Molecular Therapy
Nucleic Acids
Review

Molecular mechanisms and antisense oligonucleotide therapies of familial amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS), a progressive neurodegenerative disease, presents considerable challenges in both diagnosis and treatment. It is categorized into sporadic and familial amyotrophic lateral sclerosis (fALS); the latter accounts for approximately 10% of cases and is primarily inherited in an autosomal dominant manner. This review summarizes the molecular genetics of fALS, highlighting key mutations that contribute to its pathogenesis, such as mutations in *SOD1*, *FUS*, and *C9orf72*. Central to this discourse is exploring antisense oligonucleotides (ASOs) that target these genetic aberrations, providing a promising therapeutic strategy. This review provides a detailed overview of the molecular mechanisms underlying fALS and the potential therapeutic value of ASOs, offering new insights into treating neurodegenerative diseases.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects motor neurons.[1] Although the disease can strike at any age, the most common age is 55–75, and this disease affects 2.1–3.8 per 100,000 people in Europe.[2] However, in Saudi Arabia, there is limited research on the epidemiology of this disease. The etiology involves genetics as well as other factors, which are not fully understood.[3] Approximately 10% of patients with ALS have the familial form (fALS), which has predominantly autosomal dominant inheritance.[3] The remaining 90% of cases are sporadic ALS (sALS) with no underlying genetic etiologies.[3] Mutations known to cause endoplasmic reticulum (ER) changes that affect mitochondrial signaling contribute to the pathogenesis of fALS.[4,5] Mitochondria and the ER connect to form mitochondrion-associated membranes (MAMs).[4] MAMs include four important protein types: tethering proteins (anchoring organelles); vesicle-associated membrane proteins; regulatory proteins (regulators of the attachment of tethering proteins to organelles), such as fatty acid-coenzyme A ligase 4 (FACL4 or ACSL4); and ER-resident proteins (regulators of ER functions).[4] The most important function of ER-mitochondrion signaling is the control of intracellular Ca^{2+} homeostasis in neurons.

If this pathway is altered, in ALS for instance, by superoxide dismutase-1 (SOD1), which is a derivative of the *C9orf72* gene, ER stress results in increased levels of GRP78 (BiP) and swelling of the mitochondria, resulting in the disruption of Ca^{2+} homeostasis.[2] When the levels of misfolded proteins increase, ER functions are disrupted.[5] One of the many changes that can occur is the anchoring of BiP to misfolded proteins, which results in their accumulation in the ER and subsequent stress.[5] This ER stress induces the release of BiP, causing the misfolded proteins to attach to inositol-requiring kinase 1 and PKR-like ER kinase, which are the main mediators of ER stress.[5] ER stress induction in ALS can cause many changes, including unfolded protein response (UPR) upregulation, which increases chaperones and C/EBP-homologous protein, inducing mitochondrial apoptosis and cell death.[5]

In this review, recent findings regarding the molecular mechanisms underlying fALS and particularly the roles of mutations in three key genes (*SOD1*, *FUS*, and *C9orf72*; see Table 1 and Figure 1) as well as the potential therapeutic value of antisense oligonucleotides (ASOs) are described.

METHODS

To collect thorough and reliable knowledge on the roles of mutations in three essential genes—*SOD1*, *FUS*, and *C9orf72*—that may underlie the mechanism behind fALS, we applied a rigorous methodology for a literature review by conducting an in-depth search of articles from the English literature in peer-reviewed scientific journals. The databases searched for this purpose include PubMed, Web of Science, Cumulative Index to Nursing and Allied Health Literature (CINAHL), Scopus, and Google Scholar. Keywords were chosen to ensure the inclusion of all relevant literature such as "Amyotrophic lateral sclerosis," "familial amyotrophic lateral sclerosis," "fALS etiology," "fALS genetic factors," "fALS mutations," and "fALS therapy."

