Identification and functional analysis of novel proteins involved in retroviral silencing

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Introduction

Retroviruses insert their genome into host chromosome during the life cycle and can alter the function of genes located near their insertion site. When retroviruses infect cells at the early stage of embryogenesis, they could cause even more extensive damage by affecting subsequent development of the embryo or by transmitting damages through further generations. To prevent these dire consequences, host cells have developed a defense mechanism, termed retroviral silencing, to suppress transcription of proviruses from both exogenous and endogenous retroviruses (ERVs), especially in cells at early embryogenesis. Retroviral silencing is recapitulated *in vitro* in pluripotent cells including embryonic stem cells (ESCs), embryonal carcinoma cells (ECCs) as well as induced pluripotent stem cells (iPSCs).

Retroviral silencing is believed to be a complex process, which involves DNA methylation and histone modifications. One important regulatory element for retroviral silencing is the primer-binding site (PBS), located near the 5' end of the retrovirus. PBS, a DNA sequence where proline tRNA binds to initiate reverse transcription, also assembles silencing-associated proteins that suppress expression of the provirus. Mutating the murine leukemia virus (MLV) PBS, to PBSQ, which binds glutamine tRNA, in a recombinant virus permits expression of the provirus in ECCs and ESCs.

Reprogramming of somatic cells to iPSCs requires drastic epigenetic changes, including silencing of endogenous and exogenous retroviruses. A recent study using a Sendai virus-based reprogramming system and insertional chromatin immunoprecipitation (iChIP) identified various candidate proteins for retroviral silencing, including two RNA-binding proteins, RBP-X and RBP-Y.

In this research, I will attempt to confirm the role of these novel proteins in retroviral silencing and elucidate their molecular mechanism.

Materials and Methods

Production of retrovirus

Kusabira-orange (hKO) expressing retroviruses were produced by transfection of the plasmid into PLAT-E packaging cells. Two different retroviruses, MLV(YY-PBS-hKO) and MLV(YY-PBSQ-hKO), were produced.

Induction of retroviral silencing by reprogramming

MEFs cells were transduced with the hKO-expressing retrovirus in the presence of 8 μg/mL polybrene and were selected with puromycin for two days.

Candidate genes were knocked down by siRNA transfection in the selected MEFs. Two days after transfection, the cells were infected with a Sendai virus vector encoding EGFP, KLF4, OCT4, SOX2 and c-MYC (SeVdp(GKOSM)) to reprogram MEFs. One day after SeVdp infection, MEFs were passaged onto feeder cells and maintained at 37°C. FACS was performed at day 7 of reprogramming to measure the silencing index, which is a percentage of hKO-negative cells in the total population of SeVdp-infected cells ($EGFP^+$ cells).

Retroviral silencing in ECCs.

ECCs (F9 cells) were transfected with siRNA against each gene by lipofection. Two days after siRNA transfection, F9 cells were transduced with retrovirus 12 h after passage, followed by centrifuging at 1,500 x g for 40 min. The degree of silencing was observed by microscopy.

Results and Discussion

Involvement of RBP-X in silencing during reprogramming

MEFs were transfected with either control siRNA (siLuc) or siRNA targeting RBP-X (siRBP-X) before reprogramming. At day 7 of reprograming, retroviral silencing was observed in over 80 percent of cells transfected with siLuc. By contrast, silencing was observed in only 40 percent of cells transfected with siRBP-X (Figure 1). This confirmed that RBP-X has a role in retroviral silencing.

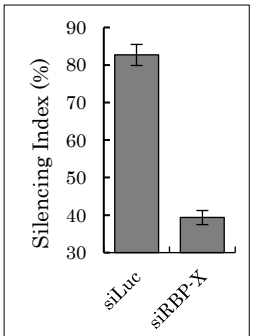

Figure 1. Silencing index at day 7 of reprogramming

Involvement of RBP-X and RBP-Y in silencing in ECCs.

To confirm if RBP-X is also required for silencing in ECCs, silencing was assessed in F9 cells. In addition to siRBP-X, an siRNA targeting RBP-Y, which is complexed with RBP-X when binding RNA, was also transfected. Then, F9 cells were transduced with retrovirus harboring either PBS or PBSQ.

The PBS retrovirus was silenced right after infection, with no observable effect from siRNA transfection. However, I observed higher and transient expression from PBSQ retrovirus after siRBP-X or siRBP-Y transfection, as compared to the siLuc control. These results suggest that silencing consists of early and late phases, which are PBS-independent and PBS-dependent, and that RBP-X and RBP-Y have a role in the former one.

Future Plans

The goal of this research is to elucidate a more detailed molecular mechanism by which these two RNA binding proteins are involved in retroviral silencing.

To achieve this, I plan to develop a method to quantitatively asses the silencing induction in F9 cells by FACS for more detailed analyses. Then, I would like to overexpress RBP-X and RBP-Y proteins, as well as their deletion mutants, to investigate their molecular function on the silencing induction. As RBP-Y has two isoforms, I also plan to determine which isoform is involved in retroviral silencing.