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Exploring VEGF-Linked Pathways: Investigating Multiple miRNAs for Their Therapeutic Potential in Angiogenesis Targets and as Biomarkers in Recurrent Glioblastoma Multiforme

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ABSTRACT

Alternative pathways frequently operate as the origins of resistance to drugs that block the vascular endothelial growth factor (VEGF) pathway. To find possible therapeutic targets and indicators, this study explored the VEGF pathway and how miRNAs control it in recurrent glioblastoma multiforme (rGBM). Differentially expressed miRNAs (DEmiRNAs) were identified by using GBM GSE profiles (GSE32466). To find pathways containing DEmiRNAs, VEGF pathway genes, and their related genes, DIANA-miRPath v3.0 and the ToppGene database were utilized. miRNAs linked to VEGF signaling pathway genes, interactional genes, and DEmiRNAs were discovered by extracting common pathways. The ability of these miRNAs to distinguish rGBM patients from those with primary GBM was assessed using ROC analysis. The study revealed that in rGBM, 30 miRNAs were significantly up-regulated and 49 miRNAs were considerably down-regulated. Among them, the VEGF pathway was connected to 22 up-regulated miRNAs and 29 down-regulated miRNAs. The MAPK pathway shared the most genes with the VEGF pathway, accounting for 1,014 of the interacting genes, which were discovered to have interactions with VEGF signaling pathway genes. Furthermore, 14 miRNAs were identified as having a great deal of potential as molecular biomarkers and therapeutic targets for rGBM. The results indicate that the VEGF pathway in rGBM is regulated by a number of interrelated pathways. The discovered miRNAs hold promise as rGBM biomarkers and therapeutic targets, offering possibilities for novel therapy strategies and aiding rGBM diagnosis and prognosis.

Keywords: VEGF, recurrent GBM, angiogenesis, signaling pathways, GSE32466, biomarker

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Introduction

Glioblastoma multiforme (GBM), the most aggressive central nervous system (CNS) tumor with the poorest prognosis, is caused by extreme and abnormal angiogenesis (1, 2). Despite multiple aggressive treatments for GBM, the outcome is unpleasant, and patients generally die of tumor recurrences (3). Angiogenesis is considered one of the main hallmarks of GBM, making this type of tumor an attractive candidate for anti-angiogenic therapies, especially during recurrence (4). The phenomenon of angiogenesis occurs through a variety of modalities, primarily involving vascular endothelial growth factor (VEGF), with other members of the VEGF family also playing significant roles. This has led to the development of anti-VEGF blocking agents for GBM treatment. However, these agents have failed to induce significant therapeutic effects (5). Bevacizumab (BEV), a monoclonal antibody against VEGF, only provides transient effects, and GBM recurrence remains unavoidable (6, 7).

Advancements in our understanding of GBM epigenetics have significantly contributed to our knowledge of tumorigenesis, development, and recurrence of GBM tumorigenesis, development, and recurrence of GBM (8). The interactions between epigenetic factors such as miRNAs and cell signaling pathways hold great potential as targets for novel therapeutic approaches (9). Numerous miRNAs can modulate mRNAs, leading to an intricate regulatory network involved in various pathways, including drug resistance, angiogenesis, and recurrence (9-12). A comprehensive literature survey of miRNAs deregulated in GBM revealed their overexpression or down regulation compared to normal brain tissue (9, 13). High-throughput technologies, such as microarrays or next-generation sequencing (NGS) methods, provided vast amounts of data that increased our insights into the roles of non-coding RNAs (ncRNAs), particullary microRNAs (14).

This in silico work aimed to identify pathways that impact the VEGF pathway. Another objective was to recognize specific miRNAs that, due to their influence on these pathways, can be reported as biomarkers in rGBM. This research strategy has been employed identifying indirect pathways controlling rGBM angiogenesis.

Materials and methods

Study design: pipeline

As shown in the pipeline, there were eight main steps based on differentially expressed microRNAs (DEmiRNAs) to identify pathways related to the genes in the VEGF signaling pathway:

1. Data collection, involving the identification DEmiRNAs based on an expression dataset.
2. Finding VEGF signaling pathway genes.
3. Determining the genes and pathways related to VEGF signaling pathway genes.
4. Figuring out how pathways talk to each other.
5. Identifying the relationships between different pathways.
6. Classifying the pathways based on their characteristics.
7. Identifying the final set of miRNAs.
8. Conducting a receiver operating characteristic (ROC) analysis to validate the miRNAs as appropriate biomarkers and significant targets.

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The pipeline consists of a systematic approach to explore and understand the connections between DEmiRNAs, VEGF signaling pathway genes, and other related pathways. By following these steps, the study aims to pinpoint potential biomarkers and important miRNAs for additional investigation and application in relative to rGBM.

Data collection sources

The miRNA expression datasets of GBM were searched using the keywords “GBM”, “recurrent GBM”, “miRNA”, “Homo sapiens” [porgn: txid9606]', and “Expression profiling by array” against the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo). One GSE profile (GSE109476) was selected and analyzed. The dataset was related to miRNA expression and was based on GPL10850 (Agilent-021827 Human miRNA Microarray (V3) (miRBase release 12.0 miRNA ID version)).

