

Highlight

A glimpse of endocrine pancreas development from single-cell analyses

Diabetes is a global public health concern in the 21st century. According to the report in the International Diabetes Federation Diabetes Atlas in 2015, 415 million adults suffer from diabetes globally. Although considerable progress has been made in the treatment of diabetes, such as insulin administration, researchers are still looking for alternative therapeutic approaches, e.g. islet transplantation from cadaveric donors, for its radical cure. However, this approach is limited due to the scarcity of donors and availability of islet cells.

To obtain sufficient supply of β -cells for transplantation, many efforts have been tried to produce endocrine pancreatic cells from human embryonic stem (hES) cells or human induced pluripotent stem (hiPS) cells *in vitro*. By using RA treatment and seeding the cells at low densities, Cai et al. (2010) established a highly efficient and reproducible protocol for generating PDX1⁺ pancreatic progenitors (PP) from hES cells. Mechanism-wise, RA treatment can inhibit hepatic differentiation of endodermal cells through inhibiting phosphorylation of the BMP downstream effector Smad1/5/8 (Cai et al., 2010). Shahjalal et al. (2014) elaborated the protocol further by developing a five-step xeno-free culture system to differentiate hiPS cells into insulin-producing cells *in vitro* efficiently. They formulated different combinations of signaling molecules, agonists, and/or inhibitors for each of the five steps to induce the differentiation of iPS cells first into definitive

endoderm (DE) cells, then in the order of primitive gut tube (PG) cells, PP, endocrine progenitors (EP), and endocrine cells (EC) (Shahjalal et al., 2014), a process mimicking the embryonic endocrine pancreas development (Yang et al., 2011; Shih et al., 2013; Gao et al., 2019).

Efficient and large-scale generation of functional, pure, and terminally differentiated islet cells from pluripotent stem cells or other differentiated cells rely on our understandings of (i) signatures of each developmental intermediate stage of the terminal differentiated cell types and (ii) identification of key regulators controlling each developmental stage *in vivo* (Figure 1). Single-cell analyses have been proven to be a powerful tool for this purpose. Single-cell RNA-seq has been used to reveal the heterogeneity of adult pancreatic islet cells or embryonic PP (Stanescu et al., 2017; Tritschler et al., 2017). In this issue of *JMGB*, Lu et al. (2019) adopted the *TgBAC(neurod1:EGFP)* transgenic reporter fish to determine the islet specification process during early organogenesis at the single-cell level.

Previously studies have revealed that the zebrafish islet originated from two anlagen: posterior dorsal pancreas and anterior ventral pancreas (Hesselson et al., 2009; Yang et al., 2011). Also in this issue of *JMGB*, Gao et al. (2019) demonstrated that depleting zebrafish Hhex will abolish the exocrine but not endocrine pancreas, further confirming the independent origin of the posterior dorsal pancreas. A number of transcription factors and signaling molecules have been identified to control the initiation and specification of the three types of endocrine pancreatic cells, namely α , β , and δ cells in zebrafish (Prince et al., 2017). By using the *TgBAC(neurod1:EGFP)* transgenic report fish, Lu et al. (2019)

successfully isolated islet cells from embryonic zebrafish at four developmental stages: 18 hours post fertilization (hpf), 22 hpf, 30 hpf, and 52 hpf, covering the processes from nascent islet cells to functionally matured zebrafish islet. Data analyses identified five clusters from 413 high-quality single-cell transcriptomes, including three clusters corresponding to α , β , and δ cells. Excitingly, the other two represent newly postulated islet precursors and proliferative progenitors.

