EZ Switch From EZH2 to EZH1

Histone Methylation Opens a Window of Cardiac Regeneration

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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).[1] 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).[2] PRC2 is essential for maintenance of histone H3K27me3 in embryonic stem cells,[3] 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.

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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.[4] In cardiac myocyte–specific conditional knockout mice, He et al[5] found that *Nkx2-5-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 group.[6] Interestingly, however, He et al[5] 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.[7]

The article by Ai et al[8] 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,[5] removal of both EZH1 and EZH2 might result in disruption of the PRC2 complex similar to the depletion of EED. Table summarizes the cardiovascular phenotypes as a result of knocking out various components of the PRC2 components.[5–9]

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 occupancy 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.[10]

Ai et al[8] extended their studies to postnatal heart regeneration. In the mouse heart, regeneration capacity rapidly declines 7 days after birth,[11] 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

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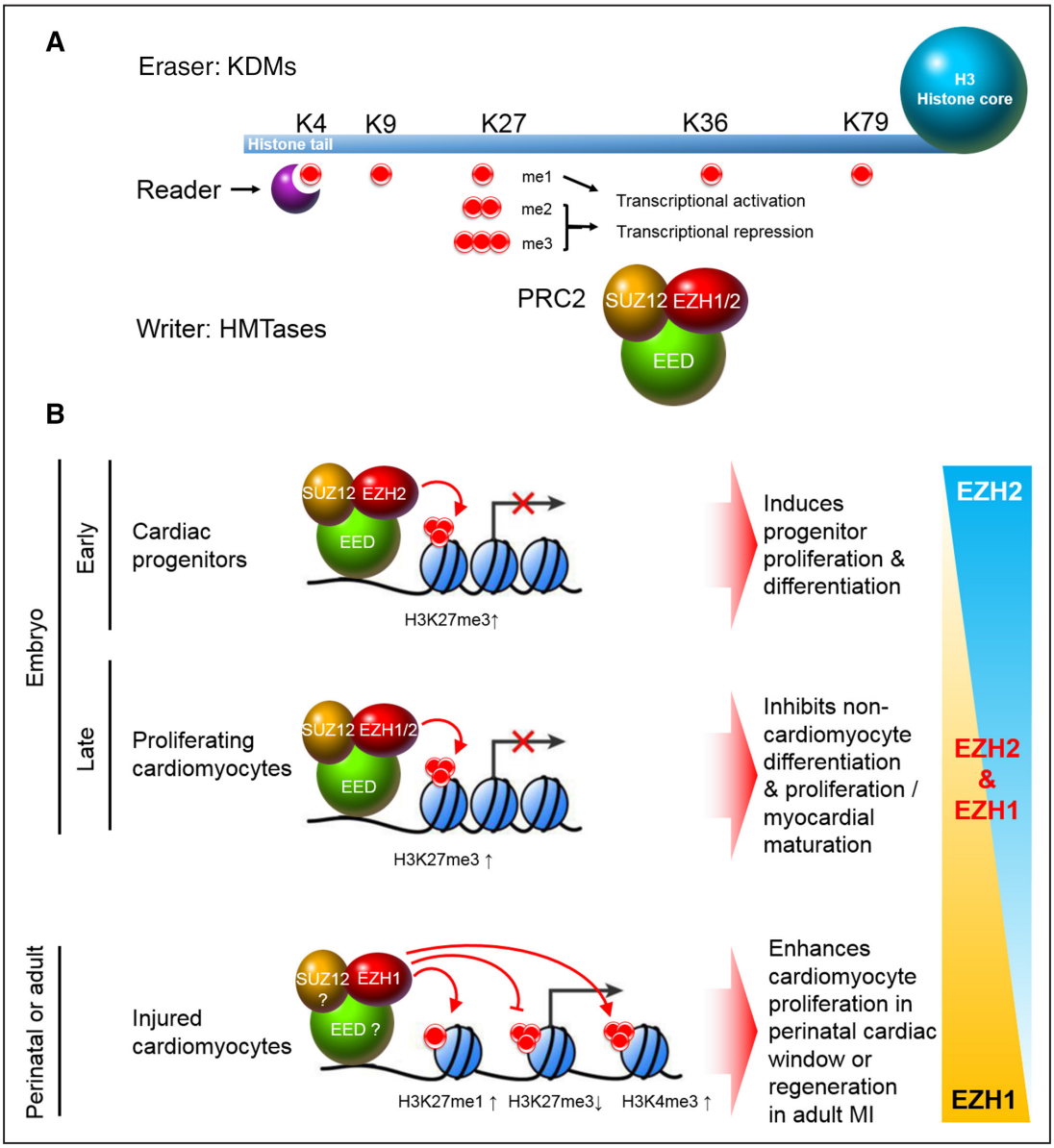

