Video Article

Surgical Injury to the Mouse Pancreas through Ligation of the Pancreatic Duct as a Model for Endocrine and Exocrine Reprogramming and Proliferation

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Abstract

Expansion of pancreatic beta cells *in vivo* or *ex vivo*, or generation of beta cells by differentiation from an embryonic or adult stem cell, can provide new expandable sources of beta cells to alleviate the donor scarcity in human islet transplantation as therapy for diabetes. Although recent advances have been made towards this aim, mechanisms that regulate beta cell expansion and differentiation from a stem/progenitor cell remain to be characterized. Here, we describe a protocol for an injury model in the adult mouse pancreas that can function as a tool to study mechanisms of tissue remodeling and beta cell proliferation and differentiation. Partial duct ligation (PDL) is an experimentally induced injury of the rodent pancreas involving surgical ligation of the main pancreatic duct resulting in an obstruction of drainage of exocrine products out of the tail region of the pancreas. The inflicted damage induces acinar atrophy, immune cell infiltration and severe tissue remodeling. We have previously reported the activation of Neurogenin (Ngn) 3 expressing endogenous progenitor-like cells and an increase in beta cell proliferation after PDL. Therefore, PDL provides a basis to study signals involved in beta cell dynamics and the properties of an endocrine progenitor in adult pancreas. Since, it still remains largely unclear, which factors and pathways contribute to beta cell neogenesis and proliferation in PDL, a standardized protocol for PDL will allow for comparison across laboratories.

Video Link

The video component of this article can be found at <http://www.jove.com/video/52765/>

Introduction

The increasing prevalence of diabetes, affecting more than 300 million people world-wide^{1,2} has boosted the search for new sources of insulin-producing beta cells, both *in vitro* and *in vivo*, to replenish the deficient beta cell mass.³ Identifying key mechanisms and factors that regulate beta cell proliferation and beta cell neogenesis, *i.e.*, the differentiation of beta cells from a non-beta cell or progenitor cell, can provide novel targets for the development of regenerative therapies in diabetes.

In the developing rodent pancreas, all of the endocrine cell types differentiate from a transient population of endocrine progenitor cells, expressing the transcription factor Neurogenin3 (Ngn3).^{4,5} In the adult rodent pancreas, under normal physiological conditions, the beta cell mass is maintained at an optimal number to meet metabolic demands. Changes in beta cell size, apoptosis and replication constitute the major mechanisms for beta cell expansion and turn-over.⁶⁻⁸ While the potential of beta cells to proliferate under normal physiological conditions is homogenous throughout the population,⁹ their proliferation rate is low and re-replication is restricted by a dynamic quiescence period or refractory period^{6,7}, influenced by age and glucose metabolism.¹⁰ Since endocrine progenitor cells have so far not been identified in the normal adult pancreas, neogenesis is thought to not contribute to normal adult beta cell growth.⁸

Therefore, the identification of a facultative endocrine progenitor cell in the adult pancreas that is expandable and capable of yielding new beta cells would provide a novel, possibly unlimited source of beta cells.

Partial duct ligation (PDL) is an animal injury model that has been described to induce beta cell neogenesis in the adult pancreas.^{11,12} In this model, the main pancreatic duct draining the pancreatic tail is surgically ligated. The resulting obstruction of exocrine drainage induces major tissue remodeling, accompanied by inflammation and acinar atrophy distal to the ligation.¹²⁻¹⁴ Within this inflammatory environment, re-expression of the endocrine progenitor marker Ngn3 is induced and the beta cell volume increases two-fold. This doubling in beta cell volume results from the generation of new beta cells from an Ngn3 expressing embryonic-type endocrine progenitor cell and from proliferation of pre-existing and newly-formed beta cells that are prone to re-duplication without "quiescence period".^{11,15}

