Regeneration and Repair of the Exocrine Pancreas

L. Charles Murtaugh and Matthew D. Keefe

Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, Utah 84112; email: murtaugh@genetics.utah.edu

Annu. Rev. Physiol. 2015. 77:229–49

First published online as a Review in Advance on October 24, 2014

The *Annual Review of Physiology* is online at physiol.annualreviews.org

This article's doi: 10.1146/annurev-physiol-021014-071727

Keywords

pancreatitis, acinar cell, inflammation, metaplasia, differentiation, caerulein, cerulein

Abstract

Pancreatitis is caused by inflammatory injury to the exocrine pancreas, from which both humans and animal models appear to recover via regeneration of digestive enzyme–producing acinar cells. This regenerative process involves transient phases of inflammation, metaplasia, and redifferentiation, driven by cell-cell interactions between acinar cells, leukocytes, and resident fibroblasts. The NFκB signaling pathway is a critical determinant of pancreatic inflammation and metaplasia, whereas a number of developmental signals and transcription factors are devoted to promoting acinar redifferentiation after injury. Imbalances between these proinflammatory and prodifferentiation pathways contribute to chronic pancreatitis, characterized by persistent inflammation, fibrosis, and acinar dedifferentiation. Loss of acinar cell differentiation also drives pancreatic cancer initiation, providing a mechanistic link between pancreatitis and cancer risk. Unraveling the molecular bases of exocrine regeneration may identify new therapeutic targets for treatment and prevention of both of these deadly diseases.

INTRODUCTION

The phenomenon of regeneration has fascinated laypeople and scientists alike since the dawn of recorded history: If a starfish can regrow an arm, or an axolotl can recover from a spinal cord injury, wouldn't it be nice for humans to do the same? Unfortunately, we are more limited than our distant relatives in our capacity to regenerate, with the liver furnishing one of the few examples in which a human organ can fully recover from profound injury. Other organs, such as the brain and the heart, exhibit little or no capacity for true regeneration, although functional compensation for injury is often possible. Next door to the liver, the pancreas presents a more mixed picture, with at least some capacity for regeneration and repair firmly established in mammalian model organisms and potentially present in humans as well.

Most studies of pancreas regeneration have focused on the endocrine pancreas, home to the insulin-producing beta (β)-cells that are destroyed in type 1 diabetes. If these cells could be efficiently regenerated, it is hoped, diabetes might be reversed. Regeneration of the exocrine pancreas, comprising digestive enzyme–producing acinar cells (the majority cell type of the organ) and a network of ductal cells that carry these enzymes to the intestine, has received increasing attention of late, particularly as experimental models of exocrine regeneration reveal a surprising capacity for cell fate plasticity that is likely relevant to cancer and diabetes. As we discuss below, exocrine pancreas injury and regeneration frequently involve changes in differentiated cell identity. When transient and reversible, cellular plasticity may be critical for regeneration; when sustained, however, this same plasticity can predispose to cancer.

PANCREATIC ANATOMY AND DEVELOPMENT

Embryonically, the mammalian pancreas derives from two patches of posterior foregut endoderm, dorsal and ventral, which evaginate, branch, and ultimately coalesce to form the mature organ. The ventral pancreas gives rise to the head of the organ, adjacent to the duodenum, whereas the dorsal pancreas gives rise to the tail, attached to the spleen. Each lobe contains a ramified ductal network that connects acinar cells, organized in clusters at the terminus of the ductal tree, to the small intestine. Although these networks arise separately in early development, they are partially interconnected in the mature organ, and the main pancreatic duct is joined by the common bile duct before entering the intestine. This last connection, in fact, makes the exocrine pancreas vulnerable to damage by gallstone obstructions, a significant cause of acute pancreatitis (1).

A detailed accounting of pancreas development is outside the scope of this review but is available from several recent sources (2, 3). Like any other complex organ, the pancreas develops through coordination of cell-cell interactions with the activity of diverse transcription factors (TFs). Among the most important advances in the pancreas development field has been to link expression of these TFs to specific cell fate decisions within the organ via the technique of Cre-loxP lineage tracing (4). Developed in mice, this approach combines deletor transgenic or knock-in strains that express Cre protein in the domain of one of these TFs, together with reporter strains in which Cre-mediated recombination activates a genetic lineage marker such as green fluorescent protein. More sophisticated inducible strategies use one of several tamoxifen-dependent CreERT (fusions of Cre to an estrogen receptor domain that binds tamoxifen) variants, which allow for temporal control of recombination based on tamoxifen treatment. Collectively, these studies have defined specific lineages within the developing pancreas on the basis of TF expression domains (2, 3). In addition, they have provided an invaluable resource for conditional knockout studies in which genes relevant to pancreatic injury, regeneration, or cancer can be manipulated selectively in different pancreatic cell types, as described below.

Lineage tracing indicates that all cell types of the pancreas arise from multipotent progenitor cells (MPCs) that coexpress the homeobox protein PDX1, the Sry-box protein SOX9, and the bHLH protein PTF1A. MPCs decline in numbers as development proceeds, and each of these TFs becomes increasingly restricted to specific cell types: PDX1 to insulin-producing β-cells, SOX9 to duct cells, and PTF1A to acinar cells. This restriction can be detected in lineage-tracing experiments utilizing inducible CreERT strains, such as *Ptf1a*^{CreERT} (5): Tamoxifen administration during early stages [e.g., embryonic day 11.5 (E11.5)] marks all endocrine and exocrine cell types, whereas later-stage activation (>E14.5) labels exocrine acinar cells almost exclusively. This shift in expression coincides with a shift in the functional role of the individual TFs: PTF1A, for example, is initially required for the specification of pancreatic MPCs, but it later functions in acinar cells as a master regulator of acinar cell–specific gene expression (2, 6).

These same inducible Cre deletor strains have been used for lineage tracing in the adult pancreas to address the question of whether this organ contains stem or progenitor cells. Although this issue is still contentious, the weight of evidence to date is negative. For example, whereas SOX9^{+} duct-like cells of the embryo give rise to β-cells and other endocrine cells, via transient intermediates that express the bHLH factor NGN3, this capacity declines precipitously after birth, as does the overall number of NGN3^{+} cells (7). Similar results have been obtained with other ductal Cre drivers as well as with acinar drivers such as *Ptf1a*^{CreERT} (5, 8–10) and collectively indicate that progenitor cell activity is restricted largely to prenatal life. Within the exocrine compartment, duct cells normally beget more duct cells and acinar cells more acinar cells, and crossover of one lineage to the other is vanishingly rare. As we discuss below, however, injury to the pancreas can cause cells to dramatically change their fate, with either positive or negative implications for long-term health of the organ and organism.

PANCREATIC INJURY AND REGENERATION: CLINICAL AND LABORATORY OBSERVATIONS

Before considering the numerous animal models of pancreas injury and regeneration, we must consider the clinical context in which these concepts are of most immediate relevance: that of pancreatitis. Pancreatitis, an inflammatory condition of the exocrine pancreas, comes in both acute and chronic varieties. Acute pancreatitis presents clinically with abdominal pain and nausea and is distinguished from other gastrointestinal illnesses primarily by detection of pancreatic enzymes, including amylase and lipase, in the circulation (1). Most cases are mild and resolve without incident within days or weeks, whereas more severe cases, particularly those defined as necrotizing, can be lethal due to multiorgan failure. Acute pancreatitis can be caused by obstructions such as gallstones or as a secondary effect of alcohol abuse or hypertriglyceridemia, as well as by less common causes such as infection or trauma. Chronic pancreatitis is most commonly associated with alcoholism and is associated with pain but not, as a general rule, with circulating pancreatic enzymes (11). Epidemiologically, chronic pancreatitis is a predisposing factor for pancreatic cancer (12); potential mechanisms connecting pancreatitis and cancer are discussed in the final section of this review.

Acute pancreatitis and chronic pancreatitis can be linked, as when recurrent inflammatory events progress to chronic disease. The absence of circulating enzymes in chronic pancreatitis is thought to reflect the atrophy of enzyme-producing acinar cells, due to persistent inflammation and fibrosis, rather than a fundamental difference in the etiology of the two conditions. Indeed, digestive enzyme activity is likely to be central to pancreatitis, as almost all mutations that increase the risk for pancreatitis occur in genes encoding acinar digestive enzymes or their inhibitors (13). The predominant paradigm for pancreatitis is that these enzymes, particularly trypsin, become inappropriately active within the acinar cells themselves or in their immediate microenvironment,

leading to autodigestion, necrosis, and inflammation. This paradigm is supported by numerous animal models, although recent findings have somewhat complicated this picture. In particular, genetic knockout of the mouse *Trypsinogen-7* gene, encoding the major trypsin enzyme of mice, reduces pancreatitis-associated necrosis but does not prevent inflammation (14). Clinically, efforts to treat pancreatitis with inhibitors of trypsin and other acinar proteases have been ineffective (15). These and other findings have led to an increasing appreciation of the role of acinar cell endoplasmic reticulum (ER) stress as a trigger for pancreatitis, in parallel with protease activation (16). Further clinical and animal model studies will be required to determine the precise contribution of these factors to pancreatitis severity.

One reason that animal models have been central to pancreatitis research for more than a century is that the events underlying the human pathology are inaccessible to direct observation. This problem applies, for example, to regeneration: Although it is generally assumed that the human pancreas regenerates from mild pancreatitis, concomitant with resolution of pain and other symptoms, direct evidence of regeneration has never been provided. Surgery for pancreatitis is relatively rare and is limited to removing damaged portions of the organ in cases of necrotizing acute or chronic disease. In other words, inferences regarding regeneration must be made from surgically resected organs that were unable to regenerate. Damaged human pancreata do exhibit increased proliferation of residual acinar tissue, as well as histological changes consistent with ongoing regeneration. But such pancreata also display rampant fibrosis, leukocyte infiltration, and epithelial structures variously termed acinar-ductal metaplasia (ADM) or tubular complexes, which may represent acinar cells that have dedifferentiated in response to chronic inflammation (17–21) (**Figure 1**). Efforts to determine the origin of ADM, and to decipher the signals that drive loss and regeneration of acinar tissue after injury, have relied on experimental animal models.

