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ORIGINAL RESEARCH

Reduced Expression of hsa-miR-338-3p Contributes to the Development of Glioma Cells by Targeting Mitochondrial 3-Oxoacyl-ACP Synthase (OXSM) in Glioblastoma (GBM)

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Background: MicroRNAs have been identified as major regulators and therapeutic targets of glioblastoma (GBM). It is thus meaningful to study the miRNAs differentially expressed (DE-miRNAs) in GBM.

Materials and Methods: We performed a meta-analysis of previously published microarray data using the R-based "metaMA" package to identify DE-miRNAs.The biological processes of the DE-miRNAs were then analyzed using FunRich. KEGG pathways of the DE-miRNAs gene targets were analyzed by mirPath V.3. Luciferase activity assay was performed to validate that *OXSM* is a direct target of *hsa-miR338-3p*. Flow cytometry was used to detect the effects of *miR-338-3p* on GBM cell proliferation, apoptosis and cell cycle.

Results: DE-miRNAs in blood and brain tissue from GBM were identified. "Type I interferon signaling pathway" and "*VEGF* and *VEGFR* signaling network" were the most significantly enriched biological processes shared by all GBM types. In KEGG pathway analysis, DE-miRNAs both in blood and tissue show altered fatty acid biosynthesis. Further validation shows *hsa-miR-338-3p* regulates fatty acid metabolism by directly targeting *OXSM* gene. In addition, our data revealed an accelerated cell cycle and an anti-apoptotic role for OXSM in glioma cells, which has not been reported. Finally, we confirmed that *hsa-miR-338-3p* inhibitor antagonized the effect of downregulation of *OXSM* on cell cycle and apoptosis of GBM cells.

Conclusion: We revealed that *hsa-miR-338-3p*, down-regulated in GBM, may affect the biogenesis and rapid proliferation of glioma cells by regulating the level of *OXSM*, providing new insights into understanding the pathogenesis of GBM and developing strategies to improve GBM prognosis.

Keywords: glioblastoma, *hsa-miR-338-3p*, *OXSM*, fatty acid metabolism

Introduction

Glioblastoma (GBM) is one of the most prevalent and highly invasive malignant tumors.[1] The overall survival (OS) time for GBM patients is 15 months and the 5-year survival rate is below 10%, even after comprehensive treatments such as surgery, radiation therapy, and chemotherapy.[2,3] This is mainly due to the ability of the tumor in infiltrating the surrounding brain tissues.

MicroRNAs (miRNAs), a class of small non-coding RNAs, play a pivotal role in RNA silencing and post-transcriptional regulation of gene expression, thereby

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taking part in numerous biological processes and biological pathways. Numerous experiments have found that miRNAs were involved in all aspects of tumor-related processes, including proliferation, apoptosis, metastasis, angiogenesis, and immune response.[4–6] Because of its high sensitivity and specificity, miRNA is very likely to become a new type of tumor diagnostic and prognostic markers and a new target for tumor treatment.[7,8] Therefore, it is meaningful to study the miRNAs differentially expressed (DE-miRNAs) in GBM including in serum or tumor tissues. In past decades, numerous studies have been conducted to understand the complex host-miRNA interactions of GBM. For example, the expression level of *hsa-miR-210-3p* in GBM tumor tissue is higher than that in normal brain tissue, and it is positively correlated with the pathological grade of patients[9] The *hsa-miR-199a-3p* inhibits glioma growth by inhibiting *AKT/mTOR* signaling pathway[10] The *hsa-miR-96* activates the *Wnt/β-catenin* pathway by targeting the *GSK3b/β-catenin* downstream tumor suppressor gene *HBP-1* and promoting the proliferation of glioma cells[11] The *hsa-miR-181b-5p* targets *IGF-1R* and inhibits glioma cell proliferation, migration, and invasion[12] The *hsa-miR-7-5p* might control growth of GBM microvasculature by regulating the *PI3K/ATK* and *Raf/MEM/ERK* pathways.[13] Although the accumulating data provide useful information about miRNAs in GBM, the identification of key miRNAs and pathways from these studies was restricted due to the limited sample size in the independent study.

Herein, we first screened several DE-miRNAs in blood or tumor tissues from GBM compared with healthy controls or adjacent normal tissues from GBM by analyzing nine independent microarray datasets downloaded from the GEO database. Then, FunRich was employed to predict the biological processes and biological pathways of DE-miRNAs in GBM. Next, KEGG pathway enrichment analysis of genes target by DE-miRNAs in GBM was conducted by mirPath V.3. Subsequently, fatty acid metabolism regulated by DE-miRNAs was further analyzed, GEPIA was used to analyze the mRNA expression related to fatty acid metabolism. In addition, *OXSM* was identified as a direct target of *has-miR-338-3p* by luciferase activity analysis. Finally, our data revealed an accelerated cell cycle and an anti-apoptotic role for *OXSM* in glioma cells, which has not been reported. Considering that *hsa-miR-338-3p* can down-regulate the expression of the target gene *OXSM*, we also investigated whether *hsa-miR-338-3p* is involved in the regulation of GBM cell function by *OXSM*. We confirmed that *hsa-miR-338-3p* inhibitor antagonized the effect of downregulation of *OXSM* on cell cycle and apoptosis of U87 and U251 cells. Our results indicate that *hsa-miR-338-3p* may affect the biogenesis and rapid proliferation of glioma cells by regulating the level of *OXSM*.

