Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Integrated genomic and molecular insights into astrocyte- and oligodendrocyte-derived amyotrophic lateral sclerosis: focus on miRNAs and extracellular vesicles

Ehsan Asghari Jafari[1], Maryam Arabi[2], Ahmad Bereimipour[3,4*]

[1] Department of Biology, School of Basic Science, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2] Medical Genomics Research Center, Tehran Medical Sciences Islamic Azad University, Tehran, Iran
[3] Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
[4] Department of Biological Sciences and BioDiscovery Institute, University of North Texas, Denton, TX, 76203, USA

Article Info

Article history:

Received: October 10, 2024
Accepted: April 02, 2025
Published: June 30, 2025

Use your device to scan and read the article online

Abstract

Motor neurons in the brain and spinal cord begin to die off in Amyotrophic lateral sclerosis (ALS), a disease that can be fatal. Molecular pathways in neurological disease, especially ALS, remain a challenge in the medical sciences. In this disease, a disorder in both astrocytes and oligodendrocytes can cause the disease to progress. This study aimed to investigate the molecular mechanisms and find key elements between these two cells in ALS with a bioinformatics perspective. In this study, using integrated and continuous bioinformatics analytics by various tools and databases, we investigated genes, protein products, and miRNAs between astrocytes and oligodendrocytes. The obtained data were involved in the Cellular senescence, actin cytoskeleton, and cell cycle signaling pathways. Then, after careful evaluation of the information, TP53, MDM2, KRAS, PTPRC, and GSK proteins were candidates, which are regulated by hsa-miR-564, hsa-miR-496-5p, hsa-miR-324-5p, hsa-miR-296-5p, and hsa-miR-4258-3p miRNAs. Finally, the four genes had a more robust and better relationship in this study between astrocyte and oligodendrocyte-derived ALS.

Keywords: MiRNAs, Astrocyte, Oligodendrocyte, Amyotrophic lateral sclerosis.

1. Introduction

An adult-onset neurodegenerative illness known as amyotrophic lateral sclerosis (ALS) is characterized by the gradual loss of motor function and, ultimately, death due to respiratory failure. ALS is the second leading cause of dementia after Alzheimer's. ALS has many neuropathological and genetic features associated with frontotemporal dementia (FTD). TDP-43, a 43-kD trans-active response (TAR) DNA-binding protein, is frequently mutated in both disorders, as are other RNA-binding proteins (RBPs)[1,2].

During the past decade, the profession has been rocked by the development and clinical use of antisense oligonucleotides (ASOs). As long as the mutant gene has a toxic function, then the toxic gene product is targeted for degradation to stop the harmful RNAs or proteins in their tracks. The strategy is straightforward and elegant. As an example, in the therapy of spinal muscular atrophy, another form of oligonucleotide is employed to prevent unwanted splicing processes in order to increase gene product synthesis[2–4].

Nearly one-tenth of instances of ALS are referred to as "family-based" (FALS), while the other two-thirds are labeled "sporadic" (SALS), meaning there is no obvious family history. It has been a major factor in our understanding of disease mechanisms because of the finding of ALS-causing mutations in families and subsequent experiments on these genes. It is possible to utilize genetic methods to identify the defective gene that is associated with people who acquire ALS and separate them from people who do not. Most of the key ALS genes have been discovered as a result of this type of research[4]. Identifying and validating ALS genes is crucial for understanding how mutations in those genes affect the symptoms experienced by ALS patients. Specifically, it's important to determine whether a mutation leads to a loss or gain of protein function, including the acquisition of toxic properties. A gene therapy technique to reactivate a gene's activity would be needed if a mutation results in disease through loss of function. A therapeutic approach aimed at reducing the quantities of the mutant gene's gene products or inhibiting its functio-

* Corresponding author.
E-mail address: ahmadlta@yahoo.com (A. Bereimipour).
Doi: http://dx.doi.org/10.14715/cmb/2025.71.6.1

nality would be more appropriate if a gain in function of the mutant gene causes sickness. Sorting this data out is essential for the development of ALS therapies[5,6].

The computing and informatics difficulties in neuroscience are numerous. These issues involve a lot of "classic" bioinformatics (such as sequence analysis) that have been applied to the field of neuroscience in new and innovative ways. The word "neuroinformatics" was coined to describe the application of computers and informatics in neuroscience, probably more than any other area. There are many facets to the field of neuroinformatics, from the creation of databases to the creation of standards, models, tools, and simulations. Bioinformatics and neuroinformatics have a lot in common when it comes to studying genes and proteins[7,8]. A large part of the fascination with neuroinformatics, on the other hand, stems from the wide variety of neuroscience studies being conducted and the potential for these studies to be more closely linked through the use of informatics. In general, the study of molecular and genetic aspects of neurological diseases has not been done transparently and accurately so far. Examining the dimensions of signal pathways, genes, protein products produced from genes, as well as regulatory elements can provide new windows for the diagnosis and treatment of this disease[9,10]. Therefore, the aim of this study was to evaluate more accurately and better the microenvironmental processes in astrocytes and oligodendrocytes in patients with ALS in order to find better methods for both the diagnosis and treatment of the disease by selecting appropriate biomarkers.

