Chapter

The Role of TDP-43 in Genome Repair and beyond in Amyotrophic Lateral Sclerosis

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Abstract

The pathology of the RNA-/DNA-binding protein TDP-43, first implicated a decade ago in the motor neuron disease amyotrophic lateral sclerosis (ALS), has been subsequently linked to a wide spectrum of neurodegenerative diseases, including frontotemporal dementia (FTD), Alzheimer's disease (AD), and related dementia-associated disorders. ALS, also known as Lou Gehrig's disease, is a progressive, degenerative motor neuron disorder, characterized by a diverse etiopathology. TDP-43 pathology, mediated by a combination of several mutations in the *TARDBP* gene and stress factors, has been linked to more than 97% of ALS patients. We recently identified, for the first time, the critical involvement of TDP-43 in neuronal genome maintenance and the repair of DNA double-strand breaks (DSBs). Our studies showed that TDP-43 regulates the DNA break-sealing activities of the XRCC4-DNA Ligase 4 (LIG4) complex in DSB repair, suggesting that loss of genomic integrity in TDP-43-associated neurodegeneration may be amenable to a DNA repair-based intervention. In this chapter, we discuss the broader aspects of TDP-43 toxicity-induced pathomechanisms, including the emerging role of TDP-43 in neuronal DSB repair and its synergistic genotoxic effects with other neurodegeneration-associated etiologies that contribute significantly to neuronal dysfunction. We also discuss potential future perspectives and underscore how unraveling the molecular basis and implications of TDP-43-induced genome instability in ALS could guide the development of neuroprotective therapies.

Keywords: TDP-43, DNA damage, DNA double-strand breaks, nonhomologous end joining, XRCC4-DNA ligase 4, ALS, stress granule, Rab11, neurodegeneration

1. TDP-43 pathology: a predominant player in ALS

Transactive response DNA-binding protein 43 (TDP-43) is a versatile 43 kDa DNA-/RNA-binding protein of the heterogeneous nuclear ribonucleoprotein (hnRNP) family. TDP-43's cytosolic aggregation and inclusion body formation are the key pathologic hallmarks of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) [1–3]. In these progressive motor neuron diseases, TDP-43 pathology manifests as the tau- and synuclein- negative and ubiquitin-positive inclusions in the anterior horn, spinal cord, neocortex, and hippocampus regions of the brain. In the last decade, tremendous scientific investigations have been carried out to understand the etiopathologies of TDP-43 toxicity in ALS and FTD. These studies demonstrated that TDP-43 pathology-linked ALS is one of the

most complex neurological diseases due to TDP-43 pathomechanisms overlapping with other ALS-causing genes, such as C9orf72 [4–6], valosin-containing protein (VCP) [7–9], fused in sarcoma (FUS) [10], optineurin (OPTN) [11], ubiquilin 2 (UBQLN2) [12, 13], and ataxin 2 (ATXN2) [14, 15]. Strikingly, TDP-43 pathology has been detected in more than 97% of sporadic ALS cases (>90%), making it a predominant player in most ALS patients.

1.1 Familial and sporadic ALS

Due to highly overlapping symptoms, determining the sporadic or familial nature of the ALS disease at its time of onset is difficult based on clinical features alone. Familial ALS (FALS) is predicted based on genetic screening for *TARDBP* mutation(s) in the patient and matching the same mutation in the family member(s) with a positive clinical/medical history of neurodegeneration and dementia in the juvenile or early stage of life. Race, age, and gender are risk factors for progression of FALS. FALS incidences vary significantly from 5 to 10% among all ALS cases. The disease is more common in Caucasian men and women than African, Asian, and Hispanic populations [16]. Notably, clusters of ALS incidences have been associated with socio-economic conditions along with race. According to Centers for Disease Control and Prevention, ALS prevalence is highest in the Midwest and Northeast of US main land (5.7 and 5.5 per 100,000 population, respectively) [17]. In Europe, the prevalence of FALS was lower in the southern part [18]. Studies show that men are at slightly higher risk than women, with an average age of onset ~60 years. Patients usually survive for 1.5–4 years after diagnosis, depending on the disease aggressiveness [19].

FALS associated pathology affects anterior horn motor neurons in the cervical and lumbosacral regions in the spinal cord, frontal cortex, and cranial nerve motor neurons in the pons and medulla segments of brainstem. Both sporadic ALS (SALS) and FALS pathologies are associated with upper and/or lower motor neurons of the spinal cord. Upper motor neuron diseases accompany degeneration of lateral corticospinal tracts leading to hyperreflexia/spasticity and cardiac arrest. Conversely, lower motor neuron death leads to muscle atrophy and denervation.

FALS cases fall into to three categories, namely, autosomal dominant, autosomal recessive, and X-linked dominant. Autosomal dominance is the most common inherited pattern among the FALS cases, where a mutation in one copy of the ALS-linked gene is sufficient to develop ALS conditions. These patients usually have a strong positive family history and their children bear 50% risk of developing ALS. However, autosomal dominance can also happen in apparently sporadic ALS (because the genetic inheritance is unknown or poor medical history) with de novo dominant mutation(s). In such cases, patients' siblings may have very low risk but children may have up to 50% risk of developing ALS. To date, more than 50 mutations in *TARDBP* gene have been identified in ALS etiopathologies [20]. In addition, a study on a German cohort of non-SOD1 FALS patients revealed mutations in *TARDBP*, suggesting *TARDBP* gene mutation screening should be crucial among non-SOD1 FALS patients [21]. The known *TARDBP* mutations in FALS are G290A, G298S, A315T, M337V, N345K, A382T, and I383V; some of the common mutations identified in SALS are D169G, G287S, G294A, Q331K, G348C, R361S, and N390S/D (**Figure 1**) [21–27]. Most of these *TARDBP* mutations are located within exon 6, as commonly found in Caucasian and European ALS patients; however, a large cohort of Han Chinese population with non-SOD1 SALS did not show any such mutations in exon 6 [28].

