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Amyotrophic Lateral Sclerosis-Associated Mutants of SOD1 Modulate miRNA Biogenesis through Aberrant Interactions with Exportin 5

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Abstract

Mutations in the *SOD1* (superoxide dismutase 1) gene are associated with amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease. By employing ascorbate peroxidase-based proximity labeling, coupled with LC–MS/MS analysis, we uncovered 43 and 24 proteins exhibiting higher abundance in the proximity proteomes of SOD1^{G85R} and SOD1^{G93A}, respectively, than that of wild-type SOD1. Immunoprecipitation followed by western blot analysis indicated the preferential binding of one of these proteins, exportin 5 (XPO5), toward the two mutants of SOD1 over the wild-type counterpart. In line with the established function of XPO5 in pre-miRNA transport, we observed diminished nucleocytoplasmic transport of pre-miRNAs in cells with ectopic expression of the two SOD1 mutants over those expressing the wild-type protein. On the other hand, RT-qPCR results revealed significant elevations in mature miRNA in cells expressing the two SOD1 mutants, which are attributed to the diminished inhibitory effect of XPO5 on Dicer-mediated cleavage of pre-miRNA to mature miRNA. Together, our chemoproteomic approach led to the revelation of a novel mechanism through which ALS-associated mutants of SOD1 perturb miRNA biogenesis, that is, through aberrant binding toward XPO5.

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Supporting Information

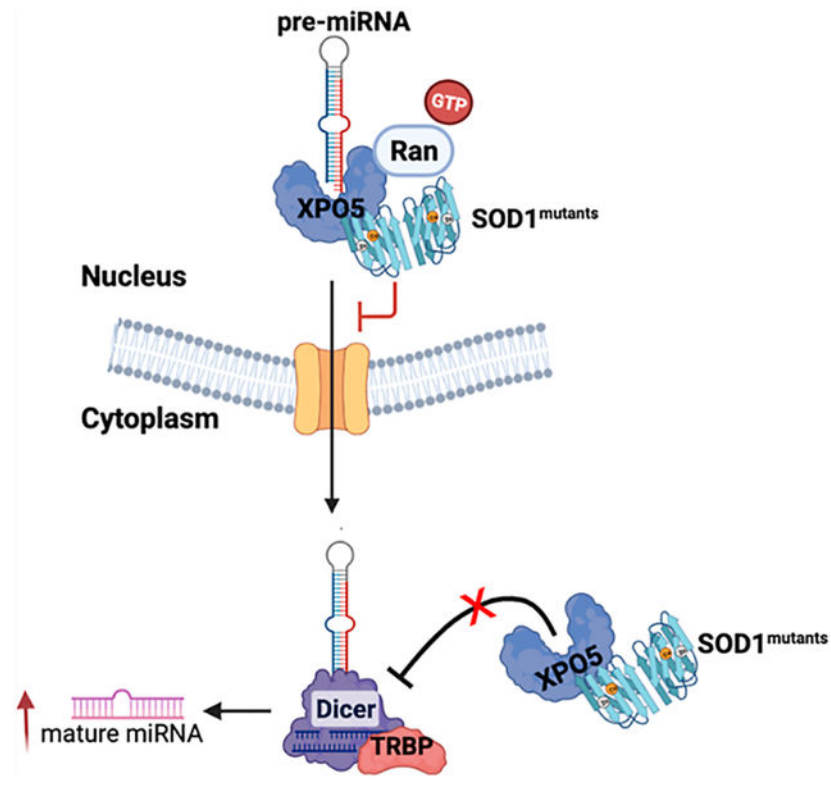
The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acscchembio.2c00591>.

List of primers used, LC–MS/MS quantification results, and gene ontology analysis results (PDF)

Complete contact information is available at: <https://pubs.acs.org/10.1021/acscchembio.2c00591>

The authors declare no competing financial interest.

Graphical Abstract



INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive, fatal neurodegenerative disorder manifested by the degeneration of motor neurons in the brain and spinal cord, resulting in paralysis and death.¹ The onset of ALS usually occurs in the late middle life at around 55 years old, starting with insidious muscle weakness and spreading to progressive muscle atrophy, with survival being limited to 2–3 years after disease onset.² Mechanisms such as mitochondria dysfunction, altered axonal transport, disturbances in RNA metabolism, inflammation, proteasome impairment, and oxidative stress have been implicated in ALS pathologies.^{1,3} More than 20 ALS-linked genes have been identified to date, including pathogenic variants of *SOD1*, *FUS*, *C9ORF72*, and *TARDBP*, which are among the most extensively studied.⁴

Approximately 10% of ALS patients are inherited (familial), among which about 20% arise from mutations in the *SOD1* gene encoding the Cu/Zn-superoxide dismutase, a free radical-scavenging enzyme protecting cells against oxidative stress.⁵ More than 200 ALS-associated mutations have been found for *SOD1*, with Gly-to-Ala mutation at position 93 (G93A) and Gly-to-Arg mutation at position 85 (G85R) being the most extensively investigated.^{6,7} Previous studies documented protein aggregation as one of the most prominent features of ALS-associated *SOD1* mutations, suggesting a loss of effective folding and degradation of the mutant *SOD1* proteins.⁸ Nevertheless, little is known about how those mutations of *SOD1* found in ALS patients modulate its interactions with other cellular proteins.

In recent years, proximity-based labeling methods, including biotin ligase BirA^{R118G} (BioID), engineered ascorbate peroxidase (APEX), and TurboID, have been widely employed to examine protein–protein interactions in the native cellular environment.^{9–11} Both BioID and APEX rely on the generation of a reactive biotin derivative to label proximal endogenous proteins.^{9,10} However, compared with BioID, APEX exhibits much faster reaction kinetics and shorter labeling time, which enable the study of short-lived cell states.¹²

In this study, we employed APEX-based proximity labeling using the improved second-generation enzyme APEX2¹⁰ in conjunction with liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis, to examine how two ALS-associated mutations of SOD1, that is, the G93A and G85R substitutions,¹² perturb the interactions of SOD1 with other cellular proteins. We uncovered that the two ALS-associated mutants elicited alterations in a number of proteins in the proximity proteome of SOD1, including a markedly elevated presence of exportin 5 (XPO5). We also demonstrated that the aberrant interactions of SOD1 mutants with XPO5 resulted in the compromised export of pre-miRNA and elevated biogenesis of mature miRNA.

EXPERIMENTAL SECTION

Cell Culture.

HEK293T cells (ATCC) and N2a cells (Thermo Fisher) were maintained in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin/streptomycin (at 10,000 U/mL, Thermo Fisher). The cells were cultured at an 80% confluency under 37 °C in a humidified atmosphere containing 5% CO₂.

