Deciphering Histone Code Enigmas Sheds New Light on Cardiac Regeneration

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Histone methylation is tightly regulated by the equilibrium between histone methyltransferases and demethylases.[1] Methylation occurs on lysine (K), arginine (R), and histidine (H) residues, without affecting histone charge. Interestingly, histone methylation plays different roles in consequence of the specific position of the methylated residue. The transcribed chromatin might, in fact, be enriched in methylation at histone 3 (H3) residues K4 (H3K4), K36 (H3K36), and K79 (H3K79), whereas methylation at K9 (H3K9), K27 (H3K27), and histone 4 (H4) K20 (H4K20) characterizes silenced chromatin regions.[2] The level of K methylation, such as mono-, di-, or trimethylation, is also important allowing for chromatin accessibility by transcription complexes. For example, monomethylation of H3K9 (H3K9me1) or H3K27 (H3K27me1) associates with active transcription, whereas their trimethylated forms (H3K9me3; H3Kme3) with silencing.[2] Histone methyltransferases belong to the larger lysine methyltransferases family whose members incorporate the conserved methyltransferase domain first identified as part of the Su(var)3 to 9, Enhancer-of-zeste, Trithorax (SET) proteins. On the contrary, histone demethylases are included into the lysine demethylases (KDMs) and grouped into 2 major classes: the Flavin adenine dinucleotide–dependent demethylases acting on mono- and di-methylated Ks and the Jumonji demethylases, depending on Fe(II) and α-ketoglutarate cofactors, selective for trimethylated Ks[1].

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Polycomb: The Switch Controller Between Pluripotency and Differentiation

Among epigenetic writers, the lysine methyltransferase polycomb repressive complexes 1 and 2 (PRC1 and PRC2) gained considerable popularity in the field for their involvement in the regulation of stem cell pluripotency, embryonal development, and cancer.[3] Recently, the interest of epigeneticists toward Polycomb has been further raised by the evidence that these complexes associate with many long noncoding RNAs being possibly implicated in gene regulation.[4] PRC1 and PRC2 are multiprotein complexes grouped under the definition of Polycomb Group responsible for histone PTMs able to condensate the chromatin.[3,5] Specifically, PRC1, through its ubiquitin ligase subunits, Ring1A and Ring1B, monoubiquitinates the histone H2A at K119 residue, whereas PRC2, via its Ezh2 and Ezh1 subunits, generates H3K27me2 or K27me3 residues.[5]

PRC2, governing cell transcription by H3K27 methylation, controls the switch between pluripotency maintenance and the start of complex differentiation programs.[6,7] Several in vivo studies based on PRC2 and PRC1 subunit knockout support this evidence. Specifically, in vivo depletion of PRC2 subunits associates with early embryonic lethality,[6] whereas PRC1 deficiency seems compatible with life although the progeny shows developmental abnormalities.[8] In vitro, studies on embryonic stem cells pointed out the pivotal role of PRC1 and PRC2 during stem cell differentiation and lineage commitment without revealing significant effects on the self-renewal process. Of note, embryonic stem cells bearing the knockout of different PRC2 subunits were still able to differentiate into the 3 germ layers although their full differentiation abilities were impaired.[7]

In a study published in the present issue of *Circulation Research*, Dal-Pra et al[9] report about the importance of downregulating PRC2 expression to reduce H3K27me3 chromatin density. A step seemingly important for the initiation of cardiac fibroblast reprogramming into cardiomyocytes. Genetic or epigenetic manipulations have been often adopted to enhance the conversion of cardiac fibroblast into cardiomyocytes. The most conventional protocols are based on the cotransduction of cardiomyogenic transcription factors, such as Gata4, Mef2c, Tbx5, and Hand2, whereas the strategy adopted by Dal-Pra et al[9] took advantage from a micro-RNA (miR) combination (miR combo), already described in 2 previous works from the same group.[10,11] MiR combo, in fact, a combination of miR-1, miR-133, miR-208 and miR499, stimulates the direct reprogramming of cardiac fibroblasts, both in vivo and in vitro, by induction of cardiac specific markers and biological functions typical of cardiomyocytes including expression of ion channels, spontaneous calcium oscillations, and contractility finally resulting in an improved cardiac performance after injury.[10,11] In spite of these relevant findings, the mechanism of action determining miR combo properties remained obscure to date. In the present study, the authors mechanistically analyzed the effect of miR combo, revealing a striking modulation of the epigenetic landscape. The analysis, performed by using a quantitative RT[2] Profiler polymerase chain reaction array specific for epigenetic chromatin modification enzymes, disclosed the sensitivity of several histone methyltransferases and histone demethylases to miR combo treatment. This result well correlated with the immunoblot analysis of the targeted methylated histone marks and brought the attention on the role of the

