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# Neural stem cells for diabetes cell-based therapy

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Diabetes mellitus is a disorder resulting from lack of either production or action of insulin signalling. It affects more than 200 million people worldwide. There is no cure for diabetes and the patients rely on exogenous supply of insulin and/or oral use of hypoglycemic substances. Type I diabetes results from the destruction of pancreatic insulin-producing beta cells in the islets of Langerhans, usually through an autoimmune reaction, while type II diabetes is associated with dysfunctional beta cells. Thus, diabetes presents an ideal model for cell therapy, since it is the lack of a single defined cell type that is largely responsible for the disease pathology. Transplantation of isolated islets or entire pancreases from cadavers to replenish the lost beta cell pool has yielded some promising results. However, limited availability of the islets, side effects of continuous immunosuppressant administration and the logistics of finding suitable donors have proven major setbacks. In the absence of an *ex vivo* culture system to expand pancreatic progenitors, alternative sources of beta cells have attracted great attention (Mishra et al, 2010).

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> *Isolation and cultivation of pancreas stem cells as renewable sources of beta cells would be a major breakthrough...*

The discovery of stem cells which have virtually unlimited self-renewal and multi-differentiation potential raised great expectations for their use in regenerative medicine. Isolation and cultivation of pancreas stem cells as renewable sources of beta cells would be a major breakthrough although their presence in the adult human pancreas remains controversial. Recent studies have highlighted the possible use of embryonic stem (ES) cells and induced pluripotent stem (iPS) cells as the source. ES cells are derived from the early developing embryo and can generate all differentiated cell types of the adult organism, including beta cells. Major ethical and logistical concerns regarding their use in the clinic have rapidly pushed iPS technology to the forefront. iPS cells can now be derived from multiple differentiated adult cells. As an alternative, a number of studies have demonstrated the use of adult hematopoietic and mesenchymal stem cells in generation of insulin-expressing 'surrogate' beta cells which improved the diabetic conditions in some clinical trials (Mishra et al, 2010). An exciting study has demonstrated that beta cells can be derived not only from stem cells but also from glucagon-expressing alpha cells of the islets (Zhou et al, 2008). Even though these sources are promising, fundamental improvements are required for their clinical use. The main drawbacks include the need of high cell numbers due to either absence of established tissue culture or efficient differentiation into insulin expressing cells, the necessity of immune suppression to prevent graft rejection and the fact that transdifferentiation into beta-like cells is mainly achieved by use of viruses and genetic manipulation.

In this issue of EMBO Molecular Medicine, Kuwabara et al (Kuwabara et al, 2011) provide evidence that neural stem cells (NSCs) isolated from diabetic patients can be used to generate surrogate beta-cells. Mammalian neurogenesis continues into adulthood in specialized regions which form a ''niche'' for the maintenance of NSCs and their neuronal differentiation (Ming & Song, 2011). The authors demonstrate the expression of insulin in the neurons of the hippocampus and the olfactory bulb, stem cells of which can generate insulin-expressing cells *in vitro*. These results are in accordance with the fact that several transcription factors and signalling pathways involved in development of the pancreas are also active during the formation of the brain regions with insulin-expressing neurons.

The identification and isolation of NSCs from the adult human brain has been seen as an exciting discovery raising hopes for their use in treatment of neurodegenerative diseases. Of note, NSCs can be isolated from the adult mouse and human olfactory bulb, a readily accessible tissue source (Pagano et al, 2000). Kuwabara et al employ these cells in a rather unexpected approach, *i.e.* by transplanting them directly into the pancreas of diabetic rats. They observe that niche signals in the pancreas can at least partially reprogram the transplanted cells into the pancreatic lineage by inducing pancreas specific transcription factor 1a (Ptf1a) and v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA). Using genetic means, the authors demonstrate that this process depends on Wnt signalling and its effector beta-catenin. The study thus reveals the ability of neural progenitors to respond to the niche signals in the pancreas and unleash their intrinsic ability to express critical regulators of pancreatic insulin production.

