Journal of Molecular Neuroscience
https://doi.org/10.1007/s12031-022-02071-1

Integrative Analysis of Motor Neuron and Microglial Transcriptomes from SOD1^{G93A} Mice Models Uncover Potential Drug Treatments for ALS

Elif Kubat Oktem[1] · Busra Aydin[2] · Metin Yazar[3,4] · Kazim Yalcin Arga[4,5]

Received: 28 May 2022 / Accepted: 19 September 2022

Abstract
Amyotrophic lateral sclerosis (ALS) is a fatal disease of motor neurons that mainly affects the motor cortex, brainstem, and spinal cord. Under disease conditions, microglia could possess two distinct profiles, M1 (toxic) and M2 (protective), with the M2 profile observed at disease onset. SOD1 (superoxide dismutase 1) gene mutations account for up to 20% of familial ALS cases. Comparative gene expression differences in M2-protective (early) stage SOD1^{G93A} microglia and age-matched SOD1^{G93A} motor neurons are poorly understood. We evaluated the differential gene expression profiles in SOD1^{G93A} microglia and SOD1^{G93A} motor neurons utilizing publicly available transcriptomics data and bioinformatics analyses, constructed biomolecular networks around them, and identified gene clusters as potential drug targets. Following a drug repositioning strategy, 5 small compounds (belinostat, auranofin, BRD-K78930611, AZD-8055, and COT-10b) were repositioned as potential ALS therapeutic candidates that mimic the protective state of microglia and reverse the toxic state of motor neurons. We anticipate that this study will provide new insights into the ALS pathophysiology linking the M2 state of microglia and drug repositioning.

Keywords SOD1 mutation · Amyotrophic lateral sclerosis · Transcriptomic · Drug repositioning · Repositioned therapeutics

Introduction

Amyotrophic lateral sclerosis (ALS) is a lethal disease of the motor neurons controlling voluntary muscle functions. The motor cortex, brainstem, and spinal cord are the areas most affected by this disease (Kaur et al. 2016). Familial ALS cases account for 5–10% of all ALS cases, and up to 20% of familial cases involve SOD1 (superoxide dismutase 1) gene mutations (Rosen et al. 1993; Sreedharan and Brown 2013). Under normal conditions, SOD1 catalyzes the inactivation of superoxide radicals into hydrogen peroxide and oxygen, providing a crucial antioxidant defense mechanism against superoxide radicals. Mutations of the SOD1 gene lead to neuronal hyperexcitability (Kubat Öktem et al. 2016), mitochondrial mutations and dysfunction, cytoskeletal abnormalities, differential gene expression, and aberrant protein interactions (Liu et al. 2004). G93A is the most studied SOD1mutation in the literature (Prudencio et al. 2009), and transgenic models of this mutation have been essential for understanding the pathophysiology of the SOD1 mutation.

Although microglia also contribute to ALS and motor neuron degeneration, the mechanisms that may influence disease progression remain controversial. Studies demonstrate that microglia, which are thought to be the resident immune cells of the brain, play a dual role in ALS. The two different profiles, M1 (toxic) and M2 (protective), are

✉ Elif Kubat Oktem
ekoktem@outlook.com

1 Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Istanbul Medeniyet University, Kuzey Yerleşkesi H Blok, Ünalan Sk. D100 Karayolu Yanyol 34700, Istanbul, Turkey

2 Department of Bioengineering, Faculty of Engineering and Architecture, Konya Food and Agriculture University, Konya, Turkey

3 Department of Genetics and Bioengineering, Faculty of Engineering and Natural Sciences, Istanbul Okan University, Istanbul, Turkey

4 Department of Bioengineering, Faculty of Engineering, Marmara University, Istanbul, Turkey

5 Genetic and Metabolic Diseases Research and Investigation Center, Marmara University, Istanbul, Turkey

observed at different stages of the disease and lead to the production of distinct effector molecules (Geloso et al. 2017). In the M1 phenotype, pro-inflammatory cytokines are secreted leading to neuronal network dysfunction, whereas the M2 phenotype is activated by anti-inflammatory cytokines that restore hemostasis (Du et al. 2017). The M1 phenotype is activated at the disease end-stage, whereas the M2 phenotype is activated at the disease onset (Liao et al. 2012). According to the defined disease stages of the transgenic SOD1 models of ALS, an age of 70–130 days after birth is considered the onset of the disease, where the initial signs of motor deficiency are observed. The end-stage of the disease originates after 150 days of postnatal age and is associated with full paralysis of the lower limbs (Lee et al. 2013).

Little is apprehended on comparative gene expression alterations of microglia and motor neurons, although transciptomic analysis of these cell types was studied distinctly (Henriques et al. 2015; Noristani et al. 2015). On the other hand, the comparative evaluation of mRNA expression profiles from the two cell types, accepted as having protective and toxic behaviors in ALS, respectively, may pave the way for development of more effective treatment strategies by virtue of drug repositioning.

We used publicly available transcriptome data and bioinformatic analyses to compare gene expression profiles in mice $SOD1^{G93A}$ motor neuron and microglia samples with $SOD1^{WT}$ samples in this study. We also present our findings in the context of drug repositioning and propose novel therapeutics to be developed for the treatment of ALS.

Materials and Methods

$SOD1^{G93A}$ Mice Microglia and Motor Neuron Data

Data from mice microglia and motor neurons were used for the study. The Gene Expression Omnibus database (NCBI-GEO) provides the transcriptome datasets (Table 1) online (Barrett et al. 2005). GSE96047, representing the gene expression profiles in mice microglia, included three control samples with $SOD1^{WT}$, three mice microglia spinal cord samples with $SOD1^{G93A}$ at 90 days of age (Noristani et al. 2015). Gene expression profiles in mice spinal cord motor neuron were provided by GSE60856, consisted of seven control samples with $SOD1^{WT}$ and seven samples with $SOD1^{G93A}$ at 15 weeks of age (Henriques et al. 2015) Although the GSE60856 dataset also consisted of $SOD1^{G93A}$ mice treated with GCSF at week 15, only the samples with $SOD1^{G93A}$ mice at week 15 (untreated with GCSF) were used to allow appropriate comparison with the microglia data.

Differential Gene Expression (DEG) Analysis

A previously constructed statistical analysis procedure (Öktem et al. 2019) was adopted in the present study to determine differentially expressed genes (DEGs). Raw data from the Affymetrix datasets were read into the statistical software R (version 4.1.0) via the Affy package (Gautier et al. 2004) and normalized via the justRMA package (Bolstad et al. 2003) in the Bioconductor platform (version Rx64 3.0.2) (Gentleman et al. 2004) using Robust Multi-Array Average (RMA). The Linear Models for Microarray Data (LIMMA) package (Smyth et al. 2005) was used to compare the normalized mRNA expression levels and to determine DEGs. The Benjamini-Hochberg correction was applied to control the false discovery rate. To determine the statistical significance of DEGs, a fold-change cut-off ≤ 0.5 (for downregulation) or ≥ 2 (for upregulation) and an adjusted p value cut-off of < 0.05 were applied.

Functional Enrichment Analysis

Pathway and functional enrichment analyses were performed using Metascape (Zhou et al. 2019) to reveal functional annotations (i.e., biological processes and molecular pathways) significantly associated with DEGs. The KEGG

Table 1 GEO transcriptome datasets

GEO reference series	Organism	Sample subsets	Array	Reference
GSE60856	Mus musculus	7 wild-type mice motor neurons at week 15 (control) 7 $SOD1^{G93A}$ mice motor neuron at week 15	Affymetrix Mice Genome 430 2.0 Array	(Henriques et al. 2015)
GSE96047	Mus musculus	3 wild-type mice microglia at day 90 (control) 3 $SOD1^{G93A}$ mice microglia at day 90	Affymetrix Mice Genome 430 2.0 Array	(Noristani et al. 2015)

SOD1 superoxide dismutase 1 gene

Pathway (Kanehisa et al. 2008), Gene Ontology (GO) Biological Processes (Ashburner et al. 2000; Carbon et al. 2021), and Reactome (Fabregat et al. 2016) databases were employed as annotation resources.

Construction of Protein–Protein Interaction (PPI) Network Around DEGs

DEGs for both datasets were used as queries for the PPI construction. Because we were interested in the ALS protective biomarkers of microglia, DEGs were selected on the basis of differences, so microglia-specific and motor neuron–specific DEGs (the number of DEGs in each group is given in Table 2) were used for the dataset-specific PPI constructions. Human orthologs of the DEGS were retrieved from Ensembl:Biomart (Smedley et al. 2015). The PPI data were retrieved from BioGrid (version 4.4.202) (Oughtred et al. 2019), and the networks were visualized using Cytoscape (version 3.7.2) (Shannon et al. 2003). Topological analysis of the networks was carried out using "Network Analyzer" tool integrated in Cytoscape. The "Cytohubba" plug-in was used to identify topological features of the network, including local (i.e., degree) and global (i.e., betweenness connectivity) metrics (Chin et al. 2014). The MCODE plug-in was used to elucidate the modules of the PPI networks (Bader and Hogue 2003).

Signature-Based Drug Repositioning Using PPI Network Module Elements

After the construction of PPI networks, modules of these networks were determined. The modules that have at least three nodes were selected for further analyses. The elements of each module were used as queries for signature-based drug repositioning analysis. Drug repositioning analysis was evaluated using the L1000CDS2 tool (Duan et al. 2016). Analyses were repeated considering two scenarios to obtain drugs that could potentially reverse the ALS M1 toxic or mimic ALS M2 protective gene expression states. The first scenario included reversing upregulated/downregulated gene expressions for motor neurons and mimicking upregulated/downregulated gene expressions for microglia. The second scenario was constructed via mimicking the upregulated microglia/downregulated motor neuron gene expressions and reversing the downregulated microglia/upregulating motor neuron gene expressions. These two scenarios aimed to reposition drugs that potentially mimic the microglia state to evaluate the protective features of the microglia gene expression and reverse the motor neuron gene expressions to avoid ALS state. Databases like DrugBank (Wishart et al. 2018) and the Comparative Toxicogenomics Database (CTD) (Davis et al. 2019) and PubChem (Kim et al. 2019) were employed to further evaluate the resultant drugs.

Identification of Anticipated Target Genes for the Repositioned Drugs

We used the Search Tool for Interactions of Chemicals (STITCH, stitch.embl.de), a protein-drug interaction database with 430,000 compounds and 9.6 million proteins, to search protein-drug interaction networks (Szklarczyk et al. 2016). This database allowed us to find the repositioned molecules along with their matching target genes.

Results

Differential Gene Expression Profiles by the SOD1^{G93A} Mutation

Gene expression changes by the SOD1^{G93A} mutation were analyzed and compared with those by SOD1^{WT} in control samples. Volcano plots were generated for each dataset and DEGs with a fold change ≤ 0.5 (for downregulation) or ≥ 2 (for upregulation) and an adjusted p value < 0.05 were identified (Fig. 1a and c). Downregulated and upregulated genes were equally distributed in both cases. We also identified 1574 microglia-specific and 865 motor neuron–specific DEGs (Table 2, Supplementary Tables 1 and 2).