Multiple models of pancreatic injury and regeneration exist, and their benefits and drawbacks have been reviewed extensively (22). Simply put, no single model encompasses all features of human pancreatitis, including fibrosis, exocrine insufficiency, and association with alcohol intake, nor is any one of these features perfectly reproduced in an animal. The choice of approach depends on the question of interest but is also dictated by what is feasible and convenient in a given model organism. As investigators increasingly resort to genetically modified mice, the injury model of choice has become caerulein-induced pancreatitis (23). Caerulein is an analog of cholecystokinin, which directly stimulates acinar enzyme production and secretion in rodents. When administered at supraphysiological levels, caerulein promotes excessive digestive enzyme production, ultimately leading to the improper activation of these zymogens within the cell by lysosomal cathepsin B (24, 25). A significant fraction of pancreatic injury can be attributed to cathepsin B–mediated production of activated trypsin, although as described above, ER stress due to digestive enzyme overexpression/misfolding can induce inflammation independently of trypsin activity, most likely by inducing NFκB-dependent cytokine synthesis (16, 26). The result, after a 1–2-day course of caerulein injection, is widespread acinar cell apoptosis and necrosis, followed by transient ADM in which acinar cells downregulate digestive enzymes and upregulate normally duct-specific proteins such as the intermediate filament cytokeratin 19 (CK19) and the TF SOX9 (27–31) (**Figure 2**). The endocrine pancreas is entirely spared during this process. Within 1–2 weeks, similar to what occurs in human acute pancreatitis, nearly all aspects of pancreatic morphology, differentiation, and function return to normal in wild-type mice. The simplicity of this experimental system has facilitated numerous insights into the cellular and molecular mechanisms of exocrine pancreas regeneration and repair.

The caerulein model most readily models acute pancreatitis, but the drug treatment period can be extended over several weeks to produce a more chronic pancreatitis–like set of phenotypes such as fibrosis and persistent ADM (32). Surgical injury models have been developed to model

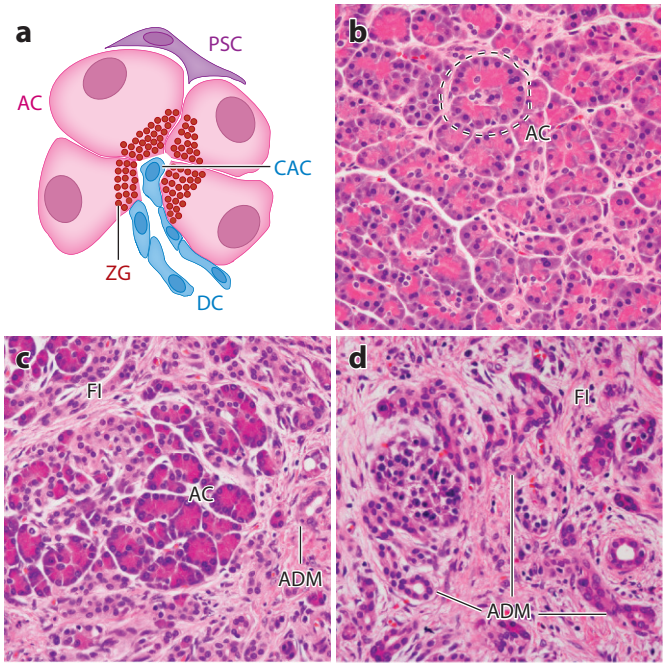

Figure 1

The normal and injured pancreas. (*a*) Schematic diagram of an acinar unit, indicating acinar cells (AC) with apically localized zymogen granules (ZG), centroacinar cells (CAC) and duct cells (DC), and quiescent pancreatic stellate cells (PSC). (*b*) Hematoxylin-and-eosin (H&E)-stained section of a normal human pancreas, with an exemplary acinar structure outlined with the dotted line. (*c*,*d*) H&E-stained sections of human chronic pancreatitis specimens of varying severity, indicating fibrosis (FI) as well as examples of putative acinar-ductal metaplasia (ADM).

additional aspects of human pancreatitis, as well as to provide independent insight into pancreatic regeneration. Pancreatic duct ligation, in which the main pancreatic duct is tied shut upstream of its junction with the bile duct, models obstructive pancreatitis and causes rapid atrophy of acinar cells (33). As with caerulein pancreatitis, duct ligation does not damage the endocrine pancreas and, in fact, provided Banting and Best with a means to isolate high levels of insulin from dog pancreata without degradation by exocrine enzymes (34). Unlike the caerulein model, there is essentially no acinar cell regeneration after duct ligation. Partial pancreatectomy has been a popular model for both exocrine and endocrine regeneration studies for decades (35, 36). In general, young rodents are capable of replacing half or more of lost acinar and islet mass after surgical resection, whereas older animals have much less regenerative ability (37, 38). Limited studies of humans indicate that regeneration occurs after resection in children, but not in adults (39–41). Altogether, experimental rodents appear more resistant than humans to severe, persistent pancreatic injury, in part because of an enhanced capacity for regeneration. Keeping in mind the limitations of animal models (and inspired by the hope that their capacity for repair could be translated to patients), we now turn to the insights these models have provided into mechanisms of exocrine pancreas regeneration.

THE CELLULAR BASIS OF EXOCRINE PANCREAS REGENERATION

Almost immediately from the point of injury, multiple cell types participate in the process of exocrine pancreas repair and regeneration. These cells include not only the acinar cell, which

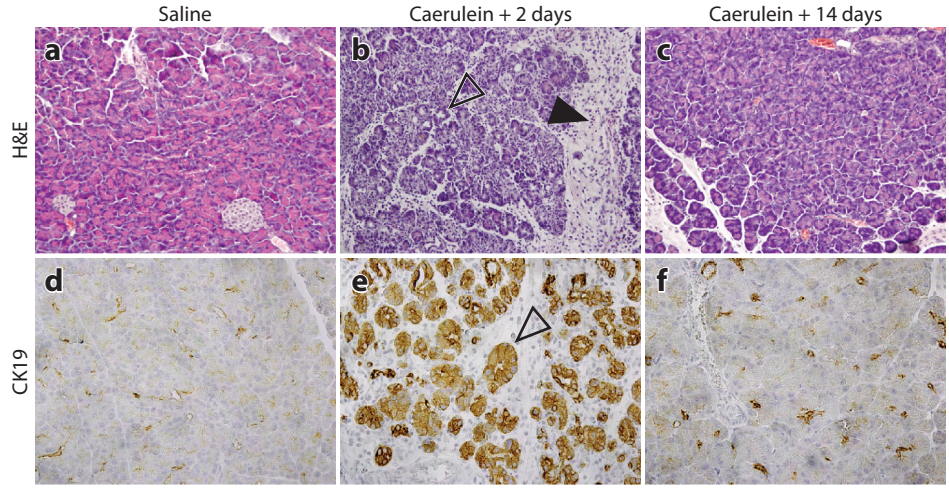

Figure 2

Caerulein-induced pancreatitis and metaplasia. Sections from mice injected with saline solution as a negative control (*a*,*d*) or with high doses of caerulein to induce pancreatitis and harvested 2 days (*b*,*e*) or 14 days (*c*,*f*) postinjection, stained with H&E (*a–c*) or by immunohistochemistry against the duct marker cytokeratin 19 (CK19) (*d–f*, *brown*). Metaplasia (*open arrowhead*) and inflammatory cell infiltration (*closed arrowhead*) are widespread at 2 days, accompanied by upregulation of CK19 in acinar cells, but are essentially undetectable when regeneration is complete at 14 days. Figure adapted from Reference 46, under a Creative Commons Attribution (CC-BY) 3.0 License (copyright held by L.C.M. and M.K.).

is both villain and victim in pancreatic injury, but also ductal epithelial cells, inflammatory cells of the immune system, and resident fibroblasts, or stellate cells. The contributions of these cells have been revealed by tissue-specific genetic manipulation, a technique that has revolutionized mouse research in general. Among the most powerful tools in the mouse genetic toolkit are lineage tracing and conditional gene knockout, both of which have been used to study the parenchymal (i.e., exocrine and endocrine) response to pancreatic injury. More conventional knockout mouse approaches, together with pharmacology and cell culture, have predominated in studies of the fibroinflammatory response to injury; as discussed below, these studies have left unresolved certain important questions about the precise role of individual nonparenchymal cell types.

Epithelial Transformations and Interactions During Injury and Regeneration

An emergent theme of adult pancreas regeneration, both exocrine and endocrine, is that like begets like: When large numbers of a specific cell type are destroyed, they are replaced by expansion of residual surviving cells rather than by cells that have newly differentiated (neogenesis) from an outside source, such as stem cells. This phenomenon was first demonstrated for insulin-producing β-cells (42, 43) and was soon afterward extended to acinar cells in damage models such as caerulein pancreatitis and partial pancreatectomy (31, 44, 45). Conversely, acinar cells generally retain their lineage commitment after injury and do not contribute to duct or endocrine cells in the course of regeneration. Nonetheless, several important caveats apply to the conclusion that acinar regeneration is driven simply by proliferation of surviving acinar cells. The first of these is technical: Comparing the fate of cells before and after injury relies on tamoxifen-inducible lineage-tracing experiments that are subject to substantial experimental variation. Acinar cells are labeled by tamoxifen treatment prior to injury, and a similar fraction of cells are labeled, after a period of

regeneration, in control and injured mice (31, 44–46). Although these results indicate that most regenerated acini derive from acini already existing at the time of injury, a minority of acini may originate via neogenesis from other cells, such as ductal cells, in numbers small enough to hide within the error bars. Results of the converse experiment, in which duct or other nonacinar cells are labeled and studied for their recruitment into the acinar lineage postinjury, have not yet been reported.

