A Commentary on Mitochondrial Dysfunction and Compromised DNA Repair in Neurodegeneration: The Emerging Role of FUS in ALS

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ABSTRACT: Mitochondrial dysfunction plays a pivotal role in the progression of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Alzheimer's, and Parkinson's disease. Recent discoveries have highlighted the involvement of DNA damage and repair processes, particularly mitochondrial DNA (mtDNA) damage, in these conditions. This commentary reflects on our recent findings, demonstrating the RNA/DNA binding protein fused in sarcoma (FUS)'s crucial role in maintaining mtDNA integrity through interactions with mitochondrial DNA ligase IIIα (mtLig3). Our studies provide direct evidence of increased mtDNA damage in ALS-linked FUS mutant cells, emphasizing the potential of targeting DNA repair pathways to mitigate neurodegeneration. Furthermore, the restoration of mitochondrial function through targeted expression of human DNA ligase 1 (Lig1) in FUS mutant models showcases the therapeutic promise of DNA repair mechanisms in neurodegenerative diseases. These insights offer new molecular understanding and open up future avenues for therapeutic interventions, particularly in FUS-associated ALS and related disorders.

KEYWORDS: ALS, DNA damage, mitochondria, DNA ligase, neurodegeneration

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Features of Mitochondrial Dysfunction Associated With Neurodegenerative Diseases

Neurodegeneration affects millions of individuals worldwide. The incidence of dementia is projected to increase from 57 million cases globally in 2019 to 153 million cases in 2050.[1] Among the various etiopathological factors associated with neurodegeneration, mitochondrial dysfunction is a central feature in most neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), Huntington's disease (HD) and Parkinson's disease (PD).[2] Mitochondria play a critical role in cellular functions including adenosine triphosphate (ATP) production, Ca^{2+} homeostasis, amino acid, nucleotide and fatty acid metabolism, iron homeostasis and apoptosis.[3] Neurodegeneration-associated mitochondrial dysfunction is observed at different disease stages and involves key processes such as mitochondrial dynamics (fission, fusion and transport), Ca^{2+} homeostasis, cell death, ATP production, reactive oxygen species (ROS) production and mitochondrial DNA (mtDNA) defects.[4]

Human mtDNA, a circular molecule of 16,569 base pairs with only 3% noncoding regions, is highly susceptible to damage due to its proximity to ROS-generating sites and mitochondria's limited DNA repair arsenal.[5] Although not all mitochondrial dysfunction arises from mutations in mtDNA, a significant portion of age-related mitochondrial dysfunction may be attributed to the accumulation of mtDNA mutations over time.[6,7] These mutations, often unique to individual cells, increase with age, and impair cellular function. Mitochondrial oxidative stress and DNA mutations are particularly damaging to terminally differentiated cells such as neurons, which primarily rely on mitochondria for energy required for synaptic activities.[8]

MtDNA damage is a common feature observed in neurons affected by major neurodegenerative diseases, including ALS, Alzheimer's, Parkinson's, and Huntington's disease. This damage is closely associated with increased production of reactive oxygen species (ROS), leading to oxidative stress, mitochondrial dysfunction, and disruptions in cellular calcium homeostasis. These mitochondrial disturbances can amplify cellular stress, weakening neuronal health and resilience.[5,9-11] Disease-specific processes, such as the accumulation of pathogenic protein aggregates and aging, exacerbate oxidative stress and contribute to mitochondrial dysfunction.[12] Pathogenic proteins can directly impair mitochondrial

membranes and proteins, disrupting electron transport.[13] Genome instability and mitochondrial dysfunction in neurodegenerative diseases are significantly influenced by nuclear DNA and mtDNA base modifications. DNA repair, especially base excision repair (BER), is crucial for maintaining the mammalian central nervous system, and its deficiency is a key factor in neuronal loss.[4,5] Additionally, altered or dysregulated DNA repair processes may directly contribute to the pathogenesis of diseases like ALS. Understanding the molecular underpinnings of mtDNA damage, repair, and mitochondrial dysfunction in neurodegenerative disorders could lead to new therapeutic targets.