Beta cell neogenesis and replication in injury models, such as pancreatectomy^{6,7,16-19} and selective ablation of beta cells²⁰ have been extensively described. However, the regenerative outcome in these models is influenced by the extent of the inflicted damage and is associated with a

decreased initial beta cell mass²¹. PDL is a surgical model in which the initial beta cell mass is not affected and beta cell neogenesis and proliferation are robustly activated. Indeed, in the pancreas of mice that underwent PDL, Ngn3 expressing cells are identified near the epithelial lining of the duct. These cells can be isolated from the ligated pancreas of Ngn3-GFP transgenic mice using fluorescence activated cell sorting (FACS) and are able to differentiate towards functional beta cells following engraftment into and *ex vivo* culture of the pancreas of E12.5 Ngn3^{-/-} mice.¹¹ Similarly, in Ngn3^{CreERT};R26^{YFP} mice in which cells that activated the Ngn3 gene are permanently labeled after tamoxifen injection, label-positive Ngn3 cell-derived beta cells are detected after PDL.¹⁵ Moreover, newly formed beta cells dilute pre-existing beta cells and preferentially locate in small islets within which beta cells show high proliferation potential.¹⁵ Ngn3 is important for beta cell expansion after PDL since decreased Ngn3 expression using target-specific short-hairpin RNA significantly decreases beta cell mass and beta cell proliferation after PDL.¹¹ Notably, the fraction of Ngn3 cell-derived beta cells and the beta cell mass after PDL critically depends on the level of Ngn3 induction¹⁵. This is in accordance with the observation that high level of Ngn3 expression is a critical step for endocrine commitment from multipotent pancreatic progenitors during pancreatic development.²² In addition, selective ablation of Ngn3 expressing cells by diphtheria-toxin administration to Ngn3^{CreERT};R26^{DTTR} mice results in decreased insulin content and reduced beta cell proliferation, especially in small islets.¹⁵

Although the induction of Ngn3 expression in duct cells after PDL has been confirmed by many^{11,15,16,23,24}, Ngn3 expression in islets cells^{24,25} and discrepancies in outcome of PDL challenged our initial observations of increased beta cell mass^{26,27}, appearance of Ngn3 expressing duct-derived endocrine progenitors^{24,26,28,29}, and increased beta cell proliferation²⁷ after PDL.³⁰

These conflicting results could, at least partially, be attributed to a combination of factors, including variations in the post-surgical time points of analysis, bodyweight, sex and age of the mice, post-operative physiological and environmental conditions and, most importantly, differences in surgical technique.³⁰ In our hands, beta cell proliferation, insulin content, beta cell volume and the number of small islets are consistently increased after PDL. Also Ngn3 mRNA consistently increases, but there are large differences in the Ngn3 mRNA expression between PDL tail pancreases, for which we have no direct explanation. We hypothesized that the level of Ngn3 mRNA might correlate with the degree of beta cell neogenesis from non-beta cells¹⁵, but this needs further substantiation. Although it does not remove all experimental variations, a standardized method for performing PDL surgery allows for better uniformity in results and opens new avenues in studying beta cell proliferation and neogenesis.

Protocol

All manipulations follow the guidelines issued by the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123 and 2010/63/EU).

1. Preparation of Work Area

1. Provide a dedicated preparation area, a surgical area and a recovery area.
2. Conduct the entire surgical procedure in a laminar flow cabinet to minimize environmental contaminants.
3. Assemble the supplies (as listed in Materials and Methods) needed on the preparation, surgical and recovery area, using proper aseptic technique.
4. Ensure that surgical tools are autoclaved prior to surgery.
5. Provide a recirculating water heating pad at a temperature of 38 °C for temperature stabilization during surgery. Cover the heating pad with a sterile waterproof pad.
6. Provide an operating microscope with a magnification of at least 6.3X.
7. Use an instrument sterilizer, such as a hot bead sterilizer, to sterilize instruments in between surgical procedures.
8. Prepare a recovery area consisting of a large cage, lined by flat paper bedding

2. Preparing the Animals for Surgery

1. For PDL and sham surgery, use 8 week old male mice, housed in standard cages and maintained on a 12 hr light/12 hr dark cycle and fed a standard rodent diet *ad libitum*.
Note: Here, we use BALB/cJrJ mice but we have also successfully used other strains and various transgenic strains.
2. Use Buprenorphine as preemptive analgesia (0.05 – 0.1 mg/kg) 30 min prior to surgery.
3. Anesthetize the mice by intraperitoneal injection of 100 mg/kg of ketamine and 5 - 10 mg/kg of xylazine.
4. Assess proper anesthetization by observing gradual loss of voluntary movement and muscle relaxation. Test the loss of reflexes by toe pinching.
5. Apply ophthalmic ointment to prevent dryness of the eyes while under anesthesia.

