Gastroenterology 2016;151:526–539

BASIC AND TRANSLATIONAL—PANCREAS

YAP1 and TAZ Control Pancreatic Cancer Initiation in Mice by Direct Up-regulation of JAK–STAT3 Signaling

Ralph Gruber,[1] Richard Panayiotou,[2] Emma Nye,[3] Bradley Spencer-Dene,[3] Gordon Stamp,[3] and Axel Behrens[1,4]

[1]Mammalian Genetics Laboratory; [2]Transcription Laboratory; [3]Experimental Histopathology, The Francis Crick Institute, Lincoln's Inn Fields Laboratory, London, UK; and [4]School of Medicine, King's College London, London, UK

See Covering the Cover synopsis on page 380; see editorial on page 393.

BACKGROUND & AIMS: Pancreatitis is the most important risk factor for pancreatic ductal adenocarcinoma (PDAC). Pancreatitis predisposes to PDAC because it induces a process of acinar cell reprogramming known as acinar-to-ductal metaplasia (ADM)—a precursor of pancreatic intraepithelial neoplasia lesions that can progress to PDAC. Mutations in KRAS are found at the earliest stages of pancreatic tumorigenesis, and it appears to be a gatekeeper to cancer progression. We investigated how mutations in KRAS cooperate with pancreatitis to promote pancreatic cancer progression in mice. **METHODS:** We generated mice carrying conditional alleles of *Yap1* and *Taz* and disrupted *Yap1* and *Taz* using a Cre-lox recombination strategy in adult mouse pancreatic acinar cells (Yap1fl/fl;Tazfl/fl;Ela1-CreERT2). We crossed these mice with LSL-KrasG12D mice, which express a constitutively active form of KRAS after Cre recombination. Pancreatic tumor initiation and progression were analyzed after chemically induced pancreatitis. We analyzed pancreatic tissues from patients with pancreatitis or PDAC by immunohistochemistry. **RESULTS:** Oncogenic activation of KRAS in normal, untransformed acinar cells in the pancreatic tissues of mice resulted in increased levels of pancreatitis-induced ADM. Expression of the constitutive active form of KRAS in this system led to activation of the transcriptional regulators YAP1 and TAZ; their function was required for pancreatitis-induced ADM in mice. The JAK–STAT3 pathway was a downstream effector of KRAS signaling via YAP1 and TAZ. YAP1 and TAZ directly mediated transcriptional activation of several genes in the JAK–STAT3 signaling pathway; this could be a mechanism by which acinar cells that express activated KRAS become susceptible to inflammation. **CONCLUSIONS:** We identified a mechanism by which oncogenic KRAS facilitates ADM and thereby generates the cells that initiate neoplastic progression. This process involves activation of YAP1 and TAZ in acinar cells, which up-regulate JAK–STAT3 signaling to promote development of PDAC in mice.

Keywords: PanINs; Pancreatic Cancer Progression; Mouse Model; Inflammation.

The most common and lethal tumor of the pancreas, pancreatic ductal adenocarcinoma (PDAC), exhibits the histologic morphology and marker expression of pancreatic duct cells. However, many of these tumors originate from the other major exocrine cell type, acinar cells, through a reprogramming process known as acinar-to-ductal metaplasia (ADM).[1] ADM is characterized by a change in marker expression: acinar cells positive for amylase also begin to express the ductal cell marker CK19. These poorly differentiated cells express the pancreatic progenitor cell markers Pdx1 and Nestin[2,3] and are important precursors of malignancy.[4] Pancreatic intraepithelial neoplasia (PanIN) lesions have a well-established histologic progression toward PDAC,[5] and can arise from ADM lesions.[1] ADM is the earliest pre-neoplastic lesion that predisposes to PDAC, making acinar-to-ductal reprogramming a crucial step in pancreatic cancer initiation.

KRas mutations are found in >90% of all PDAC cases[6] and they occur early, in low-grade pre-neoplastic lesions.[7,8] Although the importance of *KRas* mutation in the development of ADM, PanIN, and pancreatic tumors is well established,[9] the mechanisms by which oncogenic Ras leads to PDAC are not fully understood.

