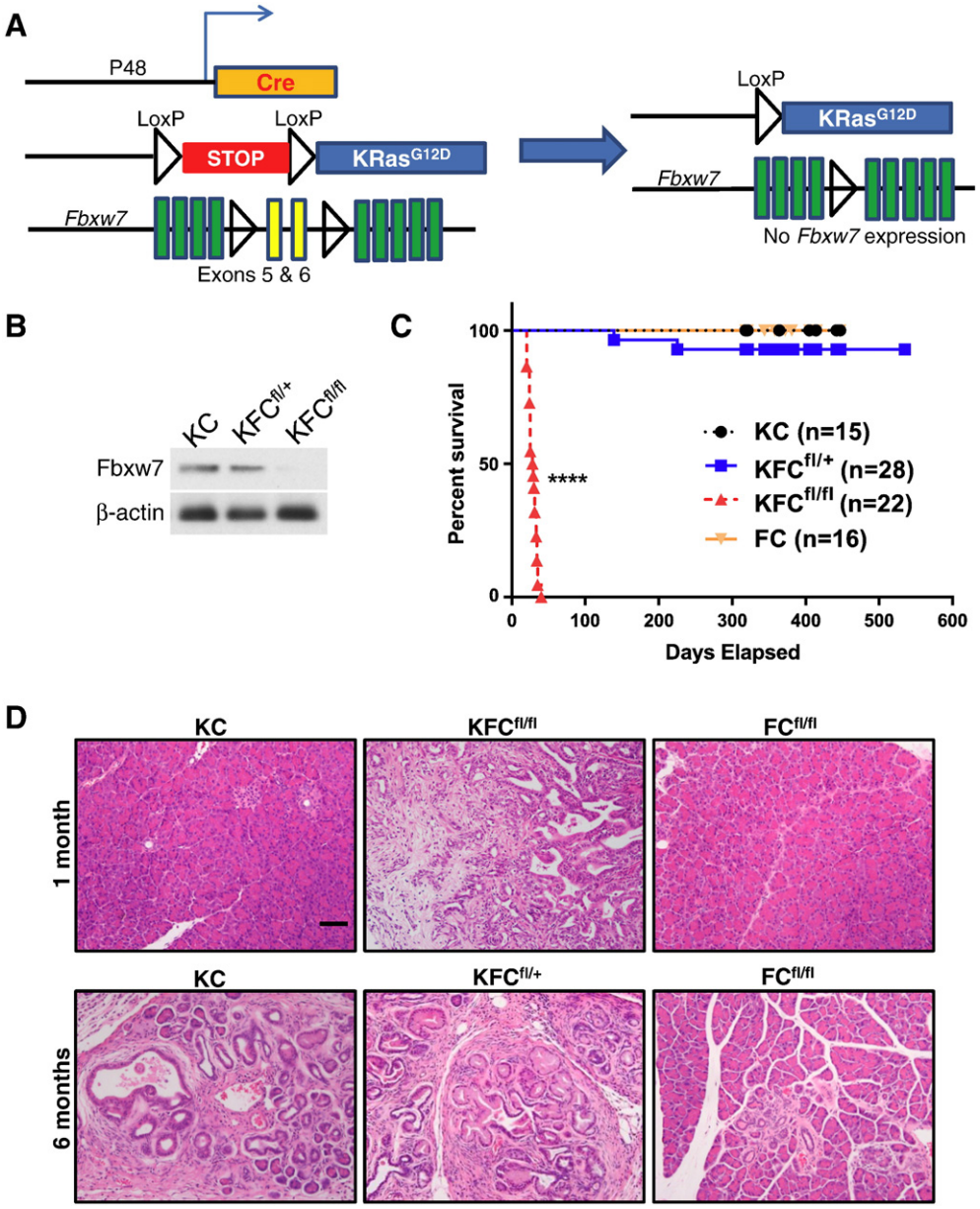

Figure 1. Deletion of mouse *Fbxw7* accelerates *Kras*^{G12D}-driven pancreatic tumorigenesis. (A) Genetic makeup of the KFC model. (B) Western blot analysis of Fbxw7 protein levels in KC, KFC$^{fl/+}$, and KFC$^{fl/fl}$ pancreata specimens from 1-week-old mice. (C) The survival of KC, FC, KFC$^{fl/+}$, and KFC$^{fl/fl}$ mice is expressed using the Kaplan-Meier method, and statistically significant differences from KC mice are shown ($P < .0001$****). (D) H&E staining of pancreata at 1 and 6 months. Scale bar, 100 μm. Each image is representative of at least three independent animals.

tumors. Consistent with the low levels of Yap observed in pancreata from KC mice (relative to KFC), exogenously expressed FBXW7 reduced Yap, c-Myc, and Notch protein levels in primary KFC-derived cells (Figure 4*A*) without substantial changes in other Fbxw7 substrates (Cyclin E, Mcl-1, mTOR; Supplementary Figure 5*A*). We then depleted Yap from KFC cells to determine its contribution to the increased growth of KFC cells and found that Yap depletion caused a significant decrease in the growth of KFC cells which was similar to the effect observed by exogenous FBXW7 expression (Figure 4*B*). Interestingly, Yap depletion was also associated with reduced c-Myc protein levels (Figure 4*A*). To further define the role of YAP in KFC$^{fl/fl}$ tumorigenesis, we utilized human pancreatic cancer cells that were deleted of FBXW7 by shRNA and then further silenced for YAP by siRNA. FBXW7 shRNA-treated Panc-1 and MiaPaCa-2 cells demonstrated increased levels of YAP and c-MYC levels without change in other FBXW7 substrates (Figure 4*C*; Supplementary Figure 5, *B* and *C*). Silencing of YAP by either of two independent siRNAs caused a reduction in c-MYC protein levels and, more importantly, significantly inhibited the growth of FBXW7-deficient human pancreatic cancer cells (Figure 4*D*, Supplementary Figure 5*D*). These results are consistent with the accelerated tumorigenesis and increased Yap levels observed in KFC$^{fl/fl}$ pancreata. Taken together, these data demonstrate that Yap accumulation is causally related, at least in part, to the accelerated growth observed in Fbxw7-deficient, *Kras* mutant pancreatic cancer cells.

