Retroviral Silencing Mechanisms in Different Types of Stem Cells

Efrat Harel, Sharon Schlesinger

Department of Animal Science, Robert H. Smith Faculty of Agriculture, Food, and Environment, The Hebrew University of Jerusalem

Introduction

Retroviruses are viruses that use RNA as their genetic material. When they infect a host cell, they insert a copy of their own genome into the host's DNA through reverse transcription. Our genome is full of these intruders – about 8% of the human and the mouse genomes are composed of endogenous retroviruses (ERV), which are derived from retroviruses that have infected germline cells millions of years ago.

Embryonic stem cells (ESC) are pluripotent stem cells, originating from the inner cell mass of the blastocyst. They are able to differentiate into all three primary germ layers: ectoderm, mesoderm and endoderm; thus, they are the cells that compose the entire embryo. Due to their critical role in the embryonic development, ESC have acquired the ability to restrict the expression of endogenous and exogenous retroviruses, using several mechanisms.

Our work is focusing on Moloney Murine Leukemia Virus (MMLV), which is silenced effectively in ESC and in Embryonal Carcinoma Cells (ECC). Silencing of this retrovirus is carried out by Trim28, which recruits an epigenetic silencing complex. Trim28 binds to the virus' LTR by two zinc finger proteins; one of them is ZFP809[1], which recognizes an 18-base pair sequence complementary to the 3' end of a cellular tRNA, called the Primer Binding Site (PBS). MLV anneals the proline tRNA to its PBS (PBS-P) and uses it as its primer for reverse transcription. This silencing mechanism is a hallmark of pluripotency, but do other stem cells also possess this unique ability? If they do, are they using the same mechanism? These questions have yet to be answered.

Methodology

Retroviral silencing in different types of stem cells:
Cancer Stem Cells (CSC) of Glioblastoma Multiforme and Trophoblast Stem Cells (TSC) were infected with MMLV, carrying either a native PBS site (PBS-P) or a mutant PBS site that anneals glutamine tRNA (PBS-Q), which is silenced less effectively. Additionally, the retroviruses were carrying an mCherry or a GFP reporter. Expression rates of the viruses were analyzed using Flow Cytometry, and compared to NIH3T3 cells (positive control) and mESC (negative control).

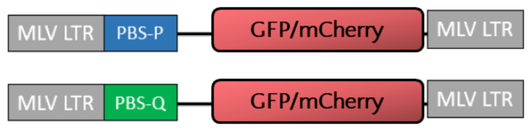

Illustration: Retroviral vectors carrying a reporter gene

Post-translational phosphorylation of Serine 824 of Trim28 effect on retroviral silencing:
GFP positive ECC cells were infected with variants of hTrim28 lentiviruses: S824A, in which serine was replaced with alanine, mimicking the unphosphorylated protein, and S824D, in which serine was replaced with aspartate, mimicking the phosphorylated protein. Cells were also infected with mTrim28 shRNA (KD), separately or together with the hTrim28 variants. GFP expression rates were later analyzed using Flow Cytometry and RT-qPCR.