Identification of DEmiRNAs

To identify DEmiRNAs, the GSE32466 dataset was analyzed by the Limma R package in Bioconductor, which was utilized to mine statistically significant DEmiRNAs based on the difference in their expression values between samples of the primary GBM and rGBM. Significant differential expression was determined as a log2-fold change $\geq |1|$ and the adjusted p-value threshold of 0.05 (15).

Definition of VEGF signaling pathway genes

In the other section, all genes involved in the VEGF signaling pathway were collected from Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (https://www. genome.jp/kegg/ pathway.html). KEGG is an information database for networks of genes and metabolic compounds. The expression levels of these genes in GBM were obtained using the Expression Atlas site (www.ebi.ac.uk/gxa).

DEmiRNA signaling pathway detection

DEmiRNAs are essential regulators in many biological pathways. To show the roles of each DEmiRNA and learn more about their functions, DIANA-miRPath v3.0 (http:// www.microrna.gr/ miRPathv3) was used. DIANA-miRPath is an online tool that integrates miRNAs with the KEGG pathway database to provide a deeper understanding of the process by which biological pathways are regulated (16). The threshold p-value of 0.05 was considered significant.

Identification of genes and signaling pathways related to the VEGF signaling pathway

The genes that had genetic interactions with the VEGF signaling pathway genes were obtained by Network analyst (https://www.networkanalyst.ca/) based on the STRING interactome. They showed a medium (400) to high (1000) confidence score. The confidence score cutoff was set at 900 and required experimental evidence. Then, signaling pathway detection was performed for interactional genes using the ToppGene tool (https://toppgene.cchmc.org/), and the adjusted p-value cutoff of 0.05 was considered significant. Furthermore, the Expression Atlas database was used (https://www.ebi.ac.uk/gxa/home) to find dysregulated gene expression of the VEGF signaling pathway genes in GBM.

Crosstalk between pathways

XTalkDB (http://www.xtalkdb.org) was used to find crosstalk between specific pairs of signaling pathways; scientific literature (17) shows how crosstalk works. “VEGF signaling pathway” was inserted into the XTalkDB to find crosstalking pathway pairs. Finally, we have a list of pathways that have inhibitory or activating effects (or both) on the VEGF signaling pathway. Detection of final pathways and class

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assessment. To deduce commonalities across the collected pathways (pathways related to DEmiRNAs, VEGF signaling pathway genes, and interactional VEGF genes, respectively), the Venny 2.1 free online tool (https://bioinfogp.cnb.csic.es/tools/venny/) was used. At last, the classes of final pathways were determined by the KEGG database.

Identification of key miRNAs

As was already said, all of the genes in the VEGF signaling pathway and all of the genes that interact with the genes in the VEGF signaling pathway were gathered. In this study, the ToppGene tool (https://toppgene.cchmc.org/) was used to find the microRNAs that target the genes mentioned by TargetScan and Tarbase. The threshold for statistical significance, determined by the Benjamini-Hochberg technique, was set at 0.05. Finally, Venny 2.1.0 was used to obtain the shared and specific DEmiRNAs across miRNAs that regulated VEGF signaling pathway genes, interactional genes, and DEmiRNA.

Validation of miRNAs (Receiver operating characteristic) ROC analysis

ROC (receiver operating characteristic) analysis was used to look at the diagnostic value of the expression of certain miRNAs in GBM patients compared to rGBM patients. The area under the ROC curve (area under the ROC curve (AUC)) was calculated to evaluate the diagnostic value of the candidate miRNAs, and the 95% confidence interval with $p < 0.05$ was considered a significance level in this study. The powerful GraphPad Prism tools (version 9.1.0) were utilized for this statistical analysis

Results

Data collection 1 and Identification of DEmiRNAs

The following are the summarized results of this in silico analysis: One microarray miRNA expression dataset, GSE32466 (GPL10850), was used in this study. The dataset contains two conditional experiments (12 primary GBM and 12 recurrent GBM; 24 samples in total). The dataset was analyzed by the Limma package. 79 DEmiRNAs: 30 up-regulated miRNAs and 49 down-regulated miRNAs. Then DEmiRNAs were used for cluster analysis; the pheatmap package from R software was used for hierarchical cluster analysis of DEmiRNAs, as shown in Figure 2.

Data collection 2: VEGF signaling pathway genes

Using the KEGG PATHWAY Database (https://www.genome.jp/kegg/pathway.html), all genes (n = 59) in the VEGF signaling pathway were collected. As shown in Table 1, the gene expression of some of these genes differs between GBM and normal brain tissue. According to the Expression Atlas site, the expression of 14 genes out of 59 genes in the VEGF signaling pathway showed increased expression, and 37 genes showed decreased expression (adjusted $p \leq 0.05$). For the remaining genes, no changes in expression were observed.

DEmiRNAs and pathway prediction

The DIANA-miRPath v.3 software was used to find the biologically significant pathways of the 79 DEmiRNAs. The DEmiRNAs were differentially expressed between the primary GBM and rGBM. Among up-regulated DEmiRNAs and according to the KEGG pathway maps, miR-497-5p was present in most pathways (34 pathways), and miR-204-5p and miR-551b-3p, in contrast, participated in only one pathway.

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On the other hand, 57 pathways were related to miR-17-5p (the most involved pathway), but only 2 pathways were related to miR-31-3p (the least involved pathway) in down-regulated DEmiRNAs.