The microglia-specific DEGs were mainly enriched in immune-related processes ($p<.0.05$), such as leukocyte migration, T cell activation, regulation of cytokines, antigen processing, response to interferon-gamma, and L13a-mediated translational silencing of ceruloplasmin expression

Table 2 Number of differentially expressed genes (DEGs)

	# of total DEGs	# of upregulated genes	# of downregulated genes
SOD1^{G93A} motor neuron	991	112	373
SOD1^{G93A} microglia	1700	243	141
Only SOD1^{G93A} motor neuron	865	88	333
Only SOD1^{G93A} microglia	1574	231	131

DEGs differentially expressed genes

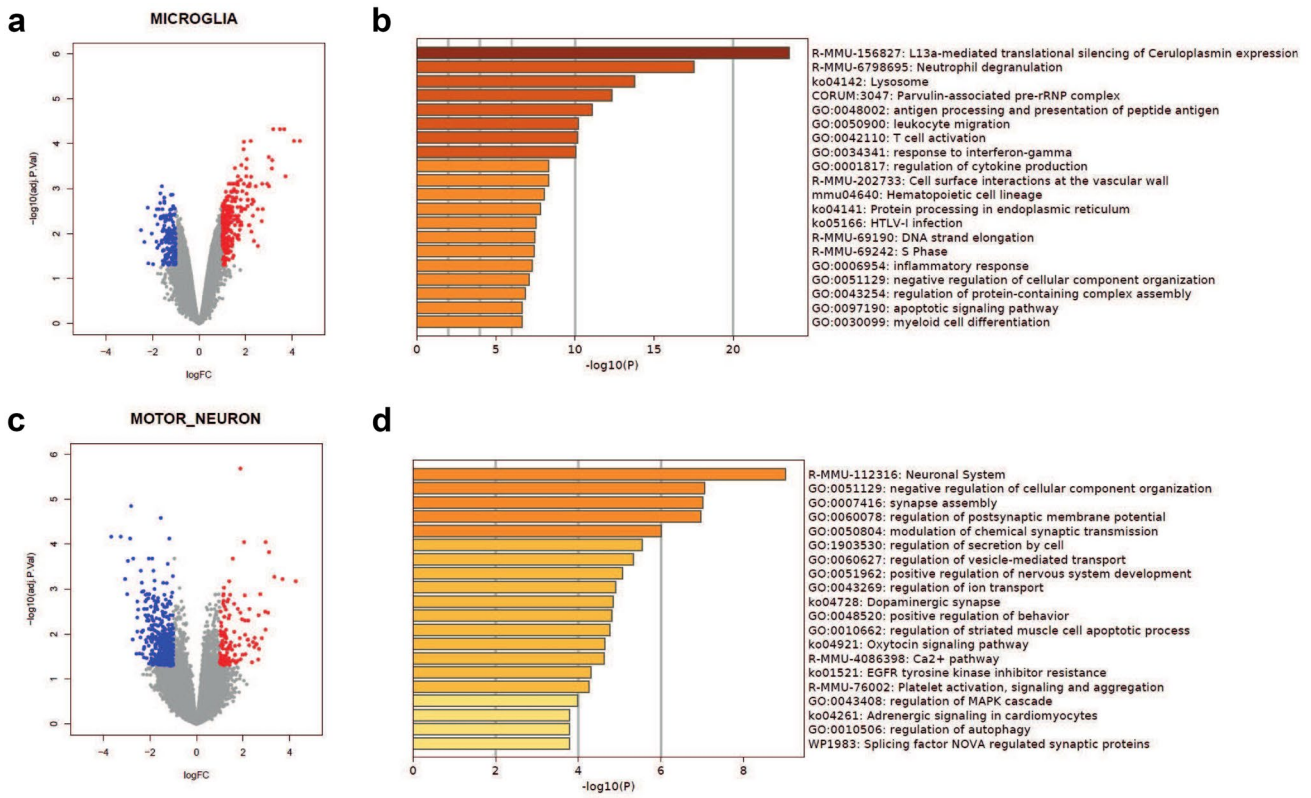


Fig. 1 Expression profiles of microglia $SOD1^{G93A}$ and $SOD1^{WT}$ transcriptome data shown in volcano plot **a** and pathway enrichment results of the DEGs represented by heatmap, the significance of the pathways illustrated by darker colors **b**. The expression profiling of motor-neuron $SOD1^{G93A}$ and $SOD1^{WT}$ is represented in the volcano plot **c**; pathway enrichment results of these DEGs are shown in a heatmap **d**

(Fig. 1b). However, motor neuron–specific enrichments occurred primarily in neuronal pathways ($p<0.05$), including regulation of ion transport synapse assembly, regulation of postsynaptic membrane potential, dopaminergic synapses, and modulation of chemical synaptic transmission (Fig. 1d).

PPI Networks and Their Modules as Potential Drug Targets

The construction of an ALS -specific PPI network based on transcriptome data specific to both motor neurons and microglia was analyzed. Both PPI networks showed scale-free topology. The motor neuron–specific PPI network consisted of 2094 nodes and 2831 edges around them, whereas the microglia-specific network included 3421 nodes and 6594 interactions between them. The microglia-specific PPI network displayed a more dense structure than the motor neuron specific network. The Cytohubba analysis using local and global metrics (degree and betweenness centrality) elucidated hub proteins. *BRCA1, MYC, EP300, FBL, NPM1, RPL10, RPL19, LMNA, FBXO6,* and *HRAS* were found as hubs for the microglia-specific PPI network, whereas *BAG3, FBXW7, FKBP5, HNRNPA1, NR3C1, PTEN, RPS6, UBC, RPL21,* and *YWHAQ* were hubs of the motor neuron–specific PPI network. Because their expression may affect all other linked proteins, these hub proteins represented key central nodes that may serve as prospective therapeutic targets (Figs. 2 and 3, Supplementary Tables 3 and 4).

After the construction of specific PPI networks, modules were obtained through the MCODE plug-in of Cytoscape to detect clusters within networks. The node score cut-off was set to 0.2 to access densely interconnected modules, and the fluff feature was adjusted to 0.0 to prevent the joining of peripheral nodes that have only one interaction. The microglia-specific PPI network and motor neuron–specific PPI network analysis revealed 20 and 12 modules with a varied number of nodes and interactions, respectively (Supplementary Table 3). The elements of each module for both networks were charted in Figs. 4 and 5. According to the results, the microglia-specific network included more crowded module structures with respect to the motor neuron–specific PPI network. The elements of every module were screened whether they are DEGs or not. If they were detected to be also DEGs for each dataset, they were selected for further drug repositioning analysis. The chosen module elements are shared in Supplementary Table 4.

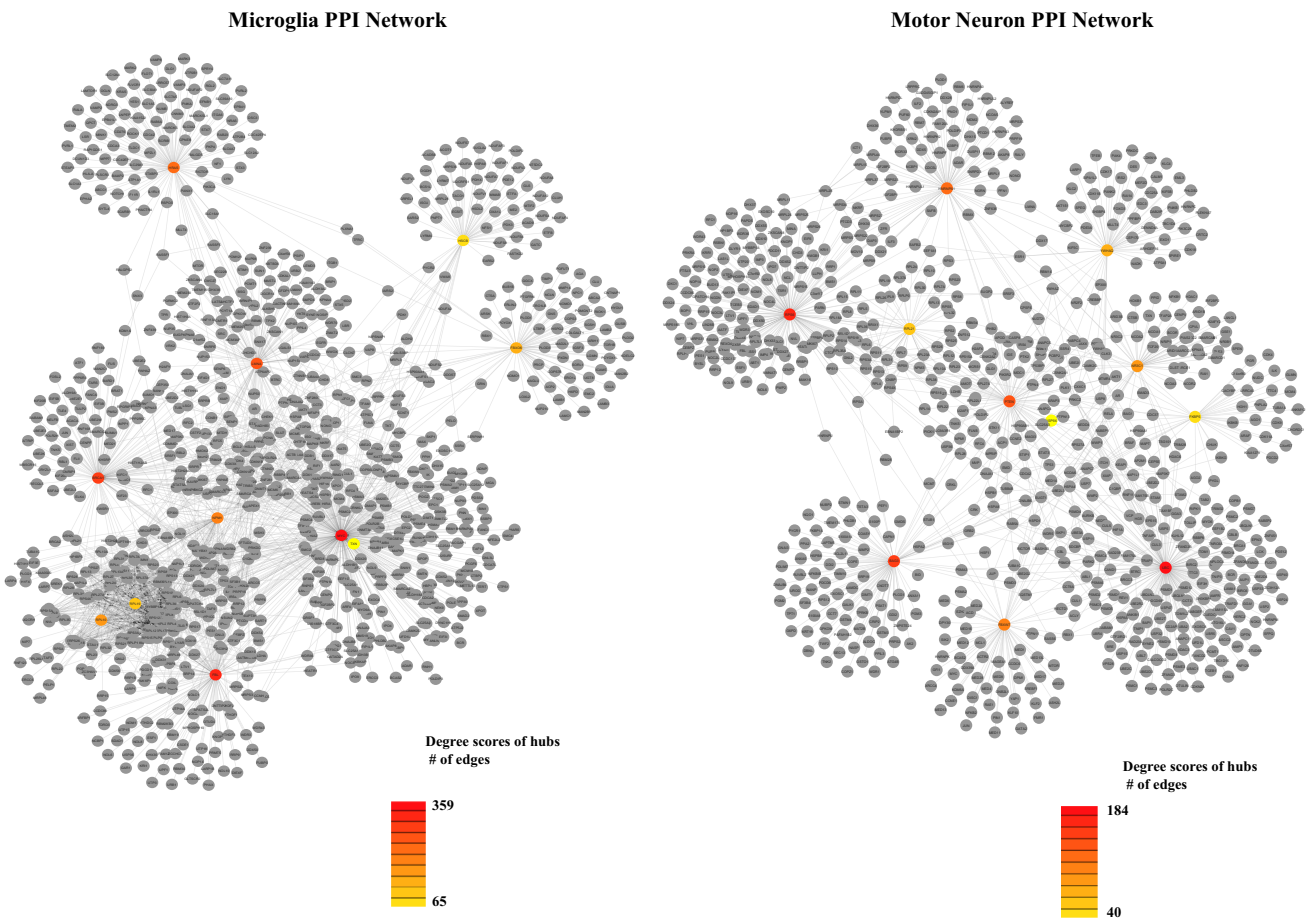


Fig. 2 The microglia- and motor neuron–specific PPI networks demonstrated DEG-based interactions. The hubs of each network were calculated according to degree metrics. The colors of the hubs represent the significance of hubs sliding through the dark to light colors

Repositioned Drug Candidates for the Treatment of ALS

The repositioned drugs have the potential to mimic the protective features of microglia or reverse the ALS situation. We introduce all repositioned small molecules based on the mentioned scenarios in the supplementary material (Supplementary Tables 5, 6, 7, and 8).

The signature-based drug repositioning using elements of significant modules revealed repositioned drug candidates. The above-mentioned two pairs of drug repositioning analyses reported 151 prospective drugs; however, we needed to narrow this colossal list to comprehend the case better. In order to do that, we intersected repositioned drug lists of reversing the scenario and mimicking the scenario (Tables 3 and 4).