A second caveat is that neither caerulein pancreatitis nor partial pancreatectomy fully ablates acinar cells: Might a more severe injury provoke an alternative form of regeneration? Such alternate regeneration has been established in the endocrine pancreas, in which near-complete destruction of β-cells, in a diphtheria toxin–based transgenic model, induced β-cell replacement via transdifferentiation of glucagon-producing α-cells (47). Recent work provides suggestive evidence of a similar phenomenon in the exocrine compartment: When acinar cells are extensively killed via a similar diphtheria toxin–based approach, regeneration of this cell type appears to depend on duct-to-acinar transdifferentiation rather than on acinar self-renewal (48). This result suggests that the capacity of duct cells to adopt an acinar identity is context dependent and is potentially inhibited, in more limited injury models, by the presence of persistent acinar cells.

As we discuss in the next section, the juxtacrine-acting Notch pathway is a candidate mediator of this inhibitory interaction, which exemplifies a third key aspect of exocrine regeneration: its dependence on intimate cell-cell communication. For example, when gap junctional communication between acinar cells is disrupted in *Connexin-32* (*Cx32*) knockout mice, the severity of caerulein-induced pancreatitis is increased, and necrosis persists through stages at which wild-type pancreata have regenerated (49). The integrity of the ductal system also determines the severity of injury to acinar cells: Mouse knockouts for the *Cftr* gene, which encodes a ductal chloride channel, exhibit increased acinar necrosis and inflammation in response to caerulein pancreatitis, modeling the susceptibility to pancreatitis of human cystic fibrosis patients (50). Spontaneous inflammation and pancreatitis-like phenotypes are observed in mice with other putatively duct-specific genetic defects, such as loss of cilia (51). The ducts can also directly control injury, for example, by producing proapoptotic Fas ligand, which induces acinar cell atrophy and metaplasia after duct ligation (52). These findings suggest that the effectiveness of regeneration, like the severity of injury, may reflect cell-cell interactions as well as acinar cell–intrinsic responses.

That ductal defects can indirectly produce acinar phenotypes also mandates caution when one is interpreting conditional knockout phenotypes in the pancreas, because acinar phenotypes in whole-pancreas knockouts may not reflect gene function in acini. Pancreas-specific knockouts typically use Cre transgenes targeted to early MPCs, such as *Pdx1-Cre* and *Ptf1a*^{Cre} (53), which induce deletion beginning in embryogenesis and extending well beyond acinar cells. Acinar injury or regeneration phenotypes observed in such conditional knockout mice may therefore reflect subtle developmental defects and/or non-acinar-autonomous gene function. A more specific strategy is to use an acinar-specific, temporally regulated Cre deletor line (54–56), although this approach has its own caveats in terms of efficiency and stringency of deletion (53). No experimental system is perfect, and the possibility for false-negative and false-positive results should always be kept in mind.

A final point regarding acinar self-renewal during regeneration concerns postinjury ADM, during which acinar gene expression declines and duct markers are induced (27–30). Although metaplastic cells normally revert rapidly to a differentiated acinar state, persistent injury can block their redifferentiation and produce a sustained loss of acinar identity (5, 44). Whether these transdifferentiated cells retain the capacity to return to a normal acinar state is unknown, as is the contribution that these cells make to chronic inflammation. Given that similar ADM is common in human chronic pancreatitis (**Figure 1**), and that acinar cells are normally a major

source of inflammatory cytokines in pancreatic injury (57–59), it is tempting to speculate that transdifferentiated acinar cells contribute to the persistence of this disease phenotype. This issue is discussed in more detail at the end of this review.

The Fibroinflammatory Contribution to Regeneration: More Questions than Answers?

At a histological level, pancreatic injury is associated with abundant infiltration of inflammatory cells and expansion of resident fibroblasts, termed pancreatic stellate cells (PSCs). These cells persist during ADM, and their disappearance or persistence correlates with the success or failure of regeneration. There has therefore been considerable interest in understanding the contribution of these cell types to pancreatic injury and regeneration, although results to date have been limited and occasionally contradictory.

Fibrosis, the excessive deposition of extracellular matrix (ECM) molecules, is a common feature of chronic pancreatitis as well as of pancreatic cancer and has generally been taken to be a contributor to disease pathogenesis (60). The cell responsible for fibrosis is the PSC, a resident pancreatic fibroblast that switches from a quiescent to an activated state in response to injury. PSCs are very similar to stellate cells of the liver, which are similarly implicated in fibrosis of that organ. Prior to injury, PSCs reside in periacinar and periductal spaces, exhibit minimal proliferation or ECM production, and are distinguished from other fibroblasts by containing small but distinctive lipid droplets (61). After injury, in response to cytokines and other factors, PSCs proliferate, upregulate myofibroblast markers such as smooth muscle actin, and produce abundant ECM such as collagen (60).

Although in vitro experiments have delineated a variety of stimuli and responses associated with PSC activation, the in vivo role of PSCs in injury and regeneration remains uncertain. In part this uncertainty is due to a historical lack of appropriate Cre drivers to manipulate PSCs in vivo, a situation that may finally be changing. The TF Nkx3.2 is expressed in the pancreatic mesenchyme of the embryo, and recombination driven by an *Nkx3.2*^{Cre} knock-in allele marks pancreatic fibroblasts before and after birth, although their contribution to PSCs specifically has not yet been determined (62). If it proves to be active in the PSC lineage, *Nkx3.2*^{Cre} could be of great utility in understanding the role of this cell type. Recombination driven by *Nkx3.2*^{Cre} begins in utero, however, and therefore may not be suitable for deleting genes that might have developmental as well as adult roles. Although inducible Cre lines for adult PSCs have not yet been described, a *Vimentin-CreERT* line has been generated for manipulation of adult hepatic stellate cells (63) and may be useful in the pancreas as well. Given the importance of epithelial-mesenchymal interactions during pancreas development (2, 62), interactions between parenchymal cells and PSCs are almost certain to be important for proper exocrine regeneration. Nonetheless, surprises may arise as the field moves from in vitro to in vivo studies: Whereas PSCs had been ascribed a protumorigenic role in pancreatic cancer, based primarily on tissue culture experiments (60), recent genetic studies suggest that PSC-driven fibrosis actually restrains tumor growth and extends life span (64, 65).

Among the first events in pancreatic injury is an influx of inflammatory leukocytes, which amplify the severity of injury (66, 67). This damage-promoting effect is mediated in part by macrophage secretion of the inflammatory cytokine tumor necrosis factor α (TNFα), which has the remarkable effect of promoting trypsinogen activation within acinar cells to amplify autodigestion injury (68). Macrophages persist during chronic pancreatitis and secrete cytokines that promote PSC-driven fibrosis (69). Other immune cell types also enter the injured pancreas and modulate its repair. *Rag1* knockout mice, which lack both B cells and T cells, exhibit abnormally

persistent macrophage infiltration and ADM in response to caerulein pancreatitis, suggesting a role for adaptive immune cells in limiting inflammation (70). In contrast, specific depletion of $CD4^+$ T cells reduces caerulein pancreatitis severity (71), highlighting the complexity of immune cell involvement in injury and repair.

This complexity is further indicated by a recent study of the role of dendritic cells (DCs) in pancreatitis. DCs are recruited to the injured pancreas with a delay relative to neutrophils and macrophages but express a similar repertoire of inflammatory cytokines (72). Inhibition of DC infiltration might therefore have been expected to reduce injury, as in the case of macrophages and neutrophils. Instead, depletion of DCs before injury results in dramatically increased severity, culminating in necrotizing pancreatitis and death of caerulein-treated mice. This phenotype was not prevented by normally effective interventions to limit injury, such as inhibition of inflammatory cytokines or ablation of neutrophils or $CD4^+$ T cells (72). These results suggest a changing role during the course of pancreatitis for what is generically termed inflammation: An early phase amplifies acinar injury, whereas a later phase promotes injury resolution, possibly by clearing cellular debris. Overall, the DC depletion phenotype is among the most profound yet described in pancreatitis research, and further investigation of DCs in repair and regeneration is clearly warranted.

MOLECULAR REGULATORS OF EXOCRINE PANCREAS REGENERATION

To date, most studies of exocrine pancreas injury models have focused either on injury, and how various factors determine its severity, or on the later process of regeneration. The former studies generally address the role of immune cells and inflammatory mediators, whereas the latter, which have become particularly abundant in the decade since developmental biologists rediscovered caerulein pancreatitis (27), tend to deal with molecular mechanisms originally implicated in pancreatic organogenesis. A notable exception is the recent study of pancreatitis in *Rag1* knockout mice, described above, which documented a macrophage-dependent regeneration defect caused by ablation of B and T lymphocytes (70). In discussing the molecular basis of exocrine pancreas regeneration, we consider inflammatory and developmental factors separately while keeping in mind that these are likely to be linked in more than trivial ways: Not only is injury a prerequisite for regeneration, but there are likely to be important feedback mechanisms between severity of injury and capacity for differentiation.

Inflammatory Signaling Pathways and Acinar Cell Regeneration

Both acute pancreatic injury and chronic pancreatic injury are associated with abundant production of inflammatory cytokines, which originate from and act on essentially every relevant cell type: acini, leukocytes, and stellate cells (66, 67). Two of these appear to be particularly important, despite contradictory data on their specific roles in injury and regeneration: TNFα, which acts primarily through the NFκB pathway, and IL-6, which acts through the JAK-STAT pathway.

