STEM CELLS

Cells produced during in vitro β -cell differentiation characterized

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Type 1 diabetes mellitus (T1DM) is characterized by the loss of insulin-producing β -cells. One line of research has focused on how to produce replacement β -cells to treat patients with T1DM, including using in vitro differentiation of human stem cells. New research published in *Nature* and led by Douglas Melton (Harvard University, USA) has brought successful therapeutic stem cell differentiation a step closer by providing a thorough characterization of the cells produced by an in vitro protocol of β -cell differentiation from human stem cells.

“A few years back, we showed that it is possible to make functional human β -cells from stem cells,” says Melton. “While this was considered by many to be a significant advance, it was not widely noted that our protocol produced about 30% β -cells and the remainder were ‘other’ cells, some of which were endocrine.” Melton and colleagues devised the current study to characterize all of the cells produced by their differentiation protocol.

This immunofluorescence image is a section of a cluster of human cells that have undergone directed differentiation from embryonic stem cells into the human islet lineage. The cluster of cells was obtained by antibody sorting (described in the paper) and is nearly pure β -cells (purple stain for insulin; blue is nuclei). The few non- β -cells are other endocrine cells (glucagon-expressing α -cells in yellow and enterochromaffin cells in teal). Image courtesy of Henry Bushnell.

The researchers analysed RNA transcripts from more than 100,000 cells undergoing in vitro β -cell differentiation using single-cell RNA sequencing. “It might be worth noting that because we control differentiation in vitro, we were able to analyse progenitors and precursors that are generally not accessible from early development in human embryos and fetuses,” notes Melton. This technique enabled the researchers to identify the different cells produced by their protocol — populations of β -cells, α -like cells, progenitors and cells that resembled pancreatic exocrine cells. In addition, they identified a previously unreported population of cells that were similar to enterochromaffin cells (enterochromaffin cells are enteroendocrine cells that secrete serotonin and are found in the digestive tract).

Further analysis of the enterochromaffin-like cells produced in the protocol revealed that they were very similar to the β -cells that were produced, but also had fundamental differences. The enterochromaffin-like cells were shown to have many of the markers of in vivo enterochromaffin cells, including the expression of certain genes and the production of serotonin. The similarities between these two cell populations suggest that there is a relationship between the fates of β -cells and enterochromaffin cells.

“The single-cell analysis enabled us to identify gene products that are expressed on the cell surface and could be used to enrich or purify desired cell types,” explains Melton. “In this case, we report on a significant enrichment

for β -cells and α -cells: insulin and glucagon-producing cells, respectively.” The researchers were able to identify CD49a as a cell surface marker of β -cells, which means that the differentiated cells can be sorted to increase the purity of β -cells. Indeed, the team used anti-CD49a staining and magnetic microbeads to label and sort β -cells. This method produced clusters that were up to 80% β -cells and less than 5% enterochromaffin cells.

The researchers also assessed the β -cells throughout the protocol period. They were able to demonstrate that the stem-cell-derived β -cells develop glucose-responsive insulin secretion functionality that is stable for 4 weeks in culture and does not need exogenous factors. They also showed that the stem-cell-derived β -cells expressed genes that are known to be key features of adult in vivo β -cells, including genes with products involved in metabolic sensing and signalling, as well as insulin synthesis, packaging and secretion.

“Because we were able to describe all the genes that come on and off during the development of human islet cells, we now have a map of the developmental history of cell types that are likely to be clinically useful,” says Melton. “This kind of description, what you might call a ‘molecular biography’, is known for few, if any, other cell types.” Melton concludes that the stage is now set for further manipulation of in vitro β -cell differentiation involving controlling the proportions of cells produced and studying the physiological functions of the cells.

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ORIGINAL ARTICLE Veres, A. et al. Charting cellular identity during human in vitro β -cell differentiation. *Nature* **569**, 368–373 (2019)

FURTHER READING Bakhti, M. et al. Modelling the endocrine pancreas in health and disease. *Nat. Rev. Endocrinol.* **15**, 155–171 (2019) | Frank, J. A. et al. Optical tools for understanding the complexity of β -cell signalling and insulin release. *Nat. Rev. Endocrinol.* **14**, 721–737 (2018)