



The Role of Mitochondrial Dysfunction and ER Stress in TDP-43 and C9ORF72 ALS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of the motor system with complex determinants, including genetic and non-genetic factors. Despite this heterogeneity, a key pathological signature is the mislocalization and aggregation of specific proteins in the cytoplasm, suggesting that convergent pathogenic mechanisms focusing on disturbances in proteostasis are important in ALS. In addition, many cellular processes have been identified as potentially contributing to disease initiation and progression, such as defects in axonal transport, autophagy, nucleocytoplasmic transport, ER stress, calcium metabolism, the unfolded protein response and mitochondrial function. Here we review the evidence from *in vitro* and *in vivo* models of C9ORF72 and TDP-43-related ALS supporting a central role in pathogenesis for endoplasmic reticulum stress, which activates an unfolded protein response (UPR), and mitochondrial dysfunction. Disruption in the finely tuned signaling between the ER and mitochondria through calcium ions may be a crucial trigger of mitochondrial deficits and initiate an apoptotic signaling cascade, thus acting as a point of convergence for multiple upstream disturbances of cellular homeostasis and constituting a potentially important therapeutic target.

Keywords: C9orf72, TDP-43, calcium homeostasis, ALS, mitochondria, endoplasmic reticulum, UPR

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is the most common type of motor neuron diseases and is characterized by progressive degeneration of upper and lower motor neurons, resulting in loss of voluntary muscle action and eventually death through respiratory failure. While the majority of cases are sporadic, ~10% of ALS is due to inheritance of a genetic mutation in an autosomal dominant pattern. To date, mutations in up to 20 genes have been associated with ALS, including superoxide-dismutase 1 (SOD1), TAR-DNA binding protein (TARDBP) and FUS (Mathis et al., 2019; McCann et al., 2020). However, the most common single mutation is a GGGGCC hexanucleotide repeat expansion in the C9ORF72 gene, which accounts for ~40% of all familial ALS and significant numbers of apparently sporadic cases, and provides a clear genetic link to frontotemporal dementia (FTD) (DeJesus-Hernandez et al., 2011; Renton et al., 2011).

There are multiple potential mechanisms through which the hexanucleotide might drive pathogenesis in C9ORF72-related disease, leading to either toxic gain of function or loss of normal function: formation of nuclear RNA foci that sequester a range of hnRNPs; production of poly-dipeptides (GA, GP, GR, PA, PR) through repeat non-ATG (RAN) translation leading to the accumulation of toxic aggregates; or haploinsufficiency due to transcriptional silencing.

While these mechanisms are not mutually exclusive, and each may operate in different phases of disease, the relative contribution of acquired toxicity and loss of normal function as initiating factors in C9ORF72-related neurodegeneration is still unclear.

Despite the genetic heterogeneity of familial ALS, abnormal accumulation of misfolded or aggregated proteins is a unifying pathological feature. The organelle mainly responsible for native folding, post-translational modifications and trafficking of many proteins is the endoplasmic reticulum (ER) (Schroder, 2008; Bernard-Marissal et al., 2015b). Quality control performed by ER-resident chaperones ensures precise folding of newly synthesized proteins and identifies unfolded or misfolded proteins, which are then targeted to specific degradation pathways (Kaushik and Cuervo, 2015). When proteostasis is disturbed through accumulation of misfolded or unfolded proteins, a stress response is triggered which is mitigated by an adaptive signaling mechanism called the “unfolded protein response” (UPR). The UPR reduces general translation and enhances the expression of specific UPR target genes, such as ER chaperones to restore protein folding and promote quality control mechanisms or degradation of irreversible misfolded proteins. Dysfunction of proteostasis significantly increases ER stress and is associated with neuronal degeneration (Scheper and Hoozemans, 2015).

The ER is found in close physical and functional connection with mitochondria and numerous lines of evidence indicate that mitochondrial dysfunction is involved in ALS. Mitochondria are the main source of cellular energy via oxidative phosphorylation and, through physical and functional interaction with the ER, they both contribute to common essential functions such as calcium homeostasis and lipid biosynthesis. Abnormalities in mitochondrial morphology have been observed in tissue from patients affected by sporadic or familial ALS, and in cellular and animal models, with defects in mitochondrial transport and morphology first demonstrated in cultured primary neurons harboring ALS causing mutations (De Vos et al., 2007; Magrane et al., 2009, 2012; Song et al., 2013). While the majority of early studies linking mitochondrial dysfunction with ALS were based on SOD1 models, it has become evident in the last decade that both functional and morphological defects in mitochondria are found in other familial ALS cases, particularly those due to mutations in TARDBP or C9ORF72.

