Stem Cell Biology and Regenerative Medicine

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Pancreatic Stem Cells

Humana Press

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Preface

The last decade has witnessed the consolidation of "regenerative medicine" as a recognized scientific field, encompassing disciplines as diverse as cell biology, immunology, developmental biology, and surgery. The report on the isolation of human embryonic stem (huES) cells by James Thomson in 1998[1] opened our eyes to a ground-breaking notion: that defective tissues could be replaced by an unlimited source of self-renewing cells with the ability to morph in vitro into any of them. The revolutionary nature of this idea is evidenced by the fact that concepts such as "regenerative medicine" or "stem cell therapies" were not in common use in the scientific literature until the late nineties. Until then, and despite reports of embryonic stem cells obtained from several animal species,[2–4] there was no identifiable organized quest for a "human tissue building block," as there was one, for example, to decipher the entirety of the human genome. In retrospect, it is as though the majority of the scientific community had not envisioned applications for these unique cells other than to create animal models for human diseases, increase livestock output, or improve the production of therapeutic proteins from transgenic animals. This seeming "unexpectedness" was further confirmed when, shortly after this breakthrough, all major scientific journals started to publish a plethora of reports on the therapeutic potential of stem cells of adult origin. Since the technology to isolate and expand adult stem cells had already been in use for a long time before Thomson's discovery, it remains surprising that very few had openly contemplated until then the idea of using adult stem cells for medical purposes. Be it as it may, the field has gone a long way throughout this past decade. Several adult stem cell types are currently in clinical trials for a variety of conditions ranging from myocardial infarction[5] to graft-versus-host disease,[6,7] and huES cells will shortly follow suit.

Among the many conditions potentially treatable by stem cells, type I diabetes (a disease where the endocrine pancreatic cells that synthesize and secrete insulin are destroyed by autoimmune processes) holds a position of privilege. Unlike many other commonly cited targets of stem cell approaches (such as Parkinson's disease or spinal cord injury), there is already an effective cell therapy for type I diabetes. Indeed, islet transplantation has been shown to completely restore normoglycemia in human patients,[8–10] and even if there is a progressive loss of function of the graft over time,[11] patients invariably report a much higher quality of life than before the

procedure.[12] Based on our experience with this therapy, it is not unreasonable to expect that any stem cell type with the ability to give rise to insulin-producing, pancreatic endocrine-like tissues will also work in a transplantation setting.

In this context, this book has been conceived with the goal of presenting the state of the art in regenerative therapies for the pancreas. First, we will briefly describe how the adult organ works (in the chapter "The Pancreas"). Then, we will thoroughly review the two physiological processes that should be recapitulated in different therapeutic settings, namely *pancreatic development* (in the chapter "Pancreatic Development") and *islet regeneration* (in the chapter "Pancreatic Regeneration"). The chapter "Stem Cell Differentiation: General Approaches" will examine the general experimental strategies used to differentiate stem cells, regardless of their origin, whereas the chapters "Embryonic Stem Cells and Pancreatic Differentiation" and "Adult Stem Cells and Pancreatic Differentiation" will focus, respectively, on the utilization of embryonic and adult stem cell types for the procurement of transplantable insulin-producing cells. The latter will include special sections on bone marrow cells, umbilical cord blood stem cells, ductal and acinar cells, and mesenchymal stem cells. The chapter "Transdifferentiation" will drift away both from the general concept of stem cell differentiation and the two islet neogenesis processes known to happen in vivo (development and regeneration), to touch upon *transdifferentiation*, an intriguing phenomenon by which terminally differentiated cells from other tissues might be induced to alter their phenotype to become islet-like cells. We will conclude with a general overview of the remaining challenges and clinical perspectives of all of the above strategies.

Despite what many may perceive as a slow pace in translating basic findings into clinical therapies for type I diabetes, the last 10 years have been very productive in terms of shaping the overall direction of the field, many times as a consequence of a trial-and-error process. Progress has been steadfast, however, and the current state of the art suggests that stem cell-based trials, perhaps combined with immunological therapies, might be just around the corner. Because type I diabetes is a complex disease, a cure will only come from a multidisciplinary effort, which will almost certainly include a strong stem cell component. It is our hope that this book will help frame the problem for researchers and clinicians alike.

