Challenging the “chromatin hypothesis” of cardiac laminopathies with LMNA mutant iPS cells
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Lamins A and C are intermediate filaments that provide structural support to the nuclear envelope and regulate gene expression. In this issue, Bertero et al. (2019. J. Cell Biol. https://doi.org/10.1083/jcb.201902117) report that although lamin A/C haploinsufficient cardiomyocytes show disease-associated phenotypes, those changes cannot be explained by alterations in chromatin compartmentalization.

Mutations in LMNA encoding lamin A/C cause “laminopathies,” a plethora of diseases, of which dilated cardiomyopathy (DCM) is the most prevalent. More than 400 LMNA mutations have been associated with laminopathies; however, a unifying genotype–phenotype link is still missing. Three hypotheses underpin the pathogenesis of laminopathies: 1) the mechanical hypothesis postulates that lamin A/C provides structural support to the nucleus; 2) the signaling hypothesis assumes that lamin A/C regulates intracellular pathways; and 3) the chromatin hypothesis supports a role for lamin A/C in controlling gene expression by modulating chromatin compartmentalization.

Positioning of interphase chromosomes is spatially defined in different compartments within the three-dimensional space of the nucleus. At the sub-megabase scale, genome-wide DNase chromosome conformation capture (HiC) analyses revealed that the eukaryotic genome is organized into structural domains referred to as topologically associated domains (TADs). TAD boundaries are specified and maintained by chromatin architectural proteins, which induce the formation of loops mediating promoter–enhancer contacts. TADs tend to segregate based on their transcriptional status, so eukaryotic genomes are divided into active (A) and inactive (B) compartments. This compartmentalization is regulated by nuclear positional constraints. Domains with high transcriptional activity are mainly found in the nuclear interior, while transcriptionally silent regions are mostly associated with nucleoli and the nuclear envelope. From a bi-dimensional point of view, chromosomes are anchored at the nuclear periphery through the nuclear lamina (NL) by lamina-associated domains (LADs), heterochromatic regions overlapping with the B compartment. The major components of the NL are lamins, divided into A type (the aforementioned lamins A and C, encoded as splice variants of the LMNA gene) and B type (products of the LMNB1 and LMNB2 genes).

Given the role of lamin A/C in regulating chromatin compartments and dynamics, it has been hypothesized that some LMNA mutations might alter cell type–specific chromatin interactions and, consequently, induce aberrant gene expression: the “chromatin hypothesis.” However, experimental testing of such a hypothesis in disease-relevant cellular models has just begun. In this issue, Bertero et al. (2019. J. Cell Biol. https://doi.org/10.1083/jcb.201902117) report that although lamin A/C haploinsufficient cardiomyocytes show disease-associated phenotypes, those changes cannot be explained by alterations in chromatin compartmentalization.

Bertero et al. started with hiPSCs from patient cells harboring an LMNA haploinsufficient mutation (R225X; causing truncation of both lamin A and C) and edited them to generate gene-corrected pluripotent stem cells (hiPSCs) differentiated into cardiomyocytes (hiPSC-CMs; Fig. 1). The authors identified CACNA1A as a disease-relevant gene. CACNA1A encodes a subunit of a neuronal-specific calcium channel, whose ectopic expression might explain the electrophysiological defects observed in mutant hiPSC-CMs. Indeed, pharmacological treatments to dampen these ectopic calcium currents helped improve the aberrant electrophysiological phenotypes described in lamin A/C mutant hiPSC-CMs. This suggests that CACNA1A may represent a therapeutic target to reverse the
electrical abnormalities in the myocardium of DCM patients.

