MECHANISTIC ANALYSIS OF THE INITIATION OF RETROVIRAL GENE SILENCING BY REPROGRAMMING FACTORS

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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, BUI PHUONG LINH describes theinvestigation of retroviral gene silencing by reprogramming factors. The summary is as follows:

（**目的　Purpose**）

Transcriptional downregulation of integrated viral genomes has been found in a variety of stem cells of mammals. This transcriptional silencing is critical to the maintenance of the genetic stability of stem cells and the genotypic damage from subsequent viral replication. Despite hundreds of genes involved in the complex of the retroviral silencing, the functions of only a few of them could be determined. The biggest question is how the silencing complex is established in the initial phase. Interestingly, by ectopic expression of reprogramming factors (RFs) (OCT4, SOX2, KLF4, and c-MYC) by retroviral vectors, differentiated cells can be reprogrammed to induced pluripotent stem (iPS)cellsand undergo retroviral silencing. Here the author utilized replication-defective and persistent Sendai virus (SeVdp)-based vectors to monitor retroviral silencing during reprogramming and clarify the initial phase of silencing by overexpression of 4 RFs to detect which factor is involved in and understand the mechanism of the retroviral silencing.

（**対象と方法　Materials and Methods**）

The author designed a unique system for detecting the retrovirus silencing by using a novel replication-defective and persistent Sendai virus (SeVdp) vector, insertional chromatin immunoprecipitation (iChIP) and mass spectrometry (MS). The SeVdp vector used for generating iPSCs was based on the Cl.151 Sendai virus strain which has mutation on genes encoding nucleocapsid (NP, P and L proteins), resulting in long term persistence in the host genome. The structural genes encoding M, F and HN involved in viral infectivity were deleted and replaced by 4 RFs (OCT4, SOX2, KLF4, and c-MYC). Therefore, SeVdp vector can continuously express 4 exogenous genes in the host cytoplasm at a constant ratio in a single cell. iChIP was developed to isolate proteins and RNAs that are bound to a specific region within the genome. In this research, the author inserted LexA binding site near the primer binding site (PBS) and combine it with the identification of immunoprecipitated proteins by mass spectrometry to determine binding patterns of provirus silencing proteins.

（**結果　Results**）

The author observed that retroviral silencing occurred at an early reprogramming stage around day 5 after overexpression of RFs without the requirement for KLF4 or the YY1-binding site in the retroviral genome. By means of iChIP, the author isolated factors assembled on the silenced provirus, including components of the inhibitor of histone acetyltransferase (INHAT) comprised of the SET oncoprotein. Knockdown of TAF-Iα, an isoform of SET oncoprotein, in mouse embryonic fibroblasts (MEFs) diminished retroviral silencing during reprogramming, and overexpression of TAF-Iα in fibroblasts reinforced retroviral silencing by a SeVdp-based vector that was otherwise defective in retroviral silencing.

（**考察　Discussion**）

Contrary to the earlier studies, the author’s results demonstrated that retroviral silencing occurs at an earlier stage, even before the acquisition of pluripotency. This discrepancy may be due to the use of the vectors to reprogram somatic cells and determine the required time for retroviral silencing.Although the YY1-binding site and PBS are known as the two important cis-elements in retroviral silencing in ES and EC cells, retroviral silencing was dependent on almost exclusively on the PBS in this study. The YY1 site may be necessaryat the late reprogramming stage when the silencing is further consolidated by DNA methylation or other mechanisms.In almost all of the provirus silencing model suggested by other researchers, the silencing complex includes the stem-cell specific transcriptional repressor TRIM28 tied to the PBS DNA through zinc finger protein 809 (ZFP809) with sequence-specific DNA-binding activity. In contrast, the author’sMS data did not detect ZFP809 on the silenced proviral DNA.However, the author found that knocking down or overexpression of SET gene can affect the silencing of retrovirus. SET is a subunit of the INHAT complex, one likely mechanism is that SET protects histones from acetylation, thereby promoting deposition of repressive marks to repress transcription of the LTR.Taken together, the SeVdp-based reprogramming systemprovided a valuable source of candidates related to the establishment of the retrovirus silencing complex.The fact that the reprogramming factors initiate the retroviral silencing is important for disclosing the silencing pathways linking the repressive complex and the transcription factors that constitute the core pluripotency network.

審査の結果の要旨
Abstract of assessment result

（**批評　General Comments**）

In the present study, the author investigatedthe mechanism of retroviral gene silencing during iPS cell generation.The author found that OCT4, SOX2 and c-MYC were sufficient for retrovirus silencing and that

INHAT complex including TAF-Iα functioned in retrovirus silencing during reprogramming.The findings in this study certainly have impact in the field of stem cells and regenerative medicine and benefit for a better understanding of retrovirus silencing in the reprogramming process.

（**最終試験の結果　Assessment**）

The final examination committee conducted a meeting as a final examination on Oct 29, 2019. 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.