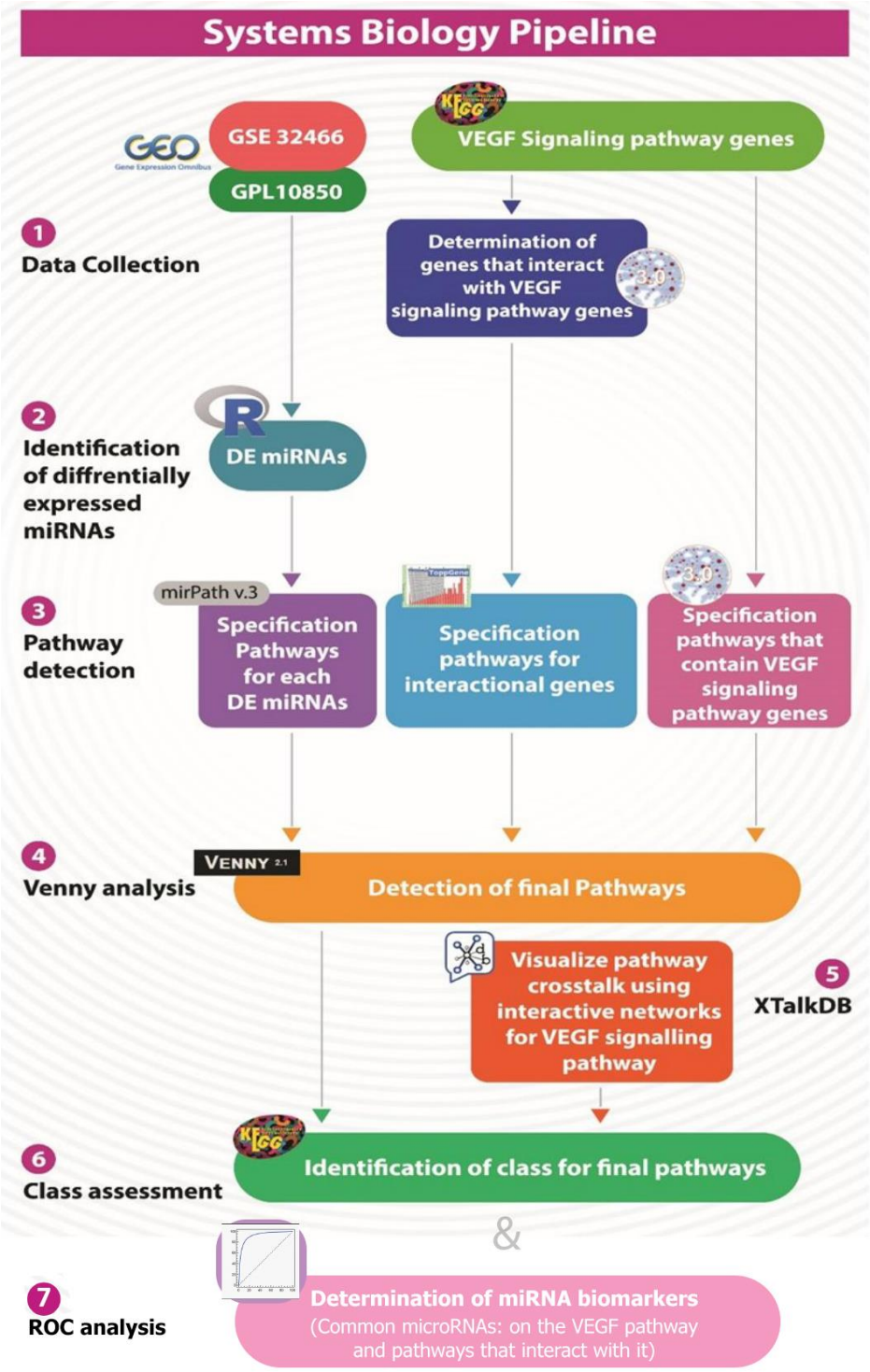

Fig.1. Schematic overview of the systems biology pipeline described in this study.

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Table 1. VEGF signaling pathway genes: Changes in the expression levels of VEGF pathway genes reported in this table are listed based on the Expression Atlas site (www.ebi.ac.uk/gxa) for GBM vs. normal. Up: up-regulation; Down: down-regulation; Unknown: There is no report on the Expression Atlas site.

