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Pancreas Phylogeny and Ontogeny in Relation to a “Pancreatic Stem Cell”

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Résumé

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 & glucagon, are produced by the dominating cell types of the pancreatic Islet of Langerhans: the insulin producing beta-cell and the glucagon producing alpha-cell. 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 ES-cells towards therapeutic beta-cells.

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 cause hyperglycemia and diabetes. Long term elevations of blood glucose leads 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 hyperinsulinemia-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 amounts in order to minimize fluctuations in blood glucose levels. The beta cells has 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 to cause acute and severe hypoglycaemia. Beta-cell specific mechanisms to commit suicide to regulate/eliminate excess

beta-cell mass is likely reflecting part of the price associated with the acquired competence to administer the potentially deadly hormone, insulin (see (1) for review).

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. 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 on we have also the islet PP-cell entering. ((2,3)). Hagfish and lampreys may have one or more endocrine buds – and later the vertebrate pancreas develop as independent ventral and dorsal buds which eventually fuse to become one organ. In the bony fish, a giant islet – known as the ‘Brockman-body’ is derived of dorsal origin, while ventral bud may give rise to acinar cells, ducts and smaller islets (4).

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 encoding the bHLH-transcription factor required for the initiation of endocrine cell maturation program (13) – a process which 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 thus 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, Pdx1 expression from *Ptf1a/p48* cis-regulatory sequences restores pancreas tissue to *Pdx1*-null mice (19). This indicates that p48 and Pdx1 together are required in the specification of the pancreatic progenitor cell.

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) has precocious formation of endocrine (alpha) cells in the pancreatic epithelium due to lack of repression of *Ngn3* (14). However within the Pdx1 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 *pxd1* domain (21).

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-cell together with the somatostatin producing delta cell 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 –and 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 mill yrs where the brain has been given 1 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 reflects 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 cells. 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 proof of concept that the restoration of an adequate beta cell mass can restore euglycemia (22). Future cell therapy of diabetes will rely on stem cell derived therapeutic beta cells (alternatively, controlled in vivo beta-cell regeneration). Cell therapy of diabetes is envisioned to prevent development of diabetes late complications – and may in some ways even be considered a cure (1).

Embryonic stem 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 developing embryo cells are going through to eventually becoming 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 (Pdx1+/Nkx6.1+/p48+) cell-stage constitutes a milestone along the developmental path. It 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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Figure 1.

Phylogenetic relationship of vertebrates is shown in colours to indicate the successive entrance of current pancreatic cell types. Figure adapted from (5).