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Review

Regulation of Cellular Identity in Cancer

Nilotpal Roy[1] and Matthias Hebrok[1,*]
[1]Diabetes Center, Department of Medicine, University of California, San Francisco, CA 94143, USA
*Correspondence: mhebrok@diabetes.ucsf.edu
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Neoplastic transformation requires changes in cellular identity. Emerging evidence increasingly points to cellular reprogramming, a process during which fully differentiated and functional cells lose aspects of their identity while gaining progenitor characteristics, as a critical early step during cancer initiation. This cell identity crisis persists even at the malignant stage in certain cancers, suggesting that reactivation of progenitor functions supports tumorigenicity. Here, we review recent findings that establish the essential role of cellular reprogramming during neoplastic transformation and the major players involved in it with a special emphasis on pancreatic cancer.

Introduction

During embryonic development, the process of cellular differentiation confers commitment of a cell toward a specific, terminally differentiated fate. Pluripotent stem cells give rise to multipotent progenitor cells, which are poised to assume properties of lineage-restricted precursors in response to intercellular and intracellular cues. Lineage-restricted precursors undergo final differentiation toward a terminally matured cell type. Traditionally, it has been accepted that once a cell has concluded its differentiation path toward a specific fate, this state is permanent and irreversible. This view of cellular maturity as an immovable state was challenged by elegant experiments first in frogs and later on mammals that demonstrated cellular plasticity of mature cells (Gurdon and Melton, 2008). The more recent discovery that adult fully differentiated cells can be genetically reprogrammed to induced pluripotent stem cells (iPS), an embryonic stem cell-like state capable of giving rise to all lineages, further refuted the dogma that the terminal differentiation state of a cell is irreversibly locked (Takahashi and Yamanaka, 2006).

Over the last few years, we have learned that cellular dedifferentiation might be a common theme in degenerative diseases, including diabetes (Puri et al., 2014, 2013; Talchai et al., 2012). Similarly, such erosion of the final differentiation state of cells has also been observed during malignant progression. Matured cells with increased plasticity have the ability to acquire some of the genotypic and phenotypic characteristics of a progenitor-like state or adopt a distinct differentiated state. In the case of cancer initiation, mutation in key regulatory genes is one of the major drivers of increased plasticity. Following an oncogenic insult, a mature cell may undergo loss of cellular identity on its way to neoplasia and maintain this abnormal plasticity through the malignant stages. Loss of cellular identity comes in two flavors: dedifferentiation, defined as loss of mature functionality, and transdifferentiation, characterized by a change in cellular identity toward a different mature cell type. Of note, dedifferentiation can precede transdifferentiation toward a distinct cellular fate (Puri et al., 2014). In this review, we will discuss how loss of the defined differentiation state is emerging as a common step toward cellular transformation in many different cancers.

Defective Differentiation States in Cancer

Emergence of a progenitor-like state promotes cellular transformation and tumor formation. This raises the question as to whether such a progenitor state can be modulated for therapeutic purposes. In other words, is it possible to revert tumor cells toward a quiescent, matured state with reduced or absent malignant potential? In a seminal study, G. Barry Pierce provided evidence that malignant cells indeed can be differentiated into benign, post-mitotic cells (Pierce and Wallace, 1971). This finding not only conceptualized the origin of differentiation therapy but also established the rationale of studying initial reprogramming of cells at the inception of cancer. The underlying theory of clinically targeting defective differentiation states by promoting maturation was successfully validated in acute promyelocytic leukemia (APML), a lethal form of hematological malignancy driven by an incomplete differentiation program. APML is characterized by reciprocal translocation of chromosome arms 15 and 17, which results in the fusion of the promyelocytic leukemia gene (*PML*) with the retinoic acid receptor gene (*RAR-a*) (Borrow et al., 1990; Larson et al., 1984). The resulting PML-RARa homodimers repress target genes essential for granulocytic differentiation, thus holding tumor cells back in a progenitor-like state. Anthracycline-based chemotherapy, which inhibits the proliferation of malignant cells, used to be the only way to treat APML, but the benefit to patients was limited and often short lived. One of the characteristics of APML is the abnormal accumulation of promyelocytes within the bone marrow of patients (Wang and Chen, 2008). This observation led to the hypothesis that a block in granulocytic differentiation caused by the fusion protein might act as a driving force for APML formation. A major breakthrough in APML research was the finding that leukemia cells can be induced to undergo full differentiation upon treatment with certain agents such as all-trans retinoic acid (ATRA) (Breitman et al., 1981, 1980). As a consequence, patients receiving ATRA exhibit a gradual transition of leukemic promyelocytes toward terminal granulocytes resulting in long-lasting and sometimes curative responses (Tallman et al., 1997; Warrell et al., 1991). This is perhaps one of the best-documented examples in which tumor cells are successfully targeted based on their defective differentiation state.

