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The role of ETV1 transcription factor in human pluripotent stem cell adhesion and human endocrine pancreas development *in vitro*.

Key words: human pluripotent stem cells, human pluripotent stem cell adhesion, cell therapy, endocrine pancreas, diabetes, beta-cell differentiation in vitro from human pluripotent stem cells

Abstract

The pancreas is a glandular organ with a vital role in the production and release of the digestive enzymes (exocrine part) and the hormones regulating glucose homeostasis (endocrine part). The pancreatic endocrine cells form islets of Langerhans, in which the most abundant cells (about 90% per islet) are α- and β-cells. The β-cells control the glucose homeostasis by secreting insulin in response to increased sugar levels in the serum. Insulin allows glucose uptake from the bloodstream by cells in peripheral tissues where the sugar is stored (hepatocytes) or used as energy source (muscle). In the opposite manner, the glucagon secreting α-cells increase blood sugar level by stimulating hepatocytes to lyse the stored glycogen and to release the glucose. Long-term disruption of glucose homeostasis may lead to a chronic, metabolic disease – diabetes mellitus. The typical hallmark of each diabetes type is decreased insulin production caused by either β-cell loss by an autoimmune attack (in type 1 diabetes) or impaired function of β-cells or insulin receptors on peripheral tissues (in type 2 diabetes). Current therapeutic strategies rely on the controlling of the carbohydrate's homeostasis by a healthy lifestyle, together with multiple insulin injections or medicines stimulating or inhibiting insulin or glucagon secretion, respectively. However, these approaches cause inconvenience for diabetes patients and do not offer a cure.

The human pluripotent stem cells (hPSCs) provide a potentially unlimited cell source for cell replacement therapies in regenerative medicine. The regeneration of β-cell mass derived from hPSC *in vitro* differentiation offers an attractive platform to restore normoglycemia in diabetes patients. Thus, better understanding of stem cell biology is required for maintaining the high quality in hPSC culture and highly efficient differentiation *in vitro* to all lineages.

Using single-cell transcriptomics, we have recently identified ETS Variant Transcription Factor 1, Etv1, as specifically expressed in a subset of cells within the mouse pancreas at embryonic day 14.5. The Etv1 expression was restricted in endocrine progenitors and newborn α- and β-cells, suggesting the Etv1 role in endocrine progenitor maturation and endocrine cell specification. Moreover, in Etv1 knock-out mice, the pancreatic islet size was decreased in newborns, supporting the developmental role of Etv1 in pancreas. Yet, the mechanisms by which Etv1 regulates pancreas development remain unknown. Besides the pancreas, ETV1 was shown to be involved in maturation of murine and hPSC-derived cardiomyocytes, terminal maturation of murine cerebellar granule cells, and development of neural crest cells in *Xenopus laevis* embryos.

Here, one of the aims of the study was to create a tool for discovering the ETV1 role in pancreatic islet development using hPSC *in vitro* differentiation to pancreatic β-cells. In the thesis, the ETV1 knock-out hPSC line was established using the CRISPR/Cas9 method. During characterization of ETV1 deficient cell lines the phenotype of enhanced cell adhesion was identified. The cell-to-matrix or cell-to-cell adhesion regulates pluripotency and self-renewal and drives pluripotency exit. However, the detailed mechanisms of the adhesion-pluripotency network remain elusive. For instance, the critical transcriptional regulators controlling the expression of adhesion genes in hPSC are still not discovered.

As part of this work, a comparative confluency analysis between ETV1 knock-out and wild-type hPSCs revealed increased confluency in ETV1 deficient cells. The whole transcriptome analysis discovered enrichment in pathways involved in cell adhesion and cell attachment to the extracellular matrix. The immunofluorescence staining confirmed the increase in adhesion protein levels, including e-cadherin,

paxillin and integrin 5□, after ETV1 ablation compared to wild-type cells. Additionally, the pathways involved in pluripotency were downregulated in ETV1 knock-out compared to wild-type hPSCs suggesting the ETV1 role in maintaining self-renewal and pluripotency in hPSCs. Therefore, together this work revealed ETV1 as novel regulator of adhesion and pluripotency in hPSCs.

Another important goal of the research was to determine the ETV1 role in human endocrine development. The spontaneous differentiation experiments using ETV1 knock-out and wild-type hPSCs revealed higher efficiency of endoderm and mesoderm differentiation in ETV1 knock-out cells. Interestingly, the directed differentiation into pancreatic □-cells resulted in a loss of the endocrine specification in ETV1 deficient cells. The single-cell RNA-sequencing was performed to determine the differences in arising cell populations in ETV1 knock-out and wild-type during pancreatic *in vitro* differentiation and discover the molecular mechanisms underlying the deficient endocrine pancreas differentiation after ETV1 ablation.

Taken together, the research provides an interesting deeper view into ETV1's double role in both pluripotency and development.