SALS has been reported in an increasing number among people exposed to lead or other heavy metals [29–31], smoking [32, 33], and pesticides [34]. Increased exposure to organochlorine pesticides, biphenyls, and brominated flame retardants

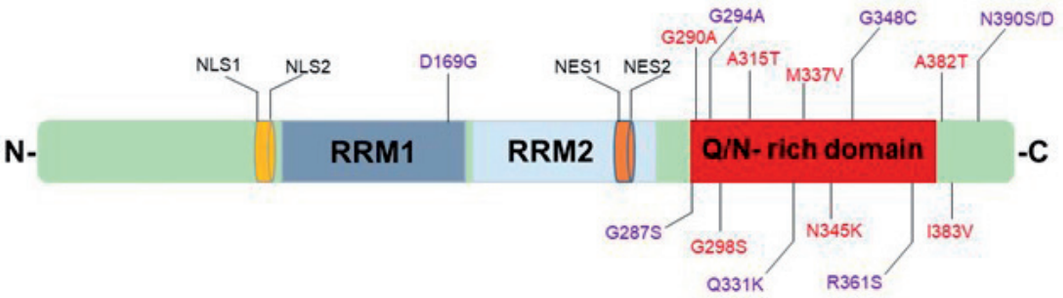

Figure 1.
TDP-43 protein's structural organization. Schematic representation of TDP-43 protein structure shows N-terminal domain of TDP-43 consists of highly ordered nucleic acid binding domains, namely, RNA recognition motifs (RRM) 1 and 2. RRM1 domain follows a bifurcated nuclear localization signal sequence (82–98aa)—NLS1 and NLS2. RRM1 domain spans from 104 to 176aa. RRM2 domain ranges from 192aa to 262aa including bifurcated nuclear export signal sequence (239–250aa)—NES1 and NES2. Later part in the C-terminal domain is mostly disordered and contains majority of the pathogenic mutational hotspots. Familial ALS-related mutations are indicated in red, and sporadic ALS-linked mutations are in blue color.

used in household furniture is associated with higher risk for ALS [35–37]. Interestingly, while smoking has been shown to predispose individuals to ALS risk, alcohol consumption did not show any effect on ALS development [38]. This suggests that diet and exposure to various environmental toxins could increase the ALS susceptibility in the vulnerable population. Most notably, US veterans from the Gulf War [39, 40] and those with head injury [41, 42] are also at a greater risk for developing SALS. By targeted sequencing of exon 6 of *TARDBP* from brain samples of US veterans with SALS, we observed several missense mutations (both reported and novel) and the association of TDP-43 Q331K mutation with DNA DSB repair impairment [22]. Another well-known incidence of sporadic ALS is the Guamanian ALS, which falls on the Parkinsonism-dementia complex spectrum (ALS/PDC). This particular type of ALS in people living in Guam and Rota islands is caused by the consumption of cyanobacteria-infested cycad fruits containing the neurotoxin β-methylamino-L-alanine (BMAA) [43, 44]. BMAA exerts neurotoxicity by incorporating into amino acid sequences and inducing tau pathology, oxidative stress, glutathione depletion, and protein aggregation [45–48]. Genome-wide analyses for ALS-/PDC-associated chromosomal loci revealed three disease-specific loci—two regions on chromosome 12 and the MAPT region on chromosome 17—in these populations [49].

1.2 Unique feature of TDP-43 pathology: dual role of loss of function and gain of toxicity

Given that TDP-43 contains a prion-like domain, its cellular content and distribution are two contributing factors for preventing the protein aggregation leading to the onset of disease. These two conditions are maintained by TDP-43's autoregulatory feedback loop mechanism. TDP-43 is mainly a nuclear protein, but it also shuttles to the cytosol, mitochondria, and other cellular organelles in response to various stimuli. In ALS/FTD pathology, TDP-43 mislocalization, due to pathogenic mutations and/or protein aggregation, is known as the primary culprit for the onset of neurodegeneration. Studies suggest that initiation of caspase-3/7 activation by TDP-43 toxicity facilitates the disease progression by cleaving mislocalized TDP-43 into 35 and 25 kDa fragments. These highly aggregation-prone fragments promote inclusion body formations with the hyperphosphorylated and polyubiquitinated C-terminal domain of TDP-43 [50, 51]. In addition to the C-terminal fragment, full-length and homo-dimerized TDP-43 also has been identified in the spinal cord

and brain of ALS patients [52, 53]. Furthermore, recent findings have highlighted that this protein aggregation enhances the aggregation propensities of other prion-like domain containing proteins. The interaction of these prion-like proteins increasing the disease complexity severalfold higher than that of single-protein toxicities [54]. TDP-43's cellular shuttling is regulated by the bifurcated nuclear localization signal (NLS) and nuclear export signal (NES) sequences (**Figure 1**), and loss of either of these signal sequences could be deleterious for cell survival. In vitro studies expressing NLS-deleted TDP-43 found accelerated cytoplasmic aggregate formation along with aberrant RNA processing defects [55, 56]. This phenomenon was further supported by in vivo studies with a transgenic mouse model and transcriptomic analysis in ALS/FTD human brain samples, which revealed significantly altered mRNA splicing of histones and aberrant chromatin remodeling following cytosolic accumulation of toxic TDP-43 [57]. Almost all of the pathogenic sporadic and familial mutations in TDP-43 induce its nuclear clearance and cytosolic sequestration to varying extents. However, in vitro studies with the FALS-linked, NLS-specific A90V mutation in TDP-43 found disease-like detergent insoluble cytosolic aggregates, confirming the crucial role of NLS/NES sequences in TDP-43 homeostasis, and further suggesting cytosolic aggregation of TDP-43 is a determining factor in motor neuron death [58, 59].

Persistent neuroinflammation is considered one of the leading causes for motor neuron death in ALS/FTD and also affects patients' therapeutic response. Innate immune responses, including microglial hyperactivation and astrogliosis, were consistently documented in human postmortem ALS human brain and spinal cord, as well as brains from ALS-model mice [60–63]. TDP-43 plays a vital role in regulation of neuroinflammation by inhibiting NF-κB activation, possibly through competitive binding to importin α3 [64]. TDP-43 depletion or nuclear loss induces extracellular secretion of TNF-α and enhanced nuclear localization of NF-κB p65 in glia and neurons [65–67]. Furthermore, inflammatory responses increase nuclear loss of TDP-43 and progression of ALS/FTD pathology.