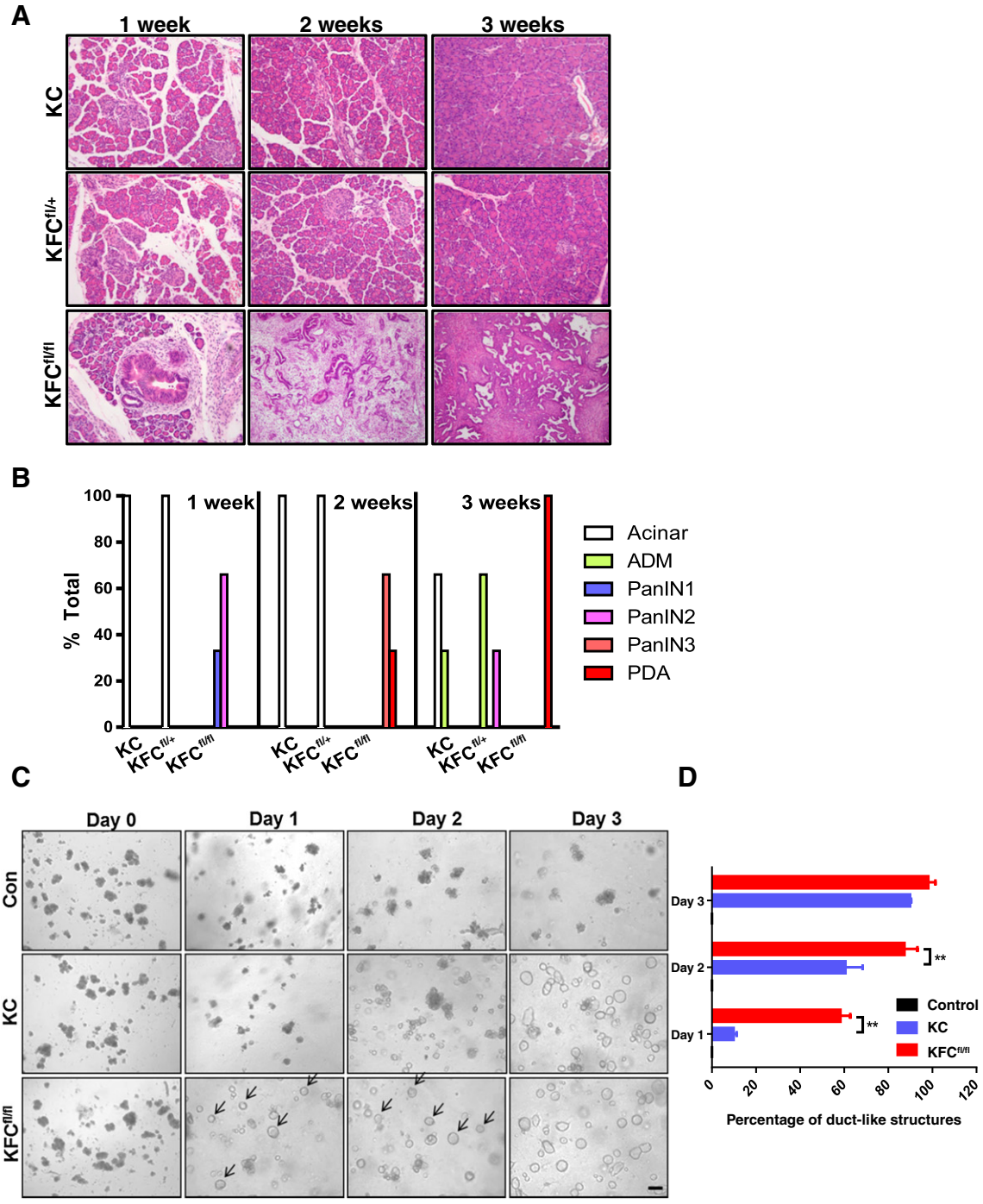

Figure 2. Pancreatic tumor progression in KFC$^{fl/fl}$ mice. (A) H&E staining of KC, KFC$^{fl/+}$, and KFC$^{fl/fl}$ pancreata at 1, 2, and 3 weeks. Scale bar, 100 μm. (B) Pathologic analysis of the lesions of KC, KFC$^{fl/+}$, and KFC$^{fl/fl}$ pancreata at 1, 2, and 3 weeks. Data represent at least three independent mice for each genotype and time point. (C) Deletion of *Fbxw7* accelerates ADM. Transmitted light images of control, KC, and KFC$^{fl/fl}$ pancreatic cell clusters in 3D culture from day 0 to 3 are shown. Scale bar, 100 μM. (D) Quantification of ductlike structures. Statistically significant differences were determined by a two-sided, unpaired Student's *t* test and are indicated ($P < .01$**).

Discussion

In this study, we show for the first time that Fbxw7 is a suppressor of *Kras* mutation-driven pancreatic tumorigenesis. The dramatically accelerated tumorigenesis observed in the KFC$^{fl/fl}$ mice was associated with accumulation of oncogenic Fbxw7 substrates such as c-Myc, Notch, and Yap. Importantly, we found that Yap expression was a driving mechanism of the accelerated tumorigenesis in response to Fbxw7 deletion given that Yap depletion attenuated growth in both KFC primary cells as well as FBXW7-deficient human pancreatic cancer cells. These findings suggest that alterations in FBXW7 may be an important contributor to human pancreatic tumorigenesis. Furthermore, our data suggest that Fbxw7 is a relatively potent suppressor of pancreatic tumorigenesis given the development of tumors by less than 1 month in response to *Fbxw7* deletion relative to

