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Peering into the Cardiomyocyte Nuclear Epigenetic State

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Epigenetic modifications including DNA methylation, chromatin remodeling, and histone acetylation or methylation regulate gene expression either by suppressing or activating target genes. Modifications such as DNA methylation and trimethylation of H3K9 and H3K27 are well-described characteristics of heterochromatin, tightly packed DNA that can repress gene expression,^{1, 2} whereas trimethylation of H3K4 and H3K36, methylation of H3K4, and acetylation of H3K27 all act to open chromatin structures and stimulate transcriptional activity.³ Cell fate and differentiation is dependent on epigenetic modifications; however, detailed analysis of cell type specific epigenetic marks and the resultant transcriptional profiles in myocardium has been largely unexplored.

Aberrant regulation of epigenetic networks during embryonic development can result in congenital heart disease, which affects up to 2% of newborns and is the most common of all birth defects.⁴ There is compelling evidence that mis-regulation of epigenetic modifiers may be responsible for many cases of congenital heart disease. A recent case-control study identified de novo causative gene mutations by investigating exome sequences of patients affected with severe congenital heart disease. Surprisingly, many mutations were involved in histone H3 lysine 4 trimethylation (H3K4me3) including a gene known to cause congenital heart disease called MLL2.⁵ Mutations in the histone methyltransferase MLL2 are well known to be the underlying cause of Kabuki syndrome, which results in congenital heart defects among an array of other phenotypic abnormalities.⁶ Deletion of MLL2 impairs embryonic stem cell proliferation and differentiation to cardiomyocytes in vitro demonstrating a critical role for histone methylation in cardiomyocyte specification.⁷

In addition to embryonic differentiation, postnatal heart development also depends on epigenetic regulation. Neonatal mice can regenerate their hearts shortly after birth but this regenerative potential is lost within the first week of life.⁸ Although the events that orchestrate postnatal organ differentiation are poorly understood, a recent study demonstrated that significant changes in the cardiac methylome occur between postnatal day

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1 and 14, the critical window of cardiomyocyte terminal differentiation and exit from the cell cycle.⁹ Adult cardiomyocytes demonstrate accumulation of the repressive histone mark H3K9me3 in E2F dependent promoters, which in part explains the chromatin modifications that lead to cell cycle exit in differentiated cardiomyocytes.¹ During cardiomyocyte differentiation the retinoblastoma (Rb) family of proteins recruits heterochromatin protein-1 to H3K9me3 promoter regions of E2F genes thereby facilitating target gene silencing.¹ In mice, conditional deletion of Rb decreased genome-wide heterochromatin and resulted in increased expression of cell cycle genes. Rb deletion enabled cell cycle activity of adult cardiomyocytes suggesting that modulating epigenetic marks may prove to be a therapeutic approach for enhancing post-mitotic cardiomyocyte cell cycle activity and heart regeneration.¹

Recent studies have begun to address the question of how the cardiomyocyte transcriptome is regulated by epigenetic modifiers. In vitro experiments profiling embryonic stem cells differentiated to cardiomyocytes revealed that histone marks are tightly coordinated through the stages of differentiation and these marks orchestrate cardiomyocyte gene expression.¹⁰ In vitro ChIP-seq experiments have the advantage of a uniform cell type in comparison to multicellular tissues, so gene expression can be linked to epigenetic marks in a given cell type. Epigenetic studies from whole tissues provide insight into tissue specific epigenetic regulation but interpretation is confounded by cell admixture. Myocardium includes fibroblasts and endothelial cells, with cardiomyocytes making up only about 60% of the cells in the adult heart.¹¹ Thus, techniques allowing transcriptional and epigenetic analysis of specific cell types within myocardium will provide a more informative picture of cardiomyocytes than whole tissue analysis.