2. Broad functions of TDP-43 in central nervous system (CNS)

2.1 RNA transactions

Extensive research in the last decade on TDP-43 toxicity in ALS has established that TDP-43 has multiple functions, including autoregulation of its levels through a negative feedback loop and controlling a diverse set of RNA-associated mechanisms, including pre-mRNA processing and splicing, micro RNA biogenesis, and RNA transport, transcription, and translation. Importantly, TDP-43 also regulates protein levels of critical RNA-binding proteins involved in RNA splicing, including SRSF1, PTB, and hnRNP L [68, 69]. TDP-43's autoregulation is solely controlled by the TDP-43-binding region (TDPBR) domain, which is comprised of several polyadenylation (pA) sites. The silent intron-7 linked to the TDPBR regulates the alternative splicing of pA sites based on the TDP-43 cellular concentration. In the steady state, pA1 allows cytoplasmic shuttling of TDP-43 mRNA for the appropriate amount of protein production. When overexpressed, TDP-43 binds threefold more to TDPBR and increases polymerase II binding at this sequence, which then processes intron-7 to remove the pA1 signal sequence and enforces the use of pA2 and pA4 sites [70]. The use of pA2/A4 sites makes the TDP-43 mRNA partially retained in the nucleus and leads to reduced production of TDP-43 protein in the cell [71]. Moreover, a recent study on TDP-43 autoregulation using the *Drosophila* model has identified human homolog transcription elongation regulator 1 (TCGER1) of

Drosophila protein GC42724 as having a role in TDP-43 mRNA alternative splicing and nucleo-cytoplasmic shuttling. TCGER1 regulates TDP-43 production through its TDPBR region [72].

TDP-43 contains two RNA recognition motifs (RRMs): RRM1 and RRM2 (**Figure 1**). Each RRM contains two highly conserved RNA recognition segments: octameric ribonucleoprotein 1 and hexameric ribonucleoprotein 2 [73]. TDP-43 RRMs bind a minimum of six UG/TG repeats, and its binding affinity increases with increasing numbers of repeats [74]. However, the binding preference is quite different between two motifs; unlike RRM1, RRM2 prefers a short stretch of UG repeats over long repeats ($(UG)_3 > (UG)_6$) [75].

Amino acid sequence analysis reveals a high degree of sequence similarities in the N-terminal domains of TDP-43 among human, mouse, rat, *Drosophila melanogaster*, and *Caenorhabditis elegans*, suggesting that TDP-43's function is crucial in these organisms. The C-terminal region of TDP-43 is predominantly disordered in its native structure, comprising a prion-like domain and several glycine-asparagine rich patches that contribute to the exon-skipping activity of TDP-43 (**Figure 1**). In vivo studies have revealed that TDP-43 regulates its splicing mechanism via its C-terminal glycine-rich domains [76]. A recent study of TDP-43 knockdown in a *Drosophila* model expressing chimeric repressor proteins demonstrated that TDP-43 in motor neurons regulates RNA splicing fidelity through splicing repression [77]. These findings are in agreement with previous studies reporting TDP-43's interaction with more than 6000 mRNAs in the mouse brain and TDP-43 depletion-induced altered splicing of ~900 mRNAs causing neuronal vulnerability (**Figure 2**) [78]. Furthermore, a recent study with ALS-TDP-43 M337V mutant knock-in mouse model has revealed mRNA splicing deregulation as the major pathological hallmark of mutant TDP-43, even in the absence of any noticeable motor deficits [79]. The mammalian TDP-43 primary transcript produces 11 alternatively spliced variants of mRNA, further supporting the functional complexity of TDP-43 [80]. As part of the hnRNP family, TDP-43 has a similar domain organization to hnRNP A1 and A2/B1 [81]. TDP-43's C-terminal domain interacts with a number of hnRNP family proteins, particularly hnRNP A2/B1 and A1, to form the hnRNP rich complex that is crucial for splicing inhibition [82]. HnRNP A1, a 34 kDa protein, is abundant in motor neurons and has been implicated in the pathomechanism of spino-muscular atrophy [83]. Mislocalized TDP-43 modulates the inclusion of exon 7B in the alternatively spliced hnRNP A1 transcript, leading to the production of an isoform with an extended prion-like domain [84–86]. Because TDP-43 and hnRNP A1 interact directly, increasing the aggregation propensity of hnRNP A1 could strongly influence TDP-43's aggregation in a synergistic pattern [87].

In addition to pre-mRNA splicing regulation, the N-terminal domain (NTD) of TDP-43 plays an essential role in protein stabilization and prevention of pathogenic cytoplasmic aggregation. In vitro studies have shown that N-terminal Leu71 and Val72 in the β7 strand region at the interface are crucial for its homodimerization into dimers and/or tetramers in a concentration-dependent manner [88]. Furthermore, single amino acid substitutions at V31R and T32R disrupt TDP-43's splicing activity and induce aggregation. The L28A mutation strongly destabilizes the TDP-43 NTD and promotes its mislocalization, and the L27A mutation increases its monomeric forms [89]. Recent findings by Chen et al. show that the K181E mutation near the TDP-43 RRM1 domain disrupts TDP-43's ability to process mRNA, induces mutant TDP-43 hyperphosphorylation, enhances detergent-resistant aggregation propensity by several fold, and leads to more wild-type TDP-43 in the nuclear and cytoplasmic inclusion bodies [90]. TDP-43 oligomerization has been reported to be the first toxic intermediate in TDP-43 proteinopathies [91]. An RNA-mediated intervention strategy showed inhibition of TDP-43 misfolding in

