The American Journal of Pathology, Vol. 171, No. 1, July 2007

DOI: 10.2353/ajpath.2007.061176

Tumorigenesis and Neoplastic Progression

Acinar Cells Contribute to the Molecular Heterogeneity of Pancreatic Intraepithelial Neoplasia

Liqin Zhu,* Guanglu Shi,* C. Max. Schmidt,† Ralph H. Hruban,‡ and Stephen F. Konieczny*

From the Department of Biological Sciences and the Purdue Cancer Center, Purdue University, West Lafayette, Indiana; the Departments of Surgery and Biochemistry and Molecular Biology,† Indiana University School of Medicine, Indianapolis, Indiana; and the Departments of Pathology and Oncology,‡ Sol Goldman Pancreatic Cancer Research Center, The Johns Hopkins University School of Medicine, Baltimore, Maryland*

A number of studies have shown that pancreatic ductal adenocarcinoma develops through precursor lesions termed pancreatic intraepithelial neoplasia (PanIN). PanINs are thought to initiate in the small ducts of the pancreas through activating mutations in the *KRAS* proto-oncogene. What remains unanswered is the identification of the individual cell type(s) that contributes to pancreatic ductal adenocarcinoma formation. To follow the cellular and molecular changes that occur in acinar and duct cell properties on $Kras^{G12D}$ expression, we took advantage of $LSL\text{-}Kras^{G12D/+}/p48^{Cre/+}$ mice, which faithfully mimic the human disease. In young animals (4 weeks), the predominant cellular alteration in the exocrine pancreas was acinar metaplasia in which individual acini consisted of acinar cells and duct-like cells. Metaplastic acinar structures were highly proliferative, expressed Notch target genes, and exhibited mosaic expression patterns for epidermal growth factor receptor, ErbB2, and pErk. This expression pattern paralleled the expression pattern detected in mouse PanINs, suggesting that mouse PanINs and acinar-ductal metaplasia follow similar molecular pathways. Indeed, immunofluorescence studies confirmed the presence of acinar cells within mPanIN lesions, raising the possibility that $Kras^{G12D}$-induced mPanINs develop from acinar cells that undergo acinar-ductal metaplasia. Identification of an acinar contribution to PanIN formation offers new directions for successful targeted therapeutic approaches to combat this disease. ***(Am J Pathol 2007, 171:263–273; DOI: 10.2353/ajpath.2007.061176)***

The development of individual cell types or organ systems progresses through restricted molecular and morphological pathways that ultimately define the endpoint phenotype. In most instances, the terminal differentiation state is static with cells primed to perform a final, highly specialized biological function. However, at times of stress, injury, or genetic alterations, cells are capable of exhibiting remarkable cellular plasticity in which tissues undergo metaplasia. Metaplasia is defined as the process by which one predominant adult cell type is replaced by a different adult cell type.[1,2] This process occurs through a variety of mechanisms, including activation and expansion of quiescent stem cells with the concomitant removal of the unwanted cell population.[3] Metaplasia can also be achieved through transdifferentiation events in which individual cells are reprogrammed to transition from one differentiated cell type into another. In instances in which this process is relatively slow, biphenotypic cells can be identified that exhibit aspects of each differentiated cell, whereas in cases of rapid conversion identification of biphenotypic cells is elusive. Metaplasia is frequently associated with an increased risk of neoplasia and is often a hallmark of many human cancers.

One cancer that shows metaplastic properties is pancreatic ductal adenocarcinoma (PDA). PDA is the fourth leading cause of cancer deaths in the United States with ~213,000 new cases diagnosed world-wide each year.[4] Despite extensive clinical efforts, the mortality of PDA patients has not significantly changed, and the 5-year survival rate (3 to 5%) remains unacceptably low. Al-

Supported by the National Institutes of Health (DK55489 to S.F.K. and National Cancer Institute Specialized Program of Research Excellence grant P50CA62924 to R.H.H.), the Department of Defense Breast Cancer Research Program (BC043093 to S.F.K.), the Phi Beta Psi Sorority Cancer Fund (to S.F.K.), Indiana University/Purdue University Collaborative Biomedical Research Pilot Grant Program (to S.F.K. and C.M.S.), the Lustgarten Foundation for Pancreatic Cancer (to S.F.K.), and the American Association for Cancer Research-Pancreatic Cancer Action Network Career Development Award in Pancreatic Cancer Research (to C.M.S.).

Accepted for publication March 22, 2007.

Address reprint requests to Stephen F. Konieczny, Department of Biological Sciences and the Purdue Cancer Center, Purdue University, Hansen Life Sciences Research Building, 201 South University St., West Lafayette, IN 47907-2064. E-mail: sfk@bio.purdue.edu.

though clinical progress has been slow, our understanding of the genetic and cellular events that precede PDA formation have been extensive and a common model for the development of PDA has emerged in which clinical, histopathological, and genetic studies have identified precursor lesions and genetic alterations that lead to PDA formation. The most common precursor lesions are known as pancreatic intraepithelial neoplasia (PanIN).[5–7] PanINs are thought to initiate in the small ducts of the pancreas and are classified from low grade to high grade (PanIN-1, PanIN-2, PanIN-3) based on the relative degree of cellular architecture and nuclear atypia. Molecular profiles for each grade of PanIN have been defined with activating mutations in the *KRAS* proto-oncogene thought to be early events in the development of PanIN-1 lesions. Additional genetic alterations occur temporally and include telomere shortening (PanIN-1), inactivation of the $p16^{INK4a}$ locus (PanIN-2), and inactivation of *Trp53*, *SMAD4/DPC4*, and occasionally *BRCA2* (PanIN-3).[5–7] These alterations cause pleiotropic effects that are reflected in the deregulation of signaling pathways controlling cell proliferation, survival, adhesion, and migration.[8,9] What remains unanswered is how these pathways generate unique pathologies within the PanIN and PDA spectrums. Equally important is identifying the individual cell types that contribute to this disease.

To study the initiation and progression events that are responsible for PDA promotion, the endogenous mouse *Kras* locus has been targeted with a $Kras^{G12D}$ allele (*LSL-*$Kras^{G12D}$).[10,11] In this model, the *LSL-*$Kras^{G12D}$ locus remains transcriptionally inert unless activated by Cre recombinase. Pancreas-specific Cre expression is provided by crossing *LSL-*$Kras^{G12D/+}$ mice to *pdx1-Cre*[10] or $p48^{Cre/+}$[12] mice, which express Cre throughout the developing and adult pancreas. *LSL-*$Kras^{G12D/+}$*/pdx1-Cre* and *LSL-*$Kras^{G12D/+}$*/*$p48^{Cre/+}$ mice develop PanINs (called mPanIN in the mouse) with 100% penetrance.[10] In all cases, the number and grade of the mPanIN lesions increase (mPanIN-1 → mPanIN-2 → mPanIN-3) with advancing age. Animals 2 to 3 months old contain primarily normal ducts (>80%), whereas the majority of the duct structures in older animals (7 to 10 months) exhibit high-grade mPanIN-3 to rare invasive and metastatic PDA.[10] Inclusion of additional genetic defects leads to more severe and rapid PDA disease.[13–15]

Although there is convincing evidence that PanINs progress to PDA as they accumulate additional genetic alterations, there remains uncertainty as to the contribution of individual cell lineages in this progression scheme. For instance, it is unknown if duct cells are solely responsible for PanIN development or whether additional cell types (acinar, islet, stellate, adult progenitor cells) may contribute to the formation of PanIN-1 lesions. Preliminary data from several groups[13,15,16] have shown that $Kras^{G12D}$- or transforming growth factor (TGF)-α-induced PanIN structures often exhibit molecular heterogeneity, suggesting that mPanIN cells are not equivalent. Indeed, these initial observations support the concept that more than one pancreatic cell lineage may participate in PanIN formation and ultimately in PDA development.

To investigate the initial changes that occur in acinar and duct cell properties on $Kras^{G12D}$ expression, we took advantage of the *LSL-*$Kras^{G12D/+}$*/*$p48^{Cre/+}$ model. Acinar-ductal metaplasia was the predominant alteration observed at 4 weeks in the pancreas. Metaplastic acinar structures were highly proliferative, expressed Notch target genes, and consisted of acinar and duct cell phenotypes. These cells also exhibited mosaic expression patterns for EGF signaling components, in which cells that retained acinar characteristics were epidermal growth factor receptor (EGFR)- and cytoplasmic ErbB2-positive but pErk-negative. In contrast, cells exhibiting a duct cell phenotype expressed high levels of pErk and nuclear ErbB2 but became EGFR-negative. Interestingly, the expression of these signaling intermediates precisely mimicked expression detected in PanIN lesions, suggesting that PanINs and acinar-ductal metaplasia follow similar molecular pathways. Indeed, early mPanIN-1 lesions consisted of both duct and acinar cell types, raising the possibility that $Kras^{G12D}$-induced PanINs develop from acinar cell lineages that undergo acinar-ductal metaplasia to generate the characteristic ductal phenotype observed in PDA.

