論文 / 著書情報
Article / Book Information

題目(和文)	ヒト多能性幹細胞から膵臓β細胞への分化誘導における亜鉛役割の解明
Title(English)	Elucidating the role of zinc in pancreatic β cell differentiation using human pluripotent stem cells
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Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生命理工学 生命理工	系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of	（ 学術 ）
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Human pluripotent stem cells (hPSCs) have a high proliferation rate and the ability to differentiate into all major somatic cell lineages, potentially for use in cell replacement therapy, drug discovery, and disease modeling. In this thesis, I focus on the importance of nutrient components in culture media to generate functional hPSC-derived pancreatic β cells to provide an alternative cell source for islet transplantation. Undifferentiated hPSCs are in an active state of methionine catabolism. Culturing hPSCs under a condition of five hours of methionine deprivation (ΔMet) resulted in a rapid decrease in intracellular S-adenosyl methionine ([SAM]i). The reduction in SAM content of the hPSCs placed the cells at a biased state for differentiation, specifically compared to complete (Compl) and other amino acid deprivation pretreated hPSCs (Shiraki et al., 2014). From the microarray results using Compl and ΔMet pretreated hPSCs, 18 common upregulated genes with significant differences, including *SLC30A1*, were observed selectively in ΔMet cells. *SLC30A1* encodes a zinc exporter localizes on the plasma membrane, ZNT1. Upregulation of ZNT1 theoretically decreases intracellular Zinc (Zn) concentration. Then, through further investigations, we found that hPSCs cultured under ΔMet actually showed a reduced intracellular protein-bound Zn content level of hPSCs. Homocysteine could replace Zn from its binding protein. I found that cell lysate from ΔMet treated hPSC contained a higher amount of homocysteine, exhibiting a lower protein-bound Zn content. From these results, I hypothesized that the elevated differentiation potency after ΔMet treatment of hPSCs might be due to the changes in Zn contents in the cells. Therefore, my research aims to understand the roles of Zn in the maintenance culture of undifferentiated hPSCs and pancreatic differentiation and to reveal the relationship between Zn and Met.

In the hPSC maintenance media, insulin (INS) supplements are usually added to sustain cell growth. However, INS contains excess Zn ions for its structural stability. To investigate the effect of Zn on hPSCs, I introduced a Zn-deprived (ΔZn) custom-made media, in which insulin growth factor 1 (IGF1) is used to substitute INS supplements. Undifferentiated hPSCs were cultured with the maintenance media supplemented with IGF1 and graded concentrations of Zn for 3 days. The cells were then sampled and analyzed for cell proliferation (EdU incorporation) and gene expression (real-time PCR). Cells cultured under 0 μM Zn exhibited lower DAPI+ cells count and decreased EdU+ cell population, indicating a reduced proliferation rate compared to control hPSCs cultured in 3 μM Zn. Furthermore, hPSCs cultured under 0, 0.5, and 1 μM Zn showed significant downregulation of cell proliferation markers, *GRB7*, and *HCK*. Compared to controls, upregulation of differentiation markers, *GATA4, PECAM, and PAX6* was also observed under low Zn conditions. The results suggest that hPSCs cultured under low Zn exhibit an increased differentiation potency.

The results suggested that ΔZn potentiates differentiation. I then investigated the effect of Zn in definitive endoderm (DE) and pancreatic differentiation. Undifferentiated hPSCs were pretreated with 5h of Compl and ΔMet, and then DE differentiation was initiated under a graded Zn concentration (0, 0.5, 3 μM). As a result, the proportion of cells expressing the pluripotency marker, OCT3/4, increased with increasing Zn. Also, adding 3 μM Zn in Compl pretreated cells reduced the proportion of cells expressing the DE marker, SOX17. While under ΔMet, DE differentiation was efficient in all three Zn concentrations. This result indicated that Zn addition reduced the efficiency of DE differentiation, and ΔMet pretreatment overrides the effects of Zn. Also, DE differentiation using eight hiPSC lines revealed that cell line-to-cell line variation could be eliminated under the 0 μM Zn condition compared to the control 3 μM Zn. Next, DE cells were differentiated into pancreatic progenitors, and endocrine cells using differentiation media under Zn added conditions. Cells cultured under 3 μM Zn

showed a higher percentage of the pancreatic marker, NKX6.1+ cells among the PDX1+ cell population compared to 0 μM Zn, suggesting that Zn and INS contribute to endocrine progenitor differentiation.

Lastly, RNA sequencing was performed to analyze the global expression profile of hPSCs cultured under graded Zn concentrations and compared with ΔMet treated hPSCs. Overall, ΔZn cultured cells exhibited changes in gene expressions related to the Met cycle, such as *MAT2A* and *DNMT3B*. The relationship between Met and Zn was also confirmed by analyzing intracellular Met-cycle metabolites in cells cultured at different Zn concentrations. Excretions of homocysteine, SAM, and S-adenosyl-homocysteine were observed under ΔZn cell culture conditions but not in control. The results suggested that PSCs cultured under ΔZn condition exhibited an altered Met metabolism, revealing the link between Met metabolism and Zn signaling.

Overall, I focused on Zn in culture media and found that Zn supports hPSCs cell growth. I concluded that Zn regulates pancreatic differentiation in a stage-dependent manner. Addition of Zn reduced DE differentiation but potentiated differentiation into pancreatic progenitor cells and further into endocrine cells. This study revealed that methionine metabolism regulates Zn mobilization in PSCs and is essential for the maintenance of pluripotency, proliferation, and differentiation of PSCs. Zn is required during late-stage pancreatic development. Utilizing the metabolic specificities of the PSCs to methionine and Zn, I established a novel differentiation procedure that is applicable to different PSC lines for generating functional pancreatic β cells. The procedures will be useful for further application in regenerative medicine.