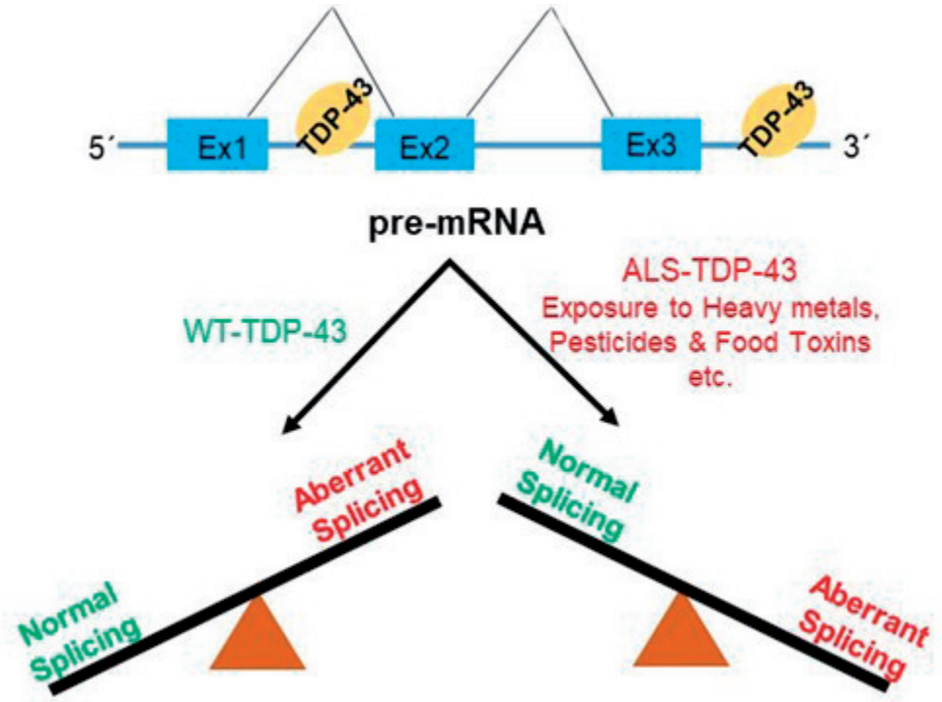

Figure 2.
TDP-43 proteinopathy causes RNA processing defects. TDP-43 being a major functional subunit of spliceosomal complex regulates the RNA maturation processes of hundreds of target genes. Many of which are directly associated with amyotrophic lateral sclerosis (ALS) and FTD. TDP-43, through its RRM domains, binds mainly to intronic and 3′ UTR-located UG-repeat sequences of pre-mRNA to modulate their splicing events. In healthy condition, TDP-43 strongly promotes normal splicing events and inhibits disease-causing aberrant alternative splicing. However, ALS/FTD-TDP-43 pathology induces aberrant splicing several folds higher than normal splicing leading to mRNA dysregulation-associated proteinopathies in motor neurons and glia. Moreover, exposure to various environmental toxins like heavy metals (Lead, iron, zinc), pesticides (organochloride compounds), and food toxins (BMAA) could cause pathogenic alterations in TDP-43 leading to its mislocalization and dysregulation of its splicing activity as a result causing sporadic ALS.

wild-type and pathogenic ALS-TDP-43 mutants, further suggesting a critical role for RRM domains in TDP-43 pathology.

MicroRNAs (miRNAs) are the master regulators of a number of vital cellular mechanisms and diseases, including neurodegeneration and genomic instability. TDP-43 regulates a subset of miRNAs by direct interaction and modulates their biogenesis through Drosha and Dicer complexes [92]. In the brain, neurexin-1 (NRXN1) controls vesicular trafficking between synaptic junctions. TDP-43 has been shown to bind mir-NID1 to suppress NRXN1 expression and thereby inhibit neuronal development and functionality [93]. Given that long noncoding RNA (lncRNA) confers a higher degree of gene expression regulation, TDP-43's increased interaction with two crucial lncRNAs, MALAT1 and NEAT1, possibly modulates the RNA metabolism dysregulation of ALS- and FTD-associated TDP-43 pathology [94, 95]. Furthermore, lncRNA gadd7, which is involved in cell cycle regulation and DDR signaling, orchestrates TDP-43's interaction with CDK6 mRNA that leads to its controlled decay [96].

2.2 Stress granules

Stress granule (SG) assembly is a dynamic, reversible process that promotes cell survival when stress factors are present. Membraneless SG organelles vary in their morphology and building composition in a cell type-specific manner [97, 98]. As long as the assembly/disassembly ratio is maintained in healthy cells, SGs act as the emergency store house for certain classes of RNA and protein molecules to protect

them from the various stress stimuli (**Figure 3**). However, altered SG assembly processes are associated with a number of human diseases, including neurodegenerative disorders and dementia [99]. Recent discoveries indicate SGs are critical players in modulating signal circuits determining cell death versus survival in response to stress exposure.

Emerging evidence suggests that TDP-43, but not FUS, regulates SG dynamics and secondary polymerization of TIA-1, which is essential for SG assembly (**Figure 3**) [100]. TIA-1 is an RNA-binding protein and classical SG marker and has more recently been considered as a candidate gene for ALS and ALS/FTD due to its rare heterozygous mutations (P362L and E384K) in the conserved amino acid residues within its low complexity domain [101]. TDP-43 increases its association with TIA-1 in a time-dependent manner in response to the osmotic and oxidative stressor, Sorbitol, in primary glial cells or other stressor-induced SGs in primary cortical neurons and astrocytes [102]. Studies have also shown aging as a crucial modulator of SG dynamics in neurodegenerative conditions. Notably, wild-type and ALS-linked pathogenic TDP-43 mutants show distinct patterns of stress formation rates and morphology. Mutant TDP-43 exhibits faster stress granule incorporation along with rapid increases in granule-size, while wild-type TDP-43 forms increasing numbers of granules with consistent size over time [99]. Apart from the TIA-1 aggregation regulation, TDP-43 also controls the mRNA level of G3BP1, an essential SG initiation factor. The loss of functional TDP-43 or pathogenic TDP-43-mediated G3BP1 mRNA depletion perturbs the interaction between SG components and processing bodies, leading to impaired storage of polyadenylated mRNAs [103]. However, different TDP-43 ALS-mutant variants exhibit differential regulation mechanisms. For instance, TDP-43 D169G does not affect SG formation mechanism, but R361S and A382T variants show loss-of-function phenotypes with

