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Review

Stem cells and the pancreas: from discovery to clinical approach

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Stem cells: present and future
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Abstract

The existence of stem cells within the adult pancreas is supported by the ability of this organ to regenerate its endocrine component in various conditions such as pregnancy and following partial pancreatectomy. Several studies have shown that progenitor or adult stem cells may reside within the pancreas and particularly in the pancreatic ducts, including acinar cells and islets of Langerhans. The discovery of human pluripotent stem cells in the pancreas, and the possibility of development of strategies for generating these, represented a turning point for the therapeutic interventions of type 1 diabetes.

Keywords

Stem cells, pancreas, type 1 diabetes.

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The history of pancreatic stem cells

The existence of stem cells in the adult pancreas is proven by the organ's capacity to regenerate its endocrine component in different conditions, such as after a partial pancreatectomy, in animals chemically made diabetic and in pregnancy. The latter is a physiological condition that requires an increase in insulin production. In a normal pregnancy the maternal endocrine pancreas shows acute and reversible adaptive changes that include: a lower glucose stimulating threshold, an increase in the synthesis and secretion of insulin and an increased rate of beta-cell proliferation. The regenerative capacity of the pancreas in pregnancy has been known for some time; already in 1978 Van Assche et al. [1] demonstrated that, in 5 pancreases from pregnant women who had died in automobile accidents, it was possible to observe an increase in the pancreas endocrine component and a significant increase in beta cells. The study conducted on a sixth woman who had died of a choriocarcinoma showed in this case that the pancreas had not undergone changes.

Subsequently, other studies confirmed the regenerative capacity of the pancreas. In 1998 [2] a study carried out on rats attempted to assess the influence of the state of nutrition on the adaptive capacity of the pancreas. In the rats these adaptations peaked on the 14th day of pregnancy: the pancreas increased its beta-cell mass almost two-fold and remained this way up to the end of gestation. It has been shown that the adaptations are controlled by the placental lactogens I and II; moreover, it was observed that these are strongly conditioned by the mother's nutritional state. Normally, the mass of islets increases regularly up to the end of gestation while in poorly fed animals it remains similar to non-pregnancy values for the entire duration of gestation. Therefore, an early state of poor nutrition has dramatic consequences on the capacity of the pancreas to satisfy the demand for insulin during pregnancy.

Later, several studies revealed that adult progenitor/stem cells may reside inside the pancreas, and more precisely in the pancreatic ducts among the acinar cells and the islets of Langherans. The clonal isolation of presumed adult pancreatic precursors has been an evasive target of many researchers who have tried to develop strategies for cell substitution in diabetes and many resources have been used in trying to identify them.

From this point on, the new techniques of research and cell identification have made it possible to study in a more and more specific and detailed way the characteristics of every cell population.

In 2003, when it was still thought that the beta cells had a limited capacity to replicate, a study by Banerjee and Bhonde [3] attempted to understand if it would be possible to create new pancreatic islets through isolation of intrainsular precursors treated with streptozotocin (natural chemical substance that is particularly toxic for the beta cells). This drug was administered to mice in which the diabetic state was then introduced. Pancreatic islets were then taken from them and cultivated in specific growth media.

Initially, the islets showed a single layer of epithelial cells and later these differentiated into clusters of islet cells. Such cells were then characterized with specific markers such as: cytokeratin-7, cytokeratin-19 and a specific marker of the PDX1 insular cell; this showed the specificity of the islets just generated by the diabetic pancreas.

Seaberg was among the first to suggest the possible existence of a pluripotent stem cell present with low frequency in the murine pancreas. In one of his studies published in 2004 [4], he succeeded in identifying precursors within ductal populations of pancreatic islets by means of an oligoclonal culture starting from the adult mouse pancreas. These cells *in vitro* proliferated to form clonal colonies that co-expressed markers of precursors of neural and pancreatic cells (PDX1, marker of ductal and acinar cells, and nestin, a marker of pancreatic, but also neuronal cells). After differentiation, the single clonal colonies produced distinct populations of neurons, glial cells, beta, alpha and delta pancreatic cells and the pancreatic exocrine and stellate cells. Furthermore, the beta-like cells just generated showed that they were capable of increasing intracellular Ca concentrations and releasing insulin when stimulated with high concentrations of glucose.

Still using a murine model, but with methods of purification with flow cytometry and clonal analysis, Suzuky et al. [5] proposed the c-Met positive cells as stem cells of the pancreas. The data in this study suggest that the interaction between c-Met (receptor kinase tyrosine present on the cell membrane) and its ligand HGF (hepatocytary growth factor) played an important role in the production of stem/progenitor cells both during development and in adult pancreatic cells. This interaction also appeared to be present in cancer-producing processes.

