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Pancreas phylogeny and ontogeny in relation to a 'pancreatic stem cell'

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Abstract

Blood glucose regulation has likely evolved during early vertebrate evolution to allow and secure the concurrent evolution of complex brains and nervous systems: an inner milieu of constant blood glucose levels through millions of years has provided an extra degree of freedom for the brain to evolve without having to *think* of getting energy supply. Key regulators of blood glucose, insulin, and glucagon are produced by the dominating cell types of the pancreatic islet of Langerhans: the insulin producing beta cells and the glucagon producing alpha cells. Interestingly, it appears that the beta cell pioneered the formation or the foundation of the pancreatic organ according to current phylogenetic insights. Such phylogenetic aspects of a pancreatic stem cell are at the end discussed in relation to directed differentiation of embryonic stem cells/ES cells towards therapeutic beta cells. ***To cite this article: O.D. Madsen, C. R. Biologies 330 (2007).***

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1. Introduction: beta cells and diabetes

The pancreatic islet beta cell is uniquely specified to produce and administer insulin to the blood circulation in response to glucose levels. The islet beta cell thus continuously monitors glucose levels, and a glucose increase following food intake is quickly counteracted by increased insulin release – and consequently insulin-induced glucose uptake in peripheral tissues. Functional beta-cell deficiency (and thereby insulin deficiency) is the hallmark of diabetes (T1 vs. T2 diabetes are characterized by a complete vs. relative deficiency of a functional beta cell mass). Insulin deficiency causes hyperglycaemia and diabetes. Long-term elevations of blood glucose lead to damaging glycosylation reactions, eventually causing devastating diabetes late complications. During fasting, glucose levels are maintained via glucagon action where low glucose stimulates glucagon release from the pancreatic islet alpha cell, which in turn stimulates glucose production by the liver. Brain function cannot be sustained during acute hypoglycaemia and unregulated excess insulin release from, e.g., even a small benign insulinoma may cause lethal hyperinsulinaemia-induced hypoglycaemia.

The islet of Langerhans has thus evolved as minute and dispersed organs within the pancreatic tissue possessing a highly specialized ability to sense glucose levels and to secrete insulin or glucagon in adequate

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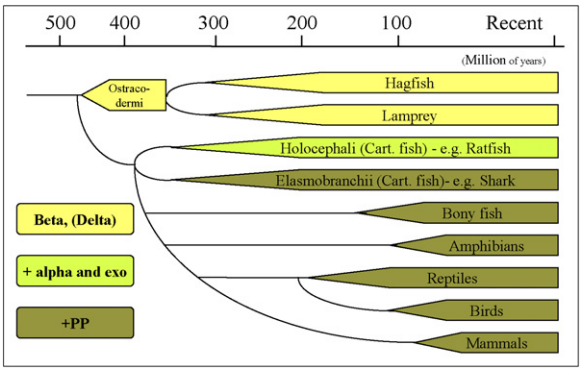

Fig. 1. Phylogenetic relationships between vertebrates are shown in different colours[1] (or grey levels) to indicate the successive entrance of current pancreatic cell types. Figure adapted from [5].

amounts, in order to minimize fluctuations in blood glucose levels. The beta cells have evolved a superior competence to mass-produce and administer insulin – a hormone that is required to sustain life (T1 diabetes patients die if not treated with insulin) – and a hormone that can be an instant killer if overdosed, causing acute and severe hypoglycaemia. Beta-cell-specific suicidal mechanisms allowing regulation/elimination of an excess beta-cell mass are likely reflecting the price to be paid for the acquired competence to administer the potentially deadly hormone, insulin (see [1] for a review).

2. The beta cell as the phylogenetic founder of pancreas

Interestingly, work pioneered by Falkmer and others indicate that pancreas phylogeny was founded by the insulin-producing beta cell (see Fig. 1). In the hagfish and lampreys (our most primitive vertebrate species of today), the first sign of 'a new organ' is found as collections of endocrine cells around the area of the bile duct connection with the duodenum. These endocrine organs are composed of 99% beta cells and 1% somatostatin-producing delta cells. Compared to the more primitive protochordates (e.g., amphioxus), this represents a stage where all previously scattered insulin-producing cells of the intestinal tissue have now quantitatively migrated to found a new organ involved in sensing blood glucose rather than gut glucose. Only later in evolution, the beta cells are joined by exocrine tissue and alpha cells (exemplified by the rat-, rabbit- and elephant-fishes). Finally, from sharks and onwards in evolution, we have the islet PP-cell entering to complete the pancreas [2,3]. Hagfish and lampreys may have one or more endocrine buds – and later the vertebrate pancreas develop as independent ventral and dorsal buds that eventually fuse to become one organ. In the bony fish, a giant islet – known as the 'Brockman-body' is derived of dorsal origin, while the ventral buds give rise to acinar cells, ducts, and smaller islets [4,5].