2. Materials and methods

2.1. GEO datasets selection and processing for identifying differentially expressed genes

In this study, bioinformatics analysis was performed. We first used the GEO database and selected the three appropriate microarray-dataset for this study. GSE26276, GSE87385 and GSE87385. After defining the groups using the GEO2R tool, we isolated the differential expression profiles of the genes and saved them in an Excel file. Then we separated the gene clusters with up and down expression and prepared them for the next step. In this part, the p value<0.05 was considered statistically significant (Figure 1 and Table 1).

2.2. Evaluation of signaling pathways and gene ontology

Genes with high and low expression in dataset were first isolated. Then the obtained common genes to the Enrichr database to examine the signaling pathways and gene ontology. After that, the KEGG library was used to analyze the signaling pathways. The ontology section was then used to evaluate the molecular functions and biological processes of high and low-expression genes. The Shiny GO database was then used to plot the communication network between the results. The p value<0.05 was considered statistically significant.

2.3. Evaluation of proteins and genes network

After evaluating the signaling pathways and gene ontology, the pathways that played a significant role in the development of neurons and ALS were selected. The relationship between their protein networks was assessed using the STRING and GeneMania databases.

2.4. Selection of candidate miRNAs and construction of miRNA-target gene networks

After nominating important genes and proteins in the evaluated pathways, to confirm and obtain more information about the amount of miRNAs, first uploaded the genes to the miRwalk database to validate and evaluate miRNAs. Then the MienTurnet and miRnet databases to design the communication network between the miRNAs and the target genes.

2.5. Evaluation of miRNAs released into extracellular vesicles

We used miRnet to initially screen for miRNAs potentially associated with extracellular vesicles. We then refined our selection by cross-referencing these candidates with the ExoCarta and Vesiclepedia databases to confirm their presence in EVs.

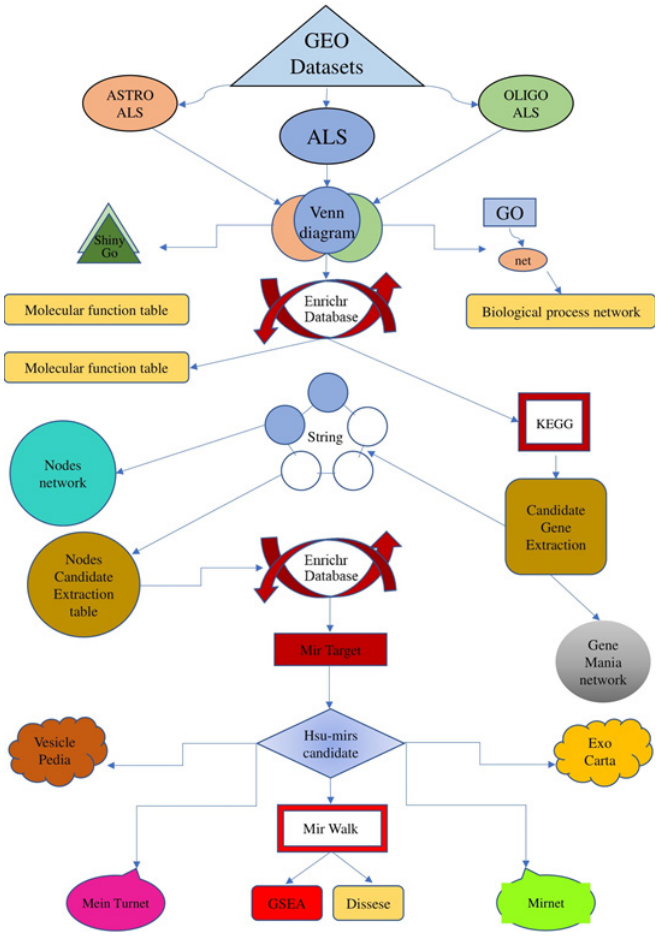

Fig. 1. Bioinformatics analysis schematic pathway.

Table 1. Information about GEO datasets.

Accession ID	Name of group	Count	Platform
GSE26276	ALS/ASTRO/OLIGO	9 Samples	GPL6244
GSE87385	ASTROCYTE	12 Samples	GPL570
GSE87385	OLIGODENDROCYTE	12 Samples	GPL570

