Title: Tissue-specific H2Bub1 chromatin marks are required for cardiomyocyte development
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Background: Chromatin remodeling gene mutations contribute to 2.3% of congenital heart disease (CHD). Monoubiquitination of histone H2B (H2Bub1) is a chromatin modification required in stem cell differentiation and cilia function. \textit{De-novo} mutations affecting the core complex required for H2Bub1 (RNF20, RNF40 and UBE2B) are enriched in CHD patients compared to controls. We investigated H2Bub1’s role in heart development, beyond determination of laterality, in mice and IPSC-derived cardiomyocytes.

Methods: To study the role of RNF20 \textit{in vivo}, we evaluated the cardiac phenotype of mice with cardiac-specific deletion of Rnf20. We then defined the genome-wide distribution of H2Bub1 marks during cardiomyocyte development \textit{in-vitro} through H2Bub1-ChIP-seq of iPSC-derived cardiomyocytes. To define the effect of abnormal H2Bub1 on cardiomyocyte development, we used CRISPR to generate mutations in RNF20 and UBE2B in iPSCs. The H2Bub1-mutant iPSCs were differentiated into cardiomyocytes and analyzed by H2Bub1 ChiP-seq, RNA-seq and functional analysis.

Results: Constitutive \textit{Rnf20}\textsuperscript{−/−} mice are pre-implantation lethal and \textit{Rnf20}\textsuperscript{+/−} mice are normal. Mice with cardiac-specific \textit{Rnf20} deletion using \textit{Nkx2.5-Cre} die by e12.5, have incomplete septation, decreased
compact myocardium and abnormal organization of the cardiac sarcomere.

ChIPseq for H2Bub1 in iPSC-derived cardiomyocytes demonstrated that H2Bub1 is dynamically regulated during differentiation: there are abundant genes with H2Bub1 marks in iPSCs and mesoderm, but only a few genes maintain their H2Bub1 mark during the transition from cardiac mesoderm to cardiac progenitors. The set of selectively maintained genes is strongly enriched for sarcomeric calcium genes, and 9/10 of these calcium genes are associated with human cardiomyopathy.

We then tested the effect of downregulation of RNF20 complex members on cardiomyocyte differentiation. Haploinsufficiency for RNF20 arrested differentiation into cardiac mesoderm. Notably, decreased Rnf20 led to a paradoxical increase in H2Bub1 at chromatin modifier genes. Depletion of UBE2B resulted in either beating cardiomyocytes or failure to differentiate into cardiac mesoderm. RNAseq of the UBE2B deficient cardiomyocytes showed decreased expression of sarcomeric genes and sarcomeric calcium signaling genes.

**Conclusions:** Our data show that in vivo, RNF20 is required for compact myocardium formation, and in-vitro, precise regulation of H2Bub1 is required for cardiomyocyte development in IPSC-derived cardiomyocytes.

**Word Count:** 342