Editorial

EZ Switch From EZH2 to EZH1
Histone Methylation Opens a Window of Cardiac Regeneration
Hyun Kook, Sang-Beom Seo, Rajan Jain

An important area of research is the functional significance of posttranslational modifications of histone proteins. The modification of histones is maintained by a balance of enzymes which place specific modifications (writers), factors which read modifications (readers), and enzymes which remove modifications (erasers). Histone methylation is regulated by histone methyltransferases (writers) and demethylases (lysine demethylases; erasers). Lysine residues can be mono- (me1), di- (me2), or tri- (me3) methylated with different resultant functions (Figure A). A connection between histone methylation and Polycomb group–mediated gene silencing was established by purification and characterization of the Polycomb repressive complex 2 (PRC2). PRC2 is essential for maintenance of histone H3K27me3 in embryonic stem cells, and enhancer of zeste 1 (EZH1) and EZH2, SUZ12, and EED are the key components of the PRC2 complex. EZH2, a histone methyltransferase, catalyzes H3K27me3, and it has been shown to be dysregulated in various cancers. EZH1 is an alternative core subunit to EZH2 in PRC2 complexes. Increasing evidence indicates that EZH2 is highly expressed in cancer stem cells and mediates cancer stem cell expansion and maintenance.

The Polycomb group family of proteins has been shown to be an important epigenetic regulator during heart development and cardiac myocyte differentiation. Mice with genetic deletion of Ezh2 die before embryonic day 7 owing to a failure of gastrulation. In cardiac myocyte–specific conditional knockout mice, He et al found that Ncx2.5-Cre–mediated deletion of Ezh2 results in a spectrum of congenital heart defects, such as failure of myocardial compaction, hypertrabeculation, and ventricular and atrial septal defects. Similar findings have also been published by independent research groups. Interestingly, however, He et al also observed that deletion of Ezh2 using cTNT-Cre, a cardiomyocyte-specific Cre driver (E2KO), did not result in congenital heart defects. Consistent with context-dependent roles of Ezh2, cardiac progenitor–specific knockout of Ezh2 in the anterior heart field using Mef2c-AHF-Cre results in normal cardiac myocyte differentiation and morphology during embryogenesis but results in cardiac hypertrophy postnatally.

The article by Ai et al in this issue of Circulation Research points to functional redundancy of EZH1 and EZH2 in the developing heart; the authors observed no abnormal cardiac phenotypes in Ezh1 knockout (E1KO) mice during embryogenesis. However, deletion of both Ezh1 and Ezh2 (double knockout [DKO]) resulted in lethality characterized by hypertrabeculation of myocardium, compact myocardial hypoplasia, and ventricular septal defects. EED is a core component of the PRC2 complex, and consistent with the DKO phenotype, conditional deletion using cTNT-Cre resulted in myocardial hypoplasia and ventricular wall thinning. Considering that EED deletion in myocytes leads to a similar phenotype, removal of both EZH1 and EZH2 might result in disruption of the PRC2 complex similar to the deletion of EED. Table summarizes the cardiovascular phenotypes as a result of knocking out various components of the PRC2 components.

The authors also found that total H3K27me3 levels were diminished in E2KO embryonic hearts but not in E1KO cardiac tissues. Interestingly, H3K27me3 was further decreased in DKO mice as compared with E2KO embryonic hearts. The number of dysregulated genes, as well as the magnitude of dysregulation, was greater in the DKO mice than in E2KO mice. Analysis of H3K27me3 promoter occupany of those dysregulated genes was consistent with a critical role for EZH1/EZH2 in the maintenance of this histone modification. Gene ontology analysis suggested that EZH1/EZH2 function by repressing noncardiac genes. Interestingly, the promoters of ≈20% of upregulated genes in DKO mice were not occupied by H3K27me3. In addition, approximately one fourth of dysregulated genes were downregulated in mutant tissues as compared with control. These effects might be caused by additional noncanonical mechanisms or by indirect effects like derepression. Interestingly, the redundancy of EZH1 and EZH2 has been reported in other tissues; chondrocyte-specific knockout of Ezh1 and Ezh2 induces failure of skeletal growth.

