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Target prediction and functional annotation analysis for potential microRNAs associated with Glioma

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ABSTRACT

Glioma is a deadly tumor that accounts for the majority of malignancies of the brain and central nervous system. Currently, the application of biomarkers in combination with clinical treatments in the diagnosis and prognosis of Glioma is the optimal trend. Among the effective biomarkers, microRNA has emerged as a novel, efficient biomarker. In this study, we used data from the Kyoto Encyclopedia of Genome and Gene Routes Database (KEGG) and bioinformatics tools for functional prediction and annotation of two Glioma-associated molecules (miR-6780a-5p and miR-6754-3p). The result indicated that 361 genes related to two molecules miR-6780a-5p and miR-6754-3p. In which, miR-6780a-5p interacts with 198 genes and miR-6754-3p interacts with 166 genes. PPI analysis (protein-protein interaction analysis) and functional enrichment analysis revealed that target molecules of two miRNAs (miR-6780a-5p, miR-6754-3p) belong to cancer signaling pathways such as MAPK (Mitogen-activated protein kinase) and WNT (Wingless-related integration site). In addition, two miRNAs (miR-6780a-5p and miR-6754-3p) affect not only Glioma but also regulation in many other cancers such as pancreatic, thyroid, and endometrial cancer. Briefly, the result has clarified the role of (miR-6780a-5p, miR-6754-3p) in regulating signaling pathway of Glioma, thereby evaluating the interaction between miRNAs, target genes and target signaling pathways, and also supporting for the selection of potential biomarkers in Glioma diagnosis.

Keywords: *Bioinformatics, Glioma, Functional annotation, microRNA, Target prediction*

1. Introduction

Glioma is a cancer that accounts for one-third of tumor cancer in the brain and in the central nervous system. Normally, Glioma originates from glial cells which include two types, non-diffusing benign tumor and diffuse (malignant) tumor, in which malignant tumor is most dangerous and easily relapses. It is most prevalent and difficult to be treated when they enter the system of neuron and nervous cells (Mondal & Kulshreshtha, 2021). Recently, the development of non-invasive biomarkers opened a new turning point for the early diagnosis and prognosis of Glioma. One of the potential biomarkers in the diagnosis of cancer is microRNA biomarker (Visone & Croce, 2009). Therefore, the evaluation of miRNA expression patterns by using Microarray and Next-generation sequencing (NGS) approach is very useful for understanding the feature of Glioma; and the characterized miRNAs can be used for non-invasive diagnosis (Dat et al., 2022; Mondal & Kulshreshtha, 2021; Murakami et al., 2014). In the previous study, two molecules (*hsa-miR-6780a-5p* and *hsa-miR-6754-3p*) were described as potential Glioma-associated miRNAs by bioinformatic tools based on post-transcriptional expression assessment of serum samples (Phi & Dat, 2022). In this study, target molecules of the two miRNAs (*hsa-miR-6780a-5p* and *hsa-miR-6754-3p*) were predicted through sequence-based matching algorithms; and these targets will be functionally annotated, data enrichment analysis, and interaction network analyzed to describe the influence of these molecules on biological processes, signaling pathways, genetic factors, and proteins in Glioma.

2. Materials and method

Predicting miRNA-targets

The online tool, MirDIP was used for miRNA-targets prediction, the result showed that nearly 152 million predicted human miRNA-targets from 30 different databases. MirDIP provided statistical analysis algorithms to identify miRNA-targets with high confidence (Tokar et al., 2018). In this study, the miRNA-targets were predicted at a high group confidence score (1%).

Function annotations for gene-targets

Gene-targets were functionally analyzed using G: Profiler (bioinformatic tool) to determine network interaction and the role of these target-genes on cancer genes, signaling pathways, genetic molecular factors, and disease phenotypes in Glioma (Raudvere et al., 2019). In this study, gene-targets were functionally annotated and analyzed with a statistical threshold of FDR ($q<0.001$) according to the method of Benjamini-Hochberg.