3. Results

3.1. Pathway enrichment analysis reveals key signaling pathways in astrocyte- and oligodendrocyte-derived ALS

Pathway enrichment analysis revealed significant dysregulation in astrocyte- and oligodendrocyte-derived ALS. Using Venn diagram analysis to identify common gene expression changes, we found 404, 247, and 133 upregulated genes common to astrocyte- and oligodendrocyte-derived ALS, astrocyte-derived ALS, and oligodendrocyte-derived ALS, respectively. Functional annotation of these upregulated gene sets indicated enrichment in pathways related to actin cytoskeleton regulation, steroid hormone biosynthesis, inositol phosphate metabolism, P53 signaling, cell cycle, cellular senescence, glioma, cAMP signaling, protein digestion and absorption, and T cell receptor signaling (Figure S1A). Conversely, we identified 221, 108, and 107 downregulated genes common to astrocyte- and oligodendrocyte-derived ALS, astrocyte-derived ALS, and oligodendrocyte-derived ALS, respectively. Pathway analysis of the downregulated gene sets showed enrichment in pathways related to regulation of lipolysis in adipocytes, insulin signaling, longevity regulation, aldosterone synthesis and secretion, glucagon signaling, HIF-1 signaling, neurodegeneration, butanoate metabolism, and tight junctions (Figure S1B).

3.2. Evaluation of gene ontology between astrocyte and oligodendrocyte derived ALS.

This section further evaluates the astrocyte and oligodendrocyte-derived ALS from the previous step and examines their molecular functions and biological processes. Accordingly, insulin-like growth factor receptor binding, hydrolase activity, hydrolyzing N-glycosyl compounds, oxidoreductase activity, acting on the CH-CH group of donors, NAD or NADP as acceptor, retinoic acid receptor binding, FATZ binding, disordered domain specific binding, water transmembrane transporter activity, hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in linear amides molecular functions in upregulated genes. Besides, pseudophosphatase activity, 15-hydroxyprostaglandin dehydrogenase (NAD+) activity, Phenanthrene 9,10-monooxygenase activity, Histone deacetylase binding, Transmembrane receptor protein tyrosine phosphatase activity, Transmembrane receptor protein phosphatase activity, and cyclohydrolase activity (Figure 2, Tables 2 and 3).

3.3. Hub proteins participated in the astrocyte and oligodendrocyte-derived ALS.

In this part of the study, a more in-depth analysis of relationship between proteins with genes associated with astrocyte and oligodendrocyte-derived ALS was performed. KRAS, GSK, IRS1, PLCH1, AKR1C3, and IPMK in astrocyte and oligodendrocyte-derived ALS were observed. TP53, MDM2, GADD45A, PKH, GAPDH, and LDHA participated in astrocyte-derived ALS and PTPRC, PTK2, MLLT4, ACADS, FZD1, and ACSM2B prominent role oligodendrocyte derived ALS (Figure 3). For more details, visit Figures S2, S3, and S4.

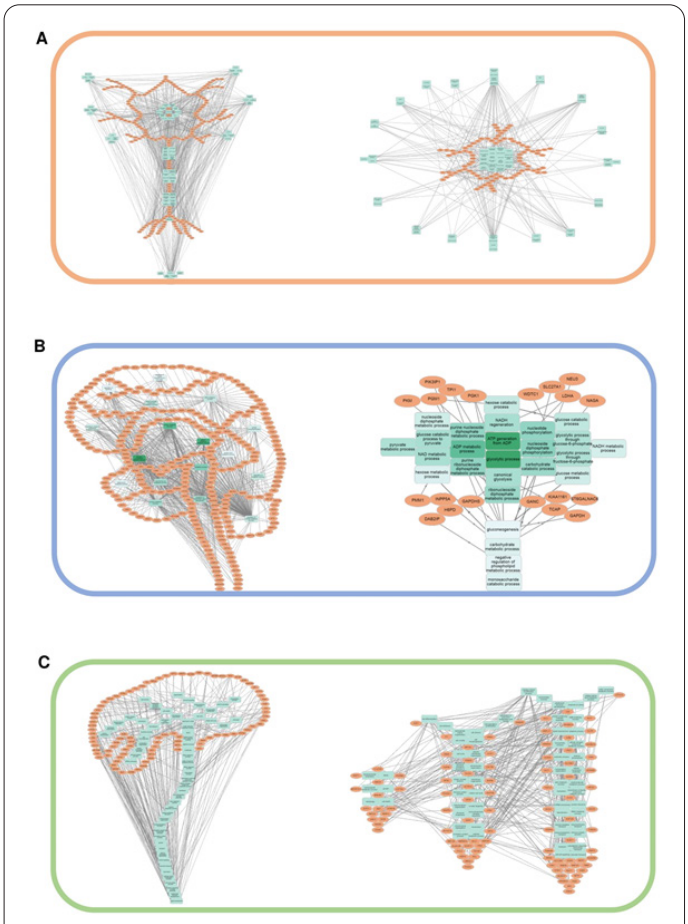

Fig. 2. Biological processes demonstrated in upregulated and downregulated genes between A: Astrocyte and oligodendrocyte-derived ALS, B: Astrocyte-derived ALS and C: Oligodendrocyte-derived ALS.