Miami, FL Juan Domínguez-Bendala

Contents

The Pancreas

Abstract The pancreas is a unique organ that encompasses both endocrine and exocrine functions. Here we review its main anatomical features, including its innervation and vasculature, the exocrine acinar tissue (which secretes a variety of enzymes that take part in food digestion), the ductal system (which collects exocrine secretions and is thought to harbor pancreatic stem cells), and the endocrine component, also termed the *islets of Langerhans*. These cell clusters, embedded in the pancreatic parenchyma, synthesize and secrete into the bloodstream some of the most important hormones involved in the maintenance of glucose homeostasis.

Keywords Endocrine • Exocrine • Islets of Langerhans • Ductal system • Beta cells • Alpha cells • Glucagon • Insulin

1 Introduction

The pancreas is a solid glandular organ in the gastrointestinal tract, with both digestive (exocrine) and endocrine functions. It is elongated (12.5–15 × 4 cm) and irregular in shape, with three poorly defined regions: The broader extremity is the *head*, and the narrower end is called the *tail*. In between is the *body*, which is connected to the head by a slender constriction, also called the *neck*. It is located transversely across the posterior wall of the abdomen, beneath the stomach, and connected to the small intestine at the duodenum[13] (Figs. 1 and 2).

2 The Ductal System

The *pancreatic duct* (also called *duct of Wirsung*) runs transversely from left to right throughout the organ, from the junction of the tail lobular ductules to the common bile duct, receiving the affluence of smaller ducts. Sometimes, a secondary duct receiving the lower head ductules (*duct of Santorini*)[14] opens into the duodenum from the neck region.

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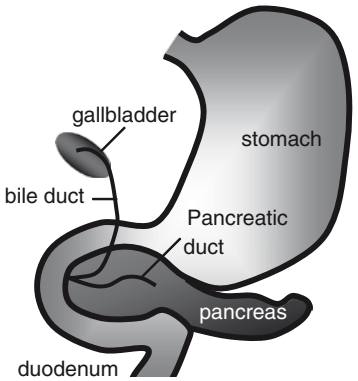


Fig. 1 The pancreas in its anatomical context

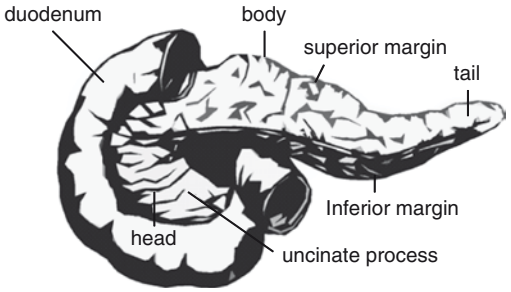


Fig. 2 Gross anatomy of the pancreas

The cells that form the ducts are typically arranged in one single layer of *ductal cells*. Probably due to their primary function of collecting and channeling pancreatic exocrine secretions, these cells express a relatively high amount of intermediate filaments, which may result in additional mechanical strength. Cytokeratins (CK) 7, 19, and 20 are the most represented in ductal cells, and as such are usually used as immunohistochemical/immunofluorescent markers of ductal tissue.[15] Other markers are the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) chloride pump and the carbohydrate antigen CA-19.9 (not to be confused with CK-19).[16] Interestingly, the pancreatic and duodenal homeobox-1 protein (Pdx1), whose expression in the adult pancreas is normally restricted to beta cells, can also be detected in ductal cells, albeit with a different phosphorylation pattern.[17] This, together with other lines of evidence to be discussed in the chapter "Stem Cell Differentiation: General Approaches," have led to the hypothesis that ductal cells might act as progenitors of beta cells during normal turnover and regeneration in the adult pancreas.

3 Vasculature

The vasculature of the pancreas and duodenum is so intertwined that it may be considered common to both (reviewed in[13,18,19]). Arteries are derived from the celiac trunk and the superior mesenteric artery. The celiac trunk arises from the front of the aorta and has three branches (left gastric artery, hepatic artery, and splenic artery). As it turns into the lesser omentum, the hepatic artery gives off the right gastric and gastroduodenal arteries. The latter extends behind the first part of the duodenum and then divides into the right gastroepiploic artery and the superior pancreaticoduodenal artery, which supplies the second part of the duodenum and the head of the pancreas.