3. Surgical Site Preparation

1. Disinfect thorax and abdomen with antiseptic chlorhexidine solution.
2. Shave an area of 2.5 cm x 1.5 cm of the abdomen.
3. Disinfect the shaven area using gauze soaked with chlorhexidine solution, then alcohol solution and a final application of chlorhexidine solution.
4. Position the animal in the surgical area so that the prepared surgical site is upwards facing the surgeon.
5. Drape the mouse using a waterproof surgical drape with an opening that leaves the disinfected abdominal region exposed while covering the rest of the body to create a sterile working field. Monitor the mouse prior to the procedure for depth of anesthesia.

4. Pancreatic Duct Ligation

1. Laparotomy
 1. Make an upper midline incision in the skin extending from the xiphoid process to the umbilicus using a sterile surgical blade. Separate the underlying linea alba and the peritoneum using sterile scissors in order to expose the upper abdominal quadrant.
 2. Prevent drying-out of the internal organs by regular sprinkling with sterile 0.9% sodium chloride.
 3. Using sterile tweezers or swab, retract the stomach superiorly, exposing the spleen and the splenic lobe (the tail region) of the pancreas.
 4. Gently retract the duodenum and part of the upper jejunum to the right upper abdominal quadrant to expose the head, neck and body region of the pancreas covered by the visceral peritoneum.
2. Ligation
 1. Locate the anatomical position of the pancreatic main duct in the neck region of the pancreas.
 2. Incise the visceral peritoneum and the gastrocolic ligament, granting access to the retroperitoneum, exposing the body and tail region of the pancreas. In order to expose the neck region, perform a Kocher maneuver. Lift the duodenum and head of the pancreas off the retroperitoneum elevating them from the inferior vena cava and aorta below.
 3. Carefully place the spatula underneath the neck region. Ligate the pancreatic duct with 6-0 Prolene thread at the left side of the portal vein that separates the gastro-duodenal and splenic lobes.
 4. Perform a second ligation in close proximity to the first ligation to ensure that the lobes are adequately separated.
 5. Ligate very carefully in order not to damage the underlying blood vessels, namely the superior pancreaticoduodenal artery, the inferior pancreaticoduodenal artery and the pancreatic part of the splenic artery.
 6. Place the organs back into the abdominal cavity.
 7. Close the incision using 4-0 polyglycol filamentous thread in a continuous suture pattern for the muscle/peritoneal layer and in a discontinuous suture pattern for the skin.

5. Sham Surgery

1. Perform all steps as described in steps 1 through 4.1.4. While the pancreas is exposed, do not perform ligation of the pancreatic duct.
2. At the end of step 4.1.4, place internal organs back into the abdominal cavity.
3. Close the incision as described in 4.2.7.

6. Post-operational Care and Monitoring

1. After the surgical procedure is complete, place the mouse in the recovery area. This consists of a cage placed on a heating pad and lined with flat paper bedding in order to maintain normal body temperature.
2. Provide nutritional support to avoid post-operative hypoglycemia by moistened food placed on the cage bottom. For this purpose, use standard rodent diet pellets soaked in water until they soften.
3. Provide fluid support by the moistened food and provide water *ad libitum*.
4. Use Buprenorphine as analgesia (0.05 – 0.1 mg/kg) twice daily for 2 days post-surgery. During the entire experimental, follow up, observe the mice for occurrence of possible signs of infection, including secretion of liquid or pus from the wound, or for physical deterioration characterized by reduction in grooming behavior and activity level, lower appetite and bodyweight loss.

