Chapter

Molecular Basis of DNA Repair Defects in FUS-Associated ALS: Implications of a New Paradigm and Its Potential as Therapeutic Target

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Abstract

Amyotrophic lateral sclerosis (ALS) is a progressive motor neuron disorder, characterized by a diverse etiopathology. While ALS is predominantly sporadic, mutations in one or more of a dozen risk factors have been linked to approximately 10% of familial ALS patients. The multifunctional RNA/DNA-binding protein fused in sarcoma (FUS) is one such protein whose autosomal dominant missense mutations were identified in a subset of familial and sporadic ALS patients. Initial studies linked FUS with both RNA-related and genome maintenance functions, yet the mechanisms and potential implications to neurodegeneration were not completely understood. We recently identified a novel function of FUS in repairing single-strand break (SSB) in the genome. FUS directly interacts and recruits XRCC1/DNA Ligase IIIα (LigIII) to DNA oxidative damage sites in a PARP1 activity-dependent manner, which facilitates optimal oxidative genome damage repair. Besides, FUS regulates DNA strand break sealing by enhancing ligation activity of LigIII. The mutation of FUS induces accumulation of oxidative DNA damage as well as DNA repair deficiency in ALS patients. The novel findings provide insights into a previously undescribed mechanism of DNA repair defect in FUS-associated neurodegeneration, and raise the pentientials of developing neuroprotective therapies by targeting DNA break ligating defects.

Keywords: FUS, neurodegeneration, DNA repair, oxidative genome damage, XRCC1-DNA ligase III

1. Fused in sarcoma (FUS): pathophysiology and links to familial and sporadic ALS

1.1 FUS and its physiological functions

The FUS/TLS (Fused in sarcoma Protein/Translocated in liposarcoma) gene encodes a 526-amino acid protein that belongs to the TET (TAF15, EWS, and TLS) family, which is implicated in multiple aspects of RNA metabolism. The FUS gene was initially identified as an oncogene in multiple cancers. FUS fuses with the transcription repressor C/EBP homologous protein 10 (CHOP) in myxoid liposarcomas. It was identified for its role as an activator of ETS-related gene (ERG) in acute myeloid leukemia [1, 2] and in Ewing's sarcoma tumors [3]. Subsequently, mutations in FUS were linked to motor neuron diseases, amyotrophic lateral sclerosis (ALS), and frontotemporal dementia (FTD) [4, 5].

FUS protein is ubiquitously expressed in both the nucleus and cytoplasm of many cell types; however, it is predominantly found in the nucleus of glial cells and neurons in the central nervous system. A small fraction of FUS shuttles between the nucleus and cytoplasm under various stimuli, for example, sodium arsenite-induced oxidative stress [6, 7]. A previous study revealed that FUS binds RNA *in vivo* to engage in nucleo-cytoplasmic shuttling [8]. Another study showed that FUS is localized to dendritic spines as an RNA-protein complex and associates with mRNA encoding an actin-stabilizer protein, which indicates a regulatory role of FUS in actin/cytoskeleton processes in dendrites [9]. Interestingly, in response to stress-induced by sorbitol, FUS redistributes to the cytoplasm and localizes to cytoplasmic stress granules [10], a complex comprised of mRNA, ribosomes, RNA-binding proteins, transcription factors and nucleases that form in response to induced stress, such as oxidative stress and heat shock to maintain the stability of selected mRNAs and protein activities [11, 12]. The redistribution of FUS on stress granules is regulated by posttranslational modification, methylation, and highly related with cell survival from the stress [10]. FUS protein contains an SYGQ-rich region at its N terminal, followed by a RGG box (RGG1), an RRM motif, a second RGG box (RGG2), a zinc finger (ZnF) motif, and a third RGG box (RGG3). The C-terminus contains a nonclassical nuclear localization signal (NLS) with conserved proline and tyrosine residues (PY-NLS). The 13 terminal amino acids (514–526) containing the NLS sequence were shown to be necessary, but not sufficient, for nuclear import of FUS [13]. FUS can bind with RNA and both single-stranded (ss) as well as double-stranded (ds) DNA [14]. FUS is associated with multiple roles in RNA processing, including splicing, transcription, and transport. Studies have highlighted that FUS has a transcriptional regulatory role in global or specialized components of transcriptional machinery [15]. A Clip-seq based study revealed that FUS regulates alternative splicing of pre-mRNAs and processing of long-intron containing transcripts, and the RNAs targeted by FUS are associated with neurogenesis and gene expression regulation; interestingly, some of FUS' mRNA targets are involved in DNA damage response and repair pathways [16].