In the current issue of *Circulation Research*, Pressil et al. describe an important new approach in the analysis of cardiomyocyte gene expression and epigenetic state and how this method can provide unique insight into myocardial biology. They isolated cardiomyocyte nuclei from rat, human, and mouse to assess nascent transcriptional landscapes and chromatin states in a cell type specific manner.¹² A major technical hurdle in isolating adult cardiomyocytes is lengthy enzymatic digestion which might change native transcriptional profiles and chromatin states. Pressil et al isolated cardiomyocyte nuclei without enzymatic digestion at cold temperatures to preserve the in vivo chromatin landscapes. The authors used an antibody against pericentriolar material 1 (PMC-1) to purify cardiomyocyte specific nuclei from nuclei derived from other cell types (Figure 1). PMC-1 has been shown to be highly specific to cardiomyocyte nuclei across several mammalian species,¹³ and Pressil et al confirmed that PMC-1 is not detected in non-myocyte nuclei in the heart. Comparison of cardiomyocyte nuclei specific gene expression data with whole heart biopsy RNAseq data verified that the isolation methods used were highly specific for cardiomyocytes. Transcripts of collagen alpha-2(I) chain (Col1a2) and angiotensin converting enzyme (Ace), markers of fibroblasts and endothelial cells respectively, were virtually undetectable in the myocyte nuclei preparations. Next, they compared cardiomyocyte nuclear RNA profiles to whole cardiomyocyte cell RNA profiles. As expected, the nuclear transcripts consisted of significantly more unspliced RNA molecules. Cytoplasmic RNA included more highly expressed genes involved with transcriptional regulation and unexpectedly also several structural genes such as alpha-myosin heavy chain and titin. On the other hand, cytoplasmic

RNA was enriched for primarily metabolic genes. In addition to epigenetic modifications, which tightly regulate RNA expression levels, post-transcriptional modification influences RNA expression and ultimately protein translation and expression. RNA splicing, polyadenylation and RNA degradation all contribute to cellular RNA levels.¹⁴ The comparative analysis between nuclear and cellular RNA demonstrated striking differences in transcript abundance in several gene categories, suggesting that whole cell transcriptional analysis may not be ideal for direct comparison of epigenetic marks because of confounding post-transcriptional modifications.

To identify the most predictive histone marks of myocyte transcriptional activity, the authors generated genome wide ChIP-seq data for the H3K36me3, a mark closely associated with transcription activation, and they integrated their analysis with previously published ChIP-seq data for several histone marks from cardiomyocyte nuclei¹⁵ and whole heart tissue (Figure 1).^{16, 17} Histone methylation and acetylation patterns of whole tissue biopsies were difficult to interpret since epigenetic landscapes from multiple cell types were included in the analysis. For example, H3K27ac in the promoter region of the non-cardiomyocyte gene Vimentin (Vim) was detected in whole heart tissues, but not in purified cardiomyocyte nuclei. H3K27ac in the vimentin promoter region of whole heart tissue suggests that this histone mark is derived from non-myocytes although the exact source for this acetylation pattern remains unclear. In purified cardiomyocyte nuclei, H3K27ac correlated most highly with nuclear RNA expression compared to other histone marks examined. A recent study demonstrated that knockdown of Brg1, a chromatin remodeling protein that regulates H3K27ac, ablated in vitro cardiomyocyte differentiation.¹⁸ Furthermore, during early heart development H3K27 acetylation regulates activation of the atrioventricular canal whereas H3K27 deacetylation represses atrioventricular canal genes and drives ventricular myocardial cell specification.¹⁹ The findings from Pressil et al also identify H3K27ac as a critical regulator of cardiomyocyte gene expression. Future studies profiling cardiomyocytes during dynamic state will provide further insight into the regulatory role of H3K27ac as cardiomyocyte phenotypes transition during development and disease.

In summary, Pressil et al provide the cardiovascular community with an important methodological advance that reveals new insight into cardiomyocyte gene transcription and epigenetic state. The nuclear isolation methods will be useful for many investigators interested in profiling nuclear and cellular RNA from cardiomyocytes during health and disease states. Unraveling the complexity of the entire heart tissue with this more focused dissection of gene expression state will likely yield unique molecular understanding of the cardiomyocytes in disease states.

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Figure 1.

Representation of the cardiomyocyte nuclear isolation protocol and downstream bioinformatics-processing scheme for histone marks. (+) Indicates permissive chromatin mark and (-) indicates repressive chromatin mark.