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Research Article

The LMNA mutation p.Arg321Ter associated with dilated cardiomyopathy leads to reduced expression and a skewed ratio of lamin A and lamin C proteins

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ABSTRACT

Dilated cardiomyopathy (DCM) is a disease of the heart muscle characterized by cardiac chamber enlargement and reduced systolic function of the left ventricle. Mutations in the LMNA gene represent the most frequent known genetic cause of DCM associated with disease of the conduction systems. The LMNA gene generates two major transcripts encoding the nuclear lamina major components lamin A and lamin C by alternative splicing. Both haploinsuffiency and dominant negative effects have been proposed as disease mechanism for premature termination codon (PTC) mutations in LMNA. These mechanisms however are still not clearly established. In this study, we used a representative LMNA nonsense mutation, p.Arg321Ter, to shed light on the molecular disease mechanisms. Cultured fibroblasts from three DCM patients carrying this mutation were analyzed. Quantitative reverse transcriptase PCR and sequencing of these PCR products indicated that transcripts from the mutant allele were degraded by the nonsense-mediated mRNA decay (NMD) mechanism. The fact that no truncated mutant protein was detectable in western blot (WB) analysis strengthens the notion that the mutant transcript is efficiently degraded. Furthermore, WB analysis showed that the expression of lamin C protein was reduced by the expected approximately 50%. Clearly decreased lamin A and lamin C levels were also observed by immunofluorescence microscopy analysis. However, results from both WB and nano-liquid chromatography/mass spectrometry demonstrated that the levels of lamin A protein were more reduced suggesting an effect on expression of lamin A from the wild type allele. PCR analysis of the ratio of lamin A to lamin C transcripts showed unchanged relative amounts of lamin A transcript suggesting that the effect on the wild type allele was operative at

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the protein level. Immunofluorescence microscopy analysis showed no abnormal nuclear morphology of patient fibroblast cells. Based on these data, we propose that heterozygosity for the nonsense mutation causes NMD degradation of the mutant transcripts blocking expression of the truncated mutant protein and an additional trans effect on lamin A protein levels expressed from the wild type allele. We discuss the possibility that skewing of the lamin A to lamin C ratio may contribute to ensuing processes that destabilize cardiomyocytes and trigger cardiomyopathy

Introduction

Dilated cardiomyopathy (DCM) is a disease of the heart muscle characterized by cardiac chamber enlargement and systolic impairment of the left ventricle. The clinical symptom of DCM is heart failure, which is often associated with arrhythmia and sudden death. Mutations in the *LMNA* gene encoding the type V intermediate filament proteins, lamin A and lamin C represent the most frequent known genetic cause of DCM [1]. The clinical feature of *LMNA* cardiomyopathy is most often progressive conduction system disease and subsequent development of heart failure, which may lead to early life-threatening arrhythmias and sudden death [1]. Mutations in the *LMNA* gene cause a group of human genetic disorders called laminopathies, which affect different tissues. Laminopathies include cardiac and skeletal myopathies, lipodystrophy, peripheral neuropathy and premature aging [2]. The first report of *LMNA* mutations as a cause of DCM was published in 1999 by Fatkin et al. [3]. Genetic investigations of these patients revealed *LMNA* mutations in 4 families, and, since this discovery, many other *LMNA* mutations have been identified in DCM patients. Lamin A and lamin C are generated by alternative splicing of the *LMNA* pre-mRNA, are identical in the first 566 amino acids and have similar structural designs [4]. However, only prelamin A undergoes posttranslational processing to produce the mature wild type lamin A [5].

Both missense mutations and mutations resulting in premature termination codon (PTC) in the *LMNA* gene have been associated with DCM and dominant negative effects as well as haploinsufficiency have been suggested as disease mechanisms [6–7]. However, these mechanisms are still not clearly established because of conflicting results. Both mouse models and human cell lines have been used to understand the cellular and physiological processes leading to DCM. Evidence from several studies has shown that mRNAs containing a PTC located >50–55 nucleotides upstream of an exon–exon junction are suggested to cause degradation of such transcripts mediated by the nonsense mediated mRNA decay (NMD) system [8]. Consistent with this, Becane et al. showed reduced expression of lamin A and lamin C in DCM patient, indicating haploinsuffiency as a disease mechanism [7]. Furthermore, Muchir et al. investigated the *LMNA* nonsense mutation; p.Y259X associated with limb girdle muscular dystrophy 1B (LGMD1B) and showed reduced level of lamins A and C in patient fibroblasts [9]. Therefore, the authors proposed haploinsuffiency as a cause for the observed phenotype. However, Geiger et al. investigated one DCM patient with the same nonsense mutation, p.Arg321Ter, as we studied and WB analysis indicated that the expression levels of lamin A and lamin C in cultured fibroblasts and myocytes were not affected and were not different from the control fibroblast despite down-regulation of the mutant transcripts mediated by NMD. When patient fibroblasts were treated with a proteasome inhibitor, a lower molecular weight band became detectable indicating the presence of the truncated protein despite very small amounts of mutant transcript [6]. The authors suggested dominant negative effects as a disease mechanism due to the presence of the truncated protein. Interestingly, Pan et al. investigated a family with autosomal cardiac disease caused by a frameshift mutation resulting in PTC in the *LMNA* gene [10]. WB analysis indicated that lamins A and C proteins were reduced more than 50% and were almost absent in cultured peripheral blood lymphocytes from the affected person. This finding indicated an effect of the mutation on the expression of lamin A and lamin C from the wild type allele.

In this study, we wanted to investigate the consequences of a representative PTC mutation (c.961C>T; p.Arg321Ter) in the *LMNA* gene in 3 DCM patients from two unrelated families and to understand the underlying disease mechanisms using a combination of different methods. Due to the limited availability of patient heart tissue, we used patient-derived skin fibroblasts expressing lamin A and lamin C as a cell model to analyze lamin A and lamin C expression in patients with DCM caused by a *LMNA* mutation. We investigated whether heterozygosity for the PTC mutation, p.Arg321Ter affects mRNA and protein levels of lamin A and lamin C in cultured fibroblasts from three DCM patients. Quantitative reverse transcriptase polymerase chain reaction

Table 1 – Clinical data of DCM patients.

Patient ID/number	*LMNA* mutation	Sex/age	Symptom	ECG	Echocardiography	Ambulatory ECG	Treatment
SKS4DCM10/1	p.Arg321Ter	M/24	CHF	SR	Moderate LVD, LVEF 0.1	n/a	Cardiac transplantation
SKS47DCM4/2	p.Arg321Ter	M/62	CHF	SR	Moderate LVD, LVEF 0.25	Paroksysmal AF	Medical therapy, ICD
SKS47DCM5/3	p.Arg321Ter	F/50	None	SR	No LVD, LVEF 0.5	Paroksysmal AF, 2° AVB (Mobitz II)	ICD

Abbreviations: AF, atrial fibrillation; AVB, atrioventricular block; CHF, congestive heart failure; ECG, electrocardiography; F, female; ICD, implantable cardioverter defibrillator; LVD, left ventricular dilation; LVEF, left ventricular ejection fraction (normal range >0.55); M, male.