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Pancreatic Regeneration: Models, Mechanisms, and Inconsistencies

Mairobys Socorro[1] and Farzad Esni[1, 2, 3, 4, 5]

1 Department of Surgery, Division of Pediatric General and Thoracic Surgery, Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center, Pittsburgh, PA 15244

2 Department of Developmental Biology, University of Pittsburgh, Pittsburgh, PA 15244

3 Department of Microbiology & Molecular Genetics, University of Pittsburgh, Pittsburgh, PA 15261

4 University of Pittsburgh Cancer Institute, Pittsburgh, PA, 15123

5 University of Pittsburgh, Department of Surgery, John G. Rangos Research Center, Pittsburgh, PA 15224,

e-mail: farzad.esni@chp.edu

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1. Introduction

While skin, liver and gut are capable to regenerate and heal, other organs such as heart and brain do not display similar regenerative capacities. The adult pancreas displays a limited capacity to regenerate, although this regenerative capacity declines with age (17, 74-76, 83). Thus, with respect to the pancreas, the uncertainty is not about the overall ability of the adult pancreas to regenerate, but rather which cells may act as cell(s) of origin in this process. For example, it is widely accepted that under physiologic conditions β-cell regeneration in the adult mouse pancreas originates from β-cell self-duplication (21, 77). However, depending on the type of injury model, it appears that new β-cells can arise from cells either residing within the ducts (1, 4, 18, 35, 91), in proximity to the ductal network (88), or from other pancreatic endocrine cells (15, 16, 78, 90). This uncertainty regarding the types of cells that may potentially give rise to new β-cells comes in part from the fact that in each experimental model of regeneration, the exact target cells and the severity of the injury are different. Here, we will first review some of the injury models that have been used to study the mechanisms leading to replacement of acinar and β-cells, followed by a discussion of the discrepancies in these reports.

2. Injury models used to study pancreatic regeneration

Throughout the years multiple models of pancreatic injury have been used by different investigators to explore the regenerative capacity of exocrine or endocrine compartments of the adult pancreas (46). Among these models, some of the commonly used are pancreatic duct ligation, partial pancreatectomy, caerulein-induced pancreatitis, alloxan- or streptozotocin-induced diabetes, and diphtheria toxin-mediated cell ablation. These models are of varying specificity, and they entail surgical, chemical or genetic methods.

Pancreatectomy (Px)

Pancreatectomey is the oldest model with which to examine the regenerative capacity of the pancreas (85). The first documented removal of the pancreas was performed on dogs by Johann C. Brunner in 1683 (30). However, it was first in 1890s that pancreatectomy was reported to result in diabetes, and hence a link between the pancreas and glucose homeostasis was established (30, 85). Px can be used to study acinar and β-cell recovery in both rats and mice; however, because of the increased islet mass this injury model has been extensively used to study β-cell regeneration (5, 10, 19, 32, 47, 75, 82-84). Partial pancreatectomy (PPx) involves resection of less than 90% (often 50%-75%) of the adult mouse or rat pancreas (19-21, 42, 43, 61, 62, 83, 86). Here, the remnant of the pancreas displays normal gross morphology. A more severe form of Px that has been performed on rats is subtotal pancreatectomy (SPx), and entails removal of 90%-95% of the gland through tissue abrasion (3, 7, 47, 61, 69). Thus, in contrast to PPx, here the acinar cells in the remnant of the pancreas undergo rapid atrophy, which results in a desmoplastic reaction (47, 51). Regardless of the extent of resection, pancreatectomy is associated with an IGF/PI3K-dependent up-regulation of Pdx1 expression in duct cells (69, 82, 83). Interestingly, the limited organ recovery that follows Px appears to be proportional to the size of the excision (3, 7, 44, 61, 63). Subtotal pancreatectomy in rats leads to ductal cell proliferation and induction of an extensive regenerative process that promotes mature duct cells to regress and re-express embryonic genes such as Pdx1, Ptf1a, and Ngn3 before differentiating to the different pancreatic cell types (47, 69). While these data imply that the duct cells might contribute to the observed increased islet mass following Px, other studies would argue against the involvement of duct cells in this process (20, 21, 43, 86). Using lineage tracing studies, independent investigators have not been able to find any evidence for β-cell neogenesis following 50%-75% PPx (21, 86). Accordingly, 50% PPx in Ngn3-GFP transgenic mice failed to induce Ngn3-expression in cells within islets or ducts (43). All together, although the potential contribution of other cell types can not be rolled out, the current literature supports the notion that the main source for acinar or β-cell regeneration that follows Px is pre-existing acinar or β-cells, respectively (20, 21, 55, 86).

