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SPOTLIGHT

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 (1). 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) (2). 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 ranscriptional 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 (2). 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 (3). 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" (4). However, experimental testing of such a hypothesis in disease-relevant cellular models has just begun (5). In this issue, Bertero et al. (6) address this question using *LMNA* mutant human induced pluripotent stem cells (hiPSCs) differentiated into cardiomyocytes (hiPSC-CMs; Fig. 1).

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 isogenic control lines. Through HiC analyses of the hiPSC-CMs, the authors found that mutant hiPSC-CMs display a high degree of separation between chromosomal territories, with increased intra-chromosomal interactions over those involving different chromosomes. This was echoed by more A–A and less A–B interactions in mutant hiPSC-CMs, suggesting that lack of functional lamin A/C reinforces the separation between active and inactive chromatin. However, chromatin compartment dysregulation was restricted to only 1.2% of the genome in mutant cells, with B to A switches more frequent than A to B transitions and concentrated in hotspots on chromosomes 5 and 19 (6).

3D-DNA FISH experiments additionally showed that the B to A shift in mutant hiPSC-CMs was accompanied by relocalization of some of the genes within these lamin A/C–sensitive domains from the nuclear periphery to the interior (Fig. 1). However, the majority of genes found in the lamin A/C–sensitive domains did not substantially change their expression in mutant cells, except for a small number (29) encoding neuronal-specific transcripts, which showed up-regulation in mutant hiPSC-CMs. Among them, 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 (6). This suggests that CACNA1A may represent a therapeutic target to reverse the

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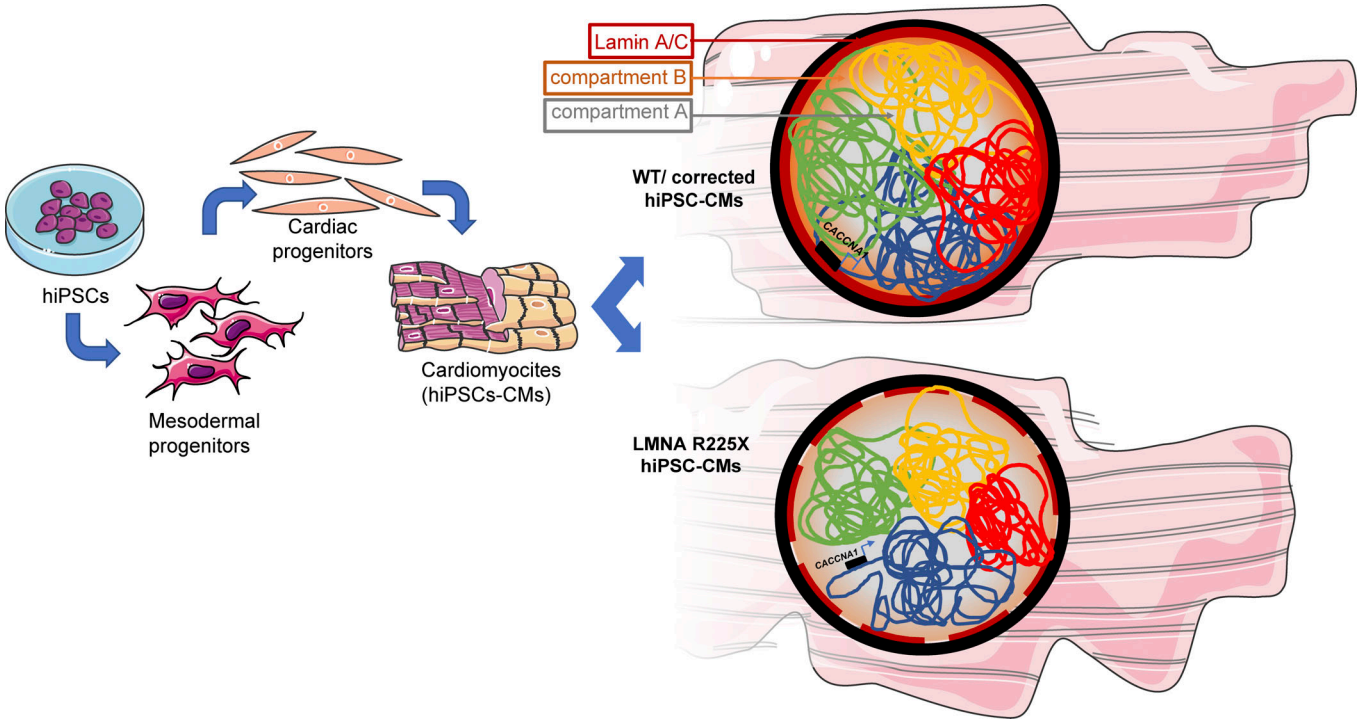

Figure 1. **Chromatin compartmentalization dynamics in healthy control (or corrected by gene editing) and LMNA haploinsufficient CMs derived from hiPSC-CMs.** The cartoon depicts the key stages of directed differentiation of hiPSCs to CMs. On the right-hand side, two CMs are magnified and their respective WT (top) and LMNA mutant (bottom) nuclei are shown. Nuclear compartmentalization in A (orange) and B (gray) domains are represented, as well as lamin A/C distribution as a red circle (solid in WT and dashed in mutant nuclei), underneath the nuclear membrane (black circle). Chromosome territories (depicted as tangled colored lines) are shown more separated in LMNA-R225X hiPSC-CMs as compared with WT/corrected cells, and the consequent dysregulation of the *CACCNA1* gene, which moves from the periphery (WT) to the nuclear interior (R225X LMNA), is highlighted. Illustration produced using SMART (Servier Medical Art; https://smart.servier.com) in accordance with a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

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

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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 (12).

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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