Figure. PRC2 complex–mediated histone methylation and differential regulation of EZH2 and EHZ1 in heart development and regeneration. **A**, Histone methylation of histone H3 and core components of PRC2 complex. **B**, Differing roles of EZH2 and EZH1 in the heart during embryogenesis and postnatally. EZH2 functions in cardiac progenitors in the early phase of cardiac development, whereas both EZH2 and EZH1 are redundantly involved in the late phase of organogenesis. EZH1 regulates cardiac myocyte proliferation and regeneration after birth. Note that a noncanonical pathway is involved in EZH1 function in the adult heart. It is unclear whether the conventional intact PRC2 complex is required in the mechanism of EZH1. EZH1 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.

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[8] 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,[11] 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).

Table. Summary of Knockout Phenotypes of PRC2 Components

Component	Knockout Type	Gene Removal at	Main Cardiac Phenotype	Reference
EED	cTNT-Cre	Cardiomyocyte in late embryo	Perinatal lethality, hypoplasia, no ASD, no VSD; cardiomyocyte proliferation ↓ in embryo	He et al[5]
SUZ12	Conventional	Whole body	Lethal in early embryo	Pasini et al[9]
EZH1	Conventional	Whole body	Normal embryonic development/reduced regeneration and impaired cardiac function in MI after birth	Ai et al[8]
EZH2	cTNT-Cre	Cardiomyocyte in late embryo	Normal	He et al[5]
EZH2	NKX2-5-Cre	Cardiac progenitor in early embryo	Perinatal lethality, hypoplasia, hypertrabeculation ASD, VSD in embryo/ fibrosis in adult	He et al[5] and Chen et al[6]
EZH2	Mef2c-AHF-Cre	Cardiac progenitor in AHF	Normal cardiomyocyte specification, differentiation, morphology in embryo/ RVH, fibrosis in adult	Delgado-Olguín et al[7]
EZH1/EZH2 (DKO)	EZH1: conventional EZH2: cTNT-Cre	EZH1: whole body EZH2: cardiomyocyte in late embryo	Lethal, hypertrabeculation, compact myocardial hypoplasia, VSD in embryo	Ai et al[8]

AHF indicates anterior heart field; ASD, anterior septal defect; DKO, double knockout; EZH1, enhancer of zeste 1; EZH2, enhancer of zeste 2; MI, myocardial infarction; PRC2, Polycomb repressive complex 2; RVH, right ventricular hypertrophy; and VSD, ventricular septal defect.

As in hematopoiesis, where switching of EZH2 to EZH1 was reported,[12] 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 H3K4me3, further suggesting an H3K27me3-independent mechanism of EZH1 action. Indeed, during hematopoiesis, EZH1 induces gene expression in an EED-independent fashion, suggesting a noncanonical role in gene expression.[12]

Several strategies for cardiac regeneration are currently under investigation. A variety of circulating or local progenitors have been studied extensively. To date, however, objective interpretations demonstrate that transplanted cells survive transiently and have been futile.[13] The use of pluripotent stem cells also has downsides, which need to be addressed prior to widespread use, including immune response, potential for teratoma formation, relative immaturity of cells, and most notably potential for arrhythmia.[14] Reprogramming of endogenous cardiac fibroblasts into cardiac myocytes by introducing defined factors represents another area of promise. For example, cocktails of heart-specific transcription factors or small molecules have been shown to reprogram mouse fibroblasts into cardiac myocytes.[15] In vivo conversion has been demonstrated in small rodent models,[15] and it will be imperative to demonstrate efficiency and efficacy in large animal models. Alternatively, selective reactivation of the proliferative capacity of cardiac myocytes in adult heart may provide an incredible therapeutic potential.

It was previously thought that nuclear, epigenetic factors are not amenable to targeting by small molecules. However, research over the last several years has yielded several small molecules in various phases of clinical use which target epigenetic factors, including inhibitors of histone deacetylases and reader activity of Bromodomain extraterminal domain proteins (Figure A). It will be interesting to determine if and how manipulation of epigenetic enzyme, including the EZH1/EZH2 and components of the Polycomb family of proteins, may impact emerging therapeutics for patients with heart failure by promoting regeneration.

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Disclosures

None.

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