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審査研究科	人間総合科学研究科
学位論文題目	Two histone variants TH2A and TH2B facilitate human iPS cell generation. （２つの異型ヒストン TH2A と TH2 はヒト iPS 細胞の作製を促進する）

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論文の内容の要旨

（**目的**）There are two major methods of reprogramming: generation of induced pluripotent stem cells (iPSCs) by overexpressing embryonic-stem-cell-specific transcription factors (OSKM: OCT4, SOX2, KLF4 and c-MYC) and somatic cell nuclear transfer (SCNT) by oocyte-specific factors. Previously, the author's group found oocyte-enriched histone variants TH2A, TH2B and the histone chaperone nucleoplasmin (NPM2) enhance the reprogramming by OSKM in mice. In addition, the author's group found mouse histone TH2A and TH2B form unstable nucleosome compared to canonical histones. In this study, the author investigates the role of mouse histone TH2A and TH2B in modulating chromatin structure. Moreover, the author analyzes the role of human TH2A, TH2B and NPM2 in human induced pluripotent stem cell generation.

（**対象と方法**）The study was performed as follows:

TH2Aand TH2B were amplified from genomic DNA by PCR and inserted into pLenti6.3 vector. Expression of TH2A and TH2B were confirmed by western blot. Lentivirus system was used to generate induced pluripotent stem cells. After indicated time, live cell staining was

performed with anti-SSEA4 and anti-TRA-1-60 antibodies to count iPSC colonies. In vitro differentiation was performed by transferred iPSCs into non-adherent plate for 3 days prior to spontaneous differentiation in adherent plate. For teratoma formation, single iPSCs were transplanted into dorsal flanks of NOD/ShiJic-scidJcl mice. Teratomas were dissected after six to nine weeks and processed to hematoxylin and eosin staining or immunostaining with antibodies for three germ layers markers. Microarray analysis was performed by Human Gene 1.0 ST Array and processed by R package Oligo.

(結果) The author found the following results:

1) The nuclei from NIH3T3 cells expressing mouse TH2A, TH2B, and NPM2 exhibited higher DNase I sensitivity than those from cells overexpressing canonical histones. However, these histones variants did not affect the total H3K9me3 level.
2) Th2a-/-Th2b-/- spermatids have defects in the deposition of TNP2 on chromatin. The level of canonical histone H2B is upregulated by a feedback mechanism.
3) Human TH2A, TH2B and NPM2 enhance the OSKM-induced reprograming of human dermal fibroblasts and umbilical vein endothelial cells.
4) In vitro and in vivo assay was performed and pluripotency of iPSCs generated by co-expression of OSKM and BAN (TH2A, TH2B, and NPM2) was proved.
5) These iPSCs gave rise to highly differentiated teratomas compared to iPSCs induce by OSKM alone.
6) Genome-wide analysis was performed and the author found TH2A, TH2B and NPM2 regulated genes which are involved in naïve stem cell stage.

(考察) Open chromatin is a hallmark of ESCs and iPSCs, in which the overall genome is hyperactive and the levels of heterochromatin are reduced compared with differentiated cells. The author speculated that TH2A/TH2B contribute to the reprogramming of somatic cells by inducing transcriptionally active open chromatin, whereas Chd1 contributes to the maintenance of an established open chromatin state in ESCs by suppressing heterochromatin formation.

The author found BAN can enhance the number of TRA-1-60 and SSEA-4 positive colony number in HUVECs about 8 folds and 3.5 folds, espectively. On the other hand, the degree of enhancement for adult dermal fibroblast is approximately 2.5 folds, indicating that HUVECs may efficiently form open chromatin than adult fibroblasts. BAN-regulated genes are overlapped with transcript signature of naïve human ESCs. Therefore, the author speculated that open chromatin structure induced by BAN may facilitate the naïve state of human ESCs or iPSCs. Indeed naïve state cells are shown to have a high cellular potency due to the genome wide low methylation levels, and low expression of lineage specific genes. Further analyses would be required to dissect the mechanisms underlie the naïve state and investigate how BAN is involved in this naïve state.

審査の結果の要旨

（**批評**） 著者は、TH2A と TH2B がどのようにクロマチン構造を開放するのか、そのメカニズムを明らかにし、将来 TH2A と TH2B さらに P-NPM2 のカクテルがリプログラミングに働き、ヒト iPS 細胞を効率的に産生する可能性について示唆する結果を示したことは、評価に値する。

平成 28 年 1 月 18 日、学位論文審査委員会において、審査委員全員出席のもと論文について説明を求め、関連事項について質疑応答を行い、最終試験を行った。その結果、審査委員全員が合格と判定した。

よって、著者は博士（医学）の学位を受けるのに十分な資格を有するものと認める。