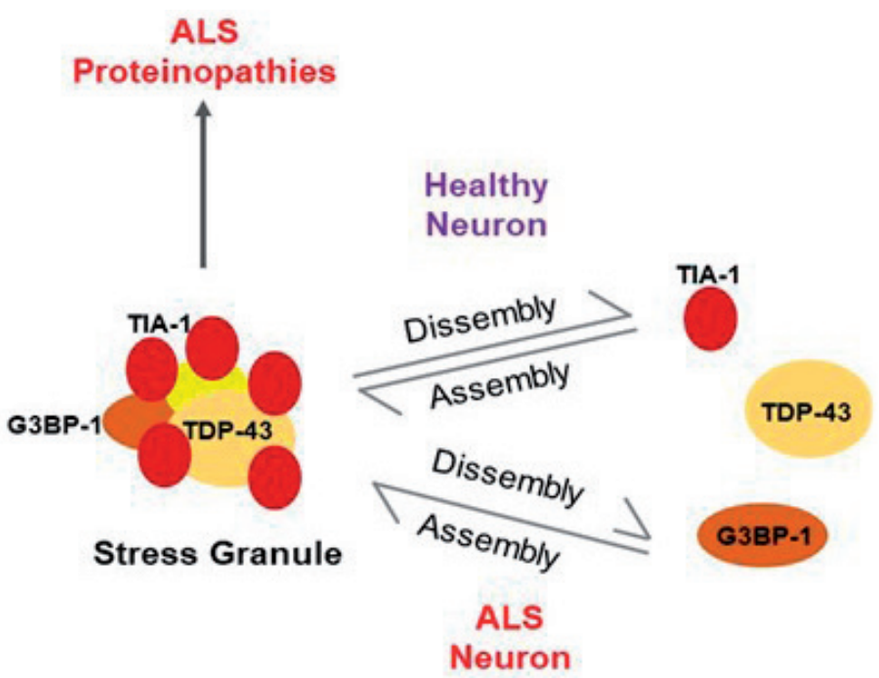

Figure 3.
TDP-43 is a critical component of stress granule. Stress granule formation, in normal condition, is a very important cellular defense mechanism to overcome stress responses. Stress granule assembly and disassembly is a reversible process in healthy cells. It's a membraneless structure consisting of stress granule marker proteins TIA-1, G3BP-1, and TDP-43 as the major structural component. Given that ALS-/FTD-associated TDP-43 pathology induces aberrant cytosolic sequestration of toxic TDP-43, it impairs the reversible nature of stress granule formation mechanism, thereby inducing the seeding mechanism for protein aggregation.

respect to SG dynamics [104, 105]. Interestingly, chronic TDP-43 liquid-liquid phase separation (LLPS) has been observed in neuronal cells and ALS-iPSc-derived motor neurons, even with only a transient stress induction from sonicated amyloid fibrils or arsenite. Early stage LLPS TDP-43 droplets are formed in the nucleus independent of conventional SG mechanisms. Cytosolic TDP-43 droplets formed by transient stress induction gradually incorporate importin-α and Nup62, leading to the mislocalization of nuclear pore complex proteins Nup107, Ran, and RanGap1. This situation inhibits nucleo-cytoplasmic shuttling and clearance of toxic TDP-43 and results in neuronal cell death [106]. Hans et al. have shown that TDP-43 hyper-ubiquitylation-mediated insolubility could happen by multiple distinct mechanisms independent of its translocation to cytosolic SGs [107]. This study shows neither endoplasmic reticulum kinase inhibitors nor translation blockers could prevent TDP-43 ubiquitylation. Moreover, the sorbitol-induced stress response involves impaired TDP-43 splicing activity, whereas sodium arsenite-induced SG formation occurs through oxidative stress, which can be quenched by the treatment with antioxidant like N-acetylcysteine. High-content screening of inhibitors for blocking pathogenic TDP-43 accumulation in SGs from ALS patient-derived iPSc-motor neurons has identified planar aromatic moieties with DNA intercalation properties as the potent small molecule therapy for ALS/FTD-TDP-43 pathology [108]. In this context, it is important to mention that TDP-43 aggregates/inclusions do not completely overlap with TDP-43 associated SGs, rather a subpopulation of those TDP-43 aggregates enter into SGs, and ALS-pathology-linked TDP-43 inclusion bodies are devoid of SGs. Notably, SGs indirectly exhibit positive feedback regulation of TDP-43 aggregation by disrupting HDAC6-mediated pathogenic TDP-43 clearance from ALS neurons and thereby accelerating TDP-43's cytosolic aggregation [109].

2.3 Novel roles of GTPases: Rab11 and RGNEF in neurodegeneration

A common feature in a number of neurodegenerative diseases, including ALS, Alzheimer's, and Parkinson's disease, is the impaired clearance and recycling of damaged and aggregated proteins from cells. Moreover, perturbation of physiological vesicle trafficking systems affects several vital mechanisms in the cells, such as efficient nutrient absorption, failure of cell-to-cell communication, the immune response, and loss of synaptic transmission [110, 111]. Rab-GTPases, first discovered in brain tissue, belong to the major subset of the Ras superfamily [112]. In neurons, Rab-GTPases primarily orchestrate vesicle sorting and trafficking between target membranes through their interactions with effector proteins (coat proteins, kinesins, and dyneins), in addition to tethering and SNARE complexes [113, 114]. Among the Rab-GTPases, Rab11 plays critical roles in trafficking, sorting, and recycling endosomal vesicles around the perinuclear region. Rab11 is transported to the cellular periphery via recycling vesicles traveling along the microtubules and directly regulates vesicular exocytosis at the plasma membrane (**Figure 4**) [115, 116]. Furthermore, Rab11 has been identified as the master regulator for the transport of neurotrophin receptors and β-integrin via axonal junctions in dorsal root ganglion neurons and is critical for their maturation, functionality, and survival [117]. Rab11 participates in different cell survival pathways in neurodegenerative diseases in response prion/prion-like protein toxicity. In the ALS-TDP-43 *Drosophila* model, TDP-43 toxicity reduces levels of the synaptic growth promoting bone morphogenetic protein (BMP) at the neuromuscular junction and increases BMP receptors in recycling endosomes and at the neuromuscular junction. This pathogenic condition leads to larval crawling defects in ALS fruit flies, which are rescued by the overexpression of Rab11 [118]. Schwenk et al. has shown that TDP-43 regulates the number of Rab11-positive recycling endosomes in dendrites (**Figure 4**) [119]. These recycling