TNFα was among the first signals to be directly implicated in pancreatitis severity (73), and it is required for macrophage stimulation of acinar cell injury (68). A recent in vitro study indicates that macrophages also utilize TNFα to promote ADM in an NFκB-dependent manner (74). NFκB has also been implicated in caerulein-induced upregulation of the CK19/*Krt19* gene (29), a sentinel indicator of ADM in vivo. It is therefore surprising that the actual role of NFκB in acinar cells in vivo remains a subject of debate. NFκB constitutes a family of five related TFs, all of which are under negative regulation through cytoplasmic sequestration by IκB proteins. Cytokines

and other stimuli activate IκB kinases (IKKs) that phosphorylate IκBs, causing their degradation and releasing active NFκBs into the nucleus to activate target genes (75). One research group has pursued the role of NFκB in pancreatitis primarily through gain-of-function experiments, overexpressing either the NFκB factor RELA/p65 or the upstream activator IKK2 specifically in acinar cells; the former manipulation exacerbated caerulein pancreatitis, whereas the latter was sufficient to induce a pancreatitis-like phenotype even without caerulein (76). In both cases, affected pancreata exhibited persistent ADM and fibrosis, supporting the conventional model of acinar cell NFκB as an inducer of inflammation, PSC recruitment, and dedifferentiation.

By contrast, another group took the loss-of-function approach of deleting *RelA* in the pancreas and observed increased severity of caerulein pancreatitis, including widespread necrotic cell death (77). Conversely, when these same investigators deleted the IκBα/*Nfkbia* gene, encoding a negative regulator of RELA, they found reduced severity of pancreatitis (78). Metaplasia and fibrosis, prominent in *RelA*-overexpressing mice, were not directly assessed in *RelA* knockouts, nor was the extent of necrosis examined in *RelA* overexpressors (76, 77). Thus, what was described as increased injury in these contrasting models may have had distinct etiologies: chronic but low-grade inflammation, associated with ADM, in the context of NFκB hyperactivation, as opposed to acute necrotizing pancreatitis in the absence of NFκB. NFκB has conflicting roles in acute injury and chronic injury in other contexts (75), and the different methodologies of these studies may also account for their distinct outcomes. As noted above, acinar-specific and pancreas-wide genetic manipulations will not necessarily have identical outcomes, and the phenotype of RELA- and IκBα-deficient pancreata may reflect roles for these genes in duct or other nonacinar cells.

Local and circulating levels of IL-6 increase dramatically in human and rodent pancreatitis, and this cytokine has diverse effects on the parenchymal and fibroinflammatory cells of the injured pancreas (66). Whereas genetic deletion of IL-6 exacerbates pancreatic injury caused by caerulein pancreatitis (79, 80), administration of an IL-6-blocking antibody, following caerulein treatment, reduces the acute severity of injury (81). Pancreas-specific deletion of the downstream *Stat3* TF has yielded similarly contradictory results, with one study reporting reduced pancreatitis severity (79) and another reporting enhanced severity, including delayed acinar regeneration (82). As with the studies of NFκB signaling, it is not obvious how these contrasting findings can be reconciled, although they suggest diverse functions for this pathway during injury and, potentially, regeneration.

Developmental Signals Regulating Pancreatic Regeneration

The field of exocrine pancreas regeneration has diversified considerably in the past decade, following a seminal paper that analyzed the process of caerulein-induced pancreatitis from a developmental biology perspective (27). Among other things, this study documented the induction, in regenerating acinar cells, of the TF PDX1, which is normally expressed at high levels in embryonic progenitor cells but is extinguished in the postnatal exocrine pancreas, as well as components of the Notch and Wnt signaling pathways. Notably, the delayed kinetics with which these developmental regulators were induced, relative to the initial wave of inflammation, suggested a role in regeneration rather than in injury (27). These striking observations, together with an appreciation for the simplicity of caerulein pancreatitis as an injury model, stimulated a flurry of studies examining the role of developmental signals and TFs in regeneration.

The Notch signaling pathway inhibits acinar development during embryonic organogenesis, in part by promoting the development of duct/islet-restricted progenitors within the pancreatic epithelium (83). Notch signaling depends on cleavage of ligand-bound Notch receptors by the gamma (γ)-secretase protease complex, releasing an intracellular fragment that can enter the

nucleus and activate target genes via interaction with the RBPJ/CSL TF. Whereas conditional deletion of *Rbpj* in uninjured adult acinar cells has no detectable effect, its deletion in terminal duct/centroacinar cells causes duct-to-acinar transdifferentiation (54), consistent with a role for Notch in suppressing acinar development in both the embryonic pancreas and the adult pancreas. By contrast, a pro-acinar role for Notch has been proposed on the basis of pancreas-wide deletion of *Notch1*, one of several family members expressed in the organ: These mice have no baseline pancreatic phenotype, and exhibit a seemingly normal initial injury response to caerulein treatment, but subsequently fail to resolve the normally transient phase of ADM (84). Acinar-derived cells retain expression of PDX1 as well as the injury marker Clusterin and exhibit increased proliferation and apoptosis compared with controls. Similar results were obtained with pharmacological inhibition of γ-secretase, administered during and following caerulein treatment. Although these findings may reflect an indirect requirement for Notch in duct or other nonacinar cells, the simplest interpretation is that Notch promotes acinar redifferentiation after injury, a role not predicted from developmental studies.

A similar regenerative role was found for the Hedgehog (HH) signaling pathway (31). HH activity is normally inhibited during pancreas development (2, 3), and pancreas-wide deletion of the HH signaling component Smoothened/*Smo* has no significant effect on the exocrine pancreas (31). During caerulein pancreatitis, however, exocrine cells upregulate expression of the ligand Sonic hedgehog (SHH) as well as its downstream target gene *Ptc1*, suggesting that injury induces autocrine/paracrine HH signaling. In pancreas-specific *Smo* knockouts, as with *Notch1* knockouts, initial caerulein-induced injury is similar to that in wild type, but acinar redifferentiation is blocked, and ADM persists at stages when regeneration should be complete. Importantly, the same result is obtained when adult mice are treated with a pharmacological SMO inhibitor and when *Smo* is deleted from adult acini via an inducible CreERT approach, confirming that the requirement for HH signaling is autonomous to mature acinar cells (31). A paradoxical aspect of this study is that HH signaling in vertebrates normally requires primary cilia (85), which are not present on acinar cells (51). Whether acinar cells develop primary cilia in response to injury, or respond to HH in a cilia-independent manner, is unknown.

Together, these studies identify important and novel roles for the Notch and HH pathways in promoting acinar redifferentiation after injury. The Hippo signaling pathway may have a similar role: Pancreas-specific deletion of the *Mst1* and *-2* genes, homologs of the *Drosophila* Hippo kinase, results in spontaneous inflammation, fibrosis, and ADM, mediated by activation of the downstream YAP TF (86, 87). It will be important to determine whether the common role of the Notch, HH, and Hippo pathways in suppressing chronic inflammation and maintaining acinar identity reflects cross talk between these cascades or whether these pathways converge at some distant downstream element. The effect of these pathways appears to be the opposite of that of the epidermal growth factor receptor (EGFR), which is necessary and sufficient for sustained acinar-ductal transdifferentiation (88, 89). This opposition offers an opportunity for genetic epistasis studies, in which analysis of double-mutant phenotypes (e.g., *Notch1*; *Egfr*) can be used to order the pathways relative to one another.

Another developmental signaling pathway, Wnt/β-catenin, affects growth rather than differentiation during regeneration. The canonical Wnt signaling component β-catenin is essential for acinar development in utero (90, 91), and its signaling activity promotes acinar proliferation after birth (46, 92, 93). Inducible deletion of β-catenin from adult acini does not affect the initial injury response to caerulein treatment, nor does it impair metaplasia or redifferentiation, but it does strongly inhibit the proliferative expansion of surviving acini (46). In this respect, then, the regenerative role of β-catenin is analogous to one of its developmental roles: promoting progenitor and acinar proliferation. Whether β-catenin promotes acinar proliferation downstream of Wnt

ligands, or via a Wnt-independent structural function (94), is currently unknown. In the injured mouse kidney, Wnt ligands secreted by infiltrating macrophages promote epithelial proliferation and regeneration (95), and a similar process may be responsible for acinar repopulation as well.

Developmental Transcription Factors Regulating Pancreatic Regeneration

An abundance of TFs have been identified as regulators of pancreas development (2, 3), and many of those implicated in exocrine development or homeostasis have been studied for their roles in pancreatic regeneration. Among these is the orphan nuclear hormone receptor NR5A2/LRH1, which is required in early progenitor cells for normal acinar differentiation (96). Deletion of *Nr5a2* from later-stage progenitor cells causes a mild and focal ADM phenotype in the mature pancreas, in which a small fraction of acini spontaneously activate duct genes and downregulate acinar genes (97). When these mice are subjected to caerulein pancreatitis, their pancreata exhibit normal injury and initiation of ADM, but acinar redifferentiation is blocked, and the organ is subject to atrophy, inflammation, and fibrosis. This phenotype thus resembles that of *Notch1* and *Smo* mutants, although direct comparisons have not been made between these models. A quite similar phenotype is also observed following acinar-specific overexpression of the TF SOX9, which is normally restricted to duct cells but is upregulated in ADM (98), indicating opposing effects of duct and acinar regulators during the resolution of injury. This opposition may facilitate epistasis studies to order these TFs within a regulatory network.