SOD1 MUTATIONS AND ASOs

Genetic mutations, notably those affecting protein folding and inducing mitochondrial stress, lead to an increase in glutamate toxicity.[6]

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Table 1. Summary of mutations in fALS

Gene	Frequency in fALS	Key features	Effects of targeted ASOs
SOD1	25%	causes ER stress by misfolding and interacting with Derlin-1, leading to superoxide radical release	specific to mRNAs, targeting mutant genes to decrease SOD1 levels
FUS	4%	causes neurodegeneration, associated with RNA splicing/transcription, and leads to paraspeckle build-up	targets the C-terminal region of FUS, influencing mutant FUS proteins
C9orf72	22.5%	leads to early-onset cognitive impairment and FTD, involves the GGGGCC hexanucleotide repeat expansion	targets sense strands of RNA, reducing RNA foci related to sense strands

This accumulation of L-glutamate has been implicated in cellular dysfunction, including oxidative stress and protein aggregation.[7] Such alterations precipitate an "excitatory state" in the central nervous system, exacerbating neurodegenerative processes.[8] In the context of ALS, these pathophysiological changes are particularly critical, as they contribute considerably to motor neuron degeneration.

The role of SOD1 in the pathogenesis of ALS is multifaceted. SOD1 mutations result in its aberrant accumulation in the ER lumen, triggering ER stress. SOD1 activates the UPR by inhibiting Derlin-1, which is crucial for removing misfolded proteins from the ER.[5] This disruption of protein homeostasis is a key factor in ALS, linking SOD1 mutations to neuronal death. Furthermore, misfolded SOD1 leads to the liberation of harmful superoxide radicals (O_2^-), potentiating oxidative damage. SOD1 mutations contribute to fALS, accounting for a notable proportion of cases.[8]

ASOs represent a promising therapeutic strategy, targeting specific mRNAs to mitigate the effects of mutant genes.[9,10] ASOs are synthetically engineered, single-stranded oligonucleotides tailored to bind to complementary sequences in target mRNAs.[8] Their design incorporates modifications to enhance nuclease resistance and increase affinity for target RNAs, ensuring greater efficacy in gene silencing. A crucial challenge for ASO therapy is the limited permeability of the blood–brain barrier (BBB).[11] Innovative strategies, such as lipid nanoparticle encapsulation, are being explored to enhance ASO delivery across the BBB.[11]

Approximately 25% of fALS cases are linked to missense mutations in Cu^{2+}/Zn^{2+} SOD1.[8] Targeting these mutations is a pivotal focus of ASO therapy.[10,12] This precision medicine approach offers the potential for personalized treatment strategies, addressing the specific genetic makeup of each patient. Smith et al. described how surgically implanted catheters in rats with SOD1 mutations can facilitate the delivery of oligonucleotides directly to the brain, demonstrating marked reductions in mutant SOD1 levels after 14 days of treatment.[13] These findings underscore the potential value of the direct administration of ASOs in the central nervous system as a viable therapeutic approach. However, the specificity of ASOs is paramount, as interactions with non-target RNAs may lead to unintended gene silencing.[14] Future research must therefore focus on optimizing ASO design for maximal specificity and minimal off-target effects.

FUSED IN SARCOMA MUTATIONS AND ASOs

Fused in Sarcoma (FUS) on chromosome 16 contributes to RNA splicing and transcription as well as RNA metabolism.[15] Mutations in FUS are associated with fALS.[16,17] This RNA-binding protein is implicated in various cellular processes, including DNA repair, RNA transport, and the regulation of gene expression. FUS mutations cause neurodegeneration in patients with ALS.[17] FUS accounts for approximately 4% of fALS cases and less than 2% of sALS cases.[16] Most *FUS* mutations in fALS are missense mutations in five arginine residues on exon 15.[17] These mutations often lead to the cytoplasmic mislocalization of FUS, contributing to motor neuron degeneration. The aggregation of mutated FUS disrupts normal cellular functions and leads to neuronal toxicity.