Materials and Methods

Mouse Strains

Conditional *LSL-*$Kras^{G12D/+}$, *pdx1-Cre*, and $p48^{Cre/+}$ strains (gifts of D. Tuveson, Cambridge Research Institute, Cambridge, UK; and C. Wright, Vanderbilt University, Nashville, TN; respectively) were intercrossed to generate *LSL-*$Kras^{G12D/+}$*/*$p48^{Cre/+}$ and *LSL-*$Kras^{G12D/+}$*/pdx1-Cre* mice as previously described.[10] Individual genotypes were confirmed by standard polymerase chain reaction (PCR) conditions using gene-specific primer sets.[10] All studies were conducted in compliance with the National Institutes of Health and the Purdue University Institutional Animal Care and Use Committee guidelines.

Histological Analysis and Immunohistochemistry

Murine tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and 4-μm serial sections prepared. Routine hematoxylin and eosin (H&E) staining was performed by standard procedures. Immunohistochemistry was accomplished with biotinylated secondary antibodies using the Elite Vectastain ABC kit and peroxidase substrate diaminobenzidine kit (Vector Laboratories, Burlingame, CA). Briefly, sections were deparaffinized, rehydrated, and antigens were retrieved using the 2100-Retriever (PickCell Laboratories, Amsterdam, The Netherlands) and antigen unmasking solution (Vector Laboratories). For keratin 19 (K19) immunolabeling, antigen retrieval was done by digesting sections with 250 μg/ml proteinase K in 2.5 mmol/L $CaCl_2$ and 10 mmol/L Tris-HCl, pH 7.5, for 6 minutes at room temperature. Samples were blocked using the MOM blocking reagent (Vector Laboratories). Primary antibodies were incubated at 4°C overnight and included rabbit amylase (1:1000;

Calbiochem, San Diego, CA), rat K19 (TROMA-3, 1:100; a gift of Rolf Kemler, Department of Molecular Embryology, Max-Planck Institute, Freiburg, Germany), rabbit EGFR (1:300; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit ErbB2 (1:200; Santa Cruz Biotechnology), rabbit phospho-p44/42 MAPK (1:100; Cell Signaling, Charlottesville, VA), rabbit Hes1 (1:2000; a gift of Tetsuo Sudo, Pharmaceutical Research Laboratories, Toray Industries, Inc., Tebiro, Kamakura, Japan), mouse Ki67 (1:100; Novocastra, Newcastle On Tyne, UK), rabbit PDX1 (1:2500; a gift of Michael Rukstalis, Department of Molecular Endocrinology, Massachusetts General Hospital, Boston, MA), and rabbit Mist1 (1:2000).[17,18] For K19/amylase costaining, the K19 and amylase signals were visualized by diaminobenzidine and 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium sequential detection following the manufacturer's (Vector Laboratories) recommendations.

Protein Immunoblot Assays

Whole cell protein extracts (50 μg) were separated on 7.5% acrylamide gels, transferred to polyvinylidene difluoride membranes, and incubated with primary antibodies against rabbit amylase (1:5000; Calbiochem), rat K19 (1:500), sheep carbonic anhydrase II (1:500; The Binding Site, Birmingham, UK), EGFR (1:1000, no. 2232; Cell Signaling), rabbit Neu/ErbB2 (1:1000, sc-284; Santa Cruz Biotechnology), and rabbit phospho-ErbBs (1:1000; Cell Signaling) including phospho-EGFR (Tyr845) (no. 2231), phospho-EGFR (Tyr992) (no. 2235), and phospho-Her2/ErbB2 (Tyr1221/1222) (6B12, no. 2243). Rabbit Hsp 90α/β (1:2000, sc-7947; Santa Cruz Biotechnology) was used as a loading control. After secondary antibody incubation, the immunoblots were developed using an enhanced chemiluminescence kit (Pierce, Rockford, IL) as per the manufacturer's instructions.

RNA Expression Analysis

Total RNA was isolated from the pancreas using the RNeasy isolation system (Qiagen, Valencia, CA). One μg of total RNA was reverse-transcribed using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). cDNA reactions were amplified with *Taq* polymerase and gene-specific primers for mouse *Hes1* (5′-TCTACACCAGCAACAGTG-3′, 5′-TCAAACATCTTTGGCATCAC-3′), *Hey1* (5′-GCGGACGAGAATGGAAACTTG-3′, 5′-GCTCAGATAACGGGCAACTTCG-3′), and *Hey2* (5′-TGAGCATTGGATTCCGAGAGTG-3′, 5′-ATACCGACAAGGGTGGGCTGATTG-3′). Target sequences were amplified within the linear range using 95°C for 40-second, 55°C for 40-second, and 72°C for 55-second conditions.

Results

PanIN in humans is often associated with lobulocentric parenchymal atrophy with three distinct cellular compartments, ducts at the center of lobules with associated PanINs, a zone of acinar-ductal metaplasia, and areas of normal, differentiated acinar cells (Figure 1A).[19,20] The acinar-ductal metaplastic structures contain both acinar (zymogen granules, intense eosinophilic staining) and duct-like cells with a mucinous cytoplasm (Figure 1B). Despite these clinical observations, the relationship between PanINs and acinar structures in disease progression remains unclear.

To study the molecular events involved in acinar-ductal metaplasia, we examined the *LSL-Kras$^{G12D/+}$/pdx1-Cre* and *LSL-Kras$^{G12D/+}$/p48$^{Cre/+}$* mouse models.[10,15] These animals develop ductal lesions (mPanINs)[21] that recapitulate the full spectrum of human PanINs, eventually progressing to rare PDA. As with the human condition, the pancreatic lobules in the *Kras$^{G12D/+}$/pdx1-Cre* and *LSL-Kras$^{G12D/+}$/p48$^{Cre/+}$* mice developed three distinct zones of cells consisting of PanINs, acinar-ductal metaplasia, and normal acinar cells (Figure 1C). Examination of the mouse acinar-ductal metaplastic units again revealed the presence of acinar cells and duct-like cells within a single structure (Figure 1D). Thus, *LSL-Kras$^{G12D/+}$* mice faithfully model the human disease and provide an opportunity to identify the earliest events that participate in acinar-ductal metaplasia and mPanIN formation.

Characterization of Acinar-Ductal Metaplastic Structures in LSL-Kras$^{G12D/+}$/p48$^{Cre/+}$ Mice

Although PanINs are considered the precursor lesions to PDA, the contribution of acinar cells and acinar-ductal metaplasia to the initial stages of disease progression has not been extensively studied, in part because of the difficulty of following early events in patients. We examined young (4 to 6 weeks) *LSL-Kras$^{G12D/+}$/p48$^{Cre/+}$* mice to investigate the earliest changes in acinar and duct cell properties that are induced on *Kras^{G12D}* expression. As expected, wild-type and control *LSL-Kras$^{G12D/+}$* pancreata (lacking Cre) contained normal ducts and acinar cells and showed no signs of activation of the *Kras^{G12D}* allele (Figure 1E). *LSL-Kras$^{G12D/+}$/p48$^{Cre/+}$* pancreata similarly exhibited large areas of normal acinar and duct cells that were indistinguishable from control animals (Figure 1F). Indeed, at this age most ductal epithelium remained normal, comprised solely of cuboidal cells with uniform, round nuclei and amphophilic cytoplasm. mPanIN lesions were rarely observed. However, 4-week *LSL-Kras$^{G12D/+}$/p48$^{Cre/+}$* samples did exhibit significant acinar metaplasia in which individual acini developed distended, open lumens and contained mucin-expressing duct-like cells (Figure 1, G and H). The duct-like cells showed a loss of zymogen granules and assumed a columnar appearance with atypical nuclei.