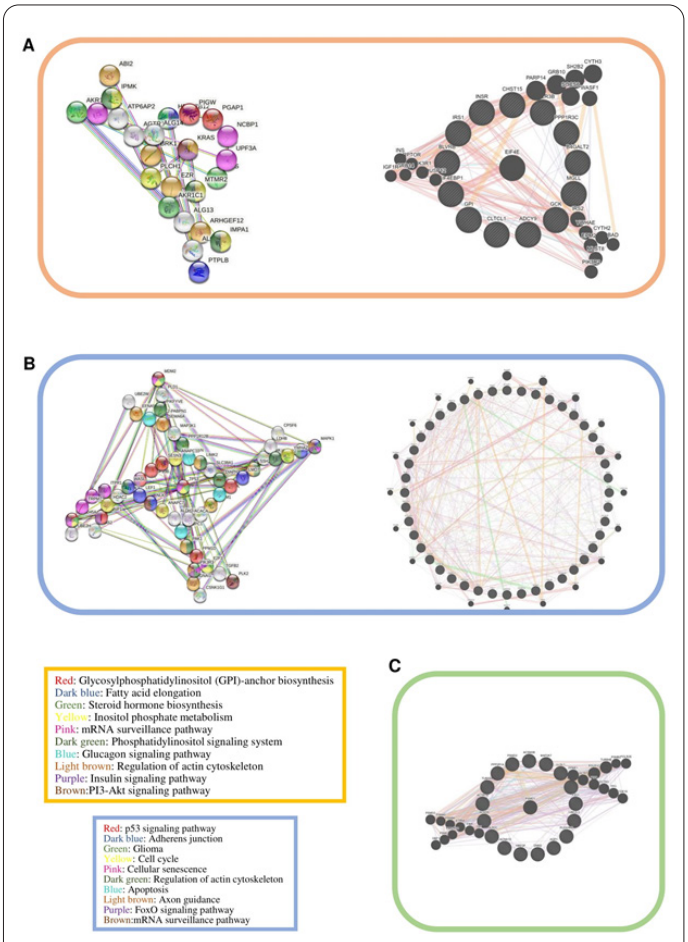

Fig. 3. Hub proteins involved in A: astrocyte and oligodendrocyte-derived ALS, B: astrocyte derived ALS and C: oligodendrocyte derived ALS.

Table 2. Molecular functions in upregulated genes between astrocyte and oligodendrocyte derived ALS.

Enrichment FDR	Pathway	Genes
	ASTRO ALS	
0.0238870892703713	Pseudophosphatase activity	*STYXL1 STYX*
0.0238870892703713	Nucleic acid binding	*SMC1L1 EZR DDX17 ALG13 POP1 RBM28 GLRX3 PC4 PTBP3 ETF1 MACF1 WDR36 NCBP1 RPS8 C1orf131 RPS27A*
0.0238870892703713	15-hydroxyprostaglandin dehydrogenase (NAD+) activity	*HPGD ABCC4*
0.0368563051655137	Bile acid binding	*AKR1C1 AKR1C3 PYGL*
0.0427100401626544	Phenanthrene 9,10-monooxygenase activity	*AKR1C1 AKR1C3*
	ALS	
1.74823173142107E-07	Nucleic acid binding	*RC3H2 CCAR1 PABPC1 CFAP20 PNN ZC3HAV1 LUC7L3 CPSF6 CHD4 SPTBN1 WDR75 TIA1*
0.0000106371561518451	Enzyme binding	*MTA3 MEF2A SUDS3 TRIM38 TRIM22 RNF217 PHF6 PPM1D TRIM27 WNK1 HACD3 RIC1 XPO5*
0.0000106371561518451	Histone deacetylase binding	*MTA3 MEF2A SUDS3 PHF6 ANKRA2 CRY1 NR2C1 KLF4 CHD4 LEF1 TP53 RBBP4 KPNA2 HDAC2*
0.00047877506032404	DNA binding	*CHD4 RBBP4 SMARCC1 HDAC2 ZNF195 CRY1 ZNF582 ZNF280C MEF2A ZNF506 KLF3 E2F3 ZNF684*
0.00047877506032404	RNA binding	*RC3H2 CCAR1 PABPC1 CFAP20 PNN ZC3HAV1 LUC7L3 CPSF6 SPTBN1 WDR75 TIA1 PRRC2C*
	OLIGO ALS	
0.0191102340088124	Transmembrane receptor protein tyrosine phosphatase activity	*PTPRO PTPRK PTPRC*
0.0191102340088124	Transmembrane receptor protein phosphatase activity	*PTPRO PTPRK PTPRC*
0.0191102340088124	Cation binding	*SOD2 ESYT2 GCH1 S100A10 MTHFD2 CYLD KDM4C CLEC4A METTL3 CYP2R1 RBM5 variant*
0.0191102340088124	Metal ion binding	*SOD2 ESYT2 GCH1 S100A10 MTHFD2 CYLD KDM4C CLEC4A CYP2R1 RBM5 variant FAT1 ZNF268*
0.0219261641679001	Cyclohydrolase activity	*MTHFD2 GCH1*

Table 3. Molecular functions in downregulated genes between astrocyte and oligodendrocyte derived ALS.