In knockin mice expressing the ALS-associated mutant FUS, including heterozygous P517L/wild-type (WT) and WT Δ14/WT mutant FUS knockin mice, within 1.5 years of observation, there were 11% fewer motor neurons in heterozygous P517L/WT mice than in WT mice. After 2 years of observation, both WT controls showed 22% progression. This experiment revealed that both mutation types can cause gliosis and neuromuscular junction denervation.[16] The study has highlighted the importance of FUS mutations in motor neuron pathology and their potential as therapeutic targets. FUS mutations also have a close relationship with subnuclear paraspeckles, RNA granules in the nuclear interchromatin space, including core and secondary proteins, assembled in non-coding RNA strands,[18] indicating that cell compartments are asymmetrical. Mutant FUS causes the build-up of nuclear paraspeckle assembly transcript 1 (NEAT1), causing an increase in the severity of ALS.[18] NEAT1 accumulation is hypothesized to contribute to the disruption of nuclear-cytoplasmic transport, a key pathological feature in ALS.

ASOs with hydrophobic 2′ sugar modifications assist with attachment to the C-terminal region of *FUS*.[15] Therefore, FUS will bind to ASOs,[15] thereby disrupting the pathological interactions of mutant FUS, potentially mitigating its toxic effects. 5-10-5 gapmer phosphorothioate (PS)-ASOs are used for numerous cytoplasmic ribonucleoproteins (RNPs). RNPs are cytoplasm stressors produced by translocated in liposarcoma/FUS.[15] FUS mutations in patients with ALS result in the removal of FUS from the nucleus and accumulation of RNP granules that are similar to normal stress granules but can be differentiated.[15] WT FUS will normally produce stress granules in response to osmotic stress.[15] The WT FUS protein functions to control which proteins enter and exit the cytoplasm and the nucleus.[19] Accordingly, FUS helps to maintain "the peace" (an equilibrium) between each compartment.[19] The recruitment of stress granules

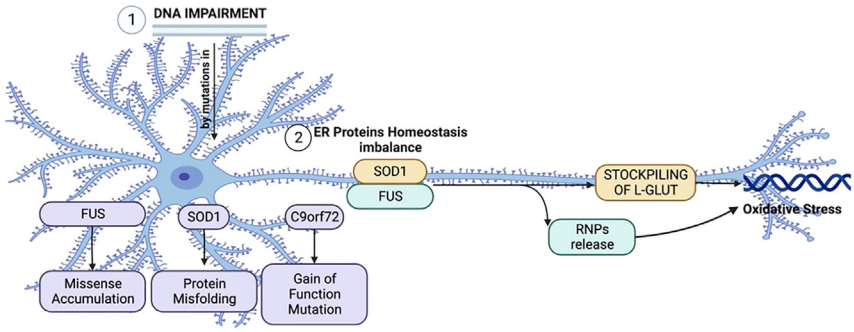

Figure 1. The gene mutations (FUS, SOD1, and C9orf72), the imbalance of ER protein homeostasis, and RNP release in fALS

is fairly rapid.[19] Using G3BP, which causes stress granules to assemble, with an immunofluorescent marker, FUS accumulation in the cytoplasm prior to stress granule formation has been observed.[19] Furthermore, sorbitol treatment results in stress granule formation containing both FUS and G3BP within an hour.[19] FUS redistribution in the cytoplasm has also been noted.[19] The use of cEt-modified PS-ASOs does not affect mutant FUS.[15] *In vitro*, the mutated FUS has high affinity to cEt-modified PS-ASO binding sites; therefore, the ASOs exert effects on proteins downstream of mutated FUS.[15] This suggests that cEt-modified PS-ASOs are a promising therapeutic strategy for targeting mutant FUS in ALS.

