Investigating the Functional Loss Mechanisms of Angiogenin Mutations in Amyotrophic Lateral Sclerosis through Computational Prediction and Experimental Validation

Abstract

Amyotrophic Lateral Sclerosis (ALS) is a fatal, progressive neurodegenerative disorder characterized by the selective degeneration of motor neurons, consequently leading to paralysis and death of the patients due to respiratory failure between 3–5 years of symptom onset. Approximately 140,000 new ALS cases are diagnosed worldwide each year. Most of the ALS cases are sporadic, while approximately 10% are familial. Currently, no therapy exists for this disorder, and the only available drug for treatment is an antiglutamatergic compound, Riluzole, which extends a patient’s lifespan by a few months but has no major effect on improving the symptoms. Although ALS is instigated by a number of factors including protein aggregation, mitochondrial dysfunction, oxidative stress, defective axonal transport, excitotoxicity and dysfunctional growth factor signalling, genetic variation remains one of the primary causes of ALS. While it is known that mutations in genes \textit{SOD1}, \textit{FUS/TLS}, \textit{TARDBP}, \textit{ANG}, and a hexanucleotide repeat expansion (GGGGCC) in \textit{C9ORF72} cause ALS most frequently, the molecular mechanisms underlying ALS pathogenesis due to these mutations are not fully understood. In order to develop a successful therapy, there is an urgent need to discover novel genes, corresponding gene mutations and decipher their role in disease manifestation.

Among the ALS causative genes, angiogenin (\textit{ANG}) was identified as one of the most frequently mutated genes in ALS patients. Previous studies have shown that mutations in the angiogenin effector (\textit{ANG}) cause loss of certain neuroprotective functions, such as ribonucleolytic activity and nuclear translocation activity, resulting in ALS. The primary objective of this work was to investigate the loss-of-function(s) mechanisms of all the \textit{ANG} mutants implicated in ALS. To achieve this, extensive all-atom molecular dynamics (MD) simulations for wild-type \textit{ANG} (WT-\textit{ANG}) and all the ALS associated \textit{ANG} mutants was carried out. MD simulations were performed to study the structural and dynamic differences in the catalytic triad (responsible for ribonucleolytic activity) and nuclear localization signal (responsible for nuclear translocation activity) residues between the WT-\textit{ANG} and mutants. It was observed that certain ALS associated \textit{ANG} mutants exhibit loss of ribonucleolytic activity due to conformational switching of the catalytic residue His114, and other mutants’ exhibit loss of nuclear translocation activity due to reduction of solvent accessible surface area (SASA) by local folding of nuclear localization signal residues ^3RRR^3.

In addition, the role of several single nucleotide polymorphisms and rare mutations found in \textit{ANG} were investigated. As a rational, experimental validation of loss-of-function(s) predictions for uncharacterized \textit{ANG} mutants through functional assay experiments were carried out. Functional assay results show that the ALS associated \textit{ANG} mutant - D22G exhibits loss of ribonucleolytic activity while the L35P mutant results in loss of both ribonucleolytic and nuclear translocation activities, thereby matching completely with simulation predictions. Further, to help clinicians and researchers for a better understanding of ALS etiology, a fast method based on implicit-solvent MD simulation was developed to determine the functional loss mechanisms of \textit{ANG} mutants as the predictions using all-atom explicit-solvent MD simulation was computationally intensive. In addition, we noticed that no web-based tool was available in the public domain to predict whether a newly identified \textit{ANG} mutation would be deleterious or benign. To achieve this, a freely available web-based tool, \textit{ANGDelMut}, was developed to determine whether a novel \textit{ANG} mutation could result in loss-of-functions and cause ALS.

In this study, using a combination of computational predictions followed by biochemical and cellular biology experiments, the molecular etiology of \textit{ANG}-associated ALS has been uncovered. The overall results of this thesis presents the underlying molecular mechanisms of loss-of-functions of \textit{ANG} mutants and provides insight into how missense mutations in \textit{ANG} affects the structure and dynamics of \textit{ANG} mutants, leading to the loss of neuroprotective functions, and thereby causing ALS. Findings from this thesis contribute to our growing understanding of how genetic mutations and polymorphisms could affect the protein functions in ALS pathophysiology, and may lead to a new, effective therapeutic intervention.