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MicroRNA-Mediated regulation of GFAP as a marker of astrocyte reactivity: An in silico study

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Abstract
Astrocytic reactivity is characterized by profound cytoskeletal remodeling, altered proteostasis, and elevated expression of glial fibrillary acidic protein (GFAP), a hallmark intermediate filament of reactive astrocytes. Rather than being regulated as an isolated molecular target, GFAP expression reflects the broader structural and stress-adaptive state of astrocytes. In this study, we employed an integrative in silico framework to identify microRNAs (miRNAs) potentially associated with astrocytic cytoskeletal and proteostatic gene networks in which GFAP serves as a structural marker of reactive astrocyte phenotypes. Protein–protein interaction analysis was used to define a GFAP-associated protein set, followed by multi-database miRNA target prediction and network-level prioritization. Ten candidate miRNAs were identified, with hsa-miR-1207-5p emerging as a broadly predicted multi-target miRNA within the GFAP-associated network. Functional enrichment analysis highlighted intermediate filament organization and suggested a potential involvement of chaperone-mediated protein quality control mechanisms. Collectively, these findings propose a network-level miRNA-associated framework relevant to reactive astrocyte biology, in which GFAP functions as a structural indicator rather than a direct regulatory endpoint, and provide a rational basis for experimental validation.

Keywords: *Astrocytes, Glial Fibrillary Acidic Protein, MicroRNAs, Intermediate Filament Proteins*

1. Introduction

Astrocytes play essential roles in maintaining central nervous system homeostasis, regulating extracellular fluid, ions, and neurotransmitter endocytosis, supporting neurons, modulating local blood flow, assisting in the regulation of interstitial fluid outflow, synaptic regulation, and neuroplasticity. Astrocytes comprise two primary components: the soma and protrusions, which can be further categorized hierarchically into branches, branchlets, and leaflets. The unique structure supports multitasking quality, so it reaches neurons, glial cells, synapses, and blood vessels. Astrocytes become a reactive form caused by disorders and damage to the central nervous system, including SCI, stroke, and neurodegeneration; therefore, this has led to changes in their morphological, molecular, and functional type. These reactive astrocytes play a critical role in maintaining the neuron, microglia, and central nervous system milieu.[1,2]

A defining molecular feature of astrocytes, particularly under pathological conditions, is the elevated level of glial asidic fibrillar protein (GFAP), which serves as a well-established marker of astrocyte reactivity and cytoskeletal reorganization. GFAP plays roles in cytoskeletal reorganization under changing conditions and is identified as characteristic of reactive astrocytes.[3,4] Furthermore, GFAP concentration in blood or CSF is frequently used in clinical studies as a substitute for astrocyte reactivity.[5] It is reported that GFAP expression level alteration occurs in several pathological conditions, including neuroinflammation, traumatic brain injury, and neurodegenerative disorders.[6,7] Therefore, GFAP is of significant importance both among researchers and clinicians as a potential therapeutic goal and diagnostic approach.[8]

MicroRNAs (miRNAs) are small regulatory,non-coding RNAs and consist of around 18–25 nucleotides. Following a layered biogenesis procedure including Drosha and Dicer-controlled processing, they generate endogenous transcripts. Mature miRNAs attach to complementary regions in target messenger RNAs (mRNAs) as part of the RNA-induced silencing complex (RISC), leading mRNA degradation or translational inhibition. Therefore, miRNAs have a regulatory role in cellular homeostasis to perform essential functions in neuronal development, plasticity, and stress adaptation through modulating gene expression at the post-transcriptional stage.[9,10] miRNAs can mediate both the regulation of their function and dysfunction in astrocytes, so alterate inflammatory, apoptotic, autophagic, and oxidative stress pathways.[1] Current literatüre reported that modification of some miRNAs affected astrogliosis and GFAP expression.[11–13] However, direct miRNA-mediated regulation of GFAP remains poorly characterized, supporting the notion that GFAP expression more likely reflects indirect modulation of astrocytic structural and stress-responsive networks.

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In light of this information, the study aims to identify miRNAs that may regulate GFAP using systematic bioinformatic analyses. Network-oriented in silico strategies integrating protein–protein interaction analysis, miRNA target prediction, and functional enrichment offer a powerful approach to prioritize miRNAs with potential regulatory relevance exhibited with evaluations.