To examine the molecular events that defined the acinar-ductal structures, *LSL-Kras$^{G12D/+}$/p48$^{Cre/+}$* tissues were immunolabeled (immunohistochemistry) for amylase (acinar-specific) and K19 (duct-specific). At 4 weeks, individual acini were identified that expressed both amylase and K19 gene products (Figure 1I). In many cases, biphenotypic cells coexpressing amylase

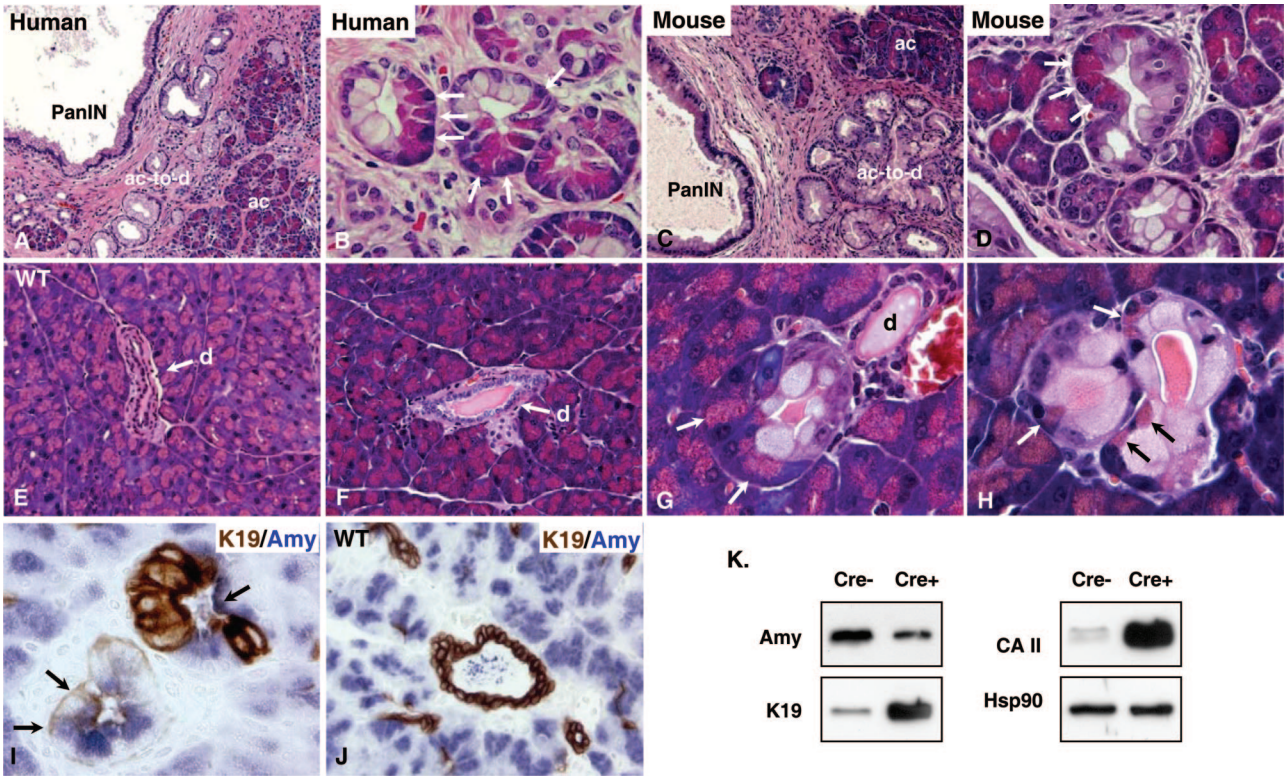


Figure 1. Acinar metaplasia is an early event in $Kras^{G12D}$ expression. **A:** PanINs in humans are often associated with lobulocentric atrophy producing three zones of cells on H&E staining—central PanIN lesions, acinar-ductal metaplasia (ac-to-d), and normal acinar (ac) cells. **B:** Higher resolution of human acinar-ductal metaplasia showing metaplastic units that are composed of acinar cells (**arrows**) and duct-like cells. **C:** *LSL-*$Kras^{G12D/+}$*/p48*$^{Cre/+}$ pancreas section containing the same three zones of exocrine cells as observed in human samples. **D:** Higher resolution images of mouse acinar-ductal metaplastic units revealing the contribution of acinar (**arrows**) and duct-like cells to these structures. **E:** H&E staining of control wild-type (WT) pancreas samples reveal normal acinar and duct (d) structures. **F:** Normal histological section from a 4-week-old *LSL-*$Kras^{G12D/+}$*/p48*$^{Cre/+}$ mouse. These areas are indistinguishable from the control sample in **E**. **G** and **H:** Early metaplastic acinar units from 4-week *LSL-*$Kras^{G12D/+}$*/p48*$^{Cre/+}$ pancreas sections revealing the contribution of acinar (**arrows**) cells and duct-like cells. **I:** An early acinar metaplasia area from a 4-week *LSL-*$Kras^{G12D/+}$*/p48*$^{Cre/+}$ sample costained for K19 (brown) and amylase (purple). These individual structures reveal biphenotypic acinar cells (**arrows**) expressing duct cell markers. Note that these structures are surrounded by normal acinar tissue. **J:** Control WT pancreas costained for K19 and amylase expression. Note the mutually exclusive expression pattern of K19 and amylase in duct and acinar cells, respectively. **K:** Immunoblot analysis on 4-month control *LSL-*$Kras^{G12D/+}$ (Cre−) and *LSL-*$Kras^{G12D/+}$*/p48*$^{Cre/+}$ (Cre+) pancreas samples. Pancreata that activate the $Kras^{G12D}$ allele exhibit a decrease in acinar cell products (amylase) and an increase in duct cell products (K19, CA-II). The Hsp90 blot serves as a loading control.

and K19 were observed. In contrast, normal ducts from wild-type or control *LSL-*$Kras^{G12D/+}$ mice never contained amylase-positive cells, and normal acinar cells never expressed K19 (Figure 1J). The molecular changes from an acinar to ductal phenotype was also observed in older animals (4 months) by immunoblot analysis in which acinar (amylase) gene products decreased and duct (K19, CA II) gene products increased on activation of $Kras^{G12D}$ expression (Figure 1K). These results demonstrate that *LSL-*$Kras^{G12D/+}$*/p48*$^{Cre/+}$ mice undergo a dramatic shift from acinar to ductal cell types on activation of the $Kras^{G12D}$ gene.

Early Acinar Metaplasia Is Associated with Cellular Proliferation, Pdx1 Expression, and Activation of Notch Signaling

The early appearance of acinar metaplasia in *LSL-*$Kras^{G12D/+}$*/p48*$^{Cre/+}$ pancreas samples suggested that metaplastic acinar cells participate in disease progression. If true, metaplastic structures should exhibit an elevated cellular proliferation index. To examine this, 4-week control *LSL-*$Kras^{G12D/+}$ and experimental *LSL-*$Kras^{G12D/+}$*/p48*$^{Cre/+}$ pancreata were analyzed for evidence of cellular proliferation. At this age, only 3.7% of acinar cells and 9.7% of duct cells from control *LSL-*$Kras^{G12D/+}$ mice expressed the cell proliferation marker Ki67 (Figure 2A). In *LSL-*$Kras^{G12D/+}$*/p48*$^{Cre/+}$ mice the proliferation rate of histologically normal acinar and of ductal cells both increased (35.1 and 37.8%) (Figure 2B). However, the proliferation indices were highest in the metaplastic acinar structures. For the earliest metaplastic events (open lumens), multiple Ki67-positive acinar cells were always identified (Figure 2, C and D), whereas only random, single Ki67-positive acinar cells were detected in age-matched control *LSL-*$Kras^{G12D/+}$ mice. As metaplastic structures grew in size, the percentage of Ki67-positive cells also increased (Figure 2, E and F). Similar results were obtained using a phospho-(ser^{10}) histone 3 antibody (data not shown). Interestingly, most proliferating cells within a single structure exhibited a duct cell phenotype whereas the few remaining Ki67-negative cells retained acinar cell characteristics (zymogens) (Figure 2, E and F). These results suggest that cells within a metaplastic acinus actively divide only after converting to a duct-like cell.

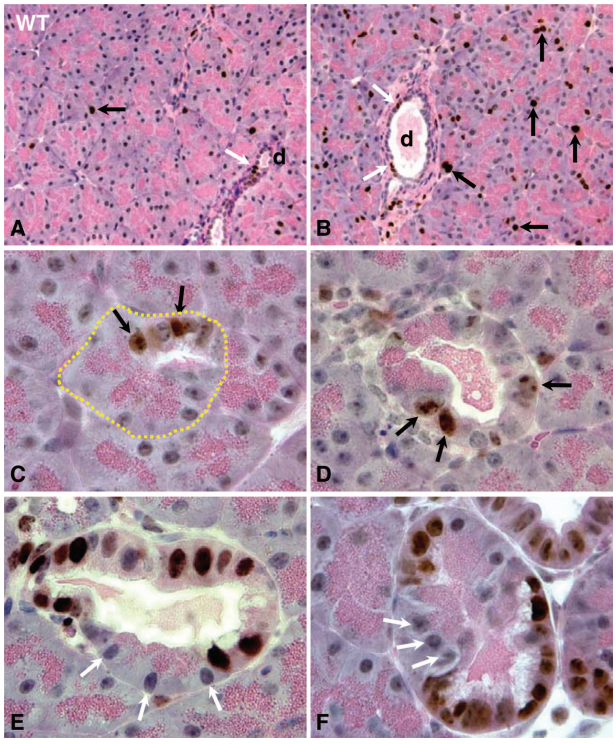


Figure 2. Ductal cells within acinar-ductal metaplastic units are highly proliferative. **A:** Control 4-week pancreas immunolabeled for Ki67. A small percentage of acinar cells (**black arrows**) and duct cells (**white arrows**) are Ki67-positive. **B:** Morphologically normal areas from a 4-week *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ mouse reveal an increase in the percentage of Ki67-expressing acinar (**black arrows**) and duct (**white arrows**) cells. d, duct. **C–F:** Early metaplastic structures from 4-week *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ mice reveal heterogeneity with respect to Ki67 expression. Whereas the acinar component (**white arrows**) within the metaplastic units remain Ki67-negative, the duct-like components (**black arrows**) are invariably Ki67-positive. Note that as the metaplastic units increase in size, the number of proliferating cells also increase. The **dotted line** in **C** highlights a single metaplastic acinar unit.