Ai et al extended their studies to postnatal heart regeneration. In the mouse heart, regeneration capacity rapidly declines 7 days after birth, which suggests a limited window in which cardiac myocyte can proliferate. The authors ligated the left anterior descending artery of E1KO or E2KO mice 2 days after birth, which leads to myocardial infarction. Three weeks later, E1KO mice demonstrated a marked increase in...
fibrotic scars concomitant with severely depressed heart function. Incorporation of EdU by cardiac myocytes was reduced at the border zone in the E1KO hearts, suggesting perhaps a decrement in proliferation by depletion of E1KO. Studies performed in E2KO mutant animals resulted in minimal fibrotic scarring, similar to that in wild-type control mice. Scar size was partially normalized in the E1KO mutant hearts by delivery of AAV9-cTNT-Ezh1 but not AAV9-cTNT-Ezh2. These experiments point to differential roles of EZH1 and EZH2 in the post–myocardial infarction response in cardiac myocytes.

Ai et al next addressed whether forced overexpression of EZH1 could extend the window in which cardiac proliferation occurs perinatally. The authors delivered cardiac myocyte–specific viral Ezh1 after myocardial infarction in P5 or P10 E1KO mice. In both cases, Ezh1 reduced fibrotic scarring and improved cardiac function. It is noteworthy that P10 is out of the window of cardiac regeneration, but EZH1 overexpression may prolong the window. In brief, (1) EZH2 shapes cardiac progenitor cell behavior; (2) EZH1 and EZH2 are redundantly required for the proliferation/differentiation of cardiac myocytes and maturation of myocardium in the relatively late phase of embryonic development; (3) EZH1 works to maintain the perinatal window of cardiac myocyte proliferation; (4) EZH1, but not EZH2, contributes to cardiac myocyte proliferation/regeneration after myocardial infarction in adult (Figure B).

Figure. PRC2 complex–mediated histone methylation and differential regulation of EZH2 and EHZ1 in heart development and regeneration. A, Histone methylation of histone H3 and core components of PRC2 complex. B, Differing roles of EZH2 and EHZ1 in the heart during embryogenesis and postnatally. EZH2 functions in cardiac progenitors in the early phase of cardiac development, whereas both EZH2 and EHZ1 are redundantly involved in the late phase of organogenesis. EHZ1 regulates cardiac myocyte proliferation and regeneration after birth. Note that a noncanonical pathway is involved in EHZ1 function in the adult heart. It is unclear whether the conventional intact PRC2 complex is required in the mechanism of EHZ1. EHZ1 indicates enhancer of zeste 1; EZH2, enhancer of zeste 2; HMTase, histone methyltransferase; KDM, lysine demethylase; MI, myocardial infarction; and PRC2, Polycomb repressive complex 2.
As in hematopoiesis, where switching of EZH2 to EZH1 was reported, this switching mechanism in the heart is intriguing. Mechanistically, the noncanonical pathway of histone modification seems to be involved in the EZH1 mechanism; EZH1 reduced H3K27me3 but increased both H3K27me1 and H3K4me3, both associated with gene expression/activation. In addition, a relatively small portion of EZH1 occupancy of promoters was co-occupied by H3K27me3. Instead, the majority of EZH1-occupied promoters were co-occupied by EZH1/EZH2 (DKO), EZH1: conventional whole body lethal in early embryo; SUZ12: conventional whole body lethal in early embryo; EZH1: cardiomyocyte in late embryo normal; EZH2: cTNT-Cre cardiomyocyte in late embryo perinatal lethality, hypoplasia, no ASD; EZH1/EZH2 (DKO): EZH1: conventional whole body hypoplasia, ASD, VSD in embryo; EZH2: cardiomyocyte in late embryo cardiomyocyte proliferation ↓ in embryo.

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Disclosures
None.

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