In adult acinar cells, NR5A2 activates target genes in collaboration with the acinar-specific PTF1-L complex, which comprises a trimer of the bHLH factor PTF1A, one of several common bHLH E-protein partners, and the CSL-like factor RBPJL (6, 96, 99). These TFs bind to and activate most acinar cell–specific genes, including their own loci, and together constitute a master regulatory network for acinar differentiation. That *Nr5a2* disruption abolishes acinar redifferentiation and repair suggests that this network is particularly critical for maintaining cell type identity in the face of injury. Among the downstream targets of NR5A2 and PTF1-L is another bHLH factor, MIST1, which is required for terminal differentiation and intracellular organization of acinar cells (100). Unlike pancreas-specific *Nr5a2* knockouts, *Mist1* null mutants exhibit increased severity of caerulein-induced pancreatitis from the onset of treatment, together with persistent fibrosis and a shift toward necrotizing pancreatitis (101). That this early phenotype is not seen in *Nr5a2* mutants as well may reflect residual *Mist1* expression in the absence of NR5A2 (96).

Other TF knockout mice that exhibit a persistent metaplasia/atrophy phenotype similar to that of *Nr5a2*, *Notch1*, and *Smo* include the Polycomb family repressors *Bmi1* (102) and *Ezh2* (103). In the case of *Ezh2* mutants, loss of regeneration is accompanied by a profound cell cycle arrest within the metaplastic epithelium, caused by derepression of the EZH2 target gene *Cdkn2a/p16*^{INK4A}. When this gene is deleted together with *Ezh2*, normal regeneration is restored, suggesting an obligate role for cell cycle progression in acinar cell redifferentiation (103). An alternative explanation is that nondividing acinar cells are lost entirely in caerulein-treated *Ezh2* knockouts, leaving behind a metaplastic epithelium that is actually of ductal origin; *Cdkn2a* deletion would then simply prevent acinar cells from being overwhelmed by apoptosis, rather than permitting redifferentiation per se. In general, the origins of the abnormal epithelial structures that develop in mutant pancreatitis models are unknown and could be addressed by combining inducible, acinar-specific Cre deletors with Cre-dependent reporter genes. Such a strategy was used in the context of β-catenin deletion to confirm that knockout cells survived injury, underwent transient metaplasia, and redifferentiated despite reduced proliferation (46).

Altogether, at least five distinct developmental genes, *Smo*, *Notch1*, *Nr5a2*, *Bmi1*, and *Ezh2*, are required for normal acinar cell regeneration, apparently acting at the level of redifferentiation

rather than that of injury. Nonetheless, the histology of these models indicates the persistence of inflammation and/or fibrosis in association with metaplastic epithelium, suggesting possible interactions between fibroinflammatory and developmental mechanisms in promoting or inhibiting regeneration. In addition, these findings suggest that acinar cells are particularly prone to cell fate changes in response to injury and that disrupting normal acinar differentiation may have profound consequences to organ and organismal health. We conclude this review by next considering these issues, particularly with an eye on future directions for the field.

LINKS BETWEEN INJURY AND REGENERATION

In addition to cell death, leukocyte recruitment, and stellate cell activation, ADM is among the first pancreatic responses to injury. ADM manifests initially as downregulation of acinar-specific genes, upregulation of duct markers such as CK19 and SOX9, and morphological remodeling of the acinar architecture to a more duct-like appearance (27–31). In wild-type animals, this process is transient and resolves as the overall morphology of the injured organ returns to normal (**Figure 2**); in several mutant backgrounds, as discussed above, acinar cells instead stably transdifferentiate into duct cells, and the organ is subject to fibrosis and persistent inflammation. To our knowledge, there are no models in which normal injury occurs without inducing ADM, nor are there any models in which ADM persists without accompanying fibrosis and inflammation. We therefore hypothesize the existence of a bidirectional linkage between acinar differentiation state and injury, one perhaps understudied due to the tendency of researchers in the pancreatitis field, with rare exceptions (70), to study injury and regeneration as separate processes.

We present a speculative model for this linkage in **Figure 3**, featuring a central role for NFκB in initiating and maintaining ADM. NFκB is rapidly activated after acinar injury, driving the recruitment of macrophages and other leukocytes that, in turn, produce TNFα and other cytokines to further activate acinar NFκB. This hyperactivation drives both acinar cell death and metaplasia, explaining the close correlation between inflammation and ADM. The role of NFκB in promoting acinar cell death is well established (66, 75); its role in ADM was only recently identified (74) but is consistent with data indicating direct regulation by NFκB of the

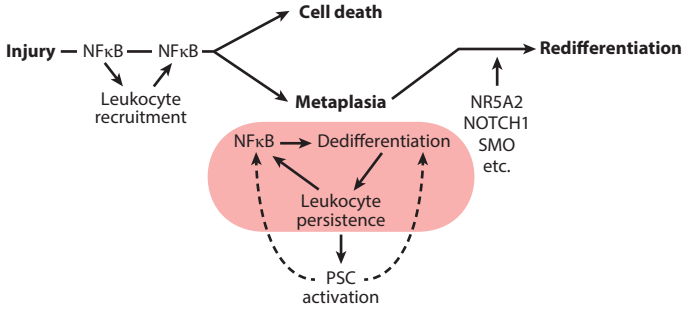

Figure 3

Model for exocrine pancreas injury and regeneration. In response to initial injury, acinar cells recruit leukocytes via NFκB-dependent cytokines, and leukocytes further stimulate NFκB in acinar cells to amplify injury and induce cell death and acinar-ductal metaplasia (ADM) (how individual cells choose between death and ADM is unknown). Dedifferentiated acinar cells continue to recruit and activate inflammatory cells, which in turn continue to stimulate acinar cell NFκB signaling. Pancreatic stellate cells (PSCs) are also activated by leukocytes and may produce additional prometaplasia signals, although the role of PSCs in vivo remains uncertain. In acute injury, developmental factors are activated to terminate this loop and induce redifferentiation, whereas chronic injury results when metaplasia cannot be resolved.

ADM markers CK19/*Krt19* (29) and *Sox9* (104). Initiation of metaplasia, we propose, establishes a positive feedback loop between NFκB, acinar dedifferentiation, and persistent leukocyte activation; in wild-type pancreata, this loop is opposed by prodifferentiation factors such as NR5A2, NOTCH1, and SMO, which promote the simultaneous resolution of inflammation and metaplasia. When these factors are absent, inflammation persists and, in turn, drives fibrosis via leukocyte–stellate cell interactions. Thus, the balance between NFκB and differentiation factors determines whether pancreatitis is acute or chronic, and the persistence of metaplastic epithelium is both a cause and an effect of chronic disease.

This model could be invalidated by identifying models in which inflammation and ADM are uncoupled; future studies should therefore address both of these processes together. In turn, the field would benefit from a more rigorous definition of metaplasia, beyond simple up- and downregulation of a limited number of genes. A useful entry point into the mechanism of metaplasia might be NR5A2 and its PTF1-L partner complex, which together activate most acinar-specific genes (99). The chronic pancreatitis–like phenotype of caerulein-treated *Nr5a2* knockout mice (97) implies that loss of NR5A2 activity contributes to the proinflammatory phenotype of metaplastic epithelium. In turn, NR5A2 and PTF1-L may be key targets of NFκB-induced metaplasia, as their inhibition would be predicted to destabilize acinar cell fate. As discussed above, NFκB has both pro- and anti-inflammatory activities in the pancreas, revealed by distinct experimental strategies, and complete ablation of NFκB activity predisposes the pancreas to a severe, necrotizing response to caerulein (75, 77). These findings may indicate an organ-protective role for transient metaplasia, perhaps mediated by recruitment of DCs (72). More close attention to ADM in necrotizing pancreatitis models, together with the use of experimental strategies permitting precise temporal control of NFκB activity, could help disentangle the conflicting roles ascribed to this pathway. The complexity of NFκB signaling in the pancreas makes it a challenging target for human pancreatitis therapy (75). In contrast, NR5A2 and SMO have relatively restricted activities in the pancreas and are amenable to pharmacological activation (105, 106), potentially providing new leads for preventing or treating pancreatitis.

ACINAR CELL PLASTICITY IN HEALTH AND DISEASE

The studies reviewed here indicate that acinar cells are capable of transiently or stably adopting duct-like characteristics, depending on genetic and environmental circumstances. This capability may be explained by insights from pancreas developmental biology: The earliest MPCs within the pancreas have an acinar-like phenotype, expressing PTF1A together with low levels of the digestive enzyme carboxypeptidase A1, and localize to distal tips within the epithelium that prefigure the anatomical position of mature acini (3, 5, 9). The embryonic acinar developmental program is thus coupled to progenitor activity, and this coupling may be reestablished in response to stress or injury.

The latent capacity of acinar cells for transdifferentiation makes them an attractive target for regenerative medicine approaches to diabetes, in which they might be reprogrammed into insulin-producing β-cells (107). Acini can be converted to a β-cell-like phenotype through viral misexpression of β-cell-specific TFs in adult mice (108); remarkably, this reprogramming is inhibited by coexpression of NR5A2, underlining the key role of this factor in maintaining acinar cell identity (109). More recent studies suggest that acinar cells could be converted into β-cells even without drastic genetic manipulation. Long-term lineage tracing of mouse acinar cells following pancreatic duct ligation revealed the presence of rare β-cells derived from acinar cells (5). The efficiency of acinar-to-β-cell conversion, which occurred via a metaplastic duct intermediate, was enhanced when operated mice were rendered diabetic by ablation of endogenous β-cells, suggesting a role for hyperglycemia in controlling differentiation. Although the number of acinar

cell–derived β-cells was too low to establish euglycemia, this study provided the first strong evidence of an endogenous pathway for acinar-to-β-cell conversion (5). Even more remarkable is a very recent study demonstrating that infusion of diabetic mice with EGF and CNTF induces widespread acinar-to-β-cell transdifferentiation, sufficient to restore normal blood sugar control in the majority of animals (110). CNTF acts through the JAK-STAT pathway, and its ability to promote β-cell neogenesis requires acinar cell *Stat3* expression. EGFR signaling and JAK-STAT signaling therefore appear to be central modulators of acinar cell plasticity, promoting ductal metaplasia in the context of pancreatitis but β-cell differentiation in the context of diabetes.