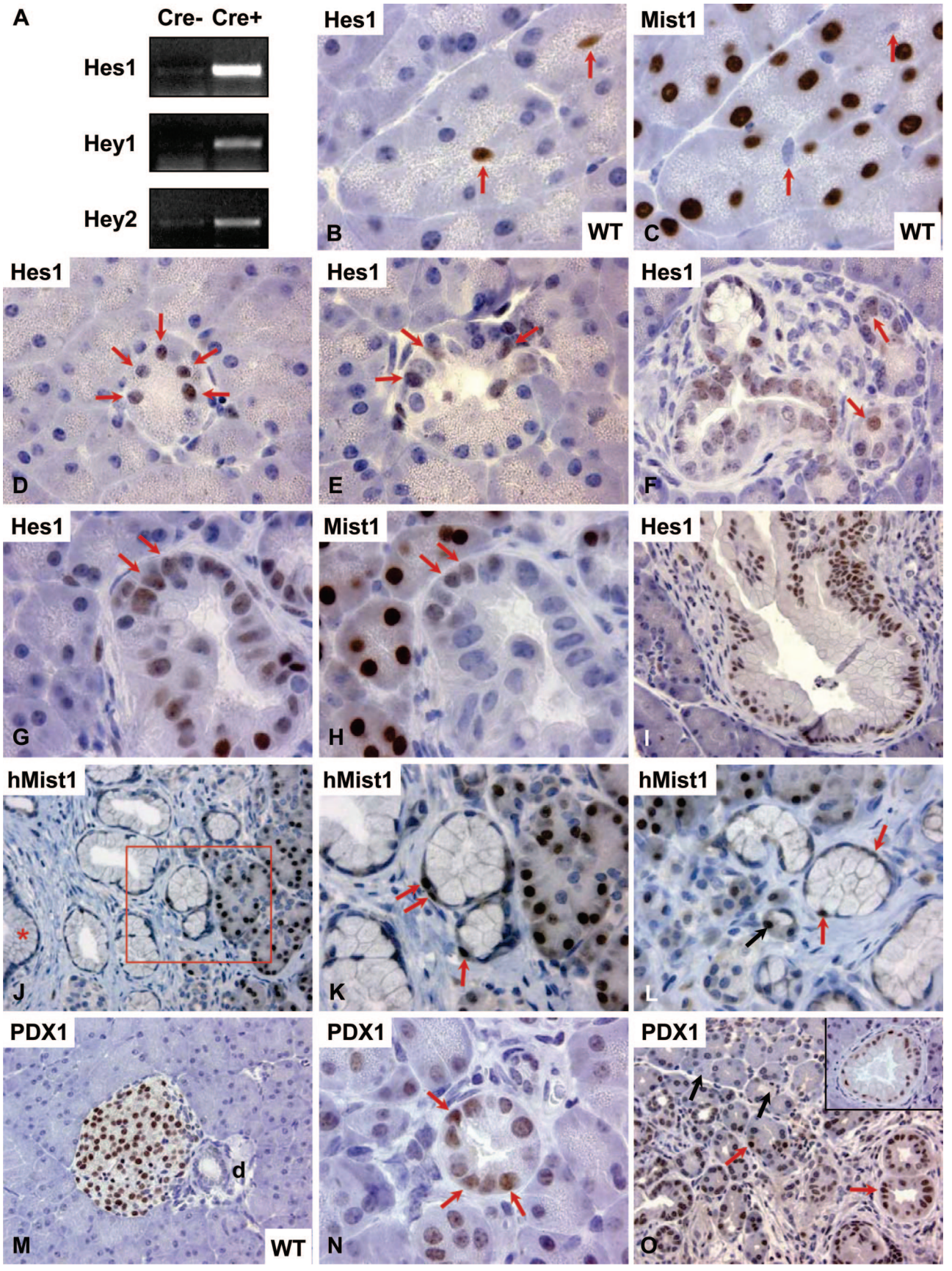
Although *Kras*^{G12D} expression is activated in all acinar cells in *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ mice,[10,12] only a subset of the cells undergo acinar-ductal metaplasia and exhibit an increased proliferation rate. Differences in specific signaling pathways probably account for some of the observed alterations in the acini. One pathway that has been implicated in pancreatic cancer progression is Notch signaling. Induction of Notch activity is thought to be an initiating event of PanIN formation and pancreatic tumorigenesis, and this pathway may be critical to maintaining transformed cells in an undifferentiated state.[16,22–25] Analysis of Notch signaling in *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ mice revealed that the Notch target genes *Hes1*, *Hey1*, and *Hey2* were transcriptionally upregulated in the presence of *Kras*^{G12D} activity (Figure 3A). Using immunohistochemistry and antibodies against Hes1 and Mist1 (an acinar-restricted transcription factor),[18,26] we next examined control *LSL-Kras*$^{G12D/+}$ and experimental *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ samples to identify the cell types involved in activation of the Notch signaling pathway. As shown in Figure 3, B and C, Hes1 expression in the control pancreas was restricted to Mist1-negative, centroacinar cells.[16] In contrast, early acinar metaplastic structures from 4-week *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ mice contained a significant number of Hes1-positive cells (Figure 3, D–F). In most cases, the differentiated acinar cells (containing zymogen granules) present in these early metaplastic structures remained Hes1-negative, whereas the duct-like cells were Hes1-positive (Figure 3E). For slightly larger acinar metaplastic structures, Hes1 protein was also detected in the surrounding acinar cell areas (Figure 3F). Indeed, Hes1 and Mist1 immunohistochemistry on adjacent serial sections revealed a subset of cells that coexpressed both proteins, confirming the acinar origin of the Hes1 expressing duct-like cells (Figure 3, G and H). Similar areas of acinar metaplasia were often found in human PDA samples in which the metaplastic units contained Mist1-positive (acinar) cells within individual ductal lesions (Figure 3, J–L). As predicted, cells within human and mouse PanINs were also Hes1-positive (Figure 3I and data not shown). These results demonstrate that *Kras*^{G12D} acinar cells and PanIN cells similarly activate the Notch signaling pathway and suggest a possible model in which acinar cells undergo a transition from Hes1^{-} → Hes1^{+} as they convert to duct-like cells.

Finally, *Hes1* expression is thought to be instrumental in maintaining transformed cells in an undifferentiated state in which pancreatic cancer precursor lesions often activate expression of embryonic genes such as *Pdx1*.[10,16] The PDX1 transcription factor is critical to pancreas development and is initially expressed in the earliest pancreatic cell lineages but becomes restricted to islet β-cells in the adult (Figure 3M).[12] In *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ mice *Pdx1* expression was activated in the early acinar metaplastic units as well as in mPanIN lesions, and this activation became more robust as acinar metaplasia progressed (Figure 3, N and O), again suggesting that both acinar-ductal metaplasia and mPanIN formation require activation of the same transcriptional network.

Activation of Specific EGF Receptor Signaling Pathways Defines Acinar and Ductal Metaplastic Properties

One hallmark of precursor ductal lesions and PDA tumor progression is the activation of specific signaling pathways that ultimately provide growth and survival advantages to the cells. In the case of PDA, the EGF, TGF-β, and Notch signaling pathways are typically maintained in a hyperactive state.[16,27] However, the cells that initially activate these components are not well defined, and many studies disagree as to which molecular markers can be used to classify the degree of cellular atypia associated with PanIN and PDA formation. These differences could reflect the difficulty in comparing molecular signatures across individual patients or species, or they could reflect normal variations in different cell types.