2. Materials and Methods

2.1. Selection of the GFAP-associated gene network

The STRING database (https://string-db.org/, Version 12.0) was used to analyze protein–protein interactions (PPIs) to identify proteins functionally associated with GFAP.[14] Interactions were retrieved for *Homo sapiens* using a high-confidence interaction score threshold (≥0.7), chosen to minimize false-positive associations while preserving biologically relevant connectivity. Only experimentally validated and curated database interactions were considered.

2.2. miRNA target prediction and integration

miRNA–target interactions were predicted using miRDB (https://mirdb.org/)[15] and miRWalk (http://mirwalk.umm.uni-heidelberg.de/).[16] For miRDB, miRNAs with prediction scores ≥80 were retained, while for miRWalk, interactions with binding probability ≥0.7 were selected. Only miRNAs predicted by both databases were included to reduce database-specific bias and enhance confidence.[17,18]

2.3. Multi-target miRNA prioritization

To identify miRNAs with potential regulatory impact at the network level, a multi-target filtering strategy was applied. Only miRNAs predicted to target at least two genes within the GFAP-associated network were retained, reflecting the concept that miRNAs exert biologically meaningful effects primarily through coordinated regulation of functionally related gene sets.[19]

2.4. Functional enrichment analysis

The Metascape platform (https://metascape.org/gp/) was used to conduct functional enrichment analysis.[20] Gene Ontology (GO) Biological Process terms and Reactome pathways were analyzed, and enrichment significance was evaluated using adjusted q-values provided by the platform.

3. Results

3.1. GFAP-Associated Cytoskeletal and Proteostatic Interaction Network

PPI analysis revealed that GFAP is embedded within a tightly connected network comprising five genes named VIM, DES, NES, HSPA8, and EEF1A1 that identified intermediate filament proteins (VIM, DES, NES) and stress-responsive components (HSPA8, EEF1A1). This network structure supports the concept that GFAP operates within an integrated cytoskeletal–proteostatic system rather than as an isolated structural element (Figure 1 and Table 1).

The protein–protein interaction network was constructed using the STRING database (version 12.0) with a high-confidence interaction score (≥0.7). The network highlights GFAP-centered interactions with cytoskeletal and stress-related proteins, including VIM, DES, NES, HSPA8, and EEF1A1, supporting their functional association within astrocytic structural networks.

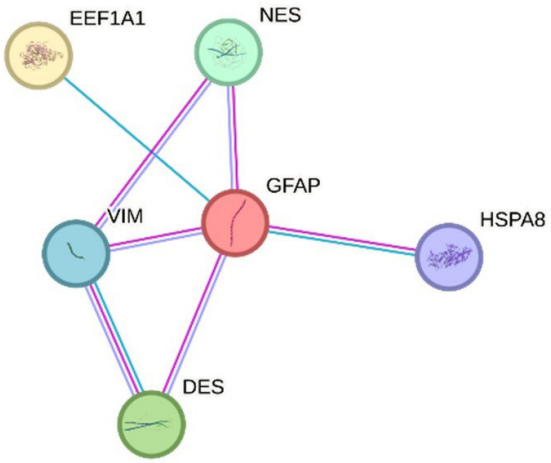

Figure 1. Protein–protein interaction network of GFAP-associated cytoskeletal genes.

Table 1. GFAP-associated cytoskeletal gene set used for in silico analysis. The table summarizes the six genes selected based on protein protein interaction analysis and literature evidence, representing structural and stress-related components of the astrocytic cytoskeleton.

Gene	Functional Role
GFAP	Astrocytic intermediate filament
VIM	Cytoskeletal remodeling
DES	Structural stability
NES	Reactive astrocyte/progenitor marker
HSPA8	Chaperone-mediated autophagy
EEF1A1	Protein synthesis / stress response

3.2. Identification of High-Confidence Network-Targeting miRNAs

Integration of miRDB and miRWalk predictions followed by multi-target filtering yielded ten high-confidence miRNAs predicted to regulate multiple components of the GFAP-associated network. Among these, hsa-miR-1207-5p was identified as the top candidate, predicted to target all six genes, suggesting a potential role as a higher-order regulator of astrocytic cytoskeletal and stress-responsive pathways (Table 2).

3.3. Functional Enrichment Highlights Chaperone-Mediated Autophagy

Functional enrichment analysis confirmed significant enrichment of intermediate filament organization, validating the biological coherence of the selected gene set. Notably, Reactome pathway analysis identified chaperone-mediated autophagy as the most significantly enriched pathway, underscoring a close functional association between GFAP-related cytoskeletal dynamics and selective protein quality control mechanisms (Figure 2).