1.2 FUS is mutated in both familial and sporadic ALS

The prevalence of ALS has been observed to be non-uniform geographically but ranges between 0.6 and 3.8 per 100,000 population worldwide. The rate of ALS incidence appears to be rising according to the recent epidemiological studies, despite the geographical differences [17]. Notably, the incidence and prevalence of ALS are greater in men than in women [18]. It has been reported that the male: female ratio is between 1 and 3 and changes in an age-dependent manner [19]. In approximately 90% of ALS cases the cause is unknown, and, as such, they are considered sporadic (SALS); while, around 10% of ALS patients have a clear family history (FALS). In 2009, two independent studies identified that FUS R521 is mutated in FALS cases and that mutation is characterized pathologically with enhanced cytoplasmic inclusions of FUS and motor neuron degeneration [4, 5]. FUS mutations have also been linked to cases of SALS [20–22]. The FUS gene is composed of 15 exons and most mutations are clustered in exon15. Exon15 encodes the NLS domain, implying a possible involvement of its nuclear import defects. These mutations/truncations include 495X, R521C/G/H/L/S, R522G, P525L, and so on. R521 is the most commonly mutated site in ALS, whereas P525 mutations are highly related with early onset and severe progress of ALS [23–25]. Mutations also occur in domains other than NLS. For example, G187S within Gly-rich domain, G399V in RGG domain, and P431L in ZnF domain [26], which is believed to induce functional defects of FUS. Autosomal dominant mutations in the gene encoding the FUS protein have been detected in approximately 5% of FALS patients and a small subset (~1%) of SALS cases.

1.3 FUS pathology in FTD

It is important to note that many ALS patients (36–51%) also exhibit cognitive impairment, with about 20% developing FTD, also called frontotemporal lobar degeneration (FTLD) [27], a disorder characterized by cognitive, behavioral, and linguistic dysfunction. The reverse is also seen, wherein patients with FTD can develop ALS [28]. FTD accounts for 10–15% of dementias, making it the second most common type of dementia for people under the age of 65, after Alzheimer's disease. The overlap between ALS and FTD indicates a likely common molecular basis between FUS and cognitive deficits. After the discovery of FUS mutation in ALS, a novel subtype of FTD with FUS pathology was identified, although no FUS mutation was seen [29]. In 2010, Oriane Broustal et al. identified three exonic FUS variants, c. 1562G > A (p.Arg521His), c. 1566G > A (p.Arg522Arg), and c.188A > G (pAsn63Ser) from 317 patients including 144 patients with familial FTD and 173 patients with FTD-ALS. Interestingly, the three variants were found only in patients with both FTD and ALS [27].

2. Multifaceted role of FUS in RNA and DNA transactions

The molecular mechanisms of FUS-ALS are complex and ambiguous, typically described by both loss of function and gain of toxicity hypotheses. Although controversial, loss of function is not entirely supported by animal models: FUS knockout mice and zebrafish do not develop ALS-like phenotypes [30–32]. As mentioned in the previous section, FUS plays multiple roles in RNA metabolism. In fact, thousands of RNA targets have been identified that bind to FUS in various cell lines and brain tissue from both patients and animal models [33]. The dysregulation and disturbance of RNA processing are considered to be one mechanism that leads to neurodegeneration. Depletion of FUS in mouse nervous system has been shown to alter the levels of splicing of over 950 mRNAs [34]; FUS knockout in neuroblastoma cells disturbs the splicing of more than 400 introns [35]. Expression of FUS P525L mutant was shown to inhibit splicing of minor introns by trapping U11 and U12 small nuclear RNAs (snRNAs) in these aggregates [35], and expression of R521G and R522G mutations influence RNA transcription and splicing but in a different way [36]. Besides, mRNA transport and stabilization are also affected by ALS-linked FUS mutations [37, 38].