To identify the signaling pathway signatures that define acinar-ductal metaplastic structures and early mPanIN lesions, we examined adjacent sections of *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ pancreata for expression of EGFR, Mist1, and pErk to determine whether mPanINs and aci-


A
Cre- Cre+
Hes1
Hey1
Hey2
Hes1
B
WT
Mist1
C
WT
Hes1
D
Hes1
E
Hes1
F
Hes1
G
Mist1
H
Hes1
I
hMist1
*
J
hMist1
K
hMist1
L
PDX1
d
M
WT
PDX1
N
PDX1
O

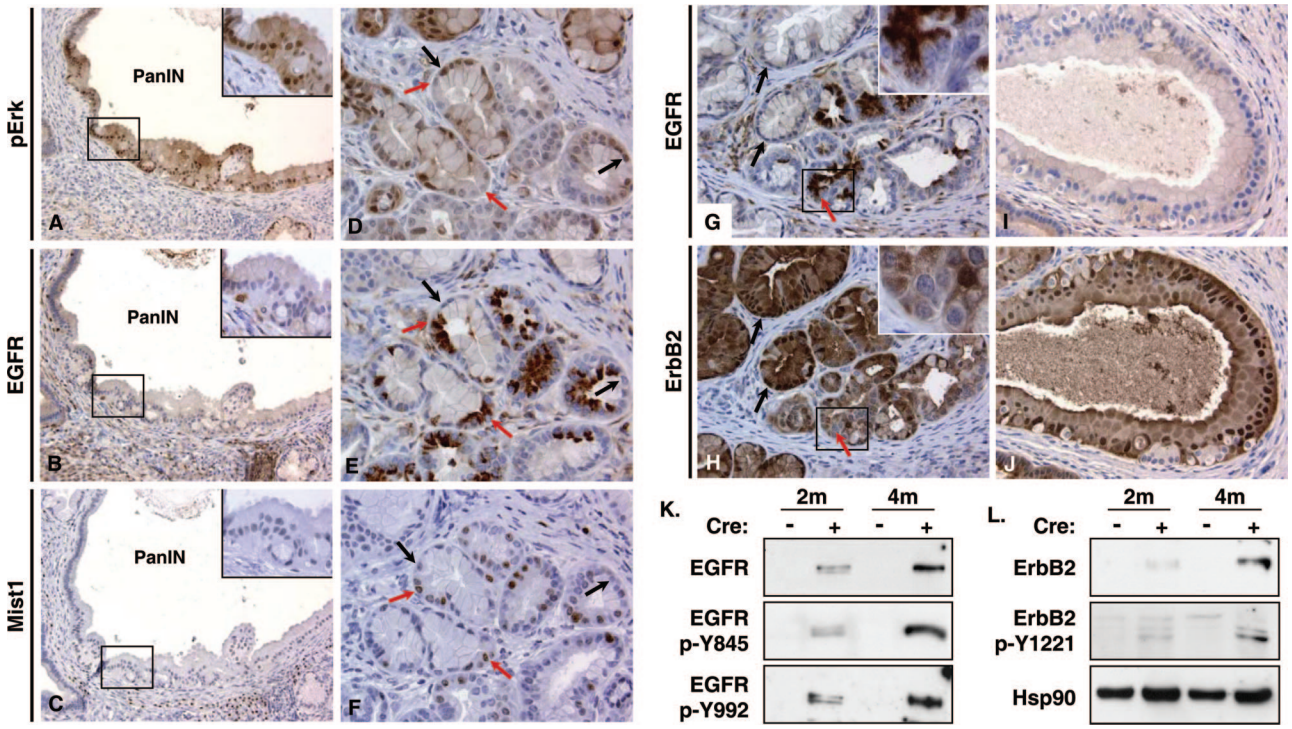


Figure 4. Acinar-ductal metaplasia leads to heterogeneity in EGFR, pErk, and ErbB2 expression profiles. **A–C:** *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ pancreas serial sections stained with anti-pErk, anti-EGFR, and anti-Mist1 reveal that mPanIN lesions are pErk-positive but EGFR- and Mist1-negative. Boxed areas are shown at higher resolution in the **insets**. **D–F:** Analysis of acinar metaplasia reveals a distinct gene expression pattern in which Mist1-positive cells (**red arrows**) coexpress EGFR, whereas Mist1-negative cells (**black arrows**) express pErk but are EGFR-negative. These results confirm that mPanINs and duct-like cells within acinar metaplastic units exhibit a similar EGF-signaling expression pattern. **G** and **H:** Serial sections from *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ pancreas samples were labeled with antibodies to EGFR and to ErbB2. EGFR and ErbB2 exhibit opposite expression patterns in acinar metaplastic structures where nuclear ErbB2 protein is restricted to the EGFR-negative duct-like cell compartment. Note that although ErbB2 protein levels are greatly elevated in all metaplastic acinar cells, nuclear ErbB2 is found only in the more advanced EGFR-negative cells. Boxed areas are shown at higher resolution in the **insets**. **I** and **J:** Similar sections as in **G** and **H** showing that mPanIN lesions are nuclear ErbB2-positive but EGFR-negative, reflecting an identical expression pattern as observed in acinar-ductal metaplastic structures. **K** and **L:** Immunoblot analysis on protein extracts isolated from control *LSL-Kras*$^{G12D/+}$ (Cre−) and *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ (Cre+) pancreas samples from 2- and 4-month animals. EGFR and ErbB2 protein levels are not detected in the control samples but dramatically increase with advancing age in the *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ animals. The EGFR and ErbB2 receptors are active in these samples as revealed by the presence of phosphotyrosine residues 845/992 and 1221, respectively. The Hsp90 blot serves as a loading control.

nar metaplastic structures followed similar gene expression patterns. Most mPanIN-1 lesions were uniformly pErk-positive but EGFR- and Mist1-negative (Figure 4, A–C). In contrast, acinar-ductal metaplastic structures coexpressed pErk, EGFR, and Mist1, but in mosaic patterns. EGFR-positive cells undergoing acinar-ductal metaplasia were typically differentiated acinar cells that were Mist1-positive but pErk-negative, whereas Mist1-negative cells expressed high levels of nuclear pErk (Figure 4, D–F). Adjacent normal acinar cells, although Mist1-positive, did not express detectable levels of EGFR or pErk (data not shown). Likewise, normal duct cells remained EGFR- and pErk-negative (data not shown). These results suggest that metaplastic acinar cells may progress to duct-like cells by transitioning from Mist1^{+}/EGFR^{+} to Mist1^{-}/EGFR^{-}/pErk^{+} cells. Interestingly, we rarely observed the previously reported EGF signaling heterogeneity described for mPanIN lesions.[15] Instead, heterogeneous expression of EGFR and pErk was mostly observed in acinar-ductal metaplastic areas that were closely associated with PanINs. Here, the Mist1^{-}/EGFR^{-}/pErk^{+} acinar-ductal metaplastic cells exhibited identical molecular signatures to those found in mPanIN lesions.

The EGFR/pErk expression profiles were surprising because elevated levels of EGFR in metaplastic acinar-ductal cells did not immediately generate the predicted

Figure 3. Hes1-negative acinar cells and Hes1-positive duct-like cells are found within acinar-ductal metaplastic structures. **A:** Semiquantitative reverse transcriptase-PCR showing that the Notch downstream target genes *Hes1*, *Hey1*, and *Hey2* are transcriptionally active in the *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ model. **B** and **C:** Adjacent sections from a WT pancreas sample immunolabeled with antibodies to Hes1 and Mist1. Centroacinar cells (**arrows**) express Hes1 but not Mist1 (an acinar cell-restricted transcription factor). By contrast, normal acinar cells are Mist1-positive but Hes1-negative. **D–F:** Early acinar metaplastic units from *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ mice reveal that Hes1 protein (**arrows**) is found in the duct-like cells. Note the normal acinar cells that surround these localized transition events. **G** and **H:** Adjacent serial sections from *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ pancreas tissue stained with anti-Mist1 and anti-Hes1. A single acinar-ductal metaplastic unit reveals cells that are both Hes1- and Mist1-positive (**arrows**), confirming that activation of Hes1 expression initiates in metaplastic acinar cells. The surrounding normal acinar cells remain Hes1-negative but Mist1-positive. **I:** A single mPanIN lesion showing that mPanIN cells express Hes1 whereas the surrounding acinar tissue is Hes1-negative. **J–L:** Human pancreas sections from PDA patients stained for hMist1 protein. The boxed-in area in **J** is shown at higher magnification in **K**. Note the Mist1-positive (acinar) cells within the metaplastic ductal lesions (**red arrows**). The **black arrow** in **L** points to an early metaplastic acinus. The extreme edge of a PanIN-2 lesion is shown in **J** (**asterisk**). **M:** A control WT section showing that PDX1 is primarily expressed in islet cells. d, duct. **N** and **O:** PDX1 expression (**red arrows**) is up-regulated during acinar-ductal metaplasia in the *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ mice. Adjacent normal acinar tissue (**black arrows**) remains PDX1-negative. PDX1 is also up-regulated in mPanINs (**inset**) in this model.