Plasmids.

Plasmids for wild-type (WT) SOD1 (plasmid #26397), SOD1^{G85R} (plasmid #26400), and SOD1^{G93A} (plasmid#26401) were obtained from Addgene. The plasmids for APEX experiments were engineered from Mito-V5-APEX2 (Addgene, plasmid #72480) by removing the region encoding the mitochondria matrix targeting sequence and by inserting NotI, EagI, EaeI, BsiEI, ApoI, EcoRI, NheI, and BmtI restriction recognition sites to generate the V5-APEX2 vector (Figure S1). The coding sequences of WT and mutant SOD1 were subsequently cloned into the NotI and NheI restriction sites of the V5-APEX2 vector on the N-terminus of the V5 tag to generate the SOD1^{WT}-APEX2, SOD1^{G85R}-APEX2, and SOD1^{G93A}-APEX2 plasmids. The SOD1^{WT}-Flag, SOD1^{G85R}-Flag, and SOD1^{G93A}-Flag for expressing SOD1 variants with three tandem repeats of the Flag tag being fused on the C-terminus were constructed by inserting the coding sequences of the SOD1 variants into the HindIII and XbaI restriction sites of the pRK7 3× Flag vector (Addgene, plasmid #8996). All constructs were confirmed by Sanger sequencing.

Cell Lysis and Proteomic Sample Preparation.

APEX labeling was performed at 24 h after the transfection of HEK293T cells with the aforementioned SOD1-APEX2 plasmids, following recently described procedures.¹³ In brief, approximately 2×10^7 cells transfected with the plasmids were incubated with 500 μ M

at 1% protein FDR. Normalized label-free quantification was used for protein quantification with a minimum ratio count of two.

The MS proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE¹⁴ partner repository with the dataset identifier PXD035452 (username: reviewer_pxd035452@ebi.ac.uk; password: oleWYQkO).

Immunoprecipitation and Western Blot.

Approximately 2×10^7 cells were harvested and lysed in CellLytic M Cell Lysis reagent supplemented with a complete protease inhibitor cocktail (Sigma-Aldrich). The lysates were subjected to rotation at 4 °C for 15 min, followed by sonication using a Qsonica sonicator q125 (1/8 probe) for 15 s three times (42% amplitude, 15 s on/15 s off). After sonication, the cell lysates were rotated for another 15 min and then centrifuged at 13,000 rpm for 10 min at 4 °C. The supernatant was incubated with prewashed anti-Flag M2 beads at 4 °C for 2 h. The beads were washed three times with PBS-T (PBS buffer containing 0.05% Tween 20), and subsequently boiled in 2× SDS-PAGE loading buffer to elute the captured proteins for western blot analysis.

Antibodies recognizing human XPO5 (Proteintech, #28628-1-AP; 1:2000), SOD1 (Proteintech, #10269-1-AP; 1:4000), V5 (Proteintech, #14440-1-AP; 1:2000), streptavidin (Thermo Scientific, #S911), and lamin B1 (Proteintech, #12987-1-AP; 1:10000) were used as primary antibodies for western blot analysis. Goat antirabbit IgG (whole molecule)-peroxidase antibody (Sigma, #A0545; 1:4000) and *m*-IgGκ BP-HRP (Santa Cruz Biotechnology, #sc-516102; 1:4000) were employed as secondary antibodies. Anti-GAPDH (Santa Cruz Biotechnology, #sc-32233; 1:10000) was used for internal control (GAPDH) to confirm an equal amount of protein loading.

Real-Time-Quantitative PCR Analysis of Expression Levels for Pre-miRNA and Mature miRNA.

Pre-miRNAs were extracted by following previously published procedures.¹⁵ Cells were seeded in six-well plates at a 35% confluence level and transfected with plasmids encoding SOD1^{WT}-Flag, SOD1^{G85R}-Flag, and SOD1^{G93A}-Flag the following day.

Cytosolic RNAs were extracted 24 h after transfection using cytoplasmic extraction reagents I and II, followed by the extraction of nuclear RNA from the cell pellet using 50 μL of nuclear extraction reagent (Thermo Scientific, #78833). Both cytosolic and nuclear RNAs were treated with 1 mL of TRIzol, followed by cDNA synthesis using a previously published method.⁸ Approximately 2 μg RNA was denatured and annealed by incubating with random hexamer-containing reverse primers of the four mature miRNAs at 70 °C for 5 min. The resulting RNA was quickly placed on ice, followed by reverse transcription at 42 °C for 1 h and 75 °C for 15 min using 100 units of M-MLV reverse transcriptase (Promega), M-MLV buffer, dNTP mixture, and RNase inhibitor. Pre-miRNA primers and Luna Universal qPCR Master Mix were subsequently added to the mixture, followed by real-time quantitative PCR (RT-qPCR) analysis on a Bio-Rad iCycler system, with the running protocol being set at 95 °C for 3 min and 55 cycles at 95 °C for 15 s, 55 °C for

30 s, and 72 °C for 45 s. The primers are listed in Table S1. The levels of pre-miRNA were normalized to that of *GAPDH* mRNA.

Mature miRNA was isolated as described previously.¹⁶ Cells were seeded in a six-well plate at a 35% confluence level and transfected with SOD1^{WT}-Flag, SOD1^{G85R}-Flag, and SOD1^{G93A}-Flag the following day. Total RNA was extracted at 24 h after transfection using TRIzol, followed by cDNA synthesis. In general, approximately 1 µg of RNA was reverse-transcribed by employing 100 units of M-MLV reverse transcriptase (Promega), 1 unit of poly(A) polymerase (New England Biolabs), and a 5'-tagged oligo(dT)₁₅ primer. After a 1 h incubation at 42 °C, the reverse transcriptase was deactivated by heating the mixture at 95 °C for 5 min. RT-qPCR experiments were performed using the Luna Universal qPCR Master Mix on a Bio-Rad iCycler system. The primers are listed in Table S1. The levels of mature miRNAs were normalized to the mRNA level of *GAPDH*.

RESULTS

APEX2 Labeling in Combination with LC–MS/MS for Assessing the Interactomes of SOD1^{WT}, SOD1^{G85R}, and SOD1^{G93A}.

In this study, we employed APEX labeling, coupled with LC–MS/MS, to explore the differences in interaction proteomes of SOD1^{WT}, SOD1^{G85R}, and SOD1^{G93A} (Figure 1a). We first optimized the amounts of plasmids employed for the transfection so as to achieve a similar level of expression for SOD1^{WT}, SOD1^{G85R}, and SOD1^{G93A}, as suggested by western blot analysis (Figure 1b). We next performed APEX labeling, and our results from western blot analysis indicated that the biotin labeling efficiencies for SOD1^{WT}, SOD1^{G85R}, and SOD1^{G93A} were similar (Figure 1c). In this vein, it is of note that western blot analysis only provided a semiquantitative assessment of protein expression.