Wnt signalling plays multiple roles during development of the mammalian brain and the pancreas. It is also of central importance for the maintenance of multiple types of adult stem cells. In the adult hippocampus, wingless-type MMTV integration site family, member 3 (Wnt3) is expressed by the radial glia-like astrocytes, which are integral components of the neurogenic niche. Interestingly, both the hippocampal astrocytes and islet alpha cells can be identified by the expression of the glial fibrillary acidic protein (GFAP). Moreover, Wnt3 is reduced in both the adult brain and pancreas in rat models of diabetes. The authors conclude that Wnt signalling provided by the adult pancreatic and neural niche cells represents the essential regulator of insulin-expression during this process. Wnt signalling regulates adult hippocampal neurogenesis via induction of NeuroD1 expression which activates important neuronal differentiation and maturation genes in granule neurons (Kuwabara et al, 2009; Lie et al, 2005). NeuroD1 has been identified as an essential regulator of the formation of hippocampus downstream of Wnt signalling pathway evidenced by a loss of hippocampal formation in NeuroD1 mutants. NeuroD1 is also part of the machinery that regulates insulin production in pancreatic beta cells. In cultured NSCs, Wnt3a stimulation induced NeuroD1 expression which — in turn — directly regulates the insulin-1 gene by binding to its promoter. Moreover, neural progenitors cultured from diabetic animals continue to express insulin, which can be dramatically up-regulated by the Wnt pathway. These findings provide a molecular mechanism for Wnt mediated activation of insulin production in neural progenitors.

Finally, the authors show that NSCs derived from diabetic rats can generate functional insulin-expressing neurons using a transplantation approach. In order to demonstrate their use in the treatment of diabetes, the authors transplant neurons isolated NSCs from STZ-induced type I diabetic or type II diabetic Goto-Kakizaki rats into the pancreas of the respective diabetic rat models. Grafted cells survive for a long period, continue to produce insulin even 10 weeks following their transplantation and increase pancreatic and plasma insulin levels accompanied by a dramatic reduction in blood glucose levels. Most importantly, removal of the transplants inverts blood glucose and insulin levels, demonstrating the long-term functionality of the transplanted cells.

A major drawback of insulin administration used by diabetic patients is the lack of minute-by-minute regulation of blood glucose levels such as can be provided by intact pancreatic beta cells. In their study, Kuwabara et al demonstrate that neural cells respond to high glucose levels by increasing insulin production *in vitro*. Indeed, transplanted animals display enhanced ability to clear increased blood glucose levels to that of the controls suggesting that glucose sensing is at least partially active in the transplants. Finally, the survival rate of diabetic rats following transplantation are comparable to the controls suggesting that excess insulin from grafted neural cells do not induce lethal hypoglycemia. It will be essential to determine the extent to which the transplanted cells can regulate insulin release and production for their employment as therapeutic agents.

The most important improvement offered by this study is the derivation of insulin-expressing cells from diabetes patients without the need for the use of genetic manipulation. Such an approach promises the generation of autologous insulin-expressing cells without concerns about their safety or the presence of pluripotent (and potentially tumorigenic) cells, and bypassing chronic dependence on immunosuppression. It will be essential to validate these results in available human NSC lines as well as patient-derived olfactory bulb NSCs. One major concern about the use of patient-derived NSCs for diabetic therapy is the lack of efficient culture methods to rapidly expand human NSCs in culture to obtain adequate numbers required for transplantation. Further refinement of culture conditions to efficiently differentiate NSCs into insulin expressing neuronal cells will further decrease the cell numbers for transplantation.

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Another aspect of this elegant study is the emphasis of a conserved Wnt signalling network among developmentally distinct tissues. The results provided by Kuwabara et al hint to the presence of a pancreatic niche capable of supporting Wnt-responsive stem cells. In the last decades, we and others have demonstrated a pivotal role for Wnt signalling in adult stem cell niches which led to establishment of human and mouse organ cultures that resemble functional organs. As an alternative to NSCs, the Wnt-dependent stem cell cultures might serve as potential sources for transdifferentiation into the pancreatic lineage. Understanding how niche signals in the pancreas influence induction of a pancreatic endocrine program might also serve to design protocols for the generation of 'surrogate' beta cells from other tissue stem cells.

  

The authors declare that they have no conflict of interest.

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