	Symbol	Description	Expression
1	VEGFA	vascular endothelial growth factor A	Up
2	HSPB1	heat shock protein family B (small) member 1	Up
3	SPHK1	sphingosine kinase 1	Up
4	RAC2	Rac family small GTPase 2	Up
5	PXN	paxillin	Up
6	NRAS	NRAS proto-oncogene, GTPase	Up
7	RAF1	Raf-1 proto-oncogene, serine/threonine kinase	Up
8	KDR	kinase insert domain receptor	Up
9	PLA2G4A	phospholipase A2 group IVA	Up
10	SH2D2A	SH2 domain containing 2A	Up
11	MAPKAPK3	MAPK activated protein kinase 3	Up
12	MAPKAPK2	MAPK activated protein kinase 2	Up
13	CDC42	cell division cycle 42	Up
14	NFATC2	nuclear factor of activated T cells 2	Up
15	PRKCA	protein kinase C alpha	Down
16	MAPK11	mitogen-activated protein kinase 11	Down
17	AKT3	AKT serine/threonine kinase 3	Down
18	PIK3CD	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta	Down
19	MAP2K2	mitogen-activated protein kinase kinase 2	Down
20	RAC3	Rac family small GTPase 3	Down
21	MAP2K1	mitogen-activated protein kinase kinase 1	Down
22	PIK3CB	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta	Down
23	HRAS	HRas proto-oncogene, GTPase	Down
24	PIK3R1	phosphoinositide-3-kinase regulatory subunit 1	Down
25	PIK3R3	phosphoinositide-3-kinase regulatory subunit 3	Down
26	NOS3	nitric oxide synthase 3	Down
27	PLCG2	phospholipase C gamma 2	Down
28	MAPK1	mitogen-activated protein kinase 1	Down
29	PRKCG	protein kinase C gamma	Down
30	SPHK2	sphingosine kinase 2	Down
31	PLCG1	phospholipase C gamma 1	Down
32	PPP3CC	protein phosphatase 3 catalytic subunit gamma	Down
33	MAPK12	mitogen-activated protein kinase 12	Down
34	PLA2G4D	phospholipase A2 group IVD	Down
35	AKT2	AKT serine/threonine kinase 2	Down
36	PIK3R2	phosphoinositide-3-kinase regulatory subunit 2	Down
37	PPP3CA	protein phosphatase 3 catalytic subunit alpha	Down
38	PLA2G4C	phospholipase A2 group IVC	Down
39	PLA2G4F	phospholipase A2 group IVF	Down
40	PLA2G4E	phospholipase A2 group IVE	Down
41	SHC2	SHC adaptor protein 2	Down

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42	PLA2G4B	phospholipase A2 group IVB	Down
43	JMJD7-PLA2G4B	JMJD7-PLA2G4B readthrough	Down
44	PPP3CB	protein phosphatase 3 catalytic subunit beta	Down
45	PTGS2	prostaglandin-endoperoxide synthase 2	Down
46	PPP3R2	protein phosphatase 3 regulatory subunit B, beta	Down
47	PRKCB	protein kinase C beta	Down
48	PPP3R1	protein phosphatase 3 regulatory subunit B, alpha	Down
49	PTK2	protein tyrosine kinase 2	Down
50	KRAS	KRAS proto-oncogene, GTPase	Down
51	MAPK3	mitogen-activated protein kinase 3	Down
52	SRC	SRC proto-oncogene, non-receptor tyrosine kinase	Unknown
53	MAPK13	mitogen-activated protein kinase 13	Unknown
54	BAD	BCL2 associated agonist of cell death	Unknown
55	MAPK14	mitogen-activated protein kinase 14	Unknown
56	AKT1	AKT serine/threonine kinase 1	Unknown
57	CASP9	caspase 9	Unknown
58	PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	Unknown
59	RAC1	Rac family small GTPase 1	Unknown

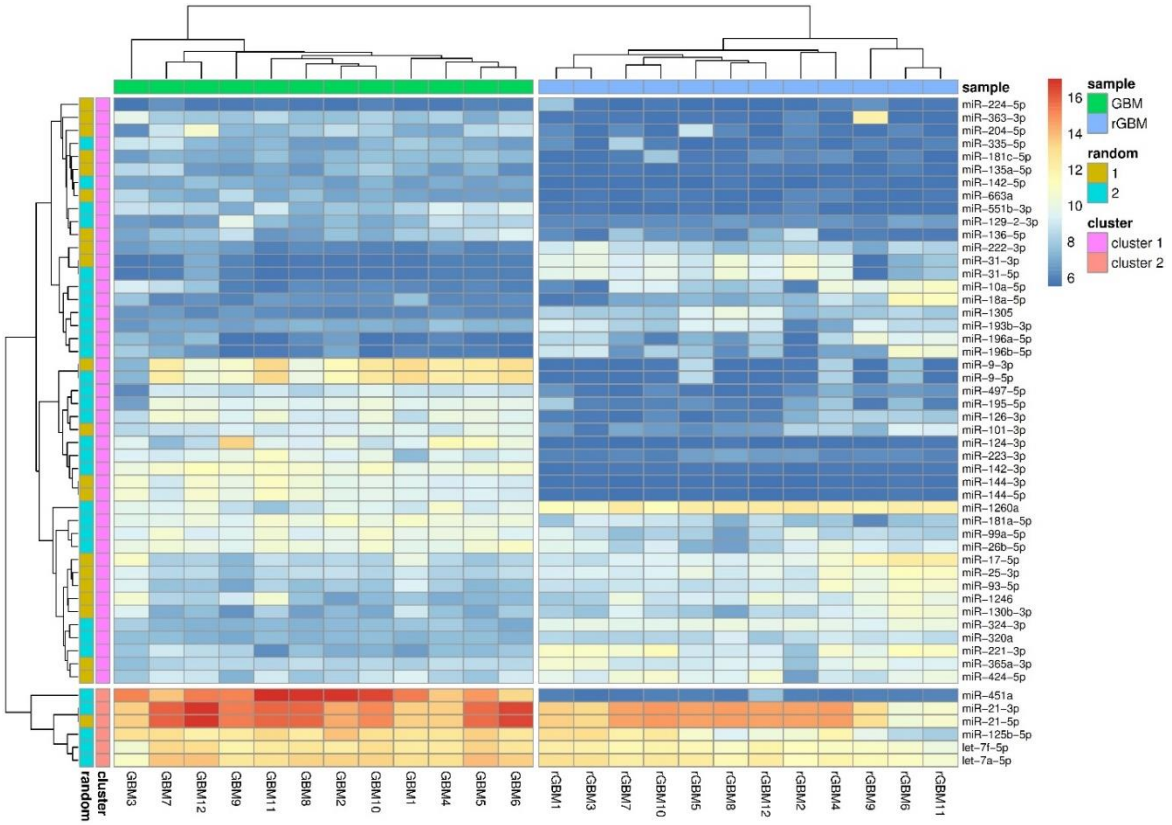

Fig.2. The heatmap corresponding to the hierarchical clustering analysis was performed using the pheatmap package. The columns are samples, and the rows are DEmRNAs. The green represents primary GBM, while the blue represents rGBM that are presented above the horizontal axis.

