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Commentaries on Cutting Edge Science

Mature Cardiomyocytes Recall Their Progenitor Experience Via Polycomb Repressive Complex 2

Aibin He, William T. Pu

Epigenetic Repression of Cardiac Progenitor Gene Expression by Ezh2 is Required for Postnatal Cardiac Homeostasis
Delgado-Olguín et al
Nat Genet. 2012;44:343–347.

Polycomb repressive complex 2 (PRC2) deposits repressive epigenetic marks that silence inappropriate gene expression programs. Recent work from Delgado-Olguín et al[1] in conjunction with studies from other laboratories[2–4] show that PRC2 activity in cardiac progenitors influences growth and gene expression of mature cardiomyocytes. These studies illustrate the power of epigenetic mechanisms to retain information about the heart's developmental history and deploy it to influence mature heart function.

Physiological and pathophysiological stresses experienced by the heart during gestation and postnatal life have long-standing effects on heart function. How does the heart remember its past experiences? Recent work by Delgado-Olguín et al[1] and concurrently published work from our group[2,3] and another laboratory[4] highlight the key role of epigenetic mechanisms in shaping myocardial function and provide a molecular mechanism that likely underpins molecular implementation of cardiac memory (Figure).

In current parlance, epigenetics refers to mechanisms active over rounds of cell division or between generations that lead to heritable gene expression changes without involving changes to the DNA sequence. Epigenetic regulatory mechanisms involve modifications to DNA (eg, DNA methylation) or histones, or higher-order packaging of DNA around nucleosomes. These epigenetic modifications govern DNA interaction with the transcriptional machinery to either stimulate or repress gene expression. One of the major epigenetic marks is trimethylation of histone H3 at lysine 27 (H3K27me3), a histone modification associated with gene repression.[5,6] H3K27me3 marks are deposited by the multisubunit histone methyltransferase PRC2. PRC2 is essential for embryonic stem cell differentiation because PRC2 loss of function prevents normal lineage specification by blocking the silencing of inappropriate gene expression programs.[7–9] Interestingly, H3K27me3 marks recruit PRC2 and stimulate its methyltransferase activity, providing a mechanism to maintain the repressive mark and to propagate it to nucleosomes newly established after DNA replication.[10] Thus, H3K27me3 marks are stably maintained and mediate long-term memory of the developmental and environmental history of a cell and its precursors.

The core of PRC2 is formed by a complex between the proteins EED and SUZ12 with either EZH2 or EZH1.[5,6] EZH2 and EZH1 are related SET domain methyltransferases. EZH2 is generally predominant in immature and proliferating cells, and EZH1 is generally predominant in differentiated and nonproliferating cells. EZH2 and EZH1 were partially functionally redundant in safeguarding embryonic stem cell identity and in executing pluripotency in embryonic stem cell differentiation.[8] Functional redundancy of EZH2 and EZH1 also was noted in the analysis of PRC2 function in hair follicle homeostasis.[11]

Delgado-Olguín et al observed that bulk H3K27me3 levels increase on differentiation of cardiac progenitor cells.[1] Inactivation of *Ezh2* specifically in cardiac progenitors of the anterior heart field, using Mef2c-AHF-Cre,[12] blocked the accumulation of H3K27me3, indicating that in these cells EZH2 is the predominant H3K27me3 methyltransferase. The loss of functional *Ezh2* did not impair cardiomyocyte specification or differentiation, nor did it disrupt cardiac morphogenesis. However, postnatal cardiomyocytes that descended from this progenitor subset, located in the right ventricle, ventricular septum, and outflow tract, were hypertrophic and expressed elevated levels of *Nppa*, *Nppb*, and *Myh7*, which are biomarkers of cardiac hypertrophy. The myocardium was also fibrotic and expressed elevated levels of the fibrotic genes *Tgfb3*, *Spp1*, and *Postn*. These results reveal a novel role of PRC2 in silencing hypertrophic and fibrotic gene programs in cardiomyocytes.

