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BASIC AND TRANSLATIONAL—PANCREAS

Identification and Manipulation of Biliary Metaplasia in Pancreatic Tumors

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BACKGROUND & AIMS: Metaplasias often have characteristics of developmentally related tissues. Pancreatic metaplastic ducts are usually associated with pancreatitis and pancreatic ductal adenocarcinoma. The tuft cell is a chemosensory cell that responds to signals in the extracellular environment via effector molecules. Commonly found in the biliary tract, tuft cells are absent from normal murine pancreas. Using the aberrant appearance of tuft cells as an indicator, we tested if pancreatic metaplasia represents transdifferentiation to a biliary phenotype and what effect this has on pancreatic tumorigenesis. **METHODS:** We analyzed pancreatic tissue and tumors that developed in mice that express an activated form of Kras ($Kras^{LSL-G12D/+}$;$Ptf1a^{Cre/+}$ mice). Normal bile duct, pancreatic duct, and tumor-associated metaplasias from the mice were analyzed for tuft cell and biliary progenitor markers, including SOX17, a transcription factor that regulates biliary development. We also analyzed pancreatic tissues from mice expressing transgenic SOX17 alone ($ROSA^{tTa/+}$;$Ptf1^{CreERTM/+}$;*tetO-SOX17*) or along with activated Kras ($ROSAtT^{a/+}$;$Ptf1a\text{-}^{CreERTM/+}$;*tetO-SOX17*;$Kras^{LSL\text{-}G12D;+}$). **RESULTS:** Tuft cells were frequently found in areas of pancreatic metaplasia, decreased throughout tumor progression, and absent from invasive tumors. Analysis of the pancreatobiliary ductal systems of mice revealed tuft cells in the biliary tract but not the normal pancreatic duct. Analysis for biliary markers revealed expression of SOX17 in pancreatic metaplasia and tumors. Pancreas-specific overexpression of SOX17 led to ductal metaplasia along with inflammation and collagen deposition. Mice that overexpressed SOX17 along with $Kras^{G12D}$ had a greater degree of transformed tissue compared with mice expressing only $Kras^{G12D}$. Immunofluorescence analysis of human pancreatic tissue arrays revealed the presence of tuft cells in metaplasia and early-stage tumors, along with SOX17 expression, consistent with a biliary phenotype. **CONCLUSIONS:** Expression of $Kras^{G12D}$ and SOX17 in mice induces development of metaplasias with a biliary phenotype containing tuft cells. Tuft cells express a number of tumorigenic factors that can alter the microenvironment. Expression of SOX17 induces pancreatitis and promotes $Kras^{G12D}$-induced tumorigenesis in mice.

Keywords: Pancreatic Cancer; Pathogenesis; Mouse Model; Signal Transduction.

Pancreatic ductal adenocarcinoma (PDA) is currently the fourth leading cause of cancer death, with an overall 4-year survival rate of 6% and an abysmal median survival of just 4 to 6 months.[1] Because symptoms appear late in disease progression and metastasis has typically occurred by the time of diagnosis, earlier detection is likely to be invaluable. Elucidation of early detection markers requires a greater understanding of early disease pathology, such as pancreatic intraepithelial neoplasia (PanIN), a proposed precursor to PDA, and acinar-to-ductal metaplasia (ADM), a process that results in the formation of highly reactive metaplastic ducts. The consistent association of ADM with PDA suggests that lesions arise as a consequence of disruption of nearby normal tissue. Recent studies implicate them as a source of PanIN.[2,3] Consistent with this, metaplastic ducts are a hallmark of chronic pancreatitis, which may be part of the reason why chronic pancreatitis is a significant risk factor for PDA.

Epithelial metaplasia is a hallmark of inflammatory and neoplastic disease in several organs. In many tissues, normal epithelium is replaced by epithelium usually confined to a developmentally related organ; this

Abbreviations used in this paper: **ADM, acinar-to-ductal metaplasia; COX, cyclooxygenase; DAPI, 4′,6-diamidino-2-phenylindole; DCLK1, double-cortin-like kinase 1; EGFR, epidermal growth factor receptor; IF, immunofluorescence; IHC, immunohistochemistry; mPanIN, murine pancreatic intraepithelial neoplasia; PanIN, pancreatic intraepithelial neoplasia; PBG, peribiliary gland; PDA, pancreatic ductal adenocarcinoma; PDG, pancreatic duct gland; YFP, yellow fluorescent protein.**

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phenomenon occurs in adenocarcinoma of the esophagus in areas of Barrett's metaplasia, where the epithelium takes on gastric and intestinal characteristics marked by CDX2 expression, or cystitis glandularis, where bladder metaplasia becomes phenotypically colon-like.[4] In the pancreas, ADM is generally described as pancreatic acinar cells being replaced by pancreatic duct cells, with no prior description of their mimicry of epithelia of related tissues.