Here we discuss recent evidence for ER and mitochondrial dysfunction associated with C9ORF72 and TARDBP mutations in the context of underlying gain of function vs. loss of function mechanisms.

CALCIUM SIGNALING BETWEEN ER AND MITOCHONDRIA

The ER takes part in multiple cellular functions, including calcium (Ca^{2+}) homeostasis, lipid and protein biosynthesis, protein folding, post translational modification and regulation of gene expression (Yoshida, 2007; Eden, 2016) reviewed in Hetz and Saxena (2017). A multitude of studies suggest that

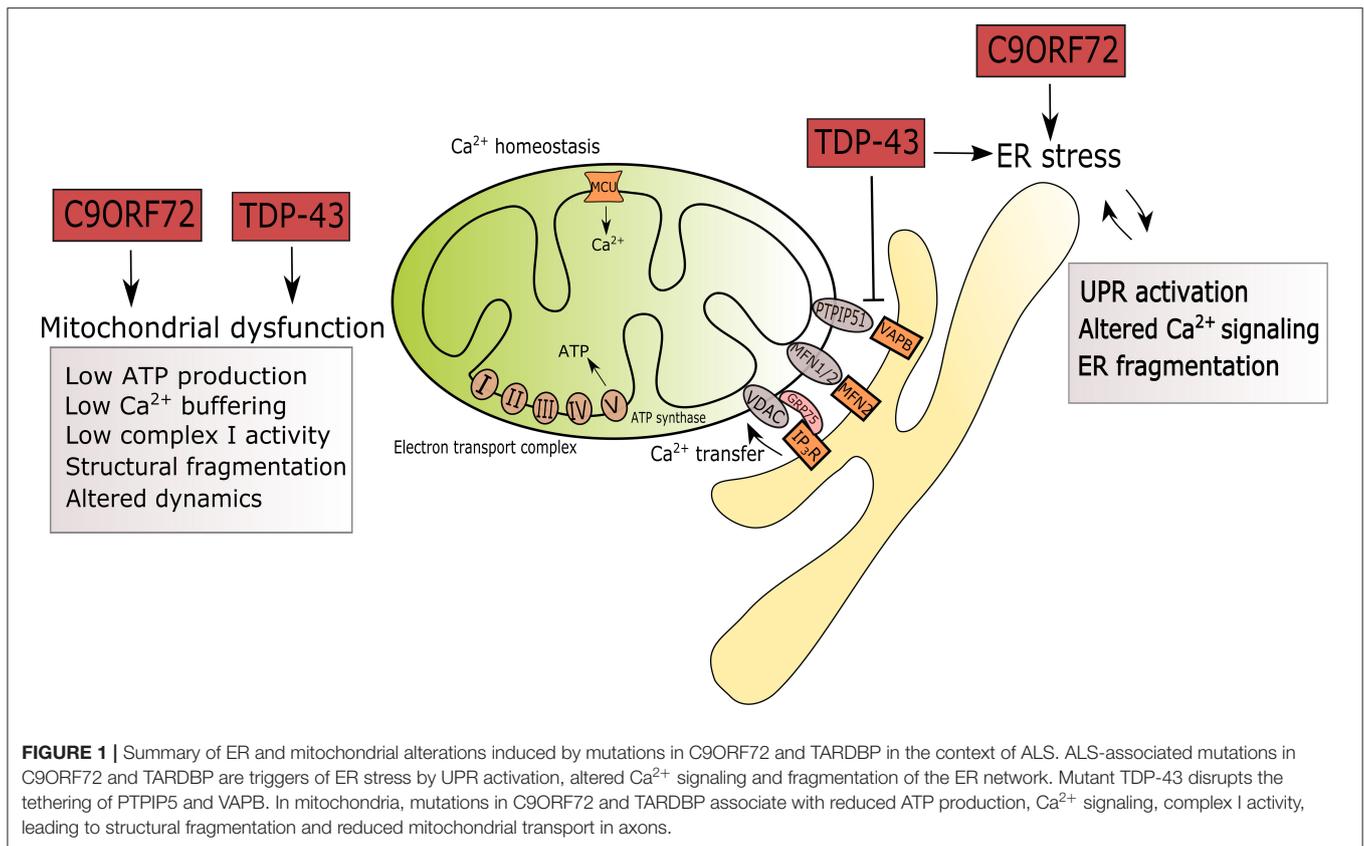
disruptions in ER proteostasis and crosstalk with mitochondria can result in neuronal degeneration and motor neurons are highly susceptible to perturbations in these pathways.

