Allele-Specific CRISPR/Cas9 Correction of a Heterozygous DNM2 Mutation Rescues Centronuclear Myopathy Cell Phenotypes.

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Abstract
Genome editing with the CRISPR/Cas9 technology has emerged recently as a potential strategy for therapy in genetic diseases. For dominant mutations linked to gain-of-function effects, allele-specific correction may be the most suitable approach. In this study, we tested allele-specific inactivation or correction of a heterozygous mutation in the Dynamin 2 (DNM2) gene that causes the autosomal dominant form of centronuclear myopathies (CNMs), a rare muscle disorder belonging to the large group of congenital myopathies. Truncated single-guide RNAs targeting specifically the mutated allele were tested on cells derived from a mouse model and patients. The mutated allele was successfully targeted in patient fibroblasts and Dnm2R465W/+ mouse myoblasts, and clones were obtained with precise genome correction or inactivation. Dnm2R465W/+ myoblasts showed an alteration in transferrin uptake and autophagy. Specific inactivation or correction of the mutated allele rescued these phenotypes. These findings illustrate the potential of CRISPR/Cas9 to target and correct in an allele-specific manner heterozygous point mutations leading to a gain-of-function effect, and to rescue autosomal dominant CNM-related phenotypes. This strategy may be suitable for a large number of diseases caused by germline or somatic mutations resulting in a gain-of-function mechanism.

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KEYWORDS: CRISPR; Cas9; allele-specific; autophagy; centronuclear myopathy; congenital myopathy; dominant mutation; dynamin; endocytosis; therapy

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