Drug repositioning revealed 5 small molecules including belinostat, auranofin, BRD-K78930611, AZD-8055, and COT-10b (Fig. 6). Table 3 and Table 4 summarize repurposed drugs with their indications, mechanisms of actions, and novelty regarding ALS. Forty percent of these 95 drugs were antineoplastic agents, 40% of them revealed unknown functions, whereas the rest were chrysotherapeutic agent.

Anticipated Target Genes for the Repositioned Drugs

Examination of potential drug-associated mechanisms using The Search Tool for Interactions of Chemicals (STITCH) revealed anticipated target genes (Fig. 7). Of the five repositioned drugs, namely, belinostat, auranofin, AZD-8055, BRD-K78930611 and COT-10b, the last two, BRD-K7890611 and COT -10b, could not be found in any study in the literature. The associated literature search provided information on the anticipated target genes of each molecule and how their modulation will affect disease progression in ALS (Tables 5, 6, 7).

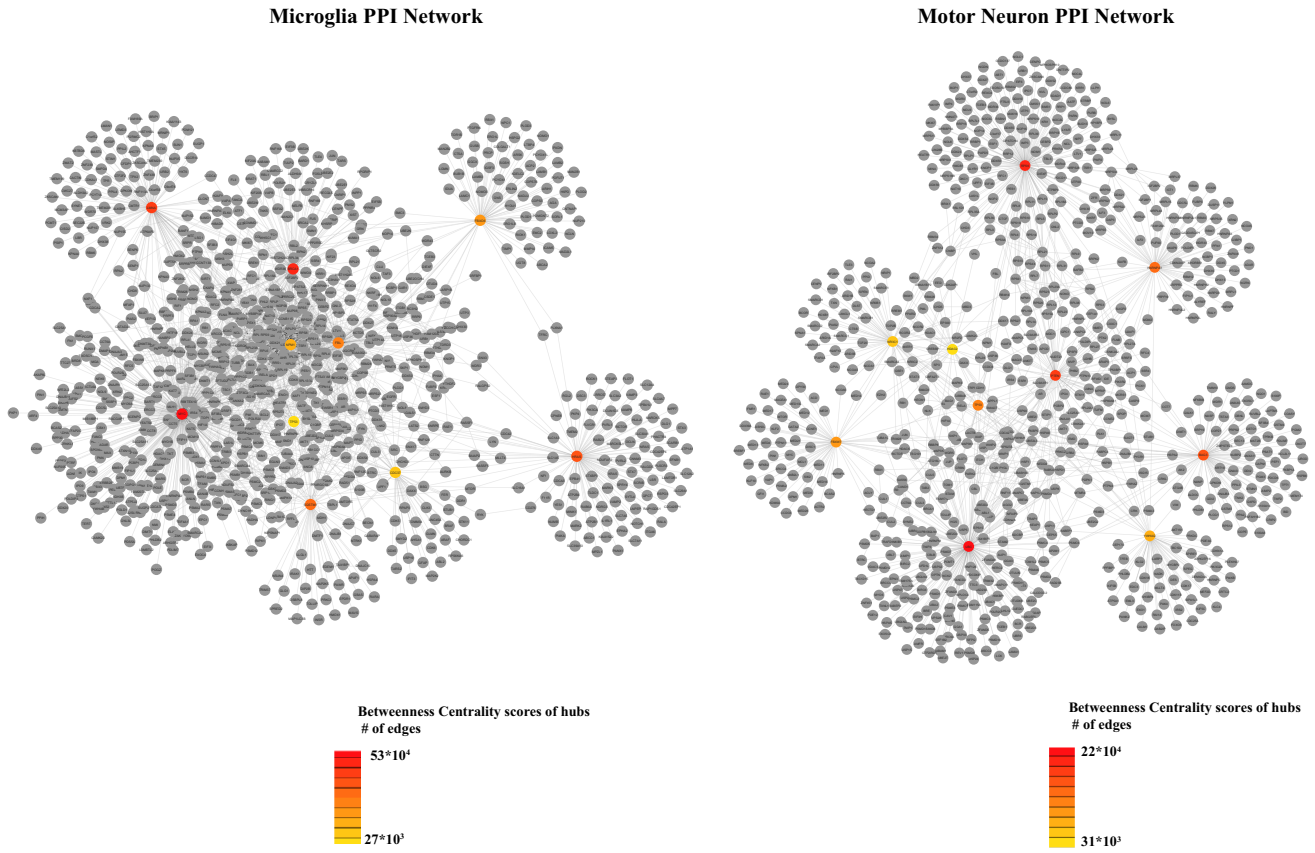


Fig. 3 The microglia- and motor neuron–specific PPI networks demonstrated DEG-based interactions. The hubs of each network were calculated according to betweenness centrality metrics. The colors of the hubs represent the significance of hubs sliding through the dark to light colors

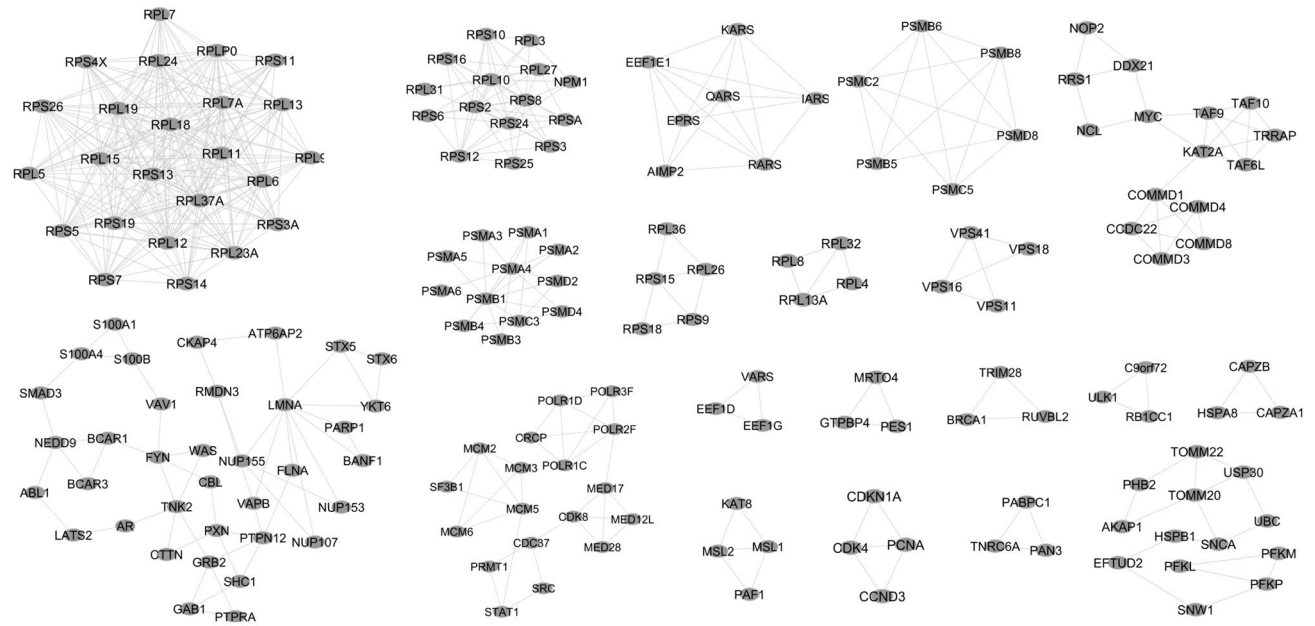


Fig. 4 The significant clusters of the microglia-specific PPI network which were used as a query for drug repositioning

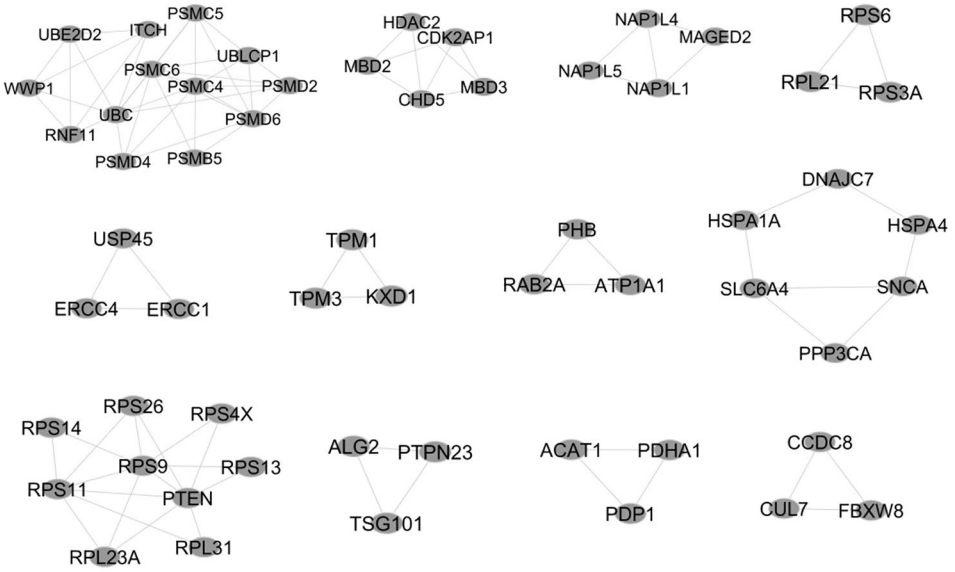


Fig. 5 The significant clusters of the motor neuron–specific PPI network which were used as a query for drug repositioning

Discussion

Microglia are immunocompetent cells that may utilize either neurotoxic (M1) or neuroprotective (M2) responses regarding their physiological environment (Appel et al. 2011; Henkel et al. 2006; Liao et al. 2012). In this study, we aimed to evaluate the discrepancies between M2 type neuroprotective microglia with motor neurons of $SOD1^{G93A}$ mice in order to find out potential target pathways and therapeutic molecules for ALS. Therefore, under this objective, we investigated transcriptome data that unraveled ALS-specific signatures then which were used for the construction of biological networks and repositioned drug candidates. We built a transcriptome-based PPI network and proposed hub genes. The signature-based drug repositioning based on the transcriptome data was performed.

Our detailed DEG analysis revealed GRN (2.2 fold), IGF1 (12. 6 fold), TREM2 (2.4 fold), SPP1 (16.8 fold), MMP12 (4.9 fold) to be significantly upregulated as M2-protective mode gene signatures of microglia in accordance with the literature (Chiu et al. 2013; Geloso et al. 2017; Noristani et al. 2015). Microglia encodes GRN (Granulin) as a survival factor (Rosen et al. 2011; Ryan et al. 2009) and shows a neuroprotective effect in ALS zebrafish models (Laird et al. 2010). Given to ALS animals, IGF1 (insulin-like growth factor 1) coded by IGF1, promotes AKT prosurvival signaling in motor neurons and enhances lifespan (Chiu et al. 2013).