Even deadlier than pancreatitis or diabetes, however, is pancreatic cancer [i.e., pancreatic ductal adenocarcinoma (PDAC)], the genesis of which is dangerously sensitive to acinar cell plasticity. Despite the ductal phenotype exhibited by PDAC cells, extensive studies in mice indicate that this tumor and its precursor lesions arise from differentiated acinar cells rather than from duct cells (98, 111, 112). Furthermore, many of the same genes and pathways that promote metaplasia and inflammation in pancreatitis promote the development of PDAC (66). These pathways include EGFR and JAK-STAT (89, 113–115), raising caution about the possible manipulation of these pathways in promoting acinar-to-β-cell conversion. Chronic pancreatitis promotes PDAC development in humans and mice (12, 56, 66), mediated in part by persistent, inflammation-driven NFκB activation (116–118). Finally, developmental genes that stabilize acinar cell fate in the face of injury, such as those encoding NOTCH1, MIST1, and NR5A2, are also required to limit the progression of acinar cell–derived PDAC (97, 119, 120). Although these studies highlight the inherent vulnerability of acinar cells to diseases associated with altered cell fate, they also raise the hope that, by understanding the mechanisms of metaplasia and transdifferentiation, new therapeutic targets can be identified with efficacy in both pancreatitis and pancreatic cancer.

SUMMARY POINTS

1. Whereas acute pancreatitis is rapidly resolved by acinar cell regeneration, chronic pancreatitis is characterized by persistent inflammation, fibrosis, and acinar-to-ductal metaplasia.
2. Interactions between acinar cells and infiltrating leukocytes, particularly macrophages and dendritic cells, determine the initial severity of injury as well as its resolution.
3. Although fibrosis driven by activated pancreatic stellate cells is a prominent feature of chronic pancreatitis, the functional role of these cells in vivo is unknown.
4. Acinar cell NFκB signaling has diverse roles in promoting inflammation and metaplasia as well as in limiting necrotic injury.
5. A variety of developmental signaling pathways and transcription factors regulate acinar cell redifferentiation after injury, and their dysfunction may contribute to chronic pancreatitis.
6. Inflammation and acinar-ductal metaplasia appear to be tightly coupled; they are potentially driven by an NFκB-based positive feedback loop between acinar cells, leukocytes, and stellate cells and are inhibited by acinar differentiation factors.
7. This feedback loop is also likely to contribute to the development of pancreatic cancer from differentiated acinar cells and may provide new drug targets for treatment of both pancreatitis and pancreatic cancer.

8. Future progress will require increased attention to the relationships between injury, differentiation, and regeneration.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Dr. Mary Bronner of the University of Utah Department of Pathology for the human pancreas images included in **Figure 1**. We are grateful to the Murtaugh lab and our colleagues in the exocrine pancreas biology field for extensive and insightful discussions. This work was supported by grant R21-CA179453 from the National Institutes of Health/National Cancer Institute to L.C.M.

LITERATURE CITED

1. Steinberg WM. 2006. Acute pancreatitis. See Ref. 121, pp. 1241–69
2. Pan FC, Wright C. 2011. Pancreas organogenesis: from bud to plexus to gland. *Dev. Dyn.* 240:530–65
3. Shih HP, Wang A, Sander M. 2013. Pancreas organogenesis: from lineage determination to morphogenesis. *Annu. Rev. Cell Dev. Biol.* 29:81–105
4. Kretzschmar K, Watt FM. 2012. Lineage tracing. *Cell* 148:33–45
5. Pan FC, Bankaitis ED, Boyer D, Xu X, Van de Casteele M, et al. 2013. Spatiotemporal patterns of multipotentiality in Ptf1a-expressing cells during pancreas organogenesis and injury-induced facultative restoration. *Development* 140:751–64
6. Masui T, Long Q, Beres TM, Magnuson MA, MacDonald RJ. 2007. Early pancreatic development requires the vertebrate Suppressor of Hairless (RBPJ) in the PTF1 bHLH complex. *Genes Dev.* 21:2629–43
7. Kopp JL, Dubois CL, Schaffer AE, Hao E, Shih HP, et al. 2011. Sox9^{+} ductal cells are multipotent progenitors throughout development but do not produce new endocrine cells in the normal or injured adult pancreas. *Development* 138:653–65
8. Solar M, Cardalda C, Houbracken I, Martin M, Maestro MA, et al. 2009. Pancreatic exocrine duct cells give rise to insulin-producing β cells during embryogenesis but not after birth. *Dev. Cell* 17:849–60
9. Zhou Q, Law AC, Rajagopal J, Anderson WJ, Gray PA, Melton DA. 2007. A multipotent progenitor domain guides pancreatic organogenesis. *Dev. Cell* 13:103–14
10. Kopinke D, Murtaugh LC. 2010. Exocrine-to-endocrine differentiation is detectable only prior to birth in the uninjured mouse pancreas. *BMC Dev. Biol.* 10:38
11. Forsmark CE. 2006. Chronic pancreatitis. See Ref. 121, pp. 1271–1308
12. Greer JB, Whitcomb DC. 2009. Inflammation and pancreatic cancer: an evidence-based review. *Curr. Opin. Pharmacol.* 9:411–18
13. Whitcomb DC. 2013. Genetic risk factors for pancreatic disorders. *Gastroenterology* 144:1292–302
14. Dawra R, Sah RP, Dudeja V, Rishi L, Talukdar R, et al. 2011. Intra-acinar trypsinogen activation mediates early stages of pancreatic injury but not inflammation in mice with acute pancreatitis. *Gastroenterology* 141:2210–17.e2
15. Singh VP, Chari ST. 2005. Protease inhibitors in acute pancreatitis: lessons from the bench and failed clinical trials. *Gastroenterology* 128:2172–74
16. Logsdon CD, Ji B. 2013. The role of protein synthesis and digestive enzymes in acinar cell injury. *Nat. Rev. Gastroenterol. Hepatol.* 10:362–70

17. Bockman DE. 1997. Morphology of the exocrine pancreas related to pancreatitis. *Microsc. Res. Tech.* 37:509–19
18. Bockman DE, Boydston WR, Anderson MC. 1982. Origin of tubular complexes in human chronic pancreatitis. *Am. J. Surg.* 144:243–49
19. Willemer S, Adler G. 1989. Histochemical and ultrastructural characteristics of tubular complexes in human acute pancreatitis. *Dig. Dis. Sci.* 34:46–55
20. Ebert M, Yokoyama M, Ishiwata T, Friess H, Buchler MW, et al. 1999. Alteration of fibroblast growth factor and receptor expression after acute pancreatitis in humans. *Pancreas* 18:240–46
21. Zimmermann A, Gloor B, Kappeler A, Uhl W, Friess H, Buchler MW. 2002. Pancreatic stellate cells contribute to regeneration early after acute necrotising pancreatitis in humans. *Gut* 51:574–78
22. Lerch MM, Gorelick FS. 2013. Models of acute and chronic pancreatitis. *Gastroenterology* 144:1180–93
23. Willemer S, Elsasser HP, Adler G. 1992. Hormone-induced pancreatitis. *Eur. Surg. Res.* 24(Suppl. 1):29–39
24. Halangk W, Lerch MM, Brandt-Nedelev B, Roth W, Ruthenbuerger M, et al. 2000. Role of cathepsin B in intracellular trypsinogen activation and the onset of acute pancreatitis. *J. Clin. Investig.* 106:773–81
25. Hofbauer B, Saluja AK, Lerch MM, Bhagat L, Bhatia M, et al. 1998. Intra-acinar cell activation of trypsinogen during caerulein-induced pancreatitis in rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* 275:G352–62
26. Sah RP, Dawra RK, Saluja AK. 2013. New insights into the pathogenesis of pancreatitis. *Curr. Opin. Gastroenterol.* 29:523–30
27. Jensen JN, Cameron E, Garay MV, Starkey TW, Gianani R, Jensen J. 2005. Recapitulation of elements of embryonic development in adult mouse pancreatic regeneration. *Gastroenterology* 128:728–41
28. Morris JP IV, Cano DA, Sekine S, Wang SC, Hebrok M. 2010. β-Catenin blocks Kras-dependent reprogramming of acini into pancreatic cancer precursor lesions in mice. *J. Clin. Investig.* 120:508–20
29. Zhong B, Zhou Q, Toivola DM, Tao GZ, Resurreccion EZ, Omary MB. 2004. Organ-specific stress induces mouse pancreatic keratin overexpression in association with NF-κB activation. *J. Cell Sci.* 117:1709–19
30. De La O J-P, Murtaugh LC. 2009. Notch and Kras in pancreatic cancer: at the crossroads of mutation, differentiation and signaling. *Cell Cycle* 8:1860–64
31. Fendrich V, Esni F, Garay MV, Feldmann G, Habbe N, et al. 2008. Hedgehog signaling is required for effective regeneration of exocrine pancreas. *Gastroenterology* 135:621–31
32. Neuschwander-Tetri BA, Bridle KR, Wells LD, Marcu M, Ramm GA. 2000. Repetitive acute pancreatic injury in the mouse induces procollagen $\alpha 1_I$ expression colocalized to pancreatic stellate cells. *Lab. Investig.* 80:143–50
33. Walker NI. 1987. Ultrastructure of the rat pancreas after experimental duct ligation. I. The role of apoptosis and intraepithelial macrophages in acinar cell deletion. *Am. J. Pathol.* 126:439–51
34. Banting FG. 1925. *Nobel lecture: diabetes and insulin*. **http://www.nobelprize.org/nobel_prizes/medicine/laureates/1923/banting-lecture.html**
35. Lehv M, Fitzgerald PJ. 1968. Pancreatic acinar cell regeneration. IV. Regeneration after resection. *Am. J. Pathol.* 53:513–35
36. Bonner-Weir S, Trent DF, Weir GC. 1983. Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. *J. Clin. Investig.* 71:1544–53
37. Watanabe H, Saito H, Rychahou PG, Uchida T, Evers BM. 2005. Aging is associated with decreased pancreatic acinar cell regeneration and phosphatidylinositol 3-kinase/Akt activation. *Gastroenterology* 128:1391–404
38. Rankin MM, Kushner JA. 2009. Adaptive β-cell proliferation is severely restricted with advanced age. *Diabetes* 58:1365–72
39. Berrocal T, Luque AA, Pinilla I, Lassaletta L. 2005. Pancreatic regeneration after near-total pancreatectomy in children with nesidioblastosis. *Pediatr. Radiol.* 35:1066–70
40. Menge BA, Tannapfel A, Belyaev O, Drescher R, Muller C, et al. 2008. Partial pancreatectomy in adult humans does not provoke β-cell regeneration. *Diabetes* 57:142–49
41. Tsiotos GG, Barry MK, Johnson CD, Sarr MG. 1999. Pancreas regeneration after resection: Does it occur in humans? *Pancreas* 19:310–13

