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

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論　　文　　概　　要（Thesis Abstract）

○ 論 文 題 目

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

（リプログラミング因子によるレトロウイルスサイレンシング開始機構）

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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, we could only determine the functions of a few of them. The biggest question is how the silencing complex was 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 cells and undergo retroviral silencing. Here we 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 involved in and understand the mechanism of the retroviral silencing.

対象と方法：

（Material and method）

We 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 for nucleocapsid (NP, P and L proteins), resulting in long term persistence in the host genome. Their structural genes encode 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 the level of a single cell. iChIP was developed to isolate proteins and RNAs that are bound to a specific region within the genome. In this research, we 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：

(Result)

We 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. iChIP enabled us to isolate factors assembled on the silenced provirus, including components of the inhibitor of histone acetyltransferase (INHAT) comprised of the SET oncoprotein. Knockdown of TAF-Ia and ANP32a in mouse embryonic fibroblasts (MEFs) diminished retroviral silencing during reprogramming, and overexpression of TAF-Ia in fibroblasts reinforced retroviral silencing by a SeVdp-based vector that is otherwise defective in retroviral silencing.

考察：

(Discussion)

Contrary to these earlier studies, our 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 retroviral vectors to reprogram somatic cells and determine the required time for retroviral silencing in their project.

Besides, as reprogramming is a reversal of normal differentiation, the YY1 site may be necessary at a 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 contains the stem-cell specific transcriptional repressor TRIM28 tied to the PBS DNA through zinc finger protein 809 (ZFP809) with sequence-specific DNA-binding activity. However, our MS data does not find ZFP809 protein bound to PBS site which can explain by the similar peptide sequences among Zinc finger protein might interfere with protein identify or during reprogramming other protein inducing by RFs help TRIM28 and other silencing related protein anchor to the PBS and facilitate silencing complex formation.

We found that knocking down or overexpression of Set gene can affect the silencing of retrovirus. Moreover, 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. In this regard, the recent demonstration of CAF1 as an important factor in silencing in ECCs. Despite its molecular mechanism is unknown, CAF1 and SET may function in a similar manner and need to be clarified in further experiments.

結論：

(Conclusion)

In conclusion, the SeVdp-based reprogramming system described here nicely complements the previous studies that used ESCs and ECCs and provide a valuable source of candidates related to the establishment of the retrovirus silencing complex. Although the silencing mechanism and its sequence of events may differ in their details, the fact that the known reprogramming factors initiate the retroviral silencing greatly facilitates the identification and mechanistic analyses of the pathways link the repressive complex and the transcription factors that constitute the core pluripotency network.