7. Evaluation of Successful PDL and Harvest of PDL Tail and Sham Tail Pancreas

1. Euthanize mice by cervical dislocation.
2. Re-shave the abdominal region to avoid carry-over of fur.
3. Open the abdominal skin and muscle layer and remove a large area of skin and muscle to obtain good access to the pancreas.
4. Using sterile tweezers or swab, the stomach is retracted superiorly, exposing the spleen and the splenic lobe (the tail region) of the pancreas.
5. Gently pull out the duodenum and part of the upper jejunum to expose the gastro-duodenal lobe (head region) of the pancreas.
Note: The ligated portion of the PDL pancreas has now reduced in size and has become almost translucent so that islets are visible as small white dots. The head portion of the pancreas is opaque pink and distinct exocrine lobuli can be observed.
6. Using sterile scissors, separate the ligated tail part of the pancreas from the spleen, by cutting along the spleen. Cut loose the connective tissue connecting the tail region of the pancreas to the internal organs.
7. Cut the PDL tail region right in front of the ligation, excluding the ligature and the tissue immediately adjacent to it from the harvested tissue.
8. To isolate sham tail tissue, follow steps 7.1 through 7.5. Using sterile scissors separate the tail region of the sham pancreas from the spleen by cutting along the spleen. Isolate the tail region by cutting into the neck region of the pancreas and cutting loose the connective tissue connecting the tail pancreas to the internal organs
Note: Both the tail and head region of the sham pancreas are opaque pink, to isolate both parts separately, cut the pancreas in the neck region.

Representative Results

PDL induces acinar atrophy and inflammation but does not affect bodyweight and glycemia

In 8 week old male BALB/c mice, the duct draining the exocrine enzymes from the tail of the pancreas is ligated while the organ's head, located adjacent to the stomach and duodenum, remains unaffected. Age, sex and weight-matched male BALB/c mice undergo sham surgery

recapitulating all steps of partial duct ligation surgery, except the ligation of the pancreatic duct. Pancreas tissue was harvested 3, 7, 14, 30 days post-surgery.

When PDL is performed correctly, mice appear healthy and do not show a significant difference in bodyweight (**Figure 1A**) or glycemia (**Figure 1B**) compared to sham-operated mice. Exocrine acinar tissue is lost gradually after PDL surgery (**Figure 2A-E**), resulting in a reduction in size and weight of the ligated portion of the PDL pancreas (**Figure 3**), from hereon called PDL tail. Three days post PDL, acinar tissue morphology becomes disrupted and acinar cells undergo apoptosis (**Figure 4**) and are likely engulfed by infiltrating CD45+ immune cells (**Figure 5**). At day 7 post PDL, many acinar lobules are replaced by fibrotic (**Figure 6**) and adipose tissue (**Figure 2C-E**) while remaining acinar cells undergo acinar-to-ductal metaplasia. By day 14 post PDL, almost all acinar lobuli are devoid of acinar cells (**Figure 2A-E**) making the PDL tail pancreas appear translucent so that islets become visible to the naked eye (**Figure 3**). Coincident with the initiation of acinar apoptosis, an increase in cell cycle activity of the ductal epithelium is observed (**Figure 7**).

PDL induces an increase in insulin+ beta cell volume

Two weeks after PDL the total beta cell volume in PDL tail has doubled as compared to non-ligated Sham tail. Beta cell volume is quantified by measuring the INS+ area in 4 μ m sections, 36 μ m apart spanning the whole tissue accounting for 10% of the total pancreas volume. PDL induces an increase in insulin content two weeks after surgery compared to non-ligated pancreas as can be shown by automated whole -tissue optical tomography (OPT).¹⁵ Since beta cell size is not changed after PDL the increase in beta cell volume is the result of an increase in beta cell number.

PDL induces an increase in beta cell proliferation and the activation of Ngn3 expression

Beta cell proliferation is analyzed by immunohistochemical (IHC) staining in pancreata harvested 7 days and 14 days after Sham or PDL surgery. The percentage of beta cells positive for proliferation marker Ki-67 is quantified by inspection of individual cells in a non-automated manner. At 7 and 14 days post PDL surgery, beta cell proliferation in PDL tail is significantly increased compared to non-ligated pancreas.

Within 3 days after PDL the expression of the embryonic islet progenitor marker Ngn3 is significantly increased in PDL tail as compared to non-ligated pancreas. Maximal levels of *Ngn3* transcript were reached within 1 week and subsequently decreased slowly. We routinely measure *Ngn3* gene activation by expressing the level of Ngn3-encoding mRNA in PDL pancreas relative to the stable *Ngn3* level in duodenum. We recently suggested that the extent of neogenesis in PDL might depend on the level of *Ngn3* gene activation.^{15,30}

Figure 1. PDL does not affect bodyweight or glycemia. Bodyweight(g) (**A**) and glycemia (mg/dL) (**B**) from sham-operated (white bars) and PDL mice (black bars) was measured at different time points (day 0, 7, 14 and 30) following surgery and did not show any significant difference between sham and PDL operated mice at any time point. Figure originally published by Xu *et al.*, 2008.¹¹ [Please click here to view a larger version of this figure.](#)