Pancreatic duct ligation (PDL)

As in the case of Px, ductal obstruction and ligation have historically been used in investigating pancreatic regeneration (85). PDL involves ligation of one of the main ducts, which leads to acinar cell death and inflammation in the area distal to the ligation. An advantage with PDL is that the unligated portion of the pancreas remains unaffected, and thus can be used as an internal control. However, the regenerative process in this model, particularly the acinar regeneration, appears to be species-dependent. PDL in rats is associated with near complete acinar recovery through a process that involves appearance of ductular structures, and their differentiation into acinar cells (7, 13). In mice, although PDL results in the formation of similar metaplastic ducts, the acinar compartment does not regenerate (13, 20, 38, 71). Lineage tracing studies in mice show that both surviving acinar cells (20) and Hnf1β-expressing duct cells (71) in the ligated part of the pancreas can contribute to the formation of these ductular structures. In other words, the tubular structures observed in the ligated part consist of acinar-derived metaplastic ducts and pre-existing ducts that have changed morphology. Similarly, in rats it is believed that pre-existing acinar cells transdifferentiate into these ductal structures (7, 13), whereas the contribution of duct cells in this process has yet to be determined.

Pancreatic duct ligation has been primarily used to provide insights on islet β-cell generation, as it is reported to stimulate β-cell regeneration in both mouse and rat (7, 13, 80, 81, 86-88, 91). Nevertheless, there is a controversy with respect

to the mechanism allowing the observed β-cell generation. While some studies favor β-cell proliferation as the main mechanism for β-cell formation after PDL (13, 38, 65, 71, 86), others support the potential contribution of non β-cells (in particular cells within or in proximity of ductal network) to β-cell neogenesis in a PDL setting (35, 60, 88, 91).

Caerulein-induced pancreatitis

Caerulein is a cholecystokinin orthologue, which when administered repeatedly at high concentrations leads to a dropout of over half of acinar compartment which can cause acute or chronic pancreatitis (25, 40, 46). In mice, the pancreas regains its normal histology within a week after caerulein treatment. The rapid regenerative process associated with this injury model has been used by many investigators to follow the course of acinar recovery (28, 33, 36, 53, 72). Lineage-tracing studies have shown that following caerulein-induced pancreatitis the surviving acinar cells contribute to the recovery of the acinar compartment (20, 28, 53, 72). Acinar regeneration in this model is through acinar-to-ductal metaplasia (ADM), a process that requires a transient reactivation of various developmental genes and signaling pathways, including notch, hedgehog and wnt (28, 36, 39, 53, 54, 64). ADM involves dedifferentiation of acinar cells into duct-like cells, proliferation of metaplastic ducts, and finally re-differentiation of duct-like cells into acinar cells (53). A first step toward transdifferentiation of one cell type to another cell type is that cells have to lose their original identity in order to acquire a new one. Accordingly, the dedifferentiation of acinar cells is associated with expression of ductal markers such as Hnf6, Sox9, and cytokeratin-19 and concomitant repression of acinar markers Ptf1a, Mist1, amylase and Cpa (64). Wnt/β-catenin signaling is one of the embryonic pathways, which is reactivated in ADMs following caerulein-induced pancreatitis (53, 54). However, for the ADMs to re-differentiate into acinar cells wnt-signaling has to be eventually downregulated, as persistent wnt/β-catenin activity leads to impaired acinar recovery (24, 53). The precise mechanism for the dynamic activity of wnt/β-catenin in ADMs is not clear, but a recent report implicates HDACs as an important epigenetic switch required for controlling nuclear β-catenin transcriptional activity (24). The transcriptional factor PDX1 has primarily been associated with the embryonic pancreas and mature β-cells in the adult pancreas. However, a new study highlights the importance of PDX1 in maintaining acinar cell identity (66). PDX1 displays similar dynamic expression as wnt/β-catenin during ADM, and accordingly its down-regulation is necessary for re-differentiation of ADM into acinar cells (66).