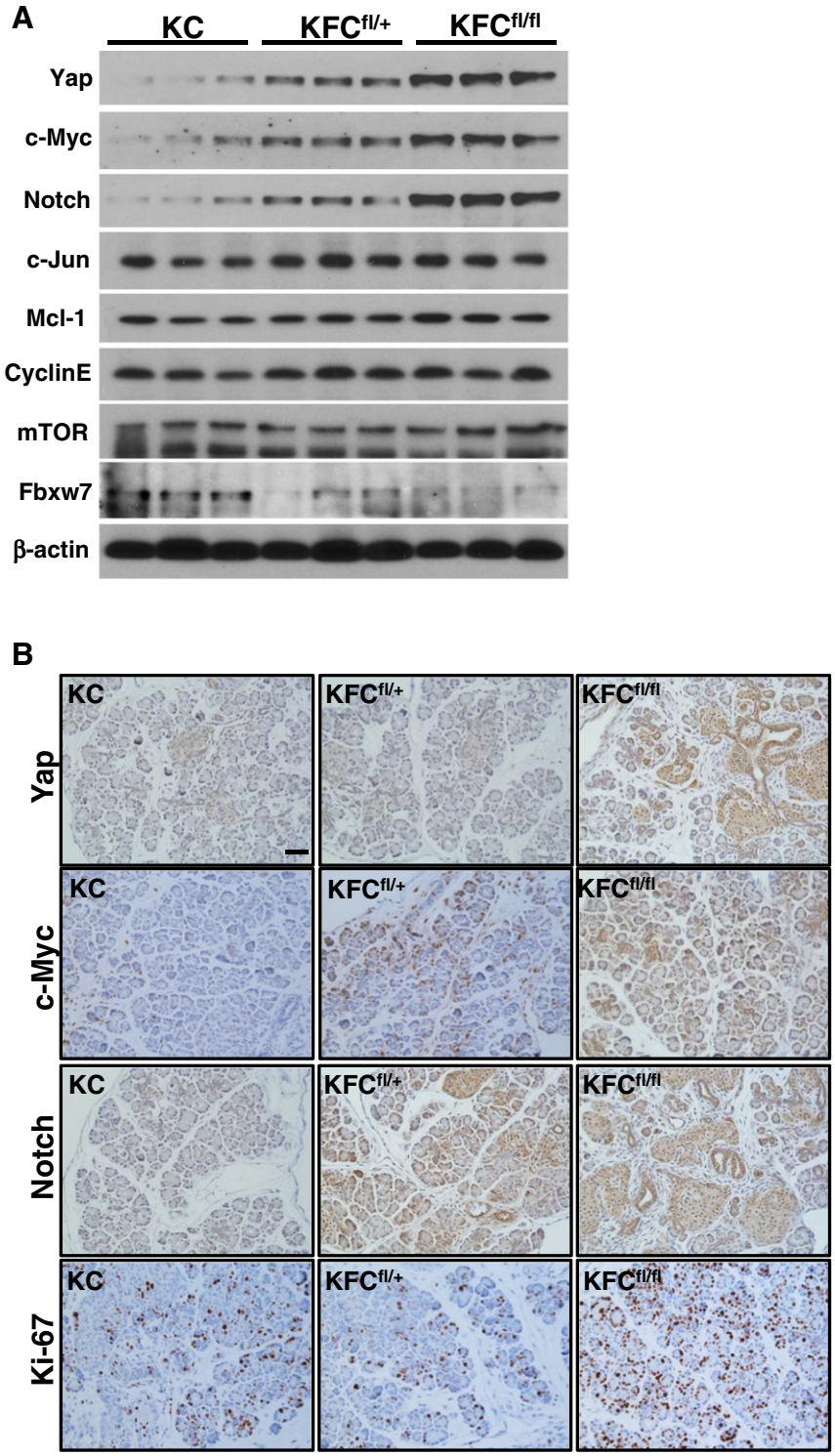

Figure 3. Analysis of Fbxw7 substrates in pancreata. (A) Western blot analysis of the indicated proteins in lysates obtained from pancreata of 1-week-old mice for each of the indicated genotypes. (B) Immunohistochemical assessment of Yap, c-Myc, Notch, and Ki67 in KC, $KFC^{fl/+}$, and $KFC^{fl/fl}$ pancreata at 1 week. Scale bar, 100 μm.

