論文 / 著書情報
Article / Book Information

題目(和文)	ヒト多能性幹細胞を用いた膵臓分化を促進する新規化合物の作用機序解明
Title(English)	Elucidation of the mechanism of action of a novel small molecule that promotes iPS cell differentiation into pancreatic β cells
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出典(和文)	学位:博士(理学), 学位授与機関:東京科学大学, 報告番号:甲第238号, 授与年月日:2025年3月26日, 学位の種別:課程博士, 審査員:粂 昭苑,白木 伸明,山口 雄輝,加納 ふみ,川上 厚志
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学位種別(和文)	博士論文
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 系 生命理工学 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of （ 理学 ）
学生氏名： Student's Name	島谷 幸宏	審査員主査： Chief Examiner	粂 昭苑

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Diabetes mellitus is caused by failure of blood glucose regulation and affects an estimated 500 million people worldwide. To address the growing need for transplantation treatment of diabetes, researchers are studying the differentiation of pluripotent stem cells into pancreatic β cells. In our laboratory, we conducted joint research to identify a compound that promotes pancreatic differentiation. We screened a chemical library of 55,000 compounds, performing the first screening, reproducibility test, dose dependency test, and final screening. We focused on one hit compound, "K-1". After chemical modification, a novel compound, "K-3," demonstrated enhanced dose dependency and effectiveness in promoting pancreatic differentiation of human induced pluripotent stem (iPS) cells. However, the mechanism of action of this novel compound was unknown. In this research, I investigated the impact of K-3 on pancreatic differentiation and the downstream of K-3 by focusing on the effective period of K-3 treatment on cell yield, differentiation efficiency, and functionality of β cells.

I performed pancreatic differentiation using a human iPS cell line to obtain pancreatic β cells following differentiation protocol (Sim et al., 2022). In this protocol, cells were cultured in 5-steps of medium; each stage represents DE (definitive endoderm, S1), PG (primitive gut tube, S2), PP (pancreatic progenitor, S3), EP (endocrine progenitor, S4), and EC (endocrine cells, S5). K-3, or DMSO, as a vehicle, is added to the differentiation medium from S3 to S5. The differentiated cells were assessed at the end of S5 to examine how much the differentiation efficiency was affected by performing flow cytometry and immunocytochemistry. K-3 treatment from S3 to S5 increased INSULIN expressing pancreatic β cell population as expected, and notably, K-3 treatment during only S3 was still enough to increase the differentiation efficiency. The pancreatic β cells were also assessed for their function to secrete INSULIN responding to the glucose concentration. K-3 treatment during only S3 exhibited enhanced function as good as K-3 treatment from S3 to S5. Additionally, the K-3 treatment increased the cell yield at the end of S5. Although the cell yield increase by K-3 treatment in S3 compared to control was insignificant, K-3 treatment in the earlier stage tended to increase the cell yield. Considering these results, I hypothesized that K-3 treatment during S3 was critical for promoting differentiation into pancreatic β cells.

To elucidate the direct target of K-3, RNA-sequencing was conducted at the S3 end, comparing with or without K-3 treatment. Genes expressed higher or less than 1.5-fold with a p-value < 0.05 were recognized as DEG (differential expression genes). Gene ontology enrichment analysis using DEGs listed GO terms related to cell development, such as "nerve development" and "type B pancreatic cell differentiation" in upregulated genes. Pancreatic and duodenal homeobox 1 (PDX1) were particularly significantly upregulated by K-3 treatment. Cells at the end of S3 were also assessed by immunocytochemistry. It turned out that anti-PDX1 staining intensity in the nuclei was stronger in the K-3 treated condition. PDX1 is a transcription factor known as a key factor for pancreatic differentiation, so K-3 treatment promotes pancreatic differentiation by upregulating PDX1 expression. Furthermore, K-3 treatment, including the S3 period, increased the percentage of PDX1 and NK6 homeobox 1 (NKX6.1) expressing cells at the end of S4 detected by immunocytochemistry. K-3 treatment increased PDX1 expression at S3, promoting differentiation efficiency into pancreatic progenitor at S4.

I further investigated the character of pancreatic β cells differentiated with K-3 treatment. According to the immunostaining result, K-3 treatment increased the population of INS+/GCG-/SST- cells. K-3 treatment directed the cell fate specifically into β cells. The gene expression of pancreatic β cells with K-3 treatment was quantified by RT-qPCR. K-3 treatment from S3 to S5 upregulated pancreatic β cell-related genes such as *PDX1*, *NKX6.1*, *INS*, and *MAFA*. On the other hand, *GCG* and *SST* were also upregulated by K-3 treatment. Other endocrine cell marker genes were upregulated in the K-3 condition because K-3 promoted differentiation into PP cells, so the total endocrine cell population increased in the K-3 condition.

In this research, I found that the mechanism of K-3 is mediated by PDX1 upregulation. This function promotes differentiation into PP cells, which leads to differentiation into pancreatic β cells. PDX1 is a key gene of pancreatic progenitor and is essential for maintaining the character of β cells. For further research, the direct target of K-3 needs to be investigated. It is important to examine whether the β cells differentiated with K-3

treatment can cure diabetes using a diabetic mice model.

備考 ： 論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。

Note : Thesis Summary should be submitted in either a copy of 2000 Japanese Characters and 300 Words (English) or 1copy of 800 Words (English).

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