42. Dor Y, Brown J, Martinez OI, Melton DA. 2004. Adult pancreatic β-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 429:41–46
43. Nir T, Melton DA, Dor Y. 2007. Recovery from diabetes in mice by β cell regeneration. *J. Clin. Investig.* 117:2553–61
44. Strobel O, Dor Y, Alsina J, Stirman A, Lauwers G, et al. 2007. In vivo lineage tracing defines the role of acinar-to-ductal transdifferentiation in inflammatory ductal metaplasia. *Gastroenterology* 133:1999–2009
45. Desai BM, Oliver-Krasinski J, De Leon DD, Farzad C, Hong N, et al. 2007. Preexisting pancreatic acinar cells contribute to acinar cell, but not islet β cell, regeneration. *J. Clin. Investig.* 117:971–77
46. Keefe MD, Wang H, De La O J-P, Khan A, Firpo MA, Murtaugh LC. 2012. β-Catenin is selectively required for the expansion and regeneration of mature pancreatic acinar cells in mice. *Dis. Models Mech.* 5:503–14
47. Thorel F, Nepote V, Avril I, Kohno K, Desgraz R, et al. 2010. Conversion of adult pancreatic α-cells to β-cells after extreme β-cell loss. *Nature* 464:1149–54
48. Criscimanna A, Speicher JA, Houshmand G, Shiota C, Prasadan K, et al. 2011. Duct cells contribute to regeneration of endocrine and acinar cells following pancreatic damage in adult mice. *Gastroenterology* 141:1451–62.e6
49. Frossard JL, Rubbia-Brandt L, Wallig MA, Benathan M, Ott T, et al. 2003. Severe acute pancreatitis and reduced acinar cell apoptosis in the exocrine pancreas of mice deficient for the Cx32 gene. *Gastroenterology* 124:481–93
50. Dimagno MJ, Lee SH, Hao Y, Zhou SY, McKenna BJ, Owyang C. 2005. A proinflammatory, anti-apoptotic phenotype underlies the susceptibility to acute pancreatitis in cystic fibrosis transmembrane regulator (−/−) mice. *Gastroenterology* 129:665–81
51. Cano DA, Sekine S, Hebrok M. 2006. Primary cilia deletion in pancreatic epithelial cells results in cyst formation and pancreatitis. *Gastroenterology* 131:1856–69
52. Crawford HC, Scoggins CR, Washington MK, Matrisian LM, Leach SD. 2002. Matrix metalloproteinase-7 is expressed by pancreatic cancer precursors and regulates acinar-to-ductal metaplasia in exocrine pancreas. *J. Clin. Investig.* 109:1437–44
53. Magnuson MA, Osipovich AB. 2013. Pancreas-specific Cre driver lines and considerations for their prudent use. *Cell Metab.* 18:9–20
54. Kopinke D, Brailsford M, Pan FC, Magnuson MA, Wright CV, Murtaugh LC. 2012. Ongoing Notch signaling maintains phenotypic fidelity in the adult exocrine pancreas. *Dev. Biol.* 362:57–64
55. Stanger BZ, Stiles B, Lauwers GY, Bardeesy N, Mendoza M, et al. 2005. *Pten* constrains centroacinar cell expansion and malignant transformation in the pancreas. *Cancer Cell* 8:185–95
56. Guerra C, Schuhmacher AJ, Canamero M, Grippo PJ, Verdaguer L, et al. 2007. Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. *Cancer Cell* 11:291–302
57. Gukovsky I, Gukovskaya AS, Blinman TA, Zaninovic V, Pandol SJ. 1998. Early NF-κB activation is associated with hormone-induced pancreatitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 275:G1402–14
58. Grady T, Liang P, Ernst SA, Logsdon CD. 1997. Chemokine gene expression in rat pancreatic acinar cells is an early event associated with acute pancreatitis. *Gastroenterology* 113:1966–75
59. Gukovskaya AS, Gukovsky I, Zaninovic V, Song M, Sandoval D, et al. 1997. Pancreatic acinar cells produce, release, and respond to tumor necrosis factor-α. Role in regulating cell death and pancreatitis. *J. Clin. Investig.* 100:1853–62
60. Omary MB, Lugea A, Lowe AW, Pandol SJ. 2007. The pancreatic stellate cell: a star on the rise in pancreatic diseases. *J. Clin. Investig.* 117:50–59
61. Apte MV, Haber PS, Applegate TL, Norton ID, McCaughan GW, et al. 1998. Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture. *Gut* 43:128–33
62. Landsman L, Nijagal A, Whitchurch TJ, Vanderlaan RL, Zimmer WE, et al. 2011. Pancreatic mesenchyme regulates epithelial organogenesis throughout development. *PLOS Biol.* 9:e1001143
63. Troeger JS, Mederacke I, Gwak GY, Dapito DH, Mu X, et al. 2012. Deactivation of hepatic stellate cells during liver fibrosis resolution in mice. *Gastroenterology* 143:1073–83.e22

64. Özdemir BC, Pentcheva-Hoang T, Carstens JL, Zheng X, Wu C-C, et al. 2014. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. *Cancer Cell* 25:719–34
65. Rhim AD, Oberstein PE, Thomas DH, Mirek ET, Palermo CF, et al. 2014. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. *Cancer Cell* 25:735–47
66. Gukovsky I, Li N, Todoric J, Gukovskaya A, Karin M. 2013. Inflammation, autophagy, and obesity: common features in the pathogenesis of pancreatitis and pancreatic cancer. *Gastroenterology* 144:1199–209.e4
67. Zheng L, Xue J, Jaffee EM, Habtezion A. 2013. Role of immune cells and immune-based therapies in pancreatitis and pancreatic ductal adenocarcinoma. *Gastroenterology* 144:1230–40
68. Sendler M, Dummer A, Weiss FU, Kruger B, Wartmann T, et al. 2013. Tumour necrosis factor α secretion induces protease activation and acinar cell necrosis in acute experimental pancreatitis in mice. *Gut* 62:430–39
69. Treiber M, Neuhöfer P, Anetsberger E, Einwächter H, Lesina M, et al. 2011. Myeloid, but not pancreatic, RelA/p65 is required for fibrosis in a mouse model of chronic pancreatitis. *Gastroenterology* 141:1473–85.e7
70. Folias AE, Penaranda C, Su AL, Bluestone JA, Hebrok M. 2014. Aberrant innate immune activation following tissue injury impairs pancreatic regeneration. *PLOS ONE* 9:e102125
71. Demols A, Le Moine O, Desalle F, Quertinmont E, Van Laethem JL, Deviere J. 2000. $CD4^+$ T cells play an important role in acute experimental pancreatitis in mice. *Gastroenterology* 118:582–90
72. Bedrosian AS, Nguyen AH, Hackman M, Connolly MK, Malhotra A, et al. 2011. Dendritic cells promote pancreatic viability in mice with acute pancreatitis. *Gastroenterology* 141:1915–26.e14
73. Denham W, Yang J, Fink G, Denham D, Carter G, et al. 1997. Gene targeting demonstrates additive detrimental effects of interleukin 1 and tumor necrosis factor during pancreatitis. *Gastroenterology* 113:1741–46
74. Liou GY, Doppler H, Necela B, Krishna M, Crawford HC, et al. 2013. Macrophage-secreted cytokines drive pancreatic acinar-to-ductal metaplasia through NF-κB and MMPs. *J. Cell Biol.* 202:563–77
75. Gukovsky I, Gukovskaya A. 2013. Nuclear factor-κB in pancreatitis: jack-of-all-trades, but which one is more important? *Gastroenterology* 144:26–29
76. Huang H, Liu Y, Daniluk J, Gaiser S, Chu J, et al. 2013. Activation of nuclear factor-κB in acinar cells increases the severity of pancreatitis in mice. *Gastroenterology* 144:202–10
77. Algul H, Treiber M, Lesina M, Nakhai H, Saur D, et al. 2007. Pancreas-specific RelA/p65 truncation increases susceptibility of acini to inflammation-associated cell death following cerulein pancreatitis. *J. Clin. Investig.* 117:1490–501
78. Neuhofer P, Liang S, Einwachter H, Schwerdtfeger C, Wartmann T, et al. 2013. Deletion of IκBα activates RelA to reduce acute pancreatitis in mice through up-regulation of Spi2A. *Gastroenterology* 144:192–201
79. Zhang H, Neuhofer P, Song L, Rabe B, Lesina M, et al. 2013. IL-6 trans-signaling promotes pancreatitis-associated lung injury and lethality. *J. Clin. Investig.* 123:1019–31
80. Cuzzocrea S, Mazzon E, Dugo L, Centorrino T, Ciccolo A, et al. 2002. Absence of endogenous interleukin-6 enhances the inflammatory response during acute pancreatitis induced by cerulein in mice. *Cytokine* 18:274–85
81. Chao KC, Chao KF, Chuang CC, Liu SH. 2006. Blockade of interleukin 6 accelerates acinar cell apoptosis and attenuates experimental acute pancreatitis in vivo. *Br. J. Surg.* 93:332–38
82. Shigekawa M, Hikita H, Kodama T, Shimizu S, Li W, et al. 2012. Pancreatic STAT3 protects mice against caerulein-induced pancreatitis via PAP1 induction. *Am. J. Pathol.* 181:2105–13
83. Afelik S, Jensen J. 2013. Notch signaling in the pancreas: patterning and cell fate specification. *WIREs Dev. Biol.* 2:531–44
84. Siveke JT, Lubeseder-Martellato C, Lee M, Mazur PK, Nakhai H, et al. 2008. Notch signaling is required for exocrine regeneration after acute pancreatitis. *Gastroenterology* 134:544–55
85. Kornberg TB. 2014. The contrasting roles of primary cilia and cytonemes in Hh signaling. *Dev. Biol.* 394:1–5