Results

Cancer Stem Cells do not silence retroviruses

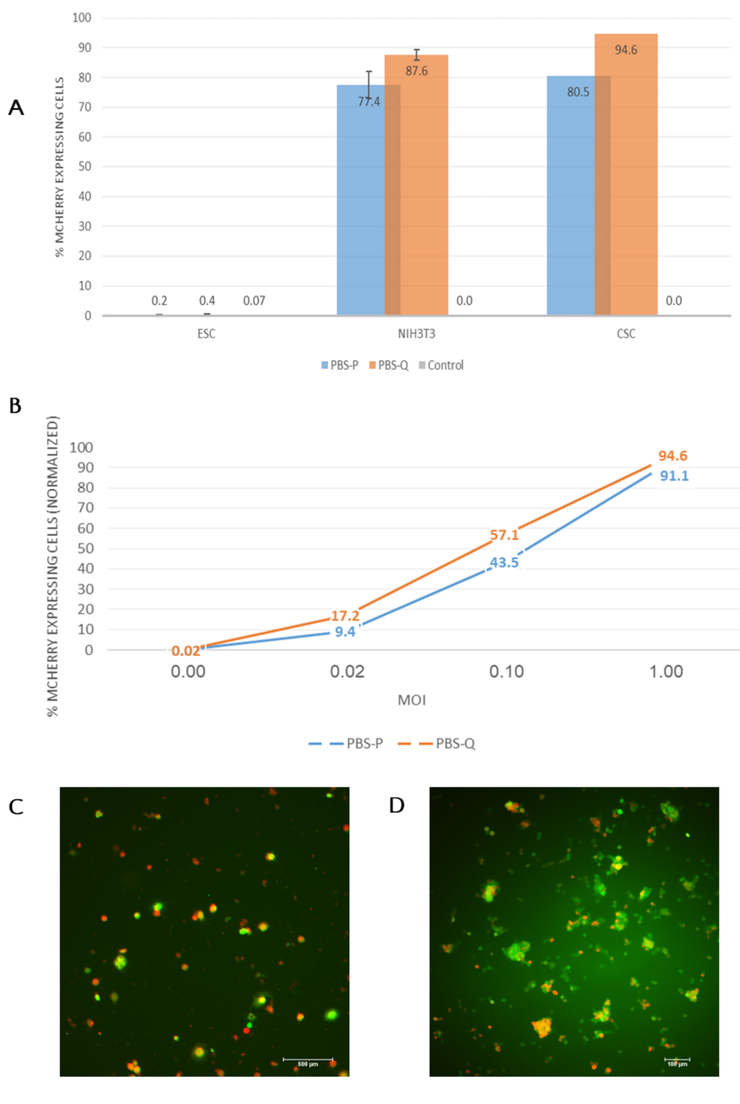

Fig. 1
(a). CSC were infected with mCherry viruses at an MOI of 1. They showed no silencing, expression rates were even higher than control cells NIH3T3. Error bars show ± SEM with n=2 (ESC, NIH3T3)
(b). CSC were then infected with the same viruses at an MOI of 0.1 and 0.02, to understand whether silencing is concealed by their high mitotic activity; but still no silencing was shown

Fluorescent microscope images:
(c). CSC infected with PBS-Q mCherry MLV (MOI 1), 24h after infection, x40
(d). CSC infected with PBS-Q mCherry MLV (MOI 0.1), 72h after infection, x100

Trophoblast Stem Cells silence retroviruses

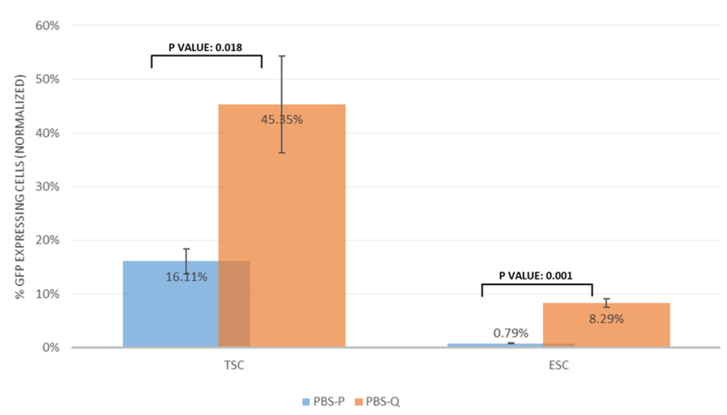

Fig. 2
TSC were infected with GFP viruses at an MOI of 0.33. Compared to negative control (ESC) and normalized to NIH3T3 expression rates, they showed significant retroviral silencing that could potentially be attributed to the Trim28-containing silencing complex. Error bars show ± SEM (n=4)

Fold change averages

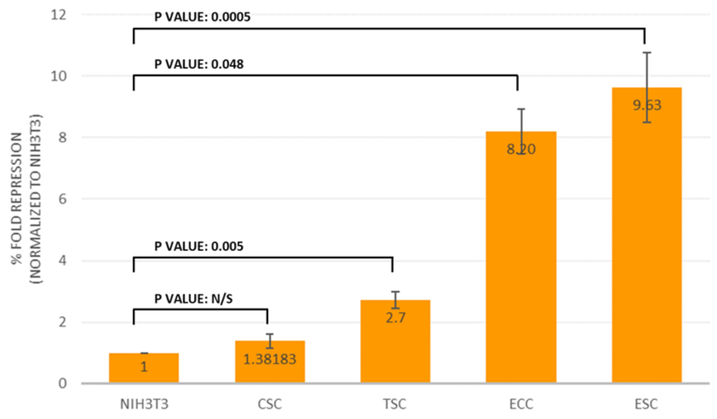

Fig. 3
NIH3T3, ESC, TSC, ECC and CSC were infected with either PBS-P or PBS-Q MMLV carrying reporter genes. This graph shows the ratio of PBS-Q/PBS-P infection efficiency in each cell line, normalized with NIH3T3 = 1. Mean ± SEM with n=2 (ECC, CSC) and n=4 (TSC, ESC)

Phosphorylation status of residue S824 on Trim28 does not affect retroviral silencing

