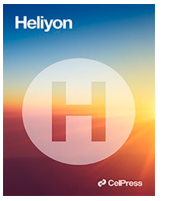
Heliyon 10 (2024) e34963

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Review article

The prion-like effect and prion-like protein targeting strategy in amyotrophic lateral sclerosis

Yang Wenzhi [a,b], Liu Xiangyi [a,b], Fan Dongsheng [a,b,1,*]

[a] Department of Neurology, Peking University Third Hospital, Beijing, China
[b] Beijing Key Laboratory of Biomarker and Translational Research in Neurodegenerative Diseases, Beijing, China

ARTICLE INFO

Keywords:
ALS
Prion-like
Protein

ABSTRACT

Pathological proteins in amyotrophic lateral sclerosis (ALS), such as superoxide dismutase 1, TAR DNA-binding protein 43, and fused in sarcoma, exhibit a prion-like pattern. All these proteins have a low-complexity domain and seeding activity in cells. In this review, we summarize the studies on the prion-like effect of these proteins and list six prion-like protein targeting strategies that we believe have potential for ALS therapy, including antisense oligonucleotides, antibody-based technology, peptide, protein chaperone, autophagy enhancement, and heteromultivalent compounds. Considering the pathological complexity and heterogeneity of ALS, we believe that the final solution to ALS therapy is most likely to be an individualized cocktail therapy, including clearance of toxicity, blockage of pathological progress, and protection of neurons.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal disease characterized by neuronal degeneration of the spinal cord and brain. In ALS, upper motor neurons and lower motor neurons are both affected, resulting in progressive motor symptoms including muscle weakness, muscle atrophy, and spasticity. The progression of symptoms is irreversible, most patients die from respiratory failure within 5 years. Pathological protein aggregation occurs in ALS, such as superoxide dismutase 1 (SOD1), C9orf72, TAR DNA-binding protein 43 (TDP-43), and fused in sarcoma (FUS), but it is still unclear if the pathogenic proteins are cause or the result of ALS pathology. Approximately 10 % of patients with ALS have a family history (fALS), and some gene variants carried by patients with fALS may increase the risk of ALS-relevant phenotypes. The remaining 90 % are sporadic (sALS). Patients with sALS have no specific pathogeny; the possible causes could be physical damage, chemical toxicity and infection, and some rare genetic mutation [1–4]. However, sALS and fALS share the same pathogenic pathway in part. Several proteins were observed in the neuronal cytoplasmic inclusions in both sALS and fALS, including TDP-43, FUS, OPTN, and p62. But in SOD1-mutant fALS, TDP-43 and FUS were not observed [5]. The development of therapeutic methods for ALS is challenging because of its complicated pathogenesis and heterogeneity. Riluzole and edaravone, are the only two drugs that have proven to be effective for ALS. The mechanism of Riluzole is not completely clear yet. In ALS treatment, Riluzole can interact with glutamate receptors directly, block the activation of glutamate receptors, and reduce the excitotoxicity of glutamate [6,7]. Edaravone can reduce oxidative stress by scavenging free radicals. The chemical name of Edaravone is 3-methyl-1-phenyl-2-pyrazoline-5-one, which is a one-electron antioxidant. Edavarone can provide

* Corresponding author. Department of Neurology, Peking University Third Hospital, Beijing, China.
E-mail address: dsfan2010@aliyun.com (F. Dongsheng).
[1] Permanent address: Huayuanbei Road, Haidian District, Beijing, China.

https://doi.org/10.1016/j.heliyon.2024.e34963
Received 18 March 2024; Received in revised form 9 July 2024; Accepted 19 July 2024
Available online 22 July 2024

electrons to reactive oxygen species (ROS), eliminate the oxidability of ROS, and protect cells from oxidative damage [8,9].

Although both Riluzole and Edaravone can protect neurons, they can only slow disease progression, and the prognosis of patients with ALS is inevitable.

In fALS, abnormal proteins encoded by mutant genes play important roles, such as SOD1, C9orf72, TDP-43, and FUS, some ALS-associated mutations are also present in sALS patients. The mechanism of neuron death in ALS is also unclear yet, the mainstream theories include loss-of-function and gain-of-toxicity. Loss-of-function theory implies that the mutation genes have no function or low function, and some important biological processes cannot be completed, which leads to the death of neurons. Gain-of-function theory implies that the mutant proteins tend to aggregate, and the aggregation proteins are neurotoxic, and hard to degrade, which leads to the death of neurons finally [10–12]. Research has revealed that pathological proteins, such as SOD1, TDP-43, and FUS show characteristics similar to prion proteins, they can destroy the structure of the normal protein and transfer it into pathological form, and the new-born pathological protein can repeat this process, which will lead to the formation of amyloid protein. Although the function of amyloid protein is still unclear, some researchers believe amyloid proteins are neurotoxic. We believe the prion-like replicating ability may play an important role in the ALS mechanism. According to this theory, the key point of blocking the ALS process is to eliminate the prion-like pathological proteins.

Some technologies have been developed to prevent the expression of pathological proteins. The production of pathological proteins can be divided into 5 steps: transcription, translation, modification, misfolding, and aggregation. In this review, we listed 6 technologies, including antisense oligonucleotides (ASO), antibody-based technology, peptides, chaperones, autophagy enhancement, and heteromultivalent compounds, which intervene in different steps in the production of pathological proteins. ASOs can prevent protein production by inhibiting transcription and translation [13–16]. Chaperones can help protein fold correctly, which inhibits the abnormal aggregation of pathological protein [17–19]. Peptides can interact with proteins and prevent proteins from aggregating [20,21]. Antibody-based technology and autophagy enchantment can enhance the ability to degrade pathological protein, an antibody-based technology called 'Trim-Away' can eliminate any endogenous proteins without prior modification [22–24], which has a potential for pathological protein elimination. Heteromultivalent compounds can inhibit aggregation of the pathological proteins and promote their dissolution.

In this review, we summarize the ALS-relevant toxic proteins, the prion-like effect of these proteins, and the prion-like protein targeting strategy. We have listed six potential technologies for ALS therapy and discussed their advantages, disadvantages, and key technical limitations.

2. The toxic proteins in ALS

2.1. Superoxide dismutase 1

SOD1 was defined as an ALS-associated gene in 1969, about 3 % of ALS cases are caused by SOD1 mutation [25]. The structure of the SOD1 protein can be divided into 3 segments. The N-terminal (residues 1–37) contains 3 domains of β-strand; Residues 38–90 include the Zn loop and two β-strand domains; C-terminal (residues 91–153) consists of 3 β-strand domains and the electrostatic loop. SOD1 dimerizes and converts the superoxide radical into molecular oxygen and hydrogen peroxide when combined with copper and zinc. The mature body of SOD1 is very stable, and the lack of copper and zinc may lead to SOD1 misfolding and aggregating, which results in function loss and toxicity [26].

SOD1-related ALS pathomechanisms may involve oxidative stress, neuroinflammation, endoplasmic reticulum (ER) stress, mitochondrial dysfunction, axonal transport disruption, axonal transport disruption, non-cell autonomous toxicity, prion-like propagation and disruption of protein homeostasis (proteostasis) [25]: 1) Oxidative stress. Reactive oxygen species generated by aerobic metabolism damaged cells by oxidizing various biomolecules including proteins and lipids. As an antioxidant enzyme, mutation of SOD1 may lead to the low biological activity of SOD1 protein, which contributes to the production of superoxide, previous study also showed the mutant SOD1 can bind to GTPase Rac1 and lead to sustained activation of NADPH oxidase 2 and overproduction of superoxide [27,28]. Superoxide can promote the transcription of various inflammatory genes leading to neuroinflammation [29]. 2) ER stress. ER, stress occurs when abnormal proteins accumulate in ER. In ER stress, the unfolded protein response (UPR) mechanism will be activated to degrade the abnormal proteins, but the UPR and prolonged ER stress may also lead to apoptosis of cells [30,31]. 3) Mitochondrial dysfunction. The mutant SOD1 can bind to membrane proteins of mitochondria, including Bcl-2 and VDCA1, disrupt the homeostasis of mitochondria and lead to mitochondrial dysfunction, which leads to release of cytochrome *c* from the mitochondrial intermembrane space and apoptosis of cells [32–34]. 4) Axonal transport disruption. Axon transport is important for highly polarized motor neurons, and axonal transport defects were observed in $SOD1^{G93A}$ mice, which suggested the SOD1 mutation may lead to progression of ALS [35,36]. 5) Non-cell autonomous toxicity of neuroglia. Non-cell-autonomous mechanism refers to motor neuron degeneration caused by the toxic effect of the surrounding glial cells, including microglia [37], astrocytes and oligodendrocytes. A study showed the mutant SOD1 in astrocytes is toxic for motor neurons, and the toxic effect exists in both sALS and fALS [38]. Another study showed the oligodendrocytes were replaced by oligodendrocyte progenitors in the early stage of disease onset. The oligodendrocyte progenitors express low-level monocarboxylate transporter 1 (MCT1), the loss of MCT1 increases the vulnerability of motor neurons [39,40]. 6) Prion-like propagation. The misfolded SOD1 has prion-like propagation. The prion-like propagation has been observed in $SOD1^{G93A}$ and $SOD1^{G37R}$ mice [41]. 7) Disturbance of proteostasis. The disturbance of proteostasis is an important biomarker for ALS, various ALS-associated proteins, including VCP, UBQLN2, OPTN, and TBK1, are involved in the proteostasis [42]. The wild-type SOD1 is highly stable, but the wild-type SOD1 recombinant protein aggregates rapidly after the stability was disrupted [43]. Mutant SOD1, including G93A, G37R, A4V, and G85R, showed a high property to aggregate and form amyloid fibrils [43,44]. The structure of SOD1

is maintained by the Cu/Zn metalation and intrasubunit disulfide bonds between Cys57 and Cys146, loss of Cu/Zn can lead to pathogenic misfolding of SOD1 [45,46].

SOD1 localizes to ER and Golgi in the cytoplasm of eukaryotic cells [47]. Previous study suggested SOD1 were transported to ER via a signal-peptide independent pathway, and the mutant SOD1 were more easily translocated into ER than wild-type SOD1 in ste24Δ hrd1Δ cells. SOD1 was secreted after signal peptide-independent translocation into ER [48]. The secreted SOD1 from neurons and glias will spread over the central nervous system via cerebrospinal fluid (CSF), and the misfolded SOD1 in CSF was proved to be neurotoxic [47,49]. The secretion of mutant SOD1 is mediated by chromogranin-A (CgA) and chromogranin-B (CgB), which are components of neurosecretory vesicles [47]. The secreted aggregating SOD1 can penetrate inside cells by micropinocytosis and lead to aggregation of the soluble mutant SOD1 in cytoplasma [50].

Although SOD1 is an important antioxidant enzyme, research suggested that the SOD1-knockout mice showed no neuron death, which implies that SOD1-associated ALS may be not caused by the function loss of SOD1. More than 160 SOD1 mutations were observed in ALS, and various SOD1 mutations increase the aggregating property of SOD1 proteins, which is believed to be associated with neuronal death. SOD1 toxicity is a common feature of SOD1-mutant fALS and may be caused by mutant SOD1-mediating protein misfolding. Previous studies have suggested that the elimination of wild-type SOD1 does not affect neuronal degeneration. However, a high-efficiency mutation, SOD1G93A, leads to cell degeneration, but SOD1 depletion didn't lead to cell degeneration [51,52]. In addition, the combination of SOD1 and Cu2+ requires a specific copper chaperone for SOD1 (CSS). The absence of the CSS has no effect on ALS onset and progression in SOD1G93A, SOD1G37R, and SOD1G85R mice [53,54].

Although the intrinsic toxicity of SOD1 aggregates remains to be determined, increasing evidence suggests that protein aggregates exhibit general toxicity unrelated to the function of proteins in their natural state. However, the pathological mechanism of SOD1 in ALS is not completely clear yet, it is currently unclear whether the dominant mechanism of SOD1-ALS is loss of normal SOD1 function or the toxicity of SOD1 aggregates.