The third branch of the celiac trunk, the splenic artery, winds along the upper border of the pancreas to the hilum of the spleen. Its most important branch to the pancreas is the dorsal pancreatic artery, which passes into the superior border of the organ near the neck. This vessel gives off a single branch to the left, the transverse pancreatic artery, and two branches to the right, toward the head of the pancreas. The splenic artery also gives rise to the pancreatica magna and a caudate branch, near the body-tail junction and in the tail region, respectively.

The splenic vein, which runs alongside the splenic artery, drains venous blood from the pancreas. It joins the superior mesenteric vein to form the portal vein, which runs into the liver.

4 Innervation

The pancreas is richly innervated by myelinated or unmyelinated nerve fibers, thick nerve bundles, and scattered intrapancreatic ganglia, which represent the intrinsic neural component of the organ.[20] The two main extrinsic components derive from the anterior and posterior branches of the vagi nerves and the splanchnic nerve trunks. The afferent system is composed of thin unmyelinated fibers that run along parasympathetic or sympathetic nerves, and is chiefly involved in sensory/pain relay to the central nervous system. One of the key features of pancreatic neurons is their ability to secrete biologically active substances, including acetylcholine, nitric oxide, and gastrin-releasing peptide, which have been associated with the regulation of endocrine/exocrine secretion.[20–26]

5 Exocrine Pancreas

Because the organ combines two completely different functions, references to exocrine and endocrine pancreas are common. Approximately 90–95% of the tissue of the organ is exocrine, and its main function is to secrete digestive enzymes into

the duodenum. This tissue is organized in cell clusters termed *acini*, and thus the exocrine pancreas is also commonly referred to as *acinar tissue*. Acini are connected to ductules by centroacinar cells, which share many markers with ductal cells (Fig. 3).

Some of the key digestive enzymes secreted by the exocrine pancreas, mainly in response to duodenally secreted cholecystokinin (CCK), are listed in Table 1. Many of these are secreted in an inactive form, becoming activated only when in contact with other proteases in the lumen of the small intestine. This mechanism of activation has evolved so that the acinar cells are not digested by the very same enzymes they harbor. In addition to these enzymes, ductal cells also secrete a bicarbonate solution, which, in a biochemical process orchestrated by the hormone secretin, helps regulate the duodenal pH after the influx of gastric acid secretions.

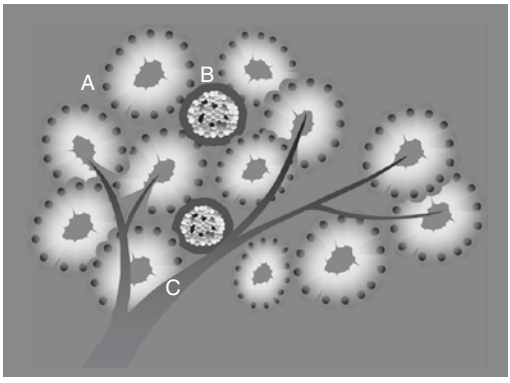


Fig. 3 Histological organization of the pancreas. The ductal system (C) collects the secretion of acinar cells (A), which are typically organized in acini around ductules. The islets of Langerhans (B) are intercalated throughout the acinar tissue

Table 1 Pancreatic exocrine enzymes

Carboxypeptidase A (CPA1)	Hydrolyzes C-terminal residues, particularly those of aromatic (A) nature
α-Amylase	Cleaves the α(1,4) glycosidic bonds of amylose (which is one of the two main components of starch), yielding maltose and dextrin
Trypsinogen	Inactive form of *trypsin*, a serine protease activated by *enterokinase* upon secretion into the small intestine. With few exceptions, it cleaves proteins at the carboxylic side of the residues lysine and arginine
Chymotrypsinogen	Inactive form of *chymotrypsin* (also called *zymogen*), activated by trypsin in the lumen of the small intestine. It cleaves proteins mostly at the carboxylic side of phenylalanine, tyrosine, and tryptophan
Pancreatic elastase (ELA-1)	Cleaves elastin, a main protein component of the connective tissue
DNAse and RNAse	Nucleases that break down nucleic acids
Pancreatic lipase	Hydrolyzes ester bonds of lipids

6 Endocrine Pancreas

The endocrine component of the pancreas is organized in cell clusters termed islets of Langerhans, first identified by the German biologist Paul Langerhans in 1869.[27] Islets are composed of at least five types of endocrine cell types, namely alpha, beta, delta, beta, PP, and epsilon, which secrete glucagon, insulin, somatostatin, pancreatic polypeptide, and ghrelin, respectively.