One of the main functions of the ER is to store high levels of Ca^{2+} , which regulate functions within the ER as well as other critical cellular functions by modulating its release into the cytosol. Ca^{2+} ions are actively transported into the ER against the gradient by the sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) pump, and sequestered by Ca^{2+} -binding proteins present in the ER. Intraluminal levels of Ca^{2+} in the ER regulate the activity of Ca^{2+} -binding chaperones, such as GRP78/BiP, GRP94 and protein-disulphide isomerase (PDI) (Coe and Michalak, 2009). In addition, GRP78/BiP is also a regulator of the Unfolded Protein Response (UPR). It is involved in the folding, assembly and translocation of newly synthesized proteins, and its association with peptides is dependent on a high Ca^{2+} concentration (Vogel et al., 1990). Calnexin and calreticulin are both Ca^{2+} binding ER chaperones involved in the quality control process where they promote proper folding of nascent proteins in a Ca^{2+} -dependent manner (Prell et al., 2013). These chaperones can also act as high-capacity Ca^{2+} stores at the mitochondria-associated membrane when Ca^{2+} is transferred to the mitochondria and genetic ablation of calreticulin was found to accelerate muscle denervation in ALS (Bernard-Marissal et al., 2015b).

CALCIUM MISCOMMUNICATION BETWEEN ER AND MITOCHONDRIA IN ALS

Calcium miscommunication between the ER and mitochondria has recently emerged as a major factor in loss of Ca^{2+} homeostasis in ALS. The membranes of the ER and mitochondria are closely connected at contact sites called mitochondria-associated ER membranes (MAM). The membranes of the two organelles are tethered by interactions between several protein complexes: Mitofusin 1 and 2 (MFN1/2); IP_3R and VDAC via GRP75; and VAPB binding to protein tyrosine phosphatase-interacting protein 51 (PTPIP51) on the outer mitochondrial membrane (reviewed in Lau et al., 2018) (Figure 1). Disruptions in the interactions between these tethering complexes have been reported in several studies in SOD1, TDP-43 and FUS-related ALS (Stoica et al., 2014, 2016).

Calcium uptake in the mitochondria is a finely tuned event with profound importance both for overall cellular homeostasis and for normal mitochondrial function. Mitochondrial Ca^{2+} uptake is dependent on mitochondrial membrane potential, which is developed and maintained by mitochondrial respiration, via the electron transport chain to reduce molecular oxygen and pump out protons. Four enzymatic complexes form the electron transport chain: NADH and succinate dehydrogenases (complex I and II), ubiquinone-cytochrome *c* reductase (complex III), and cytochrome *c* oxidase (complex IV). The proton electrochemical potential drives ATP synthase to produce energy and several mitochondrial dehydrogenases are Ca^{2+} dependent (Griffiths and Rutter, 2009).



Cytosolic Ca²⁺ is buffered by mitochondria through the mitochondrial calcium uniporter (MCU), a transmembrane protein that sits on the inner mitochondrial membrane in close connection with the ER membrane (**Figure 1**). Its activity is regulated by the EF-hand domain-containing proteins mitochondrial calcium uptake (MICU) 1 and 2 (Perocchi et al., 2010; Plovanich et al., 2013). These regulators activate or inhibit MCU by sensing the Ca²⁺ concentration in the intermembrane space (Patron et al., 2014).

RNA sequencing of iPS-derived motor neurons from patients with mutations in C9ORF72 and TDP-43 has revealed an altered balance between these two gatekeepers in ALS (Dafinca et al., 2020). In models of TDP-43 and FUS, an impairment in the communication between ER and mitochondria leads to a reduction in Ca²⁺ uptake in the mitochondria and a subsequent rise in cytosolic Ca²⁺ which may activate cellular death pathways (Stoica et al., 2014, 2016). Consistent with these results, low mitochondrial Ca²⁺ uptake was reported in C9ORF72 and TDP-43^{M337V} iPS-derived motor neurons from ALS patients, which contributed to increased neuronal death (Dafinca et al., 2020).