86. Gao T, Zhou D, Yang C, Singh T, Penzo-Mendez A, et al. 2013. Hippo signaling regulates differentiation and maintenance in the exocrine pancreas. *Gastroenterology* 144:1543–53.e1
87. George NM, Day CE, Boerner BP, Johnson RL, Sarvetnick NE. 2012. Hippo signaling regulates pancreas development through inactivation of Yap. *Mol. Cell. Biol.* 32:5116–28
88. Blaine SA, Ray KC, Anunobi R, Gannon MA, Washington MK, Means AL. 2010. Adult pancreatic acinar cells give rise to ducts but not endocrine cells in response to growth factor signaling. *Development* 137:2289–96
89. Ardito CM, Gruner BM, Takeuchi KK, Lubeseder-Martellato C, Teichmann N, et al. 2012. EGF receptor is required for KRAS-induced pancreatic tumorigenesis. *Cancer Cell* 22:304–17
90. Murtaugh LC, Law AC, Dor Y, Melton DA. 2005. β-Catenin is essential for pancreatic acinar but not islet development. *Development* 132:4663–74
91. Wells JM, Esni F, Boivin GP, Aronow BJ, Stuart W, et al. 2007. Wnt/β-catenin signaling is required for development of the exocrine pancreas. *BMC Dev. Biol.* 7:4
92. Strom A, Bonal C, Ashery-Padan R, Hashimoto N, Campos ML, et al. 2007. Unique mechanisms of growth regulation and tumor suppression upon Apc inactivation in the pancreas. *Development* 134:2719–25
93. Heiser PW, Lau J, Taketo MM, Herrera PL, Hebrok M. 2006. Stabilization of β-catenin impacts pancreas growth. *Development* 133:2023–32
94. Valenta T, Hausmann G, Basler K. 2012. The many faces and functions of β-catenin. *EMBO J.* 31:2714–36
95. Lin SL, Li B, Rao S, Yeo EJ, Hudson TE, et al. 2010. Macrophage Wnt7b is critical for kidney repair and regeneration. *PNAS* 107:4194–99
96. Hale MA, Swift GH, Hoang CQ, Deering TG, Masui T, et al. 2014. The nuclear hormone receptor family member NR5A2 controls aspects of multipotent progenitor cell-formation and acinar differentiation during pancreatic organogenesis. *Development* 141:3123–33
97. von Figura G, Morris JP IV, Wright CV, Hebrok M. 2014. Nr5a2 maintains acinar cell differentiation and constrains oncogenic Kras-mediated pancreatic neoplastic initiation. *Gut* 63:656–64
98. Kopp JL, von Figura G, Mayes E, Liu FF, Dubois CL, et al. 2012. Identification of Sox9-dependent acinar-to-ductal reprogramming as the principal mechanism for initiation of pancreatic ductal adenocarcinoma. *Cancer Cell* 22:737–50
99. Holmstrom SR, Deering T, Swift GH, Poelwijk FJ, Mangelsdorf DJ, et al. 2011. LRH-1 and PTF1-L coregulate an exocrine pancreas–specific transcriptional network for digestive function. *Genes Dev.* 25:1674–79
100. Pin CL, Rukstalis JM, Johnson C, Konieczny SF. 2001. The bHLH transcription factor Mist1 is required to maintain exocrine pancreas cell organization and acinar cell identity. *J. Cell Biol.* 155:519–30
101. Kowalik AS, Johnson CL, Chadi SA, Weston JY, Fazio EN, Pin CL. 2007. Mice lacking the transcription factor Mist1 exhibit an altered stress response and increased sensitivity to caerulein-induced pancreatitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 292:G1123–32
102. Fukuda A, Morris JP IV, Hebrok M. 2012. Bmi1 is required for regeneration of the exocrine pancreas in mice. *Gastroenterology* 143:821–31.e2
103. Mallen–St. Clair J, Soydaner-Azeloglu R, Lee KE, Taylor L, Livanos A, et al. 2012. EZH2 couples pancreatic regeneration to neoplastic progression. *Genes Dev.* 26:439–44
104. Sun L, Mathews LA, Cabarcas SM, Zhang X, Yang A, et al. 2013. Epigenetic regulation of SOX9 by the NF-κB signaling pathway in pancreatic cancer stem cells. *Stem Cells* 31:1454–66
105. Lee JM, Lee YK, Mamrosh JL, Busby SA, Griffin PR, et al. 2011. A nuclear-receptor-dependent phosphatidylcholine pathway with antidiabetic effects. *Nature* 474:506–10
106. Sinha S, Chen JK. 2006. Purmorphamine activates the Hedgehog pathway by targeting Smoothened. *Nat. Chem. Biol.* 2:29–30
107. Pagliuca FW, Melton DA. 2013. How to make a functional β-cell. *Development* 140:2472–83
108. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. 2008. In vivo reprogramming of adult pancreatic exocrine cells to β-cells. *Nature* 455:627–32
109. Li W, Nakanishi M, Zumsteg A, Shear M, Wright C, et al. 2014. In vivo reprogramming of pancreatic acinar cells to three islet endocrine subtypes. *eLife* 3:e01846

110. Baeyens L, Lemper M, Leuckx G, De Groef S, Bonfanti P, et al. 2014. Transient cytokine treatment induces acinar cell reprogramming and regenerates functional β cell mass in diabetic mice. *Nat. Biotechnol.* 32:76–83
111. De La O J-P, Emerson LL, Goodman JL, Froebe SC, Illum BE, et al. 2008. Notch and Kras reprogram pancreatic acinar cells to ductal intraepithelial neoplasia. *PNAS* 105:18907–12
112. Murtaugh LC. 2014. Pathogenesis of pancreatic cancer: lessons from animal models. *Toxicol. Pathol.* 42:217–28
113. Navas C, Hernandez-Porras I, Schuhmacher AJ, Sibilia M, Guerra C, Barbacid M. 2012. EGF receptor signaling is essential for k-*Ras* oncogene-driven pancreatic ductal adenocarcinoma. *Cancer Cell* 22:318–30
114. Fukuda A, Wang SC, Morris JP IV, Folias AE, Liou A, et al. 2011. Stat3 and MMP7 contribute to pancreatic ductal adenocarcinoma initiation and progression. *Cancer Cell* 19:441–55
115. Lesina M, Kurkowski MU, Ludes K, Rose-John S, Treiber M, et al. 2011. Stat3/Socs3 activation by IL-6 transsignaling promotes progression of pancreatic intraepithelial neoplasia and development of pancreatic cancer. *Cancer Cell* 19:456–69
116. Daniluk J, Liu Y, Deng D, Chu J, Huang H, et al. 2012. An NF-κB pathway–mediated positive feedback loop amplifies Ras activity to pathological levels in mice. *J. Clin. Investig.* 122:1519–28
117. Maier HJ, Wagner M, Schips TG, Salem HH, Baumann B, Wirth T. 2012. Requirement of NEMO/IKKγ for effective expansion of KRAS-induced precancerous lesions in the pancreas. *Oncogene* 32:2690–95
118. Maniati E, Bossard M, Cook N, Candido JB, Emami-Shahri N, et al. 2011. Crosstalk between the canonical NF-κB and Notch signaling pathways inhibits Pparγ expression and promotes pancreatic cancer progression in mice. *J. Clin. Investig.* 121:4685–99
119. Hanlon L, Avila JL, Demarest RM, Troutman S, Allen M, et al. 2010. Notch1 functions as a tumor suppressor in a model of K-ras-induced pancreatic ductal adenocarcinoma. *Cancer Res.* 70:4280–86
120. Shi G, Zhu L, Sun Y, Bettencourt R, Damsz B, et al. 2009. Loss of the acinar-restricted transcription factor Mist1 accelerates Kras-induced pancreatic intraepithelial neoplasia. *Gastroenterology* 136:1368–78
121. Sleisenger MH, Feldman M, Friedman LS, Brandt LJ, eds. 2006. *Sleisenger & Fordtran's Gastrointestinal and Liver Disease: Pathophysiology, Diagnosis, Management*. Philadelphia: Saunders