Our study provides a comprehensive evaluation of miRNA expression profiles, highlights the importance of *hsa-miR-338-3p* and *OXSM* in GBM, which will provide new insights into understanding the pathogenesis of GBM and developing strategies to improve GBM prognosis.

Materials and Methods

Microarray Data Collection

The gene expression microarray datasets with the keywords "GBM glioblastoma", "glioma", "miRNA" and "microRNA" were downloaded from Gene Expression Omnibus (NCBI) database.[14] Glioblastoma patients were considered "Cancer group" while healthy controls or adjacent normal tissues from GBM were considered "Control group". Data sets with a sample source other than serum, plasma, or brain tissue from the control group were excluded. Nine independent microarray datasets with raw data were selected and the details about these datasets are outlined in Table 1. The following information was extracted from each of the studies that were selected: GEO accession; numbers of patients and controls; sample source; platform and gene expression data. We compared microarray data for GBM (n = 332) and controls (n = 213) from the public database submissions. Three datasets included the transcriptome profiles of serum from 207 GBM and 181 controls. Six datasets included tissue from 125 GBM and 32 controls.

miRNA Expression Analysis

To identify DE-miRNAs between GBM and controls, the data collected from each eligible microarray study were imported to the R-based "metaMA" package developed by Guillemette Marot[15] which combines either p-values or modified effect sizes from different studies to find differentially expressed genes. The data were annotated after converting the probe IDs to the corresponding miRNA names. The intensity values for each probe set were log2 transformed then uploaded, processed, and annotated for data integrity. A p-value <0.05 was considered statistically significant for the analysis.

Table 1 Summary of Transcriptome Datasets Used in This Study

Study	GEO Accession	Sample Size		Sample Source	Platform
		Cancer	Control		
1	GSE93850	22	8	Serum	GPL22948 State Key Laboratory Human microRNA array 1858
2	GSE122488	12	16	Serum	GPL11154 Illumina HiSeq 2000 (Homo sapiens)
3	GSE139031	173	157	Serum	GPL21263 3D-Gene Human miRNA V21_1.0.0
4	GSE25631	82	5	Tissue	GPL8179 Illumina Human v2 MicroRNA expression beadchip
5	GSE37737	7	7	Tissue	GPL9460 Applied Biosystems Human TaqMan Low Density Array
6	GSE61710	12	5	Tissue	GPL10656 Agilent-029297 Human miRNA Microarray v14 Rev.2
7	GSE65626	3	3	Tissue	GPL19117 [miRNA-4] Affymetrix Multispecies miRNA-4 Array
8	GSE90603	16	7	Tissue	GPL21572 [miRNA-4] Affymetrix Multispecies miRNA-4 Array
9	GSE103229	5	5	Tissue	GPL18058 Exiqon miRCURY LNA microRNA array, 7th generation

Biological Process and Pathway Enrichment

Gene Ontology classification of miRNAs expressed differentially in GBM was performed with FunRich (http://www.funrich.org/), a stand-alone software tool used mainly for functional enrichment and interaction network analysis based on default background database or a customized database. miRNA id lists were uploaded and analyzed using the functional annotation chart for biological processes and pathways. Representative biological processes and pathways selected from the top significantly enriched charts are reported in the figures. A Benjamini-corrected p-value less than 0.05 was used to identify a statistically significant analysis.

KEGG pathways of genes target by DE-miRNAs in GBMs were analyzed by mirPath V.3(http://diana.imis.athena-innovation.gr/), a stand-alone software tool that can utilize predicted miRNA targets (in the CDS or 3′-UTR region) provided by the microT-CDS algorithm and miRNA interactions experimentally verified derived from TarBase.

Cell Culture and Transfection

The human GBM cell lines U87 and U251 were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China. The cells were incubated at 37°C, 5% CO2, and 10% fetal bovine serum (Invitrogen) was added to the culture medium. Transfection reagent Lipofectamine 2000 (Invitrogen) was used to transfect 20μM *has-miR338-3p* mimics or controls (GenePharma, Shanghai, China) into GBM cells according to the manufacturer's instructions.

Reverse Transcription and qRT-PCR

MiRNeasy Micro kit (QIAGEN) was used to extract miRNA from cells, and purified RNA was treated with DNase I reagent to eliminate genomic DNA contamination. RNA was reverse transcribed using Primpscript® RT kit (TAKARA, Dalian, China) according to the manufacturer's instructions. qRT-PCR was used to detect miRNA and mRNA using SYBR Premix Ex Taq ™ II (TAKARA, Dalian, China). The miRNA expression level was normalized to small nucleolar RNA (U6), and mRNA expression was normalized to *GAPDH*.