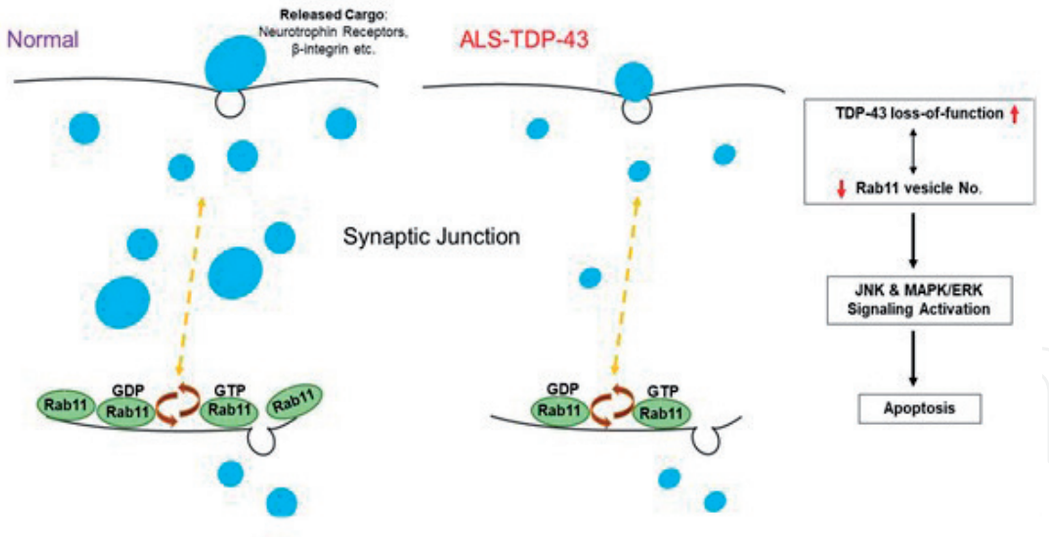

Figure 4.
TDP-43 modulates synaptic vesicle trafficking. Synaptic vesicle trafficking is a crucial mechanism for transporting macromolecules and neurotransmitters across the neuronal axon junctions. Defect in this mechanism may lead to accumulation of toxic waste products in the cells and activation of neurodegenerative conditions. Ras-GTPases like Rab11 plays a critical role in endosomal vesicle trafficking, sorting, and recycling. A study with sporadic ALS patients' brain and spinal cord samples show loss of Rab11 could be closely linked to TDP-43 proteinopathies in ALS and FTD. Loss of Rab11 affects the cargo load capacity and the size of the vesicles as well. This condition in turn activates JNK and MAPK/ERK signaling cascades leading to neuronal apoptosis.

defects in turn affect transferrin recycling in neurons and its decrease in cerebrospinal fluid of patients. In *Drosophila*, a Rab11 mutation induces JNK and MAPK/ERK signaling activation and apoptosis, resulting in defective eye and wing phenotypes [120, 121]. TDP-43 can be phosphorylated at threonine 153 and tyrosine 155 by MEK kinase, a central player in MAPK/ERK signaling pathway, in response to heat shock [122]. However, this unique phospho-TDP-43 variant is not involved in aggregate formation but is instead recruited to nucleoli for processing nucleolar-associated RNA. Further supporting the *Drosophila* model results, we have observed that sporadic ALS-TDP-43 mutations induce the loss of Rab11 in patients' brain and spinal cord compared to age-matched control samples [123]. In contrast to MAPK/ERK and Akt signaling pathways operating in parallel to activate mTORC signaling [124], we have shown that Akt and ERK signaling pathways work in a competitive manner to determine the cell fate. Moreover, in Parkinson's disease, Rab11 co-localizes with toxic α-synuclein inclusion bodies in dopaminergic neurons, and overexpression of Rab11 prevents the affected neurons from degeneration and rescues behavioral deficits [125]. In Huntington's disease, Rab11 overexpression restores synaptic dysfunction and prevents glutamate-induced cell death in neurons [126, 127]. Rab11 has also been found to co-localize with Rab7 and C9orf72 in postmortem ALS brain samples and primary cortical neurons exhibiting C9orf72-induced disrupted vesicular trafficking system in ALS [128]. These findings suggest a broader impact of TDP-43 mutation-induced Rab11 dysfunction on cellular function and survival signaling cascades.

Apart from the Rab-GTPases of Ras superfamily, small RhoA GTPases also regulate broad spectrum of cellular mechanisms including cell-to-cell communication, migration, and proliferation. Given that the mode of GTPase functionality relies on the rate of GTP to GDP turn over and vice versa, dysregulation of guanine nucleotide exchange factor (GNEF) that is responsible for such turnover has been linked to a number of diseases including neurodegeneration. Among this class of proteins with enzymatic activity, Rho GNEF (RGNEF/p190) is directly associated with neurodegenerative disease like ALS, where it not only regulates the function of GTPases

for stress survival [129] but also acts as the RNA-binding protein to modulate the stability of the crucial low molecular weight cytoskeleton protein neurofilament's mRNA by binding to its 3′ untranslated region [130]. Emerging studies on GNEF's involvement in ALS has revealed that RGNEF co-exists with TDP-43 and FUS in the neuronal cytoplasmic inclusion bodies in spinal motor neurons indicating a cross talk of protein aggregation and dysregulated cell signaling pathways in the ALS pathogenesis [131]. Micronuclei structures, containing small DNA fragments with clustered DSBs and surrounded by one lipid layer, are a strong hallmark of cellular metabolic stress, exposure to genotoxic agents, and genomic instability leading to apoptosis [132–134]. Notably, micronuclei have been observed in the ALS patient brain and spinal cord tissue samples. Studies have shown that TDP-43 interacts with RGNEF through its leucine-rich domain and forms co-aggregated structures [135]. Taken together, these findings further support the fact that genomic instability is one of the major outcomes in ALS-TDP-43 pathology leading to neuronal death.