Defective Differentiation States in Solid Tumors

Successful application of differentiation therapy has also been reported in solid malignancies such as myxoid liposarcoma (MLS), a common tumor of adipose tissue that results from an impediment in the adipogenic differentiation program. MLS is characterized by the reciprocal translocation between the *FUS* and *CHOP* genes, resulting in a fusion protein, FUS-CHOP (Crozat et al., 1993; Pérez-Losada et al., 2000; Rabbitts et al., 1993). CHOP belongs to the C/EBP transcription factor family and is involved in adipogenesis (Batchvarova et al., 1995; Ron and Habener, 1992), whereas FUS is a protein component of the nuclear riboprotein complex with an RNA binding motif (Crozat et al., 1993; Rabbitts et al., 1993). The hybrid protein functions as an abnormal transcription factor capable of deregulating CHOP target genes, including inactivation of those required for the lipogenic terminal differentiation programs (Adelmant et al., 1998). Trabectedin, a compound isolated from the sea squirt *Ecteinascidia turbinata*, promotes the transition from immature non-lipogenic spindle cells to mature lipoblasts by removing the fusion protein from promoters of genes critical for adipogenesis. As a consequence, the transcriptional block toward matured state is abolished, leading to a reduction in tumor burden (Forni et al., 2009). Thus, similar to hematological malignancies, solid tumors displaying attributes of progenitor-like states also respond to differentiation therapy.

Loss of Cellular Identity in Gastrointestinal Tumors

Chromosomal rearrangements resulting in fusion of specific genes and subsequent generation of aberrant fusion proteins evoke a defective differentiation program in both MLS and APML. However, both these malignancies display minimal additional genetic abnormalities. This brings up the pertinent question as to whether defective differentiation is commonplace in other tumor types, including those characterized by multiple "driver" mutations. If so, does loss of cellular identity only happen in cells at intermediate stages of development (like APML or MLS) or also in terminally matured cells? Genetically engineered mouse models (GEMMs) that mimic the human diseases have provided ample evidence that loss of mature cellular identity is an obligatory step for many types of solid tumor malignancies.

For example, hepatocellular carcinoma (HCC) often presents with features of a multipotent progenitor-like state arising from matured hepatocytes. Deregulation of the Hippo signaling pathway has recently been shown to transform hepatocytes into atypical duct cells and promote progression to HCC. Lineage tracing studies of hepatocytes in mice with hyper-activated Hippo pathway signaling (either through ectopic expression of the Hippo effector Yap1, or inactivation of Nf2, a factor upstream of the Hippo pathway) revealed reprogramming of hepatocytes into ductal cells bearing characteristics of progenitors (Camargo et al., 2007; Yimlamai et al., 2014). These hepatocyte-derived progenitors retained their plasticity, as they could reassume the mature hepatocyte state once normal Hippo signaling was reestablished. A recent proof-of-concept study further demonstrated that *Yap1* deletion in HCC completely blocks cell proliferation and atypical ductal cell expansion followed by gradual reactivation of a hepatocyte differentiation program (Fitamant et al., 2015). Under these conditions, HCCs change their morphology by forming nodules resembling groups of regenerative hepatocytes. Thus, the presence of dedifferentiated cells in HCCs and the loss of the malignant phenotype upon redifferentiation toward hepatocytes point to the progenitor state as critical for tumor development and maintenance.

Pancreatic Ductal Adenocarcinoma

Genetic reprogramming and loss of cellular identity are also critical early steps during the initiation of another gastrointestinal cancer type, pancreatic ductal adenocarcinoma (PDA). PDA is one of the deadliest malignancies, in large part due to the absence of specific early symptoms that would facilitate the timely diagnosis at the initial stage of the disease (Hezel et al., 2006). Thus, efforts to define the molecular mechanisms that underlie the initiation of PDA are highly relevant for early detection and timely treatment.

The pancreas is a heterogeneous organ consisting of exocrine and endocrine compartments (Puri and Hebrok, 2010). Pancreatic exocrine tissue comprises acinar cells that synthesize digestive enzymes, ductal cells that transport the acinar-derived enzymes to the duodenum, and terminal duct cells/centroacinar cells present at the interface of the acinar and ductal systems. The endocrine part consists of cellular aggregates, known as islets of Langerhans, which are composed of hormone-secreting cells. While tumors can form from the endocrine region of the pancreas, the most common cancer is the exocrine-derived PDA. PDA can arise from distinctive non-invasive lesions classified as pancreatic intraepithelial neoplasia (PanIN), mucinous cystic neoplasm (MCN), and intraductal papillary mucinous neoplasm (IPMN) (Hezel et al., 2006). Understanding the process by which normal pancreatic cells transition toward neoplasia and subsequently PDA offers the opportunity to identify early biomarkers or novel therapeutic targets that could be exploited to change the course of the disease. As discussed below, we will define different types of PDA neoplasia while summarizing what is known about the cellular transition that occurs during the development of each kind.

PanIN-Derived PDA

PanINs are a frequently diagnosed lesion type that result in aggressive PDA with a 5-year survival rate of a dismal 5% (Hezel et al., 2006). Sophisticated GEMMs have been developed that accurately mimic PanIN to PDA formation, thus allowing us to more precisely track the origin and progression of PanIN lesions. Mutations in *KRAS* serve as the oncogenic driver in 95% of PDA patients (Almoguera et al., 1988; Smit et al., 1988). In a landmark study that revolutionized the way mouse models of pancreatic cancer were generated, Hingorani and colleagues placed the human *KRAS* gene carrying an oncogenic G12D mutation that keeps the enzyme in a constitutively active state into the endogenous *Kras* locus in mice. Expression of the oncogenic version of Kras was initiated upon Cre-mediated elimination of a preceding stop cassette. Targeted expression of oncogenic Kras in pancreatic progenitor cells (around embryonic day 8.5–9) induces lesions in the mature organ recapitulating the full spectrum of human PanIN (Hingorani et al., 2003). Identifying the cellular origin for these lesions could provide critical information regarding the early detection of PDA and could suggest new therapeutic opportunities.

