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methodological approaches to detection autophagy in muscle cell line C2C12 and influence of desmin gene mutations on the formation dynamics of autophagosome

Authors:
K Sukhareva1, A Kostareva1, 1Federal Almazov Medical Research Centre, Institute of molecular biology and genetics - Saint Petersburg - Russian Federation,

Topic(s):
Basic Science - Cardiac Diseases: Cardiomyopathies

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Background: Cardiomyopathies and skeletal myopathies are known to be genetically-determined and associated with mutations in genes encoding structural or cytoskeletal proteins. Impairment of mitochondrial function and autophagy failure are often co-segregated with cardiomyopathies. However, the precise molecular mechanism describing impairment of these functions have not been described yet. Desmin gene is one of cytoskeletal-protein encoding genes, linked to several types of cardiomyopathies (dilated, restrictive, arrhythmogenic) and distal myopathies. It was demonstrated that some desmin mutations impair autophagy process leading to formation of intracellular protein aggregates. The knowledge of autophagy processes is necessary in understanding the pathogenesis and in findings of ways to induce and inhibit the autophagy process to control disease. There for the aim of our work was to study the effect of various desmin mutations on autophagy processes.

Material and methods: The evaluation autophagy process was determined using Western blot, flow cytometry and immunocytochemistry on C2C12 cell line. An identification of optimum conditions of time, temperature and concentration of the solution to cell permeabilization in various detergents was performed. We demonstrated the need to use detergent digitonin for separate assessment of LC3 protein in soluble and insoluble fractions. It was shown that washing procedures are required for the better sample quality during the sample preparation for flow cytometry and Western blot. The evaluation of the autophagy process by flow cytometry confirmed the need for prior cell fixation using paraformaldehyde.

Results: We revealed that autophagy induction and turnover differes between wt and L345P. Moreover, the number of autophagosomes is decreased in C2C12 transduced by L345P compared to WT.

Conclusion: Desmin mutations alter disclosure of the molecular mechanisms of the development of myopathies and cardiomyopathies can be adapted to identify new target genes for genetically engineered therapy and would provide progress in the treatment of this group of severe disorders.
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Supernatant

WT c  WT 2h  WT 4h  WT 6h  WT 8h  345 c  345 2h  345 4h  345 6h  345 8h

1  0.505  0.259  0.124  0.196  1  0.793  1.148  1.187  1.267