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PRC2-dependent modification H3K27me3. Specifically, miR combo was associated to a downmodulation of PRC2 followed later by the upregulation of the counterpart enzymes Kdm6A and Kdm6B. These are 2 Jumonji-domain-containing KDMs able to catalyze the removal of H3K27me2 and H3K27me3 that were associated previously to heart defects in knockout mice. Although the authors provided mechanistic insights for this effect, their results excluded the possibility of a direct miR combo effect on PRC2 and Kdm6A/B. A considerable amount of work still remains to be done before our understanding about the role of miR combo in the cardiogenic conversion of cardiac fibroblasts will be completed. Nevertheless, their experiments permitted to clarify that PRC2 downmodulation represents one of the crucial events occurring soon after 48 hours from miR combo transfection. As depicted in the Figure, this effect might be associated with the recruitment of miR Let-7c (a miR known to directly target the Ezh2 subunit of PRC2). Although delayed ≈3 to 4 days after miR combo transfection, the upregulation of Kdm6A/B has been identified as important too. This phenomenon possibly required the initial miR combo-dependent inactivation of a transcriptional repressor yet to be identified (Figure). The use of a small molecule able to interfere with lysine methyltransferases activity, the 3-deazaneplanocin A (DZNep), or that of siRNAs specific for PRC2 or Kdm6A/B further supported these evidence. In particular, the disruption of PRC2 mimicked miR combo effects on cardiac marker expression and the knockdown of Kdm6A/B expression prevented miR combo-induced reprogramming. Moreover, the authors demonstrated that cardiac reprogramming occurred only after demethylation of H3K27me3 at the gene-specific loci encoding for Tbx5, Mef2C, Gata4, and Hand2, the cardiomyogenic transcription factors essential for cardiac commitment (Figure).

Developing an Integrated Epigenetic Approach Toward a More Efficient Cardiac Repair

The genetic invasiveness and the low efficiency of exogenous expression of cardiomyogenic transcription factors render this cardiac reprogramming approach not well suitable for the clinic. However, the combination of miRNAs and chemicals, to obtain an efficient cocktail-promoting cardiac regeneration may pave the way to potentially unlimited solutions. This perspective is