Table 2. High-confidence miRNAs predicted to target the GFAP-associated cytoskeletal gene network (miRNAs were prioritized based on intersection of miRDB and miRWalk predictions and a multi-target criterion requiring regulation of at least two genes within the GFAP-associated gene set).

	miRNA	Number of targeted genes
1	hsa-miR-1207-5p	6
2	hsa-miR-3689-3p	4
3	hsa-miR-6779-5p	4
4	hsa-miR-8089	4
5	hsa-miR-3689c	4
6	hsa-miR-6819-5p	4
7	hsa-miR-6883-5p	4
8	hsa-miR-149-3p	4
9	hsa-miR-6825-5p	4
10	hsa-miR-5787	4

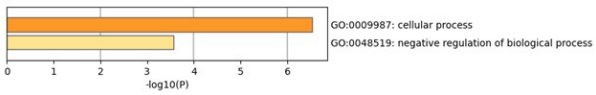

Figure 2. Functional enrichment analysis of GFAP-associated genes. (Gene Ontology and Reactome pathway analyses revealed significant enrichment in intermediate filament organization and chaperone-mediated autophagy, indicating that the GFAP-associated cytoskeletal network is closely linked to structural regulation and protein quality control mechanisms.)

4. Discussion

In this study, miRNAs a network-level miRNA-mediated framework for the indirect regulation of GFAP in astrocytes. It thought to directly or indirectly affect GFAP protein expression were identified using in silico methods. Through analysis, key proteins interacting with GFAP were determined, and miRNAs affecting these structures were identified from two different databases. Accordingly, 10 different miRNAs were found to have an effect on GFAP.

The approach, rather than focusing on direct miRNA–GFAP interactions, emphasizes coordinated regulation of cytoskeletal and proteostatic components, reflecting the complex and context-dependent nature of GFAP control. The identification of chaperone-mediated autophagy as a key enriched pathway suggests that GFAP regulation may involve modulation of cytoskeletal protein turnover under stress conditions. The prominent emergence of miR-1207-5p as a multi-target regulator raises the possibility that this miRNA may act as a higher-order modulator of astrocytic structural integrity and stress adaptability. Although miR-1207-5p has not been extensively characterized in astrocytes, its predicted broad targeting profile warrants further investigation in glial contexts.

Future studies should experimentally validate the predicted miRNA–network interactions using astrocytic models. Potential approaches include miRNA mimic or inhibitor transfection in primary astrocytes or astrocyte-like cell lines, followed by assessment of GFAP protein levels, cytoskeletal organization, and CMA activity markers such as HSPA8. Such experiments would directly test the functional relevance of the proposed network-level regulatory model.

The study is inherently limited by its computational nature and reliance on prediction databases. While the stringent multi-database and multi-target strategy enhances confidence, experimental validation is required to confirm causality. Additionally, the focused gene set may not capture all regulatory layers influencing GFAP dynamics.

5. Conclusion

This integration in silico analysis identifies candidate miRNAs potentially involved in the indirect regulation of GFAP through astrocytic cytoskeletal and chaperone-mediated autophagy pathways. By adopting a network-oriented perspective, the study provides a mechanistically interpretable framework for prioritizing miRNAs for future experimental investigation in astrocytic biology.

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Ethical approval

This study was conducted exclusively using in silico bioinformatics analyses and publicly available databases. No human participants or experimental animals were involved. Therefore, ethical approval was not required.

Authors contribution

The author solely conceived and designed the study, performed all in silico analyses, interpreted the results, and wrote and approved the final manuscript.

Conflict of interest

The author declares no conflict of interest.

Data availability

This study exclusively used publicly available datasets. No new data were generated.

