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RESEARCH HIGHLIGHT

Pancreatic differentiation from pluripotent stem cells: Tweaking the system

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Autoimmune destruction of insulin producing pancreatic β cells results in type 1 diabetes, a condition for which there is presently no cure. Clinical trials indicate that, in some instances, control of blood glucose can be restored by transplantation of cadaveric derived islets [1], raising hopes that such cell-based therapies may eventually form part of a curative treatment. However, even if the outcome of islet transplantation can be significantly improved, the availability of this treatment option will always be limited by the dearth of cadaveric islet donors. It is in this context that the derivation of β cells from human embryonic stem cells (hESCs) represents an important step toward the creation of an inexhaustible source of therapeutic replacement cells for the treatment of type 1 diabetes. Notwithstanding this promise, before *in vitro* derived β cells can be used clinically, a number of conceptual and actual impediments will need to be surmounted. These relate to the cost and efficiency of β cell generation from hESC differentiation cultures and the perennial hoary chestnut of immunity, tolerance and graft rejection.

Ideas underpinning current protocols for promoting differentiation of hESCs toward β cells have their origins in prior embryological studies, drawing on findings from work with model systems such as frogs, flies, zebrafish and mice. Using lessons learnt from these systems, researchers guide hESCs through defined differentiation pathways, following molecular signposts to ensure that cells traverse the necessary milestones of pancreatic ontogeny. These milestones correspond to recognisable developmental stages within the embryo, with initial steps taking the hESCs from the pluripotent inner cell mass-like stage through the process of mesendoderm formation. As in the embryo, this differentiation step *in vitro* is driven by factors belonging to the TGFβ and wnt families [2, 3]. These factors are proteins containing extensive disulphide bridges, making them difficult and expensive to manufacture on a large scale. Therefore, augmentation or replacement of such components with cheap small molecular weight compounds will go some way to reducing the cost of differentiation protocols. Furthermore, such compounds are likely to provide a higher degree of reproducibility than recombinant growth factors purified from either bacteria or tissue culture cells.

In this issue, the recent work of Zhang *et al*. [4] represents another step in this direction. In their differentiation protocol, mesendoderm formation is initiated by the combination of the TGFβ family ligand Activin A and the small molecular weight PI-3 kinase inhibitor, Wortmannin. Previous studies by McLean *et al*. [5] indicated that inhibition of PI-3 kinase activity in the presence of Activin signalling promoted the generation of endoderm during this first critical step of hESC differentiation. In this regard, the inclusion of Wortmannin into a complete differentiation protocol represents a small but important incremental step in the optimisation of hESC to β cell methodologies.

Following endoderm formation, most protocols include a step in which cultures are treated with retinoic acid (RA), another small molecular weight compound shown from studies in zebrafish to promote the emergence of *Pdx1*-expressing pancreatic endoderm [6]. In the new method described by Zhang and colleagues, this step is followed by a 5-day period in which cells are treated with epidermal growth factor (EGF). The primary purpose of EGF treatment is to expand the pancreatic endoderm prior to a final differentiation stage. Like RA, a role for EGF family molecules in pancreatic development has also been suggested by prior studies [7, 8]. In particular, work by Yamamoto suggested that the EGF-like molecule β-cellulin promoted islet neogenesis following alloxan injury.

As with their prior protocols, Zhang *et al*. perform a final differentiation step that includes the small molecular weight compound, Nicotinamide and the peptide Exendin-4. Like all other components, the inclusion of these molecules at this point is informed by

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earlier studies of human fetal pancreas cultures and rodent models [9, 10]. Using their optimised method, Zhang and co-workers report that by differentiation day 20, up to 25% of cells express insulin, representing a significant increase in efficiency over their previous method. As a final test of the effectiveness of the overall differentiation strategy, Zhang and colleagues demonstrate that under the same conditions, induced pluripotent stem cells (iPSCs) can also be guided down the pancreatic differentiation pathway with similar efficiencies as those achieved with hESCs.

While this report is promising, the challenges facing hESCs as a viable source of β cells still remain daunting. Human islet transplantation protocols typically require around one million islets per transplant, which represents approximately two billion β cells [11]. Even with the improvements afforded by the Zhang study, the issue of efficiency and functional maturity are important considerations that will need to be overcome before hESC-derived β cells can be viewed as a viable therapeutic option.

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