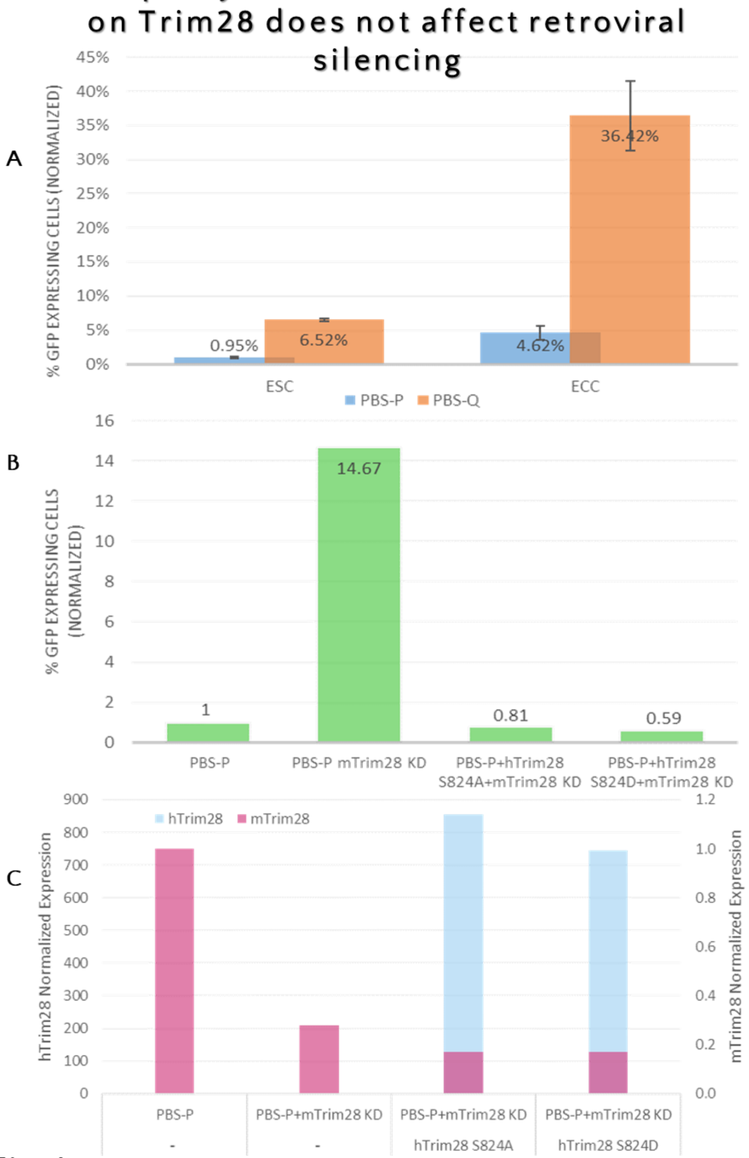

Fig. 4
(a). ECC were infected with GFP viruses at an MOI of 0.33. Compared to negative control (ESC) and normalized to NIH3T3 expression rates, they showed retroviral silencing, as previously shown[2]. Mean ± SEM (n=2).
(b). GFP positive ECC were infected with lentiviruses carrying mTrim28 shRNA (KD) and/or hTrim28 expression plasmids, mimicking the unphosphorylated protein (S824A) and phosphorylated protein (S824D). Flow cytometry analysis showed no notable differences between the hTrim28 variants, as they both "rescued" the KD phenotype. Each hTrim28+KD was normalized to its hTrim28 analogue.
(c). Same cells were analyzed using RT-qPCR, to verify insertion of hTrim28 plasmids and depletion of mTrim28 expression, following KD. Each hTrim28+KD was normalized to its hTrim28 analogue.

Conclusions

- The CSC we used did not silence retroviruses, as opposed to previous reports[3]
- TSC silence retroviruses
- Phosphorylation of S824 of Trim28 does not impair its function in the retroviral silencing complex. This accords with previous works[4]

References

1. Wolf D, Goff SP. 2009. **Embryonic stem cells use ZFP809 to silence retroviral DNAs**. Nature. 458:1201–4
2. Teich NM, Weiss RA, Martin GR, Lowy DR. 1977. **Virus infection of murine teratocarcinoma stem cell lines.** Cell. 12:973–82
3. Cuellar TL, Herzner A-M, Zhang X, Goyal Y, Watanabe C, et al. 2017. **Silencing of retrotransposons by SETDB1 inhibits the interferon response in acute myeloid leukemia.** J. Cell Biol. 216:3535–49
4. Lee A, CingÖz O, Sabo Y, Goff SP. 2018. **Characterization of interaction between Trim28 and YY1 in silencing proviral DNA of Moloney murine leukemia virus**. Virology. 516:165–75