Pancreatitis is a well-known risk factor for PDAC development in humans. Patients with hereditary pancreatitis show a 50-fold increase in pancreatic cancer incidence.[10] In mouse models, both acute and chronic inflammation of the pancreas accelerate pancreatic cancer progression.[1,11] Pancreatitis can be induced experimentally by injection of caerulein, which induces acinar cell death and inflammation.[12] In caerulein-induced pancreatitis, inflammation induces acinar cells to reprogram to form ADM lesions,[11] which may be transient, or in the presence of a $KRas^{G12D}$ mutation, persistent.[13] Transforming growth factor–α (TGFα) administration or overexpression also cooperates with $KRas^{G12D}$ to induce ADM in vitro.[3,14] Pancreatic inflammation facilitates tumorigenesis by inducing ADM.

Expression of oncogenic $KRas^{G12D}$ at endogenous levels in acinar cells triggers progression to pancreatic adenocarcinoma very inefficiently. Stronger transgenic expression of oncogenic Ras,[15] or additional oncogenic stimuli, such as

Abbreviations used in this paper: **ADM, acinar-to-ductal metaplasia; CMV, cytomegalovirus; ES, embryonic stem cell; HBSS, Hank's balanced salt solution; IL, interleukin; PanIN, pancreatic intraepithelial neoplasia; PBS, phosphate-buffered saline; PDAC, pancreatic ductal adenocarcinoma; TGFα, transforming growth factor–α.**

Most current article

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2016.05.006

mutant p53[16] or TGFα overexpression,[17] are required. Many of these additional stimuli directly or indirectly increase Ras activity, suggesting that a positive feedback loop amplifying oncogenic Ras signaling is required for PDAC progression.[18]

The reliance of established PDAC tumors on *KRas*^{G12D} expression can be alleviated by up-regulation of the transcriptional regulator YAP1.[19] YAP1 has also been implicated in progression from PanIN to PDAC,[20] but its contribution at the reprogramming stage is unclear.

Here we show that *KRas* mutation sensitizes acinar cells to reprogramming by activating YAP1 and TAZ signaling, which in turn up-regulates components of the JAK–STAT3 pathway. This increases sensitivity to inflammatory stimuli, which induces widespread ADM among *KRas*-mutant acinar cells. Importantly, inhibiting *KRas*^{G12D}- and pancreatitis-induced reprogramming by inactivating YAP1/TAZ also prevents progression to PanIN, highlighting the importance of this mechanism for pancreatic cancer initiation.

Materials and Methods

Mouse Lines

For the generation of conditional *Yap1* and *Taz/Wwtr1* knockout mice, embryonic stem (ES) cell clones carrying the respective targeted alleles were obtained from the Knockout Mouse Project (KOMP) Repository (Yap1<tm1a(KOMP)Mbp>; ES clone E08) and the European Conditional Mouse Mutagenesis (EUCOMM) Program (Wwtr1<tm1a(EUCOMM)Wtsi>; ES clone A08). Targeted ES cell clones (agouti C57BL/6 parental cell line JM8A3.N1) were injected into blastocysts of C57BL/6 background to generate chimeric mice, which were then crossed with *Flpo*-expressing mice (Tg(CAG-Flpo)1Afst; C57BL/6 background)[21] to remove the neo-selection cassette and obtain mice carrying the conditional floxed alleles Yap1<tm1c(KOMP)Mbp> and Wwtr1<tm1c(EUCOMM)Wtsi> isogenic on C57BL/6. The *Flpo*-allele was bred out and mice were maintained on a C57BL/6 background. For genotyping and validation of knockout alleles, see Supplementary Figure 4 and Supplementary Materials and Methods.

The *Stat3*$^{fl/fl}$, *Pdx1-Cre*, *R26-LSL-YFP*, *LSL-KRas*^{G12D}, and *Ela1-Cre*^{ERT2} mouse lines have been described (see Supplementary Materials and Methods).