Table 3 Repositioned drug candidates with the mimic effect of up- and downregulated genes in microglia and the reverse effect of up- and downregulated genes in motor neurons

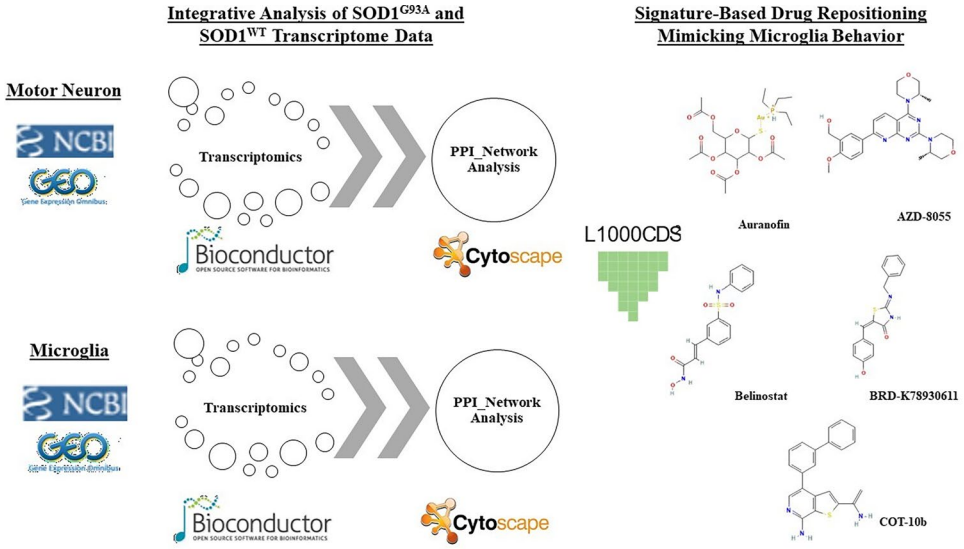
DRUG	PubChem ID	Indication	Mechanism of action	Drug usage in ALS
Belinostat	6918638	Antineoplastic agent	histone deacetylase inhibitors	NA
Auranofin	24199313	chrysotherapeutic agent	Unknown exact mechanism	NA

NA not available

Table 4 Repositioned drug candidates with mimic effect of upregulated genes in microglia/downregulated genes in motor neurons and reverse effect of downregulated genes in microglia/upregulated genes in motor neurons

Drug	PubChem ID	Indication	Mechanism of action	Drug usage in ALS
BRD-K78930611	135486338	NA	NA	NA
AZD-8055	25262965	antineoplastic agent	mTOR inhibitor	NA
COT-10b	73707373	NA	NA	NA

NA not available



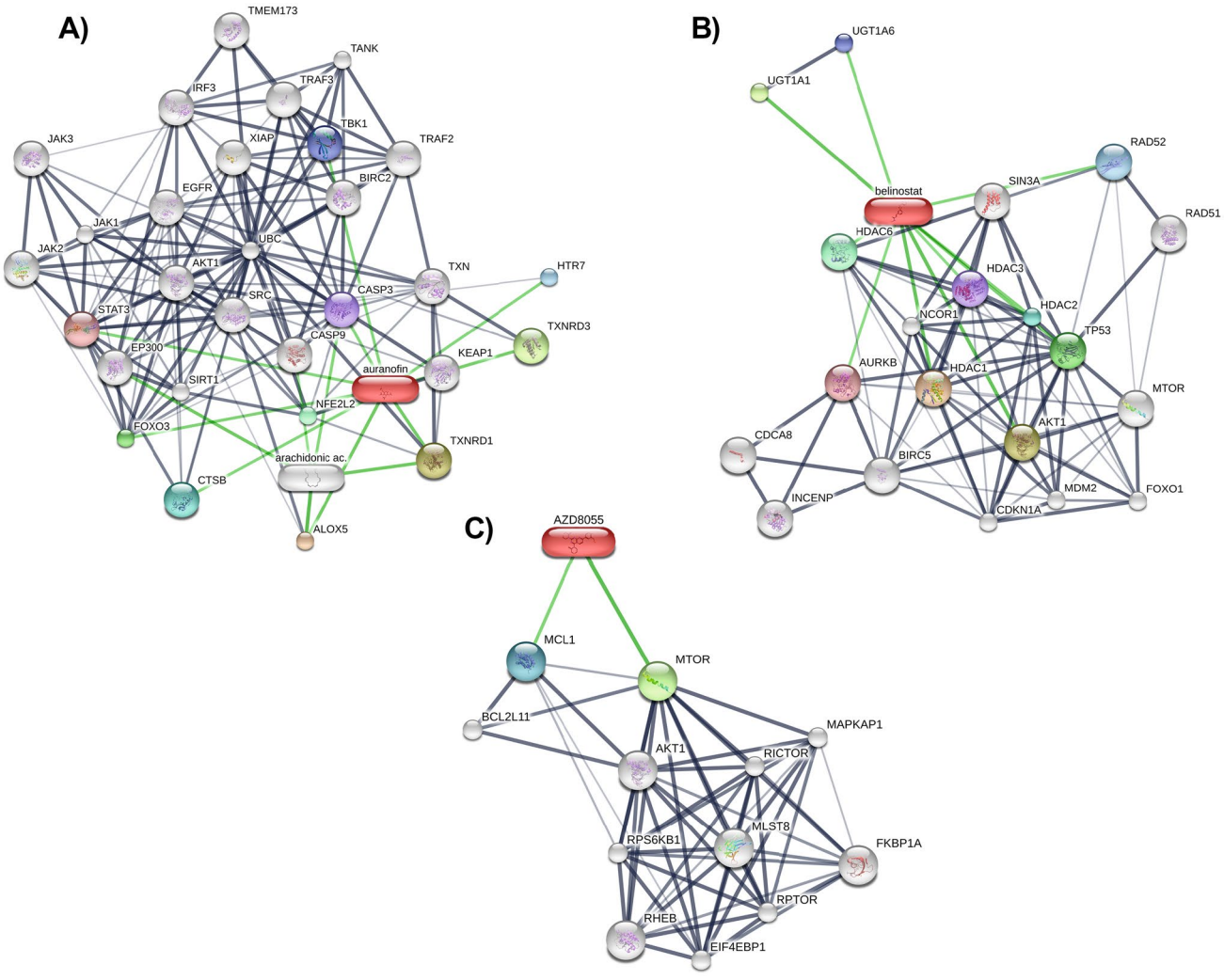


Fig. 6 Repositioned drugs related to the specific PPI network hubs

Fig. 7 Anticipated target genes of repositioned drugs based on Search Tool for Interactions of Chemicals. **A** Auranofin; **B** belinostat; **C** AZD-8055

Table 5 Anticipated targets of auranofin with its ALS association

Target symbol	Target name	Target association with ALS
UBC	Ubiquitin C	Genes encoding ubiquitin-related enzymes show altered expression in motor neurons from ALS patients (Dangoumau et al. 2021)
STAT3	Signal transducer and activator of transcription 3	A substantial increase in STAT3 is a symptom of degenerative diseases, and inhibition of STAT3 promotes motor neuron differentiation (Natarajan et al. 2014)
TRAF3	TNF receptor associated factor 3	Tax-mediated NF-B activation requires TRAF3 (Fochi et al. 2019). Alterations in NFKB signaling have been directly linked to a number of genetic and environmental risk factors for ALS (reviewed in (Källstig et al. 2021))
ALOX5	Arachidonate 5-lipoxygenase	The development of ALS is associated with oxidation products of arachidonic acid LOX sub-pathway (Trostchansky et al. 2018)
AKT1	AKT serine/threonine kinase 1	Dysfunction of the Akt signaling pathway controls the alteration in iron metabolism caused by the hmSOD1 gene mutation (Halon-Golabek et al. 2018)
SRC	SRC proto-oncogene, non-receptor tyrosine kinase	Src/c-Abl inhibitors improved in vitro survival of motor neurons generated from ALS iPSCs, making this pathway a prospective ALS therapeutic target (Imamura et al. 2017)
XIAP	X-linked inhibitor of apoptosis	XIAP was upregulated in neuro2a cells expressing mutant SOD1, reducing caspase-3 activity and cell death (Ishigaki et al. 2002)
TMEM173	Transmembrane protein 173	The mRNA level of TMEM173 in the SOD1G93A mice was markedly increased from the presymptomatic stage and peaked after disease onset (Xu et al. 2022)
FOXO3	Forkhead box O3	Activation of the DAF-16/FOXO3a pathway protects against motor neuron diseases (Mojsilovic-Petrovic et al. 2009)
TBK1	TANK-binding kinase 1	TBK1 mutations underlie ALS (Ahmad et al. 1016)
NFE2L2	Nuclear factor, erythroid 2 like 2	Modifications in two key proteins, NFE2L2 and KEAP1, involved in cellular defense against oxidative stress may influence the development of SALS (Bergström et al. 2014)
TANK	TRAF family member associated NFKB activator	The noncanonical NFKB response is controlled by TBK1, which activates the NFKB regulator TANK. Alterations in NFKB signaling have been directly linked to a number of genetic and environmental risk factors for ALS (reviewed in (Källstig et al. 2021))
CASP9	Caspase 9	Spinal motor neurons from human ALS patients showed stimulated caspase 9 (Inoue et al. 2003)
JAK2	Janus kinase 2	Activation of the JAK /STAT3 pathway was observed in reactive astrocytes from ALS patients (Shibata et al. 2009)
JAK3	Janus kinase 3	JAK3 therapy improved survival in FALS mice, indicating that certain JAK3 inhibitors may be helpful in the treatment of human ALS (Trieu et al. 2000)
CASP3	Caspase 3	Caspase-3 activation is a key component of cell death by mutant SOD1 present in motor neurons (Pasinelli et al. 2000)
EGFR	Epidermal growth factor receptor	EGFR mRNA is upregulated in the ALS patients (Romano and Bucci 2020)
IRF3	Interferon regulatory factor 3	IRF3 activation suggests that innate immunity of some form may play a role in the pathophysiology of ALS (Sakaguchi et al. 2011)
JAK1	Janus kinase 1	Activation of the JAK /STAT3 pathway was observed in reactive astrocytes from ALS patients (Shibata et al. 2009)
TXNRD1	Thioredoxin reductase 1	Thioredoxin reductase 1 haplotypes influence the development of FALS, suggesting that TXNRD1 may function as an important FALS gene (Mitchell et al. 2009)
TRAF2	TNF receptor associated factor 2	Absence of TNF-alpha receptor type 2 preserves motor neurons in a cellular model of ALS and mutant SOD1 mice (Tortarolo et al. 2015)
EP300	E1A-binding protein p300	EP300 is a pathway element for autophagy-targeted treatments for ALS (Amin et al. 2020)

Table 5 (continued)

Target symbol	Target name	Target association with ALS
TXNRD3	Thioredoxin reductase 3	Altered levels of TXNRD3 and some specific proteins of the oxidative stress pathway were detected in the brain after administration of branched-chain amino acids, suggesting a possible relationship between branched-chain amino acids and ALS (Piscopo et al. 2011)
TXN	Thioredoxin	Thioredoxin is one of several pathogenic signaling pathways associated with ALS, and recent evidence points to its important role in neurodegeneration (reviewed in (Jagaraj et al. 2021))
CTSB	Cathepsin B	Increased expression of cathepsin B was observed in ALS degenerating neurons (Kikuchi et al. 2003)
HTR7	5-Hydroxytryptamine receptor 7	The therapeutic potential of 5-HT7R signaling pathway for the treatment of neurodegenerative diseases is reviewed in (Quintero-Villegas and Valdés-Ferrer 2022)
BIRC2	Baculoviral IAP repeat containing 2	BIRC2 is a critical promising target for anti-apoptotic defense after peripheral nerve injury (Wang et al. 2012)
Arachidonic Ac	Arachidonic Acid	The development of ALS is associated with oxidation products of arachidonic acid pathway (Trostchansky et al. 2018)
KEAP1	Kelch-like ECH associated protein 1	The KEAP1-NRF2 antioxidant system is disrupted in ALS (Bono et al. 2021)
SIRT1	Sirtuin 1	The HSF1/HSP70i chaperone system is positively regulated by SIRT1, which may partially attenuate SOD1-related ALS symptoms in mice (Watanabe et al. 2014)

TREM2 (triggering receptor expressed on myeloid cell 2) deficient microglia depleted their phagocytic ability for the clearance of TDP-43 inclusions in ALS mice models, proposing a defensive role of TREM2 in TDP-43 induced ALS (Xie et al. 2022). SPP1 gene coding for secreted phosphoprotein 1 functions as an ischemic injury potential neuroprotective agent (Meller et al. 2005) with its anti-inflammatory and anti-apoptotic characteristics (Iczkiewicz et al. 2005; Knott et al. 2000; Maetzler et al. 2007; Rollo and Denhart 1996). MMP (Matrix metalloproteinase) inhibition has been found to enhance longevity in ALS mice (Lorenzl et al. 2006) and MMP12 was found to prevent pro-inflammation and encourage stimulating anti-inflammatory signaling (Mouton et al. 2018).