Cell of Origin for PanIN Lesions

Considering that mutant Kras is expressed in the entire pancreatic epithelium in the above-mentioned GEMM model, this genetic approach could not delineate as to whether a specific cell type was particularly sensitive to oncogenic Kras-mediated transformation. Therefore, to explore the cellular precursors of PanINs, several GEMMs with promoters of diverse pancreatic genes marking specific mature cell types have been generated over time to ectopically express oncogenic Kras in distinct pancreatic cell types (see Table S1 for all the relevant GEMM models and their corresponding references). Interestingly, different cell types have been attributed as the cell of origin for PanIN, and the data supporting each cell type are discussed below.

Evidence for a Ductal Origin of PanIN

Histological analysis of PDA identified associated PanINs as lesions that appeared to be in continuous contact with the existing ductal tree of the pancreas, suggesting that PanIN-derived PDA originates from duct cells. Surprisingly, constitutively expressing a different oncogenic version of Kras (KrasG12V) under control of the duct-specific *Cytokeratin 19* promoter exhibited only occasional pancreatic ductal hyperplasia (Brembeck et al., 2003). Pancreatic duct cells (PDCs) purified from these animals also did not display any difference in growth or cell-cycle distribution compared to wild-type PDCs (Schreiber et al., 2004). When oncogenic Kras (KrasG12D) was expressed via Cre-mediated recombination in adult PDCs in an inducible manner (*CK19CreERT*), a small number of mucinous metaplasia with characteristics of early PanIN lesions was observed. Conclusive evidence demonstrating that these PanINs were true lesions was not obtained, as progression toward the PDA state was not observed (Ray et al., 2011). In vitro expression of mutant KrasG12V in quiescent PDCs was found to stimulate S-phase entry, increase cell size, and cause epithelial to mesenchymal transition (Agbunag and Bar-Sagi, 2004). When these primary ductal cells expressing oncogenic Kras were transplanted orthotopically into the mouse pancreas, they formed ductal structures resembling early PanIN lesions but lacked full tumorigenic potential (Lee and Bar-Sagi, 2010; Pylayeva-Gupta et al., 2012). Thus, while activation of oncogenic Kras in PDCs was sufficient to induce mitogenic and morphogenic responses, full transformation to PDA was not observed.

More recently, organoid cultures were established from small intra-lobular ducts of normal and KrasG12D-expressing mice. Spheres containing cells expressing oncogenic Kras formed PanIN-like structures with columnar morphology upon orthotopic injection into rodent pancreas (Boj et al., 2015). Similar results were obtained with human tissue. Notably, human organoids gave rise to low and high-grade PanIN lesions within a month and progressed to full-blown PDA within several months after orthotopic transplantation into immune-compromised mice, demonstrating that human duct cells are competent to form PanIN lesions when proper oncogenic cues are present. The apparent discrepancy between this study by Boj et al. and the aforementioned mouse models that did not report efficient PanIN-PDA formation has not been addressed fully. It is possible that in vitro culture conditions are more conducive for Kras-mediated oncogenic transformation due to the lack of other supporting tissues present in vivo, and future experiments will have to resolve these issues. Furthermore, a recent report by Bailey, Leach, and colleagues presents interesting findings that do demonstrate the potential for adult PDCs to serve as progenitors for PDA in GEMMs (Bailey et al., 2015). While expression of oncogenic Kras alone in mature duct cells did not elicit neoplasia, concomitant expression of KrasG12D with two alleles of a gain-of-function mutation in *p53* (*p53R127H*) was sufficient in initiating PDA formation from mature duct cells. Of note, simultaneous expression of just one *p53R127H* allele with oncogenic Kras did not result in PDA, suggesting a requirement for a certain level of p53 activity in duct cells to promote PDA development. In addition, and somewhat peculiarly, pancreatic duct-derived neoplasia did not progress via PanIN lesions. Thus, as most autochthonous mouse models did not show the full spectrum of PanIN-PDA, the alternative hypothesis that non-duct cells might be the predominant source of PanIN-derived PDA has gained momentum.