The majority of the mutations occur in the NLS region of FUS, which induces its cytoplasmic accumulation. The widespread FUS mislocalization has been considered a hallmark of ALS [39]. Mutant, but not the wild-type FUS was shown to be assembled into stress granules in cytoplasm in response to oxidative stress or heat shock [7, 40], which potentially contributes to neurotoxicity by impairing mRNA translation [41, 42]. In fact, mutations in RNA-binding proteins (RBPs) are highly related to ALS. The interaction between mistranslocated FUS and other RBPs was recently investigated. These studies show that the cytoplasmic mislocalization caused by FUS P525L mutation impairs its interaction with other ALS-associated

RBPs including shnRNPA1, hnRNPA2B1, EWSR1, and TAF15, which facilitates the nucleation of toxic cytoplasmic FUS aggregates. In addition, high cytoplasmic FUS levels exhibit defects in protein degradation and reduced protein levels of RBPs, shedding lights on the FUS-ALS pathology linked to the homeostasis of multiple ALS-associated RBPs [43].

2.1 FUS toxicity and impaired DNA damage response (DDR) signaling

In addition to RNA dysregulation, there has been a lot of focus on the genome instability caused by FUS mutation in ALS patients since FUS was first linked to DNA damage repair by multiple studies. In human cells, one major source of genome damage is the reactive oxygen species (ROS) accumulation-induced oxidative stress. ROS that defined as a group of reactive molecules derived from oxygen including but not limited to free radicals (superoxide, O_2^-), hydroxyl radical (·OH), or non-radicals (hydrogen peroxide) can be balanced by various antioxidant systems, while the imbalance between ROS production and antioxidant defenses causes oxidative stress, leading to oxidation of lipid, protein, and DNA in cells [44]. Accumulation of oxidative DNA damge has been linked to multiple neurodegenerative diseases like Parkinson's disease (PD) and Huntington's disease (HD) [45, 46]. In addition to DNA damage, mutation in the genes of specific DNA repair pathways that lead to DNA damage repair (DDR) is another challenge for the central nervous system [47]. For example, nucleotide excision repair (NER) is defective in xeroderma pigmentosum (XP) and Cockayne syndrome (CS), base excision/single-strand break repair (BER/SSBR) defects in ataxia with oculomotor apraxia type 1 (AOA1) [48], spinocerebellar ataxia with axonal neuropathy (SCAN1) [49] and ALS [50], and defective DDR signaling and DNA double-strand break repair (DSBR) in ataxia telangiectasia (A-T) and Nijmegen break-age syndrome. FUS is found to be phosphorylated by DDR proteins ataxia-telangiectasia mutated (ATM) and DNA-dependent protein kinase (DNA-PK) in response to DSB-inducing agents. FUS was identified to interact with histone deacetylase 1 (HDAC1) in primary mouse cortical neurons, and the interaction is believed to modulate the homologous recombination (HR) and non-homologous end joining (NHEJ), two major pathways for DSB repair. Besides, FUS was shown to be recruited at DNA damage tracks induced by microirradiation (MIR) at wavelengths of 405 or 351 nm, in a PARP1-depedent manner and accompanied with PARylation by PARP1 [51–53]. Notably, MIR causes clusters of different types of DNA damage including oxidized base lesions, single-strand breaks (SSBs), and DSBs. It is generally believed that UVA (wavelength between 320 and 400 nm) predominantly induces SSBs via elevated ROS, while UVA may also induce secondary DSBs due to clustered SSBs [54]. Thus, recruitment of FUS at MIR with wavelength of 351 nm suggests its potential role in the repair of SSBs.

