Simply the right time to turn on insulin

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Have You Seen?

Title:
Simply the right time to turn on insulin

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Summary:
Recent research has made important progress in the directed differentiation of human pluripotent stem cells into insulin-producing beta-like cells *in vitro*. A new study published in this issue of The EMBO Journal reports that timely induction of NEUROG3 expression in pancreatic progenitors is a crucial checkpoint for generation of functional human beta cells.

Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia, which over time leads to long–term damage, dysfunction and failure of various organs (American Diabetes Association, 2013). Finding ways to restore the pancreatic beta cell population in patients with type 1 diabetes, or replace dysfunctional beta cells in patients with type 2 disease, remains a “holy grail” for diabetes research. Transplantation of cadaveric islets has provided the proof-of-concept that cell replacement therapy, combined with immunotherapy, represents a definitive and valuable treatment for diabetic patients, particularly for those suffering from type 1 diabetes (Ryan et al, 2005). The approach has shown to restore endogenous beta cell function and renders patients insulin-independent for a certain number of months (Ryan et al, 2005). However, the widespread application of islet cell transplantation is still challenged by the limited supply and quality of donor islets, inadequate engraftment, and deleterious effects of chronic immunosuppression. So ways to eliminate these constrains and generate vast numbers of functional, glucose-responsive insulin-producing beta cells are under intense investigation in many laboratories around the world.
Human pluripotent embryonic stem (ES) or induced pluripotent stem (iPS) not only provide tools to advance our understanding of human pancreatic development, but also offer a potential endless supply of insulin-producing cells. In the past years, great advances have been made in defining multistep protocols based on developmental paradigms to differentiate hESCs into pancreatic progenitors capable to maturate further *in vivo* (D'Amour et al, 2006 ; Rezania et al, 2012; Rezania et al, 2013; Schulz et al, 2012). Specifically, the hESC-derived pancreatic progenitors were able to differentiate *in vitro* into a heterogeneous population, including polyhormonal (insulin+, glucagon+, somatostatin+) cells, which ultimately gave rise to some glucose-responsive, insulin-secreting beta cells capable of reversing diabetes months after transplantation into mice (D'Amour et al, 2006 ; Rezania et al, 2012; Rezania et al, 2013; Schulz et al, 2012). However, full maturation of *in vitro* into *bona fide* transplantable beta cells has remained an elusive goal that

until recently has been difficult to achieve. Indeed, two recent studies focused on optimizing the maturation steps *in vitro* by systematic screen of numerous soluble factors (small molecules, cytokines and growth factors) on pancreatic progenitors (Pagliuca et al, 2014; Rezania et al, 2014). Both efforts have been successful and resulted in generating hESC-derived beta-like cells that secrete insulin in response to glucose challenge *in vitro* before being transplanted in mice (Pagliuca et al, 2014; Rezania et al, 2014). Monohormonal populations expressing beta cell transcription factors (*e.g.* NKX6.1$^+$/INSULIN$^+$ and MAFA$^+$/INSULIN$^+$ cells (Rezania et al, 2014) or NKX6.1$^+$/C-PEPTIDE$^+$ cells (Pagliuca et al, 2014) and with certain functional similarities to human beta cells were generated with an overall efficiency above 30%. Nevertheless, undesirable polyhormonal cells were still present, even though the fraction was reduced (≤15%) (Pagliuca et al, 2014; Rezania et al, 2014).

Polyhormonal cells are unwanted, as they are immature cells, expressing multiple endocrine hormones, non-glucose responsive and negative for key beta cell transcription factors, including NKX6.1, which is used to monitor the emergence of mature beta cells (Nostro et al, 2015; Rezania et al, 2013). In this issue of *The EMBO Journal*, Russ *et al.* identified the problem and provided a solution to it, demonstrating that undesirable nonfunctional polyhormonal cells result from precocious induction of the endocrine program in PDX1$^+$ pancreatic progenitors, which have not yet turned on NKX6.1 expression (Russ et al, 2015). By conducting a systematic analysis of individual components present in established hESC differentiation protocols (Rezania et al, 2012; Schulz et al, 2012), the authors found that exposure of endodermal gut tube (GT) cultures exclusively to Retinoic Acid followed by EGF and KGF treatment works the best, resulting in efficient generation of PDX1+/NKX6.1+ pancreatic progenitor (PP) cells (90%) (Russ et al, 2015) (Fig. 1). By contrast, the addition of the BMP inhibitor Noggin at these early steps, as it is routinely used in most of the current protocols, leads to premature expression of the endocrine transcription factor NEUROG3, which favors the generation of polyhormonal cells in culture (Russ et al, 2015). Yet, the inhibition of BMP signaling (by Noggin or small molecule inhibitor LDN-193189) becomes relevant afterwards, helping to push PDX1$^+$/NKX6.1$^+$ progenitors to differentiate into *bona fide* NEUROG3$^+$ endocrine progenitors (EN) (Fig. 1). Russ et al. also demonstrated that the EN cells can make it further: following an additional short time in simplified culture conditions without growth factors, they gave rise predominantly to monohormonal insulin-producing cells (> 20%) that show key features of a bona fide beta cell and to a much smaller fraction (3%) of polyhormonal cells when compared to previous reports.