Acinar Cells as Cells of Origin for PanIN

Acinar cells make up the vast majority of pancreatic epithelial cells. In support of acinar cells as the origin for PanINs, and in line with the proximity of PanIN cells with ductal cells, it has been shown that acinar cells are vulnerable toward transdifferentiation to a ductal state. Upon varying insults, acinar cells readily lose functionality and enter into an intermediate dedifferentiation state in which they abandon their identity but have not yet adopted ductal features. Subsequently, a transdifferentiation event leads to adoption of true duct-like characteristics by the enzyme-producing cells. Such a conversion is described as acinar to ductal metaplasia (ADM). Support for this notion comes from early in vitro and in vivo evidence suggesting that injury or oncogenic transformation can drive acinar cells toward a ductal phenotype (Arias and Bendayan, 1993; De Lisle and Logsdon, 1990; Grippo and Sandgren, 2000; Hall and Lemoine, 1992; Vila et al., 1994; Yuan et al., 1997). Acinar transdifferentiation into ductal metaplasia can lead to premalignant lesions as shown by EGFR, TGF-α, SV40 large T antigen, or KrasG12D expression in pancreatic acinar cells (Bockman and Merlino, 1992; Grippo et al., 2003; Ornitz et al., 1987; Sandgren et al., 1990; Tuveson et al., 2006; Wagner et al., 2001, 1998). Using *Nestin-Cre* animals, Murray Korc's group provided further support for the notion that acinar cells, or their progenitors, can give rise to neoplastic cells with ductal features (Carrière et al., 2007). Furthermore, subsequent studies using *Elastase CreERT* or *proCPA1; CreERT* transgenic mice that express Cre recombinase specifically in adult acinar cells following Tamoxifen administration confirmed that Kras activation in mature acinar cells induces PanIN lesions in a manner similar to ubiquitous Kras activation within the pancreatic epithelium (De La O et al., 2008; Gidekel Friedlander et al., 2009).

While these data collectively provided strong support that acinar cell can serve as a progenitor for PanINs, conflicting results were obtained with a different version of oncogenic Kras (KrasG12V). Ectopic expression of KrasG12V in developing acinar cells also led to spontaneous generation of PanIN and PDA, but adult pancreatic acinar cells were refractory to Kras-mediated transformation. In humans, pancreatitis and inflammation have been recognized as strong risk factors for PanIN and

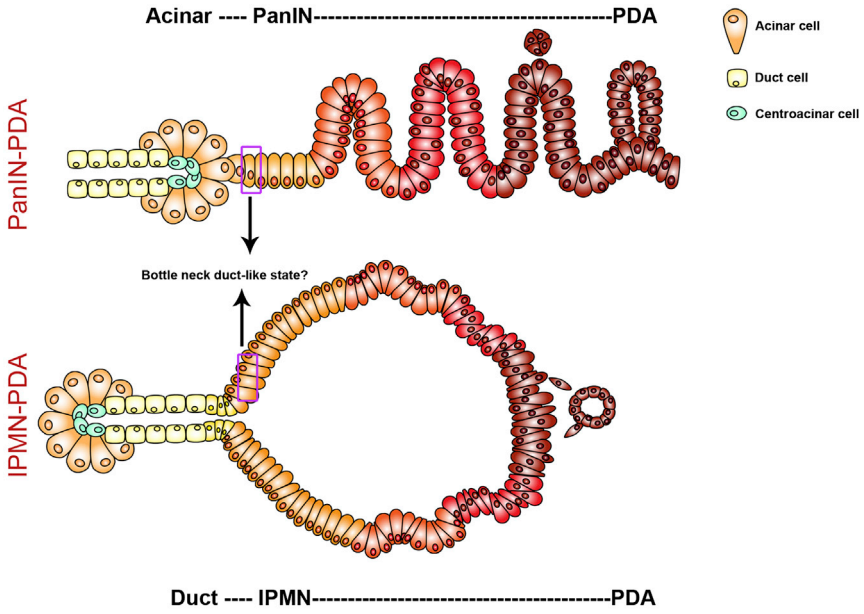


Figure 1. Transition between Cellular States during PanIN- and IPMN-Derived PDA

Acinar, ductal, or centroacinar cells can give rise to PanINs. However, based on the current observations, acinar cells have been emerged as the predominant source for PanIN-derived PDA. On the other hand, current evidence clearly indicates duct cells as the cell of origin for IPMN. Both neoplasia are characterized by the erosion of the mature differentiation state of their respective cells of origin. While acinar cells undergo transdifferentiation toward a duct-like state before PanIN formation, duct cells dedifferentiate to a lesser degree and retain aspects of the duct lineage during IPMN. Notably, both dedifferentiation programs display some features of the ductal lineage, suggesting that exocrine neoplasia might have to progress through a common "bottle neck, duct-like" state.

PDA development. Similarly, when exocrine cells were challenged with caerulein, a cholecystokinin analog capable of inducing pancreatitis associated with fibrosis and inflammation, subsequent expression of KrasG12V did induce PanIN and PDA in postnatal acinar pancreas (Guerra et al., 2007). In contrast, by using two different transgenic lines permitting ectopic expression of KrasG12D in adult acinar cells (*Elastase CreERT* or *Mist1 CreERT*), Maitra and colleagues observed spontaneous development of PanIN lesions in the absence of concurrent exocrine injury (Habbe et al., 2008). The reason for the discrepancies in the observed phenotypes remains elusive, but distinct transforming capacities between G12V and G12D may partially explain the differences. Nonetheless, the general consensus of these studies supports the notion that acinar cells respond to oncogenic Kras, either alone or in combination with inflammatory insults, by transdifferentiating toward a ductal phenotype prior to PanIN formation (Figure 1).