2.2 FUS and repair of oxidative genome damage: mechanistic insights

A comprehensive investigation by our group described the mechanistic role of FUS in BER/SSBR, a major pathway to repair oxidative DNA damage. Our study utilized multiple *in vitro* and *in vivo* model systems, including, CRISPR/Cas9-mediated FUS knockout (KO) human embryonic kidney (HEK)293 cells, FALS patient-derived induced pluripotent stem cells (iPSCs) with FUS mutations R521H and P525L, motor neurons induced from these iPSC lines, and ALS patients spinal cord tissues with FUS pathology. We discovered that the DNA integrity is substantially affected in the spinal cord tissues and the motor neurons derived from ALS patients. Both downregulation and pathological mutation of FUS were associated with DNA SSB accumulation and SSBR defects. Finally, we identified that FUS directly interacts with PARP1, XRCC1, and LigIII in response to oxidative stress and FUS facilitates the recruitment of XRCC1/LigIII to DNA damage sites in a PARP1 activity-dependent manner. FUS enhances the ligation activity of LigIII, which is critical for an optimal SSBR in neurons (**Figure 1**). The SSBR deficiency due to ALS-linked FUS mutations can be rescued by the correction of those mutations via Crisper/Cas9 technology [50]. Furthermore, FUS regulates PARP1's PARylation activity in motor neurons and thus could affect neuronal energy metabolism by uncoupling NAD^+/NADH levels.

2.3 FUS-PARP-1 interactions: a new paradigm with diverse implications

During DDR, PARP1 plays an important role in regulating DNA damage repair. In response to DNA damage, PARP1 is self-activated by its auto-PARylation and transfers ADP-ribose to create long and branched poly(ADP-ribose) on target DNA repair proteins to facilitate their recruitment; for example, PARP1 recruits XRCC1 by its PARylation. A study showed that PARP1 likely plays a major role for the poly(ADP-ribose) synthesis induced by alkylating agents, since the amount of poly(ADP-ribose) can be reduced for around 10 folds (from 60 pmol per mg of DNA to 5.85 pmol per mg of DNA) in PARP1 KO cells in the presence of MNNG [55], which activates DNA mismatch repair. Consistantly, PARP1 knockout mice are