3. Pdx-1 and other key transcription factors in pancreas formation

Interestingly, amphioxus (proto-chordate) Pdx-1 expression is already narrowed to a confined region of the gut [6]. The presence of Pdx-1 may have been instrumental for the subsequent evolutionary accumulation of beta cells in this region – as well as for the elaborate involvement of Pdx-1 as a beta-cell-specific transcriptional regulator of glucose-responsive genes. Lack of Pdx-1 in vertebrates cause pancreas agenesis [7–9]. Pdx-1 [7,8] and Nkx6.1 [10] are both transcription factors with a restricted expression within the mature pancreatic beta cell. In fact, the co-expression of those two markers in adult islets specifically identifies the beta-cell subpopulation [11]. During pancreas ontogeny, Pdx-1 is expressed within the early budding tissue as well as in the duodenum and antral stomach (albeit at lower levels). Again the co-expression of Pdx-1 and Nkx6.1 selectively specifies all of the early pancreatic progenitors found in the buds of ventral and dorsal origin [11,12]. Such progenitors need to activate *Ngn3* gene transcription to produce the bHLH-transcription factor required for the initiation of the endocrine-cell maturation program [13] – a process regulated by Notch signalling [14].

Ptf1A/p48 is an exocrine bHLH-type transcription factor involved in pancreatic enzyme gene regulation [15] and is also influenced by Notch signalling [16,17]. P48 expression is highly confined within the endoderm to the pancreatic epithelium. It was therefore not unexpected that acinar cells failed to develop in p48 null-mutant mice [18]. Endocrine cells were reported still to form, and eventually localize within the spleen (the spleen forms during condensation of the dorsal pancreatic mesenchyme) [18]. However, later lineage-tracing studies showed that most endocrine cells actually derive from p48-expressing progenitor cells – but also confirmed that some endocrine cells still form in the absence of p48 [19]. Interestingly, Pdx-1 expression from *Ptf1a/p48 cis*-regulatory sequences restores pancreas tissue to *Pdx-1*-null mice [19]. This indicates that p48 and Pdx-1 together are required in the specification of the pancreatic progenitor cell.

[1] Electronic version only.

In fact, if the *p48* expression domain is extended into the duodenal *pdx-1* domain, it will result in ectopic pancreas formation containing both endo- and exocrine cells: transgenic mice carrying the Hes1 null mutation (a down-stream effector of Notch signalling) display precocious formation of endocrine (alpha) cells in the pancreatic epithelium due to lack of repression of *Ngn3* [14]. However, within the Pdx-1 positive duodenal epithelium the lack of Hes-1 leads to focal activation of *p48* – with resulting ectopic pancreas formation [20]. Similar data were recently reported in transgenic Xenopus with pan-endodermal expression of p48, which leads to ectopic pancreas formation in the entire duodenal *pdx-1* domain [21].

4. Summary and perspective

- Pancreas phylogeny is characterized by a sequential appearance of the classical pancreatic cell types (incl. endo and exocrine) through evolution.
- The insulin-producing beta cells together with the somatostatin-producing delta cells are the first to appear in this new location.
- Only with respect to the proinsulin cell, this represents a quantitative translocation or 'migration' to a new organ, thus leaving a scattered intestinal epithelial location for good.
- However, the proglucagon cell compartmentalized itself, through differential prohormone processing (differential mRNA splicing in the fish and chicken) to ensure that glucagon became an islet hormone, while Glp-1 and -2 remained in the scattered intestinal cell type.
- This may reflect the evolutionary importance of establishing a stable 'inner milieu' by sensing blood glucose in addition to gut glucose: the new endocrine gland controls constant blood glucose throughout vertebrate evolution.
- This represents a period of >500 Myr, during which the brain has been given one extra degree of freedom to evolve in complexity – without having to 'think' of energy supply.

It may be hypothesized that the early Pdx-1+/Nkx6.1+ cells of the ontogenic pancreas reflect the phylogenetically first appearing beta cells in this region – and that these progenitors subsequently have adopted a wider differentiation potential to cover additional pancreatic cell types, including the enzyme-producing acinar cells (reflecting their subsequent appearance during phylogeny). It is highly plausible that the triple-positive cell (Pdx-1+/Nkx6.1+/p48+) found in the early pancreatic buds indeed represents a multipotent pancreatic stem cell. It may be further speculated that the early ontogenic Pdx-1+/Nkx6.1+/(p48+) pancreatic epithelial cells may constitute a source of progenitor cells carrying a phylogenetically imprinted pre-programming favouring beta-cell formation (default pathway?).

Organ donor islet transplantation has demonstrated the proof of the concept that the restoration of an adequate beta-cell mass can restore euglycaemia in patients with diabetes [22]. Future cell therapy of diabetes will rely on stem-cell-derived therapeutic beta cells (alternatively, in vivo-controlled beta-cell regeneration). Cell therapy of diabetes is envisioned to prevent the development of diabetes' late complications – and may in some ways even be considered a cure [1].

Embryonic stem (ES) cells constitute so far the only reliable source of stem cells with a proven potential (pluripotency) to become insulin-producing cells (reviewed in [1]). Recent progress in generating definitive endoderm [23] – and subsequent further maturation towards insulin-producing cells from human ES-cell cultures is a highly exciting and promising approach [24]. The strategy builds on replicating in vitro each individual step of fate-choice that the cells of the developing embryo are passing through to become glucose-sensing pancreatic beta cells. The ES cells are thus directed through a series of sequential steps designed by translating knowledge from developmental biology. Reaching the triple-positive (Pdx-1+/Nkx6.1+/p48+) cell stage constitutes a milestone along the developmental path. Achieving this stage may not only guarantee for subsequent potential towards pancreatic maturation, but may in addition provide a suitable developmental stage, which can be subjected to massive expansion [25].

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