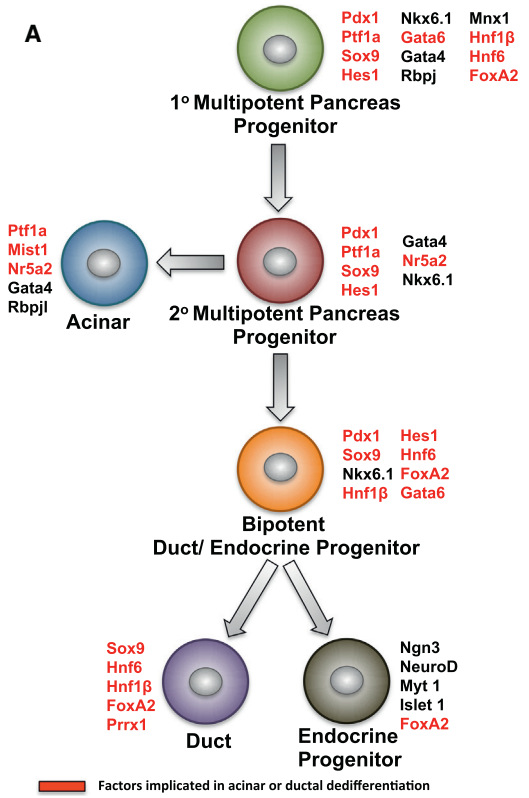
A recent study directly compared the propensity of ductal and centroacinar versus acinar cells to undergo dedifferentiation and subsequent transformation by oncogenic Kras (Kopp et al., 2012). Forced expression in either adult acinar cells (*Ptf1a Cre-ERT2*) or adult duct and centroacinar cells (*Sox9 Cre-ERT2*) induced PanIN lesions that arose predominantly from acinar but not ductal or centroacinar cells. Even when challenged with pre-neoplastic insults, such as caerulein-induced acute pancreatitis, KrasG12D-expressing duct cells showed only a low propensity to form PanIN lesions.

Centroacinar Cells as Progenitors for PanIN

In addition to the above-described acinar-centric theory of PanIN formation, others have suggested centroacinar cells as progenitors for PanIN and PDA development. Centroacinar cells display several characteristics that set them apart from other mature pancreatic cell types, including their location that is confined to the transition zone between duct and acinar cells and their propensity to express molecular markers of embryonic progenitor cells, including Notch signaling (Kopinke et al., 2011; Afelik et al., 2012; Kopinke et al., 2012). Furthermore, their ability to form replicating spheres capable of differentiating into all pancreatic cell types when cultured in vitro has supported the notion that these cells might possess adult stem cell properties, a notion that has not been confirmed in vivo (Rovira et al., 2010). However, centroacinar cells have been implicated in neoplastic transformation (Stanger et al., 2005). Elimination of Pten expression, a tumor suppressor gene, instead of ectopic expression of mutant Kras as the oncogenic driver, led centroacinar cells to transdifferentiate into ductal cells and formed mucinous metaplasia as well as PanIN lesions. An intriguing theory that arises from these findings is that the difference in the tumor-initiating event determines whether duct, centroacinar, or acinar cells serve as the origins for PanIN and PDA development.

Predisposition of Epithelial Subtypes and Cancer Stem Cell

Although the majority of studies point toward fully differentiated exocrine cells as precursors of PanIN-PDA, some reports suggest that a subset of cells exists that might be more susceptible to oncogenic transformation. While the presence of true mature pancreatic stem cells, defined as cells capable of expansion, maintenance of a progenitor phenotype, and differentiation into multiple mature cell types have not been clearly demonstrated, there is emerging evidence that distinct subsets of cells residing within the pancreatic epithelium might have higher predisposition for dedifferentiation. For example, doublecortin and Ca2/calmodulin-dependent kinase-like 1 (Dclk1) is expressed in a small percentage of duct cells, and these cells are associated with progenitor-like function (May et al., 2010). Additional studies have shown that Dclk1 high cells have enhanced PanIN sphere-forming ability, suggesting a propensity for such cells to undergo neoplastic transformation (Bailey et al., 2014). It remains to be explored as to whether Dclk1 high cells have already initiated an early dedifferentiation program that predisposes them for PanIN formation. The finding that Dclk1 is among a select group of factors, including CD24, CD44, and ESA, expressed both in early PanIN lesions and "cancer stem cells" present in fully



B

Acinar dedifferentiation

Factor	Expression
Pdx1	Up
Ptf1a	Down
Sox9	Up
Hes1	Up
Gata6	Down
Mist1	Down
Nr5a2	Down
Hnf6	Up
FoxA2	Up
Hnf1β	Up
Prrx1	Up

Ductal dedifferentiation

Factor	Expression
Pdx1	Up
Sox9	Down
Hnf6	Down
FoxA2	Down
Hnf1β	Down

Figure 2. Factors Regulating Exocrine Cell Fate Conversion

(A) During pancreas development, several transcription factors regulate the differentiation cascade that converts progenitors into fully differentiated state in a concerted, regulated manner. Many of these same factors were deregulated during cancer initiation and progression. Factors marked in red have been implicated in cellular dedifferentiation during oncogenic transformation.