2.2. Chromosome 9 open reading frame 72

It has been found that hexanucleotide (G4C2) repeat expansion (HRE) in the non-coding region of C9orf72 is the most frequent mutation in ALS. It is believed that the effects of 'gain of toxicity' and 'loss of function' exist in C9-mutant ALS. The three main alternatively spliced transcript variants of C9orf72 are V1, V2, and V3. Three transcripts produce two types of normal C9 proteins: C9–S is an isoform of 222 amino acids encoded by V1; V2 and V3 encode the same type of 481 amino acids, known as C9-L. The G4C2 HRE mutation is in the first intron of V1 and V3; for V2, the HRE is in the promoter segment; therefore, all three variants are affected by HRE. There are several theories about the mechanism of C9orf72 in ALS. The G4C2 repeat may interfere with transcription of C9orf72, which results in loss-of-function of normal C9orf72 proteins; The repeat expansion may sequester RNA binding proteins (RBP) and lead to function loss of RBPs; The expression of G4C2 HRE is ATG-independent, and the non-canonical repeat-associated non-AUG (RNA) translation products, dipeptide-repeat proteins (DPRs), have been proven to be toxic [55–57].

Little is known regarding their specific function until the specific antibody is generated. C9-L is primarily located in the cytoplasm of neurons and cerebellar Purkinje cells, whereas C9–S is in the nuclear membrane of neurons. Interestingly, C9–S relocates to the cell membranes of degenerated motor neurons under ALS conditions, the relocation of C9–S is associated with the mislocation and aggregation of TDP-43, implying that C9–S is associated with the nucleocytoplasmic transportation process [58]. Previous research has reported a decrease in C9orf72 transcript levels in patients with ALS, especially V2; however, the changes in V1 and V3 remain controversial [59–63]. In the protein level, it was confirmed that C9-L was decreased in ALS [64,65], although little is known about C9–S because of its low expression level. Only one study detected an increase in C9–S in patients with ALS. This study found that C9-L was decreased in the temporal cortex and frontal cortex of C9-ALS cases, which is consistent with previous study that C9orf72 expression is down-regulated in ALS patients. However C9–S is increased in the temporal cortex and frontal cortex of C9-ALS patients, which is unexpected [58].

There are 5 DPR species: poly-GA, poly-GP poly-GR, poly-PA and poly-PR. It has been verified that poly-GR and poly-PR are potently neurotoxic. Poly-GA also exerts toxicity. Poly-PA and Poly-GP have not shown toxicity', more studies need to be done to explore the neurotoxicity of DPRs [56,57]. The mechanisms of DPR toxicity include ER stress, inhibition of ubiquitin-proteasome system, disturbance of global translational regulation, and nucleocytoplasmic transport [66,67]. In the transgenic fly model, expression of poly-PR leads to aggregation of DPRs in nucleoli and stress granules formation by impairing global translational regulation [68]. Expression of poly-GA leads to increased ER stress, inhibition of proteasome, and dendritic branching reduction in cultured cells [69]. Poly-GA and poly-GP can cause neurotoxicity by inhibiting ubiquitin-proteasome system in cultured Neuro2a cells [70]. Expression of DRPs damaged the integrity of nucleus and nuclear membrane and disrupted the nucleocytoplasmic transport machinery. The nucleocytoplasmic transport-associated proteins, including Ran and RanGAP, were mislocalized in DPR-expressing cells [71,72].

Animal experiments in mice confirmed expression of poly-GA can lead to ALS-like symptoms, and the toxicity of poly-GA was structure-dependent, the toxicity disappeared after inhibition of fibril formation [73]. The toxic DPRs, poly-GA, poly-PA, and poly-PR, are arginine-rich, which also suggested the toxicity of DPRs is structure-dependent. In addition to DRPs, the RNA of G4C2 repeats also showed toxicity. Overexpression of G4C2 repeats leads to reduction of autophagosomal vesicles, which impair the protein degradation by inhibiting autophagy pathway [74]. Studies also showed that C9orf72 colocalized with Rab, which is associated with autophagy and endocytic transport [75]. Experiments in vitro and in vivo showed knockout of C9orf72 resulted in reduction of endocytic transport and exosome secretion, which may lead to accumulation of DPRs [76,77].

Although abundant studies revealed the potential mechanism of DPR toxicity, which mechanism takes the dominant role or occurs

first in ALS process is still unclear. The development of therapy against DPRs still needs more in-depth studies for the mechanism of DPRs. Antibodies-based treatment and inhibition of fibril formation may be potential for ALS therapy.

2.3. TAR DNA binding protein

Except for SOD1-ALS and FUS-ALS, insoluble and ubiquitinated TDP-43 inclusions were found in more than 95 % of the patients with ALS [78–80]. In addition, mutations in the TDP-43 gene can also lead to ALS [81]. TDP-43 protein consists of five regions: an N-terminal region (aa 1–105) containing a nuclear location sequence (NLS, aa 82–98), RNA recognition motif 1 (RRM1, aa 107–177), RRM2 (aa 192–259), a C-terminal region (aa 274–414) containing a glutamine/asparagine-rich domain (aa 340–360), and a glycine-rich region (aa 366–414). The C-terminal of TDP-43 is a low-complexity region associated with abnormal aggregation of TDP-43, previous studies have shown that most mutations are in exon-6, which encodes the C-terminal of the TDP-43 protein, such as A382T, M337V, A315T, and Q331K, which have also been well studied [82–86]. Evidence demonstrates that mutants Q343R and G335D promote the property of TDP-43 protein aggregation, and can increase the formation of cytoplasmic insoluble TDP-43 [87]. C-terminal fragments of the TDP-43 protein, generated by caspase and calpain proteases, are believed to play an important role in the mechanism of TDP-43 aggregation [88].

Under normal physiological conditions, TDP-43 is an RBP. Various RBPs are implicated in ALS, which implies that disturbances in the RNA metabolism may be present in ALS. Alongside its RNA-binding function, TDP-43 is also involved in RNA transportation, transcription, splicing, maturation, stabilization, and other protein-protein interactions. More than 6,000 mRNA targets were identified to associate with TDP-43, TDP-43 depletion from the nucleus may lead to mRNA splicing aberrations. In mRNA maturation and stability, TDP-43 can regulate half-life of mRNA by combining with 3′ UTR sequences of the mRNAs. TDP-43 also regulates mRNA localization and translation in the neuromuscular junctions [81]. All this information suggests the loss of normal TDP-43 function also contributes to ALS pathology.

The TDP-43 associated Frontotemporal lobar degeneration (FTLD) has been divided into 5 subtypes. Type A is characterized by dystrophic neurites (DNs) and neuronal cytoplasmic inclusions (NCis) with mutation of progranulin gene (GRN), showing behavioral variant frontotemporal degeneration (bvFTD) or non-fluent/agrammatic PPA (naPPA) in clinic. Type B is characterized by compact NCIs with mutation of C9orf72, showing ALS and FTLD in clinic. Type C is characterized by long and thick DNs in superficial cortical layers with no genetic mutation, showing FTLD in clinic. Type D is characterized by lentiform neuronal intranuclear inclusions (NIIs) in superficial and deep cortical layers and few DNs with VCP mutation, showing multisystem proteinopathy (MSP). Type E is characterized by granulofiamentous neuronal inclusions (GFNI's) and neuropil grains in superficial and deep neocortical layers and curvilinear oligodendroglial inclusions, type E can be observed in sporadic and C9orf72 mutation patients, bvFTD is related with type E in clinic [89–92]. Although the biological function of the 5 subtypes of TDP-43 and the association between them and clinical manifestation in FTLD is still unclear, and it is still unclear whether the subtypes of TDP-43 in ALS are same with FTLD, the pathological heterogeneity of TDP-43 suggests the structure or modification (ubiquitination and phosphorylation) of pathological TDP-43 may play an important role in disease progress.

The neurotoxicity of TDP-43 is still a controversial topic, it is still unclear whether the toxicity is attributed to TDP-43 aggregating or the fibrils themselves. Overexpression of wild-type TDP-43 led to motor neuron degeneration in mice and rats model, overexpression of full-length TDP-43 caused cell death in SH-SY5Y cells. Clearance of the aggregating TDP-43 can recover the normal function of TDP-43 [93,94] and ameliorate the toxicity of TDP-43 aggregates, which may be potential in ALS therapy [95–97].

2.4. Fused in sarcoma

As an RBP, FUS shares several common characteristics with TDP-43. The FUS protein includes a prion-like domain (aa 1–239), a glycine-rich region (aa 240–267), an RNA-recognition region (aa 285–371), a zinc finger (aa 422–453), two arginine-glycine-rich regions (aa 371–422, aa 454–500), and an NLS region (aa 501–526) [98]. Similar to TDP-43, FUS aggregation has also been observed in ALS. Interestingly, ALS-associated mutations in FUS are concentrated in the NLS region instead of in prion-like regions, such as the most common mutations R521C and R521G, which is in contrast to TDP-43 [78]. R521G mutation contributes to FUS mislocalization in the cytoplasm, leading to the accumulation of insoluble FUS proteins in the cytoplasm, which causes neuronal degeneration. Thus, FUS and TDP-43 may have different upstream pathological mechanisms, but they share the common feature of toxic protein aggregation. FUS mutations are rare in ALS, accounting for approximately 3 % of familial ALS cases. The function of FUS and the pathology of how mutant FUS leads to neurodegeneration remain to be revealed through further investigation.

3. The prion-like effect of TDP-43, FUS and SOD1

Prion diseases are devastating neurodegenerative disorders. The key pathology of prion disease is the conversion from the normal prion protein (PrP^{C}), dominated by α-helices, to its pathologic form (PrP^{SC}), dominated by β-sheet. Prp^{SC} can infect PrP^{C} and convert it into Prp^{SC}, which repeats this procedure. Abnormal PrP^{SC} accumulates in cells leading to disease. The structure of PrP^{C} consists of an extended intrinsically disordered N-terminal domain and a highly structured C-terminal domain. PrP^{C} mediates neurotoxic effects of prions via an interaction of its N-terminal domain with β-sheet-rich oligomeric conformers of the respective pathogenic proteins. The pathological proteins tend to aggregate, resulting in the formation of amyloid fibrils. These fibrils are neurotoxic and hard to degrade [99–102]. Although it is hard to verify whether the pathological proteins of ALS are prion-like in patients, previous studies have reported the pathological proteins of ALS, including TDP-43, SOD1, and FUS, showed prion-like properties in vitro [103–106].

As an infectious disease, the incubation of prion diseases ranges from years to decades. During the incubation period, prion proteins accumulate, eventually leading to clinical symptoms [107]. After the appearance of clinical symptoms, patients die within years. Kuru disease was first reported more than 50 years after the cessation of ritualistic mortuary feasts [108,109]. Prion diseases and ALS share many clinical manifestations and pathological mechanisms: 1) specific proteins are accumulating in these diseases. 2) Accumulating proteins can propagate and form amyloids, leading to neurodegeneration. 3) After exhibiting clinical symptoms, patients with prion disease and ALS die within up to 5 years. 4) The symptoms of ALS and prion disease originate in the central nervous system. Some researchers believe that the prion effect of the toxic protein (TDP-43 in ALS, tau in Alzheimer's disease, α-synuclein in Parkinson's disease) is the main cause of other neurodegeneration diseases, some even advocate that other neurodegeneration diseases should also be listed as prion diseases, this kind of disease is characterized by the formation of proteins inclusions in neurons that contribute to neurotoxicity [110]. In contrast to prion diseases, it is extremely difficult to determine the exact initial point of the disease process in ALS because the relationship between risk factors and ALS remains unclear, unlike the relationship between infection and prion disease. Although it has been verified the pathological proteins of ALS have prion-like effect in vitro, the relationship between the prion-like effect and ALS is still unclear. It should be cautious to interpret the results of these experiments.

