# Chapter 52
# Molecular Network Analysis of Target RNAs and Interacting Proteins of TDP-43, a Causative Gene for the Neurodegenerative Diseases ALS/FTLD

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## ABSTRACT

*TAR DNA-binding protein-43 (TDP-43) is an evolutionarily conserved nuclear protein that regulates gene expression by forming a multimolecular complex with a wide variety of target RNAs and interacting proteins. Abnormally phosphorylated, ubiquitinated, and aggregated TDP-43 proteins constitute a principal component of neuronal and glial cytoplasmic and nuclear inclusions in the brains of patients with amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), establishing a novel clinical entity designated TDP-43 proteinopathy. Although increasing evidence suggests that the neurodegenerative process underlying ALS and FTLD is attributable to a toxic gain of function or a loss of cellular function of TDP-43, the precise molecular mechanisms remain largely unknown. Recent advances in systems biology enable us to characterize the global molecular network extracted from large-scale data of the genome, transcriptome, and proteome with the pathway analysis tools of bioinformatics endowed with a comprehensive knowledge base. The present study was conducted to characterize the comprehensive molecular network of TDP-43 target RNAs and interacting proteins, recently identified by deep sequencing with next-generation sequencers and mass spectrometric analysis. The results propose the systems biological view that TDP-43 serves as a molecular coordinator of the RNA-dependent regulation of gene transcription and translation pivotal for performing diverse neuronal functions and that the disruption of TDP-43-mediated molecular coordination induces neurodegeneration in ALS and FTLD.*

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## INTRODUCTION

TDP-43 is a nuclear RNA/DNA-binding protein that is highly conserved throughout evolution. It is encoded by the TARDBP gene on chromosome 1p36.22, which was originally identified as a transcriptional repressor of the human immunodeficiency virus (HIV) gene (Ou et al., 1995). TDP-43, capable of interacting with the UG/TG repeat stretches of target RNAs/DNAs, plays a key role in the regulation of transcription, alternative splicing, mRNA stability and transport, and microRNA biogenesis (Buratti and Baralle, 2010; Lagier-Tourenne et al., 2010). Structurally, it is composed of an N-terminal domain and two highly conserved RNA-recognition motifs, RRM1 and RRM2, followed by a glycine-rich C-terminal domain (Lagier-Tourenne and Cleveland, 2009). The RRM1 domain is necessary and sufficient for the recognition of UG repeats of target RNAs, whereas the C-terminal domain that mediates protein-protein interactions plays an essential role in the regulation of splicing (Ayala et al., 2005; Buratti et al., 2005). In normal cells under physiological conditions, the vast majority of TDP-43 protein is located in the nucleus, highly enriched in nuclear bodies.

Accumulating evidence indicates that abnormally phosphorylated, ubiquitinated, and aggregated TDP-43 proteins constitute a principal component of the neuronal and glial cytoplasmic and nuclear inclusions in the brains of patients with amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-TDP) (Arai et al., 2006; Neumann et al., 2006). ALS and FTLD share substantial clinical and pathological manifestations (Mackenzie et al., 2010). ALS patients show generalized skeletal and bulbar muscle atrophy owing to progressive loss of cortical and spinal motor neurons. Up to 10% of ALS cases are caused by inheritable genetic mutations. FTLD is a cause of the second most common type of early-onset dementia. FTLD patients show behavioral changes with progressive decline in cognitive function caused by neuronal loss that chiefly affects the frontotemporal cortex. Importantly, substantial numbers of ALS patients show cognitive impairment, whereas significant numbers of FTLD patients develop symptoms of motor neuron disease. Approximately 50% of the cases of clinical FTLD exhibit TDP-43 pathology (Chen-Plotkin et al., 2010). Furthermore, TDP-43-immunoreactive inclusions accumulate, to a lesser extent, in the brains of patients with various neurodegenerative diseases, such as Alzheimer's disease (AD), dementia with Lewy bodies (DLB), Pick's disease (PiD), and the Guam parkinsonism-dementia complex (G-PDC) (Geser et al., 2009).

Because ALS and FTLD display clinicopathologically overlapping features, they have recently been categorized into a novel disease entity named TDP-43 proteinopathy (Geser et al., 2009). In TDP-43 proteinopathy, TDP-43 often translocates from the nucleus to the cytoplasm, where it forms detergent-insoluble, urea-soluble aggregates. The accumulated proteins are hyperphosphorylated, polyubiquitinated, and proteolytically cleaved to produce 25-kDa and 35-kDa C-terminal fragments (Hasegawa et al., 2008). Following exposure to stressful insults, TDP-43 promptly accumulates in the cytoplasmic stress granules (SGs) that regulate stress-induced translational arrest (Colombrita et al., 2009). Impaired dynein-mediated microtubule transport promotes the aggregation of the C-terminal fragments (Pesiridis et al., 2011). The C-terminal domain of TDP-43 contains multiple phosphorylation consensus sites, among which phosphorylation of Ser-409/410 on TDP-43 serves as the pathological hallmark of sporadic and familial ALS cases (Neumann et al., 2009). Hyperphosphorylation of TDP-43 promotes oligomerization and fibril formation *in vitro* (Hasegawa et al., 2008). Overexpression of the TDP-43 C-terminal fragment is sufficient to generate hyperphosphorylated and ubiquitinated cytoplasmic aggregates that alter the exon-splicing pattern (Zhang et al., 2009). Importantly, various