C9ORF72 MUTATIONS AND ASOs

C9ORF72 is abundant in neurons, and gain-of-function mutations account for 60% of fALS specifically (C9-ALS).[20,21] The expansion of the non-coding GGGGCC hexanucleotide repeat in the *C9orf72* gene is associated with ALS.[22] This *C9orf72* mutation accounts for 22.5% of fALS.[22] It causes the early onset of cognitive impairment, leading to frontotemporal dementia (FTD).[20] Byrne et al. evaluated 435 DNA samples from the Irish ALS Register, using polymerase chain reaction, revealing that 39 (9%) had the GGGGCC expansion.[20] The repeat expansion is bi-directionally translated, resulting in both sense and antisense *C9orf72*.[20] This results in two important products: RNA-binding proteins and chromatin modifiers. Antisense and sense RNAs will produce dipeptide repeats (DPRs), and these noncanonical repeats cause repeat-associated non-ATG-mediated translation,[10] resulting in the dysregulation of proteostasis.[10] A challenge concerning targeting the *C9orf72* mutation is the fact that it is transcribed bi-directionally.[10] The complete degradation of mutant *C9orf72* is not possible.[10] ASOs are only intended to target the sense strand, and DPRs and RNA foci will still be produced by the antisense strand.[10] ASOs can reduce RNA foci, but only those related to the sense strands. This can be considered a limitation with respect to the application of ASOs to the *C9orf72* gene mutation.[10] The *C9orf72* expansion in C9-ALS leads to motor neuron abnormalities, and these can be reversed by using ASOs targeting specific RNA foci related to the sense strands.[10,21] The *C9orf72* mutation is also associated with FTD, as it causes thalamic, posterior insula, or cerebellar atrophy.[23] Similar to fALS, FTD has an autosomal dominant familial pattern of inheritance.[23] The *C9orf72* mutation has a frequency of 11.7% in familial FTD.[22,23]

ASOs

The first ASO drug approved by the US Food and Drug Administration was fomivirsen, which targets the *CMV* gene.[24] The development of ASOs has evolved ever since.[24] RNA-targeting oligonucleotides can be identified into either double-stranded or single-stranded small interfering RNA siRNA, degrading specific RNAs.[24] In addition to the downregulation of the expression of target RNAs, ASOs can upregulate the expression of specific genes.[24] ASO drugs need to be chemically modified to ensure they exert their effect.[24]

Oligonucleotide therapies include various types, including ASOs, microRNAs, small siRNAs, and aptamers (single oligonucleotides that bind to specific proteins).[25,26] These RNAs contribute to the inhibition of posttranscriptional gene silencing.[27] Gene silencing can be enhanced using synthetic siRNAs, microRNA (miRNA) mimetics, or shRNAs.[27] The RNA-induced silencing complex will either stop the translation of the targeted RNAs via miRNAs or cleave transcripts via siRNA.[27] Once these target RNAs join the silencing complex, the interfering RNAs have the ability to silence various target RNAs.[27]

All ASOs need to be chemically modified *in vivo*.[25] The chemical modifications include a PS backbone (first-generation ASOs; Figure 2A), nucleotides with sugar modifications (2′-*O*-methyl modification of RNA [2′OMe-RNA]; second-generation ASOs; Figure 2B), and methylation of 5′ cytosines (third-generation ASOs), currently being evaluated in trials.[25,28]

The first-generation approach, PS backbone modifications, does not protect ASOs from cleavage by RNase H, necessitating high quantities of PS.[25] The second-generation approach shows a substantial improvement in oligonucleotide stability.[25] ASOs also show an increase in safety while silencing specific genes.[25] 2′OMe-RNA (e.g., 2′OMe-uridine or 2′OMe-guanosine) exhibits antagonistic interactions with immunostimulatory RNA.[30]

CONCLUSION

This review systematically explores the complex molecular landscape of fALS, underscoring the pivotal role of mutations in *SOD1*, *FUS*, and *C9orf72* in its pathogenesis. ASOs have emerged as a beacon of hope in this challenging domain, with the potential to target these specific genetic alterations. Although ASOs represent an important advancement in the treatment of fALS, there are still many challenges to overcome in clinical translation, including off-target effects, the need for delivery methods, and patient-specific responses. Ongoing research aimed at optimizing ASOs for individual genetic profiles is promising for fALS management.