Inactivation of *Ezh2* by several other cardiac Cre drivers results in phenotypes that range from overtly normal to embryonic lethal. Our group[2] and Chen et al[4] inactivated EZH2 with the Nkx2-5^{Cre} knock-in allele,[13] which is active in cardiac progenitor cells. This caused late gestational and perinatal lethality, with hypoplasia of the compact myocardium, hypertrabeculation, and septal defects. This was linked to decreased cardiomyocyte proliferation and the upregulation of the cell-cycle inhibitors *Ink4a/b*. Rare survivors exhibited decreased systolic function and myocardial fibrosis. Delgado-Olguín et al and our group also inactivated *Ezh2* in differentiated cardiomyocytes using Cre transgenes driven by

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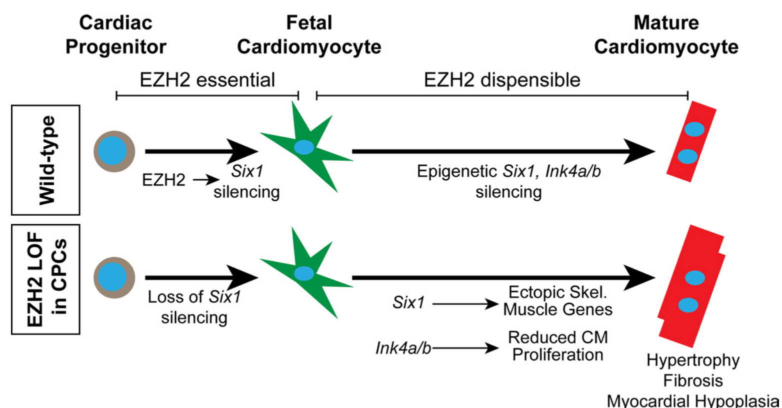

Figure. EZH2-mediated gene silencing in cardiac progenitors is required for normal regulation of adult cardiomyocyte growth and function. EZH2 deposits repressive H3K27me3 epigenetic marks in cardiac progenitor cells (CPCs), establishing a chromatin landscape that is required for silencing of *Six1*. EZH2 ablation in CPCs blocks initial deposition of these marks, derepressing *Six1* even in later stages when EZH2 activity is dispensible. SIX1 activates ectopic skeletal muscle gene expression, which contributes to adult cardiomyocyte hypertrophy and fibrosis. However, SIX1 upregulation alone is not sufficient to cause the phenotype seen in EZH2 mutants. For example, upregulation of the Ink4a/b cell cycle inhibitors likely contributes to myocardial hypoplasia.

an Nkx2-5 enhancer [Tg(Nkx2-5-Cre)][14] or by the Tnnt2 promoter (Tnnt2-Cre),[15] respectively. Unlike severe phenotypes caused by inactivation in cardiac progenitors, *Ezh2* inactivation in differentiated cardiomyocytes caused little to no overt phenotype. These results suggest that there is a crucial window during the development of cardiac progenitors in which *Ezh2* is required to establish the chromatin landscape. In differentiated cardiomyocytes, *Ezh2* becomes dispensable for normal heart development and function. Nevertheless, differentiated cardiomyocytes exhibit the aftereffects of *Ezh2* inactivation in cardiac progenitors, likely attributable to failure to establish the normal chromatin landscape required for repressing inappropriate competing gene programs. These results suggest that environmental or pathophysiological stresses also may have prolonged or delayed effects on the developing or adult heart through perturbation of the chromatin landscape.

What establishes the narrow window when *Ezh2* is required in cardiac progenitors? We gained insight into this question by inactivating the nonredundant PRC2 subunit *Eed* using Tnnt2-Cre.[2] These mutant hearts were similar to $Ezh2^{fl/fl}$::$Nkx2\text{-}5^{Cre}$ mutant hearts, indicating an ongoing requirement for PRC2 activity in differentiated cardiomyocytes. This observation suggested that *Ezh2* was dispensable in differentiated cardiomyocytes because of functional redundancy with *Ezh1*. Indeed, *Ezh1* levels increase through heart development whereas *Ezh2* levels decline, and *Ezh1* was upregulated in *Ezh2* loss of function.[2]

What key genes are directly regulated by EZH2 in the developing heart? Gene expression profiling performed by Delgado-Olguín et al revealed upregulation of a number of genes characteristic of skeletal muscle, as well as the transcription factor *Six1* and its partners *Eya1*, *Eya2*, and *Eya4*. The SIX1/EYA complex activates skeletal muscle gene expression during myoblast differentiation.[16] Interestingly, this complex also is expressed in cardiac progenitors and becomes downregulated in differentiated cardiomyocytes.[17] These data suggested the hypothesis that *Ezh2* is required for silencing *Six1* expression, so that *Ezh2* ablation causes inappropriate expression of *Six1* and skeletal muscle genes, its downstream targets, leading to cardiac hypertrophy. We found that H3K27me3 and PRC2 were highly enriched at the *Six1* promoter,[2] and Delgado-Olguín et al showed that occupancy by these factors was decreased by *Ezh2* loss of function.[1] This group went on to show that SIX1/EYA act synergistically to drive cardiomyocyte hypertrophy in cultured cardiomyocytes. Most remarkably, a two-fold reduction of ectopic *Six1* expression in $Ezh2^{fl/fl}$::Mef2c-AHF-Cre::$Six1^{+/-}$ mice normalized cardiomyocyte size and hypertrophic marker gene expression and reduced myocardial fibrosis. These data identify *Six1* as a key downstream target of PRC2-mediated gene silencing in cardiomyocytes. However, *Six1* upregulation is not sufficient to cause the heart abnormalities seen in $Ezh2^{fl/fl}$::Mef2c-AHF-Cre mice, because we observed an equal degree of *Six1* upregulation $Ezh2^{fl/fl}$::$Nkx2\text{-}5^{Cre}$ (embryonic lethal) and $Ezh2^{fl/fl}$::Tnnt2-Cre (no phenotype) hearts.[2]