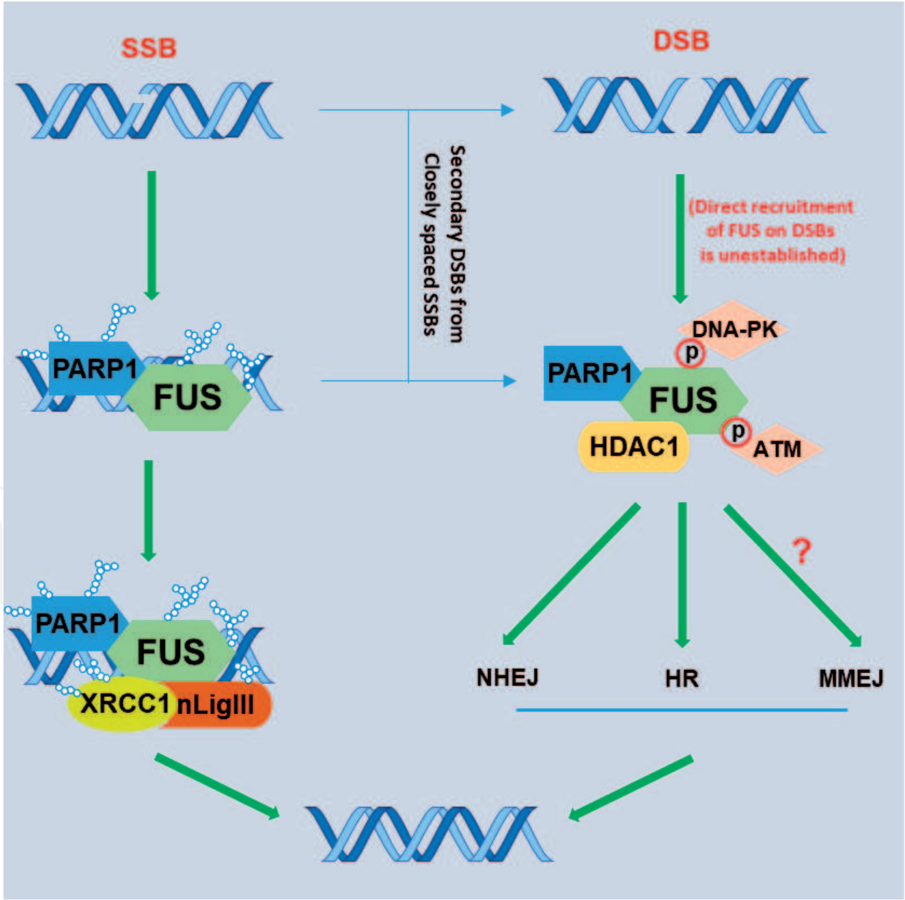

Figure 1.
Model of FUS involving DNA damage response in the nucleus. In response to DNA SSBs, FUS is PARylated and recruited to DNA damage sites by PARP1 and the recruitment facilitates the loading of XRCC1 and nuclear LigIII (nLigIII) complex, where FUS enhances the ligation activity of LigIII for an optimal SSB ligating. While in response to DSB, FUS is associated with HDAC1, which is required for an efficient NHEJ and HR-mediated DSB repair. FUS is also phosphorylated by ATM and DNA-PK, although the underlying mechanism is not known. Due to its role in the functional activation of LigII, which is involved in MMEJ-mediated DSB repair, it is likely that FUS also affects MMEJ, which needs to be investigated.

highly susceptible to DNA damaging agents and are accumulated with DNA strand breaks accompanied with genomic instability in them [56, 57].

In response to DNA damage, FUS is recruited to DNA damage site in a PARP1 activity-dependent manner [51, 52]. FUS directly binds to PAR synthesized by activated PARP1 leading to the formation of damaged DNA-rich compartments contributed by its N-terminal low-complexity domain (LCD) and C-terminal RGG domains, and the compartments are dissociated by the hydrolysis of PAR by PARG [58], which indicates that PAR polymers play an essential role for the recruitment, likely through enhancing the FUS droplet formation [59]. While the activation of PARP1 can induce shuttling of FUS from nucleus to cytoplasm, which in turn enhances FUS aggregate formation and neurodegeneration [58, 60]. Furthermore, like FUS, the other two members in TET family, TAF15 and EWSR1 are also recruited to MIR-induced DNA damage tracks depending on PARP1 activity [53], while the molecular mechanism of the cross-talk between TET family proteins and PARP-1 has not been investigated.

3. Indirect role of FUS in DDR: FUS-directed RNA transactions in DNA repair

Although, recent reports from our group and others demonstrate a complex, but the direct role of FUS in maintaining genome integrity, whether FUS RNA-binding activity plays a role in regulating the expression of DDR factors has not been investigated. FUS regulates several key steps of RNA metabolism that impairs various biological processes. CLIP-seq has been used to identify RNAs that FUS targeted in multiple studies [16, 34, 61–65], which reveals an indirect role of FUS involving in DDR by regulating RNA transcription. One report shows that FUS regulates alternative splicing of pre-mRNAs and processing of long-intron containing transcripts in HeLa cells, and FUS binds RNA encoding proteins important for DNA damage response and repair pathways. By comparing with other CLIP-based assays, a map of FUS RNA targets to DNA DSBR by NHEJ and HR is generated in the report and in which, a number of key DDR factors such as ATM, 53BP1, MRN11, NBS1 are included [16]. We recently performed RT^2 PCR arrays for DNA repair and DDR signaling pathways in CRISPR/cas9 FUS knockout (KO) cells, patient-derived FUS-mutant cells, as well as FUS-ALS patient spinal cord autopsy tissue, which revealed significant downregulation of DDR factors BRCA1, MSH complex, RAD23B, and DNA ligase 4. Notably, BRCA1 depletion has been linked to neuronal DNA DSB accumulation and cognitive defects in Alzheimer’s disease. The ubiquitin receptor RAD23 functions both in nucleotide excision repair and the proteasomal protein clearance pathway and is thus linked to amyloid load in neurodegeneration. This study provides evidence of FUS pathology-mediated perturbation in the expression of DNA repair and DDR signaling factors and thus highlights the intricate connections between FUS, genome instability, and neurodegeneration.

