Commentary

Stem Cell INVESTIGATION

MLL1: the thin red line divides naïve and primed pluripotency

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From mouse pre-implantation and early post-implantation embryos, stem cells at different pluripotent states can be captured in *in vitro* culture. For example, naïve embryonic stem cells (ESC) are derived from inner cell mass (ICM) of the blastocyst, while primed epiblast stem cells (EpiSC) are captured from the post-implantation embryo epiblast. Though both ESCs and EpiSCs are pluripotent stem cells, they have distinct characters in terms of pluripotent gene expression profile, epigenetic status, metabolic pathways, growth factor requirement, and in female, the X chromosome inactivation status (1). On the other hand, these two pluripotent states are interchangeable, once switching the culture condition of ESCs to that for EpiSCs, naïve ESCs convert to EpiSCs. The efficiency of converting EpiSCs to ESCs, however, is much low, which usually requires over-expressing pluripotency-associated genes such as *Nanog*, *Klf4*, *Esrrb*, *Tfcp2l1* and *Nr5a2* (2-6). Similar to somatic cell reprogramming to induced pluripotent stem cells, converting EpiSCs to ESCs is an epigenome-resetting process. Characterizing these epigenetic barriers represents an important approach to further improve reprogramming efficiency (7).

In the April issue of *Cell Stem Cell*, Zhang *et al.* discovered that histone H3K4 methyltransferase MLL1 is one of the major barriers that hinders EpiSCs conversion to ESCs (8). First, they analysed the expression of MLL family members during ESC differentiation, and found that expression of MLL1 was particularly up-regulated and correlated with the expression of epiblast markers, *Fgf5*, *Cer1*, etc. To understand the biological role of MLL1 during ESC differentiation, they used an inhibitor of MLL1, MM-401 in the study. Treatment of ESCs with MM-401 delayed conversion to EpiSCs, implicating MLL1's functional roles in this process.

Next, they examined the role of MLL1 in the conversion of EpiSCs to ESCs by MLL1 inhibition. Surprisingly, when EpiSCs were cultured in MM-401 with either LIF/KSR or bFGF/KSR for 72 h, the colonies acquired dome shaped morphology similar to ESCs, and stable ESC like lines were established with continued culture. Remarkably, after 72 h treatment of MM-401, 49.1% and 32.0% of cells became PECAM1^{+} in LIF/KSR and bFGF/KSR, respectively, demonstrating high conversion efficiencies. Genetically, deletion of *Mll1* in EpiSCs or knocking down *Mll1* robustly induced ESCs reversion.

In female pluripotent cells, X chromosome reactivation is one of the important criteria to differentiate naïve and primed pluripotent stem cells. Both X chromosomes are activated in ESCs, while in EpiSCs, one X chromosome is randomly inactivated (1). To assess MLL1 inhibition on X chromosome reactivation, the authors used two EpiSC lines, one is F1 hybrid $X^{Lab}X^{JF1}$ EpiSC with X^{Lab} harbors GFP transgene and truncated *Tsix* and wild-type X^{JF1} (12F); the other line has two wild type X chromosomes (9F). Upon MLL1 inhibition, at day 3, about 45% and 30% of colonies are GFP^{+} in LIF/KSR and bFGF/KSR, respectively, similar to the numbers by PECAM1 staining. For 12F EpiSCs, RNA-FISH showed loss of *Xist* coating, bi-allelic expression of X-linked genes in the converted ESCs (MLL1-rESCs), indicating the successful conversion.

The converted MLL1-rESCs were pluripotent. First, they formed mature teratomas. And then when injected into blastocysts, these cells contributed to ICM and to the germline in the chimeras.

How did MLL1 inhibition lead to the high efficient conversion from EpiSCs to ESCs? To understand the mechanism, the authors performed transcriptomic analysis. MM-401 treatment induced rapid changes of the

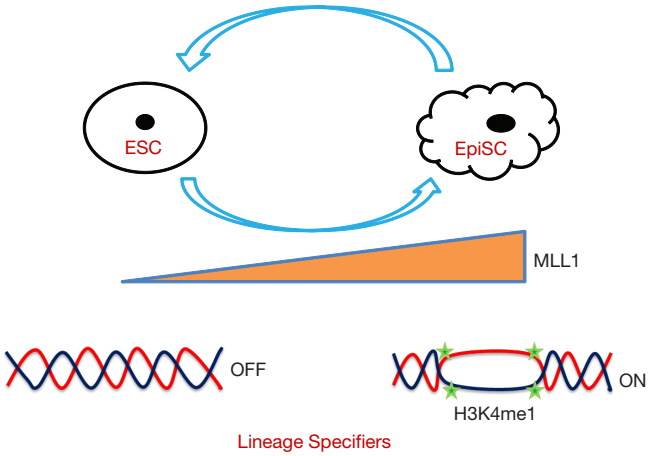

Figure 1 MLL1 in switching of pluripotent states. MLL1 is up-regulated when ESCs are differentiated. Inhibition of MLL1 in EpiSCs affects deposition of H3K4me1 on enhancers of lineage specifiers and facilitates the conversion to ESCs. ESC, embryonic stem cell; EpiSC, epiblast stem cell.

transcriptome, which eventually became similar to that of ESCs when the conversion was complete. ChIP-seq analysis identified that the majority of MLL1 binding sites in EpiSCs were in intergenic regions or introns, indicating that MLL1 functions by a mechanism via regulatory elements. Furthermore, the authors found that there were substantial differences between ESCs and EpiSCs for H3K4me1 sites (usually marking enhancers) in the genome. By combining RNA-Seq and CHIP-Seq data, they identified potential MLL1 target genes enriched with those involved in cell adhesion and development processes. Surprisingly, MLL1 did not appear to directly regulate the known pluripotency genes, demonstrating that repressing EpiSC features is a major mechanism for MLL1 inhibition induced EpiSC to ESC conversion.

The standard human embryonic stem cells (hESCs) are more similar to mEpiSCs than mESCs (9). Recently, several human naïve ESC lines have been established and characterized (10-12). It'll be interesting to examine MLL1 expression and its genome wide binding profiles in human pluripotent cells, and to test whether MLL1 inhibition in hESCs can also facilitate the conversion of hESCs to naïve hESCs. Besides its role in the conversion of different pluripotent cells, it's important to investigate whether MLL1 is involved in reprogramming mouse and human somatic cells to induced pluripotent stem cells (iPSC) by Yamanaka factors, where epigenetic resetting has an essential role (13). What are the main targets of MLL1 in somatic cells, e.g., fibroblast? Are lineage specifiers regulated by MLL1? Two of the Yamanaka factors, Oct4 and Sox2 are known to down-regulate ectodermal (ECT) and mesendodermal (ME) genes, respectively (14), will MLL1 inhibition replace one or both of these factors? Also, lineage specifiers of ECT and ME can induce pluripotency without Oct4 and Sox2 (14,15), in this milieu, will MLL1 inhibition block the reprogramming?

Overall, to convert cells from one state to another, the epigenetic barriers in the cells must be overcome to turn off the genes of the parental cell identity and to turn on those ones for establishing and maintaining the new cell identity. Zhang *et al.* set a good example to prove that by simply inhibiting the activities of an epigenetic modifier, the pluripotent state can be efficiently changed as summarized in *Figure 1*. It is anticipated that more epigenetic modifiers will be identified for their functions in the switching of cell states.

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Footnote

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