3.1. The prion-like effect of TDP-43

The sequence of the TDP-43 C-terminal resembles some prion-like domains, including the glycine-rich and Q/N-rich regions, which are found in yeast such as Cyc8, Rnq1, and Sup35 [111–113]. These regions are highly disordered, have low complexities, and are extremely hydrophobic. In the past decade, several studies have shown the self-aggregation properties of TDP-43 in vitro [85, 114–116] and in vivo [117]. In 2022, Scialò et al. found that pathological TDP-43 can accelerate the aggregation of TDP-43 substrates by rt-quic, adding to the evidence of the prion-like effect of TDP-43 [104]. Rt-quic is a technology that can detect prion-like proteins in different forms of prion and prion-like diseases. The seeds (CSF and brain homogenates of patients with ALS) accelerated the aggregation of normal TDP-43 substrates, demonstrating that pathological TDP-43 is prion-like. Another study revealed the structure of pathologic TDP-43 filaments, demonstrating that the ordered filament core of TDP-43 spans aa282–aa360, forming a double-spiral-shaped fold [118]. Research showed that the prion-like propagation of TDP-43 filaments during the accumulation of aggregated TDP-43 is consistent with the double-spiral fold structure in the frontal and motor cortices in ALS patients [104,116–119]. Despite the evidence supporting the hypothesis that TDP-43 protein exhibits prion-like characteristics in ALS, the exact relationship between this function and the disease remains unclear. Researchers believe that it is not accurate to define the prion-like effect as pathological; prion-like proteins may play a physiological function, and the prion-like effect allows them to transmit their function to other proteins. In yeast, prion-like proteins are associated with beneficial heritable phenotypes [120–124]. More research needs to be conducted to determine if that is also the function of TDP-43 in ALS.

3.2. The prion-like effect of FUS

As mentioned before, FUS shares many functional and structural characteristics with TDP-43. The prion-like domain (PLD) of FUS protein ranges from aa1 to aa239. The existing PLD allows FUS to aggregate. Using solid-state nuclear magnetic resonance (NMR), Murray et al. found that amino acids 39–95 of FUS are the core region of FUS forming amyloid, which is in parallel with β-sheet structure of prion proteins [125]. In addition, other research found multiple putative phosphorylation sites in this region, suggesting that phosphorylation may be related to the transition of FUS. However, another study found that although the accumulation and aggregation of FUS are toxic in the yeast *Saccharomyces cerevisiae*, FUS and TDP-43 showed a high propensity for co-aggregation and that FUS aggregates in yeast are not composed of amyloid, which implies that the mechanism of FUS aggregation is different from that of other amyloids. In this study, the researchers also found that the pathological aggregating FUS did not show a strong fluorescence like most amyloid fibrils under thioflavin T dye, which is a fluorescent dye that can bind amyloid fibrils and is excited at 440 nm [126]. Another study found that isolated FUS can form oligomers spontaneously, and filamentous FUS can be observed in some patients with ALS. In contrast to TDP-43, FUS aggregation relies on the arginine (glycine) domain. In addition, some FUS mutations associated with ALS do not promote aggregation [127]. The aggregation of FUS exhibits toxicity in various cell and animal models [126,128,129], the FUS mutation associated with ALS has been proven to increase the tendency of FUS aggregating [130,131].

FUS is autosomal dominant, the experiments in vitro showed the aggregations of FUS are toxic. This information may enlighten us the prion-like effect of FUS plays an important role in ALS, but the function of FUS aggregation in ALS patients is still unclear now.

3.3. The prion-like effect of SOD1

SOD1 exists as a homodimer in vivo. The structure of SOD1 consists of eight stranded β-barrel in a Greek-key fold and two large loops. One of the loops has Cu/Zn-binding affinity [132]. Interestingly, the enzymatic activity of SOD1 is extremely stable, and SOD1 can survive for more than 3000 years after mummification [133]. Some post-translation modifications increase the aggregation-prone effects of SOD1 [134,135]. Mutation of the SOD1 gene also could decrease the stability of the SOD1 protein, enhancing the aggregation of SOD1. Researchers have summarized that diverse ALS-associated SOD1 mutations have a common characteristic: the hydrophobic surface, which is buried in the interior of normal SOD1, is more likely to be exposed [136,137]. This feature is consistent with other prion-like proteins. Researchers found that aggregated SOD1 could cross the membrane, enter the cytosol, and escape ubiquitination and degradation. The aggregating SOD1 becomes 'seeds', modifying the structure of the normal soluble protein, and spreading to adjacent cells via this prion-like pattern [50]. Chattopadhyay et al. found that misfolded SOD1 can convert the normal SOD1 to

misfolded form. The misfolded SOD1 tends to aggregate, which leads to the formation of SOD1 amyloid fibrils [138,139]. It also has been shown in other studies that aggregating SOD1 can penetrate cell membranes and promote the aggregation of other soluble proteins in cells [50,136,140]. A recent study reported that SOD1 seeding activity was observed in both sALS and fALS [105], which suggested that wild-type SOD1 also has the property of aggregating and propagation.

4. The strategy targeting prion-like protein

In limb-onset ALS patients, the onset starts at the unilateral upper limb or lower limb. The common initial symptoms of ALS are weakness and muscle tremors in the distal upper limb on one side. As the condition progresses, these symptoms gradually progress to the proximal upper limb on same side. In most patients, the symptoms continuously spread from the initial site to adjacent areas, which is consistent with a prion-like spreading pattern. Therefore, theoretically, if the seeding protein is eliminated, the progression of ALS can be slowed. By referencing recent research and summarizing our knowledge, we listed the prion-like protein targeting strategies that we believe have the potential for ALS therapy and divided them into six categories: ASO, antibody-based technology, peptides, protein chaperones, autophagy enhancement, and heteromultivalent compounds. A summary of the six potential methods and their associated references is presented in Table 1, and the mechanism of these methods is shown in Fig. 1.

4.1. Antisense oligonucleotide

ASOs and RNA interference (RNAi) have been studied mostly in ALS field. Theoretically, ASO and RNAi can only lower the prion-like protein levels by blocking the transcription and translation stages and cannot help eliminate the existing aggregating protein. However, they may help in the prevention of neurodegenerative diseases. The mechanisms of ASO and RNAi are similar: they interact with the target mRNA, block the translation procedure, and reduce the expression of target proteins, and some ASOs can also target DNA directly. ASO enables the precise modulation of gene expression without editing the DNA sequence, thus avoiding additional dangers and ethical issues. Moreover, RNA drugs can be administered by intrathecal delivery, avoiding additional immunogenicity or toxicity associated with other drug delivery systems such as adenoviruses. Tofersen, an ASO, inhibits the expression of SOD1 [141]. Tofersen lowers SOD1 levels by interacting with SOD1 mRNA, activating RNase H1, and blocking SOD1 mRNA translation. Although the results of the phase 3 trial of tofersen, VALOR (NCT02623699), were negative, the results showed that tofersen can significantly reduce SOD1 concentration in the CSF of patients, suggesting that tofersen is effective at the pathological level. The results of VALOR were published in September 2022 [142]. Tofersen has been granted FDA approval in April 2023. In C9orf72-ALS, a study found that an ASO against the G4C2 repeat encoding region can effectively suppress poly-GP level in a single patient harboring mutant C9ORF72 with the G4C2 repeat expansion, the ASO was also administered via intrathecal delivery [143]. Some studies have shown that the C9orf72-targeting ASOs could reduce G4C2 repeat-containing C9orf72 mRNA and clear intranuclear RNA foci in ALS-derived motor neurons, iPSC-induced neurons, and fibroblasts [144–146]. In animal experiments, an ASO targeting C9orf72 by intracerebroventricular (i.c.v.) delivery decreased G4C2 repeat-containing C9orf72 throughout the brain and spinal cord in a mouse model [147]. However, a clinical trial of the ASO BIIB078 (NCT03626012) against G4C2 repeat-containing C9orf72 showed a negative result; patients who received BIIB078 tended to show worse clinical outcomes. In another clinic trial WVE-004 (NCT04931862), poly-GP levels decreased in CSF after WVE-e004 treatment, implying WVE-e004 is effective at the pathological level. For FUS-ALS, a study showed that ASO ION363, which targets the 6th intron of FUS, can reduce both mutant and wild-type FUS by 20%–50 % in the brain and spinal cord in the FUS P525L mutant mouse model [13]. A phase 3 clinical trial (NCT04768972) of ION363 has been approved to assess its efficacy, safety, and pharmacology and will be completed in March 2024.

4.2. Antibody-based technology

Compared to ASO therapy, antibodies can directly interact with pathological proteins. Combined with protein elimination systems such as the ubiquitin-proteasome system, antibody drugs have the potential to reverse disease progression instead of slowing it. A previous study found that 3B12A monoclonal antibody (mAB) can eliminate misfolded TDP-43 in vivo and in vitro through proteasomal and autophagy-lysosome pathways. 3B12A can bind to aggregating TDP-43 specifically, wild type TDP-43 is almost unaffected. After adding chaperone-mediated autophagy (CMA)-related signals to 3B12A, which can induce Hsp70 transcription, the clearance of

Table 1
The methods and mechanisms against prion-like protein.

Method	Mechanism	Reference
Antisense Oligonucleotide	interact with target mRNA and block the translation procedure	[13,141–147]
Antibody	bind to target protein and mediating the degradation process	[23,148–151]
Peptide	bind to target protein thought struture mach and block the aggregation of misfolded protein	[20,21,152,153]
Protein Chaperone	bind to target protein and interrupt the aggregation of prion-like protein	[19,154–162,163,164]
Autophagy Enchancement	enchance the protein degradation through activation of lysosomes	[165–169,170,171,172–174,175,176]
Heteromultivalent Compound	inhibit the aggregation of misfolded protein and mediate its degradarion	[177]

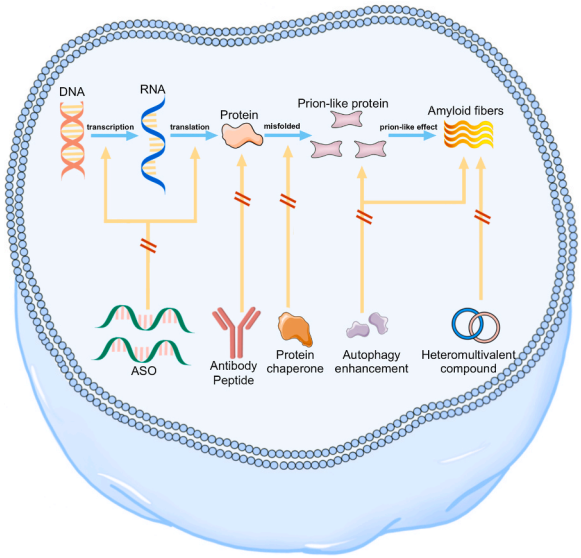


Fig. 1. The six methods and their mechanism in targeting prion-like proteins.
The mechanisms of the six methods against prion-like proteins are shown in this figure. ASOs block the transcription and translation process and inhibit the synthesis of pathological proteins. Antibodies can bind to pathological proteins and activate the degradation process. Peptides can bind to proteins and inhibit their aggregation. Protein chaperone can help proteins fold correctly, and prevent the production of abnormal proteins. Autophagy enhancement will enhance the protein degradation ability of cells, promoting the degradation of prion-like protein. Heteromultivalent compounds can interact with amyloid fiber, inhibit fiber formation, and promote its dissolution.

TDP-43 aggregation and cell viability were increased. All the results enlighten us the combination of a misfold proteins-specific intrabody and activation of proteasomal and autophagic pathways is a potential strategy for ALS therapy [178]. Another study showed a monoclonal antibody against the C-terminal domains of TDP-43 can inhibit the seeding effect of TDP-43 in vitro and reduce the phosphorylated and aggregated TDP-43 in the rNLS8 mouse model [179]. Treatment with SOD1 antibody can reduce the aggregation of misfolded SOD1, delay the onset of motor symptoms and improve survival in $SOD1^{G37R}$ or $SOD1^{G93A}$ transgenic mice model [180].