Enrichment FDR	Pathway	Genes
	ASTRO ALS	
0.00251997219642481	insulin-like growth factor receptor binding	*IRS1 INSR*
0.00329972072027439	hydrolase activity, hydrolyzing N-glycosyl compounds	*MACROD1 SARM1*
0.00417764350659283	oxidoreductase activity, acting on the CH-CH group of donors, NAD or NADP as acceptor	*TECR BLVRB*
0.00567387638375119	ubiquitin-specific protease binding	*BAG6 RAD23A*
0.00621938352769408	voltage-gated sodium channel activity	*SCN1B HCN1*
	ALS	
0.000368221934922043	nuclear receptor binding	*ASXL1 STAT5B NR4A1 MED25 RXRA HMGA1 MED16*
0.000948273368847305	retinoic acid receptor binding	*ASXL1 MED25 HMGA1*
0.0011169817513096	ankyrin binding	*FLNC SPTB PLEC*
0.00117848516901002	FATZ binding	*TCAP MYOZ3*
0.00197640009324868	disordered domain specific binding	*NUMA1 FKBP8 GAPDH*
	OLIGO ALS	
0.00191195584101257	channel activity	*AQP12B AQP4 AQP1*
0.00212823837680453	water channel activity	*AQP4 AQP1*
0.0028450121422312	water transmembrane transporter activity	*AQP4 AQP1*
0.0034919580623784	GTP binding	*RAB1B TUBA4B TUBA4A RHOQ DNM2*
0.00353196021041435	hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in linear amides	*FAAH ACY1 SIRT2*

3.4. Candidate critical miRNAs and extracellular vesicles' miRNAs in regulating astrocyte and oligodendrocyte-derived ALS.

In this part of the study, the genes involved in astrocyte and oligodendrocyte-derived ALS were to the MienTurnet database. hsa-miR-4280-3p, hsa-miR-428-5p, hsa-miR-1225-3p, hsa-miR-323b-5p, hsa-miR-184-3p, hsa-miR-423-3p, hsa-miR-515-5p, hsa-miR-3559-5p, and hsa-miR-4746-3p were selected as the most important linkages with hub genes and proteins products (Figure 4). Also showed the relationships of the other miRNAs that were significant in the miRnet database as a communication network. Then to examine the miRNAs in the extracellular vesicles, the miR-658-3p, hsa-miR-564, hsa-miR-496-5p, hsa-miR-324-5p, hsa-miR-296-5p, and hsa-miR-4258-3p were more pronounced in astrocyte and oligodendrocyte derived ALS. Also, hsa-miR-1244-3p, hsa-miR-409-3p, hsa-miR-496-3p, hsa-miR-658-3p, hsa-miR-423-3p, and hsa-miR-3146-3p were observed in miRnome ALS and extracellular vesicles (Figure 5). For more details visit Figures S5-S8.

4. Discussion

The majority of the newly discovered genes have only recently been discovered and so account for a smaller percentage of the ALS population than the previously discovered genes. Optineurin is an ALS-related gene that is directly regulated by TBK1 (TANK binding kinase 1), a protein that is necessary for autophagy and innate immune activation and is found in all cells. It has been discovered

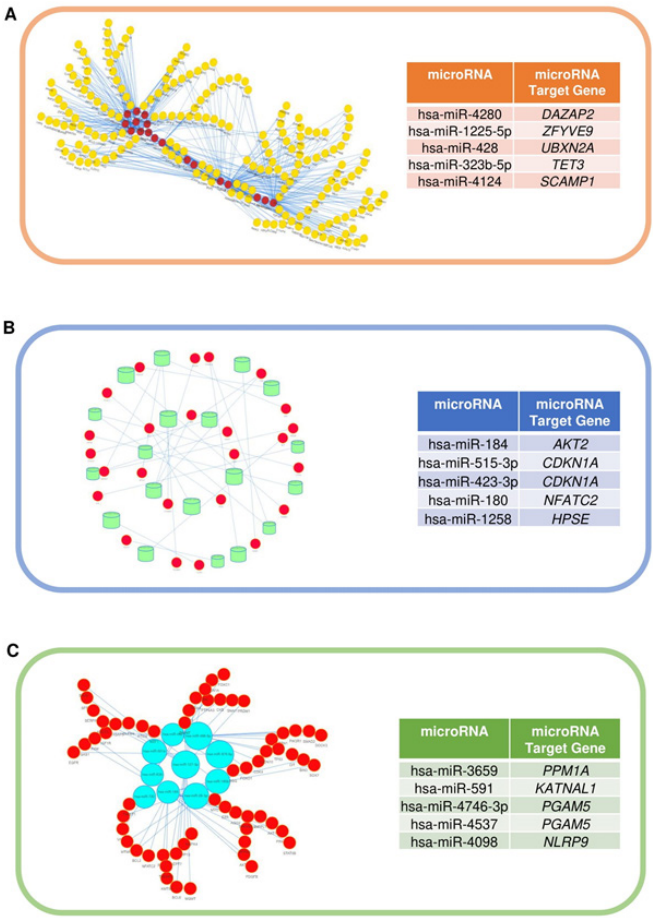

Fig. 4. Critical miRNAs could regulate hub genes, evaluated in interactive networks. A: astrocyte and oligodendrocyte-derived ALS, B: astrocyte-derived ALS and C: oligodendrocyte-derived ALS.