UPR ACTIVATION IN ALS

The UPR is activated by three stress sensors: inositol-requiring transmembrane kinase/endonuclease (IRE1), activating transcription factor 6 (ATF6) and PKR-like ER kinase (PERK). Once activated by cleavage in the ER, ATF6 translocates to

the nucleus, where it controls the transcription of target genes related to protein folding and quality control (Haze et al., 1999). IRE1 initiates splicing of the transcription factor X-Box-Binding protein 1 (XBP1), which converts it into an activator of genes responsible for protein folding, quality control and secretion of ER-associated degradation proteins (ERAD) (Calfon et al., 2002; Acosta-Alvear et al., 2007). PERK reduces protein translation in the ER by phosphorylation of the eukaryotic initiation factor 2 α (eIF2 α), decreasing the potential burden of misfolded proteins (Harding et al., 1999). Furthermore, eIF2 α activates ATF4 driving the expression of a cascade of UPR-targeted genes responsible for protein folding, autophagy and apoptosis (Tabas and Ron, 2011). Eventually, the UPR triggers attenuation of general translation and enhanced expression of genes encoding chaperones, folding enzymes, and ERAD proteins. A failure to restore ER homeostasis, leads to activation of apoptotic pathways (Malhotra and Kaufman, 2007; Krebs et al., 2015).

Evidence for the relevance of this pathway to ALS comes from studies in human post-mortem spinal cords from sporadic or familial ALS patients, where the expression of UPR pathway is significantly increased (Ilieva et al., 2007; Atkin et al., 2008; Hetz et al., 2009; Ito et al., 2009; Sasaki, 2010). Structural alterations indicative of ER stress, such as fragmentation, have also been described in the anterior horn of the spinal cord in ALS and several chaperones involved in the ER-stress response have been detected in the cerebrospinal fluid of sporadic ALS patients (Oyanagi et al., 2008; Sasaki, 2010; Vijayalakshmi et al., 2011).

Transcriptome profiles of C9ORF72-ALS human cerebellum and frontal cortex indicate activation of UPR genes as a signature of pathology, suggesting activation of ER stress (Prudencio et al., 2015). We reported increased ER stress in iPSC-derived MNs from patients with C9ORF72 mutations, followed by reduced mitochondrial membrane potential and altered mitochondrial morphology (Dafinca et al., 2016). The cells also show evidence of oxidative stress, with stress granule formation and activation of apoptosis. RNA sequencing of primary mouse neurons expressing poly(PR) identified upregulation of genes involved in ER stress, in particular the transcription factor ATF4, indicating that poly(PR) activates the UPR (Kramer et al., 2018). This in itself is reported to upregulate RAN translation, driving further production of DPRs and becoming a feed-forward loop (Zhang et al., 2014). In a different study, expression of synthetic poly(PR) induced ER stress and inhibition of the UPR increased cell survival (Wang et al., 2019). Consistent with these reports, RAN translation of the G₄C₂ expansion was also found to be enhanced by ER stress and overexpression of the repeats impaired global translation, while increasing the formation of stress granules in an eIF2 α -dependent manner (Green et al., 2017). In a recent post-mortem study in C9-FTD patients, overall levels of pPERK and peIF2 α were higher in the hippocampus of patients and they correlated with the presence of dipeptide pathology (Gami-Patel et al., 2020). In an earlier study, downregulation of PERK in the ER was shown to improve mitochondrial Ca²⁺ dynamics and restore mitochondrial elongation, highlighting the connection between UPR and mitochondrial function (Munoz et al., 2013). In cortical and spinal motor neurons from a C9ORF72 model with (G4C2)₁₈₈ repeats, excitotoxic stress and optogenetic neuronal stimulation act as promoters of RAN translation and ER stress response, leading to increased phosphorylation of eIF2 α (Westergard et al., 2019).

Cytoplasmic aggregation of TDP-43, the pathological hallmark of ALS, may be driven by activation of ER stress in motor neurons (Ayala et al., 2011; Suzuki and Matsuoka, 2012). Pharmacological induction of ER stress in neuroblastoma cells leads to TDP-43 mislocalisation and cleavage, and C-terminal fragments colocalise with PDI, potentially interfering with ER function (Walker et al., 2013). Aggregation of TDP-43 could further contribute to increasing levels of ER stress and subsequent activation of apoptosis (Suzuki et al., 2011). Moreover, ALS mutations in TDP-43 (G294A, A315T, Q331K, M337V, N390D, D169G) were shown to cause UPR upregulation in a Neuro2A cell model (Walker et al., 2013; Wang et al., 2015).