Clift et al. reported a new technology named TRIM-AWAY, which allows the elimination of any protein in cells without prior modifications, provided there is a robust antibody and antibody delivery system available. Protein degradation is achieved in three steps: 1) introduction of an antibody against the target protein; 2) recruitment of endogenous or exogenous/overexpressed TRIM21 to the antibody-bound target protein; and 3) proteasome-mediated degradation of the target protein, antibody, and TRIM21 complex [23]. We believe that this technology has great potential for neurodegenerative disease therapy. In ALS, aggregating proteins, such as TDP-43, accumulate and cannot be degraded in time by endogenous pathways. TRIM-AWAY can help cells degrade these protein inclusions, which could stop and even reverse disease progression at most underlying levels. The TRIM-AWAY system has been used to inhibit tau aggregation, and the results showed that when misfolded tau enters the cell, it can bind to the tau-specific antibody and be depleted via the TRIM21-induced ubiquitin-proteasome pathway [148]. In the C9orf72 G4C2 repeat expansion, the poly-GA based antibody interacted with TRIM21 and reduced the poly-GA level in the C9orf72 mouse model [149]. Some studies revealed that antibodies could inhibit the TDP-43 aggregation in vitro and degrade the toxic SOD1 oligomers in mice models [150,151]. Though antibody drugs show great potential for the treatment of ALS, the development of effective and safe specific antibodies is challenging. Moreover, to guarantee the efficiency of antibody transport, further research is required to develop appropriate antibody delivery systems.

4.3. Peptide

Peptide drugs have been extensively studied in recent years and peptides have been reported to have anti-amyloid effects. Two major peptide inhibitor design strategies are 1) based on the molecular recognition elements of amyloid self-assembly, and 2) based on the cross-amyloid interactions [20]. The protein-protein interaction relies on the structure of the contact interface. Peptides can selectively bind to a target protein through a structure-specific mechanism and interrupt the aggregation of prion-like proteins. A study found that the core region of Aβ fibrils, KLVFF, can bind full-length Aβ and prevent its assembly into amyloid fibrils [21]. Most of the amyloid-associated peptides are hydrophobic and prone to self-aggregation. As small molecules, they are often degraded quickly. To increase solubility and stability, peptides are modified by aminomethylation, cyclization, and D-peptides [20,152,153]. Peptides have the advantages of high specificity, low toxicity, low immunogenicity, good biocompatibility, easy availability, and low economic cost and are expected to become alternative drugs for ALS therapy [20]. However, the therapeutic effect of peptides on ALS is still a hypothesis, there is not enough evidence suggesting the therapeutic effect of peptides in ALS yet. Deeper understandings of

protein-protein interaction are needed to develop the peptide therapy for ALS.

4.4. Chaperone

The biological functions of proteins are determined by their 3-dimensional structure. Accurate protein folding relies on the complex networks of protein chaperones [19]. Hsp family is the most well-known protein chaperone family. The expression levels of Hsps are low in physical conditions and increase sharply under stress. Hsps protect cells through inhibition of cell death pathways or indirectly through activation of other pro-survival pathways, including inhibition of protein aggregation, promotion of misfolded protein degradation and maintenance of cytoskeleton [181].

Hsp104, a member of the Hsp family, plays an important role in the ability of yeast cells to survive severe heat shock. At high temperatures, accumulated aggregating proteins have been detected in mutant yeast cells lacking Hsp104 [154,155]. In the *Caenorhabditis elegans* Aβ misfolding model, the knockdown of Hsp90 and its co-chaperones leads to increased Aβ toxicity [156]. In yeast, the Hsp104 can resolubilize aggregated insoluble proteins [157]. Hsp70 recognizes target proteins in the early stages of folding via short leucine-rich hydrophobic residues. These hydrophobic regions are highly exposed in the early stages of protein translation and folding and can impair intra- and intermolecular interactions. If a target protein is misfolded, Hsp70 will induce the degradation of the misfolded protein [158–161]. This hydrophobic effect of prion-like proteins is important in neurodegenerative diseases, demonstrating the potential role of the Hsp70/Hsp90 machinery in regulating pathological protein aggregation in neurodegenerative diseases [159]. Previous studies have shown that Hsp40 and Hsp70 can bind to TDP-43 and regulate its aggregation in the nucleus of HeLa cells. Overexpression of Hsp70 and Hsp40 increased the clearance of insoluble TDP-43 in HEK cells. Moreover, a reduction in Hsp70 and Hsp40 levels is observed in the TDP-43 Q331K mutant transgenic ALS mouse model and patients with sporadic ALS [162]. Several Hsps, including Hsp27, Hsp40 and Hsp70, have been found in SOD1 inclusion in SOD1-mutant rodent model [131], the Hsps aggregates may reduce the biological available Hsps in cells.

The information above indicates that Hsps can protect neurons, and up-regulation of Hsps may be a potential approach for ALS therapy. Arimoclomol is a group of compounds, which can induce expression of HSP70 under cellular stress. In the past decade years, many studies found that Arimoclomol can enhance the expression of Hsp70 and protect neurons [162,182–186]. However, the phase III clinical trials of Arimoclomol in ALS patients, ORARIALS-01, failed to show a clinically meaningful therapeutic effect [187]. Although Arimoclomol failed, the possibility that Hsps have therapeutic potential for ALS cannot be precluded.

4.5. Autophagy enhancement

Autophagy is the process by which cells clear unnecessary intracellular components. When abnormal molecules accumulate and stress occurs, cells initiate autophagy to maintain homeostasis. Autophagy involves a double-membrane structure called the autophagosome. The autophagosome will envelop the cargo to be degraded, and fuse with the lysosome, forming the structure named 'autophagolysosome' to eliminate its content [165]. Autophagy is a two-tailed pathway. Moderate autophagy can help cells recycle the breakdown products of cytoplasmic organelles, proteins, and macromolecules. However excessive autophagy can lead to apoptosis [166,167]. Autophagy is vital for the clearance of aggregating proteins in several neurodegenerative diseases. For example, research revealed the accumulation of α-synuclein in Parkinson's disease, and Huntingtin's protein in Huntingtin's disease, are associated with increased autophagy activity [168,169]. Rapamycin is an mTOR pathway activator, which can activate autophagy. Previous research suggested promotion of autophagy by rapamycin leads to more clearance of α-synuclein and Huntingtin protein, decreasing cell toxicity [188–190]. Moreover, rapamycin-induced autophagy promotes the clearance of stress granules, which are believed to be associated with liquid-liquid phase separation in ALS [191,192]. Autophagy is activated in $SOD1^{G93A}$ mice model, treating the mice with bexarotene, a selective retinoid X receptor agonist, to enhance autophagy resulting in milder ALS pathology and delayed motor symptoms [170,171,193,194]. Activation of autophagy mediated by rapamycin showed a protected effect in $SOD1^{G93A}$ mice model [195,196]. Some studies have suggested that TDP-43 is involved in autophagosome and lysosome biogenesis during autophagy [172,173]. Additionally, TDP-43 aggregates can be eliminated by autophagy [174]. It also has been reported that the pharmacological induction of autophagy can slow down TDP-43 aggregation in a Drosophila model [95], similar results were observed in mice model [175,176,197]. Rapamycin also showed a protective effect for TPD-43 overexpressing mice model, which is mediated by activation of autophagy [176]. A phase II clinical trial of rapamycin, RAP-ALS, failed to test an effect on clinical measures due to the reduced number of samples that could be analyzed, but RAP-ALS demonstrated treatment with a low dose of rapamycin is safe for ALS patients [198].

Autophagy is a two-edged sword. Although autophagy is capable to enhance protein degradation, overactivated autophagy can cause damage to cells. A balance point between cell damage and autophagy activation must be determined before application of autophagy enhancement.

4.6. Heteromultivalent compound

Guo et al. designed a heteromultivalent platform by co-assembling cyclodextrin (CD) and calixarene (CA) amphiphiles, and two orthogonal non-covalent binding sites were distributed on the surface of the co-assembly. The heteromultivalency of this assembly allowed it to selectively bind to tyrosine-rich and lysine-rich peptides and this binding is highly stable. Heteromultivalency involves a ligand-receptor combination that is not molecular specific; more than one type of ligand interacts with more than one type of receptor simultaneously. Because of its additional binding adaptability and selectivity, the heteromultivalent molecule is more suitable than the

widely studied homomultivalent molecule for biological systems such as proteins and membranes that typically feature heterotopic contact surfaces. In this study, Guo found that this assembly can interact with Aβ42, inhibit formation, and promote dissolution of Aβ42 fibrils. The interaction between CD-CA assembly and Aβ42 is specific due to the Y and K sites and their self-optimizing matching distance with the CD and CA receptors in the co-assembly. Guo also found that the CD-CA assembly can not only inhibit the fibrils formation of Aβ42 but also dissolute the fibrils that have formed. The dissolution efficiency was measured using thioflavin T (ThT) dye, and the results showed that the dissolution efficiency was approximately 75 %. The TEM images showed that the number of fibrils was remarkably reduced. Moreover, the CA-CA assembly can better match biologically relevant heterotopic species with complex ligand sequences because the dynamic feature of self-assembly provides the platform with stronger self-adaptability. Thus, although this study only verifies the inhibition effect of CA-CA assembly for Aβ42, we believe this strategy is effective for other neurodegeneration diseases associated with pathological proteins, such as SOD1 and TDP-43 [177].

5. Discussion

By analyzing the clinical features of ALS and the prion-like effects of ALS-associated pathological proteins, we believe that prion-like protein-targeting strategies have a promising future in ALS therapy. The six strategies we listed in this paper target different stages of pathological protein production in ALS. ASO can block the transcription and translation of pathological proteins. Antibody therapy can combine with pathological proteins and promote the degradation process. Autophagy is important for the degradation of pathological proteins, but the process is non-selective. Chaperones, peptides, and heteromultivalent compounds can combined with pathological proteins and block, even reverse the aggregating process, which in turn inhibits the formation of amyloid.

ASO can only prevent the production but cannot promote degradation of the pathological proteins. In consideration of the prion-like effect of the pathological proteins, ASO may only block the pathological process, but cannot reverse it. Antibody-related technology, such as TRIM-AWAY, can promote the efficiency of protein degradation, but some technical aspects still need to be solved, such as how to transport the antibody to the target region. Autophagy is non-selective, and excessive autophagy may lead to apoptosis. Based on the current state of knowledge, we believe chaperones, peptides, and heteromultivalent compounds have the most potential to eliminate the pathological proteins of ALS, but more studies are required to confirm their effectiveness. Considering the strong heterogeneity of ALS, we believe that the final solution to ALS therapy is most likely an individualized cocktail therapy, including clearance of toxicity, blockage of pathological progress, and protection of neurons.

Ethics approval and consent to participate

Not applicable.

Consent for publication

No person's data was involved in this paper.

Data availability

No data was involved in this paper.

CRediT authorship contribution statement

Yang Wenzhi: Writing – review & editing, Writing – original draft. **Liu Xiangyi:** Writing – review & editing. **Fan Dongsheng:** Writing – review & editing, Conceptualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would also like to thank the Editors and Reviewers for their comprehensive and detailed comments which improve our manuscript significantly. Thanks Dr. Gao for the assistantance in improving the language quality of our manuscript.

References

[1] A. Al-Chalabi, O. Hardiman, The epidemiology of ALS: a conspiracy of genes, environment and time, Nat. Rev. Neurol. 9 (11) (2013) 617–628.
[2] R.H. Brown, A. Al-Chalabi, Amyotrophic lateral sclerosis, N. Engl. J. Med. 377 (2) (2017) 162–172.
[3] O. Hardiman, L.H. van den Berg, The beginning of genomic therapies for ALS, N. Engl. J. Med. 383 (2) (2020) 180–181.
[4] G.L. Nicolson, M.Y. Nasralla, J. Haier, J. Pomfret, High frequency of systemic mycoplasmal infections in Gulf War veterans and civilians with Amyotrophic Lateral Sclerosis (ALS), J. Clin. Neurosci. 9 (5) (2002) 525–529.