Network analysis for the potential targets in Glioma

Network analysis is a systematic method of analyzing data to generalize and examine interactions within biological systems. In this study, the network was performed using functional annotations based on the Kyoto Encyclopedia (KEGG) genome and gene pathway database (https://www.kegg. jp/kegg-bin/show_pathway?hsa05225). By using the algorithm, data enrichment analysis in validating miRNA target genes was achieved

based on miRNet platform (https://www.mirnet.ca/). Briefly, this is a method to identify genes that are overexpressing in the genome or to identify proteins associated with specific disease phenotypes.

The protein-protein interaction was built with data from the String database (https://string-db.org/) and the confidence level was set at 800. Freedom was set at 2.0 and the interaction score was set at 2.0 (Dat et al., 2022).

3. Results and Discussion

3.1 Predicting the target of Glioma-associated miRNAs

By looking up the homologous pairing between the miRNA sequences and the non-coding region sequences of the mRNA-targets, the result showed that two molecules (*hsa-miR-6780a-5p* and *hsa-miR-6754-3p*) were able to interact with 361 different mRNA-targets (Table 1). In which, *hsa-miR-6780a-5p* was able to interact with 198 mRNAs and *hsa-miR-6754-3p* was able to interact with 166 mRNAs. This result was consistent with many previous reports in which miRNA can regulate not only a mRNA-target but also many different mRNAs relating to cancer signaling pathway (Reddy, 2015). Furthermore, two molecules (*hsa-miR-6780a-5p* and *hsa-miR-6754-3p*) co-regulated 10 mRNAs with high target score (Target score >80) (Table 1).

TABLE 1. List of potential miRNA-targets.