Figure 2. PDL leads to a gradual loss of acinar cells. Morphological change of PDL tail pancreas revealed by haematoxylin-eosin staining at day 3 (B), 7 (C), 14 (D) and 30 (E) post surgery, compared to sham-operated tail pancreas (A). At 3 days post ligation, only a subtle disruption of the acinar tissue can be observed (B). At 7 days post ligation, acinar tissue is severely disrupted and ductal complexes have formed (C). At 14 days post ligation, few acinar cells remain and acinar lobuli are replaced by ductal structures, a fibrous network and adipocytes (indicated with an asterisk (*) in panel C and E). [Please click here to view a larger version of this figure.](#)

Figure 3. PDL tail at harvest. Sham tail (A) and PDL tail (B) harvested at day 14 post-surgery. PDL tail is dramatically reduced in size compared to Sham tail. Picture (C) shows PDL tail at day 14 post surgery *in situ*. Due to loss of acinar cells, the PDL tail appears translucent, the main pancreatic duct is clearly visible (indicated with black arrow) and islets are visible as small white dots (indicated with white arrow). [Please click here to view a larger version of this figure.](#)

Figure 4. PDL induces acinar apoptosis. Immunostaining for cleaved-Caspase 3 in PDL tail at 3, 7 and 14 days post-surgery reveals apoptotic bodies in the acinar compartment at day 3 and day 7 post surgery, while acinar cells are almost absent from PDL tail at day 14. Magnification bars are 25 μ m. Figure originally published by Xu *et al.*, 2008.¹¹ [Please click here to view a larger version of this figure.](#)

Figure 5. PDL induces immune cell infiltration. Immunostaining for the leukocyte marker CD45, showing the presence of a high number of immune cells in PDL tail 7 days after surgery compared to sham pancreas. Magnification bars are 50 μ m. [Please click here to view a larger version of this figure.](#)

Figure 6. PDL induces fibrosis. Immunostaining for alpha smooth muscle actin shows the presence of a fibrous network in PDL tail 14 days after surgery as compared to sham pancreas. Magnification bars are 50 μ m. [Please click here to view a larger version of this figure.](#)

Figure 7. PDL induces an increase in duct cell proliferation. Double immunostaining for the duct cell marker Cytokeratin 19 (CK19) and for the proliferation marker BrdU shows an increase in cell cycle activity of duct cells. Magnification bars are 25 μ m. Figure originally published by Xu *et al.*, 2008.¹¹ [Please click here to view a larger version of this figure.](#)

Discussion

In the present study, we describe in detail the methodology behind PDL, a mouse injury model to study beta cell neogenesis and proliferation and transdifferentiation of pancreatic non-beta cells. Ambiguity in data on PDL among labs stimulates the need for a standardized protocol for PDL surgery.

Critical steps in the PDL protocol include the selection of healthy, young mice for surgery. Preferably male mice should be used since unpublished data from our lab suggest that estrogen receptor signaling affects the outcome of PDL. PDL surgery induces recruitment and expression of many factors to/in the injured pancreas and dissection of this cocktail may allow the identification of factors that are necessary and sufficient to cause beta cell neogenesis and proliferation to bypass the need for surgery. However, it is important to realize that the cytokines and factors involved in this model are not yet completely characterized. Therefore, it is important to perform PDL surgery under optimal conditions since severe infection or disease of the animal can alter the occurrence of these factors and thereby affect the outcome of PDL. We advise to monitor the animals closely after PDL surgery by assessing weight, glycemia, appetite, and activity level.

Proper ligation of the pancreatic duct is the most crucial step in the protocol. The ligation needs to be secured with multiple loops to prevent incomplete ligation that may reduce the severity of the injury. However, the ligation should only affect the pancreatic duct, avoiding the underlying superior and inferior pancreaticoduodenal artery. Accidental ligation of the arteries can lead to accessory damage and to hypoxia of the pancreas with possible subsequent necrosis.