(B) Expression analysis of lineage-specifying transcription factors during acinar or ductal dedifferentiation.

developed tumors further suggests that a select sub-population of pancreatic epithelial cells could harbor progenitor functions that might be maintained over time. Cancer stem cells share the ability for unrestrained self-renewal like normal stem cells and can be identified and purified via expression analysis of specific cell surface markers such as CD24, CD44, CD133, EPCAM, ESA, c-Met, Aldh1, Lgr5, and Dclk1 (Hermann et al., 2007; Li et al., 2007; Kim et al., 2011, Sureban et al., 2011; Mizuno et al., 2013; Ischenko et al., 2014). Future studies will have to determine whether cancer stem cells truly represent a distinct cell population or whether the expression of the cellular markers and the associated renewal properties can be attained by other tumor cells under certain conditions.

Humanized Models for PDA

Current evidence indicates that all exocrine pancreas cell types possess the ability to transform into PanIN and PDA, albeit to varying degrees. While acinar cells have emerged as the predominant source for PDA from GEMM studies, one cannot disregard the possibility that murine pancreatic cell types behave differently than their human counterparts with regard to their susceptibility to oncogenic transformation. The advent of human stem cell technology has opened up opportunities to study the initiation of pancreatic neoplasia in the human context. Zaret and colleagues generated human iPS cells from primary pancreatic epithelial cells isolated from PDA, thereby developing a versatile model in which late stage tumors can be reprogrammed to recapitulate early stages of the disease (Kim et al., 2013). In another study, Muthuswamy and colleagues introduced three-dimensional culture conditions to induce differentiation of human pluripotent stem cells into exocrine organoids that can be used to model human PDA (Huang et al., 2015a). These efforts usher in an era in which human models of PDA can be exploited in connection with existing GEMMs to study early and late stages of disease initiation and progression (Kim and Zaret, 2015).

The Role of Lineage-Specifying Transcription Factors in Regulating Cellular Identity

Increasing evidence suggests that acinar dedifferentiation proceeds via an orchestrated process that involves loss of cellular identity followed by the acquisition of ductal properties. In molecular terms, the dedifferentiation program is associated with loss of acinar markers such as Nr5a2, Mist1, and Ptf1a and increased expression of ductal markers such as Sox9 and Hnf6 (Figure 2). Functional studies have shown that these transcription factors are not just markers of dedifferentiation but that they profoundly regulate acinar cell identity and prevent Kras-mediated oncogenesis. For example, Nr5a2, a member of the nuclear receptor family of intracellular transcription factors, is required for securing the fate of the enzyme-producing cells, as its deletion in the pancreas epithelium leads to destabilization of the mature acinar differentiation state and loss of regenerative capacity following acute pancreatitis (Flandez et al., 2014; von Figura et al., 2014b). In the adult pancreas, Nr5a2 co-operates with the pancreas transcription factor 1-L complex (PTF1-L) to regulate acinar-specific gene expression (Holmstrom et al., 2011). PTF1-L is a tripartite complex in which the basic helix-loop-helix protein (bHLH) protein Ptf1a serves to convey target specific gene expression. Similar to Nr5a2, deletion of Ptf1a in the pancreas also promotes rapid ADM (Krah et al., 2015). Another bHLH transcription factor, Mist1, is expressed in pancreatic acinar cells and has also been shown to maintain their function, stability, and identity (Pin et al., 2001; Shi et al., 2013). Thus, a defined set of acinar transcription factors prevents transdifferentiation toward other pancreatic cell types, most notably duct cells. Interestingly, sustained expression of these acinar-specific transcription factors appears critical in impairing oncogenic Kras-driven ADM and neoplastic transformation. In summary, these observations support the concept that erosion of the acinar differentiation

state is necessary to provide a permissive environment for Kras-driven ADM and PanIN development.

In addition to signals that maintain acinar identity, a different set of transcription factors actively promotes acinar dedifferentiation. Expression of proteins normally absent in enzyme-producing cells results in their transdifferentiation toward ductal properties. For example, Pdx1, a transcription factor mostly confined to β cells in the adult pancreas, leads to cell-autonomous acinar to ductal transition when artificially expressed in acinar cells from embryonic stages onward (Miyatsuka et al., 2006). Similarly, inappropriate expression of Sox9 in acinar cells, a transcription factor that maintains duct cell identity, destabilizes cellular identity of acinar cells, promotes expression of ductal genes, and greatly increases Kras-driven ADM. The observation that deletion of *Sox9* in KrasG12D-expressing adult acinar cells largely inhibits PanIN formation further suggests a critical function for the transcription factor in the transition toward a ductal fate (Kopp et al., 2012). Support for this notion comes from human studies that have noted upregulation of this key duct marker during ADM (Kopp et al., 2012). Prrx1, a nuclear homeodomain transcription factor upregulated during acinar dedifferentiation, binds to the *Sox9* promoter and positively regulates Sox9 expression (Reichert et al., 2013). Furthermore, Hnf6, another duct-specific transcription factor, suppresses acinar markers while inducing ductal markers such as Sox9 (Prévot et al., 2012). Thus, initiating a transdifferentiation cascade toward the duct state appears to be accomplished through activation of just a few transcription factors at the top of a hierarchical signaling cascade. Complete ductal reprogramming, however, requires the presence of oncogenic Kras. Therefore, promoting acinar transdifferentiation toward a ductal state needs to go hand-in-hand with oncogenic stimuli to induce lasting ADM, neoplasia, and PDA.

