Removing Root Cause of Fragile X Autism Spectrum Disorder by CGG Repeat Contraction via Epigenetic Approaches Involving R-loop

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Abstract

Fragile X syndrome (FXS) is one of the most common monogenic causes of autism spectrum disorders (ASD). Here I describe potential methods for correcting the genetic cause of FXS without introducing gene-editing nucleases in cellular models. In FXS patients, very long expansion of CGG trinucleotide repeats (>200 times) at the 5' UTR of the FMR1 gene causes epigenetic silencing of FMR1, a crucial gene for brain development. By investigating conditions favorable to FMR1 reactivation, I have found that certain sets of small molecules can strongly reactivate FMR1 mRNA and its protein FMRP expression in human embryonic stem (ES) and induced pluripotent stem (iPS) cells with FXS full mutation. Surprisingly, it also entails the shortening of the long CGG repeats. I traced the mechanism to a site-specific R-loop — a 3-stranded RNA-DNA structure — that is both necessary and sufficient for repeat contraction. The R-loop formation triggers DNA demethylation and FMR1 transcription, which in turn fortifies R-loop formation. Repeat contraction is specific to FMR1, restores production of FMRP protein. Collectively, these data implicate a positive feedback loop of DNA demethylation, de novo transcription, R-loop formation, and DNA repair in CGG contraction and FMR1 reactivation.