4. Conclusions and perspectives

As early as 1982, the defective DNA repair and its possible role in ALS pathology was first proposed by Bradley et al. [66], where they hypothesize that abnormal DNA in ALS may arise from a deficiency of an isozyme of a DNA repair factor. Subsequently, growing evidence suggests that defective DNA repair is a common feature of not only ALS but also several other neurodegenerative diseases, underscoring the needs of studying the implications of unrepaired DNA damage in neurons affected by neurodegeneration [67], which may lead to novel therapeutic strategies.

Our recent studies made a critical breakthrough in this direction by first time shedding lights on the molecular insights into the involvement of ALS and FTD-associated FUS and other RNA/DNA-binding proteins in specific DNA repair failure mechanisms, such as DNA ligation deficiency. While this study suggests a potential for DNA ligase complementation strategy, several key questions should be addressed to develop DNA repair-based interventions for ALS. These are: (1) The role of FUS in mitochondrial genome stability maintaining. The linkage between FUS and mitochondrial integrity has been established by a number of studies. Deng et al. demonstrated interactions between FUS and two mitochondrial proteins, mitochondrial chaperonin HSP60 and ATP synthase beta subunit ATP5B, in different studies. HSP60 mediates the translocation of FUS into mitochondria, and downregulating of HSP60 rescues mitochondrial defects and neurodegenerative phenotypes in FUS transgenic flies. While interaction between FUS and ATP5B indicates a involvement of FUS in the dysregulation of mitochondrial ATP synthesis: expression of wild-type or FUS P525L mutant disrupts the formation of the mitochondrial ATP synthase supercomplexes and suppresses the activity of ATP synthase, resulting in mitochondrial cristae loss followed by mitochondrial fragmentation [68, 69]. Nakaya and Maragkakis et al. found that expression of human FUS R495X in mouse embryonic stem cell-differentiated neurons disturbs the translation efficiency of mitochondria-associated genes and results in significant reduction of mitochondrial size [70]. Although ALS-FUS has been linked with the dysfunction of mitochondria, the role of FUS in mitochondrial genome integrity has not been explored. (2) The role of FUS in microhomology-mediated end joining (MMEJ) repair. We have established a relationship between FUS and XRCC1/LigIII, which is required for an optimal SSBR. While LigIII, together with XRCC1 and PARP1, also participates in the MMEJ-mediated DSB repair pathway, the role of which in primary neurons is unknown (**Figure 1**). We hypothesized that the MMEJ contributes DSBR in motor neurons and the loss of FUS may affect MMEJ and lead to genomic instability, which we are currently pursuing. (3) The role of FUS in maintaining genome integrity in astrocytes. Recent studies have shown that expression of ALS-linked mutant FUS and other ALS causative factors in astrocytes induces motor neuron death [71–73]. It will, therefore, be critical to investigate FUS toxicity-induced ligation activity defects in astrocytes and the collateral influence on motor neurons. (4) Whether DNA ligase I (LigI) rescues FUS mutant-mediated LigIII defects. Mammalian cells express three DNA ligases including ligase IV (LigIV), LigIII, and LigI. LigIV specifically participates in NHEJ-mediated DSB repair; LigIII has nuclear and mitochondrial isoforms. Both the nuclear and mitochondrial isoforms have ~98% similarity and function in BER/single-strand break repair (SSBR); LigI has been shown to functionally overlap with LigIII and is believed a back-up of LigIII, however, the level of LigI expression in non-cycling, postmitotic cells like neurons is negligible due to lack of replication-associated repair. Very likely, the induction of LigI into motor neurons with FUS pathology can rescue the LigIII ligation activity defects caused by FUS mutation.

Addressing these critical follow-up questions is an unmet need in the FUS-ALS field, which will help to develop a mechanism-based DNA-repair-targeted therapy. With recent emerging studies, the stage is set for such a paradigm shift.

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