In other words, the positive c-Met clonal colonies generated both ductal and acinar pancreatic endocrine cells, thus representing common progenitors for this cell line defined as pancreatic stem cells (PSCs).

The c-Met cells were able to generate daughter cells that co-expressed specific markers for other organs of endoderm origin such as the stomach, liver and intestine. In an immunohistochemical study on the neonatal pancreas with anti c-Met antibodies, these positive c-Met cells were found at the duct level at < 1% (compared to the total of duct cells), in the acinar tissue at < 0.5% (compared to the total of acinar cells) but also in the vascular endothelium at < 10% (compared to the total of endothelial cells).

All these studies thus led to the hypothesis according to which if there is a PSC it must reside at the ducts level. Subsequently, many other studies have been based on the preparation of pancreatic tissues enriched with duct cells so as to create new pancreatic islets and some have been successful.

Eberhardt's group [6] succeeded in isolating cells from adult pancreatic islets, immortalizing them and demonstrating the co-expression of nestin and Isl-1, factors that indicated the early state of maturation of these cells. In the development of the pancreas, the mesenchymal cells express Isl-1 (a transcription factor essential for the generation of endocrine cells) and nestin. The role of these cells in the adult pancreas is not yet clear, but they may be quiescent pancreatic mesenchymal cells capable of differentiating into pancreatic endocrine cells or hepatocytary cells (if properly stimulated).

The Xiao team [7] isolated a monoclonal line of epithelial cells derived from pancreas islets of an aborted fetus. These cells were positive for PDX1, glucagon, nestin and cytokeratin 19 and negative for CD34, CD45, CD44 and insulin in the immunohistochemical analysis. Moreover, the transplantation of stem monoclonal pancreatic islets into the subcapsular kidney region of rats with diabetes (induced by streptozotocin) can reduce the glucose levels in their blood. The study of the markers expressed by these isolated cells led to the finding that adult mesenchymal stem cells possessed stem properties correlated with pancreatic, epithelial and nervous tissue. These cells behave as actual multipotent cells and possess a strong potential for differentiation.

Another population of stem cell progenitors taken from pancreatic tissue appears to be that of the stellate cells. Many studies carried out on stem cells have shown that some of them were characterized by the expression of the ABCG2 transporter (a protein that belongs to the ABC superfamily; it acts as a xenobiotic transporter, which plays a role in resistance to several drugs); for this reason some research teams concentrated on the study of this transporter. A study by Mato's team [8] attempted to identify an ABCG2-positive population of pancreatic cells and tried to see if these cells exhibited properties typical of progenitor cells.

In the adult pancreas this transporter has been found in the primitive hematopoietic stem cell and is located in the pancreatic islets around the acinar cells. With the use of drugs (such as mitoxantrone) that inhibit growth of deficient cells of this transporter, the existence of a progenitor population contained in the exocrine component of the pancreas has been identified.

It has been demonstrated that the ABCG2+ cells correspond to a population of pancreatic cells known as "stellate cells". Although the origin of these cells is still the subject of debate (since they present characteristics of mesenchymal, neuronal and muscle cells), their main characteristic is that of having a strong capacity for differentiation. The mitoxantrone-resistant cells obtained were made to differentiate in special conditions and showed that they were capable of producing populations able to secrete insulin.

Therapeutic approach

The discovery of human pluripotent stem cells (HPSC) has opened the way to the generation of replacement cells and tissues in the laboratory. These may be used in disease treatment and for the screening of drugs. Recent research has moved the study of stem cells in this direction through the development of strategies for generating cells that would otherwise be difficult to obtain. Some authors, among whom the researchers at Harvard University led by Douglas Melton, aim to implant beta cells responsible for insulin production directly in the organism.

To that end, a study conducted in 2013 [9] compared the transcriptome of HPSC-derived pancreatic cells produced *in vitro* with that of human fetal and adult beta cells. The purpose was to see if there were variations that may have depended on the use of different stem cell lines. These transcriptional analyses were made possible by coloring antibodies (for insulin), followed by cell separation so as to arrive at a relatively pure population of cells that could be prepared. One conclusion drawn from these results is that there is a high degree of similarity between INS+ cells (secreting insulin) derived from the three different lines of pluripotent stem cells.

