doi 10.18699/CRISPR-2025-69

Playing with X-chromosome epigenetics in human primed and naïve pluripotent stem cells

Mhd.A. Arssan*, A.I. Shevchenko, I.S. Zakharova
Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia
** arsanmhdamin@yandex.ru*

Key words: human pluripotent cells; naïve and primed pluripotency; X chromosome epigenetics; SRC kinase signaling

Human pluripotent stem cell (hPSC) lines bearing two X chromosomes maintain them in a heterogeneous epigenetic state. Primed hPSCs mirroring late post-implantation epiblast at gastrulation onset display one active (Xa) and one inactive (Xi) X chromosomes. However, Xi often became partially re-activated, showing what is termed an 'eroded state' (Xe) that invariably passes on to differentiation derivatives, causing a deleterious imbalance in gene dosage. Naïve hPSCs reflecting early pre-implantation epiblast show two Xa, while previous Xi or Xe is not complete reactivated chromatin and remembers it was inactive. Differentiation of naïve hPSCs results in skewed X chromosome inactivation favouring the former Xi. Furthermore, not all naïve media permit X chromosome inactivation after hPSC differentiation. Therefore, strategies are needed to control the X chromosome epigenetic states to ensure that naïve and primed hPSCs have no limitations for biomedical applications.

In this study, we found that the SRC kinase inhibitor (SRCi) is essential for competence to restart X chromosome inactivation (XCI). SRCi is a key component in the media that enables naïve hPSCs to regain its ability to undergo biased XCI upon differentiation. Furthermore, we demonstrated that SRCi can trigger XCI in primed hPSC lines, restoring the normal Xi state. Interestingly, in this case, XCI restarts via a transient stage when inactive markers are appeared on both X chromosomes. We speculate that the reason for skewed XCI is that the X chromosomes in naïve hPSCs do not become epigenetically equivalent. Indeed, we found that naïve hPSCs with epigenetically equivalent X chromosomes can trigger random XCI during differentiation. Moreover, we demonstrated that the capacity to reproduce random XCI is restored when naïve hPSCs are shifted to the totipotent state. This transition presumably erases the epigenetic memory of the former Xi and reestablishes the ability to implement the mechanisms of counting and random X-chromosome choice upon XCI during differentiation. Overall, we have developed several approaches to regulate the epigenetic state of the X chromosome in both naïve and primed hPSCs. These approaches will contribute to the stability of hPSC lines and their differentiated derivatives, providing reliable results for biomedical research and paving the way for hPSC use in regenerative medicine.

Funding: The work was supported by the State project of the Institute of Cytology and Genetics, FWNR-2022–0015.