The combination of APEX labeling with LC–MS/MS analysis revealed substantial (by at least 1.5-fold) enrichments of 43 and 24 proteins in the proximity proteomes of SOD1^{G85R} and SOD1^{G93A}, respectively, relative to SOD1^{WT} (Figure 1d,e). In addition, gene ontology analysis of the biological processes for the enriched proximity proteins of the two mutants showed perturbations in several important pathways, including tRNA aminoacylation for protein translation, regulation of macro-autophagy, phosphorylation, vesicle-mediated transport, ER to Golgi vesicle-mediated transport, and RNA splicing (Figure S2). In this vein, NOP56 was found to be enriched in the proximity proteome of SOD1^{G85R} only and was a previously reported ALS-associated gene that is involved in ribosomal subunit assembly and pre-rRNA processing (Figure 1e).^{17,18} Moreover, several proteins involved in RNA metabolism, such as XPO5, NONO, SRSF6, and YTHDC1, were detected only in the proximity proteomes of SOD1^{G85R} and SOD1^{G93A} but not that of SOD1^{WT}. SEC23A and SEC23B, which are intracellular trafficking proteins and previously shown to interact with SOD1^{G85R},¹⁹ were also detected in our APEX labeling experiments (Tables S2 and S3).

Our results also showed that 221 and 426 proteins are enriched in the proximity proteomes of SOD1^{WT}, relative to SOD1^{G85R} and SOD1^{G93A}, respectively (Figure 1d). Gene ontology analysis for the enriched proteins showed several important pathways, including cytoplasmic translation, stress granule assembly, nucleosome assembly, chromatin silencing, mRNA

destabilization, and so forth (Figure S2). Along this line, several ribosomal proteins, including RPS10, RPS13, RPS14, RPS17, RPL23A, RPL26, and RPL27A, are enriched in the proximity proteome of SOD1^{WT} only (Figure 1e).

XPO5 Displays Preferential Interactions with SOD1^{G85R} and SOD1^{G93A} over WT SOD1.

XPO5 is one of the most pronouncedly enriched proximity proteins of SOD1^{G85R} and SOD1^{G93A}, and it is worth further investigation owing to its importance in small RNA transport and miRNA maturation.^{20,21} We monitored the peak areas for one of the unique peptides of XPO5, that is, DPLLLAIIPK, identified from the proximity proteomes of SOD1^{WT}, SOD1^{G85R}, and SOD1^{G93A}. We found that the peak areas observed in the selected-ion chromatograms for the [M + 2H]²⁺ ion of the peptide obtained for the proximity proteome samples of SOD1^{G85R} and SOD1^{G93A} were much larger than that of SOD1^{WT}, indicating stronger interactions of XPO5 with SOD1^{G85R} and SOD1^{G93A} than with SOD1^{WT} (Figure S3a). In this vein, the sequence of the XPO5 peptide was supported by its MS/MS (Figure S3b).

To further validate the aforementioned findings, we constructed SOD1^{WT}-Flag, SOD1^{G85R}-Flag, and SOD1^{G93A}-Flag by fusing three tandem repeats of the Flag epitope tag to the C-terminus of the SOD1 variants, and performed co-immunoprecipitation using anti-Flag beads, followed by western blot analysis. Our results revealed much stronger interactions between XPO5 and SOD1^{G85R}-Flag/SOD1^{G93A}-Flag than SOD1^{WT}-Flag in both HEK293T and N2a neuroblastoma cells (Figure 2a–d). It is of note that there was a band detected at the XPO5 location in the control pull-down samples for HEK293T and N2a cells without plasmid transfection or with transfection of empty plasmid; nonetheless, the intensity of that band in these control samples is much weaker than what we observed for the mutant SOD1-Flag pull-down samples. Together, the above MS data and western blot results substantiate that XPO5 interacts preferentially with SOD1^{G85R} and SOD1^{G93A} than with SOD1^{WT}.

ALS-Associated SOD1 Mutants Modulate the Nucleocytoplasmic Transport of Pre-miRNA and miRNA Maturation.

miRNAs are essential single-stranded noncoding RNA molecules that function in RNA silencing and gene expression regulation.²² One of the major established functions of XPO5 is to transport pre-miRNA from the nucleus to the cytosol, and the transported pre-miRNA is further processed into mature miRNA by Dicer.²⁰ Thus, we next asked whether the ectopic expression of ALS-associated mutants of SOD1 perturbs the nucleocytoplasmic transport of pre-miRNAs. Toward this end, we monitored several representative pre-miRNAs that are highly expressed in mice and humans,^{23–25} including miR-let7b, miR-30a, and miR-143. Our results revealed that, when compared with cells with the ectopic expression of WT SOD1, the ectopic expression of the two mutants of SOD1 results in elevated distributions of pre-miR-let7b, pre-miR-30a, and pre-miR-143 in the nuclei, which are accompanied by their diminished presence in the cytosol (Figure 3a). In this regard, we assessed the purities of the nuclear and cytoplasmic fractions by western blot analysis. We found that, while the cytoplasmic fraction has ~9.5% contamination of nuclear proteins (as reflected by the lamin B signal), the nuclear fraction is free of contamination from the cytoplasmic fraction (Figure S4). Together, the above results suggest that the augmented interactions between mutant

SOD1 proteins and XPO5 suppress the latter's function in exporting pre-miRNA from the nucleus to the cytosol.

After export into the cytosol, pre-miRNAs are subjected to cleavage by Dicer to generate mature miRNAs.²⁶ Thus, we also examined how ectopic expressions of the WT and the two ALS-linked mutants of SOD1 modulate the levels of mature miRNAs. Our result from RT-qPCR analysis revealed that the relative levels of mature miR-30a, miR-let7b, miR-100, and miR-15a were significantly higher in cells with the ectopic expression of the SOD1 mutants than in cells expressing SOD1^{WT} ($p < 0.05$) (Figure 3b).

