BASIC AND TRANSLATIONAL—PANCREAS

Early Requirement of Rac1 in a Mouse Model of Pancreatic Cancer

IRINA HEID,* CLARA LUBESEDER–MARTELLATO,* BENCE SIPOS,‡ PAWEL K. MAZUR,* MARINA LESINA,* ROLAND M. SCHMID,* and JENS T. SIVEKE*

*II. Medizinische Klinik Klinikum rechts der Isar, Technische Universität München, Munich; and ‡Department of Pathology, University Hospital Tübingen, Tübingen, Germany

See editorial on page 427.

BACKGROUND & AIMS: Pancreatic ductal adenocarcinoma (PDAC) is a fatal disease without effective chemopreventive or therapeutic approaches. Although the role of oncogenic Kras in initiating development of PDAC is well established, downstream targets of aberrant Ras signaling are poorly understood. Acinar-ductal metaplasia (ADM) appears to be an important prerequisite for development of pancreatic intraepithelial neoplasia (PanIN), a common precursor to PDAC. RAS-related C3 botulinum substrate 1 (Rac1), which controls actin reorganization, can be activated by Ras, is up-regulated in several human cancers, and is required for cerulein-induced morphologic changes in acini. We investigated effects of loss of Rac1 in Kras-induced pancreatic carcinogenesis in mice. **METHODS:** Using a Cre/lox approach, we deleted *Rac1* from pancreatic progenitor cells in different mouse models of PDAC and in mice with cerulein-induced acute pancreatitis. Acinar epithelial explants of mutant mice were used to investigate the role of Rac1 in vitro. **RESULTS:** Rac1 expression increased in mouse and human pancreatic tumors, particularly in the stroma. Deletion of *Rac1* in $Kras^{G12D}$-induced PDAC in mice reduced formation of ADM, PanIN, and tumors and significantly prolonged survival. Pancreatic epithelial metaplasia was accompanied by apical-basolateral redistribution of F-actin, along with basal expression of Rac1. Acinar epithelial explants that lacked Rac1 or that were incubated with inhibitors of actin polymerization had a reduced ability to undergo ADM in 3-dimensional cultures. **CONCLUSIONS: In mice, Rac1 is required for early metaplastic changes and neoplasia-associated actin rearrangements in development of pancreatic cancer. Rac1 might be developed as a diagnostic marker or therapeutic target for PDAC.**

Keywords: Genetically Engineered Mice; Ductal Cell; Cytoskeleton; Signaling.

Invasive pancreatic ductal adenocarcinoma (PDAC) is the most frequent and highly lethal type of pancreatic cancer. One of the reasons for the high lethality of PDAC is the very late and difficult diagnosis due to absence of early symptoms and of robust diagnostic markers. To date, 3 main forms of precursor lesions have been described: pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm, and mucinous cystic neoplasm. The progression of PanIN lesions from PanIN1 to PanIN3, the carcinoma in situ, is the most common and best-characterized model for development of PDAC (for review, see Hezel et al[1]). Nevertheless, the initiation process, the cell of origin, as well as the signaling events leading to precursor occurrence are still poorly understood.

Morphologic similarities suggest PanINs derive from ductal cells.[2] However, recent lineage tracing studies confirmed that premalignant lesions can arise from differentiated acinar cells in part through a reprogramming mechanism named acinar-ductal metaplasia (ADM).[3] Along this process, acinar cells react to various stimuli, including Notch signaling, growth factors, and inflammation or cellular damage, thereby reducing expression of exocrine markers and developing into tubular structures with ductal properties. One of the best-known pathways involved in ADM is epidermal growth factor receptor (EGFR) signaling. Exposure of acinar cells to the EGFR main ligand transforming growth factor (TGF)-α strongly induces ADM in vitro and in vivo.[4–9] In addition, aberrant activation or overexpression of oncogenic $Kras^{G12D}$ results in occurrence of ADM.[5,10] This process is dramatically accelerated when oncogenic $Kras^{G12D}$ is combined with overexpression of TGF-α[11] or activation of Notch signaling.[3] Finally, repeated injections of cerulein, a pancreatitis-inducing cholecystokinin (CCK) analogue, have been used in several studies to investigate the role of ADM in initiation and progression of PDAC.[12–14] Thus, EGFR-, Notch-, and/or Kras-activation driven as well as inflammation-induced transdifferentiation of acinar cells to a

Abbreviations used in this paper: ADM, acinar-ductal metaplasia; BrdU, bromodeoxyuridine; CK19, cytokeratin 19; CytD, cytochalasin D; EGFR, epidermal growth factor receptor; LatA, latrunculin A; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; Rac1, RAS-related C3 botulinum substrate 1; TGF, tumor growth factor; WT, wild-type.

0016-5085/$36.00
doi:10.1053/j.gastro.2011.04.043

ductal-like phenotype is believed to be a crucial step in the initiation of pancreatic carcinogenesis.