3. DNA binding and role in DNA transactions

TDP-43 was first identified and characterized as the transcriptional regulator of HIV-1 gene expression by binding to long terminal repeat TAR DNA motifs [136]. In contrast, a separate study found that when exposed to HIV-1 infection, HIV-1 viral production could occur in T cells and macrophages, even in the absence of TDP-43 protein [137]. As an RNA-/DNA-binding protein, TDP-43 also interacts with specific TG and non-TG repeat containing DNA sequences through its RRM domains, with each domain having specific interaction affinities and DNA conformation requirements [138]. TDP-43 has been found to regulate the cyclin-dependent kinase 6 (CDK6) transcript and protein levels through its binding to the highly conserved long-stretch of GT repeats in CDK6 gene sequence. This binding leads to CDK6 upregulation and thereby increases phosphorylation of retinoblastoma proteins pRb and pRb2/p130 [139]. TDP-43 also acts as the transcriptional repressor and/or insulation regulator for the spatiotemporal regulation of the ACRV1 (SP-10) gene [140, 141]. TDP-43 binds to two GTGTGT motifs in the promoter core region through its N-terminal RRM1 domain during spermatogenesis. In vivo studies reveal that, unlike the wild-type variant, mutations in the GTGTGT motifs in the −186/+28 promoter region leads to premature reporter gene expression in the meiotic spermatocytes [142]. Previously, TDP-43 has been shown to bind both double-stranded DNA as well as single-stranded DNA, with a higher affinity toward single-stranded DNA through binding its RRM1 domain [143, 144]. Qin et al. reported that TDP-43's N-terminal domain is indispensable for its physiological and proteinopathy functions and showed that the N-terminus maintains a highly ordered structure that equilibrates the C-terminal disordered structure by acquiring a novel ubiquitin-like fold that directly binds single-strand DNA [145]. More recently, we also documented in vitro studies on TDP-43's affinity for binding free double-stranded DNA ends, instead of binding a partially or completely blocked terminus [146].

4. TDP-43 in DNA damage response (DDR) and repair

Induction of DNA damage and dysregulated damage response are critical factors for neuronal death in ALS and other neurodegenerative diseases [147, 148]. Studies also suggest endogenous DNA breaks, including DSBs, are routinely generated and repaired in healthy neurons and are essential for the regulation of neuronal gene

expression [149, 150]. Besides the transcriptional regulation by RRM domains of TDP-43, no other DNA interactions have been reported for TDP-43. Based on an interactome study targeting TDP-43 in human cells, Freibaum et al. identified the DNA repair protein, Ku70, as one of the interacting partners of TDP-43 [151]. Furthermore, we found that TDP-43's interaction with Ku70 was modulated by DNA damage induction in neuronal cells. Notably, TDP-43 showed pathway-specific roles in DNA DSB repair via direct interaction with the classical nonhomologous end joining (NHEJ) factors XRCC4 and DNA Ligase 4 (LIG4) complex, but not with single-strand break repair factors XRCC1 and DNA Ligase 3 (LIG3) complex. Further experimental studies revealed TDP-43's interaction with a number of NHEJ proteins, including DNA-PKcs, 53BP1, polymerase lambda, XRCC4-like factor (XLF), and the DNA damage response (DDR) factors phosphorylated ATM and histone H2AX (γH2AX). Interestingly, TDP-43 depletion in neurons elicited hyperactivation of DDR signaling without affecting the recruitment of activated DDR proteins to genome damage sites and inhibited the docking, but not assembly, of DNA ligation complex factors (XRCC4, LIG4, and XLF) at the break sites. This suggests that TDP-43 has a crucial role in maintenance of genomic integrity by efficiently completing the rate-limiting DSB sealing step (**Figure 5**). Consistent with these results, neuronal cells with TDP-43 downregulation exhibited delayed DSB repair kinetics and a higher population of apoptotic cells due to the persistent accumulation of unrepaired DSBs than controls. In a correlative study of postmortem human sporadic ALS brain and cervical spinal cord samples, we determined that samples with extensive TDP-43 aggregation and/or fragmentation had greater staining for DSB markers (γH2AX and TUNEL) than their age-matched controls [146].