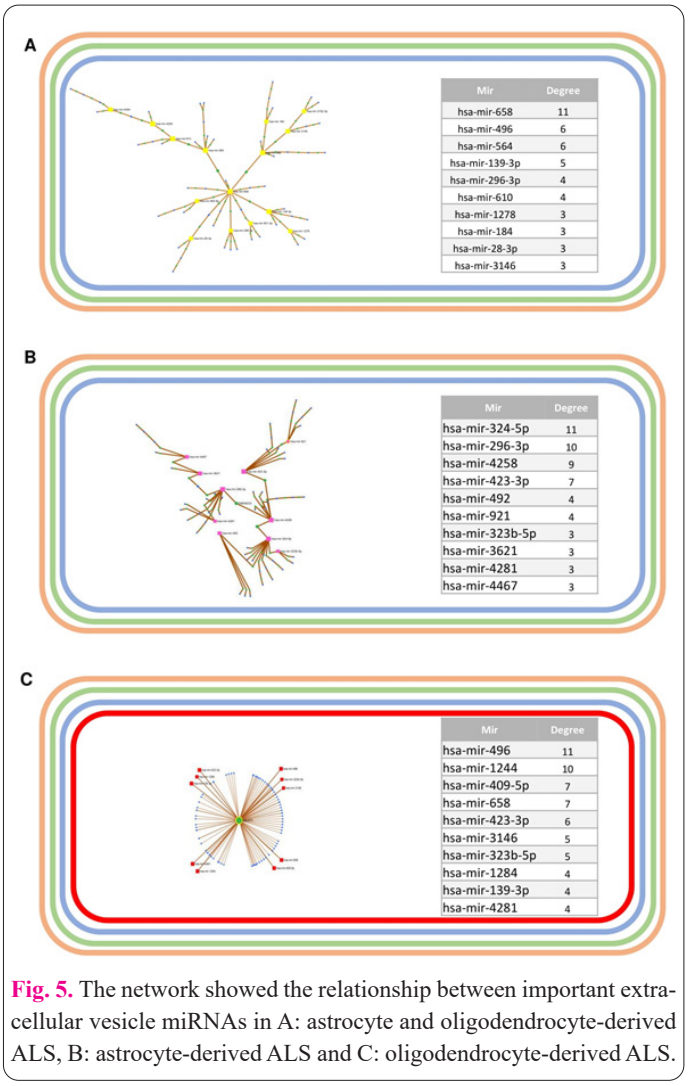

Fig. 5. The network showed the relationship between important extracellular vesicle miRNAs in A: astrocyte and oligodendrocyte-derived ALS, B: astrocyte-derived ALS and C: oligodendrocyte-derived ALS.

that a number of single amino acid deletions have occurred; it is probable that these are responsible for decreasing phosphorylation[11,12]. After conducting a thorough examination of people suffering from ALS, researchers discovered evidence that the ALS gene TUBA4A was responsible for their condition, which was then confirmed in a second investigation. There have been cases of spinal onset with characteristic upper and lower motor neuron involvement in patients with probable mutations, albeit only a few have shown cognitive impairment or frontal lobe degeneration[13,14].

Recent investigations have discovered that loss of function mutations in the C-terminal cargo binding domain are associated with Lou Gehrig's disease (ALS). It is possible that mutation carriers cause axonal transport to be disturbed in some way, but this has not been demonstrated. ANXA11 is involved in the formation of vesicles and the anchoring of mRNA for axonal transport[15,16]. ALS researchers discovered a link between the gene and the disease after searching for recurrently changed genes or variants in 50 family members who had the disease. TIA mutations are known to produce a distal myopathy with vacuoles in the limbs, which has been known for a long time (just like other ALS genes VCP, MATR3, and HNRNPA2B1)[17,18].

According to the findings of the research, CCNF carriers are more likely than not to also have clinical FTD in addition to their ALS. However, it was the segregating mutation (p.S621G) that exhibited the most severe effect, resulting in increased mortality of spinal cord neurons as

well as a shortened length of motor axons in the laboratory[19,20]. No recurrent or disease-associated variations have been discovered in other cohorts, despite the presence of additional rare and novel variant types in this study's sample population. The neuropathological and clinical characteristics of mutation carriers have not yet been studied in great detail. Despite the fact that DNAJC7 haploinsufficiency is thought to be the reason for the drug's effects, no functional studies have been done[21,22].

