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Review

Engineering pancreatic tissues from stem cells towards therapy

Yoshinobu Takahashi [a], Takanori Takebe [a, b, c, d, *], Hideki Taniguchi [a, b, **]

[a] Department of Regenerative Medicine, Yokohama City University Graduate School of Medicine, Kanazawa-ku 3-9, Yokohama, Kanagawa, 236-0004, Japan
[b] Advanced Medical Research Center, Yokohama City University, Kanazawa-ku 3-9, Yokohama, Kanagawa, 236-0004, Japan
[c] PRESTO, Japan Science and Technology Agency, 4-1-8, Honcho, Kawaguchi, Saitama, 332-0012, Japan
[d] Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati, 3333 Burnet Avenue, Cincinnati, OH, 45229- 3039, USA

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ABSTRACT

Pancreatic islet transplantation is performed as a potential treatment for type 1 diabetes mellitus. However, this approach is significantly limited due to the critical shortage of islet sources. Recently, a number of publications have developed protocols for directed β-cell differentiation of pluripotent cells, such as embryonic stem (ES) or induced pluripotent stem (iPS) cells. Decades of studies have led to the development of modified protocols that recapitulate molecular developmental cues by combining various growth factors and small molecules with improved efficiency. However, the later step of pancreatic differentiation into functional β-cells has yet to be satisfactory *in vitro*, highlighting alternative approach by recapitulating spatiotemporal multicellular interaction in three-dimensional (3D) culture. Here, we summarize recent progress in the directed differentiation into pancreatic β-cells with a focus on both two-dimensional (2D) and 3D differentiation settings. We also discuss the potential transplantation strategies in combination with current bioengineering approaches towards diabetes therapy.

Contents

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; BMP, bone morphogenic protein; ES, embryonic stem; FGF, fibroblast growth factors; IBMIR, instant blood-mediated reaction; ILV, indolactam V; Ngn3, neurogenin 3; PEG, polyethylene glycol; PI3K, phosphatidylinositol-3 kinase; PIPAAm, poly-N-isopropylacrylamide; PVA, polyvinyl alcohol; Pdx1, pancreatic and duodenal homeobox 1; Ptf1a, pancreatic transcription factor 1a; VEGF, vascular endothelial growth factor; iPS, induced pluripotent stem.

* Corresponding author. Department of Regenerative Medicine, Yokohama City University Graduate School of Medicine, Kanazawa-ku 3-9, Yokohama, Kanagawa 236-0004, Japan.
** Corresponding author. Department of Regenerative Medicine, Yokohama City University Graduate School of Medicine, Kanazawa-ku 3-9, Yokohama, Kanagawa 236-0004, Japan.
E-mail addresses: ttakebe@yokohama-cu.ac.jp (T. Takebe), rtanigu@yokohama-cu.ac.jp (H. Taniguchi).
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1. Introduction

Pancreatic β-cells are responsible for producing the only hypoglycemic hormone, namely insulin. Whole pancreas or pancreatic islet transplantation is a radical treatment for severe diabetic patients, mostly due to the lack of pancreatic β-cells. However, the number of donors has never been enough, and a possible solution to this problem is regenerative medicine.

Generally, it is difficult to expand adult derived pancreatic β-cells *in vitro*. To provide alternative cells for transplantation, many attempts have been made worldwide to develop a method for inducing the self-renewing multipotent stem cells into functional pancreatic β-cells. Among the many types of stem cells, two types of pluripotent cells are of particular interest in the development of such methods, the ES cells, which are derived from embryos that developed from fertilized eggs, and the iPS cells, which are derived from the reprogramming of human skin cells and other cells. Strategies for directed differentiation into β-cells mostly rely on aspects of physiological development of endocrine pancreas.

This article outlines pancreatic development and differentiation and explains methods for the differentiation of ES and iPS cells into pancreatic β-cells. Furthermore, future tasks are discussed based on the findings of our experiments and the role of bioengineering technologies in regenerative medicine.