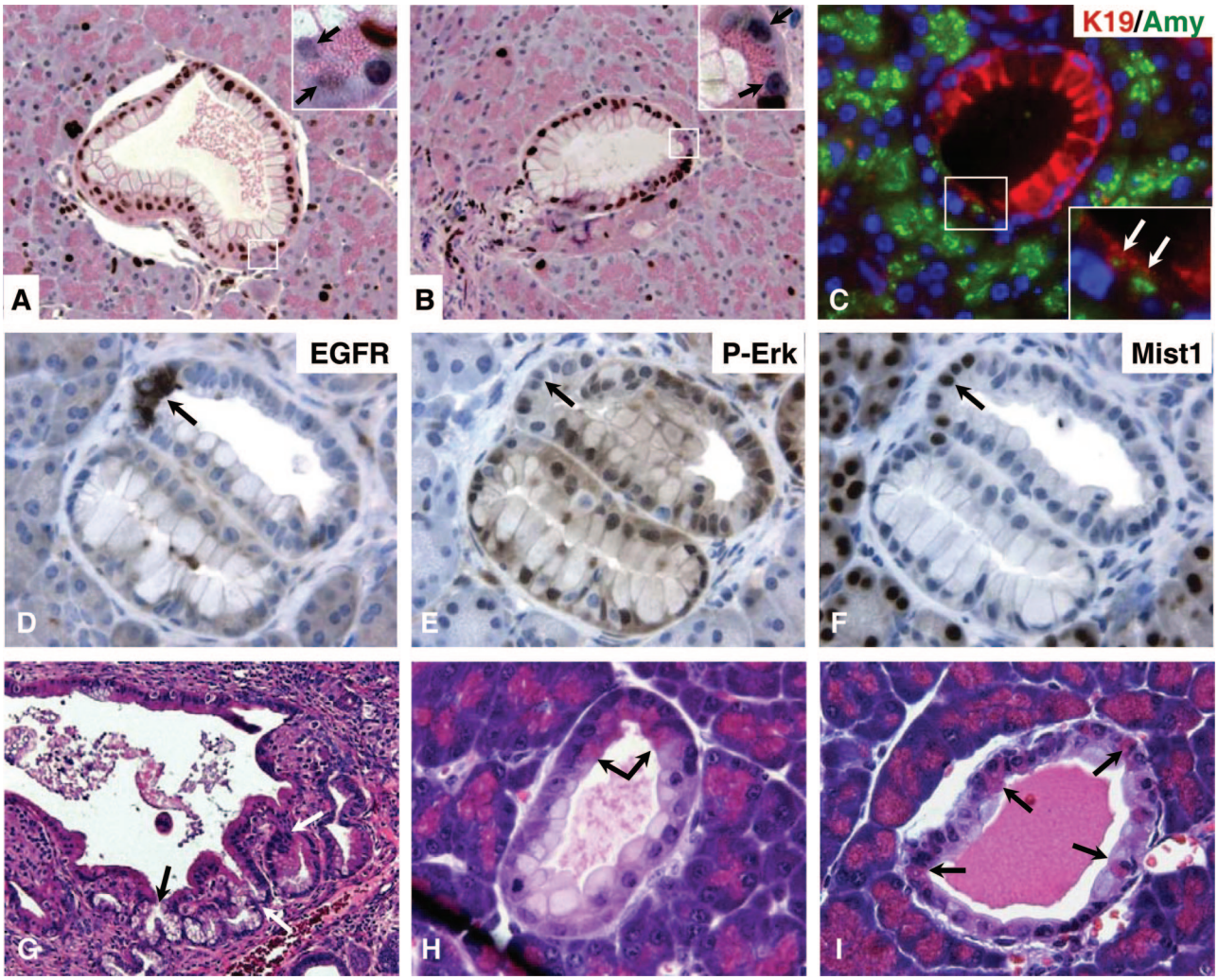


Figure 5. Acinar cells contribute to the molecular heterogeneity of mPanINs. **A** and **B:** Ki67 labeling of mPanIN-1 lesions from *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ mice revealing the high percentage of proliferating cells in these structures. Examination of mPanIN lesions often reveal a subset of Ki67-negative cells (**boxed areas**), which exhibit acinar cell properties and zymogen granules (**arrows** in **inset**). **C:** Amylase and K19 coimmunofluorescence showing a single mPanIN-1 lesion that contains cells coexpressing both products (**inset**). **D–F:** Serial sections from *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ pancreas samples were stained with antibodies to EGFR, pErk, and Mist1. EGFR and pErk exhibit opposite expression patterns in mPanIN lesions where Mist1-positive PanIN cells are EGFR-negative but pErk-positive. **G:** H&E staining of a large mPanIN lesion that is associated with areas of acinar-ductal metaplasia. **Arrows** indicate areas where acinar metaplasia is part of the mPanIN epithelial cell layer. **H** and **I:** Acinar-ductal/mPanIN hybrid structures from a 4-week *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ pancreas. These structures have properties that are common to both acinar-ductal metaplasia and mPanINs and are composed of both duct-like cells and acinar cells. The **arrows** point to several acinar cells (zymogen-positive) that are within these early acinar-ductal/mPanIN-1 hybrid structures.

activation of the downstream MAPK pathway (pErk). MAPK activity was detected only in mPanIN ductal cells or in acinar-ductal metaplastic cells that exhibited a duct-like cell phenotype. One possibility for the absence of MAPK activity in EGFR-positive cells could be that EGFR was inactive. However, this seems unlikely because *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ pancreata contained elevated levels of phospho-EGFR protein (Figure 4K).

To evaluate other members of the EGF receptor family, we next examined ErbB2 expression. As shown in Figure 4H, acinar metaplastic units universally expressed elevated levels of cytoplasmic ErbB2 protein. However, accumulation of nuclear ErbB2 was restricted to the ductal component of acinar-ductal metaplasia, and this nuclear expression was inversely correlated with EGFR (and Mist1) expression (Figure 4, G and H). Nuclear ErbB2 has been shown to be hyperphosphorylated[28] and to function as a transactivator of cancer-related genes,[29] and immunoblots confirmed the elevated levels of phospho-ErbB2 in *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ samples (Figure 4L). Thus, ErbB2-mediated signaling is likely different in the acinar and ductal components of acinar-ductal metaplasia. As predicted, mPanINs exhibited the same cytoplasmic and nuclear ErbB2 expression pattern (Figure 4, I and J). We conclude that EGF pathway signatures define individual metaplastic acinar and duct cell properties.

Early mPanIN Lesions Exhibit Acinar Cell Properties

The early appearance of acinar-ductal metaplasia in *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ pancreata and the identical molecular signature of the ductal component within

these lesions to mPanIN-1 structures prompted us to examine the possibility that acinar cells contributed to mPanINs in these animals. Centrally located mPanIN-1 structures were composed of columnar cells with basally located nuclei and abundant supranuclear mucin. As expected, most of the mPanIN cells were Ki67-positive, indicative of their proliferative potential (Figure 5, A and B). However, single cells within these structures often remained Ki67-negative. The Ki67-negative cells invariably exhibited acinar cell properties, including the presence of eosin-reacting zymogen granules (Figure 5, A and B, insets). High-resolution immunofluorescence also revealed individual biphenotypic mPanIN cells that coexpressed both K19 and amylase (Figure 5C). When we examined the expression of EGFR, pErk, and Mist1, the Mist1-positive mPanIN acinar cells were $EGFR^{+}/pErk^{-}$ (Figure 5, D–F), reflecting the previously described mPanIN molecular heterogeneity.[15] Thus, the presence of metaplastic acinar cells within mPanIN-1 lesions accounts for mPanIN heterogeneity.

The results described above confirmed that acinar-ductal metaplasia and early mPanIN lesions exhibit similar cellular and molecular properties. Examination of *LSL-Kras$^{G12D/+}$/p48$^{Cre/+}$* pancreata uncovered many areas where acinar-ductal metaplastic structures were in direct association with well-developed mPanINs (Figure 5G). In these examples, the duct-like cells within metaplastic structures were clearly part of the mPanIN epithelial cell layer, demonstrating that acinar cells become incorporated into pre-exiting mPanIN structures or that acinar-ductal metaplasia progresses to produce mPanIN lesions. Although we cannot distinguish between these alternative pathways at this time, hybrid acinar metaplasia/mPanIN-like structures that exhibited properties of both cell types were often observed in early *LSL-Kras$^{G12D/+}$/p48$^{Cre/+}$* mice (Figure 5, H and I), raising the possibility that acinar cells undergoing acinar-ductal transitions have a significant role in mPanIN formation and PDA development.

Discussion

PDAs develop from histologically and genetically well-characterized precursor lesions, the PanINs. PanIN lesions usually harbor molecular alterations that include activating or inactivating mutations in the *KRAS*, *p16^{Ink4a}*, *p19^{Arf}*, and *p53* genes.[5–7,14] The morphological and molecular signatures associated with PanINs and PDA suggest that duct cells are responsible for these lesions, but it remains unknown if other pancreatic cell types (acinar, islet, or stem cells) might also contribute to the pathogenesis of this disease.