Other models of pancreatitis

In addition to the aforementioned caerulein-induced pancreatitis, other rodent models commonly used to study acute pancreatitis entail ductular bile salt infusion, duct obstruction, the choline-deficient ethionine supplemented diet (CDE), or administration of basic amino acids such as L-arginine. These injury models have been extensively described and reviewed by Lerch and Gorelick elsewhere (46).

Alloxan or streptozotocin-induced diabetes

Alloxan and streptozotocin (STZ) are used to induce diabetes by chemical ablation of pancreatic β-cells. Alloxan was first described in early 1800, but its diabetogenic property was reported in 1943, and since then alloxan treatment has been used as an experimental model for diabetes (73). STZ was initially used as a chemotherapeutic agent in pancreatic islet cell tumors and other malignancies (45), but since its discovery as a diabetogenic agent in 1963, it has been widely used in diabetes research (26). Alloxan and STZ are both toxic glucose analogues that preferentially accumulate in insulin producing β-cells via the Glut2 glucose transporter (45). Diabetes as the result of alloxan or STZ-treatment is not associated with β-cell regeneration (73, 86). Because of the absence of

spontaneous β-cell recovery, these models have been useful tools to study a given treatment on β-cell regeneration. In addition, alloxan- or STZ-treatment can be combined with pancreatic duct ligation to study the effect of hyperglycemia on the regenerative process in the ligated portion of the pancreas (13, 16, 60). Here, while the combination of alloxan- or STZ-treatment and PDL in mice led to transformation of glucagon-producing α-cells or acinar cells into β-cells (16, 60), no such α-to β-cell conversion could be found when rats were subjected to a combined PDL and STZ-treatment (13).

Diphtheria toxin-mediated cell ablation

A relatively new method which enables cell-specific ablation is transgenic activation of the diphtheria toxin cell death pathway using a cell-specific promoter (8, 59). Mature diphtheria toxin (DT) is composed of subunits A and B (DTA and DTB) (31, 79). DT binds a toxin receptor on the cell surface of toxin-sensitive cells and is endocytosed (23, 29, 67). Upon entry into the cytoplasm, the DTA subunit is released and it catalyzes the inactivation of elongation factor 2, resulting in termination of all protein synthesis, with rapid apoptotic death of the target cell (11, 34). The toxicity of DTA is sufficiently high that only one molecule of DTA in the cytosol may be enough to kill the cell (89). The DT receptor (DTR) is a membrane-anchored form of the heparin binding EGF-like growth factor (HB-EGF precursor) (56). The human and simian HB-EGF precursors bind DT and function as toxin receptors, whereas HB-EGF from mice and rats do not bind the toxin and therefore remain insensitive to DT (52). Thus, transgenic expression of the simian or human DTR in mice can render naturally DT-resistant mouse cells DT-sensitive (14, 37, 67). Recently, a mouse strain was generated ($R26^{DTR}$), in which a loxP-flanked STOP cassette and the open reading frame of simian DTR had been introduced into the *ROSA26* locus (11). In the $R26^{DTR}$ strain, the gene encoding DTR is under the control of the potent Rosa promoter, but DTR expression is dependent first on Cre-recombinase removal of the STOP cassette (11). Following Cre-recombinase activity, the DTR-expressing cells, i.e. cells expressing Cre, and all of their progeny, are viable and function normally. However, these cells are rapidly killed upon DT administration. Noteworthy, the HB-EGF is no longer active as an EGFR ligand, as transgenic lines expressing DTR in different pancreatic lineages do not display any abnormal phenotype (17, 18). In the adult pancreas, DTR/DTA-mediated β-cell ablation has been used to study regeneration following α- or β-cell specific losses (15, 17, 57, 70, 78), acinar (17, 18), or acinar and endocrine cell ablation (17, 18).