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Genes and pathways related to the VEGF signaling pathway genes

Interactional genes (1014 genes) were found to have interactions with 59 genes of the VEGF signaling pathway by Network Analyst. In addition, all significant pathways of interactional genes were identified, and according to the enrichment analysis, 170 significant pathways were recognized for interactional genes by ToppGene. Cancer Pathways had the most interactional genes (180 genes), while Sphingosine Degradation had the fewest interactional genes (3 genes).

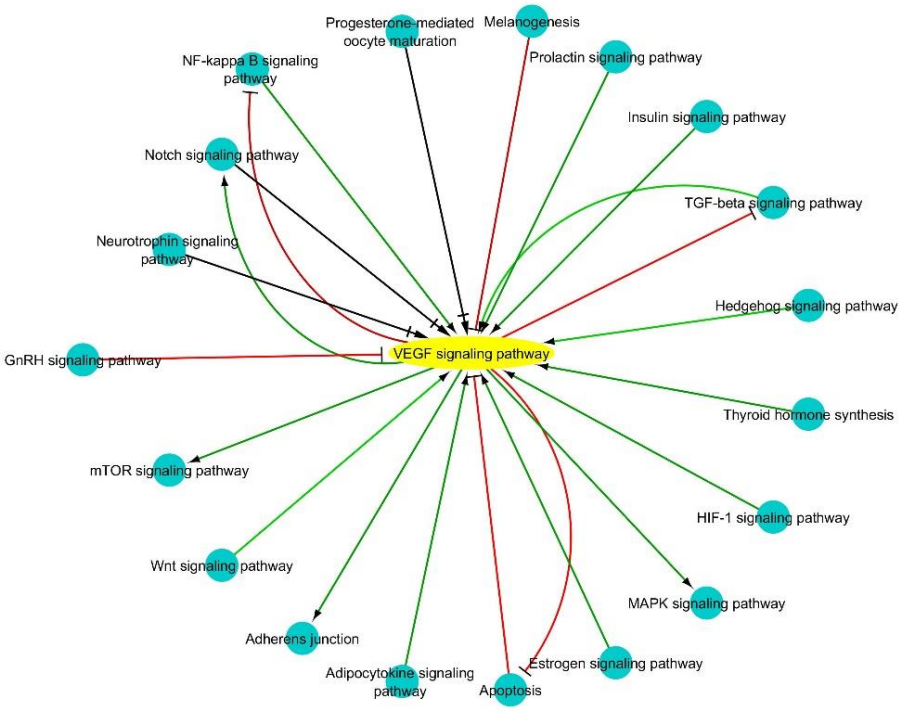

Fig.3. Crosstalk between VEGF signaling pathway and other pathways: Inhibition, activation and dual effects have been represented by red, green and black edges respectively.

Crosstalk analysis

XTalkDB (http://www.xtalkdb.org/home) demonstrated all pathways that inhibited or activated VEGF signaling pathway. Some signaling pathways had inhibition and activation effects (dual effects) on the VEGF signaling pathway, such as neurotrophin signaling pathway, Notch signaling pathway, and Progesterone-mediated oocyte maturation. In contrast, some pathways had only one-way effects (activation or inhibition) on the VEGF signaling pathway. Reciprocally, the VEGF signaling pathway activated or inhibited some pathways. All of these crosstalks are shown in Figure 3.

Identification of classes for final pathways

A class assessment of all finalized signaling pathways was performed by the KEGG pathway database. All 91 finalized signaling pathways were eventually classified into 24 different classes; “signal transduction” and “cancer: specific types” are the two largest classes in this study. Fifteen pathways were related to “signal transduction”. Some of these pathways were involved in the angiogenesis process, such as Hippo, Transforming growth factor beta (TGF-beta), tumor necrosis factor (TNF), mechanistic target of rapamycin (mTOR), and other signaling pathways. Moreover, 20 pathways were involved in “cancer: specific types”

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and cancer overview classes. On the other hand, “Cell motility” and “Circulatory system”, and others were the lowest classes determined in this study. Details of the assigned classes to the final related pathways are shown in Figure 4. The classification shown in Figure 4 is based on the frequency of related signaling pathways.