To pinpoint additional genes that are directly regulated by *Ezh2* via H3K27me3-mediated repression, we examined genes differentially expressed in $Ezh2^{fl/fl}$::$Nkx2\text{-}5^{Cre}$ in comparison with $Ezh2^{fl/+}$::$Nkx2\text{-}5^{Cre}$ heart ventricle by high throughput RNA sequencing (RNA-seq).[2] We also determined H3K27me3 and *Ezh2* chromatin occupancy genomewide by chromatin immunoprecipitation coupled with high throughput sequencing (ChIP-seq).[2] Interestingly, the majority (757/819) of H3K27me3-occupied genes were not differentially expressed in *Ezh2* loss of function. This likely reflects redundant mechanisms that maintain gene silencing, combined with *Ezh1* genetic redundancy. Using stringent criteria, we identified 52 genes that were upregulated in *Ezh2* loss-of-function and occupied by H3K27me3 and Ezh2. These genes were highly overrepresented for transcriptional regulators (23/52). A majority of them were not normally expressed in the heart, consistent with the idea that EZH2 deposition of H3K27me3 marks silences ectopic gene expression programs. Among these genes were the cardiac progenitor transcription factor *Isl1* (also reported as upregulated by Delgado-Olguín et al), the transcription factor *Six1*, and the cell-cycle inhibitors *Ink4a/b*, the upregulation of which likely contributed to myocardial hypoplasia of mutant hearts. Decreased myocyte number also may have indirectly contributed to cardiomyocyte hypertrophy observed by Delgado-Olguín et al.

The preponderance (432/511) of differentially expressed genes were not occupied by either H3K27me3 or EZH2, suggesting either indirect regulation or regulation through noncanonical, H3K27me3-independent mechanisms. *Nppa*, *Nppb*, and *Myh7*, genes suggested by Delgado-Olguín et al to be directly repressed by EZH2, were not directly occupied by

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H3K27me3 or EZH2 in our genome-wide study. Several other differentially expressed cardiac genes, such as *Myl7*, *Bmp10*, and *Myh6*, also were not directly occupied by either H3K27me3 or EZH2. We recently reported a novel mechanism of PRC2 transcriptional regulation in which EZH2 directly binds and methylates the key cardiac transcription factor GATA4.[3] GATA4 methylation impairs its recruitment of and acetylation by p300, thereby repressing its transcriptional potency. GATA4 regulates several cardiac genes, including *Nppa*, *Nppb*, *Myh6*, and *Myh7*, suggesting that this noncanonical PRC2 mechanism may contribute to differential gene expression in EZH2 loss of function. Further work will be required to identify other transcriptional regulators methylated by PRC2, and to determine the extent to which this mechanism accounts for EZH2-mediated transcriptional repression in vivo.

Growing data point to the importance of epigenetic mechanisms–DNA methylation, nucleosome positioning, and covalent histone modifications–in regulating cardiac development.[18] However, relatively less is known about the continued requirement of chromatin-modifying activities in the adult heart and their participation in postnatal heart disease. If adult cardiomyocytes are largely nonproliferative, then might there be reduced nucleosome turnover and, hence, reduced requirement for these enzymes to maintain the chromatin landscape? Although these questions remain unanswered for now, addressing these knowledge gaps has direct clinical relevance, because chromatin-modifying enzymes such as PRC2 are major therapeutic targets in cancer and other diseases. Understanding the function of these protein complexes in the adult heart will be essential for anticipating potential cardiac toxicity. Furthermore, epigenetic changes also are likely to contribute to irreversible perturbations of cardiac gene expression and function in the diseased heart. If this is the case, then normalizing the chromatin landscape may be a therapeutically desirable goal. The recent studies of cardiac PRC2 function provide insights into the molecular basis of cardiac memory and lay the foundations for future investigations into the role of epigenetic mechanisms in congenital heart disease and adult heart disease.

Disclosures

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