Structure Elucidation and Structure-Based Multimodal Targeting of Pathogenic TDP-43 Isoforms

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TAR DNA-binding protein 43 (TDP-43), a nucleic acid binding protein involved in RNA processing, is predominantly localized in the nucleus under normal conditions. In neurodegenerative diseases, mainly amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), and Alzheimer's disease (AD), TDP-43 is mislocalized to the cytoplasm. Cytoplasmic mislocalization disrupts TDP-43's canonical functions, resulting in cytotoxicity. Truncated, alternatively spliced TDP-43 isoforms may play causative roles in TDP-43 mislocalization and subsequent cytotoxicity. Such isoforms, known as shortened TDP-43s (sTDP-43s) are upregulated by neuronal hyperactivity, common amongst neurodegenerative diseases. Our study begins to investigate the structure-based targeting of a particular sTDP-43 isoform (sTDP-43$_{1\text{-}298}$), both at the protein and RNA level. At the protein level, structural analysis of sTDP-43$_{1\text{-}298}$ and sTDP-43$_{1\text{-}298}$:RNA complexes is pertinent to the development of rationally designed therapeutics. Cryo-electron microscopy (cryo-EM) is an advantageous tool to achieve this. Using cryo-EM, a potential three-dimensional volume of sTDP-43$_{1\text{-}298}$ has been produced, with an average resolution of 4.01 Å. Further structural refinement and molecular model-building is required for validation. At the RNA level, degradation of sTDP-43$_{1\text{-}298}$ mRNA prior to translation could potentially have therapeutic effects. Using available RNA-sequencing data, mRNA sequences for wild-type TDP-43 and sTDP-43 isoforms were aligned. Alignment resulted in the discovery of a unique region in the 5'-UTR of sTDP-43$_{1\text{-}298}$ mRNA, which could potentially serve as a target for RNA-degrading therapies (RIBOTACs). Two-dimensional and three-dimensional structure predictions were generated for this unique region. Structural confirmation will be done via 1D ^{1}H NMR and selective 2′ hydroxyl acylation analyzed by primer extension (SHAPE).

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