In the same study the genes expressed by the human PSCs (*in vitro*) were compared to those expressed by the fetal and adult beta cells. For this, a new method called MARIS (Method for Analyzing RNA following Intracellular Sorting) was employed for the RNA analysis. The analysis showed that the maturation of human beta cells, between the 16th gestational month and adulthood, is characterized by changes in expression of 643 genes, of which 39 are transcription factors. The differentially expressed genes found in this study may find a use as genetic markers in defining a mature human beta cell.

The production of pancreatic beta cells producing insulin from stem cells *in vitro* may provide an unprecedented source of cells leading to the development of drugs and the transplantation of pancreas cells in diabetes. However, the cells producing insulin generated previously from HPSC lack many functional characteristics for being considered beta cells. In a more recent study, Melton's group [10] reported a scalable protocol of differentiation capable of generating hundreds of millions of glucose-reactive beta cells produced by HPSC *in vitro*. These cells present markers in the mature beta cells, a flow of Ca^2+ in response to glucose and insulin inside granules of secretion. Furthermore, the amount of secreted insulin is comparable to that secreted by adult beta cells in response to an increase of serum glucose.

Immediately after transplantation, these cells are capable of secreting human insulin in the mouse serum and determine a marked regulation of glucose. The transplantation of these cells also improves the hyperglycemia in the diabetic mice. It was thus demonstrated that a large number of stem beta cells can be directly produced by HPSC *in vitro* and that these behave as true beta cells both *in vitro* and *in vivo*.

An even more recent study by the D'Amour team [11] describes the dynamic remodelling of chromatin, mediated by Polycomb proteins (proteins that preserve cell identity), which orchestrates the pancreatic differentiation of human embryonic stem cells (hESC). Embryonic development is a process characterized by rapid alternation of cell states derived from dynamic variations in the programs of gene expression. Such changes in gene expression are activated by a rapid evolution of environmental stimuli that impact on cell states and alter the chromatin structure and gene transcription. Growing evidence suggests that changes in the specific modifications of the histones, especially trimethylation of the H3 histone to lysine 4 and 27, may play a role in coordinating this highly regulated process.

Such studies have led to a better definition of PSCs and this has contributed to the development of more and more efficient methods for the treatment of diabetes by means of transplantations.

An innovation in the field of transplantations is represented by the transplantation of pancreatic cells generated from stem cells.

In a study conducted by Schulz (the D'Amour team) [12], we find a description of a stepwise process that represents an ideal therapeutic candidate for the treatment of type 1 diabetes based on the use of hESC. These cells were transferred onto gel foam disks (Vetspon®, Novartis Animal Health of the United States, Inc, cat#96001) and later grafts were created.

This process allows acquisition of a standardized cell line and a differentiated stepwise process with the possibility of cryopreserving pancreatic aggregates in a terminal state so that their function is maintained *in vivo*. The insular-like tissue generated was grafted on the epididymis fat pad (EFP) of 240 male mice: of these, 228 (95%) showed evidence of the taking root of the cells. They remained healthy for at least 3 months and exhibited a functional response to glucose stimulation. To better examine the therapeutic potential of the grafted pancreatic progenitors, the endogenous beta cells were eliminated approximately 4 to 5 months after transplantation and complete protection against post-streptozotocin hyperglycemia was observed in each of the 15 animals tested.

This approach represents the first demonstration of a practical system for the production of a treatment of type 1 diabetes based on hESC that may mitigate many of the obstacles in clinical experimentation.

Therefore, pancreatic grafts capable of perceiving glycemia values and producing insulin have been generated. The demonstration of the efficacy and safety of these grafts requires clinical studies now being performed. In any case, this work has led to the creation of a stepwise process that goes from a single expanded stem cell to the production of a glucose-sensitive cell population that produces insulin. The generated material can be grafted through specific devices with the reproducibility and compatibility necessary for industrial production. Moreover, the development of an all-compatible neo-pancreas derived from the transplantation of pancreatic progenitors, with strategies for lasting vascularization and immunoisolation or microencapsulation, is foreseen. Indeed, the most important problems concerning experimentation on humans are first of all connected with the conversion of stem cells into true functioning cells of the pancreas, but also with how to avoid the immune system which attacks all transplanted cells. At present, clinical trials conducted by the D'Amour team are under way. These researchers have devised a solution to elude the immune system. This consists of a capsule in a polyethylene net that is filled with approximately 40 million immature cells of the pancreas. The purpose of the capsules is to block the T cells of the immune system, which are too large to pass through the thick net and at the same time to allow the transplanted cells to receive nourishment from the blood flow, as well as sensing and regulating the blood sugar level.

Declaration of interest

The Authors declare that there is no conflict of interest.

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