Role of Oncogenic Kras in Cellular Reprogramming

As oncogenic Kras is central to pancreatic cancer initiation, delineating the function of the various Kras-driven pathways in the dedifferentiation process has been a major research focus. Using a mouse model that permitted pancreas-specific, temporally regulated and reversible expression of mutant Kras, Magliano and colleagues showed that oncogenic Kras reversibly altered normal epithelial differentiation following tissue damage (Collins et al., 2012). Inactivation of Kras in established acinar-derived PanINs leads to their redifferentiation into acinar cells and eventually PanIN regression. Oncogenic Kras signaling in PDA primarily involves three major pathways: the (1) Raf/MEK/ERK, (2) PI3K/Pdk1/Akt, and (3) Ral guanine nucleotide exchange factor pathways (Eser et al., 2014). Active MAPK signaling is required to maintain the KrasG12D-driven dedifferentiated acinar state in PanIN lesions. Conversely, pharmacological inhibition of MAPK pathway effector MEK1/2 drives PanIN cells to re-differentiate into acinar cells (Collins et al., 2014). Several other MAPK pathway factors that are required for Ras transformation such as Protein kinase C iota, serine threonine kinase Protein kinase D1, and immediate early response family IER3 have also been shown to be required for Kras-mediated reprogramming of enzyme-producing cells (Garcia et al., 2014; Liou et al., 2015; Scotti et al., 2012). Cell-autonomous PI3K and PDK1 signaling, the second Kras regulated pathway, serves as a key effector of oncogenic Kras-driven ADM (Eser et al., 2013; Ying et al., 2011). Likewise, Ral-GDS, a component of the third arm of Ras effector signaling, has been implicated in the early dedifferentiation program. Ral1 target Rac1 mediates F-actin redistribution and actin reorganization, which is required for morphologic changes of acinar cells undergoing ductal metaplasia (Heid et al., 2011). Thus, all three signaling cascades downstream of oncogenic Kras are involved in aspects of ADM and PanIN formation.

In addition, several other pathways in the Ras interactome, such as STAT3 and EGFR signaling, have been shown to play an integral part in the ADM process (Corcoran et al., 2011; Fukuda et al., 2011; Lesina et al., 2011;Siveke et al., 2007;Ardito et al., 2012). Summarily, available evidence reinforces the concept of a coordinated erosion process in mature acinar cells preceding the oncogenic transformation driven by mutant Kras toward PDA.

Embryonic Signaling Pathways Involved in Acinar Reprogramming

Oncogenic Kras-mediated cellular dedifferentiation often involves hijacking of signaling pathways that play a crucial role in pancreatic organogenesis such as Notch, Wnt, Hedgehog (Hh), and small non-coding RNA (Dessimoz et al., 2005; Hebrok et al., 2000; Heiser et al., 2006; Jensen, 2004; Murtaugh et al., 2005; Wells et al., 2007; Lynn et al., 2007). These pathways play critical functions during oncogene-driven acinar dedifferentiation (Morris et al., 2014; Prévot et al., 2013; Wang et al., 2014; Fendrich et al., 2008). For example, activated Notch signaling and oncogenic Kras co-operate in promoting rapid reprogramming of acinar cells into a duct-like phenotype (De La O et al., 2008; Greer et al., 2013). In contrast to the positive role of Notch during ADM, β-catenin, a critical mediator of the canonical Wnt signaling cascade, inhibits Kras-dependent reprogramming of acinar cells (Heiser et al., 2008; Morris et al., 2010). Thus, ADM is regulated by positive and inhibitory signals from embryonic pathways that guide exocrine pancreas formation.

Epigenetic modifiers and chromatin regulators too have been implicated in oncogenic Kras-driven loss of acinar identity. For example, Brg1, an ATP-dependent catalytic subunit of the SWI/ SNF chromatin remodeler complex, is required for oncogenic Kras-driven ductal metaplasia (von Figura et al., 2014a). Likewise, Sirtuin 1, a NAD-dependent histone deacetylase, regulates ADM. Sirt1-mediated deacetylation of β-catenin and acinar-specific transcription factor Ptf1a leads to a reduction of the expression of both factors and subsequently to a loss of the mature acinar state, thus placing Sirt1 atop a hierarchical cascade that controls mature acinar identity (Wauters et al., 2013).

Finally, several environmental stimuli such as smoking and chronic alcohol exposure, posited to be risk factors for PDA, have also been implicated in ADM (Huang et al., 2015b). For example, smoking, one of the major risk factors for PDA formation, induces dedifferentiation of acinar cells by activating AKT-ERK-MYC signaling (Hermann et al., 2014). This cascade inhibits the activity of Gata6, a transcription factor previously shown to play an important role in maintaining the adult acinar cell compartment (Martinelli et al., 2015). Summarily, these data demonstrate how oncogenic cues collaborate with a complex

network of genomic and epigenomic regulators and erode the matured acinar state. More importantly, identifying these critical nodes of deregulation provides us with an opportunity to clinically target the defective differentiation states of tumor cells.

