CRISPR-Cas9-based Strategy for Excision of the CGG Repeat Expansion from the FMR1 gene in Fragile X-linked conditions

Natalia Stepczak1 , Adam Ciesiolka1 , Krzysztof Sobczak1 1Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland

Background: Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) and Fragile X-associated Primary Ovarian Insufficiency (FXPOI) are disorders caused by the expansion of CGG trinucleotide repeats (CGGexp) located in the 5'UTR region of the FMR1 gene. The length of CGGexp varies from patient to patient but remains within the range of 55 to 200 repeats. This results in elevated FMR1 mRNA levels and repeat-associated non-AUG (RAN) translation of a toxic protein product – FMRpolyG – containing a long polyglycine tract. Decreased levels of FMRP, a protein encoded by the FMR1 gene, essential for the maintenance of normal synaptic plasticity, are also observed. Method: CRISPR-Cas9 technology-based removal of CGGexp from FMR1 can be considered as a potential therapeutic strategy for FXTAS and FXPOI. We identified sgRNAs targeting regions upstream and downstream of CGGexp and generated "triple" genetic constructs containing two sgRNAs (enabling simultaneous expression of CGGexp) as well as the Cas9-encoding sequence. These constructs were transfected to human HEK293 cells expressing an FMR1 transgene containing either 16, 33, 85, or 95 CGG repeats. Results: It was determined that five pairs of sgRNAs delivered the most promising effects, as they did not disturb the level of neither endogenous FMR1 mRNA nor FMRP protein but contributed to a decline in FMRpolyG levels. Conclusion: These results serve as preliminary evidence supporting the use of this CRISPR-Cas9-based strategy for neutralization of the CGGexp mutation. A future application of these sgRNAs lies in their therapeutic use, which will need to be verified in appropriate in vivo models. Another is the generation of FXTAS patient-derived iPSC isogenic control cell lines, which would enable closer monitoring of disease-related molecular and cellular pathomechanisms.