However, little is known about the role of Kras and EGFR downstream targets in induction of ADM/PanIN and progression of PDAC. One potential candidate is RAS-related C3 botulinum substrate 1 (Rac1), an EGFR and Kras effector molecule. Rac1 belongs to the Rho family of small guanosine triphosphatases.[15] Guanosine triphosphatases function as molecular switches between inactive guanosine diphosphate– and active guanosine triphosphate–bound form, receiving extracellular signals from growth factor receptors and integrins and transducing them to the intracellular target.[16] The best-described function of Rac1 is the engagement in actin cytoskeleton rearrangements and cellular motility.[17–19] Specifically, activated Rac1 causes the uncapping of the fast-growing end of actin filaments and induces rapid actin polymerization. In a mouse model of chemically induced pancreatitis, Rac1 was proven necessary for CCK-induced acinar morphologic changes, F-actin redistribution, and amylase secretion.[20] Additionally, several studies reveal that Rac1 is implicated in control of cell cycle, growth and survival,[21,22] Ras-induced transformation,[23,24] and resistance to chemotherapy.[25] Overexpression of Rac1 has been detected in human patient samples of breast, gastric, testicular, oral squamous cell, lung, and pancreatic cancer (70%).[26,27] In addition, the crucial role of Rac1 in the tumor initiation process has been described in mouse models of colorectal,[28] skin,[29] and lung[30] cancer, showing that *Rac1* overexpression promoted and ablation of *Rac1* decreased tumor progression. Here we describe the phenotype of conditional *Rac1* deletion in well-characterized $Kras^{G12D}$-induced murine models of PDAC and acute pancreatitis. We provide evidence for an important role of Rac1 and actin cytoskeleton rearrangements during ADM, underlining the importance of Rac1 in the development of pancreatic precursor lesions.

Materials and Methods

Mouse Strains and Experimental Pancreatitis

$Ptf1a^{wt/Cre}$, $Kras^{wt/LSL\text{-}G12D}$, $Trp53^{wt/LSL\text{-}R172H}$, $Rac1^{fl/fl}$, and $Rosa26^{wt/LSL\text{-}LacZ}$ mouse strains have been described before.[4,11,31–33] Mice were of mixed 129SV/C57BL/6 background. The genotypes are listed in Figure 2*F*. Acute pancreatitis was induced by administration of 8 hourly intraperitoneal injections of cerulein (10 μg/kg body wt) over 2 consecutive days as described previously.[34] Pancreata were analyzed 24 and 72 hours after the last injection. All experiments were performed according to the guidelines of the local animal use and care committees.

Statistical Analyses

Kaplan–Meier curves were calculated using the survival time for each mouse from all littermate groups and log-rank test for significance. For all other analyses, the unpaired 2-tailed Student *t* test was performed with $P < .05$ considered as significant.

Supplementary Data

Detailed description of additional procedures and supplementary figures are provided in the Supplementary Materials and Methods.

Results

Increased Rac1 Expression in Development and Progression of PDAC

To determine whether Rac1 is involved in the initiation and/or progression of $Kras^{G12D}$-induced PDAC, we crossed $Kras^{wt/LSL\text{-}G12D}$ to $Ptf1a^{wt/Cre}$ mice (referred to as $Kras^{G12D}$) as previously described.[11] Increased expression of total Rac1 in pancreatic whole tissue was detected approximately at the age of 4 weeks, when few ductal-like metaplastic structures associated with some scattered PanINs start to develop (Figures 1*A* and *B* and 2*A*). The levels of active, guanosine triphosphate–bound form of Rac1 in $Kras^{G12D}$ pancreata at this time point were similar to wild-type (WT) controls (Figure 1*A*). The amount of total Rac1 was significantly increased during disease progression on both messenger RNA and protein levels (Figure 1*B* and *C*). Immunofluorescence analysis of WT pancreata showed weak cytoplasmic acinar expression of Rac1 (Figure 1*D* and *E*). Interestingly, Rac1 expression in ADM revealed an increased basal localization suggesting Rac1 activation, while PanINs showed almost no positivity (Figure 1*D* and *E*). Strong Rac1 expression was notable in the PanIN and PDAC surrounding stroma in murine and human samples (Figure 1*D* and Supplementary Figure 2*C* and *D*).

Loss of Rac1 Reveals No Major Pancreatic Abnormalities

Rac1 is ubiquitously expressed during pancreas development in ductal, acinar, endocrine, and mesenchymal cells. For pancreatic ablation of *Rac1*, we crossed previously described $Rac1^{fl/fl}$[33] with $Ptf1a^{wt/Cre}$ lines to generate mice with pancreatic *Rac1* deficiency (referred to as *Rac1ko*; Figure 2*F*). *Rac1ko* mice were viable and born at the expected Mendelian ratio. Successful Cre-mediated recombination was assessed by polymerase chain reaction and X-Gal staining using a $Rac1ko;Rosa26R^{LSL\text{-}LacZ}$ reporter line (Supplementary Figure 1*A* and data not shown). Immunofluorescence staining and Western blot analysis revealed loss of Rac1 in pancreata of 4-week-old animals (Supplementary Figure 1*B*).

Rac1ko pancreata revealed a grossly normal morphology up to 18 months of age (Supplementary Figure 1*C*). We noted a minor impairment in the endocrine function in 4-week-old *Rac1ko* mice (Supplementary Figure 1*D*), which persisted in older animals (data not shown). However, neither weight nor size of *Rac1ko* mice were altered compared with WT littermates, and we observed similar staining patterns of exocrine and endocrine proteins (Supplementary Figure 1*D*). Thus, we conclude that deletion of *Rac1* results in no major pancreatic abnormalities. To test if this mild phenotype was due to reconstitution by similar factors, quantitative reverse-transcription polymerase chain reaction