DISCUSSION

ALS is a fatal neurodegenerative disease, and ALS patients manifest with the progressive loss of motor neuron functions, with about 2 to 3 years of survival time after the disease onset in most cases.² Neurons of ALS patients display abnormal protein aggregation, disturbance of proteostasis, and dysregulations in RNA metabolism.^{1,8,27} In the latter respect, perturbations in miRNA biogenesis, alternative splicing, and RNA transport are now considered the major mechanisms underlying ALS pathogenesis. Many studies have substantiated that ALS-associated mutations in *TDP43* and *FUS*, which are RNA-binding proteins, can alter RNA metabolism.^{28,29} Additionally, ALS-linked mutants of SOD1 were shown to perturb indirectly the RNA metabolism by modulating the functions of TDP43.³⁰ *SOD1* was the earliest identified gene mutated in ALS, and its mutations account for 20% of familial ALS and 5% of apparently sporadic ALS.^{31,32} SOD1^{G85R} and SOD1^{G93A} are among the most well-studied ALS-associated mutations in SOD1, and both mutants are known to elicit protein aggregation and misfolding in cells.⁸ To date, the protein interactomes for these two SOD1 mutants remain largely unexplored.

Here, we employed APEX labeling and label-free quantification using LC-MS/MS to examine the proximity proteomes for SOD1^{WT}, SOD1^{G85R}, and SOD1^{G93A}. We found that, when compared with SOD1^{WT}, 43 and 24 proteins were enriched in the proximity proteomes of SOD1^{G85R} and SOD1^{G93A}, respectively (Figure 1d). GO analysis of the proteins preferentially enriched in the proximity proteomes of the two SOD1 mutants revealed their involvement in several important biological pathways (Figure S2).

Among the differentially enriched proximity proteins, XPO5 exhibits preferential binding with the two mutants of SOD1 (Figure S2). We validated the proteomic results by immunoprecipitation, followed by western blot analysis, where both SOD1^{G85R} and SOD1^{G93A} display much more robust interactions with XPO5 than SOD1^{WT} in HEK293T cells (Figure 2a,b). Similar findings were made for N2a mouse neuroblastoma cells (Figure 2c,d). These results together revealed that XPO5 interacts preferentially with SOD1^{G85R} and SOD1^{G93A} than with SOD1^{WT}. In this regard, it is important to note the limitation of the immunoprecipitation method; that is, it does not support that the interactions between SOD1 mutants and XPO5 are direct. Future studies can be conducted using other techniques, for example, surface plasmon resonance and GST pull-down assay using purified recombinant proteins, to further examine whether XPO5 interacts directly with the SOD1 variants.

XPO5 is known to promote the nucleocytoplasmic transport of small endogenous RNAs, including pre-miRNAs.²⁰ Our real-time qPCR results revealed increased nuclear distributions and attenuated cytoplasmic distributions of pre-miRNAs, including pre-miR30a, pre-miR-let7a, and pre-miR-143 in cells expressing the two ALS-associated mutants of SOD1 than those expressing the WT protein (Figure 3a). This result supports that SOD1^{G93A} and SOD1^{G85R} may sequester XPO5, thereby rendering it unavailable for facilitating the export of pre-miRNAs from the nucleus to the cytosol (Figure 3c).

Transported pre-miRNAs in the cytosol are subjected to maturation by a multiprotein complex comprising of Dicer and its partners, Argonaute 2 (AGO2), TAR RNA-binding protein 2, and interferon-inducible double-stranded RNA-dependent protein kinase activator.³³ miRNAs play vital roles in many crucial biological processes, including, among others, gene silencing, RNA editing, RNA methylation, and RNA decay.³⁴ Recent studies showed that ALS-associated SOD1 mutations could perturb miRNA biogenesis and mRNA stability²⁸ and several miRNAs may serve as potential diagnostic biomarkers for ALS.³⁵ Thus, we also examined the consequences of the SOD1 mutations on miRNA maturation. Interestingly, we found that mature miR-30a, miR-let7b, miR-100, and miR-15a are present at higher levels in cells expressing the mutant SOD1 proteins than those expressing the WT counterpart (Figure 3b). At first glance, this finding appears to be incongruent with the fact that the expression of mutant SOD1 confers diminished levels of pre-miRNA in the cytosol for Dicer processing. However, aside from supporting the cytoplasmic export of pre-miRNAs, XPO5 exerts an inhibitory effect on Dicer when forming a complex with pre-miRNA and Ran-GTP.²¹ Thus, the sequestration of XPO5 by SOD1 mutants forges the elevated activity of the Dicer complex, which leads to augmented miRNA maturation (Figure 3c). Previous studies revealed aberrant miRNA biogenesis in ALS-related pathologies,^{33,36,37} and our results are in agreement with these studies. Importantly, our discovery of the interaction between XPO5 and the two mutants provides mechanistic insights into the roles of ALS-associated SOD1 mutants in miRNA biogenesis.

Apart from the identification of proteins exhibiting increased enrichment with the SOD1^{G85R} and SOD1^{G93A}, our APEX labeling coupled with LC-MS/MS analysis also results in the identifications of 221 and 426 proteins being enriched in the proximity proteome of SOD1^{WT} compared with SOD1^{G85R} and SOD1^{G93A}, respectively (Figure 1e). Gene ontology analysis of the proteins exhibiting increased presence in the proximity proteome of SOD1^{WT} showed the enrichment of several pathways, including stress granule formation, chromatin silencing, protein folding, chromatin silencing, and so forth (Figure S2). For instance, G3BP1 and TIA1 are important in stress granule formation,^{38,39} and ARL2 and CDC37 are essential for protein folding^{40,41} Future studies are needed to examine if the two SOD1 mutants elicit the loss of function of these pathways, which may also contribute to the development of ALS-associated pathologies.

In conclusion, we characterized comprehensively how two common ALS-linked mutations of SOD1 modulate its interaction proteomes and we uncovered, for the first time, XPO5 as an interaction partner of SOD1^{G85R} and SOD1^{G93A}. The enhanced interaction between XPO5 and the SOD1 mutants led to attenuated nucleocytoplasmic transport of pre-miRNA and augmented levels of mature miRNAs. It will be interesting to explore, in the future,

how altered interactions with other cellular proteins conferred by the two SOD1 mutants contribute to the etiology of ALS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