Acute Pancreatitis Induction

Acute pancreatitis in C57BL/6 mice was induced by 8 intraperitoneal injections of caerulein (Sigma-Aldrich, St Louis, MO) dissolved in phosphate-buffered saline (PBS) given at 1-hour intervals on 2 consecutive days at a dose of 50 μg/kg body weight per injection (high dose). Other strains received a similar treatment schedule except with 6 injections at a dose of 40 μg/kg body weight per injection (low dose). Control animals received injections of PBS only.

Gene Set Enrichment Analysis

Gene set enrichment analysis was performed on the published transcription profile of C57BL/6 Jackson mice treated with caerulein or PBS[22] and on the transcription profile of mouse liver organoids overexpressing YAP1,[23] with the software developed by the Broad Institute of the Massachusetts Institute of Technology and Harvard University. The settings are listed in Supplementary Materials and Methods.

Primary Acinar Cell Culture

Mice were sacrificed by cervical dislocation, the pancreas was dissected out and transferred to ice-cold Hank's balanced salt solution (HBSS) supplemented with penicillin/streptomycin (Sigma-Aldrich). Pancreata were cut into small pieces and digested with 2 mg/mL Collagenase P (Roche Diagnostics, Indianapolis, IN) in HBSS for 15 minutes at 37°C. Cells were washed 3 times with HBSS supplemented with 5% fetal bovine serum and then filtered through 500-μm and 105-μm nylon meshes (Spectrum Laboratories, Rancho Dominguez, CA). The cell suspension was layered on top of 30% fetal bovine serum in HBSS, centrifuged at 1000 rpm for 2 minutes and the cell pellet was resuspended in acinar cell culture medium ([Waymouth's medium; Life Technologies, Carlsbad, CA] supplemented with 1% fetal bovine serum, penicillin/streptomycin [Sigma-Aldrich], 1 μg/mL dexamethasone [Sigma-Aldrich] and 100 μg/mL soybean trypsin inhibitor [Sigma-Aldrich]). The cell suspension was then infected with adenoviruses either obtained from the University of Iowa Carver College of Medicine (Ad5-GFP and Ad5-Cre-GFP), at a concentration of 1.25×10^7 plaque-forming units/mL, or generated by cloning (Ad-GFP, Ad-GFP-YAP1-5SA, and Ad-GFP-TAZ-S89A), at a concentration of 5×10^5 plaque-forming units/mL. Acinar cells were incubated with adenoviruses for 1 hour at 37°C. Six-well tissue culture plates were coated with an 800 μL layer of collagen solution (4 mg/mL rat tail collagen; BD Biosciences, San Jose, CA), 10% 10× Waymouth's medium (Sigma-Aldrich) and 0.02 mol/L NaOH; Sigma-Aldrich). The cell suspension was mixed 1:1 with collagen solution and plated onto the collagen layer. The acinar cell/collagen mix was allowed to solidify for 1 hour at 37°C before adding medium. Medium was changed on day 1 and day 3 after the isolation. Quantifications were done on day 5 after isolation. To harvest the cells, the collagen matrix was digested with 1 mg/mL collagenase P (Roche Diagnostics) diluted in HBSS for 30 minutes at 37°C.

Production of Recombinant Adenoviruses

The vectors harboring the coding sequences of the phosphorylation mutants of YAP1 (5SA) and TAZ (S89A), kindly provided by S. Piccolo, were used to subclone the YAP1 and TAZ complementary DNAs in the pAd-Track-cytomegalovirus (CMV) vector (Addgene, Cambridge, MA; #16405). This results in the expression of YAP1/TAZ under the control of the CMV promoter and pAd-Track-CMV empty vector was used as control. The pAd-Track-CMV contains a GFP complementary DNA under control of a second CMV promoter. Recombinant adenoviruses expressing GFP, YAP1-5SA in combination with GFP or TAZ-S89A in combination with GFP were generated following the protocol as described.[24] Briefly, human HEK293A cells were co-transfected with pAd-Easy-1 (Addgene #16400) together with pAd-Track-CMV, pAd-Track-CMV-YAP1-5SA, or pAd-Track-CMV-TAZ-S89A. Adenoviruses were harvested 14 days after transfection. To generate higher viral titer, fresh HEK293A were infected with the adenoviruses and grown for 7 days before harvesting and this process was repeated 3 times.