Mechanistic studies demonstrated that ER stress led to casein kinase 1-dependent phosphorylation of TDP-43, followed by cytosolic aggregation (Nonaka et al., 2016; Hicks et al., 2020). Similar conclusions were reached in mutant FUS models, which showed ER stress and PDI positive aggregates (Farg et al., 2012). Both wild-type and ALS-mutant FUS and TDP-43 perturbed ER-mitochondria associations, accompanied by changes to the VAPB-PTPIP51 interaction and abnormal Ca²⁺ signaling between the two organelles (Stoica et al., 2014).

DEFICIENT MITOCHONDRIAL BIOENERGETICS IN ALS

In addition to a role in Ca²⁺ buffering, mitochondria primarily act as a source of energy for the majority of cellular processes. Functional mitochondrial changes, such as membrane hyperpolarisation, increased ATP production and respiration are detected in C9ORF72 patient fibroblasts, along with morphological changes, such as the frequent presence of mixed populations of elongated, short mitochondria (Onesto et al., 2016). In the same study, TDP-43^{A382T} fibroblasts showed a fragmented mitochondrial network along with decreased membrane potential (Onesto et al., 2016). Our group has reported reduced membrane potential in motor neurons derived from iPSC cells of C9ORF72 ALS patients and reduced mitochondrial Ca²⁺ buffering capacity in both C9ORF72 and TDP-43^{M337V} iPSC-MNs from ALS patients (Dafinca et al., 2016, 2020). Furthermore, a recent study demonstrated abnormalities in the electron chain machinery in human iPSC-derived MNs from C9ORF72 patients, where low basal respiration and maximal mitochondrial respiration were detected (Mehta et al., 2021). A reduction in bioenergetics in C9ORF72 iPSC-derived motor neurons was correlated with lowered expression of complexes I and IV of the mitochondrial electron transport chain. Consistent with these findings, Wang et al. recently showed that C9ORF72 acts as a mitochondrial-inner-membrane-associated protein that regulates oxidative phosphorylation by stabilizing TIMMDC1, an essential component for the assembly of mitochondrial complex I (Wang et al., 2021). In their study, they also demonstrate that C9ORF72 haploinsufficiency and loss of function leads to a reduction in mitochondrial complex I activity in patient-derived neurons from C9ORF72-ALS.

The most convincing direct link between C9ORF72 and mitochondrial dysfunction has been demonstrated so far by overexpression of poly-dipeptides. Arguing for a toxic gain of function, cellular and animal models overexpressing poly-dipeptides, in particular poly(GR), have consistently shown mitochondrial alterations. Patient motor neurons derived from C9ORF72 ALS iPSC cells show upregulation of the p53 pathway, high levels of DNA damage during long-term culture, followed by production of reactive oxygen species and increased mitochondrial potential (Lopez-Gonzalez et al., 2016). An interactome analysis of poly(GR) showed an abundance of mitochondrial ribosomal proteins, indicating preferential binding to the mitochondria where it is likely to induce oxidative stress. Poly(GR) expression in healthy neurons recapitulated these phenotypes. In a mouse model expressing poly(GR)₈₀, compromised mitochondrial morphology was also detected with preferential binding of the dipeptide to the complex V subunit of the mitochondrial ATP-synthase (ATP5A1), inducing its ubiquitination and degradation, which is consistent with reduced levels of ATP5A1 in patient brains (Choi et al., 2019). Interestingly, poly(GR) has been suggested to act as a mitochondrial targeting signal and it can be translated in close proximity to the mitochondrial surface. Frequent stalling of its

translation triggers ribosome-associated quality control and C-terminal extension which leads to potentially toxic aggregations of poly(GR) on mitochondria (Li et al., 2020).