[5] F. Fecto, H.-X. Deng, T. Siddique, Discovering the connection between familial and sporadic amyotrophic lateral sclerosis: pathology trumps genetics, Future Neurol. 5 (5) (2010) 625–628.
[6] A. Doble, The pharmacology and mechanism of action of riluzole, Neurology 47 (6 Suppl 4) (1996) S233–S241.
[7] B. Oskarsson, T.F. Gendron, N.P. Staff, Amyotrophic lateral sclerosis: an update for 2018, Mayo Clin. Proc. 93 (11) (2018) 1617–1628.
[8] L. Xu, Y. Gao, M. Hu, Y. Dong, J. Xu, J. Zhang, et al., Edaravone dexborneol protects cerebral ischemia reperfusion injury through activating Nrf2/HO-1 signaling pathway in mice, Fundam. Clin. Pharmacol. 36 (5) (2022) 790–800.
[9] S. Amekura, M. Nakajima, M. Watanabe, M. Saitoh, S. Iida, Y. Yamamoto, et al., 4-Cl-edaravone and (E)-2-chloro-3-[(E)-phenyldiazenyl]-2-butenoic acid are the specific reaction products of edaravone with hypochlorite, J. Clin. Biochem. Nutr. 67 (2) (2020) 159–166.
[10] W. Pang, F. Hu, Cellular and physiological functions of C9ORF72 and implications for ALS/FTD, J. Neurochem. 157 (3) (2021) 334–350.
[11] S.J. Kaur, S.R. McKeown, S. Rashid, Mutant SOD1 mediated pathogenesis of amyotrophic lateral sclerosis, Gene 577 (2) (2016) 109–118.
[12] G. Kim, O. Gautier, E. Tassoni-Tsuchida, X.R. Ma, A.D. Gitler, ALS genetics: gains, losses, and implications for future therapies, Neuron 108 (5) (2020) 822–842.
[13] V.A. Korobeynikov, A.K. Lyashchenko, B. Blanco-Redondo, P. Jafar-Nejad, N.A. Shneider, Antisense oligonucleotide silencing of FUS expression as a therapeutic approach in amyotrophic lateral sclerosis, Nat. Med. 28 (1) (2022) 104–116.
[14] J.S. Eisen, J.C. Smith, Controlling morpholino experiments: don't stop making antisense, Development 135 (10) (2008) 1735–1743.
[15] C.F. Bennett, Therapeutic antisense oligonucleotides are coming of age, Annu. Rev. Med. 70 (2019) 307–321.
[16] S.T. Crooke, B.F. Baker, R.M. Crooke, X.H. Liang, Antisense technology: an overview and prospectus, Nat. Rev. Drug Discov. 20 (6) (2021) 427–453.
[17] F.U. Hartl, A. Bracher, M. Hayer-Hartl, Molecular chaperones in protein folding and proteostasis, Nature 475 (7356) (2011) 324–332.
[18] O. Genest, S. Wickner, S.M. Doyle, Hsp90 and Hsp70 chaperones: collaborators in protein remodeling, J. Biol. Chem. 294 (6) (2019) 2109–2120.
[19] Y.E. Kim, M.S. Hipp, A. Bracher, M. Hayer-Hartl, F.U. Hartl, Molecular chaperone functions in protein folding and proteostasis, Annu. Rev. Biochem. 82 (2013) 323–355.
[20] V. Armiento, A. Spanopoulou, A. Kapurniotu, Peptide-based molecular strategies to interfere with protein misfolding, aggregation, and cell degeneration, Angew Chem. Int. Ed. Engl. 59 (9) (2020) 3372–3384.
[21] L.O. Tjernberg, J. Naslund, F. Lindqvist, J. Johansson, A.R. Karlstrom, J. Thyberg, et al., Arrest of beta-amyloid fibril formation by a pentapeptide ligand, J. Biol. Chem. 271 (15) (1996) 8545–8548.
[22] D. Clift, W.A. McEwan, L.I. Labzin, V. Konieczny, B. Mogessie, L.C. James, et al., A method for the acute and rapid degradation of endogenous proteins, Cell 171 (7) (2017) 1692–1706 e18.
[23] D. Clift, C. So, W.A. McEwan, L.C. James, M. Schuh, Acute and rapid degradation of endogenous proteins by Trim-Away, Nat. Protoc. 13 (10) (2018) 2149–2175.
[24] J. Zeng, A.F. Santos, A.S. Mukadam, M. Osswald, D.A. Jacques, C.F. Dickson, et al., Target-induced clustering activates Trim-Away of pathogens and proteins, Nat. Struct. Mol. Biol. 28 (3) (2021) 278–289.
[25] Y. Hayashi, K. Homma, H. Ichijo, SOD1 in neurotoxicity and its controversial roles in SOD1 mutation-negative ALS, Adv Biol Regul 60 (2016) 95–104.
[26] L.Q. Wang, Y. Ma, H.Y. Yuan, K. Zhao, M.Y. Zhang, Q. Wang, et al., Cryo-EM structure of an amyloid fibril formed by full-length human SOD1 reveals its conformational conversion, Nat. Commun. 13 (1) (2022) 3491.
[27] R.J. Ferrante, S.E. Browne, L.A. Shinobu, A.C. Bowling, M.J. Baik, U. MacGarvey, et al., Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis, J. Neurochem. 69 (5) (1997) 2064–2074.
[28] M.M. Harraz, J.J. Marden, W. Zhou, Y. Zhang, A. Williams, V.S. Sharov, et al., SOD1 mutations disrupt redox-sensitive Rac regulation of NADPH oxidase in a familial ALS model, J. Clin. Invest. 118 (2) (2008) 659–670.
[29] M. Mhatre, R.A. Floyd, K. Hensley, Oxidative stress and neuroinflammation in Alzheimer's disease and amyotrophic lateral sclerosis: common links and potential therapeutic targets, J Alzheimers Dis 6 (2) (2004) 147–157.
[30] T. Nagata, H. Ilieva, T. Murakami, M. Shiote, H. Narai, Y. Ohta, et al., Increased ER stress during motor neuron degeneration in a transgenic mouse model of amyotrophic lateral sclerosis, Neurol. Res. 29 (8) (2007) 767–771.
[31] S. Saxena, E. Cabuy, P. Caroni, A role for motoneuron subtype-selective ER stress in disease manifestations of FALS mice, Nat. Neurosci. 12 (5) (2009) 627–636.
[32] S. Guareschi, E. Cova, C. Cereda, M. Ceroni, E. Donetti, D.A. Bosco, et al., An over-oxidized form of superoxide dismutase found in sporadic amyotrophic lateral sclerosis with bulbar onset shares a toxic mechanism with mutant SOD1, Proc. Natl. Acad. Sci. U. S. A. 109 (13) (2012) 5074–5079.
[33] A. Israelson, N. Arbel, S. Da Cruz, H. Ilieva, K. Yamanaka, V. Shoshan-Barmatz, et al., Misfolded mutant SOD1 directly inhibits VDAC1 conductance in a mouse model of inherited ALS, Neuron 67 (4) (2010) 575–587.
[34] J. Liu, C. Lillo, P.A. Jonsson, C. Vande Velde, C.M. Ward, T.M. Miller, et al., Toxicity of familial ALS-linked SOD1 mutants from selective recruitment to spinal mitochondria, Neuron 43 (1) (2004) 5–17.
[35] S. Pun, A.F. Santos, S. Saxena, L. Xu, P. Caroni, Selective vulnerability and pruning of phasic motoneuron axons in motoneuron disease alleviated by CNTF, Nat. Neurosci. 9 (3) (2006) 408–419.
[36] D.A. Bosco, G. Morfini, N.M. Karabacak, Y. Song, F. Gros-Louis, P. Pasinelli, et al., Wild-type and mutant SOD1 share an aberrant conformation and a common pathogenic pathway in ALS, Nat. Neurosci. 13 (11) (2010) 1396–1403.
[37] S. Boillee, K. Yamanaka, C.S. Lobsiger, N.G. Copeland, N.A. Jenkins, G. Kassiotis, et al., Onset and progression in inherited ALS determined by motor neurons and microglia, Science 312 (5778) (2006) 1389–1392.
[38] A.M. Haidet-Phillips, M.E. Hester, C.J. Miranda, K. Meyer, L. Braun, A. Frakes, et al., Astrocytes from familial and sporadic ALS patients are toxic to motor neurons, Nat. Biotechnol. 29 (9) (2011) 824–828.
[39] S.H. Kang, Y. Li, M. Fukaya, I. Lorenzini, D.W. Cleveland, L.W. Ostrow, et al., Degeneration and impaired regeneration of gray matter oligodendrocytes in amyotrophic lateral sclerosis, Nat. Neurosci. 16 (5) (2013) 571–579.
[40] T. Philips, A. Bento-Abreu, A. Nonneman, W. Haeck, K. Staats, V. Geelen, et al., Oligodendrocyte dysfunction in the pathogenesis of amyotrophic lateral sclerosis, Brain 136 (Pt 2) (2013) 471–482.
[41] J.I. Ayers, S. Fromholt, M. Koch, A. DeBosier, B. McMahon, G. Xu, et al., Experimental transmissibility of mutant SOD1 motor neuron disease, Acta Neuropathol. 128 (6) (2014) 791–803.
[42] M.E. Cicardi, L. Marrone, M. Azzouz, D. Trotti, Proteostatic imbalance and protein spreading in amyotrophic lateral sclerosis, EMBO J. 40 (10) (2021) e106389.
[43] R. Chia, M.H. Tattum, S. Jones, J. Collinge, E.M. Fisher, G.S. Jackson, Superoxide dismutase 1 and tgSOD1 mouse spinal cord seed fibrils, suggesting a propagative cell death mechanism in amyotrophic lateral sclerosis, PLoS One 5 (5) (2010) e10627.
[44] N. Shvil, V. Banerjee, G. Zoltsman, T. Shani, J. Kahn, S. Abu-Hamad, et al., MIF inhibits the formation and toxicity of misfolded SOD1 amyloid aggregates: implications for familial ALS, Cell Death Dis. 9 (2) (2018) 107.
[45] F. Arnesano, L. Banci, I. Bertini, M. Martinelli, Y. Furukawa, T.V. O'Halloran, The unusually stable quaternary structure of human Cu,Zn-superoxide dismutase 1 is controlled by both metal occupancy and disulfide status, J. Biol. Chem. 279 (46) (2004) 47998–48003.
[46] M.A. Hough, J.G. Grossmann, S.V. Antonyuk, R.W. Strange, P.A. Doucette, J.A. Rodriguez, et al., Dimer destabilization in superoxide dismutase may result in disease-causing properties: structures of motor neuron disease mutants, Proc Natl Acad Sci U S A. 101 (16) (2004) 5976–5981.
[47] M. Urushitani, A. Sik, T. Sakurai, N. Nukina, R. Takahashi, J.P. Julien, Chromogranin-mediated secretion of mutant superoxide dismutase proteins linked to amyotrophic lateral sclerosis, Nat. Neurosci. 9 (1) (2006) 108–118.
[48] A. Hosomi, C. Okachi, Y. Fujiwara, Human SOD1 is secreted via a conventional secretion pathway in Saccharomyces cerevisiae, Biochem. Biophys. Res. Commun. 666 (2023) 101–106.