The typical view of the islet cellular architecture has been shaped based on early observations on the mouse pancreas, where beta cells cluster preferentially at the core of the structure and alpha, delta, and PP cells (epsilon were not discovered until recently) are peripherally arranged[28–31] (Fig. 4). In all species studied, beta cells are the most abundant (50–80%), followed by alpha cells (20–50%). The remaining cell types are scarcely represented. However, there are significant differences between species both in islet cell composition and cytoarchitecture. Thus, in human and nonhuman primates, alpha cells are scattered throughout the islet rather than concentrated in the periphery. The percentage of beta cell homotypic interactions (those of cells apposing to cells of the same type) is approximately 29% in human islets, compared with 71% in mouse.[32] Considering the complex nature of cell-to-cell communication in the endocrine pancreas, such architectural differences were expected to have functional implications. This was indeed the case, according to a recent report[32]: the oscillations in membrane potential and $[Ca_2^+]$ in response to high glucose concentrations in rodent islets were coordinated, so that the entire islet displays a synchronous oscillatory response. This did not happen in human islets, where these responses were not synchronized. These findings suggest a correlation between the pattern of cell distribution (clustered vs. scattered) and islet cell function. Additional studies, however, are needed to further clarify such possible association.

Islets are richly vascularized, to ensure the efficient secretion of endocrine hormones into the bloodstream. A classic study revealed a nearly twofold increase in both internal volume and microvascular surface area for the blood vessels within the islet compared with the surrounding exocrine tissue.[33] The additional importance of islet vasculature to maintain an adequate oxygen supply is revisited in the chapter "Stem Cell Differentiation: General Approaches."

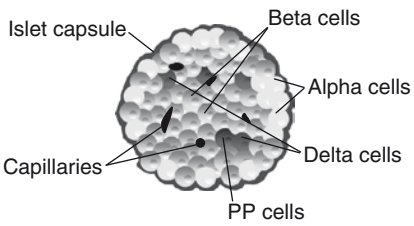


Fig. 4 Typical architecture of a murine islet. Beta cells are the most abundant, and are usually located at the core of the islet. Alpha cells, in contrast, tend to lie at the periphery. PP and delta cells are less abundant and interspersed among the other two endocrine cell types

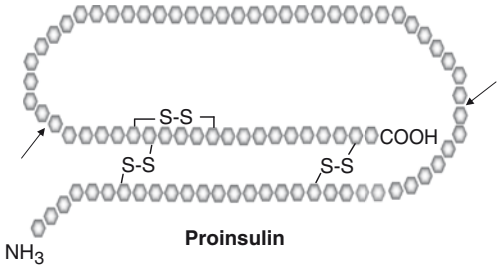
Even Langerhans himself noticed in his doctoral thesis the abundant innervation of islets,[27] which has been confirmed in most species examined thus far. Neural elements tend to coalesce both in the periphery (peri-insular plexus) and in the core (intra-insular plexus) of the islets. The physiological importance of neural activity on islet metabolism is very well documented (reviewed in[26]), but its description is beyond the scope of this book.

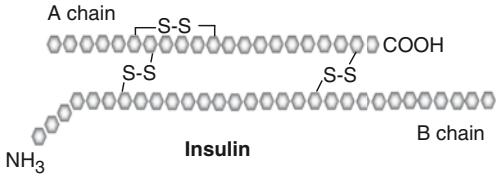
7 Glucose Metabolism

The endocrine component of the pancreas is responsible for the maintenance of glucose homeostasis (70–100 mg/dl), chiefly by means of the secretion of the hormones insulin (beta cells) and glucagon (α cells) to the bloodstream. Insulin is a two-chain polypeptide generated by the cleavage of a precursor protein, termed proinsulin (Fig. 5). Beta cells secrete this peptide in response to a variety of stimuli, including parasympathetic signals,[36] the incretins glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1),[37] cholecystokinin (CCK),[38] glucagon, and, of course, high blood glucose levels. Glucagon (Fig. 6), in contrast, is secreted by α cells when insulin levels are high and/or blood glucose is low. Figures 7 and 8 show some of the factors that affect the release of these hormones. Insulin acts by activating systemically the cellular uptake of glucose, thereby reducing blood sugar levels. In the liver, this is followed by a conversion of glucose into glycogen, a complex carbohydrate resulting from the sequential addition of single glucose molecules (Fig. 9). This process is catalyzed by the enzyme glycogen synthase. Glycogen is the main repository of glucose of the organism.[39,40]

When sugar levels are low, α cells counteract the effects of insulin by secreting glucagon. The major effect of glucagon in the liver is the catabolism of glycogen, a process called glycogenolysis.[41,42] The long chains of glycogen are progressively converted into glucose by glycogen phosphorylase, debranching enzymes and phosphoglucomutase. Another glucagon-stimulated pathway is gluconeogenesis, which converts noncarbohydrate substrates into glucose. The combined action of these processes results in a net increase of available circulating glucose (Fig. 10).