To examine which cells are involved in these events, we focused our efforts on the initial morphological alterations that developed after *Kras^{G12D}* expression in *LSL-Kras$^{G12D/+}$/p48$^{Cre/+}$* mice. Our studies revealed that acinar-ductal metaplasia was the earliest prominent change to occur in *Kras^{G12D}* pancreata where metaplastic cells repressed acinar cell characteristics (*amylase^{+}*, *Mist1^{+}*) and activated expression of the duct cell marker genes *K19* and *CA-II*. Within these structures, biphenotypic cells were often identified that exhibited characteristics of both acinar and duct cells, suggesting that acinar cells undergo transdifferentiation to a duct cell phenotype in response to *Kras^{G12D}* expression. Although our studies have not directly examined the lineage relationship between metaplastic acinar and duct cells, studies from Means and colleagues[30] have shown that acinar cells directly transdifferentiate into duct-like cells in the presence of TGF-α. Whether *Kras^{G12D}*-expressing acinar cells exhibit elevated TGF-α levels has not been examined. However, we have shown that metaplastic acinar cells in *LSL-Kras$^{G12D/+}$/p48$^{Cre/+}$* mice were highly proliferative and exhibited activated EGF signaling pathways that were differentially associated with cells exhibiting an acinar or duct cell phenotype. Single cell analysis revealed mosaic patterns of EGFR, ErbB2, and pErk protein accumulation such that EGFR was expressed in early metaplastic acinar cells that exhibited acinar cell characteristics whereas nuclear ErbB2 and pErk expression was restricted to the duct-like cells. The mosaic expression pattern of these markers was reminiscent of previous studies showing that mPanINs consisted of a heterogeneous cell population.[13,15] Although the basis for the PanIN cell heterogeneity is not known, our results suggest it is attributable to the presence of acinar cells within mPanINs.

The fate of acinar cells within mPanINs remains elusive. Although these cells do not maintain a differentiated acinar phenotype, there is no evidence for acinar-restricted cell death within early mPanIN structures (unpublished results). Instead, we propose that acinar-ductal metaplastic structures participate in the formation of mPanIN lesions and that the participating acinar cells undergo an acinar-ductal conversion as mPanINs progress from mPanIN-1 → mPanIN-3 (Figure 6). In this model, acinar cells activate a molecular switch where acinar gene products are repressed, and metaplastic ductal gene products are expressed. A key event in this conversion is activation of the Notch signaling pathway and expression of the Notch downstream target genes *Hes1*, *Hey1*, and *Hey2*. Notch activity is known to prevent acinar cell differentiation,[31] and Notch is required for the tumor-initiating effects of TGF-α.[16] Thus, mPanIN acinar cells ultimately activate the Notch signaling pathway to initiate an acinar-ductal metaplasia regulatory cascade. Once activated, the cells become highly proliferative as they expand into mPanINs and ultimately into PDA. This molecular switch may also occur in the human condition because acinar-ductal metaplasia is often associated with human PanIN lesions.[19,20]

One prediction of our model is that biphenotypic cells consisting of acinar and duct cell markers should be found within metaplastic structures. Indeed, individual cells that coexpress acinar (Mist1, amylase) and mPanIN duct (Hes1, K19) gene products have been identified, reflecting a possible transition phase as the cells adopt a ductal phenotype. Biphenotypic cells have also been reported in acinar cells undergoing transdifferentiation in TGF-α-treated three-dimensional cultures.[30] Interest-

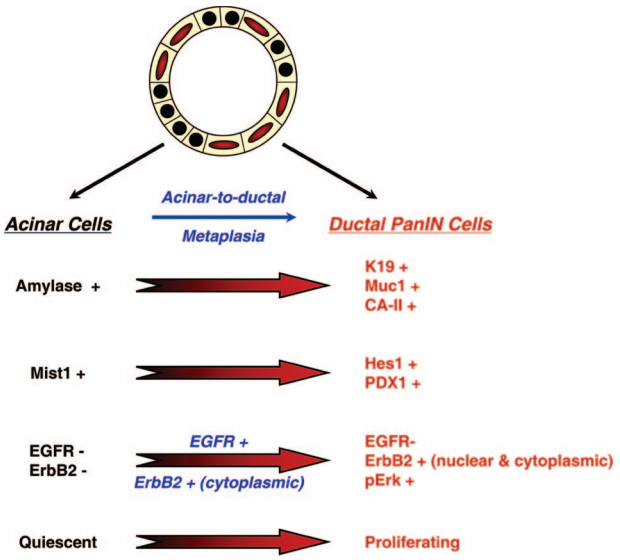


Figure 6. Model of acinar-ductal conversion in mPanINs. Acinar cells can be identified in mPanIN-1 structures by several molecular markers, including expression of the acinar gene products amylase and Mist1. These cells remain proliferatively quiescent. During acinar-ductal metaplasia, the cells undergo a switch from acinar cell properties to duct cell properties, activating expression of K19, Muc1, and CA-II. During this transition, the Hes1 and PDX1 transcription factors are similarly up-regulated. There is also a change in EGF signaling activity in which acinar cells are EGFR-, ErbB2-, and pERK-negative. At early stages of metaplasia, EGFR and cytoplasmic ErbB2 protein can be detected. As these cells transition to duct-like cells they repress EGFR expression and accumulate nuclear ErbB2 and pErk while entering a proliferative phase. Molecularly, the ductal component of acinar metaplastic units are identical to what is detected in PanIN lesions, suggesting that acinar cells convert to mPanIN cells during this process.

ingly, we have observed near identical biphenotypic cells in *Mist1*$^{KrasG12D/+}$ mice in which expression of *Kras*^{G12D} is restricted to differentiated acinar cells.[32] *Mist1*$^{KrasG12D/+}$ mice exhibit acinar-ductal metaplasia that eventually develops into pancreatic cancers with mixed acinar and ductal phenotypes.

Although our data support a model in which mature acinar cells contribute to PanIN formation, other interpretations are plausible. Metaplastic structures in 4-week mice could represent early PanINs that developed from small ducts. In this case, the presence of acinar cells and biphenotypic cells within metaplastic lesions would imply that duct cells undergo a transdifferentiation event to generate an acinar cell phenotype on *Kras*^{G12D} expression. Although formally possible, duct-to-acinar transdifferentiation has not been widely reported. In addition, targeted expression of *Kras*^{G12V} to cells of the ductal compartment failed to produce mPanINs or PDA in the *K19*$_{pr}$*-Kras*^{G12V} mouse model.[33] Thus, although activation mutations within the *Kras* allele are critical to PanIN initiation, expression of *Kras*^{G12V} in duct cells does not recapitulate the phenotype observed in *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ mice.

Another possibility is that metaplastic structures originate from adult progenitor cells (or a reserve cell population) that might be sensitive to Kras^{G12D} signaling. In this instance, Kras^{G12D} could activate a proliferation program and alter specific terminal differentiation pathways so that a subset of cells progress toward a duct cell phenotype, other cells progress toward an acinar cell phenotype, and still others exhibit aspects of both differentiated lineages (biphenotypic). Eventually, the duct cell phenotype would dominate and PanIN and PDA lesions would be produced. In this model, the *LSL-Kras*^{G12D} locus would be activated in the specific progenitor or reserve cell population. Both *p48*$^{Cre/+}$ and *pdx1-Cre* are expressed early in pancreas development (~E9.5), before commitment of individual pancreas lineages, and studies using the ROSA26 reporter line have shown that all acinar, duct, and islet cells in newborn mice contain the Cre-mediated recombined locus.[10,12] Thus, early expression of Cre from either locus would likely activate *LSL-Kras*^{G12D} in a quiescent progenitor cell population as well. In this regard, the *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ model does not perfectly mimic the human condition because all *LSL-Kras*$^{G12D/+}$/*p48*$^{Cre/+}$ pancreas cells contain an activated *Kras* allele, whereas only a small population of cells likely acquires the *Kras*^{G12D} mutation in humans. Thus, caution needs to be exercised when extrapolating data from mouse models to patients. Nonetheless, our studies have shown that pancreas samples from PDA patients also exhibit acinar-ductal metaplastic structures that contain both acinar and duct cell phenotypes. Future lineage-tracing studies in mice will be required to fully investigate the ability of mature acinar cells or other cell types (duct, stem, reserve) to participate in mPanIN and PDA development. Toward this goal, we are generating inducible Mist1-Cre mouse lines that will allow us to test the importance of acinar cells to PDA development and to determine the fate of individual cells as mPanINs progress to metastatic PDA. The identification of the initiating cells will be an important advancement for early diagnostics and targeted therapeutic approaches to battle this disease.

Note

In agreement with our study, Guerra et al[34] recently demonstrated that expression of endogenous *Kras*^{G12V} in acinar/centroacinar cells generates PanIN and invasive PDA through a possible acinar-ductal metaplasia pathway.

Acknowledgments

We thank Dave Tuveson for generously supplying *LSL-Kras*$^{G12D/+}$ mice and *LSL-Kras*$^{G12D/+}$/*pdx1-Cre* tissue samples; Chris Wright for supplying the *p48*$^{Cre/+}$ mice; Rolf Kemler, Michael Rukstalis, and Tetsuo Sudo for generously providing reagents used in this study; and Mariano Barbacid for communicating results before publication.