While the absence of NEK1 function or missense mutations has not yet been demonstrated, it is probable that the accumulation of DNA damage will contribute directly to cell damage and death in the absence of NEK1. LGALSL is a gene that encodes a protein that is related to galectins. However, the exact function of this protein is not known. The gene was discovered by the use of whole-exome sequencing. In comparison to the rest of the cohort, patients with LGALSL mutations experienced symptoms at an earlier age (by 13 years on average). ALS or ALS-FTD mutations have not been reported to be handed down via families[23,24].

GLT8D1 has been found as a glycosyltransferase with an unknown function that has been associated with Lou Gehrig's disease (ALS) using whole-exome sequencing and candidate gene sequencing. In a study of patients who had two rare missense mutations in the ARPP21 and GLT8d1 genes, researchers uncovered a haplotype that was associated with ALS. The researchers hypothesized that the two mutations might have a synergistic influence on one another's performance. As in the instance of LGALSL, compelling evidence would indicate an etiology of ALS that had not previously been recognized[25,26].

In the present study, our main focus was on the study of molecular events in astrocytes and oligodendrocytes. Which also comprehensively referred to the signal pathways, the relationship between protein products and their regulators, as well as their presence in extracellular vesicles. In the following, we examined some genes and proteins. Finding these genes is likely to open new windows in the treatment of ALS.

The GSK protein produced by this gene is a serine-threonine kinase that is a member of the glycogen synthase kinase subfamily, and it is expressed in the liver. Glucose homeostasis is negatively regulated by this protein, which is also implicated in energy metabolism, inflammation, ER stress, mitochondrial dysfunction, and apoptotic pathways. It has been shown that mutations in this gene are linked to Parkinson's disease and Alzheimer's disease. FUS-linked ALS mutations cause cytoplasmic clumps in neurons[27]. In FUS-associated ALS, they are thought to be vital. Cytoplasmic FUS aggregate clearance and breakdown in neurons may be an ALS treatment option. FUS-associated ALS is still poorly understood at the molecular level. We show that GSK-3 can modulate FUS-induced toxicity. Affecting the retina, motor neurons, and mitochondria, RNAi-mediated Shaggy knockdown in FUS-expressing flies decreases faulty phenotypes. Results also showed that saggy depletion reduced cytoplasmic FUS aggregates. Co-overexpression of Slimb, an F-box protein, lowered levels of FUS proteins, demonstrating that Slimb is required for suppressing FUS-induced toxicity in Drosophila. With GSK-3 inhibitors, the SCFSlimb-mediated FUS degradation process may be changed in the real world, which could show a new way to protect the brain[28]. Adults are most likely to develop ALS. Transient TDP-43 hyperphosphorylation clumps are detected in the cytoplasm of ALS patients. GSK-3 phosphorylates TDP-43. The spinal cord and brain tissues of ALS patients are more active. It is possible that Tideglusib, an in-house non-ATP competitive GSK-3 inhibitor, could be repurposed as an ALS therapeutic alternative. Thus, we evaluated Tideglusib's efficiency in multiple ALS models, both ex vivo and in vitro. We detected increased GSK-3 activity, TDP-43 phosphorylation, and cytoplasmic TDP-43 accumulation in lymphoblasts from sporadic ALS patients. Inhibition of the dephosphorylation of TDP-43 by Tideglusib was reduced in ALS lymphoblasts in a human neuroblastoma model. The continuous oral Tideglusib medication lowered TDP-43 phosphorylation in the Prp-hTDP-43A315T mouse spinal cord. A phase II clinical trial for Tideglusib is planned by the end of the year. Phosphorylated GSK-3 (GSK-3) disrupts brain function, causing cell damage and disease. The GSK-3 isozymes have some overlap or compensatory activity. They are vital because GSK-3 evolved differently from other GSKs. Knockout mice and isozyme inhibitors may assist. They also demonstrated our lack of understanding of isozyme dynamics[28,29]. Strong GSK-3 inhibitors could treat GSK-3 hyperactivity. Which is better: isozyme inhibitors or GSK-3 inhibitors It was the same with GSK-3 knockdown (postnatal). And it may help treat FXS. It may help to stop both enzymes. To protect neurons, suppress one GSK-3 isozyme. Both GSK-3 isozymes may be more clinically efficacious. Most drugs can't pass the Blood Brain Barrier (BBB). Endothelial cells in the brain filter chemicals, peptides, and proteins. Recently, new strategies for delivering brain medications have emerged. The polymeric nanoparticles can carry drugs. It will lead to CNS therapy. Few GSK-3 inhibitors are approved. It's a new inhibitor family with a new mechanism. They may help treat neurodegenerative disorders. Most medications are unable to cross the BBB (BBB). The brain's endothelial cells act as a filter for tiny chemicals, peptides, and proteins. Various brain medication delivery methods have lately been discovered. Polymeric nanoparticles have the potential to be used as drug carriers. These new ideas will lead to CNS therapy[30–32].