Taken together, Bertero et al. (6) show that although mutant hiPSC-CMs recapitulate the electrophysiological and contractile aberrations observed in cardiac laminopathy, lamin A/C haploinsufficiency induces only minimal switches in chromatin compartmentalization, a finding at odds with the chromatin hypothesis. Nonetheless, it cannot be excluded that transcriptional changes observed in mutant cells might instead be explained by different levels of chromatin organization caused by LMNA mutations, which do not necessarily entail A/B compartmentalization. Recently, a four-dimensional genome conformation has been described to reinforce silencing of developmental genes in differentiating human adipose stem cells (ASCs) (7). This study revealed both constitutive and variable long-range interactions between different TADs, referred to as TAD cliques, which represent a higher-order organization of heterochromatic TADs in the B compartment. Notably, during ASC differentiation, TAD cliques increase in size and become more strongly associated with the peripheric B compartment (7). This chromatin rearrangement is not accompanied by an evident A to B switch, reminiscent of what Bertero et al. observed. Thus, it is tempting to speculate that instead of promoting a compartment switch, LMNA haploinsufficiency might impact the dynamics of TAD assembly into large cliques at the nuclear periphery. Consistently, deletion of all nuclear lamins in mouse embryonic stem cells does not disrupt the overall TAD structure but rather affects TAD–TAD interactions (8).

In agreement with this, LMNA haploinsufficiency was recently found to induce relevant LAD alterations in hiPSC-CMs carrying a different frameshift LMNA mutation (5) (although lamins appear to be dispensable for LAD-mediated organization of mouse embryonic stem cell genome [9]). Most of the promoters found in LADs from control hiPSC-CMs displayed a more accessible open chromatin than isogenic mutant cells, with consequent aberrant transcriptional activation of PDGF pathway genes, which the authors associated with DCM pathogenesis (5). Interestingly, several PDGF pathway transcripts appear dysregulated also in data presented by Bertero et al. (6).

These findings favor the hypothesis that LMNA mutations can affect chromatin organization, which may impact inter-TAD assembly at the NL, rather than A/B switches. Identifying the factors that mediate such higher-order organization will be an interesting line of research in the future. One appealing possibility is that lamin A/C might exert this function cooperating with other epigenetic modifiers. Interestingly, lamin A/C mediates Polycomb repressive complex 2 (PRC2) recruitment to specific loci (10), and K219T-LMNA has recently been shown to induce aberrant PRC2 targeting and silencing of genes associated with the conduction defects of cardiac laminopathy in hiPSC-CMs (11). LMNA mutations might dysregulate transcriptional programs via defective Polycomb recruitment and epigenetic silencing. Future
comprehensive epigenetic profiling of histone marks and chromatin-modifying enzymes, as well as chromatin accessibility assays, in LMNA mutant humanized models will likely help to shed light on the mechanism conferring lamin A/C sensitivity to those disease-relevant genes. The local epigenetic state of these loci likely dictates their susceptibility, as local chromatin features determine transcriptional silencing in LADs.

Finally, recent work showed that nuclear shape abnormalities of LMNA-related muscular dystrophies can be modeled with high fidelity upon skeletal myogenic differentiation of LMNA mutant hiPSCs (13). However, this phenotypic readout appears to be more variable in hiPSC-CMs; although Bertero et al. (6) and Lee et al. (5) differentiated hiPSCs into CMs using the same protocol (14), only Lee et al. detected abnormal nuclear structures. Notably, Salvarani et al. (11) also did not detect nuclear shape defects in LMNA mutant CMs (albeit using a different protocol), in contrast with previous observations on primary cells and mature adult CMs from affected patients with the same K219T mutation. Even though these discrepancies could be ascribed to the different LMNA genotypes of the bi-dimensional hiPSC-CM cultures of the three studies, it is likely that a platform mimicking the three-dimensional tissue architecture and stresses to which CMs are subjected in vivo might be required to consistently unravel nuclear shape abnormalities in cardiac muscle, as was the case for skeletal muscle (13, 15).

In conclusion, by rigorously challenging the chromatin hypothesis, this interesting study by Bertero et al. sets the stage for future work testing the same paradigm in CMs with different LMNA mutations. It also stimulates the field to challenge the “structural” and “signaling” hypotheses in CMs and other hiPSC derivatives, taking advantage of the opportunities provided by several available protocols to differentiate LMNA mutant hiPSCs in most (if not all) tissues affected in laminopathies.

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