Functional enrichment results of case-specific DEGs revealed that microglia samples enriched mostly in immune system–associated pathways (Fig. 1b), whereas motor neuron–specific enrichments demonstrated nervous system–related biological processes (Fig. 1d). Microglia are essential innate immune cells of the brain, so immune system–associated pathways were expected to be enriched in microglia samples. Also, L13a-mediated translational silencing of ceruloplasmin expression was enriched in microglia samples. The upregulation of this protein is associated with neurodegenerative diseases (Virit et al. 2008; Wolf et al. 2006), implying a neuroprotective pathway for microglia samples. Synapse transmission and ion transport mechanisms such as regulation of postsynaptic membrane potential, modulation of chemical synaptic transmission, and regulation of ion transport and Ca^{+2} were affected mostly in motor neurons which is consistent with the persistent inward current theory of ALS. According to this theory, the disproportion of the functional ion channels leads to the spontaneous contractions of the muscles in ALS and could be one of the major reasons for this disease (Bostock et al. 1995; ElBasiouny et al. 2010; Öktem et al. 2019). Analysis of protein-protein interactions revealed important nodes that were then used for drug repositioning analysis to predict novel drugs for ALS.

The term "drug repositioning" refers to the discovery of new therapeutic applications for medications that have already been approved by the US Food and Drug Administration for another use. Drug repositioning is more attainable and affordable than innovative drug development due to the shorter research and testing periods and lower costs, allowing patients to receive new medications sooner (Roder and Thomson 2015). Edaravone and riluzole are the FDA-approved ALS treatments although the exact action mechanism of these drugs is unclear. This situation motivated us to reposition potential drugs for this disease. Related to the hubs for specific PPI networks, our drug repositioning analysis revealed five molecules including belinostat, auranofin, BRD-K78930611, AZD-8055, and COT-10b (Figure 6, Tables 3, and 4). Among them, belinostat is a histone deacetylase (HDAC) inhibitor and received expedited approval from the FDA for the treatment of relapsed or refractory peripheral T cell lymphoma (PTCL) in 2014 (Lee et al. 2015). Alterations in histone acetylation, either target specific or general, are linked to ALS (Bennett et al. 2019). Also, transgenic ALS mice expressing human mutations in the SOD1 gene revealed

Table 6 Anticipated targets of belinostat with its ALS association

TP53	Tumor protein p53	TP53 is overexpressed in ALS patients. (Eve et al. 2007)
INCENP	Inner centromere protein	INCENP, a member of the chromosomal passenger complex, plays a crucial role in mitosis. (Honda et al. 2003)
MDM2	MDM2 proto-oncogene	Increased MDM2 transcript levels in ALS motor neuron progenitors were associated with binding to p53 and controlling its transcriptional activity/ stability through an autoregulatory feedback mechanism.(von Grabowiecki et al. 2016)
HDAC3	Histone deacetylase 3	The importance of preclinical models of histone deacetylases and their inhibitors is discussed in (Klingl et al. 2020)
RAD51	RAD51 recombinase	RAD51 plays a role in DNA damage response. Decreased levels of RAD51 in the nucleus lead to slower repair of double-strand breaks, which may be associated with neurodegeneration in ALS (Konopka and Atkin 2022)
AURKB	Aurora kinase B	Dysregulated axonal transport of mitochondria has been linked to neurogeneration. AURKB is one of the three targets that should be activated for enhanced mitochondrial transport (Shlevkov et al. 2019)
HDAC1	Histone deacetylase 1	The importance of preclinical models of histone deacetylases and their inhibitors is discussed in (Klingl et al. 2020)
SIN3A	SIN3 transcription regulator family member A	SIN3A is associated with both HDAC1 and HDAC2 and regulates transcription (Klingl et al. 2021)
HDAC2	Histone deacetylase 2	The importance of preclinical models of histone deacetylases and their inhibitors is discussed in (Klingl et al. 2020)
MTOR	Mechanistic target of rapamycin	Mutant SOD1 in human-derived astrocytes resulted in enhanced activation of the MTOR pathway (Granatiero et al. 2021a)
FOXO1	Forkhead box O1	FoxO proteins are discovered as potential therapeutic targets for neurological diseases, including ALS (reviewed in (Maiese 2015))
CDCA8	Cell division cycle associated 8	A component of a chromosomal passenger complex called CDCA8 is necessary for the bipolar mitotic spindle's stability.(Gassmann et al. 2004)
UGT1A1	UDP glucuronosyltransferase family 1 member A1	Riluzole is metabolically degraded in vivo by glucuronidation in ALS patients (Van Kan et al. 2008)
BIRC5	Baculoviral IAP repeat containing 5	Expression of BIRC5 was found to be decreased in mutant SOD1 motor neurons (Kirby et al. 2011)
HDAC6	Histone deacetylase 6	The importance of preclinical models of histone deacetylases and their inhibitors is discussed in (Klingl et al. 2020)
RAD52	RAD52 homolog, DNA repair protein	Impaired DNA repair may be a key factor in the development of neurodegenerative diseases such as ALS (reviewed in (Coppedè and Migliore, 2010))
UGT1A6	UDP glucuronosyltransferase family 1 member A6	Riluzole is metabolically degraded in vivo by glucuronidation in ALS patients (Van Kan et al. 2008)
NCOR1	Nuclear receptor corepressor 1	NCOR1 and HDAC3 together repress transcription (Killoy et al. 2022)
CDKN1A	Cyclin dependent kinase inhibitor 1A	Dysregulation of CDKN1A is reported in the muscles of ALS mice (Manzano et al. 2013)

elevated levels of muscle HDAC 4 and its regulator microRNA-206 (Bruneteau et al. 2013). In addition to their promise for treating neurodegenerative illnesses, HDAC inhibitors have been shown to have significant neuroprotective features. For instance, HDAC inhibitors have a positive effect in alleviating neurodegenerative disorders by enhancing cell survival and reducing neuronal death through various mechanisms (Shukla and Tekwani 2020). Among them, 4-phenylbutryrate treatment corresponded to higher survival rates, reduced clinical disability in ALS (Ryu et al. 2005). Also, injection of valproic acid into $SOD1^{G86R}$ mutant mice dramatically reduced motor neuron death and maintained normal levels of histone acetylation (Rouaux et al. 2007). Lastly, application of trichostatin A improved muscle atrophy and neuromuscular junction denervation in $SOD1^{G93A}$ transgenic ALS mice (Yoo and Ko 2011). Auranofin is a gold compound approved for the treatment of rheumatoid arthritis in 1985 and has recently been studied as a possible treatment for a variety of human disorders, including cancer (Onodera et al. 2019) and neurological diseases (Madeira et al. 2013). Low micromolar quantities of auranofin (0.2–5 mol/L) have been demonstrated to reach the CNS, demonstrating that sufficient auranofin could pass the blood-brain barrier (Madeira et al. 2014). Considering particularly ALS, Oberstadt et al. reported that 25 mM auranofin drastically reduced TDP-43 interaction (Oberstadt et al. 2018). These studies indicate that this drug could be beneficial in neurodegenerative illnesses including Alzheimer's disease, Parkinson's disease, and ALS. The other drug,

Table 7 Anticipated Targets of AZD-8055 with its ALS association

FKBP1A	FK506 binding protein 1A	FK506 binding protein 1A was downregulated in spinal cord motor neurons from ALS patients (Jiang et al. 2005)
MLST8	MTOR associated protein, LST8 homolog	Mutant SOD1 in human-derived astrocytes resulted in enhanced activation of the MTOR pathway, which promotes cell proliferation (Granatiero et al. 2021a)
RICTOR	RPTOR independent companion of MTOR complex 2	Mutant SOD1 in human-derived astrocytes resulted in enhanced activation of the MTOR pathway (Granatiero et al. 2021a)
EIF4EBP1	eukaryotic translation initiation factor 4E binding protein 1	ALS $SOD1^{G93A}$ astrocytes showed constant MTOR activation that strongly stimulated two crucial MTOR effectors, including EIF-4EBP1 (Granatiero et al. 2021b)
BCL2L11	BCL2 like 11	Increased cytosolic distribution of Bcl-2 was associated with reports of neuronal death ALS (reviewed in (Akhtar et al. 2004))
RPTOR	regulatory associated protein of MTOR complex 1	Part of the mTOR complex encoded by RPTOR is lost when TDP-43 loses its function, which increases autophagy (Sullivan et al. 2017)
RHEB	Ras homolog enriched in brain	Research on the therapeutic potential of AAV1-RHEB (S16H) transduction by studying its neuroprotective effects and processes in the adult brain hippocampus is reviewed in (Moon et al. 2020)
MTOR	mechanistic target of rapamycin	Mutant SOD1 in human-derived astrocytes resulted in enhanced activation of the MTOR pathway (Granatiero et al. 2021a)
MCL1	Apoptosis Regulator, BCL2 Family Member	The expression patterns of several members of the BCL-2 family that regulate apoptosis were shown to be altered in SOD1 transgenic mutant mice and ALS patients (Hetz et al. 2007)
MAPKAP1	Mitogen-Activated Protein Kinase Associated Protein 1	MAPK member inhibitors have undergone testing as potential ALS treatments (reviewed in (Sahaan and Zhang 2021))
RPS6KB1	Ribosomal Protein S6 Kinase B1	Decreased mTOR activity and downregulation of RPS6KB1/p70S6, a direct target of mTOR, were detected in the mouse VCPR155H model ALS (Ching and Weihl 2013)
AKT1	AKT serine/threonine kinase 1	Dysfunction of the Akt signalling pathway controls the alteration in iron metabolism caused by the hmSOD1 gene mutation (Halon-Golabek et al. 2018)

AZD-8055, is a novel ATP-competitive inhibitor of mTOR kinase activity and causes considerable growth suppression in xenografts covering a wide spectrum of human tumor types (Chresta et al. 2010). BRD-K78930611 and COT-10b could not be found in the literature for any study. The Search Tool for Interactions of Chemicals (STITCH, stitch.embl.de) revealed the anticipated target genes for our repositioned drugs. We found information about the anticipated target genes of each molecule and how their regulation affects disease progression in the corresponding literature search on ALS.

