Recapitulation of Elements of Embryonic Development in Adult Mouse Pancreatic Regeneration

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Background & Aims: **The mammalian pancreas has a strong regenerative potential, but the origin of organ restoration is not clear, and it is not known to what degree such a process reflects pancreatic development. To define cell differentiation changes associated with pancreatic regeneration in adult mice, we compared regeneration following caerulein-induced pancreatitis to that of normal pancreatic development.** ***Methods:*** **By performing comparative histology for adult and embryonic pancreatic markers in caerulein-treated and control pancreas, we addressed cellular proliferation and differentiation (amylase, DBA-agglutinin, insulin, glucagon, β-catenin, E-cadherin, Pdx1, Nkx6.1, Notch1, Notch2, Jagged1, Jagged2, Hes1), hereby describing the kinetics of tissue restoration.** ***Results:*** **We demonstrate that surviving pancreatic exocrine cells repress the terminal exocrine gene program and induce genes normally associated with undifferentiated pancreatic progenitor cells such as Pdx1, E-cadherin, β-catenin, and Notch components, including *Notch1*, *Notch2*, and *Jagged2*. Expression of the Notch target gene *Hes1* provides evidence that Notch signaling is reactivated in dedifferentiated pancreatic cells. Although previous studies have suggested a process of acino-to-ductal transdifferentiation in pancreatic regeneration, we find no evidence to suggest that dedifferentiated cells acquire a ductal fate during this process.** ***Conclusions:*** **Pancreatic regeneration following chemically induced pancreatitis in the mouse occurs predominantly through acinar cell dedifferentiation, whereby a genetic program resembling embryonic pancreatic precursors is reinstated.**

The adult pancreas controls blood glucose homeostasis as well as intestinal nutrient uptake through the activities of the endocrine islets of Langerhans and the exocrine pancreatic cells, respectively. These highly specialized and distinct pancreatic mature cell types are known to originate from a common embryonic progenitor cell type that exists during normal pancreatic development.[1] In the mouse, such progenitor cells are defined by their expression of Pdx1,[2] p48,[3] and Nkx6.1,[4] as well as active Notch signaling.[5,6] However, as cell-fate determination occurs during embryogenesis, this particular cell type becomes more and more difficult to visualize and seems to disappear. Therefore, the persistence of any such remaining progenitor cells into adulthood remains controversial.

Following insult, the adult pancreas is challenged to regenerate, and it is important to understand the mechanisms by which such cellular replenishment can occur because this may lead to new treatment modalities, eg, for pancreatitis, and may pave the way for transdifferentiation of specific pancreatic cells such as the insulin producing β-cells. Several experimental methods such as partial pancreatectomy,[7] duct ligation,[8,9] and transgenic overexpression of IFN-γ,[10] among others have been investigated as models for inducing a pancreatic regenerative response. The insults initiating the regenerative response in each of these models are very different, making identical regenerative programs difficult to expect. Nevertheless, a common conclusion of the above studies is that neogenesis of pancreatic cells occurs from ducts. These conclusions are generally based on the observations of (1) an increased mitotic activity of pancreatic ductal cells posttreatment and (2) the scattered distribution of endocrine cells in the regenerating pancreas, both next to and within pancreatic ducts. Unfortunately, the lack of defined markers for duct cells and a general absence of lineage tracing data have not allowed for the establishment of direct evidence. Except for the finding of reactivation of Pdx1 gene expression in both ducts[7] or ductal-type cells derived from normal exocrine cells in culture,[11] most studies have not attempted to rigorously compare such possible progenitor cells to those of the normal embryonic pancreas. Therefore, it is unknown whether the pancreatic ductal cell had dedifferentiated into a precursor type from which transdiffer-

Abbreviation used in this paper: DBA, dolichus biflorus agglutinin; FOV, field-of-vision; MMP7, matrix metalloproteinase 7; pHH3, phosphorylated histone H3; SSC, saline sodium citrate.

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entiation occurred. In addition, it is possible that these cells could have derived from the exocrine compartment through an acino-ductal transformation process.