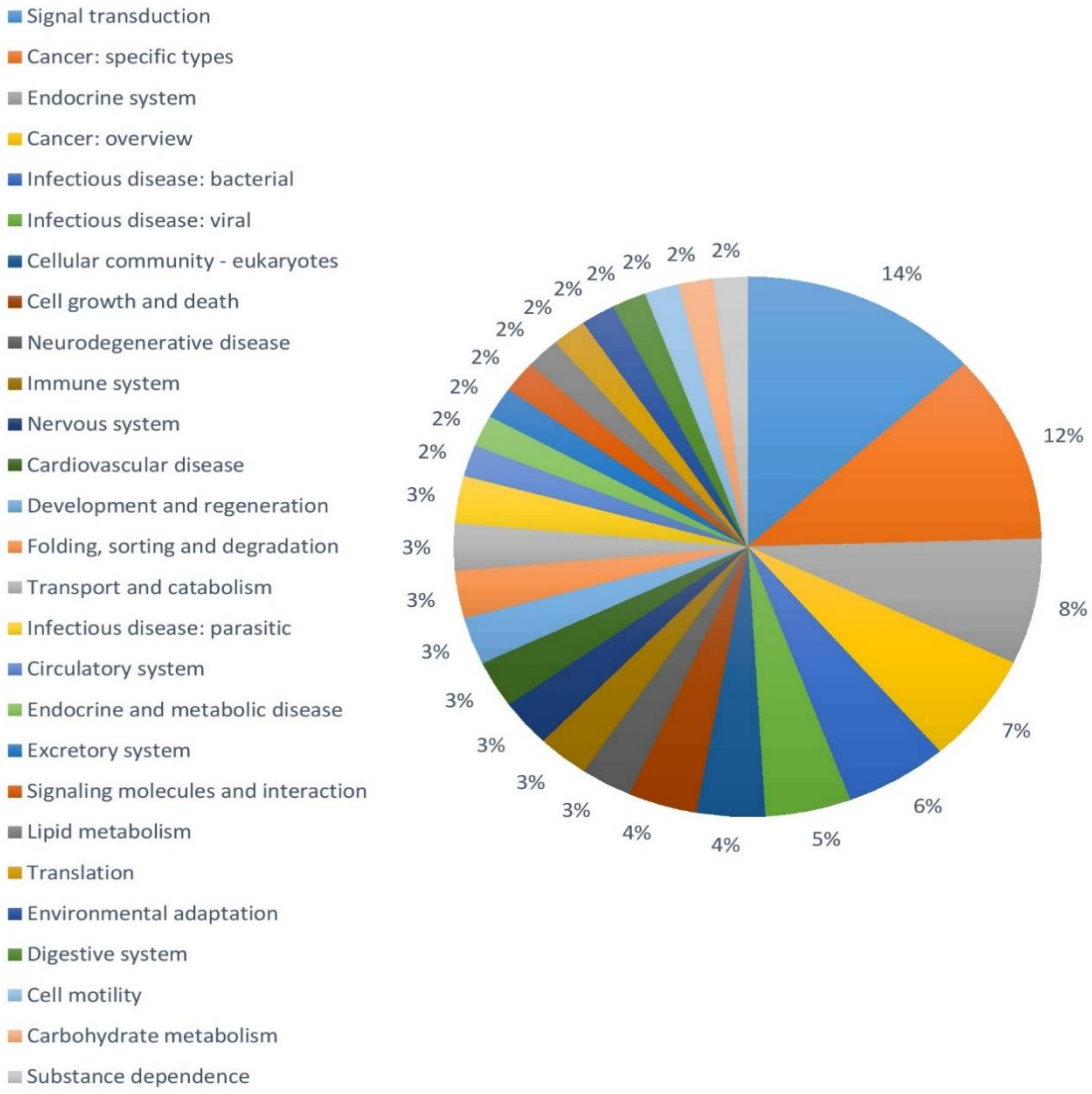

Fig.4. Classification of all finalized pathways: This percentage is sorted out by the number of pathways associated with each class. The percentages here are related to the variety of pathways in each class.

Determination of miRNA biomarkers

The total number of predicted miRNAs for VEGF signaling pathway genes was 55, while the total number of predicted miRNAs for genes that interact with VEGF signaling pathway genes was 186. In other words, both TargetScan and Tarbase have given their final approval to each of the 55 and 158 miRNAs. In the end, the Venny 2.1 tool was utilized to conduct a comparison between the previously mentioned miRNAs and DEmiRNA. As a result, 14 shared and specific miRNAs were obtained. In addition to their significance,

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these miRNAs functioned as regulators of genes involved in the VEGF signaling pathway and its interacting genes (Table 2).

Table 2. DEmiRNAs that target VEGF signaling pathway genes and genes interacting with VEGF signaling pathway genes. Some of these miRs were known for their functions in other cancers.

	Micro-RNA id	Up/ Down Regulated in rGBM than primary GBM	Functions effects in GBM (or other cancers)
1	hsa-miR-221-3p	Up	Cell growth (glioma)
2	hsa-miR-365a-3p	Up	Cell growth and apoptosis, invasion
3	hsa-miR-93-5p	Up	Migration and invasion
4	hsa-miR-17-5p	Up	Cell proliferation and invasion
5	hsa-miR-424-5p	Up	Regulation of VEGF expression
6	hsa-miR-146b-5p	Down	Inhibits glioma cell migration and invasion
7	hsa-miR-497-5p	Down	DNA damage response
8	hsa-miR-195-5p	Down	Suppresses tumorigenicity and regulates G1/S transition
9	hsa-miR-181a-5p	Down	Functioned as tumor suppressors which triggered growth inhibition, induced apoptosis and inhibited invasion in glioma cells
10	hsa-miR-135a-5p	Down	Inhibits initiation of epithelial-mesenchymal transition; inhibits tumor cell proliferation
11	hsa-miR-125b-5p	Down	Inhibits Cell Proliferation and Induces Apoptosis
12	hsa-miR-204-5p	Down	Inhibits cancer progression
13	hsa-miR-340-5p	Down	Multifunational
14	hsa-miR-21-5p	Down	Glioma growth