hsa-miR-6780a-5p						hsa-miR-6754-3p					
ADIPOQ	PRKACA	CBX5	SULT1E1	PLEC	SYT15	GAS7	RNF165	TAB2	ZNF676	TNKS	THAP12
ZXDA	ASXL2	PAPOLG	NOL9	PVR	DNAJC24	MIDEAS	ZNF160	NPEPPS	SLC9A2	PRX	PKP4
PPT2	MYLK3	EEF2K	APOL6	RUSC1	RALB	CXADR	RBM8A	DAG1	TC2N	PLEKHA6	GALNT11
CYP20A1	SYNGR1	PHACTR4	STK4	MAGIX	TULP4	CDX2	SLC16A12	CNOT2	TP53TG3B	PSME4	ZNF486
SDC3	RDH10	IRGQ	NUP43	WDR55	COX18	WDR26	KIAA0513	NFAM1	SLC4A8	MYNN	YOD1
RIMS3	XPO4	DDX6	CD164	GSK3B	LYN	C15orf40	RALGPS1	GOLGA3	MAPK14	NASP	TNRC6B
SLC35F6	GDI1	RAB5B	PPIA	MAVS	MPV17L	NKAIN2	RBSN	LMTK2	JCAD	PARVG	ZNF714
MOB3A	RBM19	ZNF490	EXOSC6	TBC1D30	NUDT19	SYT2	ZNF765	BTBD9	PRR5L	RC3H1	TAF1D
ZNF384	TBC1D20	APOBEC3F	MTX3	PIK3C2B	PACS1	CMTM4	ZNF813	SLC1A3	ZDHHC18	SV2B	SNX8
PDGFA	PNPO	LEPROT	CASP10	SENP5	JAK3	SHTN1	NXN	AKAP8	ZFX	AGPAT3	GALNT10
SEMA4D	ODAPH	FOXP4	RBMS2	C19orf47	SCN4B	IFITM10	SH3TC2	TSFM	ARFRP1	KPNA4	ZDHHC3
ZNF548	LRPAP1	C17orf75	FIBCD1	FBXL20	CHP1	OPA3	TPM3	ZNF106	TP53TG3	NFATC4	SPAG11B
YWHAQ	SERPINB9	ELK1	CLCC1	NRF1	AAK1	HSPA1A	TACC1	FRAT2	ARRDC4	MEPE	MYLK3
ZNF609	GRM5	HOOK3	FURIN	ARGFX	RFT1	AQP1	ALDH7A1	PRR14L	CENPJ	ASF1A	ASRGL1
GAB2	SRGAP3	CSNK2A1	MS4A10	TOM1L2	DNAJC8	HK2	ABHD3	ZNF621	AKT2	SGMS1	FREM2
SRGAP1	IGSF8	MTFMT	SYP	PPP1R11	RPS6KA2	CYTH1	TMEM129	FUT1	UNC13A	ZNF100	PPM1H
F11R	NSL1	CORO2B	ZNF793	LDLRAP1	ACADSB	AP5B1	CALM1	GNB4	RNF125	CDC7	POU2F2
PLEKHA5	PTPN18	KATNAL1	RPRD2	NAV1	HDAC2	LMO4	DDX6	CLTC	ARMCX3	FKBP15	ZNF99
MDM4	ERBB3	MYO10	RPRD1B	VGLL3	RPH3AL	MECP2	FKBP1A	TP53TG3C	C12orf29	KCNC4	SIK1
TAPBP	NRIP3	CYP1A2	PHLPP2	PRKCA	OSBPL1A	ANKIB1	PSKH1	SOX6	TF	SMAD3	PEX26
TEF	MSMO1	EFNA5	UTP25	PAQR4	SAMD4A	XKR4	HNRNPK	KMT2D	H3-5	CALCR	PCF11
CHMP7	JPH2	GNL3L	GNPNAT1	TXN2	PDPN	QTRT2	IL17RD	CYP20A1	NAT8L	BAG1	DUSP18
ANKS1B	DUSP8	RRP15	GLG1	CTDSPL	PARVG	UBR1	ACBD5	FMNL2	NPAS3	IL22RA1	DCAF4L2
F2RL2	SP8	NDST1	AR	TMTC1	ZNF587	BOK	CLCN5	ATRX	GDF11	ABI2	EPB41
ZNF674	TLK1	BAZ2A	GPI	TRIB1	OAS3	TIMP3	SLC22A2	CYTH2	DIO2	C4orf3	LHFPL2
DEPDC5	ARSB	IGF2BP1	TCTN2	METTL7A	MESD	OBI1	LMNA	UBE2D2	NKPD1	CCPG1	
ARHGAP26	FOXRED2	ARID2	MDM2	PCBD2	RAB3D	SMAD4	QKI	COX15	LYRM2	RASSF2	
ANKRD52	PHC3	ZNF814	TUB	NOS1	TMEM120B	ERG	GALM	CLU	RBM33		
ORAI2	PARD6B	DTX4	ANKRD40	TRPV1	SMU1						
TRAPPC2	ACSL6	NTRK3	IRAK3	KAT6A	EIF5A2						
EFCAB2	VPS13B	MPP2	TAF8	ITPRIPL2	ZC3HAV1						
SUSD6	GLDN	BCL9	GCFC2	CCL22	CPSF7						
CERS6	OR7D2	SH3BP2	FBXO10	ZNF655							

Note: The mRNAs highlighted are co-regulated by hsa-miR-6780a-5p and hsa-miR-6754-3p.

3.2 The role of miRNA-induced gene-target in Glioma

Functional annotation and data enrichment analysis showed that the gene-targets of (*hsa-miR-6780a-5p* and *hsa-miR-6754-3p*) performing many molecularly role such as: protein binding and 93 biological processes. These biological processes relating to the regulation of cellular process, regulation of localization, intracellular transport, regulation of primary metabolism, and biological signals (Figure 1). In addition, these gene-targets affecting six cellular components such as: cytoplasm, cell junctions, cytosol, membrane of organelle, nucleoplasm and endomembrane systems. Interacting with the human protein system, these gene-targets also affecting 69 protein groups in different organs such as the cerebellum and cerebral cortex, placenta, caudate, prostate, and thyroid gland. (Table 2).