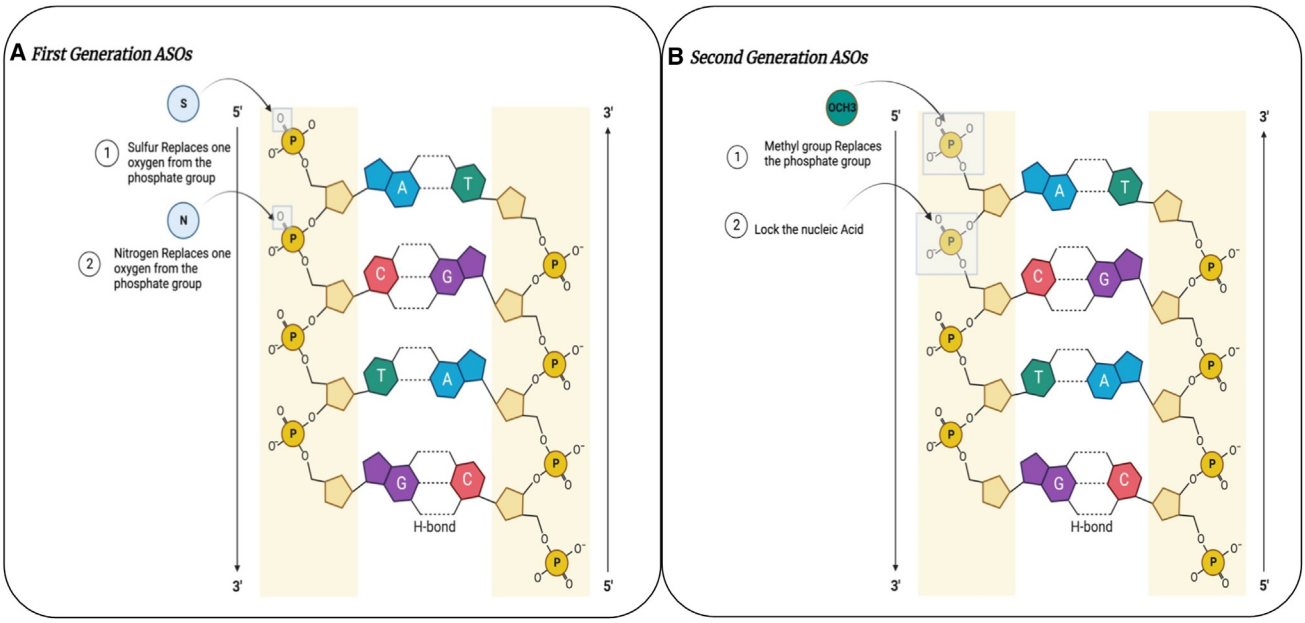

Figure 2. Two essential mechanisms by which ASOs inhibit gene expression to treat fALS

ASOs inhibit gene expression to treat fALS (A) by directing RNase H to cleave pre-mRNAs in the nucleus and (B) by engaging the RNA-induced silencing complex to degrade mRNA significantly in the cytoplasm. Both mechanisms result in reduced protein production.[29]

In conclusion, this review not only enhances our understanding of the genetic basis of fALS but also positions ASOs as a critical component in the evolving landscape of neurodegenerative disease treatment. As research progresses, these molecular insights are expected to translate into effective and personalized therapeutic strategies, ultimately altering the course of fALS.

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AUTHOR CONTRIBUTIONS

H.A.D., conceptualization, software, visualization, writing – review & editing, and supervision; B.A.Q., writing of the first draft, conceptualization, software, and drawing. Both authors have read and agreed to the published version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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