ROC analysis

The ROC curve was employed for examining the candidate miRNAs forecast accuracy. AUC was computed and used to review these miRNAs' diagnostic values. ROC curves and AUC values are demonstrated in Figure 5. The miRNAs with a $p < 0.05$ and an $AUC > 0.7$ were chosen as promising differentiating biomarkers between two groups, GBM and rGBM. The range of AUC values calculated in this study is between 0.79 and 1, which indicates high diagnostic power. According to the results obtained from the ROC analysis, the expression levels of selected miRNAs showed excellent diagnostic values (Table 3).

Discussion

Angiogenesis, VEGF, VEGFR, and HIF-1 pathways are all affected by dysregulation of genes and pathways in rGBM, contributing to cancer development. Targeting these dysregulated genes holds promise for improved treatment management and intervention (18, 19).

Neuro-oncology has advanced in diagnosis and targeted therapy thanks to various biomarker categories, including CT perfusion, multiparametric MR imaging, MRI, PET, and theranostics in precision medicine

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(20). Among these biomarkers, miRNAs are one of the types of potent molecular biomarkers currently utilized in cancer diagnosis procedures, either independently or in combination with other biomarkers. For example, miR-21 is well-recognized as an oncomir; however, there is limited research on other microRNAs (21).

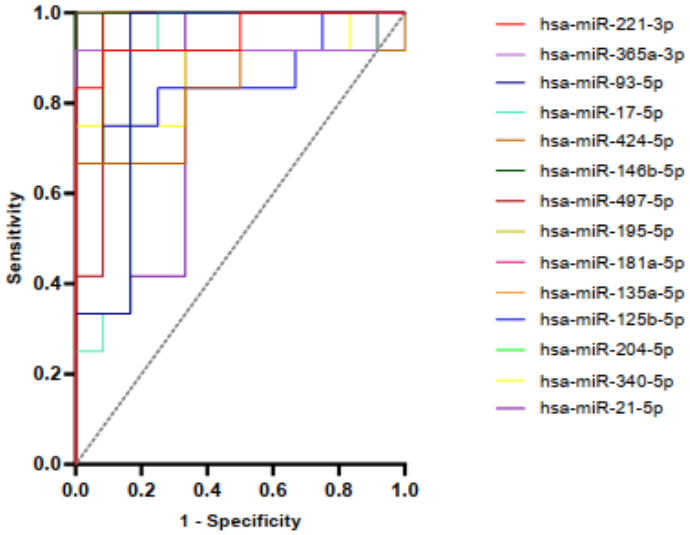

Fig.5. ROC (receiver operating characteristic): 14 potential miRNAs' ROC curves to assess the candidate miRNAs' diagnostic efficacy, the area under the curve (AUC) was calculated. An AUC score greater than 0.7 denotes a diagnostic test's moderate to good discriminatory ability. The 14 candidate miRNAs' AUC values, which ranged from 0.79 to 1, indicated that they might be used as biomarkers to identify rGBM.

Table 3. ROC analysis results for 14.

Symbol	AUC	Std. Error	95% CI	P value	Sensitivity	95% CI	Specificity	95% CI	Optimal Criterion
hsa-miR-221-3p	0.95	0.04	0.86 -1.00	0.00	0.91	0.64-0.99	0.91	0.64-0.99	>8.82
hsa-miR-365a-3p	0.92	0.07	0.77-1.00	0.00	0.91	0.64- 0.99	0.91	0.64- 0.99	>8.72
hsa-miR-93-5p	0.88	0.07	0.73 -1.00	0.00	1.00	0.75-1.00	0.83	0.55- 0.97	>8.64
hsa-miR-17-5p	0.87	0.08	0.71 -1.00	0.00	0.91	0.64-0.99	0.83	0.55- 0.97	>8.84
hsa-miR-424-5p	0.81	0.09	0.63 -1.00	0.00	0.66	0.39-0.86	1.00	0.75- 1.00	>9.47
hsa-miR-146b-5p	1.00	0.00	1.00 -1.00	0.00	1.00	0.75-0.86	0.91	0.64- 0.99	< 6.73
hsa-miR-497-5p	0.95	0.04	0.85 - 1.00	0.00	1.00	0.75-1.00	0.91	0.64-0.99	<7.71
hsa-miR-195-5p	0.97	0.03	0.91 -1.00	0.00	1.00	0.75-1.00	0.91	0.64- 0.99	<8.66
hsa-miR-181a-5p	1.00	0.00	1.00 -1.00	0.00	1.00	0.75-1.00	0.91	0.64- 0.99	<9.60
hsa-miR-135a-5p	1.00	0.00	1.00 -1.00	0.00	1.00	0.75-1.00	0.91	0.64- 0.99	<6.38
hsa-miR-125b-5p	0.85	0.08	0.69 - 1.00	0.00	0.75	0.46- 0.91	0.91	0.64-0.99	<12.22
hsa-miR-204-5p	0.90	0.07	0.75 - 1.00	0.00	0.91	0.64-0.99	0.91	0.64-0.99	<6.59
hsa-miR-340-5p	0.87	0.07	0.72 - 1.00	0.00	0.75	0.46-0.91	1.00	0.75-1.00	<6.53
hsa-miR-21-5p	0.79	0.09	0.59 -0.98	0.01	1.00	0.75- 1.00	0.66	0.39- 0.86	<15.02