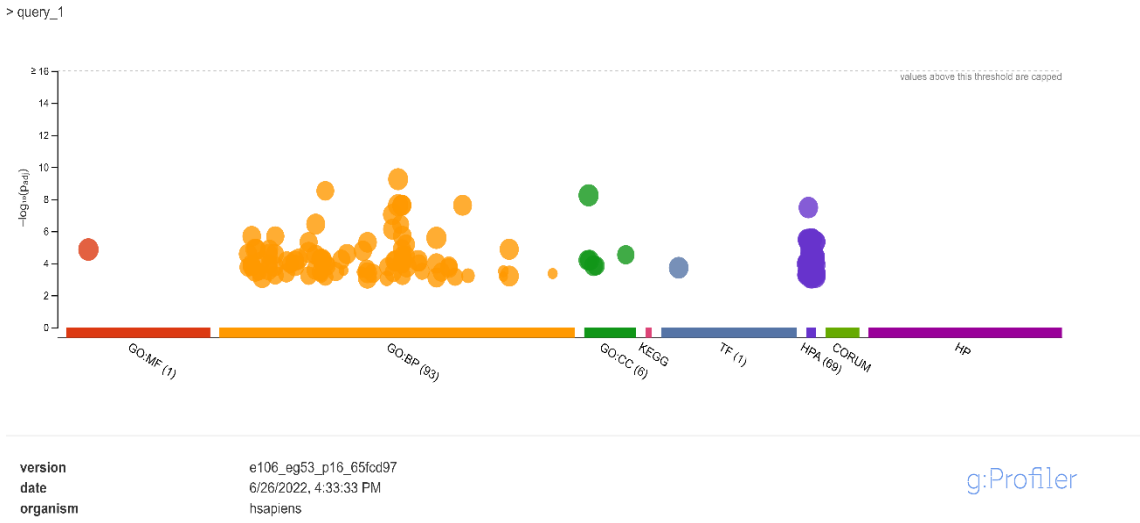

Figure 1. G profiler plot showing functional annotations for target genes in Glioma.

TABLE 2. Top 10 biological processes and enriched proteins associated with target genes.

Source	**Term**	**ID Term**	**p** value	**FDR**
GO:MF	protein binding	GO:0005515	1.38E-05	1.38E-05
GO:BP	regulation of cellular process	GO:0050794	5.67E-10	5.67E-10
GO:BP	regulation of localization	GO:0032879	3.03E-09	3.03E-09
GO:BP	regulation of primary metabolic process	GO:0080090	2.39E-08	2.39E-08
GO:BP	localization	GO:0051179	2.39E-08	2.39E-08
GO:BP	regulation of nitrogen compound metabolic process	GO:0051171	2.39E-08	2.39E-08
GO:BP	regulation of biological process	GO:0050789	2.39E-08	2.39E-08
GO:BP	positive regulation of biological process	GO:0048518	8.99E-08	8.99E-08
GO:BP	regulation of cellular metabolic process	GO:0031323	3.61E-07	3.61E-07
GO:BP	regulation of transport	GO:0051049	3.73E-07	3.73E-07
GO:BP	positive regulation of cellular process	GO:0048522	7.50E-07	7.50E-07
GO:CC	cytoplasm	GO:0005737	5.73E-09	5.73E-09
GO:CC	bounding membrane of organelle	GO:0098588	2.94E-05	2.94E-05

GO:CC	nucleoplasm	GO:0005654	6.38E-05	6.38E-05
GO:CC	cytosol	GO:0005829	6.38E-05	6.38E-05
GO:CC	cell junction	GO:0030054	0.000144	0.000144
GO:CC	endomembrane system	GO:0012505	0.000144	0.000144
HPA	cerebral cortex	HPA:0100000	3.32E-08	3.32E-08
HPA	placenta	HPA:0380000	3.04E-06	3.04E-06
HPA	epididymis; glandular cells	HPA:0180051	3.23E-06	3.23E-06
HPA	epididymis	HPA:0180000	3.23E-06	3.23E-06
HPA	prostate; glandular cells	HPA:0390051	3.23E-06	3.23E-06
HPA	prostate	HPA:0390000	3.23E-06	3.23E-06
HPA	caudate	HPA:0080000	3.23E-06	3.23E-06
HPA	cerebellum	HPA:0090000	3.53E-06	3.53E-06
HPA	thyroid gland; glandular cells	HPA:0590051	4.54E-06	4.54E-06
HPA	cerebral cortex; glial cells	HPA:0100121	4.54E-06	4.54E-06