Mitochondria have also recently emerged as a target of TDP-43 (Magrane et al., 2014; Onesto et al., 2016; Wang et al., 2016, 2017; Izumikawa et al., 2017; Davis et al., 2018; Gautam et al., 2019). Abnormal accumulations of mitochondria have been described in spinal cord motor neurons of mutant TDP-43 transgenic mice and overexpression of mutant or wild-type TDP-43 in cultured motor neurons triggered similar mitochondrial morphology and transport abnormalities to those found in SOD1 mice (Shan et al., 2010; Wang et al., 2013). Overexpression of mutant TDP-43^{Q331K} and TDP-43^{M337V} in neuroblastoma cells and in primary motor neurons leads to mitochondrial depolarisation (Hong et al., 2012; Lu et al., 2012; Wang et al., 2013). Furthermore, in TDP-43^{G298S} and TDP-43^{A382T} patient fibroblasts, complex I activity was decreased, along with reduced ATP levels and oxygen consumption (Wang et al., 2016).

RNA sequencing of the axonal compartment in motor neurons of a TDP-43 knockdown mouse revealed dysregulation of many transcripts relevant for mitochondrial function and translation (Briese et al., 2020). Of note, mitochondrial ATP synthase beta-subunit (ATP5B) was downregulated, which has previously been shown to bind to FUS in cellular and animal ALS models (Deng et al., 2018). In this study, TDP-43 knockdown led to fewer intact mitochondria in axons and reduced mitochondrial potential compared to healthy motor neurons. In a recent report, aggregated TDP-43 with ALS-associated mutations was shown to bind to and sequester a subset of nuclear encoded mitochondrial DNA, including ATP5B, while increasing expression of a different subset of mitochondrial DNA and thereby inducing a global imbalance in the mitochondria (Zuo et al., 2021). The data available linking TDP-43 mutations and mitochondrial deficiencies indicate that mitochondrial dysfunctions can occur both as a result of toxic gain of function, by direct interaction, or by loss of function, possibly at the transcriptional level.

MITOCHONDRIAL TRANSPORT DEFECTS IN ALS MOTOR NEURONS

The proper distribution of mitochondria in neurons is essential for healthy neuronal function and this is supported by the fact that pathology in neurodegenerative diseases often correlates with defects in mitochondrial intracellular localization (Chang et al., 2006; Rui et al., 2006; Schon and Przedborski, 2011; Reddy et al., 2012). Mitochondria provide the ATP necessary to actively transport mRNAs, proteins and organelles throughout the cells, in addition to its role in Ca²⁺ buffering and metabolite synthesis (Rui et al., 2006). This distribution is coordinated by microtubule-based transport mediated mainly by the motor proteins kinesin-1 and dynein, along with their adaptors (Schnapp and Reese, 1989; Pilling et al., 2006). Interestingly, defects in several MAM-associated proteins (MFN2, VAPB, SIGMAR1) were shown to impair axonal transport of mitochondria either by interfering with the attachment of mitochondria to motor proteins, or through

an increase in cytosolic Ca²⁺ levels that leads to reduced anterograde/retrograde transport (Wang and Schwarz, 2009; Misko et al., 2012; Morotz et al., 2012; Bernard-Marissal et al., 2015a).

While defects in axonal transport have long been associated with ALS, evidence of mitochondrial transport deficits in C9ORF72 models has only recently emerged. In iPS-derived MNs from C9ORF72 patients, fast axonal transport of mitochondria was found to be impaired, with no differences reported in either axonal mitochondrial counts or transcript levels of mitochondrial tRNA (Mehta et al., 2021). In this study, a major contributing factor to the deficit in transport was a reduction in basal and maximal mitochondrial respiration and PGC1 α overexpression, which increases mitochondrial biogenesis, was able to improve the transport deficit.

In a TDP-43^{A315T} mutant mouse, the earliest disease-related event observed was a reduction in retrograde mitochondrial axonal transport, which later led to accumulation of mitochondria in axon terminals and fragmentation (Magrane et al., 2014). Both SOD1 and TDP-43 have been found to bind to the mitochondrial outer membrane, suggesting that they may physically interfere with mitochondria and potentially impair its transport (Vande Velde et al., 2008; Wang et al., 2013).

