Dynamic changes in the cardiac methylome during post-natal development

C.B. Sim,[1] M. Ziemann,[2] A. Kaspi,[2] KN. Harikrishnan,[2] J. Ooi,[2] I. Khurana,[2] L. Chang,[2] J.E. Hudson,[1] A. El-Osta[2] and E.R. Porrello,[1] [1]School of Biomedical Sciences, The University of Queensland, St Lucia, QLD 4072, Australia and [2]Epigenetics in Human Health and Disease Laboratory, Baker IDI Heart and Diabetes Institute, Melbourne, VIC 3004, Australia.

Epigenetic modifications have emerged as central players in the coordination of gene expression networks in many biological processes. While epigenetic alterations including histone modifications have been studied during heart development, the role of DNA methylation in heart development remains largely unknown. The goal of this study was to determine whether DNA methylation plays an important role in guiding transcriptional changes during the first two weeks of post-natal mouse heart development, which is an important period for cardiomyocyte maturation, loss of proliferative capacity and loss of regenerative potential (Porello *et al.*, 2011,2013).

In this study we demonstrate for the first time that DNA methylation events are associated with changes in cardiac gene expression during neonatal heart development. Our findings indicate that DNA methylation levels are not static during post-natal heart development but rather undergo dynamic alterations during neonatal life. Specifically, we have identified two post-natal waves of DNA methylation in the rodent heart involving increased site-specific methylation from P1 to P14, followed by a global decrease in genomic methylation levels after P14. Gene expression profiling (RNA-seq), enzymatic DNA methylation assays and genome-wide sequencing of methylated DNA (MBD-seq) identified 2545 differentially methylated regions (DMRs) in the mouse heart from P1 to P14. The vast majority (~80%) of DMRs were hypermethylated between P1 and P14 and these hypermethylated regions were associated with transcriptional shut down of important developmental signaling pathways, including Hedgehog, bone morphogenetic protein (BMP), transforming growth factor β (TGFβ), fibroblast growth factor (FGF) and Wnt/β-catenin signaling. Importantly, DMRs were highly enriched for MyoG, Tbox and Smad2/3/4 transcription factor binding sites, suggesting that interplay between myogenic transcription factors and the epigenetic machinery might contribute to the regulation of gene expression networks during neonatal cardiac differentiation. Notably, post-natal inhibition of DNA methylation with 5-aza-2′-deoxycytidine (5aza-dC; 1mg/kg/day) through subcutaneous injection induced a marked increase (~3-fold) in cardiomyocyte proliferation and ~50% reduction in the percentage of binucleated cardiomyocytes in mouse hearts, suggesting that DNA methylation is required for neonatal cardiac maturation. This study provides novel evidence for widespread alterations in DNA methylation during post-natal heart maturation and suggests that cardiomyocyte cell cycle arrest during the neonatal period is subject to regulation by DNA methylation.

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