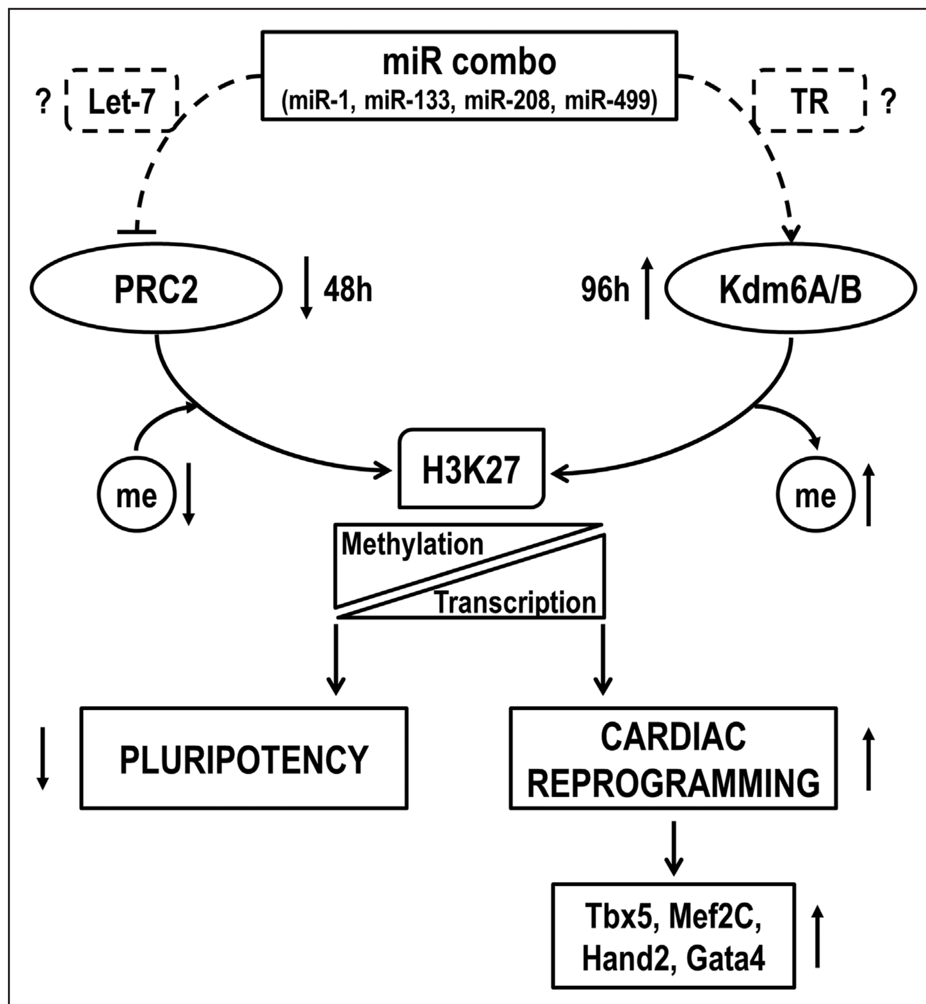

Figure. Schematic illustration of miR combo mechanism of action. Dal-Pra et al[9] report about the miR combo property of downmodulating polycomb complex 2 (PRC2). This effect was followed by a delayed increasing of lysine demethylases 6A/B (Kmd6A/B). As a consequence, the chromatin density of histone H3 lysine 27 trimethylation was reduced. To explain this phenomenon, the authors hypothesized the recruitment of miR Let-7c causing PRC2 downmodulation at 48 h (**left** dashed line) and the repression of a yet to be identified transcriptional repressor (TR) at the origin of the transcriptional activation of Kmd6A/B occurring after 96h (**right** dashed line). Hence, miR combo stimulated cardiac reprogramming by an indirect transcriptional activation of the known cardiomyogenic factors Tbx5, Mef2C, Hand2, and Gata4.

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well envisaged by the study of Dal-Pra et al[9] and in those of others as recently enlighten by the study of Cao et al.[12] In the latter, after screening a library of 89 small molecules and several rounds of cocktail tuning, the authors identified 9 chemical compounds (9C) able to elicit functions, electrophysiological properties, and transcriptomic and epigenetic features typical of cardiac cells. Intriguingly, the 9C-induced chromatin relaxation at loci crucial for cardiogenesis by a dynamic loss of H3K27me3 in favor of a gain in H3K4me3 and H3K27 acetylation. The consequent chromatin decondensation put in the spotlight-specific genomic regions, such as those encoding for Tbx5, Mef2C, Gata4, and Hand2, which became accessible to transcription factors important for the cardiogenic conversion of fibroblasts. The remarkable mechanistic similarities between 9C and miR combo shed new light on the importance of a histone code modification, H3K27me3, to date poorly associated with cardiac-regenerative processes. The regulation and function of H3K27me3 and that of its writers, readers, and erasers may then well represent a chromatin enigma to be solved and exploited for the identification of new and more effective approaches to cardiac repair.

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Disclosures

None.

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