[49] E. Tokuda, Y.I. Takei, S. Ohara, N. Fujiwara, I. Hozumi, Y. Furukawa, Wild-type Cu/Zn-superoxide dismutase is misfolded in cerebrospinal fluid of sporadic amyotrophic lateral sclerosis, Mol. Neurodegener. 14 (1) (2019) 42.
[50] C. Munch, J. O'Brien, A. Bertolotti, Prion-like propagation of mutant superoxide dismutase-1 misfolding in neuronal cells, Proc. Natl. Acad. Sci. U. S. A. 108 (9) (2011) 3548–3553.
[51] M.E. Gurney, H. Pu, A.Y. Chiu, M.C. Dal Canto, C.Y. Polchow, D.D. Alexander, et al., Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation, Science 264 (5166) (1994) 1772–1775.
[52] L.I. Bruijn, M.K. Houseweart, S. Kato, K.L. Anderson, S.D. Anderson, E. Ohama, et al., Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1, Science 281 (5384) (1998) 1851–1854.
[53] P.C. Wong, D. Waggoner, J.R. Subramaniam, L. Tessarollo, T.B. Bartnikas, V.C. Culotta, et al., Copper chaperone for superoxide dismutase is essential to activate mammalian Cu/Zn superoxide dismutase, Proc Natl Acad Sci U S A. 97 (6) (2000) 2886–2891.
[54] V.C. Culotta, L.W. Klomp, J. Strain, R.L. Casareno, B. Krems, J.D. Gitlin, The copper chaperone for superoxide dismutase, J. Biol. Chem. 272 (38) (1997) 23469–23472.
[55] E. Braems, B. Swinnen, L. Van Den Bosch, C9orf72 loss-of-function: a trivial, stand-alone or additive mechanism in C9 ALS/FTD? Acta Neuropathol. 140 (5) (2020) 625–643.
[56] J. Smeyers, E.G. Banchi, M. Latouche, C9ORF72: what it is, what it does, and why it matters, Front. Cell. Neurosci. 15 (2021).
[57] R. Balendra, A.M. Isaacs, C9orf72-mediated ALS and FTD: multiple pathways to disease, Nat. Rev. Neurol. 14 (9) (2018) 544–558.
[58] S. Xiao, L. MacNair, P. McGoldrick, P.M. McKeever, J.R. McLean, M. Zhang, et al., Isoform-specific antibodies reveal distinct subcellular localizations of C9orf72 in amyotrophic lateral sclerosis, Ann. Neurol. 78 (4) (2015) 568–583.
[59] V.V. Belzil, P.O. Bauer, M. Prudencio, T.F. Gendron, C.T. Stetler, I.K. Yan, et al., Reduced C9orf72 gene expression in c9FTD/ALS is caused by histone trimethylation, an epigenetic event detectable in blood, Acta Neuropathol. 126 (6) (2013) 895–905.
[60] M. DeJesus-Hernandez, I.R. Mackenzie, B.F. Boeve, A.L. Boxer, M. Baker, N.J. Rutherford, et al., Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS, Neuron 72 (2) (2011) 245–256.
[61] I. Gijselinck, T. Van Langenhove, J. van der Zee, K. Sleegers, S. Philtjens, G. Kleinberger, et al., A C9orf72 promoter repeat expansion in a Flanders-Belgian cohort with disorders of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum: a gene identification study, Lancet Neurol. 11 (1) (2012) 54–65.
[62] P. Fratta, M. Poulter, T. Lashley, J.D. Rohrer, J.M. Polke, J. Beck, et al., Homozygosity for the C9orf72 GGGGCC repeat expansion in frontotemporal dementia, Acta Neuropathol. 126 (3) (2013) 401–409.
[63] J.L. Jackson, N.A. Finch, M.C. Baker, J.M. Kachergus, M. DeJesus-Hernandez, K. Pereira, et al., Elevated methylation levels, reduced expression levels, and frequent contractions in a clinical cohort of C9orf72 expansion carriers, Mol. Neurodegener. 15 (1) (2020) 7.
[64] A.J. Waite, D. Baumer, S. East, J. Neal, H.R. Morris, O. Ansorge, et al., Reduced C9orf72 protein levels in frontal cortex of amyotrophic lateral sclerosis and frontotemporal degeneration brain with the C9ORF72 hexanucleotide repeat expansion, Neurobiol. Aging 35 (7) (2014) 1779 e5–e13.
[65] P. Frick, C. Sellier, I.R.A. Mackenzie, C.Y. Cheng, J. Tahraoui-Bories, C. Martinat, et al., Novel antibodies reveal presynaptic localization of C9orf72 protein and reduced protein levels in C9orf72 mutation carriers, Acta Neuropathol Commun 6 (1) (2018) 72.
[66] B.D. Freibaum, J.P. Taylor, The role of dipeptide repeats in C9ORF72-related ALS-FTD, Front. Mol. Neurosci. 10 (2017) 35.
[67] P.H. Smeele, G. Cesare, T. Vaccari, ALS' perfect storm: C9orf72-associated toxic dipeptide repeats as potential multipotent disruptors of protein homeostasis, Cells 13 (2) (2024).
[68] X. Wen, W. Tan, T. Westergard, K. Krishnamurthy, S.S. Markandaiah, Y. Shi, et al., Antisense proline-arginine RAN dipeptides linked to C9ORF72-ALS/FTD form toxic nuclear aggregates that initiate in vitro and in vivo neuronal death, Neuron 84 (6) (2014) 1213–1225.
[69] M.H. Schludi, S. May, F.A. Grasser, K. Rentzsch, E. Kremmer, C. Kupper, et al., Distribution of dipeptide repeat proteins in cellular models and C9orf72 mutation cases suggests link to transcriptional silencing, Acta Neuropathol. 130 (4) (2015) 537–555.
[70] M. Yamakawa, D. Ito, T. Honda, K. Kubo, M. Noda, K. Nakajima, et al., Characterization of the dipeptide repeat protein in the molecular pathogenesis of c9FTD/ALS, Hum. Mol. Genet. 24 (6) (2015) 1630–1645.
[71] S. Ryan, S. Rollinson, E. Hobbs, S. Pickering-Brown, C9orf72 dipeptides disrupt the nucleocytoplasmic transport machinery and cause TDP-43 mislocalisation to the cytoplasm, Sci. Rep. 12 (1) (2022) 4799.
[72] K. Zhang, J.G. Daigle, K.M. Cunningham, A.N. Coyne, K. Ruan, J.C. Grima, et al., Stress granule assembly disrupts nucleocytoplasmic transport, Cell 173 (4) (2018) 958–971 e17.
[73] Y.J. Zhang, T.F. Gendron, J.C. Grima, H. Sasaguri, K. Jansen-West, Y.F. Xu, et al., C9ORF72 poly(GA) aggregates sequester and impair HR23 and nucleocytoplasmic transport proteins, Nat. Neurosci. 19 (5) (2016) 668–677.
[74] K.M. Cunningham, K. Maulding, K. Ruan, M. Senturk, J.C. Grima, H. Sung, et al., TFEB/Mitf links impaired nuclear import to autophagolysosomal dysfunction in C9-ALS, Elife 9 (2020).
[75] M.A. Farg, V. Sundaramoorthy, J.M. Sultana, S. Yang, R.A. Atkinson, V. Levina, et al., C9ORF72, implicated in amytrophic lateral sclerosis and frontotemporal dementia, regulates endosomal trafficking, Hum. Mol. Genet. 23 (13) (2014) 3579–3595.
[76] M.A. Farg, V. Sundaramoorthy, J.M. Sultana, S. Yang, R.A.K. Atkinson, V. Levina, et al., C9ORF72, implicated in amytrophic lateral sclerosis and frontotemporal dementia, regulates endosomal trafficking, Hum. Mol. Genet. 26 (20) (2017) 4093–4094.
[77] Y. Aoki, R. Manzano, Y. Lee, R. Dafinca, M. Aoki, A.G.L. Douglas, et al., C9orf72 and RAB7L1 regulate vesicle trafficking in amyotrophic lateral sclerosis and frontotemporal dementia, Brain 140 (4) (2017) 887–897.
[78] T.J. Kwiatkowski Jr., D.A. Bosco, A.L. Leclerc, E. Tamrazian, C.R. Vanderburg, C. Russ, et al., Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis, Science 323 (5918) (2009) 1205–1208.
[79] C. Vance, B. Rogelj, T. Hortobagyi, K.J. De Vos, A.L. Nishimura, J. Sreedharan, et al., Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6, Science 323 (5918) (2009) 1208–1211.
[80] I.R. Mackenzie, E.H. Bigio, P.G. Ince, F. Geser, M. Neumann, N.J. Cairns, et al., Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations, Ann. Neurol. 61 (5) (2007) 427–434.
[81] A. Prasad, V. Bharathi, V. Sivalingam, A. Girdhar, B.K. Patel, Molecular mechanisms of TDP-43 misfolding and pathology in amyotrophic lateral sclerosis, Front. Mol. Neurosci. 12 (2019) 25.
[82] W. Guo, Y. Chen, X. Zhou, A. Kar, P. Ray, X. Chen, et al., An ALS-associated mutation affecting TDP-43 enhances protein aggregation, fibril formation and neurotoxicity, Nat. Struct. Mol. Biol. 18 (7) (2011) 822–830.
[83] R. Mutihac, J. Alegre-Abarrategui, D. Gordon, L. Farrimond, M. Yamasaki-Mann, K. Talbot, et al., TARDBP pathogenic mutations increase cytoplasmic translocation of TDP-43 and cause reduction of endoplasmic reticulum Ca(2)(+) signaling in motor neurons, Neurobiol. Dis. 75 (2015) 64–77.
[84] S. Mitsuzawa, T. Akiyama, A. Nishiyama, N. Suzuki, M. Kato, H. Warita, et al., TARDBP p.G376D mutation, found in rapid progressive familial ALS, induces mislocalization of TDP-43, eNeurologicalSci 11 (2018) 20–22.
[85] B.S. Johnson, D. Snead, J.J. Lee, J.M. McCaffery, J. Shorter, A.D. Gitler, TDP-43 is intrinsically aggregation-prone, and amyotrophic lateral sclerosis-linked mutations accelerate aggregation and increase toxicity, J. Biol. Chem. 284 (30) (2009) 20329–20339.
[86] A.K. Chen, R.Y. Lin, E.Z. Hsieh, P.H. Tu, R.P. Chen, T.Y. Liao, et al., Induction of amyloid fibrils by the C-terminal fragments of TDP-43 in amyotrophic lateral sclerosis, J. Am. Chem. Soc. 132 (4) (2010) 1186–1187.
[87] L.L. Jiang, J. Zhao, X.F. Yin, W.T. He, H. Yang, M.X. Che, et al., Two mutations G335D and Q343R within the amyloidogenic core region of TDP-43 influence its aggregation and inclusion formation, Sci. Rep. 6 (2016) 23928.
[88] M. Polymenidou, C. Lagier-Tourenne, K.R. Hutt, S.C. Huelga, J. Moran, T.Y. Liang, et al., Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43, Nat. Neurosci. 14 (4) (2011) 459–468.