IPMN-Derived PDA

Besides the vast majority of PDAs being derived from PanIN lesions, there are a small number of PDA cases, which emerge from other neoplastic subtypes such as mucinous cystic neoplasm (MCN) and intraductal papillary mucinous neoplasm (IPMN). Mouse models that faithfully recapitulate human MCN are limited, thus precluding proper identification of their cellular origin (Izeradjene et al., 2007). One study suggested MCN to be formed from acinar cells; however, whether that process involves any dedifferentiation step remains to be explored (Sano et al., 2014).

IPMN formation has been observed in several transgenic mouse models, including those with concomitant activation of TGF-alpha, stimulation of Gs-alpha subunit GNAS, deletion of *Brg1*, or attenuated Smad4 expression in the context of oncogenic Kras (Bardeesy et al., 2006; Siveke et al., 2007; Taki et al., 2015; von Figura et al., 2014a). Of particular interest is the observation that despite distinct morphological and gene expression signatures of IPMN and PanIN lesions, the resulting tumors are both classified as PDA. This raises the question of whether the early steps of IPMN and PanIN initiation are shared. To delineate the cellular origin of IPMN, Cre recombinase-driven expression of oncogenic *Kras* and elimination of *Brg1* was directed specifically to adult duct or acinar cells. Acinar-driven animals did not display any IPMN lesions, suggesting limited potential for enzyme-producing cells to undergo such neoplasia. In contrast, IPMN-like progenitor duct atypia as well as occasional IPMN formation were found in mice with oncogenic Kras/elimination of Brg1 in duct cells (von Figura et al., 2014a). Considering that IPMN lesions retain ductal characteristics, the recent finding that pancreatic duct cells also lose their mature differentiation state *en route* to oncogenic transformation was somewhat unexpected (Roy et al., 2015). Duct-derived IPMN cells were found to lose expression of many adult ductal markers such as HNF1β, Hnf6, and Sox9. Loss of Sox9 expression appears to be particularly important for duct identity, a hypothesis that was confirmed upon ectopic expression of Sox9 that prevented IPMN formation.

These observations point to the deterioration of cellular identity as a shared and critical process during PanIN and IPMN formation. While acinar cells undergo transdifferentiation toward a "duct-like" state before PanIN formation is initiated, duct cells dedifferentiate to a lesser degree and retain aspects of the duct lineage during IPMN development. Notably, both dedifferentiation programs display some features of the ductal lineage, raising the tantalizing hypothesis that exocrine neoplasia might have to progress through a common "bottle neck, duct-like" state (Figure 1). Defining in detail the dedifferentiated cells emerging from either acinar or duct cells should enable us to tailor therapeutic regimens for PDA arising from divergent neoplastic lesions.

Therapeutic Implications

A central question emerges from the above studies: is it possible to reverse the dedifferentiated state of pancreatic cancer cells and sensitize them to chemotherapy? Successful application of retinoic acid for APML or trabectedin for MLS indeed holds promise for "differentiation-targeted" therapy. One can argue that APML or MLS, unlike gastrointestinal cancers, are single karyotype diseases caused by a characteristic fusion gene that drives their tumorigenicity. However, similar arguments could be made for oncogenic Kras, the predominant oncogene for PDA. Fortunately, with the development of sophisticated GEMM, we are now equipped to analyze the different developmental stages during carcinoma progression. The information gathered from these mouse models will inform us about the critical nodes of tumor development that can be targeted. For instance, during pancreatic cancer progression, the progenitor genes *Pdx1* and *Hnf4a* are upregulated. JQ1, a bromodomain inhibitor, represses expression of these genes and activates mature duct markers (Roy et al., 2015). As a consequence, JQ1 reduced IPMN-PDA tumor burden in animals, possibly through reactivation of a cellular differentiation program. In support of the broader use of such an approach, a recent report showed that by overexpressing the bHLH protein E47, neoplastic cells reverted to an acinar state with reduced PanIN tumorigenicity (Kim et al., 2015). Perhaps the most important evidence comes from the use of MAPK inhibitors, which directly antagonize the oncogenic Kras pathway. When the MAPK-ERK pathway was inhibited pharmacologically in Kras-driven animal models, neoplastic cells were able to reverse to their mature acinar differentiation state (Collins et al., 2014). Thus, strengthening or reestablishing the cellular identity may be exploited as a novel therapeutic avenue for PDA treatment.

In their widely cited "Hallmarks of Cancer" review, Weinberg and Hanahan identified several characteristics shared by most cancer cells, such as replicative immortality, evasion of apoptosis, escape from immune cell recognition, and aberrant cellular energetics (Hanahan and Weinberg, 2011). Current therapeutic interventions attempt to target the principal nodes of the signaling pathways that lead to the disruption of these specified hallmarks of cancer. However, in light of new and emerging evidence, a "defective dedifferentiation state" may be a hallmark of many cancers that can be targeted therapeutically with the goal of reverting tumor cells toward a normal or more benign state.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one table and can be found with this article online at http://dx.doi.org/10.1016/j.devcel.2015.12.001.

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