The complex interplay of these and other hormones[37,38] in the maintenance of glucose homeostasis is beyond the scope of this chapter. However, it is important to stress that their secretion, far from responding to the clear-cut situations schematized above (high or low glucose) is exquisitely regulated at several levels. As an example, it has been recently shown that the activation of alpha cells depends not only on an initial stimulus (low sugar), but also on a decrease of the suppressive effect of beta cell-derived secretions such as insulin, γ-aminobutyric acid (GABA) or zinc. In addition, a positive autocrine feedback exerted by glutamate, which is also secreted by alpha cells, is required for effective glucagon release (Fig. 11).[43]





C-peptide

A chain: Gly-Ile-Val-Glu-Gln-Cys-Cys-Thr-Ser-Ile-Cys-Ser-Leu-Tyr-Gln-Leu-Glu-Asn-Tyr-Cys-Asn

B chain: Phe-Val-Asn-Gln-His-Leu-Cys-Gly-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys-Gly-Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Thr

Fig. 5 Insulin derives from proinsulin, a molecule that is cut twice during its maturation to give rise to insulin and C-peptide. The two insulin chains are kept together by disulfide bonds. The amino acid sequence of the two chains is indicated below[34]

His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr

Fig. 6 Structure and amino acid sequence of glucagon[35]

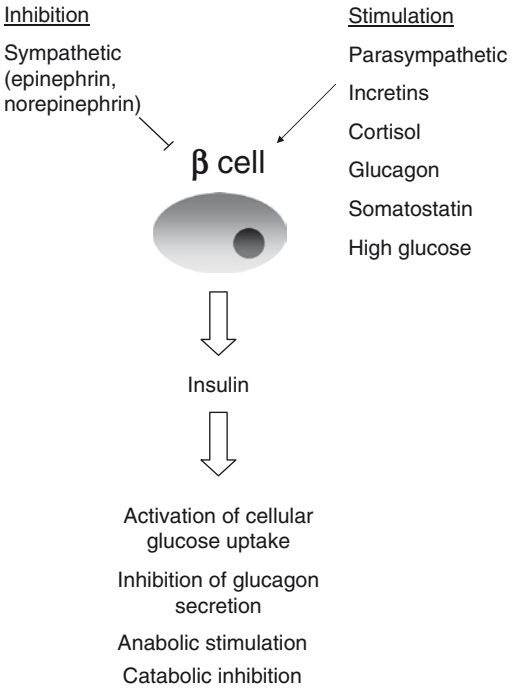


Fig. 7 Factors affecting insulin secretion

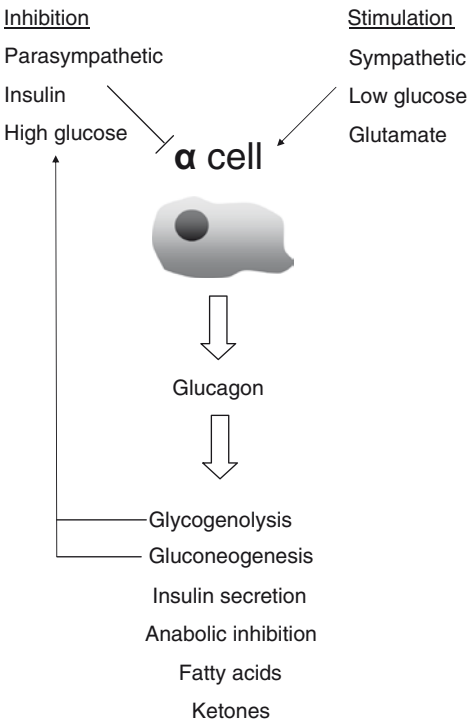


Fig. 8 Factors exerting an influence on alpha cell activity