The corresponding literature search on the anticipated target genes of the repositioned drugs revealed that almost all of these genes are associated with ALS and neurodegenerative disease mechanisms. Due to the lack of experimental validation, the drug repositioning approach utilized in this research is labeled as level 0 in the drug repositioning evidence level (DREL) classification (Oprea and Overington 2015; Vogrinc and Kunej 2017). According to this five-level classification, level 0 refers to predictions without in vitro or in vivo evaluations, while level 4 refers to well-documented clinical outcomes at doses within safety limits for the repositioned molecule. Studies with a DREL-0 score, on the other hand, should not be overlooked as they often provide unique bioinformatic methods for drug repositioning as well as new anticipated compounds for additional experimental validation. There are studies in the literature that use DREL-0 to predict various diseases (Craddock et al. 2015; Grover et al. 2015; Xu and Wang 2016; Zhang et al. 2015). In addition, in breast and prostate cancer, several success stories have been published using a similar approach, validating the efficacy of newly manufactured drugs with an in vitro cell assay (Turanli et al. 2019, 2019). It was also found that multiomics-based in silico predictions showed strong correlation with in vitro experiments (Turanli et al. 2018). Provided that additional in vitro and in vivo experiments are performed to determine the protective effects of the repositioned compounds on motor neurons, this research has the potential to improve the efficacy of existing treatments through the discovery of new therapeutics associated with ALS.

All in all, systems biology–oriented transcriptome analysis of $SOD1^{G93A}$ mutation have revealed disease signatures regarding protective and neurotoxic aspects and integrative analysis of biological networks aided us to repurpose potential drug candidates for the construction of innovative and precise treatment modalities for ALS.

Conclusion

In this study, integrative transcriptome-based SOD1^{G93A} mutation analyses revealed that microglia and motor neuron samples demonstrated different transcriptional profilings. Emphasizing the protective effects of microglia cells on ALS onset, we mimic the expression profiles of those cells and tried to reverse the expression profiles of the motor-neuron samples. As a result, we repurposed five drugs, belinostat, auranofin, BRD-K78930611, AZD-8055, and COT-10b. The presented transcriptome-based data integration provided prioritization and repurposing of the candidate drugs that might be potential candidate therapeutics for ALS.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12031-022-02071-1.

Author Contribution EKO conceived the original idea, conceptualized the study, performed analysis, prepared figures and tables, and wrote the manuscript. BA performed analysis, prepared figures and tables, and wrote the manuscript. MY performed analysis and prepared figures and tables. KYA conceptualized the study, supervised the study and improved the final version of the manuscript. All authors reviewed and approved the final version of the manuscript.

Declarations

Ethical Approval Not applicable; the study did not have any participants and relevant information.

Consent to Participate Not applicable; the study did not have any participants and relevant information.

Consent for Publication All authors consent for publication in Journal of Molecular Neuroscience.

Competing Interests The authors declare no competing interests.

References

Ahmad L, Zhang SY, Casanova JL, Sancho-Shimizu V (1016) Human TBK1: a gatekeeper of neuroinflammation. Trends in Mol Med 22:511–27

Akhtar RS, Ness JM, Roth KA (2004) Bcl-2 family regulation of neuronal development and neurodegeneration. Biochimica Et Biophysica Acta - Molecular Cell Research 1644:189–203. https://doi.org/10.1016/j.bbamcr.2003.10.013

Amin A, Perera ND, Beart PM, Bradley TJ, Shabanpoor F (2020) Amyotrophic lateral sclerosis and autophagy: dysfunction and therapeutic targeting. Celss 9:2413

Appel S, Zhao W, Beers D, Henkel J (2011) The Microglial-Motoneuron dialogue in ALS. Acta Myologica 4–8

Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM et al (2000) Gene ontology: tool for the unification of biology. Nat Genet 25:25–29. https://doi.org/10.1038/75556

Bader GD, Hogue CW (2003) An automated method for finding molecular complexes in large protein interaction networks

Barrett T, Suzek TO, Troup DB, Wilhite SE, Ngau W-C, Ledoux P et al (2005) NCBI GEO: mining millions of expression profiles—database and tools. Nucleic Acids Res 33:D562–D566

Bennett SA, Tanaz R, Cobos SN, Torrente MP (2019) Epigenetics in amyotrophic lateral sclerosis: a role for histone post-translational modifications in neurodegenerative disease. Transl Res 204:19–30. https://doi.org/10.1016/j.trsl.2018.10.002

Bergström P, von Otter M, Nilsson S, Nilsson AC, Nilsson M, Andersen PM et al (2014) Association of NFE2L2 and KEAP1 haplotypes with amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration 15:130–137. https://doi.org/10.3109/21678421.2013.839708

Bolstad BM, Irizarry RA, Astrand M, Speed TP (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. 19

Bono S, Feligioni M, Corbo M (2021) Impaired antioxidant KEAP1-NRF2 system in amyotrophic lateral sclerosis: NRF2 activation as a potential therapeutic strategy. Neurodegeneration 16

Bostock H, Sharief MK, Reid G, Murray NMF (1995) Axonal ion channel dysfunction in amyotrophic lateral sclerosis. 118

Bruneteau G, Simonet T, Bauché S, Mandjee N, Malfatti E, Girard E et al (2013) Muscle histone deacetylase 4 upregulation in amyotrophic lateral sclerosis: potential role in reinnervation ability and disease progression. Brain 136:2359–2368. https://doi.org/10.1093/brain/awt164

Carbon S, Douglass E, Good BM, Unni DR, Harris NL, Mungall CJ et al (2021) The Gene Ontology resource: enriching a gold mine. Nucleic Acids Res 49:D325–D334. https://doi.org/10.1093/nar/gkaa1113

Chin C-H, Chen S-H, Wu H-H, Ho C-W, Ko M-T, Lin C-Y (2014) cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol 8(Suppl 4):S11. https://doi.org/10.1186/1752-0509-8-S4-S11

Ching JK, Weihl CC (2013) Rapamycin-induced autophagy aggravates pathology and weakness in a mouse model of VCP-associated myopathy. Autophagy 9:799–800. https://doi.org/10.4161/auto.23958

Chiu IM, Morimoto ETA, Goodarzi H, Liao JT, O'Keeffe S, Phatnani HP et al (2013) A neurodegeneration-specific gene-expression signature of acutely isolated microglia from an amyotrophic lateral sclerosis mouse model. Cell Rep 4:385–401. https://doi.org/10.1016/j.celrep.2013.06.018

Chresta CM, Davies BR, Hickson I, Harding T, Cosulich S, Critchlow SE et al (2010) AZD8055 is a potent, selective, and orally bioavailable ATP-competitive mammalian target of rapamycin kinase inhibitor with in vitro and in vivo antitumor activity. Can Res 70:288–298. https://doi.org/10.1158/0008-5472.CAN-09-1751

Coppedè F, Migliore L (2010) DNA repair in premature aging disorders and neurodegeneration. Curr Aging Sci 3:3–19

Craddock TJA, Harvey JM, Nathanson L, Barnes ZM, Klimas NG, Fletcher MA et al (2015) Using gene expression signatures to identify novel treatment strategies in gulf war illness. BMC Med Genom 8. https://doi.org/10.1186/s12920-015-0111-3

Dangoumau A, Marouillat S, Coelho R, Wurmser F, Brulard C, Haouari S et al (2021) Dysregulations of expression of genes of the ubiquitin/SUMO pathways in an in vitro model of amyotrophic lateral sclerosis combining oxidative stress and SOD1 gene mutation. Int J Mol Sci 22:1796

Davis AP, Grondin CJ, Johnson RJ, Sciaky D, McMorran R, Wiegers J et al (2019) The comparative toxicogenomics database: update 2019. Nucleic Acids Res 47:D948–D954

Du L, Zhang Y, Chen Y, Zhu J, Yang Y, Zhang HL (2017) Role of microglia in neurological disorders and their potentials as a therapeutic target. Mol Neurobiol 54:7567–7584. https://doi.org/10.1007/s12035-016-0245-0

Duan Q, Reid SP, Clark NR, Wang Z, Fernandez NF, Rouillard AD et al (2016) L1000CDS2: LINCS L1000 characteristic direction signatures search engine. Npj Systems Biology and Applications 2:16015. https://doi.org/10.1038/npjsba.2016.15

ElBasiouny SM, Schuster JE, Heckman CJ (2010) Persistent inward currents in spinal motoneurons: important for normal function but potentially harmful after spinal cord injury and in amyotrophic lateral sclerosis. Clin Neurophysiol 121:1669–1679. https://doi.org/10.1016/j.clinph.2009.12.041

Eve DJ, Dennis JS, Citron BA (2007) Transcription factor p53 in degenerating spinal cords. Brain Res 1150:174–181

Fabregat A, Sidiropoulos K, Garapati P, Gillespie M, Hausmann K, Haw R et al (2016) The Reactome pathway Knowledgebase. Nucleic Acids Res 44:D481–D487. https://doi.org/10.1093/nar/gkv1351

Fochi S, Bergamo E, Serena M, Mutascio S, Journo C, Mahieux R et al (2019) TRAF3 Is Required for NF-κB pathway activation mediated by HTLV tax proteins. Front Microbiol 10:1302

Gassmann R, Carvalho A, Henzing AJ, Ruchaud S, Hudson DF, Honda R et al (2004) Borealin: a novel chromosomal passenger required for stability of the bipolar mitotic spindle. J Cell Biol 166:179–191

Gautier L, Cope L, Bolstad BM, Irizarry RA (2004) Affy - Analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 20:307–315. https://doi.org/10.1093/bioinformatics/btg405

Geloso MC, Corvino V, Marchese E, Serrano A, Michetti F, D'Ambrosi N (2017) The dual role of microglia in ALS: Mechanisms and therapeutic approaches. Front Aging Neurosci 9. https://doi.org/10.3389/fnagi.2017.00242

Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S et al (2004) Open access bioconductor: open software development for computational biology and bioinformatics. 5

Granatiero V, Sayles NM, Savino AM, Konrad C, Kharas MG, Kawamata H et al (2021a) Modulation of the IGF1R-MTOR pathway attenuates motor neuron toxicity of human ALS SOD1G93A astrocytes. Autophagy 17:4029–4042

Grover MP, Ballouz S, Mohanasundaram KA, George RA, Goscinski A, Crowley TM et al (2015) Novel therapeutics for coronary artery disease from genome-wide association study data. BMC Med Genom 8. https://doi.org/10.1186/1755-8794-8-S2-S1

Halon-Golabek M, Borkowska A, Kaczor JJ, Ziolkowski W, Flis DJ, Knap N et al (2018) hmSOD1 gene mutation-induced disturbance in iron metabolism is mediated by impairment of Akt signalling pathway. J Cachexia Sarcopenia Muscle 9:557–569. https://doi.org/10.1002/jcsm.12283

