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Advancements in Genetic Engineering

ISSN: 2169-0111

Commentary

Prime Editing for Correction of Frameshift Mutations in Cystic Fibrosis Transmembrane Conductance Regulator

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DESCRIPTION

Cystic Fibrosis (CF) represents one of the most common autosomal recessive disorders in Caucasian populations, primarily resulting from mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene. While the ΔF508 deletion accounts for approximately 70% of CF alleles, frameshift mutations comprising insertions or deletions (indels) represent another significant category affecting protein function. Prime editing technology, utilizing a modified Cas9 nickase fused to reverse transcriptase, offers unprecedented precision for correcting small insertions and deletions without requiring double-strand breaks or donor DNA templates.

Among the over 2,000 identified *CFTR* mutations, frameshift mutations represent a particularly severe class, leading to premature stop codons, truncated proteins, and complete loss of channel function. These mutations result in chronic respiratory infections, pancreatic insufficiency, and reduced life expectancy. While some therapies target specific mutation types, there remains a critical need for precise and broadly applicable genetic correction strategies-especially for frameshift variants.

Prime editing, a next-generation genome editing technology, offers a powerful and versatile solution for correcting frameshift mutations in the *CFTR* gene. Unlike traditional CRISPR-Cas9 editing, which relies on double-strand breaks and homology-directed repair, prime editing uses a catalytically impaired Cas9 (H840A nickase) fused to a reverse transcriptase and a prime editing guide RNA (pegRNA) to directly write new genetic information into the genome. This allows for the precise insertion, deletion, or substitution of nucleotides without causing significant off-target effects or relying on cell cycle-dependent repair pathways.

In the context of cystic fibrosis, prime editing holds exceptional promise for restoring proper reading frames and functional *CFTR* protein expression in patient-derived cells. This introduction explores the molecular basis, therapeutic potential, and current progress in applying prime editing to correct frameshift mutations in *CFTR*, highlighting its transformative potential for curative gene therapy in cystic fibrosis.

This investigation focused on correction of two prevalent frameshift mutations: the 1677delTA mutation in exon 10 and the 2184insA mutation in exon 13 of the *CFTR* gene. Primary human airway epithelial cells were isolated from CF patients harboring these mutations and cultured at air-liquid interface to maintain physiological characteristics. Prime editing constructs incorporated the PE3-SpRY variant, which demonstrates expanded Protospacer Adjacent Motif (PAM) compatibility and enhanced editing efficiency. Prime editing guide RNAs (pegRNAs) were designed with optimized Primer Binding Site (PBS) lengths of 10-13 nucleotides and Reverse Transcriptase template (RT template) lengths of 12-15 nucleotides.

Nucleofection-mediated delivery of prime editing ribonucleoprotein complexes achieved correction efficiencies of 31% for the 1677delTA mutation and 27% for the 2184insA mutation, as determined by targeted amplicon sequencing. Importantly, prime editing demonstrated superior precision compared to traditional HDR-based approaches, with minimal insertion-deletion byproducts detected at target sites. The editing process successfully restored the correct reading frame, enabling production of full-length *CFTR* protein as confirmed by Western blot analysis.

Functional characterization using Ussing chamber measurements revealed restoration of chloride transport activity in corrected cells, with cAMP-stimulated chloride current reaching 68% of wild-type levels. Forskolin-induced swelling assays demonstrated recovery of *CFTR*-mediated fluid transport, indicating functional protein restoration. Importantly, corrected cells maintained normal epithelial morphology and tight junction integrity, as assessed by transepithelial electrical resistance measurements.

CONCLUSION

Prime editing technology enables precise correction of frameshift mutations in *CFTR*, offering a promising therapeutic approach for CF patients with indel mutations. The high editing efficiency, minimal byproduct formation, and functional restoration of chloride transport demonstrate the clinical

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Received: 28-Feb-2025, Manuscript No. MAGE-25-38114; **Editor assigned:** 03-Mar-2025, PreQC No. MAGE-25-38114 (PQ); **Reviewed:** 17-Mar-2025, QC No. MAGE-25-38114; **Revised:** 24-Mar-2025, Manuscript No. MAGE-25-38114 (R); **Published:** 31-Mar-2025, DOI: 10.35841/2169-0111.25.14.406

Citation: Bhanot F (2025). Prime Editing for Correction of Frameshift Mutations in Cystic Fibrosis Transmembrane Conductance Regulator. Adv Genet Eng. 14:406.

potential of this approach. This work establishes prime editing as a viable precision medicine tool for treating genetic disorders caused by small insertions and deletions. Long-term culture studies confirmed editing stability over multiple passages, with no evidence of reversion mutations or cellular dysfunction.