latency periods of 2 to 9 months in other $Kras^{G12D}$ models (e.g., $p16^{Ink4a}$, *P53*, and *Dpc4*) [21–23].

Although *FBXW7* is most frequently mutated in colorectal, uterine, and bladder cancers, it is also a significantly mutated gene in pancreatic cancers [8,9]. *FBXW7* mutations are generally heterozygous, missense, loss-of-function mutations occurring in the WD domain that function in a dominant negative manner [13,24]. There are however emerging evidence also to suggest that FBXW7 is altered by other mechanisms in pancreatic cancers including by reduced gene expression and heightened protein degradation [11]. It is important for future studies to define the spectrum of genetic and posttranslational alterations of FBXW7 in both primary and

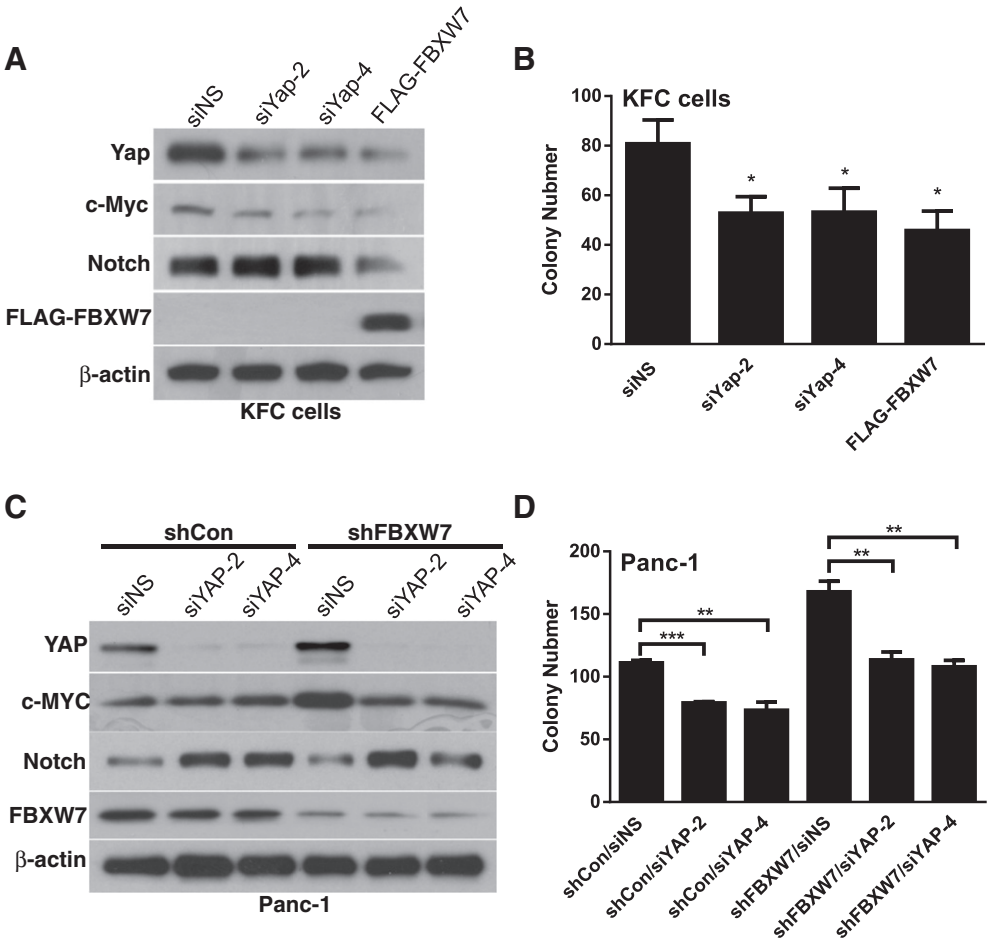

Figure 4. Depletion of Yap rescues growth in Fbxw7-deficient mouse and human pancreatic cancer cells. (A) Western blot analysis of c-Myc, Yap, and Notch proteins in KFC primary tumor cells with depletion of Yap. FLAG-FBXW7 overexpression in KFC tumor cells was used as a control. (B) The growth of KFC primary cells was measured with or without Yap depletion by colony forming assays. (C) Western blot detection of YAP, c-MYC, Notch, and FBXW7 levels in Panc-1 cells with or without FBXW7 and/or YAP depletion. (D) The growth of Panc-1 cells with or without FBXW7 and/or YAP depletion was assessed by colony formation assays. Images are representative of at least two independent experiments (A, C). Data are expressed as the mean ± SE of three independent experiments, and statistical significance is indicated ($P < .05$*, < 0.01**, or <0.001***) (B, D).

metastatic human pancreatic cancers. In addition, given the frequency of *KRAS* and *FBXW7* mutations in colorectal cancers, studies to investigate their effects on intestinal tumorigenesis are warranted.

Prior studies have shown that Fbxw7 is a haploinsufficient tumor suppressor [7,25]. In mice with *P53* deletion, heterozygous *Fbxw7* deletion resulted in the development of multiple tumor types [25]. Similarly, intestinal tumorigenesis in APC mice was accelerated by heterozygous deletion of *Fbxw7* [7]. In the current study, we found that homozygous deletion of *Fbxw7* produced a dramatic