

Zbtb7a and Zbtb7b: Opening naïve loci to reprogram ESCs

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SUMMARY Bone morphogenetic protein 4 (BMP4) was recently reported to confer reprogramming capability to embryonic stem cells (ESCs) by reactivating naïve pluripotency genes *via* Zbtb7a and Zbtb7b. A visual reporting system was developed to first identify BMP4 as a driver for the primed-to-naïve transition (PNT). In addition, two specific inhibitors were identified as significantly improving the efficiency of PNT (~80% transition) within 8 days. The Zbtb7 family members were first introduced in the context of PNT and stem cell fate decision-making. These findings provide valuable information on acquiring naïve pluripotent stem cells for regenerative medicine.

Keywords BMP4, fate decision-making, PNT, reprogramming

The primed-to-naïve transition (PNT) between embryonic stem cells (ESCs) and epiblast stem cells (EpiSCs) is finely tuned by a set of transcription factors (TFs) and chemicals (1). Naïve pluripotency is defined as the self-renewal stage of ESCs, which gives rise to a whole embryo that expresses genes such as octamer-binding transcription factor 4 (Oct4), SRY-box transcription factor 2 (Sox2), Nanog homeobox (Nanog), Kruppel-like factor 4 (Klf4), and estrogen-related receptor beta (Esrrb). Primed pluripotency means that epiblast stem cells (EpiSCs) are capable of trans-lineage differentiation when genes are activated soon after implantation, such as fibroblast growth factor 4 (Fgf4), fibroblast growth factor 5 (Fgf5), and SRY-box transcription factor 17 (Sox17). PNT could serve as a good model to reveal the determination of cell fate. The process of inducing pluripotency is slow and inefficient, which are the most significant bottlenecks. A recent study by Yu *et al.* offered new insights into the relationship between chromatin remodeling and pluripotency maintained by bone morphogenetic protein 4 (BMP4) (2).

BMP4 is a secreted signaling molecule that belongs to the transforming growth factor-beta (TGF- β) superfamily. Previous studies indicated that Bmp4-deficient mice suffered embryonic lethality due to delayed epiblast growth as early as embryonic day 6.5 (E6.5). Despite mounting evidence suggests that BMP4 enables the exit of ESCs from pluripotency and drives their differentiation, no study has reported a need for BMP4 in reorganizing chromatin. BMP4 and retinoic acid are reported to team up with p63 to

contribute to increase DNA methylation and to reduce chromatin accessibility (3). Gene expression is closely linked to chromatin accessibility, so whether BMP4 regulates chromatin interaction during PNT has yet to be determined.

To elucidate the mechanisms by which BMP4 determines the fate of ESCs, Yu *et al.* developed a visual reporting system to first identify BMP4 as a driver for PNT. In addition, small molecules were screened to identify those enhancing PNT. Among 606 compounds, two small molecules were identified: the enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) inhibitor EPZ-6438 (tazemetostat) and the DOT1-like histone lysine methyltransferase (DOT1L) inhibitor EPZ-5676 (pinometostat). According to clonal analysis, which indicated the capacity for reprogramming, EPZ-6438 and EPZ-5676 induced PNT with sufficient efficiency either alone or in combination. When the two inhibitors were combined, a highly efficient induction system was created; it facilitates up to 80% transition within 8 days. Subsequently, an assay for targeting accessible-chromatin with high-throughput sequencing (ATAC-seq) provided compelling evidence that Zbtb7a and Zbtb7b were the key regulators of cell fate. These findings provide valuable information on acquiring naïve pluripotent stem cells for regenerative medicine.

A point worth noting is that a unique chromatin structure is involved in sustaining naivety. During the BMP4-induced PNT (BiPNT) of ESCs, a precise coordination of chromatin accessibility dynamics (CAD) occurs: a substantial number of loci specific

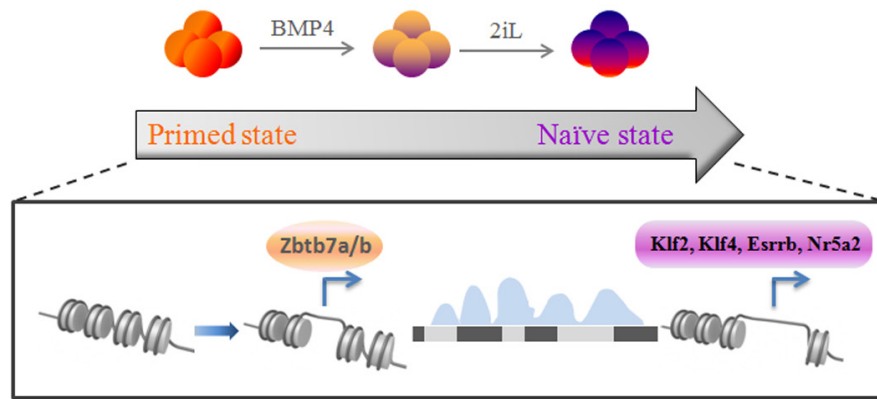


Figure 1. Rapid reprogramming of BMP4 to induce naïve stem cells allows chromatin remodeling induced by the regulation of transcription factors. Induced expression of a Zn finger/BTB domain containing protein 7a or b (*Zbtb7a* and *Zbtb7b*) is a crucial early response during BMP4-induced naïve-primed pluripotent transition (PNT). *Zbtb7a* and *Zbtb7b* play a critical role in opening chromatin loci to express core pluripotent factors such as *Klf2*, *Klf4*, *Esrrb*, and *Nr5a2*.