Mitochondrial DNA Repair and Role of Non-canonical Proteins in ALS

Originally, it was perceived that mitochondria lacked efficient DNA repair mechanisms,[14] as early studies indicated that UV-induced pyrimidine dimers were not repaired in mtDNA. However, subsequent studies demonstrated the localization of several nuclear-encoded DNA repair proteins within mitochondria, associated with different DNA repair pathways.[4] Notably, ALS-associated RNA/DNA binding proteins fused in sarcoma (FUS) and tar DNA binding protein 43 (TDP-43) have been found to play crucial roles in maintaining nuclear genome integrity by participating in DNA damage repair processes.[15-18]

FUS is recruited to DNA damage sites in a PARP1-dependent manner, where it facilitates the recruitment of XRCC1/DNA ligase III (Lig3), both critical for efficient DNA repair.[16] Similarly, TDP-43 is a key component of the non-homologous end joining (NHEJ) mediated DNA double-strand break repair machinery, acting as a scaffold for the recruitment of XRCC4-Lig4 complex.[15] While these proteins have studied primarily for their roles in nuclear genome damage repair, their emerging functions in maintaining mitochondrial genome stability are now of growing interest. Notably, mitochondria, unlike the nucleus, have a limited repertoire of backup DNA repair pathways. For example, the mitochondrial isoform of Lig3 is the sole DNA ligase responsible for both mitochondrial genome replication and repair. XRCC1 serves as a scaffold for Lig3 in the nuclear genome, however, studies have reported that XRCC1 does not localize to mitochondria.[19,20] This raises the possibility that FUS might serve as a scaffolding factor for mitochondrial Lig3.[5]

Furthermore, PARP1 is known to localize to mitochondria,[5] but its precise role in mitochondrial genome repair is still under investigation. Some studies suggest that PARylation, a post-translational modification catalyzed by PARP1, may influence mitochondrial DNA stability, with evidence of PARP1 interacting with DNA polymerase (Pol γ) and potentially coordinating the repair of mtDNA damage.[21] However, while our previous studies demonstrated that FUS's role in nuclear genome repair is dependent on PARP1 activity,[16] it remains unclear whether a similar PARP1-FUS interaction exists in mitochondria. Notably, there is some ambiguity surrounding the role of PARP1 in mitochondrial DNA repair, as recent studies have suggested that mitochondrial PARylation may largely be dependent on another ADP ribosyl transferase, Neuralized-like protein 4 (NEURL4).[22,23] This highlights the need for further investigation into the potential roles of TDP-43, FUS, and PARP1 in maintaining mitochondrial genomic stability, as their interactions in this context are still not fully understood.

FUS and Mitochondria: An Emerging Connection

FUS localization to mitochondria has been shown to induce mitochondrial fragmentation, and interfere with endoplasmic reticulum (ER)-mitochondrial contacts.[4] Although pathological FUS mutants alter mitochondrial mRNA expression, there was no direct evidence linking FUS to mtDNA stability until recently.[24] In our recent study, we provided the first direct evidence of increased DNA damage linked to FUS pathology.[5] Using FUS mutant patient-derived primary cells including fibroblasts and iPSC-derived motor neurons, FUS pathology mice models, and ALS patient spinal cord samples, we uncovered 2 major findings. First, we showed that FUS localizes to mitochondria, and under oxidative stress, its recruitment increases. FUS in mitochondria interacts with mtLig3 and enhances its activity, a process essential for repairing mtDNA and maintaining function of mitochondria, as evidenced by decreased mtDNA integrity and membrane potential in FUS knockout (KO) cells. Second, in the context of FUS proteinopathy, mutant FUS (R521H, P525L) variants exhibit increased mitochondrial localization but reduced interaction with mtLig3, resulting in deficient mtDNA repair. Elevated levels of mitochondrial FUS in ALS patient-derived cells correlate with increased mtDNA damage and repair defects. Mutant FUS disrupts the recruitment of Lig3 to mtDNA, resulting in accumulated mutations and mitochondrial dysfunction. FUS KO models further demonstrate reduced mitochondrial membrane potential, underscoring the critical role of FUS in maintaining mitochondrial health.

Mitochondrial-Targeted DNA Ligase 1 and the Potential for DNA Repair-Based Therapeutics

DNA repair can be a double-edged sword. When it is defective, it can lead to diseases such as cancer and drug resistance.[25,26] However, in terminally differentiated cells such as neurons, it is widely accepted that the expression of DNA repair proteins decreases with age. This raises the question whether enhancing DNA repair activity in neurons or aged cells could be an effective strategy to counteract the age-associated decline in DNA repair function.[27] Previous studies by Simsek et al showed that Lig3 is an essential gene due to its mitochondrial function. Their research tested other DNA ligases to determine if they