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REFERENCES

- (1). Taylor JP; Brown RH; Cleveland DW Decoding ALS: from genes to mechanism. *Nature* 2016, 539, 197–206. [PubMed: 27830784]
- (2). Kiernan MC; Vucic S; Talbot K; McDermott CJ; Hardiman O; Shefner JM; Al-Chalabi A; Huynh W; Cudkovic M; Talman P; Van den Berg LH; Dharmadasa T; Wicks P; Reilly C; Turner MR Improving clinical trial outcomes in amyotrophic lateral sclerosis. *Nat. Rev. Neurol* 2021, 17, 104–118. [PubMed: 33340024]
- (3). Ferraiuolo L; Kirby J; Grierson AJ; Sendtner M; Shaw PJ Molecular pathways of motor neuron injury in amyotrophic lateral sclerosis. *Nat. Rev. Neurol* 2011, 7, 616–630. [PubMed: 22051914]
- (4). Renton AE; Chiò A; Traynor BJ State of play in amyotrophic lateral sclerosis genetics. *Nat. Neurosci* 2014, 17, 17–23. [PubMed: 24369373]
- (5). Boillée S; Velde CV; Cleveland DW ALS: A Disease of Motor Neurons and Their Nonneuronal Neighbors. *Neuron* 2006, 52, 39–59. [PubMed: 17015226]
- (6). Matsumoto G; Stojanovic A; Holmberg CI; Kim S; Morimoto RI Structural properties and neuronal toxicity of amyotrophic lateral sclerosis-associated Cu/Zn superoxide dismutase 1 aggregates. *J. Cell Biol* 2005, 171, 75–85. [PubMed: 16216923]
- (7). Raoul C; Estévez AG; Nishimune H; Cleveland DW; deLapeyrière O; Henderson CE; Haase G; Pettmann B Motoneuron death triggered by a specific pathway downstream of Fas: Potentiation by ALS-linked SOD1 mutations. *Neuron* 2002, 35, 1067–1083. [PubMed: 12354397]
- (8). Ross CA; Poirier MA Protein aggregation and neurodegenerative disease. *Nat. Med* 2004, 10, S10–S17. [PubMed: 15272267]
- (9). Kim DI; Jensen SC; Noble KA; Kc B; Roux KH; Motamedchaboki K; Roux KJ An improved smaller biotin ligase for BioID proximity labeling. *Mol. Biol. Cell* 2016, 27, 1188–1196. [PubMed: 26912792]
- (10). Lam SS; Martell JD; Kamer KJ; Deerinck TJ; Ellisman MH; Mootha VK; Ting AY Directed evolution of APEX2 for electron microscopy and proximity labeling. *Nat. Methods* 2015, 12, 51–54. [PubMed: 25419960]
- (11). Branon TC; Bosch JA; Sanchez AD; Udeshi ND; Svinkina T; Carr SA; Feldman JL; Perrimon N; Ting AY Efficient proximity labeling in living cells and organisms with TurboID. *Nat. Biotechnol* 2018, 36, 880–887. [PubMed: 30125270]
- (12). Hung V; Udeshi ND; Lam SS; Loh KH; Cox KJ; Pedram K; Carr SA; Ting AY Spatially resolved proteomic mapping in living cells with the engineered peroxidase APEX2. *Nat. Protoc* 2016, 11, 456–475. [PubMed: 26866790]
- (13). Liu X; Yang Y-Y; Wang Y HSP90 and Aha1 modulate microRNA maturation through promoting the folding of Dicer1. *Nucleic Acids Res* 2022, 50, 6990–7001. [PubMed: 35736213]
- (14). Perez-Riverol Y; Bai J; Bandla C; García-Seisdedos D; Hewapathirana S; Kamatchinathan S; Kundu DJ; Prakash A; Frericks-Zipper A; Eisenacher M; Walzer M; Wang S; Brazma A; Vizcaíno JA The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics evidences. *Nucleic Acids Res* 2022, 50, D543–D552. [PubMed: 34723319]
- (15). Qin F; Shi X; Li H Separation of nuclear and cytoplasmic fractions for chimeric RNA characterization. *Methods Mol. Biol* 2020, 2079, 167–175. [PubMed: 31728970]

- (16). Balcells I; Cirera S; Busk PK Specific and sensitive quantitative RT-PCR of miRNAs with DNA primers. *BMC Biotechnol* 2011, 11, 70. [PubMed: 21702990]
- (17). Nel M; Mavundla T; Gultig K; Botha G; Mulder N; Benatar M; Wu J; Cooley A; Myers J; Rampersaud E; Wu G; Heckmann JM Repeats expansions in ATXN2, NOP56, NIPA1 and ATXN1 are not associated with ALS in Africans. *IBRO Neurosci. Rep* 2021, 10, 130–135. [PubMed: 34179866]
- (18). Todd TW; McEachin ZT; Chew J; Burch AR; Jansen-West K; Tong J; Yue M; Song Y; Castanedes-Casey M; Kurti A; Dunmore JH; Fryer JD; Zhang Y-J; San Millan B; Teijeira Bautista S; Arias M; Dickson D; Gendron TF; Sobrido M-J; Disney MD; Bassell GJ; Rossoll W; Petrucelli L Hexanucleotide Repeat Expansions in c9FTD/ALS and SCA36 Confer Selective Patterns of Neurodegeneration In Vivo. *Cell Rep* 2020, 31, 107616. [PubMed: 32375043]
- (19). Atkin JD; Farg MA; Soo KY; Walker AK; Halloran M; Turner BJ; Nagley P; Horne MK Mutant SOD1 inhibits ER-Golgi transport in amyotrophic lateral sclerosis. *J. Neurochem* 2014, 129, 190–204. [PubMed: 24134191]
- (20). Kim Y-K; Kim B; Kim VN Re-evaluation of the roles of DROSHA, Exportin 5, and DICER in microRNA biogenesis. *Proc. Natl. Acad. Sci. U.S.A* 2016, 113, No. E1881.
- (21). Zeng Y; Cullen BR Structural requirements for pre-microRNA binding and nuclear export by Exportin 5. *Nucleic Acids Res* 2004, 32, 4776–4785. [PubMed: 15356295]
- (22). Bartel DP MicroRNAs: Target recognition and regulatory functions. *Cell* 2009, 136, 215–233. [PubMed: 19167326]
- (23). Zhao C; Sun G; Li S; Lang M-F; Yang S; Li W; Shi Y MicroRNA let-7b regulates neural stem cell proliferation and differentiation by targeting nuclear receptor TLX signaling. *Proc. Natl. Acad. Sci. U.S.A* 2010, 107, 1876–1881. [PubMed: 20133835]
- (24). Maciejak A; Kostarska-Srokosz E; Gierlak W; Dluzniewski M; Kuch M; Marchel M; Opolski G; Kiliszek M; Matlak K; Dobrzycki S; Lukasik A; Segiet A; Sygitowicz G; Sitkiewicz D; Gora M; Burzynska B Circulating miR-30a-5p as a prognostic biomarker of left ventricular dysfunction after acute myocardial infarction. *Sci. Rep* 2018, 8, 9883. [PubMed: 29959359]
- (25). Lagos-Quintana M; Rauhut R; Lendeckel W; Tuschl T Identification of Novel Genes Coding for Small Expressed RNAs. *Science* 2001, 294, 853–858. [PubMed: 11679670]
- (26). Ha M; Kim VN Regulation of microRNA biogenesis. *Nat. Rev. Mol. Cell Biol* 2014, 15, 509–524. [PubMed: 25027649]
- (27). Rozas P; Bargsted L; Martínez F; Hetz C; Medinas DB The ER proteostasis network in ALS: Determining the differential motoneuron vulnerability. *Neurosci. Lett* 2017, 636, 9–15. [PubMed: 27150076]
- (28). Butti Z; Patten SA RNA dysregulation in amyotrophic lateral sclerosis. *Front. Genet* 2018, 9, 712. [PubMed: 30723494]
- (29). Pham J; Keon M; Brennan S; Saksena N Connecting RNA-modifying similarities of TDP-43, FUS, and SOD1 with microRNA dysregulation amidst a renewed network perspective of amyotrophic lateral sclerosis proteinopathy. *Int. J. Mol. Sci* 2020, 21, 3464. [PubMed: 32422969]
- (30). Jeon GS; Shim YM; Lee DY; Kim JS; Kang M; Ahn SH; Shin JY; Geum D; Hong YH; Sung JJ Pathological modification of TDP-43 in amyotrophic lateral sclerosis with SOD1 mutations. *Mol. Neurobiol* 2019, 56, 2007–2021. [PubMed: 29982983]
- (31). Rosen DR; Siddique T; Patterson D; Figlewicz DA; Sapp P; Hentati A; Donaldson D; Goto J; O'Regan JP; Deng HX; Rahmani Z; Krizus A; McKenna-Yasek D; Cayabyab A; Gaston SM; Berger R; Tanzi RE; Halperin JJ; Herzfeldt B; Van den Bergh R; Hung WY; Bird T; Deng G; Mulder DW; Smyth C; Laing NG; Soriano E; Pericak-Vance MA; Haines J; Rouleau GA; Gusella JS; Horvitz HR; Brown RH Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 1993, 362, 59–62. [PubMed: 8446170]
- (32). Kiernan MC; Vucic S; Cheah BC; Turner MR; Eisen A; Hardiman O; Burrell JR; Zoing MC Amyotrophic lateral sclerosis. *Lancet* 2011, 377, 942–955. [PubMed: 21296405]
- (33). Emde A; Eitan C; Liou L-L; Libby RT; Rivkin N; Magen I; Reichenstein I; Oppenheim H; Eilam R; Silvestroni A; Alajajian B; Ben-Dov IZ; Aebischer J; Savidor A; Levin Y; Sons R; Hammond SM; Ravits JM; Möller T; Hornstein E Dysregulated miRNA biogenesis downstream of cellular

