IPSC-based models for investigating mechanisms primary cardiomyopathies: focus on LMNA gene and its pathophysiological role in the human cardiomyocyte.

Primary cardiomyopathies (CMPs) are diseases of the myocardium, characterized by mechanical and/or electrical dysfunctions of the heart that usually exhibits inappropriate ventricular hypertrophy or dilatation. CMPs represent a common cause of heart failure and the most frequent cause of heart transplantation, and their prevalence it’s exponentially increasing. Among the diverse phenotypes (hypertrophic, dilated, restrictive and others), the dilated form is the more common. Dilated CMP (DCM) is defined by the presence of left ventricular dilatation and contractile dysfunction. Contemporary studies using genetic screening suggest that up to 40% of DCM is genetically determined. So far, more than 50 genes have been implicated in DCM; among the others, mutations of those encoding cytoskeletal, sarcomeric, desmosomal and nuclear envelope proteins account for up to 35% of cases. Notably, mutations in the gene encoding Lamin A/C (LMNA) are among the major causes of DCM and have been found in 4-7% of DCM cases, with variable expressivity and age-dependent penetrance.

Lamin A/C are nuclear lamina proteins with a key role in maintenance of the shape and mechanical stability of the nucleus; they also regulate many cellular processes, including gene expression, DNA replication and repair, chromatin organization, and response to biomechanical stress. Despite the knowledge recently gained on these proteins, studies on the pathophysiological mechanisms underlying defective Lamin A/C in human cardiomyocytes (CMs) and cardiac disease are still incomplete.

Within the last five years, we have focused our studies on the investigation of human models of LMNA-dependent CMP developed using induced pluripotent stem cells (iPSCs) of patients carrying diverse LMNA mutations (i.e. p.K219T; p. R190E). Indeed, CMs differentiated from iPSCs in vitro have been extensively demonstrated as valuable tool for dissecting mechanisms of disease, as well as for pharmacological testing.

Starting from a comprehensive electrophysiological analysis, we found that, compared to family-matched healthy controls (CNTR), CMs carrying the K219T-LMNA mutation (LMNA-CMs) display significant changes in key action potential (AP) properties (i.e. maximal upstroke velocity, AP amplitude and overshoot); these defects were associated with a reduction of the peak sodium currents and a diminished conduction velocity, measured in strands of electrically-coupled CMs. Biochemical studies showed significant downregulation of the sodium channel Nav1.5 in LMNA-CMs, which was accompanied by increased binding of Lamin A/C to the promoter on SCN5A, the channel's gene; binding of the Polycomb repressive complex 2 (PRC2) protein SUZ12 and deposition of the related repressive histone mark H3K27me3 were also increased at the SCN5A locus, which in this setting was preferentially localized to the nuclear periphery. In support to this, we found that the mutation increases the affinity of Lamin A/C for PRC2, resulting in their augmented physical interaction and consequently in the persistence of a repressive environment on SCN5A. Correction of the mutation by CRISPR/Cas9-mediated gene editing resulted in the re-establishment of sodium current density and SCN5A expression, supporting a mechanism by which mutated Lamin A/C cooperates with PRC2 in downregulating SCN5A, leading to decreased sodium current density and slower conduction velocity. This mechanism may underlie the conduction abnormalities usually associated with LMNA-cardiomyopathy.

Consistently, results from a transcriptional profiling of LMNA-CMs showed more than 1400 genes modulated in LMNA-CMs vs CNTR, encoding proteins with key functions in cardiac conduction and muscle contraction. Assessment of both contraction force and intracellular calcium dynamics revealed a significant decrease of cluster shortening and defects of intracellular calcium response to inotropic stimulation in LMNA-CMs. Biochemical studies confirmed the significant reduction of the expression of contractility genes (as TNNCI,
TRIM63), and found an increased binding of Lamin A/C to their promoters, together with the H3K27me3 and H3K9me3 marks, as previously shown at SCN5A locus. Altogether, our findings support that chromatin remodelling driven by defective Lamin A/C may be at the basis of both contractile and cardiac conduction defects in LMNA-CMs, eventually sustaining cardiac dysfunctions occurring in patients with LMNA-cardiomyopathy.

References (selected on the topic)


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