3.3 Network interaction and signaling pathway of miRNA-induced genes

The formation of the cancer phenotype is an interaction between many oncogenes and tumor suppressor genes. In which, miRNAs play a role in regulating oncogenes and tumor suppressor genes; also miRNAs interact with each other (miRNA-miRNA interaction) and with other transcription factors. These interactions contribute to network interaction. In this study, to evaluate the interaction between the target genes (hsa-*miR-6780a-5p* and hsa-*miR-6754-3p*), transcription factors and miRNAs in the regulation of the transcription expression leading to the Glioma phenotype, a interaction network was built through mirNet web-based platform, the result showed that 307 genes, 104 transcription factors and 99 other miRNAs were involved in the regulatory network in the Glioma. The PPI (protein-protein interaction) graph was built with 500 nodes including genes, miRNAs, transcription factors and 5446 sugar-molecules. In which, the node-interactions were performed by many edges (Figure 2). The result of functional analysis showed that this interaction network participated in the regulation of important signaling pathways in cancer formation and progression such as: MAPK (Map-kinase), neuron, WNT (Wingless-related integration site), cell cycle, and lymphocyte B receptor (Table 3).

Target gene analysis for network interaction showed that P-value was $1.75e^{-127}$ (Figure 3). This means that many miRNAs involving in regulating this network with highly enriched miRNAs which playing key roles such as *hsa-miR-1-3p*; *hsa-miR-16-5p*; *hsa-miR-124-3p*. This result was consistent with the many publications in which *hsa-miR-124-3p* was reported to participate in the regulation of FRA-2 expression, leading to inhibit cell cycle and EMT metabolism through Cyclin D1 and Cyclin E1 factors in Glioma (Tokar et al., 2018). Similarly, overexpression of *hsa-miR-16-5p* inhibited proliferation and induced apoptosis in various Glioma cell lines through targeting apoptosis regulators (Visone & Croce, 2009)

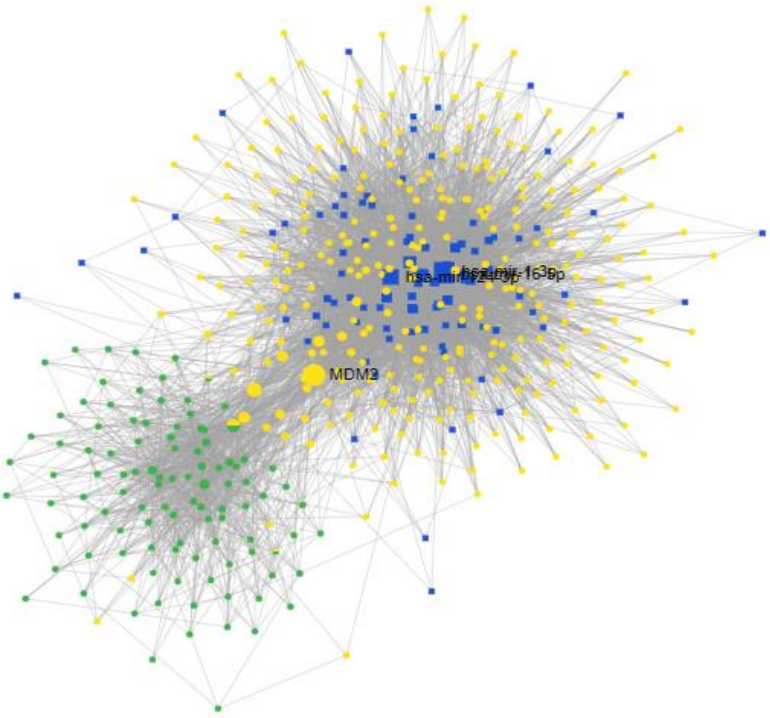

Figure 2. PPI interaction network between target genes (green: TF; yellow: mRNA; blue: miRNA).