- stress and ALS-causing mutations: a new mechanism for ALS. *EMBO J* 2015, 34, 2633–2651. [PubMed: 26330466]
- (34). Ha M; Kim VN Regulation of microRNA biogenesis. *Nat. Rev. Mol. Cell Biol* 2014, 15, 509–524. [PubMed: 25027649]
- (35). Parisi C; Arisi I; D'Ambrosi N; Storti AE; Brandi R; D'Onofrio M; Volonté C
Dysregulated microRNAs in amyotrophic lateral sclerosis microglia modulate genes linked to neuroinflammation. *Cell Death Dis* 2013, 4, No. e959.
- (36). Parisi C; Arisi I; D'Ambrosi N; Storti AE; Brandi R; D'Onofrio M; Volonté C
Dysregulated microRNAs in amyotrophic lateral sclerosis microglia modulate genes linked to neuroinflammation. *Cell Death Dis* 2013, 4, No. e959.
- (37). Zhang T; Wu YC; Mullane P; Ji YJ; Liu H; He L; Arora A; Hwang HY; Alessi AF; Niaki AG; Periz G; Guo L; Wang H; Elkayam E; Joshua-Tor L; Myong S; Kim JK; Shorter J; Ong SE; Leung AKL; Wang J FUS regulates activity of microRNA-mediated gene silencing. *Mol. Cell* 2018, 69, 787–801. [PubMed: 29499134]
- (38). Yang PG; Mathieu C; Kolaitis RM; Zhang PP; Messing J; Yurtsever U; Yang ZM; Wu JJ; Li YX; Pan QF; Yu JY; Martin EW; Mittag T; Kim HJ; Taylor JP G3BP1 Is a tunable switch that triggers phase separation to assemble stress granules. *Cell* 2020, 181, 325–345. [PubMed: 32302571]
- (39). Mackenzie IR; Nicholson AM; Sarkar M; Messing J; Purice MD; Pottier C; Annu K; Baker M; Perkerson RB; Kurti A; Matchett BJ; Mittag T; Temirov J; Hsiung GYR; Krieger C; Murray ME; Kato M; Fryer JD; Petrucelli L; Zinman L; Weintraub S; Mesulam M; Keith J; Zivkovic SA; Hirsch-Reinshagen V; Roos RP; Züchner S; Graff-Radford NR; Petersen RC; Caselli RJ; Wszolek ZK; Finger E; Lippa C; Lacomis D; Stewart H; Dickson DW; Kim HJ; Rogava E; Bigio E; Boylan KB; Taylor JP; Rademakers R TIA1 mutations in amyotrophic lateral sclerosis and frontotemporal dementia promote phase separation and alter stress granule dynamics. *Neuron* 2017, 95, 808–816. [PubMed: 28817800]
- (40). Bhamidipati A; Lewis SA; Cowan NJ ADP ribosylation factor-like protein 2 (Arl2) regulates the interaction of tubulin-folding cofactor D with native tubulin. *J. Cell Biol* 2000, 149, 1087–1096. [PubMed: 10831612]
- (41). Young JC; Agashe VR; Siegers K; Hartl FU Pathways of chaperone-mediated protein folding in the cytosol. *Nat. Rev. Mol. Cell Biol* 2004, 5, 781–791. [PubMed: 15459659]