IMPLICATIONS OF ER-MITOCHONDRIA CROSSTALK FOR SYNAPTIC TRANSMISSION

Synaptic transmission is an essential neuronal process and dysfunctional synapses are a major feature of several neurodegenerative disorders, including ALS. A recent study demonstrated that synaptic activity increases ER-mitochondria contacts and, conversely, loss of ER-mitochondria tethering mediated by VAPB-PTPIP51 leads to reduction of synaptic transmission (Gomez-Suaga et al., 2019). Recycling of synaptic vesicles is an energetically-demanding process which is under the tight control of Ca²⁺ signaling and the loss of connectivity between the ER and mitochondria may lead to impairment in synaptic transmission through a loss of Ca²⁺ communication and a reduction in ATP production (Brini et al., 2014). This highlights the importance of ER-mitochondria connectivity and how its disruption in ALS motor neurons may have widespread detrimental consequences on critical neuronal functions, ultimately leading to neuronal death.

CONCLUSIONS

Despite genetic heterogeneity, functional decline at the cellular level appears to follow similar pathways in ALS. A valuable therapeutic target would therefore be an upstream event that occurs in the majority of affected ALS neurons and recent evidence points to ER stress and mitochondrial dysfunction as a potential nodal point in ALS pathology which can be targeted by drugs. Since TDP-43 pathology is found in 98% of ALS cases, including in C9ORF72-related ALS, critically evaluating the connection between TDP-43 pathology, ER-stress

and mitochondrial dysfunction is important for identifying therapeutic targets.

Studies in which the ER stress response has been manipulated in an attempt to ameliorate ALS phenotypes are promising but inconclusive. Inhibition of eIF2 α phosphatase reduced the phenotypes in TDP-43 mutant animal models (Vaccaro et al., 2013). However, neither genetic inhibition of the UPR via ablation of PERK, nor genetic UPR enhancement via ablation of GADD34, had a beneficial effect in mutant SOD1 mice (Dzhashvili et al., 2019). More recent studies in SOD1-iPSC and mouse models demonstrated that MNs are more sensitive to ER stress and identified a number of modifiers, including TUDCA, a bile acid derivative which is currently undergoing clinical trials in ALS (Thams et al., 2019; Paganoni et al., 2020).

Most gain of function studies in C9ORF72 have used models expressing one of 5 poly-dipeptides that can result from RAN translation, with the majority focusing on the arginine-rich dipeptides (polyGR or polyPR). These dipeptides were shown to be toxic in various cellular and animal models, but the reliance on overexpression should prompt caution in interpreting these studies as supporting a disease mechanism mediated by mitochondrial dysfunction. Models which activate cell death pathways for whatever reason will always involve mitochondria as an active player, and this may be secondary and non-specific. It is therefore imperative to use models expressing poly-dipeptides at physiological levels, either with an inducible system or by using smaller repeat sizes under controlled expression to avoid activation of non-specific stress responses and apoptosis.

While most studies indicate that mitochondrial dysfunction occurs as a toxic gain of function, e.g., through poly(GR), C-terminal cleaved TDP-43, or TDP-43 aggregation, there is also evidence that loss of normal TDP-43 function can induce mitochondrial dysfunction. These mechanisms are not exclusive and could converge to initiate the dysfunction observed in ALS motor neurons. A loss of normal TDP-43 from the nucleus could negatively affect the transcription of mitochondrial proteins, while a toxic gain of function of cleaved TDP-43 in the cytoplasm may interfere with mitochondrial function.

Given recent evidence from animal and cellular models of C9ORF72, mitochondrial dysfunction may be mediated by TDP-43 pathology which becomes the driver of mitochondrial deficits and ER stress in combination with arginine rich poly-dipeptides that associate directly with these organelles.

In summary, evidence is growing that deficient interactions between ER and mitochondria are involved in neurodegeneration, including in ALS. In support of this hypothesis, mutations in several proteins involved in the communication between ER and mitochondria are associated with genetic forms of ALS (VAPB, Sigma1R) (Nishimura et al., 2004; De Vos et al., 2012). An interactome study performed in neuronal cells identified C9ORF72 enrichment in the mitochondrial fraction due to increased interaction with members of the mitochondrial outer membrane (Blokhuys et al., 2016). The possibility of C9ORF72 directly interacting with the function of MAMs remains to be explored. Due to their close functional and physical connection, a better understanding of disease mechanisms in ALS could be achieved by studying the ER and mitochondria as a functional unit, rather than separately. Irrespective of how cellular stress originates in MNs, it is clear that modulating the ER stress response and mitochondrial dysfunction in MNs are promising therapeutic avenues for ALS, whether sporadic or familial.

AUTHOR CONTRIBUTIONS

The article was conceived, planned, revised by KT and RD. RD was responsible for the first draft with contributions from PB. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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