3. Inconsistencies in understanding pancreatic regeneration

A brief look at table 1 highlights the inconsistencies that currently exist in the literature regarding the regenerative capacity of the adult mouse pancreas. For example, there is a complete recovery of the acinar compartment within a week after caerulein-induced pancreatitis, whereas there is principally no acinar regeneration following PDL. Additionally, α-cells can differentiate into β-cells, however this plasticity has been observed only upon total β-cell ablation but not following partial loss of β-cell mass. Variations between different studies are likely due to the disparities in the nature, extent, and perhaps more importantly the severity of injury used. In this review, we will argue that the combined effects of these parameters not only may determine whether or not regeneration would occur, but also dictate which cell type(s) should contribute to this process.

The nature of injury

Here, the question is not so much about whether the injury is chemically, mechanically or genetically induced, but rather what kind of cell death does it trigger? Apoptosis is programmed cell death generally associated with retention of plasma membrane integrity, condensation and cleavage of nuclear and cytoplasmic proteins and

Table 1.

Injury	Nature	Extent	Severity	Regeneration	References
Px	Surgical resection	Exocrine and Endocrine	50-90% resection	Limited organ recovery	1, 2, 6, 21-40
PDL	Apoptosis	Acinar cells	Area distal to the ligation	β-cells	12, 13, 32, 34, 41-48
Alloxan	Necrosis	β-cells	Vast majority	No	15, 33, 61, 62
STZ	Necrosis	β-cells	Dose dependent	No	33, 41, 61, 62
Caerulein	Necrosis	Acinar cells	< 50%	Yes	18, 32, 49-60
DT/DTR	Apoptosis	Exocrine and/or endocrine	Promoter-dependent	Yes/No	5, 10, 14, 17, 78, 79

cell shrinkage or the formation of apoptotic bodies (27). Apoptosis is a highly coordinated process which requires significant amount of energy, and therefore relies on mitochondrial respiration and ATP production (22). Necrosis, on the other hand is invoked in response to external stimuli and ATP-deficiency (22). Pancreatic injury and ensuing regeneration invariably depend on proper clearance of the dead cells (17). Because of its nature (loss of cytoplasmic membrane integrity, cellular fragmentation and release of lysosomal and granular contents into surrounding extracellular space), necrosis does not allow for proper removal of cell organelles, and as the result it is followed by reactive inflammation (2, 12, 22, 50, 58, 68). In contrast, apoptosis involves debriding the tissue without generating massive inflammation that is usually induced by the degeneration of dead cells (22). The effect of apoptosis or necrosis on regeneration is perhaps best manifested when one compares β-cell regeneration following STZ (or alloxan) treatment with DT-mediated β-cell ablation. In these two models, the target cells (β-cells) as well as the degree of β-cell loss (75-80% sub-optimal condition for STZ, or 75% β-cell ablation using PdxCreERT) are similar (17, 21). Interestingly, STZ-induced necrosis is accompanied with a massive inflammatory response and the absence of β-cell regeneration, whereas DT-induced apoptosis leads to almost complete β-cell mass recovery (57).

Overall, compared to the necrosis, apoptosis provides an environment that would favor β-cell regeneration. Acinar regeneration appears to be less sensitive to the nature of injury, as robust acinar tissue recovery have been reported in both necrotic (caerulein) as well as apoptotic (DT-mediated) environments (17, 18, 28, 33, 36, 53, 54, 72).