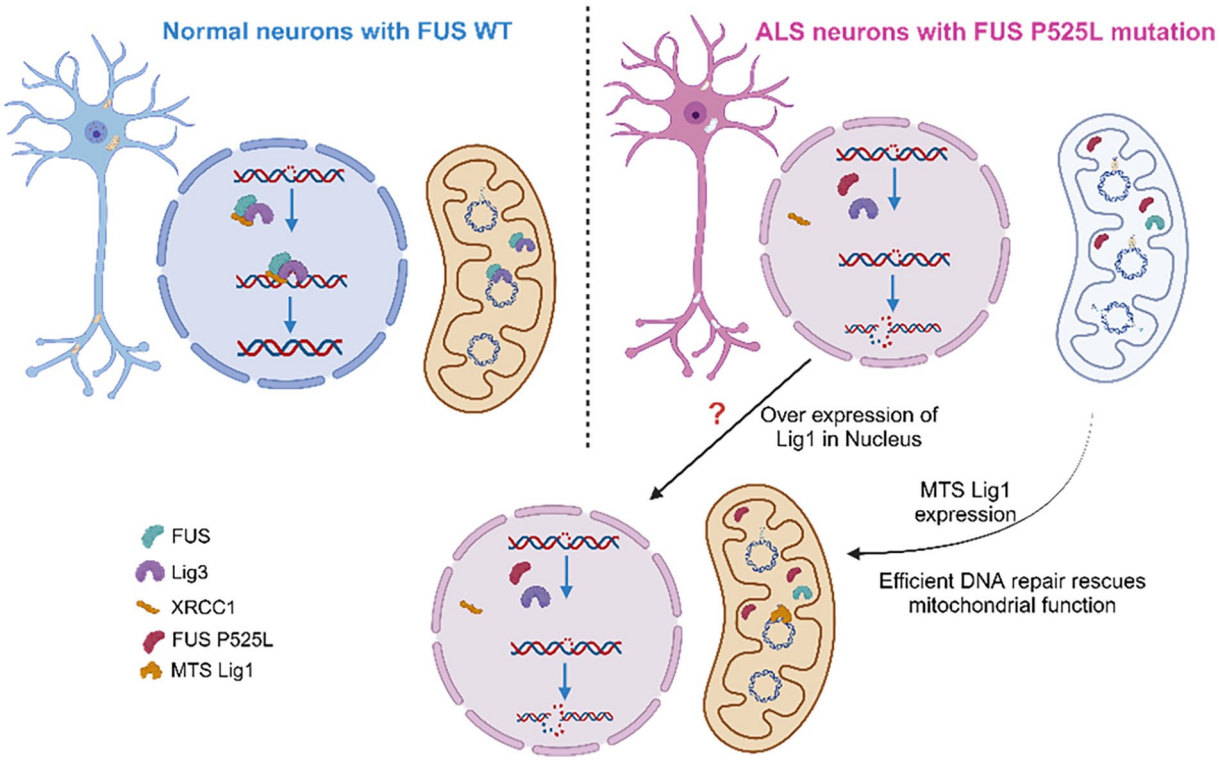

Figure 1. Schematic representation of our recent study,[5] which provide new molecular insights into the role of FUS in mitochondrial genome maintenance and the implications of FUS mutations in ALS. FUS is required for optimal mtDNA ligation in healthy neurons, facilitating efficient DNA repair. However, familial mutations in ALS causes mtDNA ligation defects, leading to overall mitochondrial dysfunction. Expression of mitochondria-targeted Lig1 (MTS Lig1) rescues mtDNA repair and restores function.
Abbreviations: ALS, amyotrophic lateral sclerosis; FUS, fused in sarcoma.

could replace Lig3's role in mitochondria. They found that Lig1, an algal ligase containing only the catalytic core, *E. coli* LigA which uses NAD+ as a cofactor, can function in mitochondria.[20] In our study, we demonstrated the rescue of mtDNA repair and mitochondrial membrane potential after expression of human nuclear Lig1 in mitochondria in the context of FUS pathology (Figure 1).

Significance and Future Directions

Our study highlights the potential of targeting DNA repair pathways as a therapeutic strategy for ALS. By enhancing DNA repair efficiency in motor neurons, we could reduce neuronal loss and slow disease progression. This approach may pave the way for developing ALS treatments that focus on underlying molecular mechanisms. The broader implications of these findings extend to other neurodegenerative diseases characterized by similar DNA repair impairments, potentially opening new research and therapeutic opportunities in neurodegeneration. While further studies are necessary to validate the therapeutic potential of our findings, the targeted delivery of Lig1 to mitochondria represents a significant step toward addressing unmet medical needs in FUS-associated ALS and other disorders associated with mitochondrial dysfunction.

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