[89] M. Neumann, E.B. Lee, I.R. Mackenzie, Frontotemporal lobar degeneration TDP-43-immunoreactive pathological subtypes: clinical and mechanistic significance, Adv. Exp. Med. Biol. 1281 (2021) 201–217.
[90] I.R. Mackenzie, M. Neumann, Reappraisal of TDP-43 pathology in FTLD-U subtypes, Acta Neuropathol. 134 (1) (2017) 79–96.
[91] I.R. Mackenzie, M. Neumann, A. Baborie, D.M. Sampathu, D. Du Plessis, E. Jaros, et al., A harmonized classification system for FTLD-TDP pathology, Acta Neuropathol. 122 (1) (2011) 111–113.
[92] S. Porta, Y. Xu, T. Lehr, B. Zhang, E. Meymand, M. Olufemi, et al., Distinct brain-derived TDP-43 strains from FTLD-TDP subtypes induce diverse morphological TDP-43 aggregates and spreading patterns in vitro and in vivo, Neuropathol. Appl. Neurobiol. 47 (7) (2021) 1033–1049.
[93] S.H. Ignatius, F. Wu, D. Harrich, L.F. Garciamartinez, R.B. Gaynor, Cloning and characterization of a novel cellular protein, tdp-43, that binds to human-immunodeficiency-virus type-1 tar DNA-sequence motifs, J. Virol. 69 (6) (1995) 3584–3596.
[94] C.F. Sephton, S.K. Good, S. Atkin, C.M. Dewey, P. Mayer, J. Herz, et al., TDP-43 is a developmentally regulated protein essential for early embryonic development, J. Biol. Chem. 285 (9) (2010) 6826–6834.
[95] L. Cragnaz, G. Spinelli, L. De Conti, E.A. Bureau, J. Brownlees, F. Feiguin, et al., Thioridazine reverts the phenotype in cellular and Drosophila models of amyotrophic lateral sclerosis by enhancing TDP-43 aggregate clearance, Neurobiol. Dis. 160 (2021) 105515.
[96] E. Buratti, F.E. Baralle, Characterization and functional implications of the RNA binding properties of nuclear factor TDP-43, a novel splicing regulator of CFTR exon 9, J. Biol. Chem. 276 (39) (2001) 36337–36343.
[97] C.F. Sephton, B. Cenik, B.K. Cenik, J. Herz, G. Yu, TDP-43 in central nervous system development and function: clues to TDP-43-associated neurodegeneration, Biol. Chem. 393 (7) (2012) 589–594.
[98] B. Portz, B.L. Lee, J. Shorter, FUS and TDP-43 phases in health and disease, Trends Biochem. Sci. 46 (7) (2021) 550–563.
[99] T. Kawasaki, K. Tsukiyama, P.H. Nguyen, Disassembly of amyloid fibril with infrared free electron laser, Int. J. Mol. Sci. 24 (4) (2023).
[100] A. Lorenzo, B.A. Yankner, Amyloid fibril toxicity in Alzheimer's disease and diabetes, Ann. N. Y. Acad. Sci. 777 (1996) 89–95.
[101] K.E. Marshall, R. Marchante, W.F. Xue, L.C. Serpell, The relationship between amyloid structure and cytotoxicity, Prion 8 (2) (2014) 192–196.
[102] C.J. Sigurdson, J.C. Bartz, M. Glatzel, Cellular and molecular mechanisms of prion disease, Annu. Rev. Pathol. 14 (2019) 497–516.
[103] S.A. Polido, C. Stuani, A. Voigt, P. Banik, J. Kamps, V. Bader, et al., Cross-seeding by prion protein inactivates TDP-43, Brain 147 (1) (2024) 240–254.
[104] C. Scialo, T.H. Tran, G. Salzano, G. Novi, C. Caponnetto, A. Chio, et al., TDP-43 real-time quaking induced conversion reaction optimization and detection of seeding activity in CSF of amyotrophic lateral sclerosis and frontotemporal dementia patients, Brain Commun 2 (2) (2020) fcaa142.
[105] J.K. Mielke, M. Klingeborn, E.P. Schultz, E.L. Markham, E.D. Reese, P. Alam, et al., Seeding activity of human superoxide dismutase 1 aggregates in familial and sporadic amyotrophic lateral sclerosis postmortem neural tissues by real-time quaking-induced conversion, Acta Neuropathol. 147 (1) (2024) 100.
[106] S.N. Rhoads, Z.T. Monahan, D.S. Yee, F.P. Shewmaker, The role of post-translational modifications on prion-like aggregation and liquid-phase separation of FUS, Int. J. Mol. Sci. 19 (3) (2018).
[107] M.D. Geschwind, Prion diseases, Continuum 21 (6 Neuroinfectious Disease) (2015) 1612–1638.
[108] K.J. Baldwin, C.M. Correll, Prion disease, Semin. Neurol. 39 (4) (2019) 428–439.
[109] R.C.C. Mercer, D.A. Harris, Mechanisms of prion-induced toxicity, Cell Tissue Res 392 (1) (2022) 81–96.
[110] L.I. Grad, S.M. Fernando, N.R. Cashman, From molecule to molecule and cell to cell: prion-like mechanisms in amyotrophic lateral sclerosis, Neurobiol. Dis. 77 (2015) 257–265.
[111] S.W. Liebman, Y.O. Chernoff, Prions in yeast, Genetics 191 (4) (2012) 1041–1072.
[112] O.D. King, A.D. Gitler, J. Shorter, The tip of the iceberg: RNA-binding proteins with prion-like domains in neurodegenerative disease, Brain Res. 1462 (2012) 61–80.
[113] B.K. Patel, J. Gavin-Smyth, S.W. Liebman, The yeast global transcriptional co-repressor protein Cyc8 can propagate as a prion, Nat. Cell Biol. 11 (3) (2009) 344–349.
[114] Y. Furukawa, K. Kaneko, S. Watanabe, K. Yamanaka, N. Nukina, A seeding reaction recapitulates intracellular formation of Sarkosyl-insoluble transactivation response element (TAR) DNA-binding protein-43 inclusions, J. Biol. Chem. 286 (21) (2011) 18664–18672.
[115] L.L. Jiang, M.X. Che, J. Zhao, C.J. Zhou, M.Y. Xie, H.Y. Li, et al., Structural transformation of the amyloidogenic core region of TDP-43 protein initiates its aggregation and cytoplasmic inclusion, J. Biol. Chem. 288 (27) (2013) 19614–19624.
[116] T. Nonaka, M. Masuda-Suzukake, T. Arai, Y. Hasegawa, H. Akatsu, T. Obi, et al., Prion-like properties of pathological TDP-43 aggregates from diseased brains, Cell Rep. 4 (1) (2013) 124–134.
[117] S. Porta, Y. Xu, C.R. Restrepo, L.K. Kwong, B. Zhang, H.J. Brown, et al., Patient-derived frontotemporal lobar degeneration brain extracts induce formation and spreading of TDP-43 pathology in vivo, Nat. Commun. 9 (1) (2018) 4220.
[118] D. Arseni, M. Hasegawa, A.G. Murzin, F. Kametani, M. Arai, M. Yoshida, et al., Structure of pathological TDP-43 filaments from ALS with FTLD, Nature 601 (7891) (2022) 139–143.
[119] J. Brettschneider, K. Del Tredici, J.B. Toledo, J.L. Robinson, D.J. Irwin, M. Grossman, et al., Stages of pTDP-43 pathology in amyotrophic lateral sclerosis, Ann. Neurol. 74 (1) (2013) 20–38.
[120] J. Shorter, S. Lindquist, Prions as adaptive conduits of memory and inheritance, Nat. Rev. Genet. 6 (6) (2005) 435–450.
[121] G.A. Newby, S. Lindquist, Blessings in disguise: biological benefits of prion-like mechanisms, Trends Cell Biol. 23 (6) (2013) 251–259.
[122] A.K. Chakravarty, D.F. Jarosz, More than just a phase: prions at the crossroads of epigenetic inheritance and evolutionary change, J. Mol. Biol. 430 (23) (2018) 4607–4618.
[123] D.F. Jarosz, J.C.S. Brown, G.A. Walker, M.S. Datta, W.L. Ung, A.K. Lancaster, et al., Cross-kingdom chemical communication drives a heritable, mutually beneficial prion-based transformation of metabolism, Cell 158 (5) (2014) 1083–1093.
[124] Z.H. Harvey, A.K. Chakravarty, R.A. Futia, D.F. Jarosz, A prion epigenetic switch establishes an active chromatin state, Cell. 180 (5) (2020) 928–940 e14.
[125] D.T. Murray, M. Kato, Y. Lin, K.R. Thurber, I. Hung, S.L. McKnight, et al., Structure of FUS protein fibrils and its relevance to self-assembly and phase separation of low-complexity domains, Cell 171 (3) (2017) 615–627 e16.
[126] D. Kryndushkin, R.B. Wickner, F. Shewmaker, FUS/TLS forms cytoplasmic aggregates, inhibits cell growth and interacts with TDP-43 in a yeast model of amyotrophic lateral sclerosis, Protein Cell 2 (3) (2011) 223–236.
[127] Z. Sun, Z. Diaz, X. Fang, M.P. Hart, A. Chesi, J. Shorter, et al., Molecular determinants and genetic modifiers of aggregation and toxicity for the ALS disease protein FUS/TLS, PLoS Biol. 9 (4) (2011) e1000614.
[128] T. Murakami, S.P. Yang, L. Xie, T. Kawano, D. Fu, A. Mukai, et al., ALS mutations in FUS cause neuronal dysfunction and death in Caenorhabditis elegans by a dominant gain-of-function mechanism, Hum. Mol. Genet. 21 (1) (2012) 1–9.
[129] J. Scekic-Zahirovic, O. Sendscheid, H. El Oussini, M. Jambeau, Y. Sun, S. Mersmann, et al., Toxic gain of function from mutant FUS protein is crucial to trigger cell autonomous motor neuron loss, EMBO J. 35 (10) (2016) 1077–1097.
[130] A. Patel, H.O. Lee, L. Jawerth, S. Maharana, M. Jahnel, M.Y. Hein, et al., A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation, Cell 162 (5) (2015) 1066–1077.
[131] T. Nomura, S. Watanabe, K. Kaneko, K. Yamanaka, N. Nukina, Y. Furukawa, Intranuclear aggregation of mutant FUS/TLS as a molecular pathomechanism of amyotrophic lateral sclerosis, J. Biol. Chem. 289 (2) (2014) 1192–1202.
[132] J.S. Valentine, P.A. Doucette, S. Zittin Potter, Copper-zinc superoxide dismutase and amyotrophic lateral sclerosis, Annu. Rev. Biochem. 74 (2005) 563–593.
[133] U. Weser, R. Miesel, H.J. Hartmann, Mummified enzymes, Nature 341 (6244) (1989) 696.
[134] A. Nordlund, M. Oliveberg, SOD1-associated ALS: a promising system for elucidating the origin of protein-misfolding disease, HFSP J. 2 (6) (2008) 354–364.
[135] P.H. Ren, J.E. Lauckner, I. Kachirskaia, J.E. Heuser, R. Melki, R.R. Kopito, Cytoplasmic penetration and persistent infection of mammalian cells by polyglutamine aggregates, Nat. Cell Biol. 11 (2) (2009) 219–225.
[136] C. Sibilla, A. Bertolotti, Prion properties of SOD1 in amyotrophic lateral sclerosis and potential therapy, Cold Spring Harbor Perspect. Biol. 9 (10) (2017).