A sub-optimal PDL can occur due to improper ligation of the duct. This leads to a remnant of acinar tissue, as evidenced at day 7 and 14 after PDL surgery. We advise not to include sub-optimal PDL samples into the analysis, since this can lead to variation in analyzed parameters such as beta cell proliferation and Ngn3 expression. Notably, the level of Ngn3 expression correlates with the level of beta cell neogenesis.¹⁵ [Hansen M.T., *et al.*, in preparation]. A successful PDL can be recognized by the visible reduction in size and transparency of the tissue compared to a non-ligated pancreas, phenomena which are clearly visible at 7 and 14 days post-surgery. Histological analysis should reveal fibrotic tissue, increased number of ductal structures, increased ductal and endocrine proliferation and recruitment of immune cells. RT-qPCR should reveal an expression of Ngn3 in PDL tail of at least 20% of the expression level in duodenum.

When analyzing proliferation in a non-automated manner by individual inspection of cells, the duct and endocrine cell proliferation in PDL leads to very reproducible results. The induction of Ngn3 expression, however, varies between experiments, indicating that this process may be more sensitive to physiological and environmental factors. Since PDL induces recruitment and/or activation of many cells and factors, identification of the factor(s) responsible for a certain process in PDL is challenging.

As a model in diabetes research, PDL does not require removal of pancreatic tissue or of beta cell reduction, as compared to pancreatectomy, alloxan or streptozotocin injection. Therefore, animals usually maintain good glycemic control after PDL surgery. It can be expected that beta cell proliferation and activation of an Ngn3-expressing progenitor is stimulated by locally produced signals originating from the inflammatory responses and from acinar cell death. Therefore, PDL is an injury model that can serve as an analytical tool to study signaling pathways involved in beta cell formation. Moreover, the experimental model of PDL may be useful beyond diabetes research: while being artificial, the inflammatory nature of PDL mimics human pathological conditions and has therefore been used as a model to study pancreatitis³¹, formation of adenocarcinoma³² and neoplasia.³³

Disclosures

The authors have nothing to disclose.