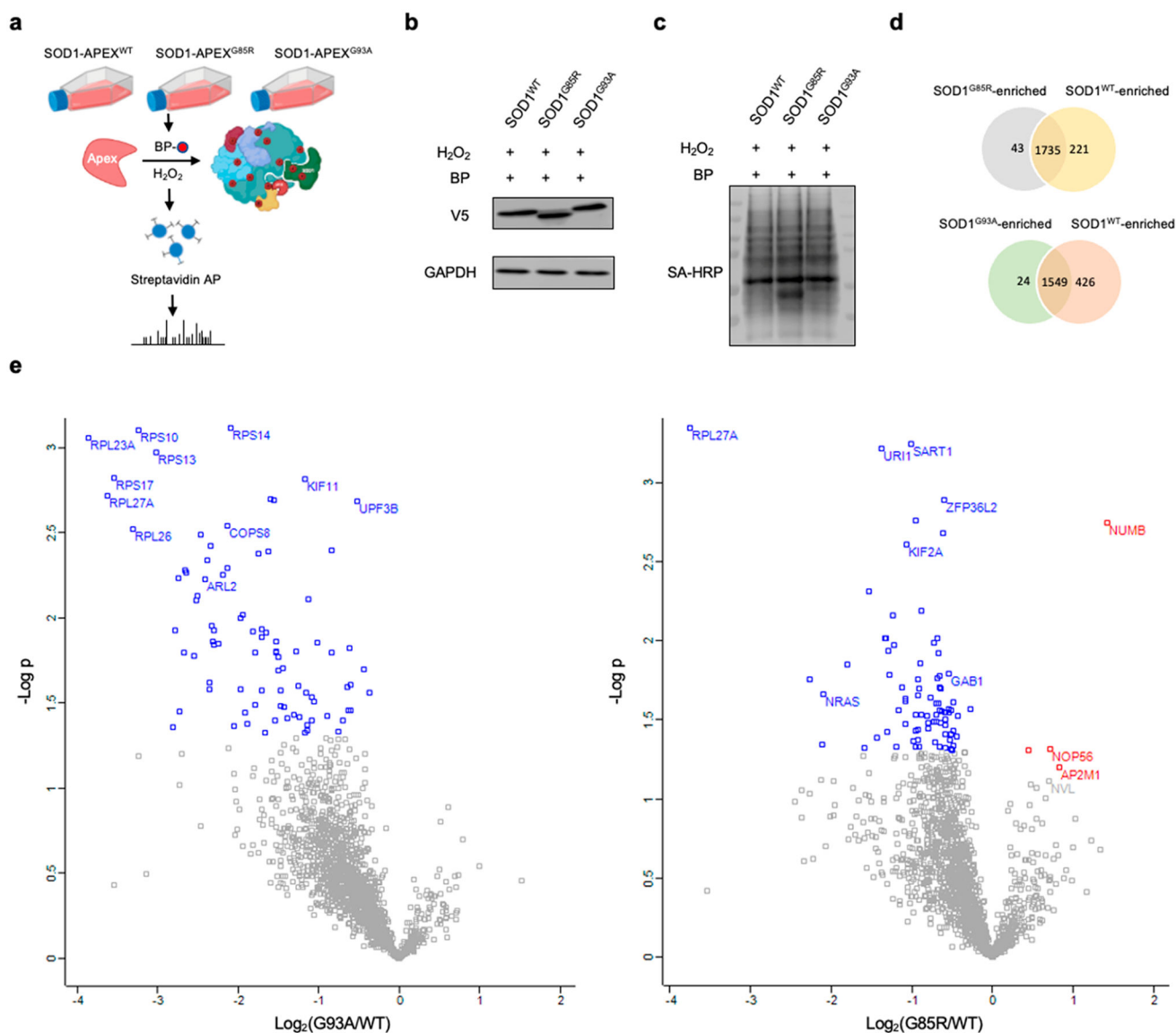


Figure 1. Combination of APEX2 labeling with LC-MS/MS revealed XPO5 as a candidate interaction partner of SOD1^{G85R} and SOD1^{G93A}. (a) Schematic diagram showing the workflow for APEX labeling. “BP” and “AP” represent biotin phenol and affinity pull-down, respectively. (b) Western blot showing a similar level of expression for different SOD1-APEX2 variants in HEK293T cells. (c) Western blot indicated similar APEX labeling efficiencies for SOD1^{WT}, SOD1^{G85R}, and SOD1^{G93A}. Cells were transfected with plasmids encoding APEX2-conjugated SOD1^{WT}, SOD1^{G85R}, and SOD1^{G93A} for 24 h, followed by treatments with biotin phenol for 30 min and then with H₂O₂ for 1 min. “SA-HRP” represents streptavidin-horseradish peroxidase. (d) Venn diagrams comparing the proximity proteomes of SOD1^{G85R} vs SOD1^{WT} and SOD1^{G93A} vs SOD1^{WT}. In total, 1999 proteins were identified. (e) Volcano plots displaying fold changes for the proteins being pulled down with streptavidin beads. The *p* values were calculated by using paired, two-tailed Student’s

t -test. The red and blue labels designate those with statistically significant fold changes ($p < 0.05$).