Henkel JS, Beers DR, Siklós L, Appel SH (2006) The chemokine MCP-1 and the dendritic and myeloid cells it attracts are increased in the mSOD1 mouse model of ALS. Mol Cell Neurosci 31:427–437. https://doi.org/10.1016/j.mcn.2005.10.016

Henriques A, Kastner S, Chatzikonstantinou E, Pitzer C, Plaas C, Kirsch F et al (2015) Gene expression changes in spinal motoneurons of the SOD1G93A transgenic model for ALS after treatment with G-CSF. Front Cell Neurosci 8:1–12. https://doi.org/10.3389/fncel.2014.00464

Hetz C, Thielen P, Fisher J, Pasinelli P, Brown RH, Korsmeyer S et al (2007) The proapoptotic BCL-2 family member BIM mediates motoneuron loss in a model of amyotrophic lateral sclerosis. Cell Death Differ 14:1386–1389. https://doi.org/10.1038/sj.cdd.4402166

Honda R, Korner R, Nigg EA (2003) Exploring the functional interactions between Aurora B, INCENP, and survivin in mitosis. Mol Biol Cell 14:3325–3341

Iczkiewicz J, Rose S, Jenner P (2005) Increased osteopontin expression following intranigral lipopolysaccharide injection in the rat. Eur J Neurosci 21:1911–1920. https://doi.org/10.1111/j.1460-9568.2005.04009.x

Imamura K, Izumi Y, Watanabe A, Tsukita K, Woltjen K, Yamamoto T et al (2017) The Src/c-Abl pathway is a potential therapeutic target in amyotrophic lateral sclerosis. Sci Trans Med 1–10

Inoue H, Tsukita K, Iwasato T, Suzuki Y, Tomioka M, Tateno M et al (2003) The crucial role of caspase-9 in the disease progression of a transgenic ALS mouse model. EMBO J 22:6665–6674

Ishigaki S, Liang Y, Yamamoto M, Niwa J-I, Ando Y, Yoshihara T et al (2002) X-Linked inhibitor of apoptosis protein is involved in mutant SOD1-mediated neuronal degeneration. J Neurochem 82:576–584

Jagaraj CJ, Parakh S, Atkin JD (2021) Emerging evidence highlighting the importance of redox dysregulation in the pathogenesis of amyotrophic lateral sclerosis (ALS). Front Cellular Neurosci 14

Jiang YM, Yamamoto M, Kobayashi Y, Yoshihara T, Liang Y, Terao S et al (2005) Gene expression profile of spinal motor neurons in sporadic amyotrophic lateral sclerosis. Ann Neurol 57:236–251. https://doi.org/10.1002/ana.20379

Källstig E, McCabe BD, Schneider BL (2021) The links between als and nf-κb. Int J Mol Sci 22. https://doi.org/10.3390/ijms22083875

Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M et al (2008) KEGG for linking genomes to life and the environment. Nucleic Acids Res 36:D480–D484. https://doi.org/10.1093/nar/gkm882

Kaur SJ, McKeown SR, Rashid S (2016) Mutant SOD1 mediated pathogenesis of Amyotrophic Lateral Sclerosis. Gene 577:109–118. https://doi.org/10.1016/j.gene.2015.11.049

Kikuchi H, Yamada T, Furuya H, Doh-Ura K, Ohyagi Y, Iwaki T et al (2003) Involvement of cathepsin B in the motor neuron degeneration of amyotrophic lateral sclerosis. Acta Neuropathol 105:462–468. https://doi.org/10.1007/s00401-002-0667-9

Killoy KM, Harlan BA, Pehar M, Vargas MR (2022) NR1D1 downregulation in astrocytes induces a phenotype that is detrimental to cocultured motor neurons. FASEB J 36:e22262

Kim S, Chen J, Cheng T, Gindulyte A, He J, He S et al (2019) PubChem 2019 update: improved access to chemical data. Nucleic Acids Res 47:D1102–D1109. https://doi.org/10.1093/nar/gky1033

Kirby J, Ning K, Ferraiuolo L, Heath PR, Ismail A, Kuo S-W et al (2011) Phosphatase and tensin homologue/protein kinase B pathway linked to motor neuron survival in human superoxide dismutase 1-related amyotrophic lateral sclerosis. Brain 134:506–517

Klingl YE, Pakravan D, Van Den BL (2021) Opportunities for histone deacetylase inhibition in amyotrophic lateral sclerosis. Br J Pharmacol 178:1353–1372. https://doi.org/10.1111/bph.15217

Klingl YE, Pakravan D, Van Den Bosch L (2020) Opportunities for histone deacetylase inhibition in amyotrophic lateral sclerosis. British J Pharmacol 178:1353–72. https://doi.org/10.1111/bph.v178.6/issuetoc

Knott C, Stern G, Wilkin GP (2000) Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2. Mol Cell Neurosci 16:724–739. https://doi.org/10.1006/mcne.2000.0914

Konopka A, Atkin JD (2022) DNA damage, defective DNA repair, and neurodegeneration in amyotrophic lateral sclerosis. Front Aging Neurosci 14:786420. https://doi.org/10.3389/fnagi.2022.786420

Kubat Öktem E, Mruk K, Chang J, Akin A, Kobertz WR, Brown RH (2016) Mutant SOD1 protein increases Nav1.3 channel excitability. J Biol Phys 42:351–70. https://doi.org/10.1007/s10867-016-9411-x

Laird AS, van Hoecke A, de Muynck L, Timmers M, van den Bosch L, van Damme P et al (2010) Progranulin is neurotrophic in vivo

and protects against a mutant TDP-43 induced axonopathy. PLoS ONE 5. https://doi.org/10.1371/journal.pone.0013368

Lee HZ, Kwitkowski VE, del Valle PL, Ricci MS, Saber H, Habtemariam BA et al (2015) FDA approval: belinostat for the treatment of patients with relapsed or refractory peripheral T-cell lymphoma. Clin Cancer Res 21:2666–2670. https://doi.org/10.1158/1078-0432.CCR-14-3119

Lee JD, Kamaruzaman NA, Fung JNT, Taylor SM, Turner BJ, Atkin JD et al (2013) Dysregulation of the complement cascade in the hSOD1G93A transgenic mouse model of amyotrophic lateral sclerosis. J Neuroinflamm 10. https://doi.org/10.1186/1742-2094-10-119

Liao B, Zhao W, Beers DR, Henkel JS, Appel SH (2012) Transformation from a neuroprotective to a neurotoxic microglial phenotype in a mouse model of ALS. Exp Neurol 237:147–152. https://doi.org/10.1016/j.expneurol.2012.06.011

Liu J, Lillo C, Andreas Jonsson P, vande Velde C, Ward CM, Miller TM et al (2004) Toxicity of Familial ALS-Linked SOD1 Mutants from Selective Recruitment to Spinal Mitochondria mitochondria as the basis for their selective toxicity in ALS. 43

Lorenzl S, Narr S, Angele B, Krell HW, Gregorio J, Kiaei M et al (2006) The matrix metalloproteinases inhibitor Ro 26–2853 extends survival in transgenic ALS mice. Exp Neurol 200:166–171. https://doi.org/10.1016/j.expneurol.2006.01.026

Madeira JM, Bajwa E, Stuart MJ, Hashioka S, Klegeris A (2014) Gold drug auranofin could reduce neuroinflammation by inhibiting microglia cytotoxic secretions and primed respiratory burst. J Neuroimmunol 276:71–79. https://doi.org/10.1016/j.jneuroim.2014.08.615

Madeira JM, Renschler CJ, Mueller B, Hashioka S, Gibson DL, Klegeris A (2013) Novel protective properties of auranofin: Inhibition of human astrocyte cytotoxic secretions and direct neuroprotection. Life Sci 92:1072–1080. https://doi.org/10.1016/j.lfs.2013.04.005

Maetzler W, Berg D, Schalamberidze N, Melms A, Schott K, Mueller JC et al (2007) Osteopontin is elevated in Parkinson's disease and its absence leads to reduced neurodegeneration in the MPTP model. Neurobiol Dis 25:473–482. https://doi.org/10.1016/j.nbd.2006.10.020

Maiese K (2015) FoxO proteins in the nervous system. Anal Cellular Pathol 2015. https://doi.org/10.1155/2015/569392

Manzano R, Toivonen JM, Calvo AC, Oliván S, Zaragoza P, Rodellar C et al (2013) Altered in vitro proliferation of mouse SOD1-G93A skeletal muscle satellite cells. Neurodegener Dis 11:153–164

Meller R, Stevens SL, Minami M, Cameron JA, King S, Rosenzweig H et al (2005) Neuroprotection by osteopontin in stroke. J Cereb Blood Flow Metab 25:217–225. https://doi.org/10.1038/sj.jcbfm.9600022

Mitchell J, Morris A, de Belleroche J (2009) Thioredoxin reductase 1 haplotypes modify familial amyotrophic lateral sclerosis onset. Free Radical Biol Med 46:202–211. https://doi.org/10.1016/j.freeradbiomed.2008.09.041

Mojsilovic-Petrovic J, Nedelsky N, Boccitto M, Mano I, Georgiades SN, Zhou W et al (2009) FOXO3a is broadly neuroprotective in vitro and in vivo against insults implicated in motor neuron diseases. J Neurosci 29:8236–8247. https://doi.org/10.1523/JNEUROSCI.1805-09.2009

Moon GJ, Shin M, Kim SR (2020) Upregulation of neuronal Rheb(S16H) for hippocampal protection in the adult brain. Int J Mol Sci 21. https://doi.org/10.3390/ijms21062023

Mouton AJ, Rivera Gonzalez OJ, Kaminski AR, Moore ET, Lindsey ML (2018) Matrix metalloproteinase-12 as an endogenous resolution promoting factor following myocardial infarction. Pharmacol Res 137:252–258. https://doi.org/10.1016/j.phrs.2018.10.026

Natarajan R, Singal V, Benes R, Gao J, Chan H, Chen H et al (2014) STAT3 modulation to enhance motor neuron differentiation in human neural stem cells. PLoS ONE 9. https://doi.org/10.1371/journal.pone.0100405

Noristani HN, Sabourin JC, Gerber YN, Teigell M, Sommacal A, Vivanco MDM et al (2015) Brca1 is expressed in human microglia and is dysregulated in human and animal model of ALS. Mol Neurodegener 10:34. https://doi.org/10.1186/s13024-015-0023-x

Oberstadt M, Stieler J, Simpong DL, Römuß U, Urban N, Schaefer M et al (2018) TDP-43 self-interaction is modulated by redox-active compounds Auranofin, Chelerythrine and Riluzole. Sci Reps 8. https://doi.org/10.1038/s41598-018-20565-0

Öktem EK, Yazar M, Gulfidan G, Arga KY (2019) Cancer Drug Repositioning by Comparison of Gene Expression in Humans and Axolotl (Ambystoma mexicanum) During Wound Healing. OMICS 23:389–405. https://doi.org/10.1089/omi.2019.0093

Onodera T, Momose I, Kawada M (2019) Potential Anticancer Activity of Auranofin. 67

Oprea TI, Overington JP (2015) Computational and practical aspects of drug repositioning. Assay Drug Dev Technol 13:299–306. https://doi.org/10.1089/adt.2015.29011.tiodrrr