One cell type absent from the exocrine pancreas is the tuft cell. Tuft cells are a type of solitary chemosensory cell and are found in multiple organs but are prevalent in the developmentally related common bile duct and murine pancreatobiliary duct (the segment of duct following the intersection of the main pancreatic duct and the bile duct before fusion with the duodenum) and associated peribiliary glands (PBGs). Solitary chemosensory cells are part of the diffuse chemosensory system and are analogous to taste cells, although they do not aggregate in buds. Solitary chemosensory cells are believed to link chemosensation of intraluminal content to local control of absorptive and secretory processes as well as central nervous system activity.[5]

In our analysis of pancreatic metaplasia, we have discovered that the aberrant genesis of pancreatic tuft cells is common in $Kras^{G12D}$-induced pancreatic disease and is accompanied by epithelial expression of SOX17, a master control factor of biliary development and differentiation.[6] Lineage tracing showed that pancreatic tuft cells transdifferentiate from adult acinar cells and express a full array of markers associated with mature tuft cells found in other tissues. ADM and murine PanINs (mPanINs) consistently contained a $PDX1^{+}/SOX17^{+}$ cell population reminiscent of the common pancreatobiliary progenitor.[6] Forced expression of SOX17 in adult pancreas was sufficient to induce acinar cell transdifferentiation into a tuft cell–containing metaplasia, accompanied by a chronic pancreatitis–like phenotype, including fibrosis and an adaptive immune response. Transgenic expression of SOX17 in concert with oncogenic K-ras expression enhanced the degree of transformation of normal pancreas, suggesting that SOX17-induced metaplasia was fully susceptible to Kras-induced transformation. We conclude that pancreatic ADM found in PDA represents transdifferentiation to a biliary phenotype and contributes to disease progression through the assumption of a pancreatobiliary progenitor cell phenotype.

Materials and Methods

Mouse Strains

LSL-$Kras^{G12D/+}$, $Ptf1a^{Cre/+}$, $Ptf1a^{Cre\text{-}ERTM/+}$, *tetO-SOX17*, *MT-Tgfa*, and $ROSA^{tTa/+}$ strains have been described previously and were genotyped accordingly.[7–12] $ROSA^{YFP}$ mice were obtained from Jackson Laboratories (Bar Harbor, ME). Experiments were conducted in accordance with the Office of Laboratory Animal Welfare and approved by the institutional animal care and use committees at Stony Brook University and the Mayo Clinic.

Mouse Tissue Microarrays

Custom 5-mm tissue microarrays were assembled by a hand corer and precast recipient molds. PDAs from 10 *LSL-*$Kras^{G12D}$*;*$P53^{R172H/+}$*;*$PDX1^{Cre/+}$ mice were included. Adjacent PanIN-containing tissues were included for 5 tumors, and distant metastases from several organ sites were included for 5 other tumors.

Human Samples

Distribution and use of all human samples was approved by the institutional review boards of Vanderbilt University Medical Center and the Mayo Clinic.

Induction of Experimental Pancreatitis

Cerulein-induced pancreatitis was achieved by treating mice twice daily with 250 μg/kg cerulein (Sigma-Aldrich, St Louis, MO) for 7 days and allowing mice to recover for 1 day. Metaplasia was induced in the *MT-Tgfa* strain by administration of 25 mmol/L $ZnSO_4$ in drinking water for 3, 6, or 9.5 months.

Overexpression of SOX17

Adult acinar cell–specific overexpression of SOX17 was accomplished by treating 6- to 12-week-old mice with 5 daily doses of 5 mg/kg tamoxifen administered through oral gavage to $ROSA^{tTa/+}$*;*$Ptf1a^{CreERTM/+}$*;tetO-SOX17* mice with 6 weeks of recovery. The identical protocol was used with $ROSA^{tTa/+}$*;*$Ptf1a^{CreERTM/+}$*;tetO-SOX17;*$Kras^{LSL\text{-}G12D;+}$ mice.

Tumor quantitation was performed by scanning 4 random H&E-stained slides from each pancreata with an Aperio slide scanner (Vista, CA). Aperio ImageScope software was used to delineate and quantitate areas flanked by fibrosis and containing metaplasia or neoplasia compared with total tissue area.

Immunostaining

Tissues were harvested and fixed overnight in 4% paraformaldehyde. Immunohistochemistry (IHC) was performed as previously described.[13] Slides were counterstained with hematoxylin and photographed on an Olympus BX41 light microscope (Olympus, Tokyo, Japan). Immunofluorescence (IF) was performed as previously described with some modifications (Supplementary Methods).[14]

Electron Microscopy

Tissue was prepared for electron microscopy by perfusion of mice with 2% paraformaldehyde/2.5% electron microscopy–grade glutaraldehyde in 0.1 mol/L phosphate-buffered saline, pH 7.4. Samples were viewed with a Tecnail2 BioTwinG[2] transmission electron microscope (FEI, Hillsboro, OR) at 80 kV. Digital images were acquired with an XR-60 CCD digital camera system (Advanced Microscopy Techniques, Woburn, MA).

Quantitation of Tuft Cells

DCLK1 IHC was performed on paraffin-embedded tissue from 11 *LSL-*$Kras^{G12D}$*;*$Ptf1a^{Cre/+}$ mice ranging in age from 4 months to 1 year using a Discovery XT autostainer (Ventana Medical Systems, Tuscon, AZ). A minimum of 20 images at 40× were acquired per slide and lesions staged. Tuft cells were quantitated as $DCLK1^{+}$ cells per number of nuclei per lesion. For quantitation in *MT-Tgfa* mice, DCLK1 IHC was performed