References

1. Li WC, Yu WY, Quinlan JM, Burke ZD, Tosh D: The molecular basis of transdifferentiation. J Cell Mol Med 2005, 9:569–582
2. Slack JM, Tosh D: Transdifferentiation and metaplasia—switching cell types. Curr Opin Genet Dev 2001, 11:581–586

3. Tosh D, Slack JM: How cells change their phenotype. Nat Rev Mol Cell Biol 2002, 3:187–194
4. Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, Feuer EJ, Thun MJ: Cancer statistics, 2004. CA Cancer J Clin 2004, 54:8–29
5. Hruban RH, Adsay NV, Albores-Saavedra J, Compton C, Garrett ES, Goodman SN, Kern SE, Klimstra DS, Kloppel G, Longnecker DS, Luttges J, Offerhaus GJ: Pancreatic intraepithelial neoplasia: a new nomenclature and classification system for pancreatic duct lesions. Am J Surg Pathol 2001, 25:579–586
6. Hruban RH, Goggins M, Parsons J, Kern SE: Progression model for pancreatic cancer. Clin Cancer Res 2000, 6:2969–2972
7. Hruban RH, Wilentz RE, Maitra A: Identification and analysis of precursors to invasive pancreatic cancer. Methods Mol Med 2005, 103:1–13
8. Schneider G, Siveke JT, Eckel F, Schmid RM: Pancreatic cancer: basic and clinical aspects. Gastroenterology 2005, 128:1606–1625
9. Prasad NB, Biankin AV, Fukushima N, Maitra A, Dhara S, Elkahloun AG, Hruban RH, Goggins M, Leach SD: Gene expression profiles in pancreatic intraepithelial neoplasia reflect the effects of Hedgehog signaling on pancreatic ductal epithelial cells. Cancer Res 2005, 65:1619–1626
10. Hingorani SR, Petricoin EF, Maitra A, Rajapakse V, King C, Jacobetz MA, Ross S, Conrads TP, Veenstra TD, Hitt BA, Kawaguchi Y, Johann D, Liotta LA, Crawford HC, Putt ME, Jacks T, Wright CV, Hruban RH, Lowy AM, Tuveson DA: Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell 2003, 4:437–450
11. Jackson EL, Willis N, Mercer K, Bronson RT, Crowley D, Montoya R, Jacks T, Tuveson DA: Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. Genes Dev 2001, 15:3243–3248
12. Kawaguchi Y, Cooper B, Gannon M, Ray M, MacDonald RJ, Wright CV: The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. Nat Genet 2002, 32:128–134
13. Aguirre AJ, Bardeesy N, Sinha M, Lopez L, Tuveson DA, Horner J, Redston MS, DePinho RA: Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. Genes Dev 2003, 17:3112–3126
14. Bardeesy N, Aguirre AJ, Chu GC, Cheng KH, Lopez LV, Hezel AF, Feng B, Brennan C, Weissleder R, Mahmood U, Hanahan D, Redston MS, Chin L, Depinho RA: Both p16(Ink4a) and the p19(Arf)-p53 pathway constrain progression of pancreatic adenocarcinoma in the mouse. Proc Natl Acad Sci USA 2006, 103:5947–5952
15. Hingorani SR, Wang L, Multani AS, Combs C, Deramaudt TB, Hruban RH, Rustgi AK, Chang S, Tuveson DA: Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. Cancer Cell 2005, 7:469–483
16. Miyamoto Y, Maitra A, Ghosh B, Zechner U, Argani P, Iacobuzio-Donahue CA, Sriuranpong V, Iso T, Meszoely IM, Wolfe MS, Hruban RH, Ball DW, Schmid RM, Leach SD: Notch mediates TGF alpha-induced changes in epithelial differentiation during pancreatic tumorigenesis. Cancer Cell 2003, 3:565–576
17. Pin CL, Bonvissuto AC, Konieczny SF: Mist1 expression is a common link among serous exocrine cells exhibiting regulated exocytosis. Anat Rec 2000, 259:157–167
18. Pin CL, Rukstalis JM, Johnson C, Konieczny SF: The bHLH transcription factor Mist1 is required to maintain exocrine pancreas cell organization and acinar cell identity. J Cell Biol 2001, 155:519–530
19. Detlefsen S, Sipos B, Feyerabend B, Kloppel G: Pancreatic fibrosis associated with age and ductal papillary hyperplasia. Virchows Arch 2005, 447:800–805
20. Brune K, Abe T, Canto M, O'Malley L, Klein AP, Maitra A, Volkan Adsay N, Fishman EK, Cameron JL, Yeo CJ, Kern SE, Goggins M, Hruban RH: Multifocal neoplastic precursor lesions associated with lobular atrophy of the pancreas in patients having a strong family history of pancreatic cancer. Am J Surg Pathol 2006, 30:1067–1076
21. Hruban RH, Adsay NV, Albores-Saavedra J, Anver MR, Biankin AV, Boivin GP, Furth EE, Furukawa T, Klein A, Klimstra DS, Kloppel G, Lauwers GY, Longnecker DS, Luttges J, Maitra A, Offerhaus GJ, Perez-Gallego L, Redston M, Tuveson DA: Pathology of genetically engineered mouse models of pancreatic exocrine cancer: consensus report and recommendations. Cancer Res 2006, 66:95–106
22. Jensen J, Pedersen EE, Galante P, Hald J, Heller RS, Ishibashi M, Kageyama R, Guillemot F, Serup P, Madsen OD: Control of endodermal endocrine development by Hes-1. Nat Genet 2000, 24:36–44
23. Murtaugh LC, Stanger BZ, Kwan KM, Melton DA: Notch signaling controls multiple steps of pancreatic differentiation. Proc Natl Acad Sci USA 2003, 100:14920–14925
24. Apelqvist A, Li H, Sommer L, Beatus P, Anderson DJ, Honjo T, Hrabe de Angelis M, Lendahl U, Edlund H: Notch signalling controls pancreatic cell differentiation. Nature 1999, 400:877–881
25. Jensen JN, Cameron E, Garay MV, Starkey TW, Gianani R, Jensen J: Recapitulation of elements of embryonic development in adult mouse pancreatic regeneration. Gastroenterology 2005, 128:728–741
26. Zhu L, Tran T, Rukstalis JM, Sun P, Damsz B, Konieczny SF: Inhibition of Mist1 homodimer formation induces pancreatic acinar-to-ductal metaplasia. Mol Cell Biol 2004, 24:2673–2681
27. Jaffee EM, Hruban RH, Canto M, Kern SE: Focus on pancreas cancer. Cancer Cell 2002, 2:25–28
28. Xie Y, Hung MC: Nuclear localization of p185neu tyrosine kinase and its association with transcriptional transactivation. Biochem Biophys Res Commun 1994, 203:1589–1598
29. Wang SC, Lien HC, Xia W, Chen IF, Lo HW, Wang Z, Ali-Seyed M, Lee DF, Bartholomeusz G, Ou-Yang F, Giri DK, Hung MC: Binding at and transactivation of the COX-2 promoter by nuclear tyrosine kinase receptor ErbB-2. Cancer Cell 2004, 6:251–261
30. Means AL, Meszoely IM, Suzuki K, Miyamoto Y, Rustgi AK, Coffey Jr RJ, Wright CV, Stoffers DA, Leach SD: Pancreatic epithelial plasticity mediated by acinar cell transdifferentiation and generation of nestin-positive intermediates. Development 2005, 132:3767–3776
31. Hald J, Hjorth JP, German MS, Madsen OD, Serup P, Jensen J: Activated Notch1 prevents differentiation of pancreatic acinar cells and attenuate endocrine development. Dev Biol 2003, 260:426–437
32. Tuveson DA, Zhu L, Gopinathan A, Willis NA, Kachatrian L, Grochow R, Pin CL, Mitin NY, Taparowsky EJ, Gimotty PA, Hruban RH, Jacks T, Konieczny SF: Mist1-KrasG12D knock-in mice develop mixed differentiation metastatic exocrine pancreatic carcinoma and hepatocellular carcinoma. Cancer Res 2006, 66:242–247
33. Brembeck FH, Schreiber FS, Deramaudt TB, Craig L, Rhoades B, Swain G, Grippo P, Stoffers DA, Silberg DG, Rustgi AK: The mutant K-ras oncogene causes pancreatic periductal lymphocytic infiltration and gastric mucous neck cell hyperplasia in transgenic mice. Cancer Res 2003, 63:2005–2009
34. Guerra C, Schuhmacher AJ, Canamero M, Grippo PJ, Verdaguer L, Perez-Gallego L, Dubus P, Sandgren EP, Barbacid M: Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. Cancer Cell 2007, 11: 291–302