

Gene Editing Using CRISPR-Cas9 to Target DKK1 in Alzheimer's Disease: Exploring a Novel Therapeutic Strategy

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Abstract

Aim: To investigate the feasibility of using the *Staphylococcus aureus* CRISPR-Cas9 system to target and edit the DKK1 gene in vitro as a potential therapeutic strategy for Alzheimer's disease (AD), where DKK1 upregulation contributes to amyloid- β -mediated neurodegeneration.

Methods: Five guide RNA sequences targeting exon 1 of the DKK1 gene were designed using CRISPR software and screened for potential off-target effects using BLAST. Guide sequences were cloned into the pX601 vector containing the *S. aureus* Cas9 cassette. Plasmid integrity and guide insertion were confirmed by restriction digest and Sanger sequencing. N2a murine neuroblastoma cells were transfected with pX601 constructs, with GFP plasmids used to assess transfection efficiency. Gene editing was evaluated by PCR amplification of the target region followed by T7 Endonuclease I (T7EI) mismatch cleavage analysis.

Results: Sequencing confirmed successful insertion of all five guide RNAs into the pX601 vector. GFP fluorescence demonstrated successful transfection of N2a cells. T7EI analysis identified insertion/deletion mutations consistent with CRISPR-mediated editing of DKK1, with guide RNAs 1 and 3 demonstrating the highest editing efficiency across repeat experiments.

Conclusions: This study demonstrates successful in vitro CRISPR-Cas9-mediated targeting of DKK1, supporting its potential as a therapeutic target in Alzheimer's disease. Further studies are required to evaluate editing efficiency, specificity and long-term functional effects in neuronal models and in vivo before clinical translation.

Index Terms

CRISPR-Cas9; Gene Editing; Alzheimer's Disease; DKK1; Neurodegeneration; Wnt Signalling; *Staphylococcus aureus* Cas9; N2a Cells; T7 Endonuclease I; Translational Medicine.