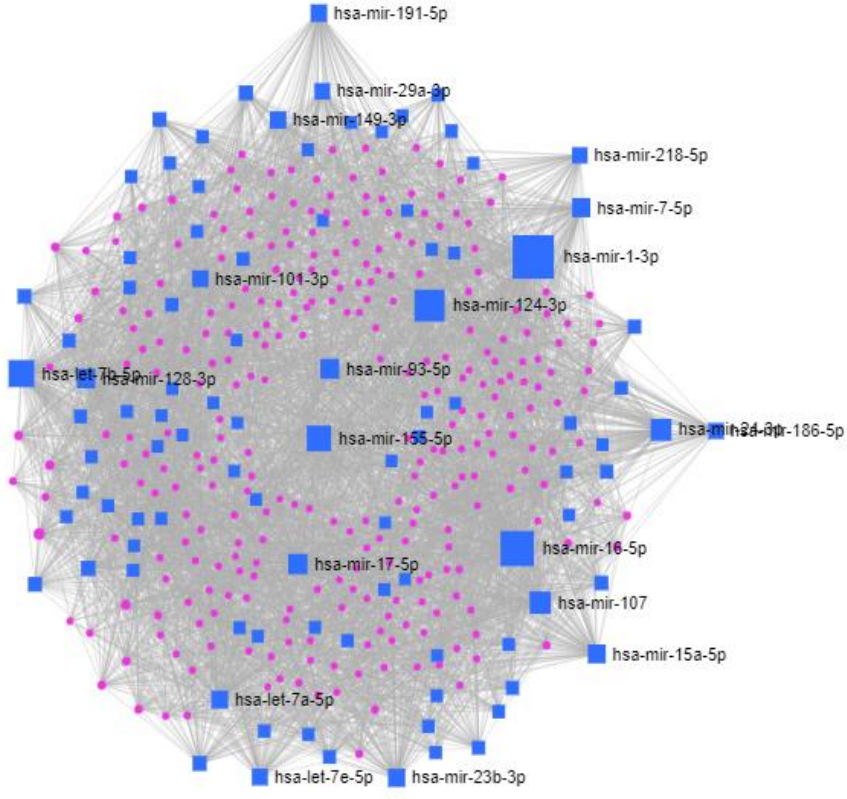

Figure 3. Interaction module between target genes and miRNAs in Glioma.

TABLE 3. Signaling pathways of network participation (KEGG).

KEGG pathway	**P value**	**FDR**
Pathways in cancer	3.49E-10	3.49E-08
Prostate cancer	6.93E-08	3.465E-06
Epstein-Barr virus infection	8.37E-07	0.000025
HTLV-I infection	0.000001	0.000025
Chronic myeloid leukemia	2.58E-06	4.317E-05
MAPK signaling pathway	2.59E-06	4.317E-05
Transcriptional misregulation in cancer	0.0000188	0.0002413
Colorectal cancer	0.0000193	0.0002413
Neurotrophin signaling pathway	0.0000311	0.000341
Cell cycle	0.0000341	0.000341
Wnt signaling pathway	0.0000463	0.0004209
Osteoclast differentiation	0.0000931	0.0007758
Endometrial cancer	0.000427	0.0032846
Influenza A	0.000557	0.00386
B cell receptor signaling pathway	0.000579	0.00386
Amphetamine addiction	0.000892	0.005575
Epithelial cell signaling in Helicobacter pylori infection	0.001	0.0058824
Pertussis	0.0012	0.0064737
Hepatitis C	0.00123	0.0064737
Measles	0.00144	0.007
Pancreatic cancer	0.00147	0.007
Thyroid cancer	0.00171	0.0077727
Acute myeloid leukemia	0.00208	0.0090435
Cytosolic DNA-sensing pathway	0.00329	0.0137083
Notch signaling pathway	0.00356	0.01424
T cell receptor signaling pathway	0.00388	0.0149231
RIG-I-like receptor signaling pathway	0.00439	0.0158214
Glioma	0.00443	0.0158214
Apoptosis	0.00476	0.0164138
Leishmaniasis	0.00537	0.0174194
Herpes simplex infection	0.0054	0.0174194
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	0.00574	0.0179375
Cocaine addiction	0.0114	0.0345455
Melanogenesis	0.015	0.0431429
Amoebiasis	0.0151	0.0431429
Dopaminergic synapse	0.0173	0.0480556

