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RESEARCH HIGHLIGHT

hESC-derived pancreatic progenitors

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Successful derivation of pancreatic progenitors from human embryonic stem cells (hESCs) *in vitro* and further differentiation towards functional β cells *in vivo* may create the possibility of using hESC-derived pancreatic progenitors (PPs), instead of derived β cells, as an alternative transplantable source in β cell replacement therapy. Here we discuss present approaches, as well as future alternatives, in the fields of basic and clinic research on β cell differentiation, derivation and transplantation.

Type 1 diabetes is a chronic metabolic disease characterized by the loss of β cells in the pancreas. Patients can benefit from transplantation of islet cells harvested from donor pancreas to restore a functional β cell mass. Since the number of pancreas donors is far too low and cannot satisfy the requirements of most diabetes patients, this has stimulated research worldwide to find alternative and sustainable sources of β cells. One of the most promising sources is hESCs, which can proliferate for long periods in culture and generate progenies of the three mammalian germ layers.

Many efforts have been made to derive various differentiated cell types from hESCs, including pancreatic endocrine cells that are of endoderm origin. Following the successful generation of definitive endoderm (DE) from hESCs by treatment with Activin A, implementation of the knowledge obtained from *in vivo* embryonic pancreas development into differentiation procedures *in vitro*, resulted in the efficient production of pancreatic cells by several laboratories. Although this success represents a breakthrough in terms of generating PPs from hESCs, the proportion of insulin-expressing cells that can be obtained *in vitro* is still low. This is may be due to the lack of knowledge about the specific signals required for the final pancreatic differentiation stage and to the fact that these cells are not comparable to genuine mature β cells since they are polyhormonal and only mildly or even non-responsive to glucose challenge [1]. To circumvent this bottleneck, hESC-derived PPs were grafted in immunodeficient mice and their behaviors were followed for several weeks. The *in vivo* environment not only improved the differentiation of endocrine progenitors and insulin-expressing cells, but also promoted their further maturation into glucose-responsive β-like cells [2, 3]. All these results have hinted the importance of hESC-derived PPs as an alternative source for diabetes cell therapy. Thus, developing technologies towards increasing the number of PPs have become a priority. Expansion of the PPs, in turn, might also be helpful for further elucidating the mechanism underlying endocrine differentiation, as well as allowing for chemical screenings of new growth factors and small molecules to streamline the process.

Strategies to obtain a large number of progenitor cells include the scalable culture and differentiation process, as well as the expansion of stage-specific differentiated progenitors (DE, PPs) (Table 1) [4-7]. Direct proliferation of PPs after pancreas commitment from hESCs would be perhaps the most economic and efficient way. It can reduce the costs generated by driving the amplified uncommitted cells towards pancreatic lineage with a large amount of inducing factors. It can increase the purity of the PPs by avoiding generation of other unexpected cell types during differentiation from DE. Recently, Melton and colleagues [7] have studied the proliferation features of ESC-derived DE and PPs by co-culturing them with distinctive mesenchymal cell lines derived from human adult pancreas, mouse embryonic and adult pancreas and other adjacent organs. Besides the establishment of two types of mesenchymal cell lines, which were particularly responsible for the proliferation of DE, they also showed that the number of NGN3+ pancreatic endocrine progenitors was upregulated by co-culturing with human pancreas- or E13.5 mouse pancreas-derived mesenchymal cell lines. Transplantation of these expanded DE and their derived PPs induced β cell derivation as efficiently as transplantation of unpassaged cells.

Given that a sufficient number of PPs can be obtained, some limitations have to be overcome before it becomes clinically applicable. For instance, are the expanded PPs able to differentiate *in vivo* as efficiently as shown for DE? It is known that maintenance of FGF10 expression in the embryonic mouse pancreas, a growth factor reported to promote the proliferation of PPs before terminal cell commitment, leads to a permanent loss of NGN3 expression and endocrine cells [8]. These data implies that continuous amplification of PPs *in vitro* might cause loss of endocrine competence after transplantation.

Other issues include potential teratoma formation after transplantation, as well as immune system rejection.

