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学位の種類	博士（　医学　）
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審査研究科	人間総合科学研究科
学位論文題目	Analysis of X Chromosome Reactivation during Reprogramming （リプログラミング時の X 染色体再活性化の解析）
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論文の内容の要旨
Abstract of thesis

【目的 Purpose】

Induced pluripotent stem cells (iPSCs) are applicable tools for modeling of diseases, drug development and transplantation medicine. However, several issues regarding its generation will limit its potentials in medicine, such as a low number of high quality iPSCs. Previous studies have shown that the epigenetic state of X chromosomes in female iPSCs is closely linked to pluripotency, an indicator for high quality of iPSCs. Female mouse iPSCs with two active X chromosomes (XaXa) exhibit higher pluripotency than iPSCs with one active and one inactive X chromosome (XaXi). In order to visualize the X chromosome status in living cells and analyze the mechanism of X chromosome reactivation (XCR), the author established a novel live cell imaging system of XCR.

【対象と方法 Materials and Methods】

The CRISPR/Cas9 system was used to generate mouse embryonic stem cell (mESC) lines that carry the *EGFP* and humanized Kusabira Orange (*hKO*) genes inserted into an intergenic site near either the *Syap1* or *Taf1* gene on both X chromosomes. Female mESCs were transfected with two different donor template plasmids, each of which contains one of the fluorescent protein genes and a drug resistant gene between the homologous sequences in the targeted locus, together with the expression vector of Cas9 and a guide RNA. After selection of drug-resistant mESC clones that expressed two fluorescence proteins (EGFP+/hKO+), PCR analysis of their genome DNA was performed to confirm that these mESC clones have the expected inserts at the targeted loci of both X chromosomes. In order to visualize X chromosome status in living cells, candidate mESC clones were differentiated through embryoid bodies

(EBs) formation and their derived differentiated single-color cells were reprogrammed to generate iPSCs to monitor X chromosome inactivation (XCI) and X chromosome reactivation (XCR), respectively.

【結果 Results】

The author observed that the female EGFP+/hKO+ ESC clones express only one of the two fluorescent proteins (EGFP+ or hKO+) upon differentiation, indicating that the inserted fluorescent protein genes are subject to XCI. Furthermore, when the single-color somatic cells were reprogrammed into iPSCs, the iPS colonies displayed double colors (EGFP+/hKO+). These results indicate that this system can also detect XCR during reprogramming in a predicted manner. Interestingly, the author found out a correlation between the extent of XCR and the level of pluripotency of iPSCs. Colonies with complete XCR expressed higher levels of pluripotency marker genes than those with partial XCR. It indicates that this system provides a simple method for distinguishing high and low quality iPSCs.

【考察 Discussion】

The author established a novel detection system of XCR, which can be utilized for visualizing the X chromosome status in living cells (tracking XCI upon differentiation; monitoring XCR during reprogramming). The author discusses that this detection system of XCR during reprogramming provides a simple method for isolating high quality iPSCs, which are promising materials for regenerative therapy research.

審査の結果の要旨
Abstract of assessment result

【批評 General Comments】

The author successfully established female ESCs that carry the *EGFP* and *hKO* genes inserted into an intergenic site near either the *Syap1* or *Taf1* gene on both X chromosomes. The *EGFP* and *hKO* genes allowed live cell monitoring of XCI during ESC differentiation and XCR during somatic cell reprogramming. Therefore, the female ESCs become an important tool for high-throughput screenings for factors and culture conditions that promote the acquisition of pluripotency by iPSCs.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on Aug 31, 2018. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.