Editorial

Stem Cell INVESTIGATION

Bmi1-mediated epigenetic signature acts as a critical barrier for direct reprogramming to mature cardiomyocytes

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Direct lineage reprogramming of specialized cell types has eliminated some of the traditional problems regarding the epigenetic instability of induced pluripotent stem cells (iPSCs) (1). The adult heart has limited regenerative potential and the predominant response after cardiac injury is dysfunctional fibroblast proliferation. Direct reprogramming of induced cardiomyocytes (iCM) is an increasingly promising option for further development of novel treatments for heart pathologies. The technology is still inefficient and the main mechanisms involved in reprogramming are largely unknown; nonetheless, RNAi screens, single-cell analysis and simpler genetic methods are beginning to focus on epigenetic status as a key barrier (2).

To address this question, Zhou *et al*. used a loss-of-function screening for epigenetic regulators with an important role in iCM (3). The authors used neonatal cardiac fibroblasts from a transgenic α-muscle heavy chain (αMHC)-GFP mouse to evaluate the combined reprogramming efficiency of Mef2c/Gata4/Tbx5 (MGT) transcriptional factors. iCM were defined, after 10 days of culture, by αMHC (GFP+) and/or cTnT+ expression.

The study found that one out of three knockdown epigenetic regulators (e.g., Ing1, Plu1) reduced reprogramming efficiency, confirming the necessity of histone acetylation for efficient iCM reprogramming. In addition, two main epigenetics-associated proteins were identified as barriers to iCM reprogramming. One was the lymphoid-specific helicase (Hells/Lsh/Smarca6), a SWI/SNF complex protein, and the polycomb transcription factor Bmi1, an essential component of the polycomb repressive complex 1 (PRC1). Knockdown of these proteins promoted a 6- and 10-fold increase, respectively, in the percentage of iCM generation. shBmi1-iCM showed the highest iCM reprogramming efficiency, a significant increase in α-actinin expression, assembled in sarcomeres, and a two-fold increase in beating iCM. These data demonstrate that Bmi1 knockdown greatly enhances MGT-mediated iCM reprogramming of neonatal fibroblasts. Zhou *et al*. (3) also demonstrated that this positive effect of Bmi1 knockdown is sustained by several other combinations of reprogramming transcription factors. They further confirmed that shBmi1 derepressed Gata4 activity during reprogramming substituting the exogenous Gata4 requirement during the process. The shBmi1 effect is not limited to embryonic or neonatal cell lines, but also promoted iCM reprogramming in adult mouse fibroblasts and adult mouse $CD31^+$ endothelial cells, which suggests adult cardiac cell plasticity; this highlights Bmi1 as a key molecule that limits cardiomyogenesis in adult mice (4). Bmi1 knockdown does not appear to improve direct reprogramming in all somatic lineages, however, because it does not increase neuron-reprogramming efficiency.

The positive effect provoked by Bmi1 knockdown is confirmed mechanistically (for both αMHC and cTnT expression) as early as three days after cotransduction, and is efficient when the vector is introduced early in iCM reprogramming (three days before or after MGT transduction). The data also suggest that Bmi1 suppresses iCM generation independently of its role in regulating its downstream effectors involved in cell proliferation (p16Ink4a, p19Arf and p53). Together with the catalytic ring finger proteins Ring1A and Ring1B, Bmi1 inhibits target gene expression through monoubiquitination of histone H2A at lysine 119 (H2AK119ub), either dependent on or

independent of the PCR2 complex. Direct target analysis during iCM reprogramming found strong Bmi1 binding peaks at the regulatory regions of critical cardiogenic genes in unreprogrammed fibroblasts; these binding sites overlapped with H3K4me3 (epigenetic active mark) or H3K27me3 (repressive mark)-occupied sites. ChIP-qPCR experiments (chromatin immunoprecipitation-quantitative real-time PCR) showed that these Bmi1-bound cardiac loci were also co-occupied by H2AK119ub, Ring1B and Ezh2 (a key component of the PRC2 complex), suggesting a repressive chromatin state. Following Bmi1 depletion, H2AK119ub was completely removed and H3K4me3 levels were moderately increased at the cardiogenic loci. All these results strongly suggest that the increase in epigenetic active marks and the reduction in epigenetic repressive marks correlate with Bmi1 knockdown and derepression of cardiogenic gene expression, to promote iCM yield and maturation.

Bmi1 was previously found necessary for reprogramming iPSC (5), and Bmi1 expression is one of the shared features of many adult stem cell compartments (6). It is thus logical to hypothesize that Bmi1 expression is relevant for maintaining multipotent precursors, but its downregulation must be critical for bypassing this stage, thus facilitating the onset of differentiation and maturation programs (at least for iCM). Importantly, these effects are cell type and reprogramming type specific, not only for Bmi1 but also for other author-tested epigenetic regulatory factors like *Plu1*, suggesting functional redundancy and compensation between epigenetic complexes.

In conclusion, Zhou *et al*. (3) establishes that there is an essential epigenetic barrier for efficient, direct reprogramming of fibroblast to iCM, in which Bmi1 is a central element. As the authors discuss, given the functional conservation of many epigenetic regulators between mouse and man, it is likely that removing similar epigenetic barriers in human fibroblasts will lead to improved efficiency of human iCM generation, which must be further evaluated in clinical studies. The authors are to be congratulated for adding a new and exciting dimension to the area of genetic reprogramming.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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