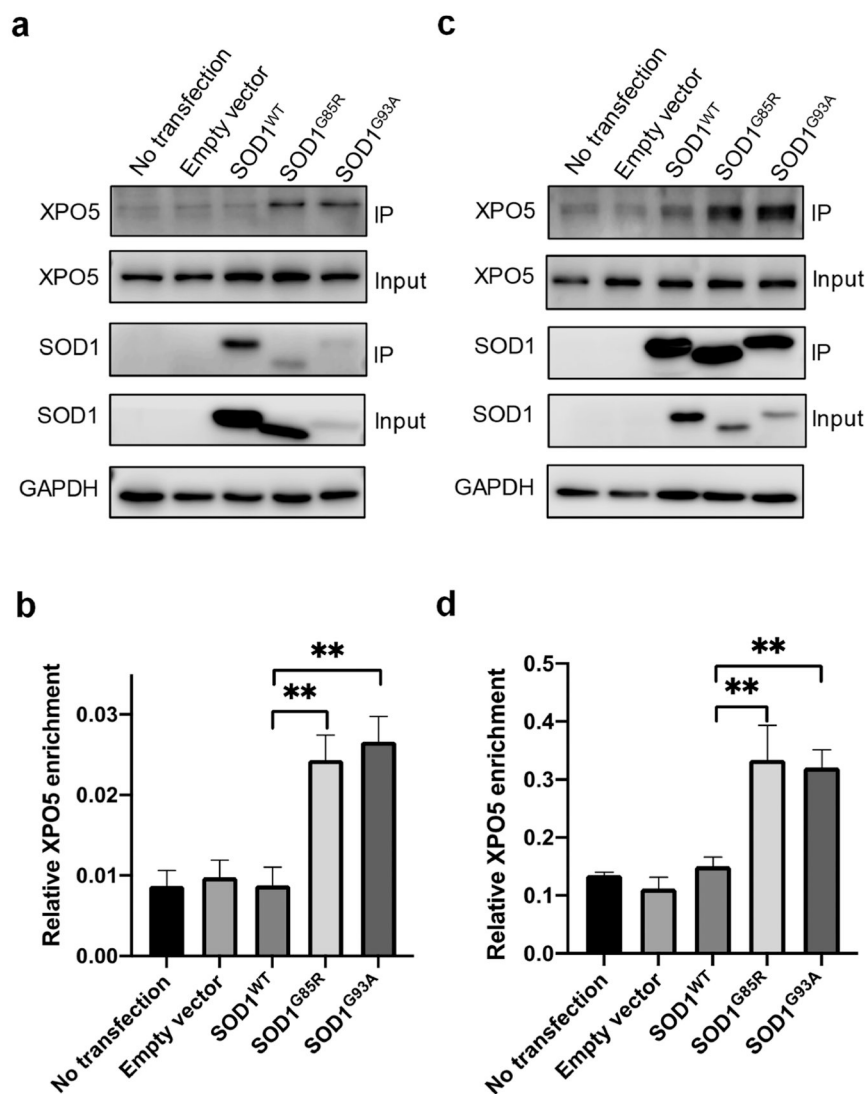


Figure 2. Immunoprecipitation followed by western blot analysis revealed stronger interactions between XPO5 and SOD1^{G85R}/SOD1^{G93A} than SOD1^{WT} in HEK293T and N2a cells. (a,c) HEK293T and N2a cells were transfected with the empty vector (pRK7), SOD1^{WT}-Flag, SOD1^{G85R}-Flag, and SOD1^{G93A}-Flag for 24 h, and the resulting lysates were incubated with anti-Flag beads for 2 h. Western blot was used to monitor the interaction between XPO5 and the three Flag-tagged proteins. (b,d) The relative enrichment of XPO5 (a,c) was quantified using Image Studio based on the band intensities of XPO5 in the IP samples and normalized to those of XPO5 in the corresponding input samples ($n = 3$). The p values were calculated using one-way ANOVA: *, $0.01 < p < 0.05$; **, $0.001 < p < 0.01$; ***, $p < 0.001$.

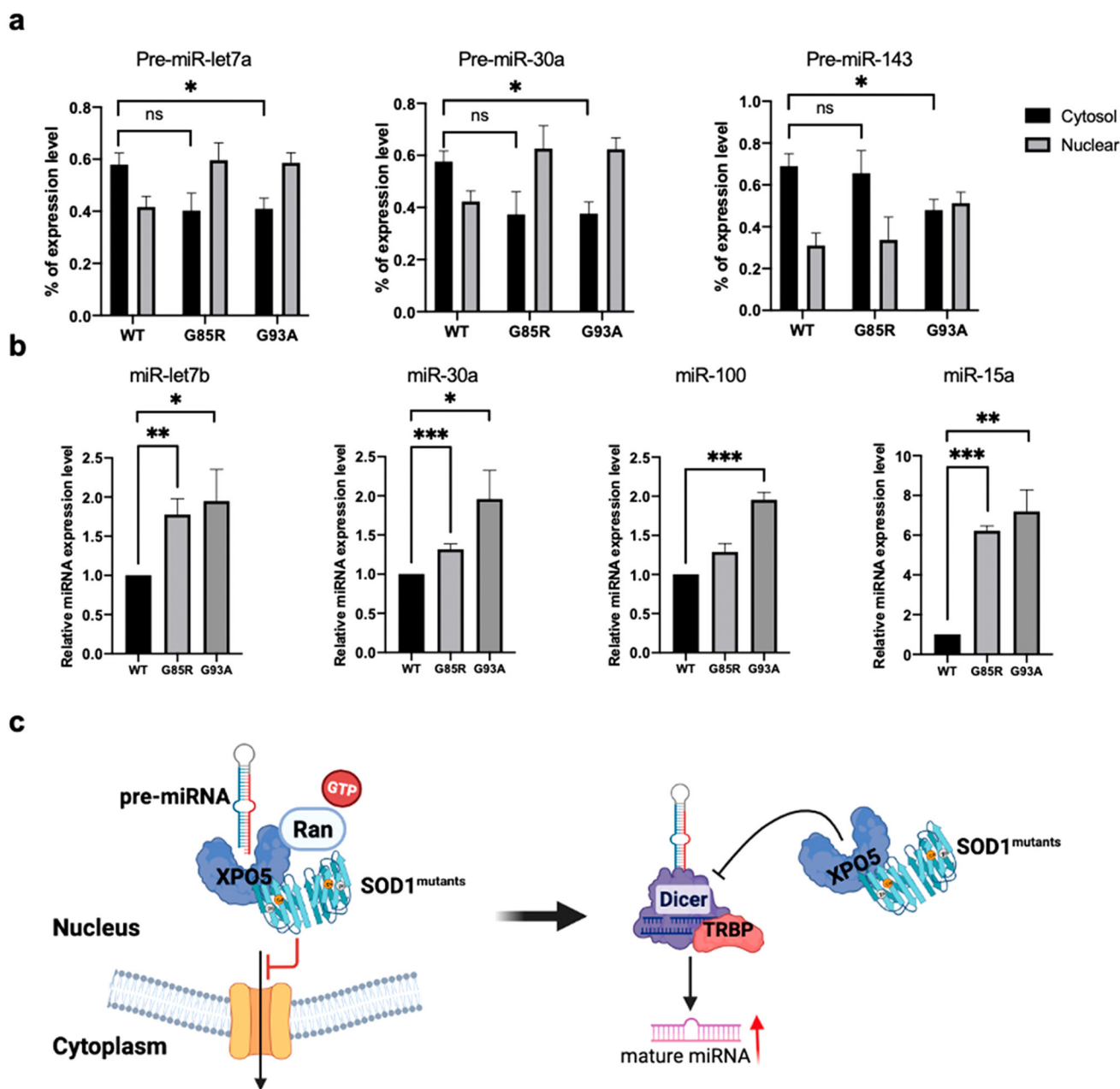


Figure 3.

Ectopic expression of the G85R and G93A mutants of SOD1 perturbs the nucleocytoplasmic transport of pre-miRNAs and the maturation of miRNAs. (a) RT-qPCR results showing increases in the nuclear levels and decreases in the cytosol levels of pre-miR-let7a, pre-miR-30a, and pre-miR-143 in cells expressing SOD1^{G93A} and SOD1^{G95R} compared to those expressing the WT protein. (b) RT-qPCR results depicting substantially increased levels of mature miR-30a, miR-let7b, miR-100, and miR-15a in cells with the ectopic expression of SOD1^{G85R} and SOD1^{G93A} than those with SOD1^{WT}. (c) Schematic diagram illustrating the inhibitory effect of ALS-associated SOD1 mutants on XPO5-mediated nucleocytoplasmic transport of pre-miRNAs and on diminished inhibitory effects of XPO5 toward the Dicer-

mediated cleavage of pre-miRNAs to mature miRNAs. All data were normalized to the mRNA level of *GAPDH*. The *p* values were calculated using unpaired, two-tailed Student's *t*-test: ns, not significant ($p > 0.05$); *, 0.01 $p < 0.05$; **, 0.001 $p < 0.01$; ***, $p < 0.001$.

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