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References

1. Fan Y, Huang H, Shao J, Huang W. MicroRNA-mediated regulation of reactive astrocytes in central nervous system diseases. *Front Mol Neurosci*. 2023;15:1061343. doi:10.3389/FNMOL.2022.1061343

2. Jurga AM, Paleczna M, Kadluczka J, Kuter KZ. Beyond the GFAP-Astrocyte Protein Markers in the Brain. *Biomolecu-*

les. 2021;11(9):1361. doi:10.3390/BIOM11091361

3. Tiwari A, Rout S, Deep P, Sahu C, Samal PK. The Dual Role of Astrocytes in CNS Homeostasis and Dysfunction. *Neuroglia*. 2025;6(4):38. doi:10.3390/NEUROGLIA6040038

4. Stanca S, Rossetti M, Bongioanni P. Astrocytes as Neuroimmunocytes in Alzheimer's Disease: A Biochemical Tool in the Neuron–Glia Crosstalk along the Pathogenetic Pathways. *International Journal of Molecular Sciences.* 2023;24(18):13880. doi:10.3390/IJMS241813880

5. De Bastiani MA, Bellaver B, Brum WS, et al. Hippocampal GFAP-positive astrocyte responses to amyloid and tau pathologies. *Brain Behav Immun*. 2023;110:175-184. doi:10.1016/J.BBI.2023.03.001

6. Middeldorp J, Hol EM. GFAP in health and disease. *Prog Neurobiol*. 2011;93(3):421-443. doi:10.1016/J.PNEUROBIO.2011.01.005

7. de Reus AJEM, Basak O, Dykstra W, van Asperen J V., van Bodegraven EJ, Hol EM. GFAP-isoforms in the nervous system: Understanding the need for diversity. *Curr Opin Cell Biol*. 2024;87:102340. doi:10.1016/J.CEB.2024.102340

8. Zheng X, Yang J, Hou Y, Shi X, Liu K. Prediction of clinical progression in nervous system diseases: plasma glial fibrillary acidic protein (GFAP). *European Journal of Medical Research 2024 29:1*. 2024;29(1):51-. doi:10.1186/S40001-023-01631-4

9. Bartel DP. Metazoan MicroRNAs. *Cell*. 2018;173(1):20-51. doi:10.1016/j.cell.2018.03.006

10. Piccinno E, Aloisio M, Scalavino V, et al. Exploring miRNA Research in Colorectal Cancer: Insights from a Bibliometric Analysis. *Pharmaceutics*. 2025;17(8):1084. doi:10.3390/PHARMACEUTICS17081084/S1

11. Wang CY, Yang SH, Tzeng SF. MicroRNA-145 as one negative regulator of astrogliosis. *Glia*. 2015;63(2):194-205. doi:10.1002/GLIA.22743;ISSUE:ISSUE:DOI

12. Fan Y, Wang W, Li W, Li X. miR15a inhibits cell apoptosis and inflammation in a temporal lobe epilepsy model by downregulating GFAP. *Mol Med Rep*. 2020;22(4):3504-3512. doi:10.3892/MMR.2020.11388/ABSTRACT

13. Pogue AI, Cui JG, Li YY, Zhao Y, Culicchia F, Lukiw WJ. Micro RNA-125b (miRNA-125b) function in astrogliosis and glial cell proliferation. *Neurosci Lett*. 2010;476(1):18-22. doi:10.1016/J.NEULET.2010.03.054

14. Szklarczyk D, Gable AL, Nastou KC, et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res*. 2021;49(D1):D605-D612. doi:10.1093/NAR/GKAA1074

15. Chen Y, Wang X. miRDB: an online database for prediction of functional microRNA targets. *Nucleic Acids Res*. 2020;48(D1):D127-D131. doi:10.1093/NAR/GKZ757

16. Dweep H, Gretz N. miRWalk2.0: a comprehensive atlas of microRNA-target interactions. *Nature Methods.* 2015;12(8):697-697. doi:10.1038/nmeth.3485

17. Öztan G, İşsever H, İşsever T. The Role of miRNAs in the Differential Diagnosis of Alzheimer's Disease and Major Depression: A Bioinformatics-Based Approach. *Int J Mol Sci*. 2025;26(17):8218. doi:10.3390/IJMS26178218/S1

18. Al-Asadi S, Mansour H, Ataimish AJ, Al-Kahachi R, Rampurawala J. MicroRNAs Regulate Tumorigenesis by Downregulating SOCS3 Expression: An In silico Approach. *Bioinform Biol Insights*. 2023;17. doi:10.1177/11779322231193535;CTYPE:STRING:JOURNAL

19. Yuka SA, Yilmaz A. Network based multifactorial modelling of miRNA-Target interactions. *PeerJ*. 2021;9:e11121. doi:10.7717/PEERJ.11121/SUPP-5

20. Zhou Y, Zhou B, Pache L, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun*. 2019;10(1). doi:10.1038/S41467-019-09234-6