AUC: Area under the ROC Curve; CI: Confidence Interval

The purpose of this study was to introduce and evaluate VEGF-linked pathways and the diagnostic or therapeutic potential of obtaned miRNAs as biomarkers in rGBM. Our research has provided significant new

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insights into the dysregulated genes, miRNAs, and signaling pathways associated with the VEGF signaling pathway in rGBM. However, it is critical to discuss possible limitations in our approach and any challenges we encountered, as well as to offer a more comprehensive analysis of our results. In contrast, our study's discovery of dysregulated miRNAs in rGBM relative to primary GBM is consistent with earlier research. According to ROC analysis, 14 miRNAs show potential as biomarkers for identifying rGBM; however, further studies are required to determine their sensitivity, specificity, and clinical relevance. Some of the miRNAs identified in our analysis, such as miR-497-5p and miR-17-5p, have also been reported in studies examining the expression profiles of miRNAs in GBM (22, 23). The importance of these miRNAs in the pathophysiology of GBM is underscored by the consistent findings. Specifically, concerning the VEGF signaling pathway, our research revealed dysregulated genes within this pathway that could contribute to the pathogenesis of rGBM. These results align with previous research indicating alternations in VEGF pathway genes in GBM (24). The convergence between our study and other research emphasizes the significance of the VEGF pathway in the biology of GBM, suggesting it as a promising treatment target. Targeting dysregulated genes or pathways holds great possibility in the treatment of cancer, and therapeutic approaches based on these findings could greatly benefit cancer treatment plans.

These interventions target the genes or signaling pathways implicated in the initiation and progression of cancer. They can be created using small chemicals, antibodies, or gene-based therapies. However, there are difficulties to consider including off-target effects, resistance mechanisms, and the intricacy of cancer heterogeneity. The outcomes of cancer patients could potentially be improved through developments in precision medicine and research (25, 26). In our study, pathway analysis identified interactions between the VEGF signaling pathway and other pathways related to rGBM. It is noteworthy that we discovered a relationship between the VEGF pathway and the mitogen-activated protein kinase (MAPK) pathway, which has been previously mentioned in other research (27).

The discovery of these pathway connections emphasizes the complexity of GBM biology and highlights the need for comprehensive therapy strategies that can simultaneously target multiple pathways. Our findings can be supplemented by further studies on gene expression, the relationship between gene expression and these microRNAs in this cancer, and functional studies. Regarding the significance of molecular biomarkers, more research is required. For instance, it is still necessary to determine whether a biomarker is indeed important and meaningful and whether it can be applied for all patients, given the variability of their conditions. Consequently, a significant amount of time may pass between a biomarker's introduction and its use in conjunction with or as a replacement for other biomarkers.

Our research also revealed potential biomarkers among the dysregulated miRNAs that may aid in rGBM diagnosis and prognosis. This result aligns with recent investigations that have identified miRNA biomarkers with utility in GBM diagnosis and prognosis (23). The similarities between our findings and those of others research highlight the potential value of miRNA biomarkers for clinical treatment and patient classification in rGBM. Our study has various advantages, including the utilization of bioinformatics methods to find new treatment targets and biomarkers in rGBM, as well as the integration of multiple datasets.

However, it is essential to acknowledge certain limitations in our study. To establish the functional roles of the identified miRNAs and pathways, additional experimental validation is required. Initially, our study

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focused on *in silico* analysis and bioinformatics predictions. Furthermore, the heterogeneity of rGBM and the specific dataset employed in this investigation may limit the generalizability of our findings. In summary, our research offers important new understandings of the dysregulated miRNAs, genes, and signaling pathways related to the VEGF signaling pathway in rGBM. In summary, our research provides valuable new insights into the the dysregulated miRNAs, genes, and signaling pathways related to the VEGF signaling pathway in rGBM. The strength of our findings is highlighted by their agreement with earlier studies, which also emphasize the significance of the identified miRNAs, genes, and pathways in the pathogenesis of rGBM.

The petential treatment targets and biomarkers discovered in this work show promise for the development of personalized medicine techniques in rGBM, but further confirmation is required. Future research should concentrate on these directions while considering multi-modal therapy approaches that target interconnected pathways for better patient outcomes. To enhance the translational potential of our findings, larger-scale investigations involving diverse patient groups and the incorporation of experimental techniques should be pursued. By addressing these issues, we can pave the way for more efficient therapies and better clinical outcome for rGBM patients.

In conclusion, the resistance to anti-angiogenesis therapies that target the vascular endothelial growth factor (VEGF) pathway is influenced by interaction with other pathways and the proteins that regulate them. Through our research, we identified 14 microRNAs that have the potential to be utilized as biomarkers and therapeutic targets. This study showed how several pathways that can affect the VEGF pathway. The 14 microRNAs identified from these pathways may hold significant value as molecular biomarkers or therapeutic targets for patient with rGBM. Further study and validation are necessary to fully realize their clinical potential.

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