Gene ontology (GO) analyses revealed that proliferative progenitors were characterized by expressing categories of genes including ‘ribonucleoprotein complex biogenesis’, ‘DNA replication’, and ‘cell cycle’. Importantly, in addition to known transcription factors *neurod1*, *isl1*, *pax6b*, and *nkx2.2a*, they identified 12 transcription factors and certain epigenetic regulators that were specifically enriched in the progenitor population (Lu et al., 2019). It is worth pointing out that, through analyzing cell cycle marker genes, the authors classified the progenitors into G1/S cells, which are presumed to be at the cross-road for differentiation, G2/M cells for proliferation, and the quiescent population of unknown function. As expected, the ratio of the progenitor cells decreased sharply after 18 hpf, a characteristic of the posterior dorsal pancreas (Hesselson et al., 2009; Yang et al., 2011).

More excitingly, Lu et al. (2019) defined a population of cells as islet precursors, which are at an intermediate state of differentiation comparing with terminally differentiated α , β , and δ cells, suggesting the existence of heterogeneous and lineage-primed pancreatic precursor cells, a phenomenon that has not yet been reported. They found that lineage-primed precursors formed by increasing the expression of

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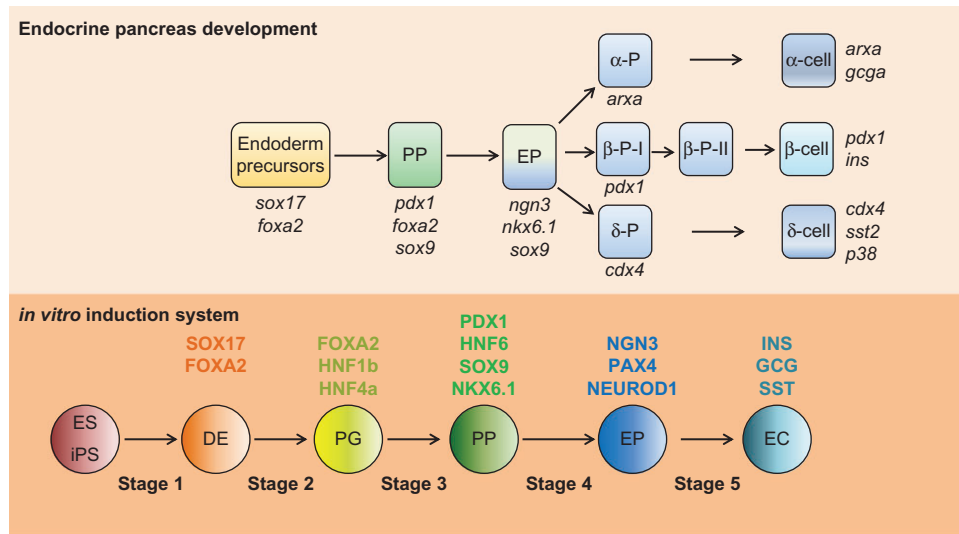


Figure 1 Molecular timeline for the development of endocrine pancreatic cells *in vivo* and *in vitro*. Top panel: molecular timeline for the stepwise *in vivo* development of terminal differentiated EC starting from DE based on current knowledge and the single-cell analyses reported by Lu et al. (2019). Lower panel: generation of β -like cells by mimicking the *in vivo* developmental process of the endocrine pancreas starting from pluripotent stem cells. Key molecular regulators or markers for each stage are indicated. α -P, α precursor; β -P, β precursor; δ -P, δ precursor.

lineage-specific transcription factors and differentiated into terminal cells by maintaining the level of these transcription factors while gradually enhancing metabolic and physiological functions.

In summary, single-cell analyses by Lu et al. (2019) have established a molecular timeline for the development of cells in the endocrine pancreas *in vivo* that provides significant insights into the mechanisms of stepwise islet cell proliferation, differentiation, and maturation (Figure 1). These findings will not only help us to understand the interesting observation of reduction of pancreatic β -cell dedifferentiation after gastric bypass surgery in diabetic patients or rats (Qian et al., 2014) but also offer guidance to refine the protocols for generation of β -cells from pluripotent stem cells or differentiated cells (Figure 1). Meanwhile, these findings will provide a benchmark for evaluating the quality of the induced PP.

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