Oughtred R, Stark C, Breitkreutz B-J, Rust J, Boucher L, Chang C et al (2019) The BioGRID interaction database: 2019 update. Nucleic Acids Res 47:D529–D541

Pasinelli P, Houseweart MK, Brown RH, Cleveland DW (2000) Caspase-1 and-3 are sequentially activated in motor neuron death in Cu, Zn superoxide dismutase-mediated familial amyotrophic lateral sclerosis. PNAS 97:13901–13906

Piscopo P, Crestini A, Adduci A, Ferrante A, Massari M, Popoli P et al (2011) Altered oxidative stress profile in the cortex of mice fed an enriched branched-chain amino acids diet: Possible link with amyotrophic lateral sclerosis? J Neurosci Res 89:1276–1283. https://doi.org/10.1002/jnr.22655

Prudencio M, Hart PJ, Borchelt DR, Andersen PM (2009) Variation in aggregation propensities among ALS-associated variants of SOD1: Correlation to human disease. Hum Mol Genet 18:3217–3226. https://doi.org/10.1093/hmg/ddp260

Quintero-Villegas A, Valdés-Ferrer SI (2022) Central nervous system effects of 5-HT7 receptors: a potential target for neurodegenerative diseases. Mol Med 28

Roder C, Thomson MJ (2015) Auranofin: Repurposing an Old Drug for a Golden New Age. Drugs in R and D 15:13–20. https://doi.org/10.1007/s40268-015-0083-y

Rollo EE, Denhart DT (1996) Differential effects of osteopontin on the cytotoxic activity of macrophages from young and old mice. Immunology 88:642–647

Romano R, Bucci C (2020) Role of EGFR in the Nervous System. Cells 9:1887

Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A (1993) Mutations in CuZn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362:59–62

Rosen EY, Wexler EM, Versano R, Coppola G, Gao F, Winden KD et al (2011) Functional Genomic Analyses Identify Pathways Dysregulated by Progranulin Deficiency. Implicating Wnt Signaling Neuron 71:1030–1042. https://doi.org/10.1016/j.neuron.2011.07.021

Rouaux C, Panteleeva I, René F, de Aguilar JLG, Echaniz-Laguna A, Dupuis L et al (2007) Sodium valproate exerts neuroprotective effects in vivo through CREB-binding protein-dependent mechanisms but does not improve survival in an amyotrophic lateral sclerosis mouse model. J Neurosci 27:5535–5545. https://doi.org/10.1523/JNEUROSCI.1139-07.2007

Ryan CL, Baranowski DC, Chitramuthu BP, Malik S, Li Z, Cao M et al (2009) Progranulin is expressed within motor neurons and promotes neuronal cell survival. BMC Neurosci 10:130. https://doi.org/10.1186/1471-2202-10-130

Ryu H, Smith K, Camelo SI, Carreras I, Lee J, Iglesias AH et al (2005) Sodium phenylbutyrate prolongs survival and regulates expression of anti-apoptotic genes in transgenic amyotrophic lateral sclerosis mice. J Neurochem 93:1087–1098. https://doi.org/10.1111/j.1471-4159.2005.03077.x

Sahaan TG, Zhang KE (2021) Mitogen-Activated Protein Kinase Pathway in Amyotrophic Lateral Sclerosis. Biomedicines 9:969

Sakaguchi T, Irie T, Kawabata R, Yoshida A, Maruyama H, Kawakami H (2011) Optineurin with amyotrophic lateral sclerosis-related mutations abrogates inhibition of interferon regulatory factor-3 activation. Neurosci Lett 505:279–281. https://doi.org/10.1016/j.neulet.2011.10.040

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D et al (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13:2498–2504. https://doi.org/10.1101/gr.1239303

Shibata N, Kakita A, Takahashi H, Ihara Y, Nobukuni K, Fujimura H et al (2009) Activation of signal transducer and activator of transcription-3 in the spinal cord of sporadic amyotrophic lateral sclerosis patients. Neurodegener Dis 6:118–126. https://doi.org/10.1159/000213762

Shlevkov E, Basu H, Bray M-A, Sun Z, Wei W, Apaydin K et al (2019) A High-Content Screen Identifies TPP1 and Aurora B as Regulators of Axonal Mitochondrial Transport. Cell Rep 28:3224–3237.e5. https://doi.org/10.1016/j.celrep.2019.08.035

Shukla S, Tekwani BL (2020) Histone Deacetylases Inhibitors in Neurodegenerative Diseases, Neuroprotection and Neuronal Differentiation. Front Pharmacol 11. https://doi.org/10.3389/fphar.2020.00537

Smedley D, Haider S, Durinck S, Pandini L, Provero P, Allen J et al (2015) The BioMart community portal: An innovative alternative to large, centralized data repositories. Nucleic Acids Res 43:W589–W598. https://doi.org/10.1093/nar/gkv350

Smyth GK, Ritchie M, Thorne N, Wettenhall J (2005) LIMMA: linear models for microarray data. In Bioinformatics and Computational Biology Solutions Using R and Bioconductor. Stat Biol Heal

Sreedharan J, Brown RH (2013) Amyotrophic lateral sclerosis: Problems and prospects. Ann Neurol 74:309–316. https://doi.org/10.1002/ana.24012

Sullivan PM, Zhou X, Hu F (2017) Autophagy-Lysosome Dysfunction in Amyotrophic Lateral Sclerosis and Frontotemporal Lobar Degeneration. Lysosomes - Associated Diseases and Methods to Study Their Function, InTech. https://doi.org/10.5772/intechopen.69371

Szklarczyk D, Santos A, von Mering C, Jensen LJ, Bork P, Kuhn M (2016) STITCH 5: Augmenting protein-chemical interaction networks with tissue and affinity data. Nucleic Acids Res 44:D380–D384. https://doi.org/10.1093/nar/gkv1277

Tortarolo M, Vallarola A, Lidonnici D, Battaglia E, Gensano F, Spaltro G et al (2015) Lack of TNF-alpha receptor type 2 protects motor neurons in a cellular model of amyotrophic lateral sclerosis and in mutant SOD1 mice but does not affect disease progression. J Neurochem 135:109–124

Trieu VN, Liu R, Liu XP, Uckun FM (2000) A specific inhibitor of Janus kinase-3 increases survival in a transgenic mouse model of amyotrophic lateral sclerosis. Biochem Biophys Res Commun 267:22–25. https://doi.org/10.1006/bbrc.1999.1905

Trostchansky A, Mastrogiovanni M, Miquel E, Rodríguez-Bottero S, Martínez-Palma L, Cassina P et al (2018) Profile of Arachidonic Acid-Derived Inflammatory Markers and Its Modulation by Nitro-Oleic Acid in an Inherited Model of Amyotrophic Lateral Sclerosis. Front Mol Neurosci 11

Turanli B, Grøtli M, Boren J, Nielsen J, Uhlen M, Arga KY et al (2018) Drug repositioning for effective prostate cancer treatment. Front Physiol 9. https://doi.org/10.3389/fphys.2018.00500

Turanli B, Karagoz K, Bidkhori G, Sinha R, Gatza ML, Uhlen M et al (2019) Multi-omic data interpretation to repurpose subtype specific drug candidates for breast cancer. Front Gen 10. https://doi.org/10.3389/fgene.2019.00420

Turanli B, Zhang C, Kim W, Benfeitas R, Uhlen M, Arga KY et al (2019) Discovery of therapeutic agents for prostate cancer using genome-scale metabolic modeling and drug repositioning. EBioMedicine 42:386–396. https://doi.org/10.1016/j.ebiom.2019.03.009

Van Kan HJM, Van Den Berg LH, Groeneveld GJ, van der Straaten R, van Vught PWJ, Lie-A-Huen L et al (2008) Pharmacokinetics of riluzole: evidence for glucuronidation as a major metabolic pathway not associated with UGT1A1 genotype. Biopharm Drug Dispos 29:139–144

Virit O, Selek S, Bulut M, Savas HA, Celik H, Erel O et al (2008) High ceruloplasmin levels are associated with obsessive compulsive disorder: A case control study. Behav Brain Funct4. https://doi.org/10.1186/1744-9081-4-52

Vogrinc D, Kunej T (2017) Drug repositioning: computational approaches and research examples classified according to the evidence level. Discoveries 5:e75. https://doi.org/10.15190/d.2017.5

von Grabowiecki Y, Abreu P, Blanchard O, Palamiuc L, Benosman S, Mériaux S et al (2016) Transcriptional activator TAp63 is upregulated in muscular atrophy during ALS and induces the pro-atrophic ubiquitin ligase Trim63. Elife 5:e10528. https://doi.org/10.7554/eLife.10528

Wang Y, Tang X, Yu B, Gu Y, Yuan Y, Yao D et al (2012) Gene Network Revealed Involvements of Birc2, Birc3 and Tnfrsf1a in Anti-Apoptosis of Injured Peripheral Nerves. PLoS ONE 7. https://doi.org/10.1371/journal.pone.0043436

Watanabe S, Ageta-Ishihara N, Nagatsu S, Takao K, Komine O, Endo F et al (2014) SIRT1 overexpression ameliorates a mouse model of SOD1-linked amyotrophic lateral sclerosis via HSF1/HSP70i chaperone system. Mol Brain 7. https://doi.org/10.1186/s13041-014-0062-1

Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR et al (2018) DrugBank 5.0: A major update to the DrugBank database for 2018. Nucleic Acids Res 46:D1074–82. https://doi.org/10.1093/nar/gkx1037

Wolf FG, dos Santos LOE, Philippi PC (2006) Plasma copper, iron, ceruloplasmin and ferroxidase activity in schizophrenia. Schizophr Res 86:167–171. https://doi.org/10.1016/j.schres.2006.05.027

Xie M, Liu YU, Zhao S, Zhang L, Bosco DB, Pang Y-P et al (2022) TREM2 interacts with TDP-43 and mediates microglial neuroprotection against TDP-43-related neurodegeneration. Nat Neurosci 25:26–38. https://doi.org/10.1038/s41593-021-00975-6

Xu R, Wang QQ (2016) A genomics-based systems approach towards drug repositioning for rheumatoid arthritis. BMC Genomics 17. https://doi.org/10.1186/s12864-016-2910-0

Xu X, Zhang J, Li S, Al-Nusaif M, Zhou Q, Chen S et al (2022) Bone Marrow Stromal Cell Antigen 2: Is a Potential Neuroinflammation Biomarker of SOD1G93A Mouse Model of Amyotrophic Lateral Sclerosis in Pre-symptomatic Stage. Front Neurosci 15. https://doi.org/10.3389/fnins.2021.788730

Yoo YE, Ko CP (2011) Treatment with trichostatin A initiated after disease onset delays disease progression and increases survival in a mouse model of amyotrophic lateral sclerosis. Exp Neurol 231:147–159. https://doi.org/10.1016/j.expneurol.2011.06.003

Zhang M, Luo H, Xi Z, Rogaeva E (2015) Drug repositioning for diabetes based on "omics" data mining. PLoS ONE 10. https://doi.org/10.1371/journal.pone.0126082

Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O et al (2019) Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun 10:1523. https://doi.org/10.1038/s41467-019-09234-6