4. Conclusion

Nowaday, applying miRNAs biomarker in the diagnosing and predicting a cancer type is interesting tendency. In Glioma, two molecules *hsa-miR-6780a-5p* and *hsa-miR-6754-3p* are modeled as potential biomarker for Glioma diagnosis based on expression value by bioinformatics and microarray analysis. This study generally supports the role of the target genes of these two molecules as well as the interaction between miRNAs-mRNA and signaling pathways in Glioma. These results contribute to experimental evaluation of samples collected from patients, and support for clinical diagnosis.

References

Dat V. H. X., Nhung B. T. H., Chau N. N. B., Cuong P. H., Hieu V. D., Linh N. T. M., & Quoc N. B. (2022). Identification of potential microRNA groups for the diagnosis of hepatocellular carcinoma (HCC) using microarray datasets and bioinformatics tools. *Heliyon*, *8*(2), e08987.

Krell A., Wolter M., Stojcheva N., Hertler C., Liesenberg F., Zapatka M., Weller M., Malzkorn B., & Reifenberger G. (2019). *MiR-16-5p* is frequently down-regulated in astrocytic Gliomas and modulates Glioma cell proliferation, apoptosis and response to cytotoxic therapy. *Neuropathology and Applied Neurobiology*, *45*(5), 441–458.

Luo L., Chi H., & Ling J. (2018). *MiR-124-3p* suppresses Glioma aggressiveness via targeting of Fra-2. *Pathology-Research and Practice*, *214*(11), 1825–1834.

Mondal I., & Kulshreshtha R. (2021). Potential of microRNA based diagnostics and therapeutics in Glioma: a patent review. *Expert Opinion on Therapeutic Patents*, *31*(1), 91–106.

Murakami Y., Tanahashi T., Okada R., Toyoda H., Kumada T., Enomoto M., Tamori A., Kawada N., Taguchi Y. H., & Azuma T. (2014). Comparison of hepatocellular carcinoma miRNA expression profiling as evaluated by next generation sequencing and microarray. *PloS One*, *9*(9), e106314.

Phi N. B., & Dat V. H. X. (2022). Detection Potential microRNAs Associated With Glioma. Proceedings of the Scientific Research Day for Lectures - Thu Dau Mot University, *1*(1), 249–252.

Raudvere U., Kolberg L., Kuzmin I., Arak T., Adler P., Peterson H., & Vilo J. (2019). G:Profiler: a web server for functional enrichment analysis and conversions of gene lists. *Nucleic Acids Research*, *47*(W1), W191–W198.

Reddy K. B. (2015). MicroRNA (miRNA) in cancer. *Cancer Cell International*, *15*(1), 1–6.

Tokar T., Pastrello C., Rossos A. E. M., Abovsky M., Hauschild A.-C., Tsay M., Lu R., & Jurisica I. (2018). mirDIP 4.1—integrative database of human microRNA target predictions. *Nucleic Acids Research*, *46*(D1), D360–D370.

Visone R., & Croce C. M. (2009). MiRNAs and cancer. *The American Journal of Pathology*, *174*(4), 1131–1138.