[137] C. Munch, A. Bertolotti, Exposure of hydrophobic surfaces initiates aggregation of diverse ALS-causing superoxide dismutase-1 mutants, J. Mol. Biol. 399 (3) (2010) 512–525.
[138] M. Chattopadhyay, A. Durazo, S.H. Sohn, C.D. Strong, E.B. Gralla, J.P. Whitelegge, et al., Initiation and elongation in fibrillation of ALS-linked superoxide dismutase, Proc Natl Acad Sci U S A. 105 (48) (2008) 18663–18668.
[139] D. Eisenberg, M. Jucker, The amyloid state of proteins in human diseases, Cell 148 (6) (2012) 1188–1203.
[140] C. Munch, A. Bertolotti, Self-propagation and transmission of misfolded mutant SOD1: prion or prion-like phenomenon? Cell Cycle 10 (11) (2011) 1711.
[141] A. McCampbell, T. Cole, A.J. Wegener, G.S. Tomassy, A. Setnicka, B.J. Farley, et al., Antisense oligonucleotides extend survival and reverse decrement in muscle response in ALS models, J. Clin. Invest. 128 (8) (2018) 3558–3567.
[142] T. Miller, M. Cudkowicz, P.J. Shaw, P.M. Andersen, N. Atassi, R.C. Bucelli, et al., Phase 1-2 trial of antisense oligonucleotide tofersen for SOD1 ALS, N. Engl. J. Med. 383 (2) (2020) 109–119.
[143] H. Tran, M.P. Moazami, H. Yang, D. McKenna-Yasek, C.L. Douthwright, C. Pinto, et al., Suppression of mutant C9orf72 expression by a potent mixed backbone antisense oligonucleotide, Nat. Med. 28 (1) (2022) 117–124.
[144] C. Lagier-Tourenne, M. Baughn, F. Rigo, S. Sun, P. Liu, H.R. Li, et al., Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration, Proc. Natl. Acad. Sci. U. S. A. 110 (47) (2013) E4530–E4539.
[145] D. Sareen, J.G. O'Rourke, P. Meera, A.K. Muhammad, S. Grant, M. Simpkinson, et al., Targeting RNA foci in iPSC-derived motor neurons from ALS patients with a C9ORF72 repeat expansion, Sci. Transl. Med. 5 (208) (2013) 208ra149.
[146] C.J. Donnelly, P.W. Zhang, J.T. Pham, A.R. Haeusler, N.A. Mistry, S. Vidensky, et al., RNA toxicity from the ALS/FTD C9ORF72 expansion is mitigated by antisense intervention, Neuron 80 (2) (2013) 415–428.
[147] J. Jiang, Q. Zhu, T.F. Gendron, S. Saberi, M. McAlonis-Downes, A. Seelman, et al., Gain of toxicity from ALS/FTD-Linked repeat expansions in C9ORF72 is alleviated by antisense oligonucleotides targeting GGGGCC-containing RNAs, Neuron 90 (3) (2016) 535–550.
[148] W.A. McEwan, B. Falcon, M. Vaysburd, D. Clift, A.L. Oblak, B. Ghetti, et al., Cytosolic Fc receptor TRIM21 inhibits seeded tau aggregation, Proc Natl Acad Sci U S A. 114 (3) (2017) 574–579.
[149] L. Nguyen, F. Montrasio, A. Pattamatta, S.K. Tusi, O. Bardhi, K.D. Meyer, et al., Antibody therapy targeting RAN proteins rescues C9 ALS/FTD phenotypes in C9orf72 mouse model, Neuron 105 (4) (2020), 645-62.e11.
[150] J.C. Esposto, S. Martic, Phosphorylated TAR DNA-binding protein-43: aggregation and antibody-based inhibition, Biochim. Biophys. Acta, Mol. Basis Dis. 1867 (12) (2021) 166234.
[151] Q.X. Dong, J. Zhu, S.Y. Liu, X.L. Yu, R.T. Liu, An oligomer-specific antibody improved motor function and attenuated neuropathology in the SOD1-G93A transgenic mouse model of ALS, Int. Immunopharm. 65 (2018) 413–421.
[152] D.J. Gordon, R. Tappe, S.C. Meredith, Design and characterization of a membrane permeable N-methyl amino acid-containing peptide that inhibits Abeta1-40 fibrillogenesis, J. Pept. Res. 60 (1) (2002) 37–55.
[153] S.A. Sievers, J. Karanicolas, H.W. Chang, A. Zhao, L. Jiang, O. Zirafi, et al., Structure-based design of non-natural amino-acid inhibitors of amyloid fibril formation, Nature 475 (7354) (2011) 96–100.
[154] D.A. Parsell, S. Lindquist, The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins, Annu. Rev. Genet. 27 (1993) 437–496.
[155] J.R. Glover, S. Lindquist, Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins, Cell 94 (1) (1998) 73–82.
[156] C.D. Link, Expression of human beta-amyloid peptide in transgenic Caenorhabditis elegans, Proc Natl Acad Sci U S A. 92 (20) (1995) 9368–9372.
[157] D.A. Parsell, A.S. Kowal, M.A. Singer, S. Lindquist, Protein disaggregation mediated by heat-shock protein Hsp104, Nature 372 (6505) (1994) 475–478.
[158] S. Rudiger, L. Germeroth, J. Schneider-Mergener, B. Bukau, Substrate specificity of the DnaK chaperone determined by screening cellulose-bound peptide libraries, EMBO J. 16 (7) (1997) 1501–1507.
[159] F.U. Hartl, M. Hayer-Hartl, Converging concepts of protein folding in vitro and in vivo, Nat. Struct. Mol. Biol. 16 (6) (2009) 574–581.
[160] C.A. Dickey, A. Kamal, K. Lundgren, N. Klosak, R.M. Bailey, J. Dunmore, et al., The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins, J. Clin. Invest. 117 (3) (2007) 648–658.
[161] P. Muller, R. Hrstka, D. Coomber, D.P. Lane, B. Vojtesek, Chaperone-dependent stabilization and degradation of p53 mutants, Oncogene 27 (24) (2008) 3371–3383.
[162] H.J. Chen, J.C. Mitchell, S. Novoselov, J. Miller, A.L. Nishimura, E.L. Scotter, et al., The heat shock response plays an important role in TDP-43 clearance: evidence for dysfunction in amyotrophic lateral sclerosis, Brain 139 (Pt 5) (2016) 1417–1432.
[163] M. Udan-Johns, R. Bengoechea, S. Bell, J. Shao, M.I. Diamond, H.L. True, et al., Prion-like nuclear aggregation of TDP-43 during heat shock is regulated by HSP40/70 chaperones, Hum. Mol. Genet. 23 (1) (2014) 157–170.
[164] Y.J. Zhang, T.F. Gendron, Y.F. Xu, L.W. Ko, S.H. Yen, L. Petrucelli, Phosphorylation regulates proteasomal-mediated degradation and solubility of TAR DNA binding protein-43 C-terminal fragments, Mol. Neurodegener. 5 (2010) 33.
[165] L.C. Gomes, L. Scorrano, Mitochondrial morphology in mitophagy and macroautophagy, Biochim. Biophys. Acta 1833 (1) (2013) 205–212.
[166] S.N. Chan, B.L. Tang, Location and membrane sources for autophagosome formation - from ER-mitochondria contact sites to Golgi-endosome-derived carriers, Mol. Membr. Biol. 30 (8) (2013) 394–402.
[167] N. Mizushima, B. Levine, A.M. Cuervo, D.J. Klionsky, Autophagy fights disease through cellular self-digestion, Nature 451 (7182) (2008) 1069–1075.
[168] M. Baba, S. Nakajo, P.H. Tu, T. Tomita, K. Nakaya, V.M. Lee, et al., Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies, Am. J. Pathol. 152 (4) (1998) 879–884.
[169] K.B. Kegel, M. Kim, E. Sapp, C. McIntyre, J.G. Castano, N. Aronin, et al., Huntingtin expression stimulates endosomal-lysosomal activity, endosome tubulation, and autophagy, J. Neurosci. 20 (19) (2000) 7268–7278.
[170] K. Castillo, M. Nassif, V. Valenzuela, F. Rojas, S. Matus, G. Mercado, et al., Trehalose delays the progression of amyotrophic lateral sclerosis by enhancing autophagy in motoneurons, Autophagy 9 (9) (2013) 1308–1320.
[171] V. Crippa, D. Sau, P. Rusmini, A. Boncoraglio, E. Onesto, E. Bolzoni, et al., The small heat shock protein B8 (HspB8) promotes autophagic removal of misfolded proteins involved in amyotrophic lateral sclerosis (ALS), Hum. Mol. Genet. 19 (17) (2010) 3440–3456.
[172] J.K. Bose, C.C. Huang, C.K. Shen, Regulation of autophagy by neuropathological protein TDP-43, J. Biol. Chem. 286 (52) (2011) 44441–44448.
[173] Z. Ying, Q. Xia, Z. Hao, D. Xu, M. Wang, H. Wang, et al., TARDBP/TDP-43 regulates autophagy in both MTORC1-dependent and MTORC1-independent manners, Autophagy 12 (4) (2016) 707–708.
[174] Q. Xia, H. Wang, Z. Hao, C. Fu, Q. Hu, F. Gao, et al., TDP-43 loss of function increases TFEB activity and blocks autophagosome-lysosome fusion, EMBO J. 35 (2) (2016) 121–142.
[175] S.J. Barmada, A. Serio, A. Arjun, B. Bilican, A. Daub, D.M. Ando, et al., Autophagy induction enhances TDP43 turnover and survival in neuronal ALS models, Nat. Chem. Biol. 10 (8) (2014) 677–685.
[176] I.F. Wang, B.S. Guo, Y.C. Liu, C.C. Wu, C.H. Yang, K.J. Tsai, et al., Autophagy activators rescue and alleviate pathogenesis of a mouse model with proteinopathies of the TAR DNA-binding protein 43, Proc Natl Acad Sci U S A. 109 (37) (2012) 15024–15029.
[177] Z. Xu, S. Jia, W. Wang, Z. Yuan, B. Jan Ravoo, D.S. Guo, Heteromultivalent peptide recognition by co-assembly of cyclodextrin and calixarene amphiphiles enables inhibition of amyloid fibrillation, Nat. Chem. 11 (1) (2019) 86–93.
[178] Y. Tamaki, A. Shodai, T. Morimura, R. Hikiami, S. Minamiyama, T. Ayaki, et al., Elimination of TDP-43 inclusions linked to amyotrophic lateral sclerosis by a misfolding-specific intrabody with dual proteolytic signals, Sci. Rep. 8 (1) (2018) 6030.
[179] T. Afroz, E. Chevalier, M. Audrain, C. Dumayne, T. Ziehm, R. Moser, et al., Immunotherapy targeting the C-terminal domain of TDP-43 decreases neuropathology and confers neuroprotection in mouse models of ALS/FTD, Neurobiol. Dis. 179 (2023) 106050.
[180] M. Maier, T. Welt, F. Wirth, F. Montrasio, D. Preisig, J. McAfoose, et al., A human-derived antibody targets misfolded SOD1 and ameliorates motor symptoms in mouse models of amyotrophic lateral sclerosis, Sci. Transl. Med. 10 (470) (2018).

[181] B. Kalmar, C.H. Lu, L. Greensmith, The role of heat shock proteins in Amyotrophic Lateral Sclerosis: the therapeutic potential of Arimoclomol, Pharmacol. Ther. 141 (1) (2014) 40–54.
[182] B. Kalmar, S. Novoselov, A. Gray, M.E. Cheetham, B. Margulis, L. Greensmith, Late stage treatment with arimoclomol delays disease progression and prevents protein aggregation in the SOD1 mouse model of ALS, J. Neurochem. 107 (2) (2008) 339–350.
[183] D. Kieran, B. Kalmar, J.R. Dick, J. Riddoch-Contreras, G. Burnstock, L. Greensmith, Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice, Nat. Med. 10 (4) (2004) 402–405.
[184] M. Ahmed, C. Spicer, J. Harley, J.P. Taylor, M. Hanna, R. Patani, et al., Amplifying the heat shock response ameliorates ALS and FTD pathology in mouse and human models, Mol. Neurobiol. 60 (12) (2023) 6896–6915.
[185] M.E. Cudkowicz, J.M. Shefner, E. Simpson, D. Grasso, H. Yu, H. Zhang, et al., Arimoclomol at dosages up to 300 mg/day is well tolerated and safe in amyotrophic lateral sclerosis, Muscle Nerve 38 (1) (2008) 837–844.
[186] M. Benatar, J. Wuu, P.M. Andersen, N. Atassi, W. David, M. Cudkowicz, et al., Randomized, double-blind, placebo-controlled trial of arimoclomol in rapidly progressive SOD1 ALS, Neurology 90 (7) (2018) e565–e574.
[187] M. Benatar, T. Hansen, D. Rom, M.A. Geist, T. Blaettler, W. Camu, et al., Safety and efficacy of arimoclomol in patients with early amyotrophic lateral sclerosis (ORARIALS-01): a randomised, double-blind, placebo-controlled, multicentre, phase 3 trial, Lancet Neurol. 23 (7) (2024) 687–699.
[188] B. Ravikumar, R. Duden, D.C. Rubinsztein, Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy, Hum. Mol. Genet. 11 (9) (2002) 1107–1117.
[189] T. Shintani, D.J. Klionsky, Autophagy in health and disease: a double-edged sword, Science 306 (5698) (2004) 990–995.
[190] J.L. Webb, B. Ravikumar, J. Atkins, J.N. Skepper, D.C. Rubinsztein, Alpha-Synuclein is degraded by both autophagy and the proteasome, J. Biol. Chem. 278 (27) (2003) 25009–25013.
[191] J.R. Buchan, R.M. Kolaitis, J.P. Taylor, R. Parker, Eukaryotic stress granules are cleared by autophagy and Cdc48/VCP function, Cell 153 (7) (2013) 1461–1474.
[192] Z. Monahan, F. Shewmaker, U.B. Pandey, Stress granules at the intersection of autophagy and ALS, Brain Res. 1649 (Pt B) (2016) 189–200.
[193] J. Riancho, M. Ruiz-Soto, M.T. Berciano, J. Berciano, M. Lafarga, Neuroprotective effect of bexarotene in the SOD1 mouse model of amyotrophic lateral sclerosis, Front. Cell. Neurosci. 9 (2015) 250.
[194] J. Riancho, M.T. Berciano, M. Ruiz-Soto, J. Berciano, G. Landreth, M. Lafarga, Retinoids and motor neuron disease: potential role in amyotrophic lateral sclerosis, J. Neurol. Sci. 360 (2016) 115–120.
[195] L. Li, X. Zhang, W. Le, Altered macroautophagy in the spinal cord of SOD1 mutant mice, Autophagy 4 (3) (2008) 290–293.
[196] X. Zhang, L. Li, S. Chen, D. Yang, Y. Wang, X. Zhang, et al., Rapamycin treatment augments motor neuron degeneration in SOD1(G93A) mouse model of amyotrophic lateral sclerosis, Autophagy 7 (4) (2011) 412–425.
[197] C.W. Cheng, M.J. Lin, C.K. Shen, Rapamycin alleviates pathogenesis of a new Drosophila model of ALS-TDP, J. Neurogenet. 29 (2–3) (2015) 59–68.
[198] J. Mandrioli, R. D'Amico, E. Zucchi, S. De Biasi, F. Banchelli, I. Martinelli, et al., Randomized, double-blind, placebo-controlled trial of rapamycin in amyotrophic lateral sclerosis, Nat. Commun. 14 (1) (2023).