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References

- Shaw, J. E., Sicree, R. A., & Zimmet, P. Z. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract.* **87**, 4-14 (2010).
- Danaei, G. *et al.* National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet.* **378**, 31-40 (2011).
- Borowiak, M., Melton, D. A., . How to make beta cells? *Current opinion in Cell Biology.* **21**, p. 727 – 732 (2009).
- Gradwohl, G., Dierich, A., LeMeur, M., & Guillemot, F. neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *Proc Natl Acad Sci U S A.* **97**, 1607-1611 (2000).
- Gu, G., Dubauskaite, J., & Melton, D. A. Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development.* **129**, 2447-2457 (2002).
- Dor, Y., Brown, J., Martinez, O. I., & Melton, D. A. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature.* **429**, 41-46 (2004).
- Teta, M., Rankin, M. M., Long, S. Y., Stein, G. M., & Kushner, J. A. Growth and regeneration of adult beta cells does not involve specialized progenitors. *Dev Cell.* **12**, 817-826 (2007).
- Bonner-Weir, S. Perspective: Postnatal pancreatic beta cell growth. *Endocrinology.* **141**, 1926-1929, doi: 10.1210/endo.141.6.7567 (2000).
- Brennan, K., Huangfu, D., & Melton, D. All beta cells contribute equally to islet growth and maintenance. *PLoS Biol.* **5**, e163 (2007).
- Salpeter, S. J., Klein, A. M., Huangfu, D., Grimsby, J., & Dor, Y. Glucose and aging control the quiescence period that follows pancreatic beta cell replication. *Development.* **137**, 3205-3213 (2010).
- Xu, X. *et al.* Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell.* **132**, 197-207 (2008).
- Wang, R. N., Kloppel, G., & Bouwens, L. Duct- to islet-cell differentiation and islet growth in the pancreas of duct-ligated adult rats. *Diabetologia.* **38**, 1405-1411 (1995).
- Yasuda, H. *et al.* Cytokine expression and induction of acinar cell apoptosis after pancreatic duct ligation in mice. *Journal of interferon, & cytokine research : the official journal of the International Society for Interferon and Cytokine Research.* **19**, 637-644 (1999).
- Van Gassen, N. *et al.* Macrophage dynamics are regulated by local macrophage proliferation and monocyte recruitment in injured pancreas. *European journal of immunology.*, doi: 10.1002/eji.201445013 (2015).
- Van de Casteele, M. *et al.* Neurogenin 3+ cells contribute to beta-cell neogenesis and proliferation in injured adult mouse pancreas. *Cell Death Dis.* **4**, e523 (2013).
- Xiao, X. *et al.* No evidence for beta cell neogenesis in murine adult pancreas. *The Journal of clinical investigation.* **123**, 2207-2217, doi: 10.1172/JCI66323 (2013).
- Ackermann Misfeldt, A., Costa, R. H., & Gannon, M. Beta-cell proliferation, but not neogenesis, following 60% partial pancreatectomy is impaired in the absence of FoxM1. *Diabetes.* **57**, 3069-3077, doi: 10.2337/db08-0878 (2008).
- Lee, C. S., De Leon, D. D., Kaestner, K. H., & Stoffers, D. A. Regeneration of pancreatic islets after partial pancreatectomy in mice does not involve the reactivation of neurogenin-3. *Diabetes.* **55**, 269-272, doi: 10.2337/db05-1300 (2006).
- Bonner-Weir, S., & Sharma, A. Pancreatic stem cells. *The Journal of pathology.* **197**, 519-526, doi: 10.1002/path.1158 (2002).
- Nir, T., Melton, D. A., & Dor, Y. Recovery from diabetes in mice by beta cell regeneration. *The Journal of clinical investigation.* **117**, 2553-2561 (2007).
- Bouwens, L., & Rooman, I. Regulation of pancreatic beta-cell mass. *Physiol Rev.* **85**, 1255-1270 (2005).
- Wang, S. *et al.* Neurog3 gene dosage regulates allocation of endocrine and exocrine cell fates in the developing mouse pancreas. *Developmental biology.* **339**, 26-37, doi: 10.1016/j.ydbio.2009.12.009 (2010).
- Pan, F. C. *et al.* Spatiotemporal patterns of multipotentiality in Ptf1a-expressing cells during pancreas organogenesis and injury-induced facultative restoration. *Development.* **140**, 751-764, doi: 10.1242/dev.090159 (2013).
- Kopp, J. L. *et al.* Sox9+ ductal cells are multipotent progenitors throughout development but do not produce new endocrine cells in the normal or injured adult pancreas. *Development.* **138**, 653-665, doi: 10.1242/dev.056499 (2011).
- Wang, S. *et al.* Sustained Neurog3 expression in hormone-expressing islet cells is required for endocrine maturation and function. *Proc Natl Acad Sci U S A.* **106**, 9715-9720, doi: 10.1073/pnas.0904247106 (2009).
- Kopinke, D. *et al.* Lineage tracing reveals the dynamic contribution of Hes1+ cells to the developing and adult pancreas. *Development.* **138**, 431-441, doi: 10.1242/dev.053843 (2011).

27. Rankin, M. M. *et al.*. beta-Cells are not generated in pancreatic duct ligation-induced injury in adult mice. *Diabetes*. **62**, 1634-1645, doi: 10.2337/db12-0848 (2013).
28. Solar, M. *et al.*. Pancreatic exocrine duct cells give rise to insulin-producing beta cells during embryogenesis but not after birth. *Dev Cell*. **17**, 849-860, doi: 10.1016/j.devcel.2009.11.003 (2009).
29. Furuyama, K. *et al.*. Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. *Nat Genet*. **43**, 34-41, doi: 10.1038/ng.722 (2011).
30. Van de Casteele, M. *et al.*. Partial duct ligation: beta-cell proliferation and beyond. *Diabetes*. **63**, 2567-2577, doi: 10.2337/db13-0831 (2014).
31. Zhang, J., & Rouse, R. L. Histopathology and pathogenesis of caerulein-, duct ligation-, and arginine-induced acute pancreatitis in Sprague-Dawley rats and C57BL6 mice. *Histology and histopathology*. **29**, 1135-1152 (2014).
32. Martinez-Romero, C. *et al.*. The epigenetic regulators Bmi1 and Ring1B are differentially regulated in pancreatitis and pancreatic ductal adenocarcinoma. *The Journal of pathology*. **219**, 205-213, doi: 10.1002/path.2585 (2009).
33. Prevot, P. P. *et al.*. Role of the ductal transcription factors HNF6 and Sox9 in pancreatic acinar-to-ductal metaplasia. *Gut*. **61**, 1723-1732, doi: 10.1136/gutjnl-2011-300266 (2012).