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論文 / 著書情報
Article / Book Information

題目(和文)	メチオニン欠乏によるヒストン修飾変化を介したヒトiPS細胞分化促進機構の解明
Title(English)	
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種別(和文)	論文要旨
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（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 ）
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Introduction

Human pluripotent stem cells (PSCs) possess the capacity for unlimited self-renewal and the potential to differentiate into cells derived from the three germ layers. Recent progress in this field has successfully generated many functionally differentiated cell types, which are expected to be useful as alternative cell sources for cell replacement therapy, disease modeling, and drug discovery.

Our group and others have previously reported that pluripotent stem cells exhibit a unique metabolic state in which they exhibit a high requirement for S-adenosyl Met (SAM), which is generated through methionine (Met) and threonine metabolism. Short-term deprivation of Met from culture media triggers a decrease in H3K4me3 modification and widespread induction of stress responses in human PSCs (hPSCs), rendering hPSCs in a biased state for differentiation. Hence, potentiation of differentiation was observed upon exposure to inductive signals. In contrast, long-term Met deprivation leads to cell death.

In hPSCs, genomic regions marked with both H3K4me3 and H3K27me3, the so-called bivalent domains, are essential for maintaining the pluripotency and self-renewal capacity of PSCs. During developmental progression, the epigenetic state of the enhancer determines developmental competence. The en masse acquisition of a poised chromatin state leads to lineage-specific target gene activation.

We have previously reported that Met deprivation could be useful for hPSCs production to eliminate undifferentiated pluripotent cells in culture. Here, we conducted further analysis of hPSCs in 3D suspension culture. In this dissertation, I examined the changes in H3K4me3 and H3K27me3 and the impact of Met deprivation, i.e., the mechanism underlying the potentiation of differentiation.

Results and Discussion

1. Met deprivation results in a rapid decrease in intracellular SAM level and H3K4me3 modification in 3D cultures.

To confirm whether undifferentiated PSCs in 3D spheroid cultures would respond similarly to Met deprivation, we tested both monolayer (2D) and sphere (3D) cultures. Undifferentiated hiPSCs cultured in 2D or 3D were subjected to 5 h of Met deprivation, followed by metabolomic

analysis. Significant decreases in SAM and MTA levels upon Met deprivation were confirmed in PSCs cultured in 2D and 3D, with a less substantial impact in 3D culture than in 2D. Although Met cycle metabolites were reduced to a lesser extent in 3D culture than in 2D culture, H3K4me3 decreased significantly in 3D culture. Other histone modifications were reduced, albeit to a lesser extent or not affected. Thus, the reduction in H3K4me3 by Met deprivation is specific among the histone modifications.

2. Short-term Met deprivation induces H3K4me3 loss in transcriptional regulator genes involved in pluripotency, the TGF-β signaling pathway and cholesterol biosynthesis.
We then carried out chromatin immunoprecipitation sequencing (ChIP-seq) of H3K4me3 or H3K27me3 modifications and RNA-seq analyses to determine the genomic loci affected by Met deprivation. Met deprivation induced broad H3K4me3 reduction. TSS region of key pluripotent genes such as *NANOG, POU5F1*, and TGF signal pathway genes are specifically impacted upon 5hr Met deprivation. The gene expression levels of these genes are also decreased. Our results indicate that Met deprivation in hPSCs potentiates their exit from the pluripotency state by altering the epigenetic modifications of genes involved in the TGF-β pathway and core pluripotency network through a synergistic H3K4me3 loss.
Genes involved in cholesterol biosynthetic process are most affected by Met deprivation in both H3K4me3 profiles and gene expression. Since lipid biosynthesis is indispensable for hPSC proliferation, cholesterol biosynthesis inhibition may be one of the main causes of cell cycle arrest and apoptosis under Met deprivation.

3. Met deprivation upregulates lineage-specific genes by resolving bivalency during early hiPSC differentiation.
We evaluated human iPSCs that initiated endoderm differentiation with Activin A and CHIR99023 for 24 hours after the short-term Met deprivation. GO analysis of upregulated genes in Met deprived differentiated cells revealed the enrichment for genes involved in early mesendoderm differentiation. Key transcriptional regulators for the definitive endoderm state were reported to be bivalent definitive endoderm genes, which resolved the H3K27me3 mark upon endoderm formation. Among the upregulated genes in Met-deprived cells, the important genes for development, including *GATA6, CER1, EOMES, MESP1, BMP2, ERBB4, and LEF1,* had reduced H3K27me3 marks, whereas H3K4me3 levels remained unchanged. We also confirmed an increase in GATA6- and p-SMAD2-positive cells upon Met deprivation, indicating that endoderm differentiation was potentiated. These results indicated that Met deprivation potentiates cell differentiation. Culturing hiPSCs without Met can potentiate differentiation, and this methodology can unveil the initial genes involved in both gene expression and histone modification in the early differentiation stage.

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).