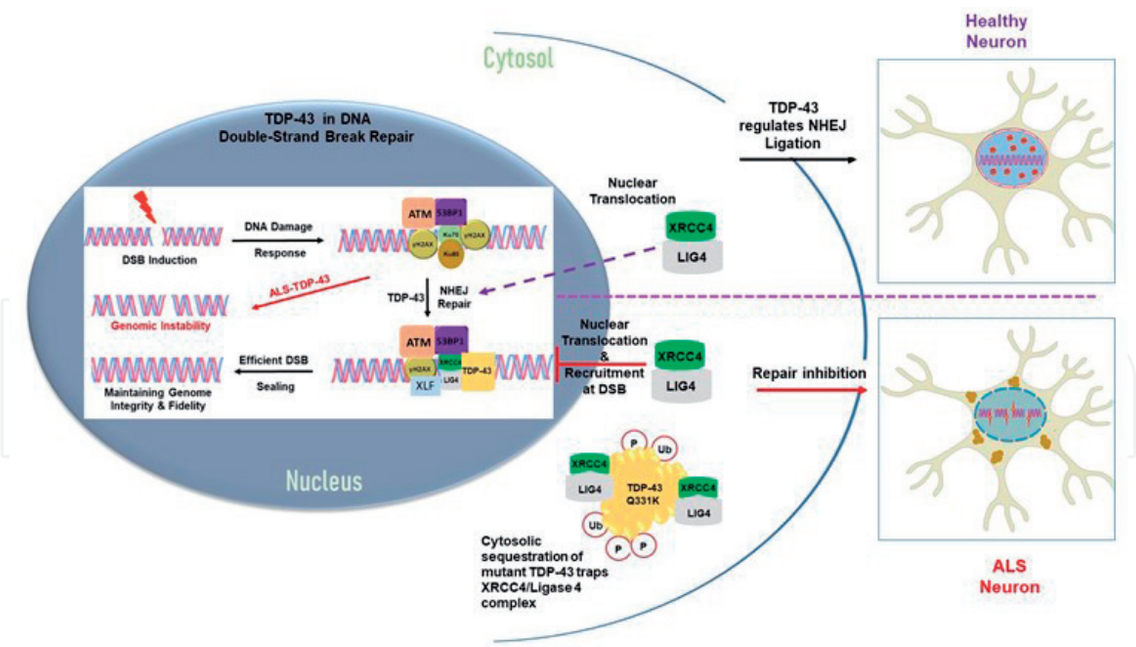

Figure 5.
TDP-43's scaffolding role in NHEJ repair mechanism of nuclear genome. In response to DNA damage induction, TDP-43 gets recruited at the DSB sites and participates in relaying DDR signaling through interaction with p-ATM (Serine1981), p-histone H2AX (Serine139), p-53BP1 (Serine1778), and Ku70/80 heterodimer. More importantly, TDP-43 regulates the most rate-limiting step of the DNA DSB repair pathway—DNA DSB end ligation, through its interaction with DNA ligation complex XRCC4-ligase 4 at the break sites. However, pathogenic mutations in TDP-43 related to ALS and FTD cause enhanced cytosolic mislocalization of TDP-43, thereby inhibiting the DNA damage-induced translocation of XRCC4-ligase 4 complex from cytosol to nucleus. Either of TDP-43 loss-of-function or ligation complex translocation inhibition causes DNA DSB repair impairment leading to nuclear genome instability in ALS/FTD motor neurons.

4.1 Mutant TDP-43 and defective DSB repair in neurons

Efficient sealing of DNA DSBs, which are the most lethal form of DNA damage, is critical for maintaining genome integrity and fidelity. In the majority of the SALS cases, the total amount of TDP-43 does not change significantly, but a significant proportion of the protein mislocalizes to the cytosol and causes ALS/FTD pathophysiology similar to disease-linked mutant TDP-43. We recently linked the TDP-43 Q331K mutation with genome instability and DSB repair defects. In studies with conditionally expressed TDP-43 Q331K mutant, cultured neurons exhibited a strong nuclear clearance phenotype and a higher amount of DNA damage at basal level without the effect of any external DNA damaging agents than control cells. Further investigations showed that TDP-43 Q331K trapped XRCC4 and LIG4 in the cytosol and inhibited their translocation to the nucleus in response to DNA damage induction. These findings suggest that TDP-43 is not only involved in the recruitment of the DNA ligation complex at genomic break sites but also regulates the damage-dependent nuclear translocation of DNA repair proteins (**Figure 5**). These impaired TDP-43 functions in the disease condition leading to genomic instability and neurodegeneration [22].

5. A double whammy of DNA damage induction and defects in their repair in TDP-43-ALS

We and others have observed that the most salient hallmarks of ALS/FTD-TDP-43 pathology, the protein aggregation and inclusion body formation in the cytosol, increase oxidative stress in affected neurons [22, 48, 152, 153]. 4-hydroxynonenal (HNE) increases as an oxidative stress indicator in sporadic ALS patients' brain, spinal cord, and serum. HNE induces oxidative damage to a broad range of cysteine-containing proteins, including TDP-43, through cysteine oxidation. Oxidized TDP-43 mislocalizes from the nucleus to form insoluble cytosolic aggregates in ALS [154]. Such nuclear clearance and cytosolic increase may cause a gain-of-toxicity, leading to aberrant mRNA processing, which in turn exerts a direct DNA destabilization through impaired DSB repair and an indirect repair inhibition by negatively regulating mRNA splicing of DNA repair proteins. Furthermore, the unrepaired DNA DSBs leads to persistent DDR signaling activation through an ATM-mediated signaling cascade that promotes neuroinflammation. This inflammatory response may induce oxidative genome damage and enhanced TDP-43's nuclear clearance, exacerbating the ALS/FTD conditions in a feed forward pattern. Moreover, dysregulation of metal homeostasis has been observed in ALS patients' brain/spinal cord tissues. In the presence of cellular oxidative stress, zinc induces TDP-43's mislocalization and cytoplasmic inclusions [155, 156]. Given that zinc is a crucial metal co-activator for several transcription factors, trapping of zinc in TDP-43 aggregates could globally affect gene activation patterns, including those for DNA repair and response-associated factors, leading to genomic instability.

6. Conclusions: TDP-43-ALS, a case for DNA repair-targeted therapy?

Emerging studies from our laboratory and other groups have shown that in addition to its RNA processing and miRNA biogenesis functions, TDP-43 acts as a key component of the NHEJ pathway for DSB repair in neurons, and its pathological clearance from the nucleus leads to the DSB repair defects seen in ALS and other TDP-43-linked neurodegenerative diseases. These newly discovered paradigms link

TDP-43 pathology to impaired DNA repair and suggest potential avenues for DNA repair-targeted therapies for TDP-43-ALS and related motor neuron diseases. Future studies should focus on a comprehensive delineation of the molecular mechanisms involved in order to develop efficiently targeted interventions. In addition, the implications of these defects in neuronal and glial functions in the CNS of TDP-43-ALS patients and the role of TDP-43 in maintaining genome integrity in non-neuronal brain cells, including glia and astrocytes, are important lines of investigation. By comparing the DNA repair role of TDP-43 in post-mitotic vs. cycling cells, we could learn important mechanistic insights on the selective vulnerability of neurons in ALS.

Recently, an increasing number of studies have demonstrated the role of RNA/DNA-binding proteins in DNA repair. We previously documented the involvement of hnRNP-U, a member of the hnRNP family, in oxidative damage repair in the human genome and its role as a molecular switch between DSB repair and oxidative damage repair when these complex damages occur in a clustered fashion [157, 158]. We also discovered that another RNA-/DNA-binding protein, fused in sarcoma/translocated in liposarcoma (FUS/TLS), linked to the ALS-FUS subtype, participates in break sealing during DNA SSB repair [10]. Because both TDP-43 and FUS influence the final DNA break/sealing step of repair, further investigations in DNA repair mechanisms are critical to developing clinically effective strategies for ameliorating the genome instability of ALS-TDP-43.

Acknowledgements

Research in Hegde laboratory is supported by grants from the National Institute of Neurological Disorders and Stroke (NINDS) and National Institute of Aging (NIA) of the National Institute of Health (NIH) under the award numbers R01NS088645, RF1NS112719, and R03AG064266 and the Houston Methodist Research Institute funds. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies. The authors thank members of the Hegde Laboratory—Haibo Wang, Manohar Kodavati, Pavana Hegde, Velmarini Vasquez, and Erika Guerrero for assistance.

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