Overall, these findings highlight the importance of precisely recapitulating the temporal induction of early pancreatic progenitor fate (PDX1 > NKX6.1 > NEUROG3) in order to generate fully differentiated beta cells *in vitro*. Hence, Russ et al. identifies a first major bottleneck along the stepwise progression of hESCs toward pancreatic beta cell fate in the early specification stage – prior to the maturation step – (Russ et al, 2015). Similarly to the mouse embryo, in which the competence of pancreatic progenitors to generate distinct endocrine cell types changes throughout development (Johansson et al, 2007), also in hES-derived endoderm the timely induction of NEUROG3 at a

precise time point is required for beta cell specification. Finally, precise manipulation of the appropriate signaling pathways at key developmental stages appears to be the “recipe for success” to overcome this bottleneck *in vitro*. In particular, this study underscores the existence of versatile functions of the BMP signaling during directed differentiation of hESCs into pancreatic fate, which is in close agreement with *in vivo* observations in vertebrate embryos (Chung et al, 2010; Wandzioch & Zaret, 2009) but at odd with most of the *in vitro* protocols currently used (D'Amour et al, 2006 ; Nostro et al, 2015; Pagliuca et al, 2014; Rezania et al, 2014; Rezania et al, 2012; Rezania et al, 2013). Further clarification on the distinct temporal activities of BMP signaling, how BMP influences NGN3 expression at the molecular level and how to achieve timely but also uniform NEUROG3 activation may enhance the efficiency of monohormonal mature beta cells in directed differentiation of hESCs. The simplified differentiation protocol of Russ *et al.* provides an *in vitro* platform highly amenable for studying these questions in human beta cell specification and maturation.

Now that significant progresses have been made towards making hESC-derived cells with beta cell traits, a comprehensive and comparative analysis of 1) the various strategies and 2) of the phenotype of the different beta cell surrogates with human beta cell counterparts found in the body would help to unify the efforts and create a framework for boosting future cell therapy treatment for diabetes.

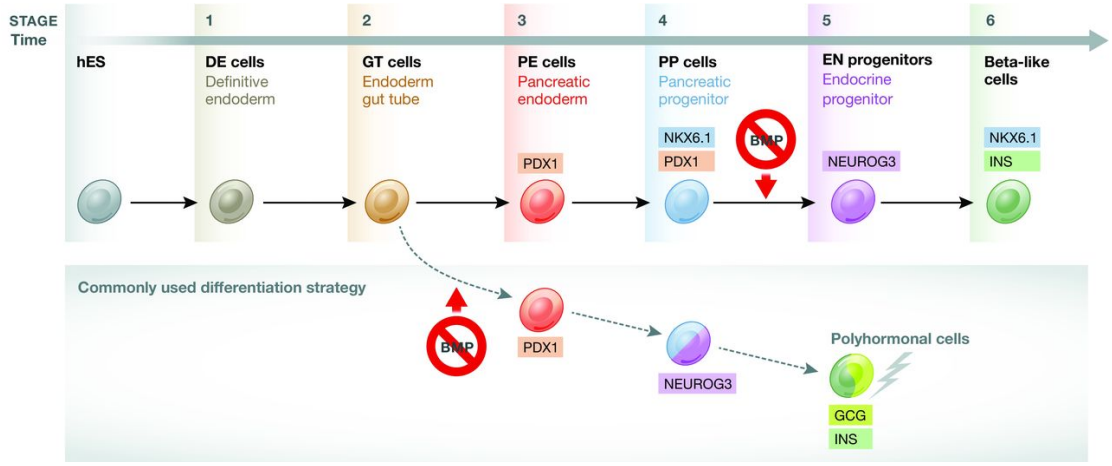

Figure 1. Directed differentiation of hES cells into mature beta-like cells. Simplified diagram describing the new strategy reported by Russ *et al* (Russ et al, 2015) for *in vitro* differentiation of hESCs into pancreatic beta-like cells. Addition of BMP inhibitors at pancreatic progenitor stage (PP) ensures precise temporal activation of NEUROG3 and favors generation of monohormonal insulin-secreting cells *versus* polyhormonal cells. Commonly used differentiation strategy is boxed in light grey, wherein earlier inhibition of BMP signaling leads to premature endocrine commitment and higher number of polyhormonal cells.

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