This latter issue becomes yet more pertinent considering the amount of data that have been obtained regarding the remodeling of the exocrine pancreas in or following pancreatitis. In humans, pancreatitis may be acute or chronic, in which case pancreatic function deteriorates over time, and the exocrine cell space is replaced by fibrotic deposits. Diabetes may occur. Little is known of the histologic process of regeneration following human pancreatitis. Yet, in late-stage chronic pancreatitis, pancreatic epithelial cells often appear as a ductular network surrounded by fibrotic tissue. The origin of such ductular complexes may be either surviving ducts or dedifferentiated exocrine cells, although this has not been formally demonstrated. Such histologic change is probably the result of a failure in completing a regenerative program. In the case of human acute pancreatitis, which generally does not lead to irreversible damage, it is not known how local regions of the pancreas recover.[12]

Experimental rodent models of acute pancreatitis have revealed a remarkable regenerative capability of the pancreas. Caerulein-induced pancreatitis is recognized to be a fully reversible process, despite the fact that the insult can lead to an almost complete depletion of pancreatic exocrine function. In the caerulein model, short-term caerulein administration at supramaximal levels leads to severe pancreatitis because of cathepsin-B-mediated, intrapancreatic trypsinogen activation and autodigestion.[13] Most cells of the exocrine pancreas are lost during this process, but full pancreatic function is restored in approximately 1 week following caerulein administration as the result of a powerful regenerative response.

In the rat, regeneration of the caerulein-treated pancreas has been suggested to occur through an acino-ductal change of exocrine cells, followed by redifferentiation back toward the exocrine phenotype.[14] Similar conclusions have also been reached in studies of taurocholate-induced, acute necrotizing pancreatitis,[15] as well as during human pancreatitis.[16] Such an acino-ductal change is also observed during in vitro culture of pancreatic acini.[17] Furthermore, during experimental pancreatic cancer elicited by pancreatic expression of transforming growth factor (TGF)-α in metallothionein-TGF-α transgenic mice, a similar acino-ductal metaplasia occurs prior to tumor development,[18] and this effect appears to be mediated by Notch signaling.[19] Whereas most pancreatic cancers in humans are referred to as *ductal adenocarcinomas*, studies suggest that pancreatic cancer may originate from initial acinar cell hyperplasia, transiently forming a pancreatic intraepithelial neoplasia (PanIN),[20] from which further progression to the ductal adenocarcinoma can occur.[21,22]

We have here investigated the process of caerulein-induced pancreatitis in mice. We focused our studies to determine the cellular origin of this regenerative process and asked whether this process might reflect normal pancreatic development. We find that, through a rapid dedifferentiation process, surviving exocrine cells induce a gene expression pattern highly reminiscent of embryonic pancreatic progenitor cells. This behavior is transient, and the majority of the cells redifferentiate within a few days as functional exocrine cells. Strikingly, the existing ductal network does not contribute significantly to regeneration nor do we find evidence that the dedifferentiated exocrine cells acquire a ductal fate. Also, throughout the process, the existing islets of Langerhans do not enter mitosis, scatter, or otherwise display signs of remodeling. Thus, our data suggest that mature exocrine cells of the pancreas harbor pancreatic progenitor-cell properties unlocked during organ regeneration.

Materials and Methods

Mice and Caerulein Treatment

FVB male and female mice (22–25 g, 6–8 weeks of age) were used for this study. Each experimental group contained 4 phosphate-buffered saline (PBS)-injected control mice and 12 mice receiving caerulein (n = 3/group: d1, d3, d5, d7). Three independent and identical setups were performed (caerulein obtained from American Peptide Co, Sunnyvale, CA); also, 2 independent, dose-response experiments evaluating the effect of a total caerulein dose of 0 to 32 μg/mouse administered over 2 days in a total of 20 mice. The final day of caerulein injection is defined as d0. For time course studies, caerulein was administered as 8 daytime doses (10 μg/mL), 0.2 mL, 1 hour apart over 2 consecutive days. Mice were inspected hourly for signs of ataxia or other signs of disturbance within the period of injection and 3 times daily thereafter. BrdU (20 mg/kg) was injected intraperitoneally (IP) 2 hours before harvesting.

Tissue Preparation and Embedding

For frozen-section immunohistochemistry, the tissue was fixed in 4% paraformaldehyde (PFA) before transfer into 30% sucrose in PBS at 4°C until equilibration then transferred into a 1:1 mix of 30% sucrose in PBS:OCT for 1–2 hours at 4°C and finally into 100% optimal cutting temperature compound (OCT) for 1 hour at 4°C before embedding in OCT. Seven-μm sections were stored at −80°C. Paraffin sections were cut at 4 μm and stored at room temperature until use.

Immunohistochemistry

Frozen sections were dried. Sections were microwave treated 2 × 5 minutes in 0.01 mol/L citrate buffer, pH 6,