Table 1 Overview of different studies related to the expansion of stage-specific progenitors during pancreas differentiation from hESCs

Study	Cell types for expansion	Methods	Results
Schulz *et al*. 2012 [6]	hESCs	High density, single cell bank of hESCs were expanded in cell factory and reaggregated in suspension culture for pancreas differentiation.	A practical scale-up system was developed and a clinical grade cell preparation was provided.
Cheng *et al*. 2012 [4]	Definitive endoderm	Cells were expanded on matrigel and MEF feeder in serum-free medium containing BMP4, bFGF, EGF, and VEGF.	Definitive endoderm cell line with self-renewal was established.
Sneddon *et al*. 2012 [7]	Definitive endoderm	Co-culture with pancreatic mesenchymal cells from adult mouse islet fraction or E18.5 mouse pancreas.	Definitive endoderm cells were passaged several times with full retention of their developmental potential towards pancreas.
	Pancreatic progenitor	Co-culture with pancreatic mesenchymal cells from adult human islet fraction or E13.5 mouse pancreas.	Pancreatic proendocrine NGN3+ cells were passaged several times *in vitro*.
Jiang *et al*. 2007 [5]	Pancreatic progenitor	EGF supplementation.	Proliferation for 1 week.

Since not every single hESC is induced towards the desired lineage during differentiation, it is virtually impossible to obtain 100% of hESCs converted into endoderm and subsequently PPs in culture. Therefore, a teratoma could develop even from a small subpopulation of undifferentiated cells present in hESC-derived PP preparations [2, 3]. This problem may be resolved by purification of PPs from a mixture of differentiated cell types, which entails the discovery of surface markers that are specifically expressed by these cells. Currently, CD24 and CD142 are proposed as surface markers for the identification of PPs, but further validation is still needed to prove their specificity [9].

Another issue to deal with is the rejection of foreign grafted cells by the host immune system. In the clinical practice, a combination of immunosuppressive drugs is administered to patients who receive donor-isolated islet transplantation in order to prevent graft rejection. However, these drugs result in metabolic or organ-specific side effects, some of which may be life threatening. Recently, encapsulation of islet cells within an alginate matrix was developed, and the encapsulated islets could survive and function normally after transplantation. The alginate capsules have pores that allow for passage of nutrients but not immune cells. An alternative approach to overcome the immune rejection caveat would entail the use of induced pluripotent stem cells (iPSCs). They present several features comparable with embryonic stem cells. By using PPs differentiated from an iPSC line generated from the patients' own somatic cells, there would be no need for life-long immunosuppressive therapy. However, it is noteworthy that notwithstanding the advantages of using iPSCs, their safety is still a matter of concern. iPSCs are mainly derived by integration of reprograming factors into the genome using virus-based delivery methods. The viral origin of the vectors and the random integration of ectopic genes into the genome constitute a major safety issue. Some recent studies aim at avoiding genomic modifications of the reprogrammed cells by using protein transduction of reprogramming factors, by injecting their messenger RNAs or by using episomal vector systems. Although iPSCs are similar to hESCs in terms of morphology, self-renewal and pluripotency, the existence of subtle differences cannot be ruled out. Indeed, recent studies have demonstrated that iPSCs retain an epigenetic memory of their cell type of origin in terms of DNA methylation and histone modifications [10]. This epigenetic memory might influence the differentiation of a given cell line. On the plus side, these iPSCs are easier to differentiate into the cell type of its origin with high efficiency. On the other hand, they might not be efficiently induced into other cell types without attenuating these epigenetic differences. As already mentioned for hESCs, PPs and insulin-expressing cells have also been successfully derived from human iPSCs, but the efficiency of terminal differentiation is still low.

In summary, the studies performed up to date on the expansion of hESC-derived PPs provide us with new tools and perspectives on cell replacement therapy in diabetes. Successful derivation and amplification of PPs will contribute to identify the specific, but yet unknown, signals for endocrine differentiation and maturation. Together with the resolution of the above-mentioned problems, it is believed that procurement of hESC-derived PPs will help

progress for future cell therapy applications in diabetes.

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