2. Pancreatic development (Fig. 1)

The pancreas is an organ of endodermal origin that functions as an exocrine gland secreting digestive enzymes into the duodenum and also as an endocrine gland secreting blood sugar-regulating hormones into the blood. Pancreas initially develops by budding from the embryonic endoderm at the junction of foregut and midgut. Pancreas development is very elaborate and involves specific time- and space-dependent activation of transcription factors and signaling molecules [1–4]. Pancreatic development in mice starts at embryonic day 8.0 upon induction of pancreatic progenitor cells from pancreatic and duodenal homeobox 1 (Pdx-1) -positive cells in the foregut endoderm [5–10] (Fig. 1a). Then, humoral factors such as activin and fibroblast growth factors (FGF) are secreted from the notochord, upon which pancreatic progenitor cells proliferate to form both the dorsal and ventral buds (Fig. 1b) [11–15]. Activin, FGF as well as retinoic acid [16,17] signaling was demonstrated to inhibit sonic hedgehog homolog, thereby, providing a critical signaling cue for the initiation of pancreatic fate specification [12,18,19]. Later on this pre-patterned pancreatic endoderm develops and differentiates into insulin producing cells (Fig. 1c). Lammert and colleagues [20–22] showed that the removal of aortic endothelial cells impaired pancreatic differentiation in Xenopus embryos as well as enhanced differentiation by introducing ectopic vascularization in mice, suggesting important role of endothelium for endocrine pancreas differentiation. Later study using mouse suggested that endothelial cell instruction can be waived in the initiation of Pdx1 expression in endoderm, however, they are essential for emergence of dorsal pancreatic buds and maintenance of Pdx1 expression through the crucial pancreatic transcription factor 1a (Ptf1a) induction [23]. The components of buds are pancreatic progenitor cells that differentiated from endodermal epithelial cells. Even after bud formation, these cells, surrounded by a mesenchyme, continue dividing so that buds can further grow symmetrically. Understandings of epithelial–mesenchymal interactions between pancreatic progenitor cells and pancreatic mesenchymal cells during the above process are extremely important in recapitulating pancreatic development in culture. At embryonic day 14.5, the ventral bud moves to lie on the dorsal side of the dorsal bud upon rotation of the stomach and duodenum, and the two buds eventually fuse (Fig. 1d). This is followed by pancreatic duct formation, and the ductal network formation starts upon branching. In parallel to the pancreatic duct formation, the duct cells give rise to pancreatic α and β-cells, and mature islets with a core-mantle structure are formed immediately prior to birth (Fig. 1e). The induced β-cells produce vascular endothelial growth factor (VEGF), then attract blood vessels, and potentiate insulin expression by recruiting a denser vasculatures compared with surrounding exocrine components [20]. After birth, mesenchymal cells differentiate into perivascular cells around the vascular networks of the islets, thereby contributing to the long-term stability of the vascular networks. In addition, vascular endothelial cells are in a close relationship with the islet endocrine cells throughout the

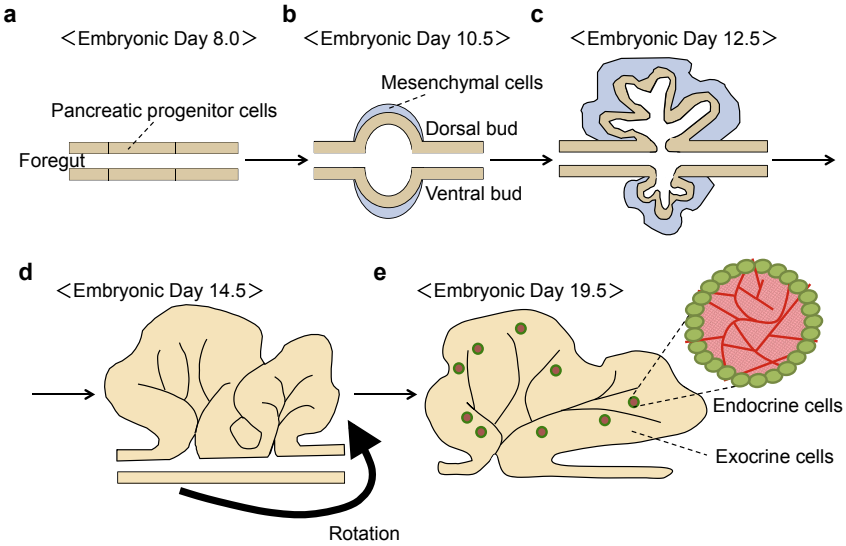

Fig. 1. Mouse pancreas development and endocrine cell differentiation.