The extent of injury

Another factor that may influence the regenerative process is the extent of injury. In other words, how many different cell types are affected by the insult? In addition to the ductal, acinar and five different hormone-producing endocrine cell types, the adult pancreas is home to numerous endothelial, stellate and neuronal cells. The type of injured cells is important as recent reports have shed light on the role of macrophages in inducing acinar-to-ductal metaplasia, as well as promoting β-cell, or acinar cell regeneration (9, 17, 49, 87). Macrophages appear to have differential functions in diverse phases of regeneration, first to debride the tissue following injury, and secondly to convert injury signals into lineage-specific regenerative signals (6, 17, 48). One exception to this rule is PDL, which as mentioned earlier is associated with acinar atrophy. Thus, one would expect that this model would lead to acinar regeneration, but instead it stimulates β-cell regeneration. Clearly, the effect of PDL on non-acinar cells residing in the ligated part cannot be ruled out. Therefore, it is possible that the combined regenerative signals released by macrophages (as the result of

engulfing damaged acinar- and non-acinar cells) would serendipitously create an environment that would promote β-cell regeneration instead of acinar. In fact, combined PDL and β-cell ablation by STZ has been reported to enhance acinar to β-cell transdifferentiation (60).

Based on our current understanding of the involvement of macrophages in regeneration, simultaneous ablation of many cell types would make direct interpretation of cellular mechanism of regeneration difficult. Thus, cell-type specific ablation may be a better method for analyzing the in vivo function of cells during regeneration, which can be achieved by using streptozotocin, alloxan (for β-cells) or caerulein (for acinar cells). Alternatively, transgenic activation of the diphtheria toxin (DTR/DTA) cell death pathway, which depending on the promoter, can target one specific (for example Elastase promoter for acinar cell ablation) or more than one cell type (Pdx1 promoter to target all pancreatic epithelial cells) (17, 18).

The severity of injury

Mounting evidence suggests that the severity of injury is perhaps one of the most important elements that dictate whether the mechanism for repair should include replication of pre-existing cells, or neogenesis from other cell types. DT-mediated cell ablation has been used by number of investigators to vary the extent and the severity of injury, while keeping other variables (such as the nature of the injury) relatively constant. Collectively, it appears that regardless of cell type, as long as ablation of a specific cell type does not reach near 100%, the mechanism for regeneration mainly involves the pre-existing cells (17, 21, 28, 53, 72, 78). Therefore, following 75% ablation of β-cells, surviving β-cells proliferate to generate new β-cells, whereas complete loss of insulin-producing cells promotes conversion of other endocrine cell types into β-cells (15, 17, 21, 78). Consistently, acinar cell recovery following caerulein-induced pancreatitis is through pre-existing acinar cells. However, near complete loss of both acinar as well as endocrine cells stimulates cells within the ductal compartment to form new acinar and endocrine cells (17, 18). One could also argue that the extent of the surgical intervention may be important also in the pancreatectomy setting, and could explain discrepancies in some reports describing absence or vigorous pancreatic regeneration after partial or subtotal pancreatectomy, respectively (3, 21, 47). However, as mentioned earlier unlike PPx, subtotal pancreatectomy is associated with acinar atrophy and a desmoplastic reaction. Of note, this inflammatory reaction has been reported to be important for the robust regeneration that follows SPx, as inhibition of the inflammation prevented regeneration (7, 41). Therefore, it is likely that the inconsistencies between PPx and SPx are due to the presence or the absence of inflammation rather than the extent of injury.

4. Conclusions

Pancreatic regeneration relies on a complex interaction between cells that provide necessary regenerative signals and cells that are receptive to those signals. As discussed here, the nature, extent and the severity of injury are three important parameters that determine whether tissue recovery is achieved. β-cell regeneration seems to be more sensitive to the nature of injury than acinar regeneration. Finally, the extent of injury determines which cell types would respond to these regenerative signals.

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6. References

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