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Epigenetic priming and GATA4 activation upon lamin A/C loss-of-function results in aberrant cardiovascular cell fate and function

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In my PhD work, I studied how chromatin tethering to the nuclear lamina controls cardiovascular cell fate choices, development and function. I found that ablation of lamin A/C rather than lamin B1 in mouse embryonic stem cells (mESCs) results in precocious activation of a transcriptional program promoting cardiomyocyte versus endothelial cell fate. This was accompanied by premature cardiomyocyte differentiation, cell cycle withdrawal and abnormal contractility. I next corroborated my findings in vivo using *Lmna*+/+ (control), *Lmna*+/- and *Lmna*-/- mice. Expression analysis revealed significant upregulation of cardiac progenitor and cardiomyocyte marker genes in dissected pharyngeal mesoderm and hearts of E8.5 embryos as well as E9.5 hearts upon *Lmna* ablation consistent with my in vitro cell culture-based studies. Histological examination unveiled non-compaction cardiomyopathy in both *Lmna*+/− and *Lmna*−/− embryos. Further, I also found precocious CM differentiation and premature binucleation coupled to CM cell cycle withdrawal during fetal heart development.
Using a combination of RNA-seq, ATAC-seq, Hi-C and 3D FISH, I next studied the effect of lamin A/C loss on the three-dimensional (3D) chromatin organization and gene expression. ATAC-seq revealed a widespread increase in chromatin accessibility across the genome, as well as at genes upregulated upon lamin A/C loss already in pluripotent stem cells. Moreover, Hi-C and 3D FISH experiments showed that around 8% of chromatin compartments switched from active A to inactive B comprtments and vice versa as a result of lamin A/C depletion and were highly associated with lamina-associated domains (LADs). Cardiac genes within lamin A/C LADs such as *Gata4*, *Isl1*, *Mef2c* and *Ttn* relocalized from the repressive nuclear periphery to the active nulcear interior upon lamin A/C loss of function. Importantly, Gata4 was activated by lamin A/C loss and Gata4 silencing or haploinsufficiency rescued the aberrant cardiovascular cell fate choices induced by lamin A/C deficiency.
In addition, I uncovered divergent functions of lamin A/C in naïve pluripotent stem cells and cardiomyocytes, which have distinct contributions to the transcriptional alterations of patients with *LMNA*-associated cardiomyopathy. In naïve pluripotent stem cells, lamin A/C keeps cell differentiation and cardiac morphogenesis genes silent, such as *Gata4/6*, *Bmps*, *Ryr2*, *Wnts*, *Myl4*, etc. Upon lamin A/C LOF, these genes are ectopically expressed in mESCs or later stage. Whereas, in CMs lamin A/C specifically regulates genes involved in cardiac contraction and sarcomere organization.
In summary, my results showed that epigenetic alterations in ESCs play a crucial role in *LMNA*-related cardiomyopathies and disruption of lamin A/C-dependent chromatin architecture in ESCs is a primary event in *LMNA* loss-of-function cardiomyopathy.
Still, an important question remains: how do different and specific *LMNA* mutations result in phenotypic diversity? Environmental factors, such as diet, exercise, and stress, as well as age, sex, and other comorbidities, might also contribute to the phenotypic variability in patients with pathogenic *LMNA* mutations. Identifying cell-type-specific interacting partners for nuclear lamins and the effect of lamin mutations on these interactions would also be important in understanding the wide-ranging clinical phenotypes and may pinpoint druggable protein–protein interfaces for therapeutic applications. Given the important role of lamin A/C in heart development and CM differentiation, developmental changes in asymptomatic-at-birth *LMNA* patients might result in late changes in heart structure and function, warranting further investigation.