In the present study, our main focus was on the study of molecular events in astrocytes and oligodendrocytes. We comprehensively addressed the signaling pathways, protein-regulator relationships, and presence in extracellular vesicles relevant to these molecular events. In the following, we examined some genes and proteins. Finding these genes is likely to open new windows in the treatment of ALS. KRAS mutations are connected to intellectual impairment. Reduced GABAergic inhibitory neuron development and function in mutant KRAS mice has been linked to behavioral issues. The role of excitatory neurons in adult behavioral deficits is uncertain. Adult mice with constitutively active mutant KRASG12V in excitatory or inhibitory neurons have poor spatial memory. KRASG12V enhanced inhibitory GABAergic synaptic transmission. When KRASG12V is expressed in inhibitory neurons in the hippocampus, picrotoxin may restore long-term potentiation. KRASG12V destroyed excitatory neurons, possibly contributing to significant behavioral deficits. These cholinergic neurons govern learning and memory[33]. Diabetes mellitus is associated with insulin resistance, or reduced neuronal metabolism and insulin

signaling (AD). Insulin resistance in BFCN was expected early in AD. Insulin resistance is detected in the medial septum of 3-month-old 3Tg-AD mice with AD histopathology and cognitive impairments. It was made with lots of insulin. In both circumstances, NGF decreases insulin resistance. Nutrient growth factor promotes IRS1, c-Fos, and glucose metabolism[34]. Activated IRS1 on the NGF receptor TrkA is unaffected. Medial septum insulin resistance develops months before neocortex and hippocampal insulin resistance. In 3Tg-AD mice, it activates TrkA/IRS1. Treatments that target NGF and insulin receptors downstream of the insulin receptor can help reduce cognitive decline in AD and diabetes-related brain insulin. Resistant insulin changes IRS-1 expression and phosphorylation. Because it's unknown. Insulin resistance in peripheral organs and the brain may result from these interactions. Wnt/catenin affects IRS-1 and insulin signaling in primary neurons. Wnt3a, a Wnt agonist, boosts insulin signaling in neurons. Rat IRS-1 expression is increased in rat primary neurons by Wnt3a. Wnt3a increases insulin-mediated glucose absorption. Treatment with Wnt3a, CHIR99021, or both increases IRS-1 gene transcription and protein expression in rat primary neurons. Wnt/catenin induces IRS-1 expression. TCF4 should be linked to the 529–516 bp human IRS-1 promoter. To prevent insulin resistance, Wnt/-catenin modulates IRS-1 and insulin signaling. Neuroinflammation occurs after an ischemic stroke. Arginine is a non-essential amino acid that reduces acute inflammation[35]. Arginine also reduces inflammation in ischemic brain tissue and cultured microglia following OGD injury. In a rat model of ischemia/reperfusion (I/R) injury, HIF-1 and LDHA levels increase, while arginine therapy reduces their expression. These findings suggest that arginine protects against ischemic neuron death by suppressing the HIF-1/LDHA-mediated inflammatory response.

These findings suggest that arginine may be able to protect the brain from damage caused by cerebral ischemia by lowering the HIF-1 and LDHA-mediated inflammatory response in microglia[36]. PSP has pathological and clinical Parkinson's disease symptoms. It's vital to distinguish PD from PSP. While PD and PSP biomarkers have come a long way, further biomarkers for these disorders are critically needed. 138 Parkinson's Disease Biomarkers Program participants were examined for the diagnostic potential of DUSP8 and PTPRC phosphatases. This is not the case for DUSP8 sufferers. These two biomarkers correlated, indicating they might be used together to stratify patients according to illness severity and progression. Overall, PTPRC expression may be a PD biomarker. Biomarkers like PTPRC should be researched throughout time[37]. Also, this paper published as a preprint in research Square[38].

In summary, our integrated bioinformatics analysis of astrocyte- and oligodendrocyte-derived ALS has revealed key signaling pathways and potential therapeutic targets. We identified TP53, MDM2, KRAS, PTPRC, and GSK as central proteins regulated by hsa-miR-564, hsa-miR-496-5p, hsa-miR-324-5p, hsa-miR-296-5p, and hsa-miR-4258-3p miRNAs, highlighting a robust regulatory network in ALS pathogenesis. These findings provide a foundation for future investigations into the roles of these genes and miRNAs in ALS and suggest potential targets for therapeutic intervention.

Competing interests

The authors declare that they have no competing interests.

Data availability

In the present study, we use in silico analysis via repository online databases. Also, we used GEO databases and selected microarray datasets. Then clusters up and downregulated genes. After that, we assessed the signaling pathways, gene ontology, protein network and miRNAs respectively.

Authors' contribution

E.A. M.A. A.B. Participated in study design, data collection and evaluation, drafting and statistical analysis; Contributed extensively to the interpretation of the data and the conclusion and figure design. All authors performed editing and approved the final version of this paper for submission, also participated in the finalization of the manuscript and approved the final draft.

Acknowledgements

We thank all colleagues in the core facilities for their support.

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