to the primed state close over the first 3 days and then more loci related to the naïve state reopen over the next 5 days under 2 iL. In addition, the combined results of ATAC-seq, gene ontology (GO) analysis, and chromatin immunoprecipitation sequencing (ChIP-seq) suggested that *Zbtb7a*, *Zbtb7b*, and *Zbtb7c* were directly targetable genes capable of independently initiating PNT. *Zbtb7a*, *Zbtb7b*, and *Zbtb7c* belong to the POZ/BTB and Kruppel (POK) family of transcription factors that are capable of context-dependent regulation of cell development as well as tumorigenesis. A rescuing experiment indicated that *Zbtb7a* and *Zbtb7b* were needed to activate naïve genes such as *Nanog*, *Klf2*, *Esrrb*, and *Nr5a2*. These findings provide a comprehensive picture of *Zbtb7a* and *Zbtb7b* in altering the pluripotency gene network and determining cell fate (Figure 1). Recently, Liu *et al.* (4) provided a molecular roadmap of reprogramming for pluripotency at the single-cell level. They suggested that the trophoblast-lineage-specific regulatory program was involved in reprogramming human somatic cells. Their findings suggest the existence of sub-branches as alternative pathways for determination of cell fate.

Several studies have indicated that chromatin-modifying enzymes play vital roles during the epigenetic reprogramming of pluripotency. The EZH2 inhibitor tazemetostat (EPZ-6438) and the DOT1L inhibitor pinometostat (EPZ-5676) have proven to be effective in treating a variety of solid tumors and hematological malignancies. Numerous studies have indicated that chromatin-modifying enzymes both silence domains and reactivate loci during the epigenetic reprogramming of pluripotency. Tazemetostat facilitates reprogramming mainly by preventing the acquisition of an epithelial-like phenotype. Interestingly, tazemetostat and pinometostat exhibit similar potency in reversing the mesenchymal-epithelial transition (MET) in different cancers (5). Generally speaking, these results indicate that selective chromatin modifiers can generate naïve stem cells in a more efficient manner with fewer exogenous

transcription factors.

The differentiation of ESCs into cardiovascular lineages has provided important insights into their regulation during heart development. In embryos, the lateral/cardiac mesoderm originates from the middle of the primitive streak. Cardiac progenitor cells (CPCs) from the lateral/cardiac mesoderm differentiate into cardiomyocytes (CMs), cardiac fibroblasts (CFs), vascular smooth muscle cells (VSMCs), and vascular endothelial cells (VECs), producing the heart. The specific transitions require sequential expression of distinct transcription factors and proteins that facilitate the change in state from pluripotency to mesoderm and then to a cardiovascular fate. A recent study found that T-box transcription factor 6 (*Tbx6*) directs cardiovascular differentiation (6). Mounting evidence suggests that during the cardiovascular differentiation of ESCs, key transcription factors determine multiple cell fates. Such as, *Nkx2.5*, *GATA-binding protein 4* (*Gata4*), *Mef2c*, *Tbx2*, *Tbx3*, *Tbx5*, and *connexin 43* (*CX43*) direct the differentiation of cardiomyocytes; *Gata6* and *nuclear factor erythroid 2-related factor 3* (*Nrf3*) direct the differentiation of VSMCs; *hyaluronan and proteoglycan link protein 1* (*Hapln1*) and *high-mobility group AT-hook protein 1* (*Hmgal1*) direct the differentiation of VECs; and *CX40* and *troponin I type 1* (*TNNI1*) direct the differentiation of CFs. In addition, growth factors such as *WNT*, *Nodal/Activin*, *BMP*, *platelet-derived growth factor* (*PDGF*), *vascular endothelial growth factor* (*VEGF*), and *neuregulin 1* (*NRG1*) are involved in complex regulatory networks to determine cell fates. Moreover, epigenetic modification, such as histone acetylation and methylation, DNA methylation, ubiquitination, and *N(6)-methyladenosine* (*m6A*) modification, has been found to play a vital regulatory role in naïve pluripotency and differentiation, possibly providing a bridge between extracellular cues, chromatin remodeling, and signaling in cardiogenesis (7-10). Comprehensive analysis with multi-omics such

as single-cell RNA sequencing (scRNA-seq) and spatial transcriptomic profiling may help to map the landscape of temporal and spatial changes in the expression of genes specific to cardiovascular lineage commitment. All of these efforts will help to improve efficiency in deciding cell fate and to strictly control the delivery of ESCs to the heart. Comprehensive molecular and functional analysis of systems for differentiation of ESCs will provide a valuable foundation for disease modeling, regenerative medicine, and drug discovery to treat cardiovascular diseases.

In summary, a study by Pei and his colleagues has provided a new understanding and entry point for studying the chemical regulation of changes in cell fate. Their work will help to guide research on the acquisition of naïve pluripotent stem cells in human or other systems. Since a global epigenetic reorganization occurs during differentiation, more specific inhibitors need to be identified to obtain genetically stable and self-renewing naïve stem cells. Moreover, an analysis of roadmaps using multimodal single-cell assays may help to identify pluripotent subpopulations of stem cells. In addition, determining the molecular pathways that regulate stem cell renewal may provide a deeper understanding and facilitate advances in embryology.

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