acceleration of pancreatic tumorigenesis that was not observed under heterozygous conditions. However, consistent with a haploinsufficient function of Fbxw7, $KFC^{fl/+}$ mice did have an increased frequency of PanIN lesions relative to KC mice (Figure 2*B*), leading to the development of PDA in a subset of these mice (7%). Taken together, these results are consistent with a haploinsufficient effect of Fbxw7 on PanIN development but clearly demonstrate that the most profound effects of Fbxw7 on $Kras^{G12D}$-induced tumorigenesis require homozygous deletion.

Our findings implicate Yap accumulation as being causally related, at least in part, to the accelerated growth observed in Fbxw7-deficient, *Kras* mutant pancreatic cancer cells. Furthermore, it is also possible that YAP accumulation is associated with the observed genomic instability, as other studies have shown that YAP can promote genomic instability [26]. Interestingly, we also found c-Myc, another established Fbxw7 substrate, consistently elevated in $KFC^{fl/fl}$ pancreata and FBXW7-deficient human pancreatic cancer cells. Although it is conceivable that direct regulation of c-Myc by Fbxw7 could be a mechanism of tumorigenesis, Yap depletion led to reduced expression of c-Myc (Figure 4, *A* and *C*, and Supplementary Figure 5*C*). This finding, together with a recent report demonstrating YAP-dependent c-MYC expression [27], suggests that c-Myc may function downstream of Yap in $Kras^{G12D}$-induced pancreatic tumorigenesis. Future studies are required to fully define the mechanisms of Fbxw7-mediated regulation of Yap and c-Myc in pancreatic tumorigenesis.

In conclusion, this study demonstrates that Fbxw7 suppresses $Kras^{G12D}$-induced pancreatic tumorigenesis via a Yap-dependent mechanism. These findings suggest that YAP and the Hippo pathway should be explored as a therapeutic strategy in pancreatic cancers with

FBXW7 alterations. Furthermore, based on the genomic instability associated with FBXW7 deletion [14,15], investigation of therapeutic strategies exploiting DNA damage repair pathways is warranted for cancers with FBXW7 alterations.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neo.2016.08.009.

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