Luciferase Activity Assay

According to the target prediction, the *OXSM* 3′UTR contained two appropriate sequences with *has-miR338-3p* binding sites (358–368 and 437–445 nt). A 3′UTR fragment containing the putative binding site of *hsa-miR-338-3p* was cloned into the GP-miRGLO vector. Lipofectamine 2000 (Invitrogen) was used to transfected empty vector plasmid (GP-miRGLO) or *OXSM* 3′UTR reporter gene (wild type, MUT-1, MUT-2), into U87 and U251 cells. Drosophila and Renilla luciferase activity was measured using the Dual-Luciferase reporter assay (Promega) at 24 h after transfection.

Cell Cycle Assay

Cell cycle phases were determined using the BD Cycletest™ Plus DNA Reagent Kit (BD Biosciences) according to the instructions provided by the manufacturer. In brief, GBM cells were cultured for 48 h after transfection. After transfection, the distribution of DNA content was determined using a BD LSR II flow cytometer and analyzed using the FlowJo software.

Detection of Apoptosis

After transfection (48 h), GBM cells were stained with PE-conjugated anti-Annexin V and 7-AAD (Biolegend). The

cells were analyzed using a BD LSR II flow cytometer and the FlowJo software.

Statistical Analysis

Data analysis was performed using SPSS 21.0 and GraphPad Prism Version 5.0 software. A p-value <0.05 was considered statistically significant.

Result

MiRNAs Expressed Differentially in Blood and Brain Tissue from Controls

First, we identified the miRNAs expressed differentially in blood from controls. According to the results of our analysis, 390 miRNAs in blood were identified to be expressed differentially between GBM and controls. Among the 390 miRNAs, 190 miRNAs were upregulated and 200 were downregulated. The downregulated miRNAs included circulating miRNAs that have been reported to be associated with GBM disease progression or pathogenesis, such as *hsa-miR-128*.[16] There were 317 DE-miRNAs in brain tissues between GBM and controls across microarray datasets. Among the 317 miRNAs, 195 miRNAs were upregulated and 122 miRNAs were downregulated. The upregulated miRNAs include *hsa-miR-155-5p*, which can promote proliferation, invasion, migration, and inhibit apoptosis of glioma cells.[17–19] The miRNAs expressed differentially in blood and tissue are shown in Supplemental Table 1.

Next, we investigated whether some different miRNAs were shared when comparing different types of samples between GBM and controls. The Venn diagram shows that 78 miRNAs have changed significantly in blood and tissue samples. Among them, 23 up-regulated miRNAs and 18 down-regulated miRNAs showed the same variation trend (Figure 1). Twenty-three upregulated miRNAs and the 18 downregulated miRNAs shared in all sample types are shown in Table 2.

MiRNAs Functional Classification and Pathway Assignment

Gene Ontology classification analysis was carried out for 390 DE-miRNAs in blood or 317 DE-miRNAs in brain tissues. Following GO analysis, the "TRAIL signaling pathway", "*VEGF* and *VEGFR* signaling network" and "*IFN-gamma* pathway" were significantly enriched for miRNAs expressed differentially in blood and tissue (Figure 2A and B). KEGG's PATHWAY database integrates current knowledge in molecular interaction networks. In this study, we analyzed KEGG pathways of 78 DE-miRNAs both in blood and tissue (Figure 2C), revealing that "Fatty acid biosynthesis" was significantly enriched.

Fatty Acid Metabolism Regulated by miRNAs

It has become increasingly clear that patients with high-risk/relapsed tumors are intimately linked to metabolic abnormalities, as well as abnormal expression of multiple factors and signaling pathways related to tumor metabolism. Fatty acid biosynthesis has been reported to play important roles in the pathogenesis of multi cancers.[20]

In the KEGG pathway analysis, DE-miRNAs in both blood and tissues may affect fatty acid biosynthesis. By constructing a network diagram of the DE-miRNAs and fatty acid biosynthesis, we found that *hsa-miR-338-3p*, *hsa-miR-19b-3p*, *hsa-miR-15b-5p*, *hsa-miR-202-5p*, *hsa-miR-338-5p*, *hsa-miR-5011-5p*, *hsa-miR-3065-3p*, *hsa-miR-4528*, *hsa-miR-195-5p*, *hsa-miR-196a-5p*, *hsa-miR-483-3p*, *hsa-miR-19a-3p*, *hsa-miR-138-2-3p*, *hsa-miR-4530*, *hsa-miR-330-3p*, and *hsa-miR-362-5p* may regulate fatty acid metabolism by regulating its predicted or validated target genes (Figure 3A). Among them, *hsa-miR-4528*, *hsa-miR-19a-3p*, *hsa-miR-19b-3p*, *hsa-miR-195-5p*, and *hsa-miR-15b-5p* were up-regulated in both blood and tissues of GBM, while *hsa-miR-338-3p*, *hsa-miR-330-3p*, *hsa-miR-338-5p*, and *hsa-miR-138-2-3p* were down-

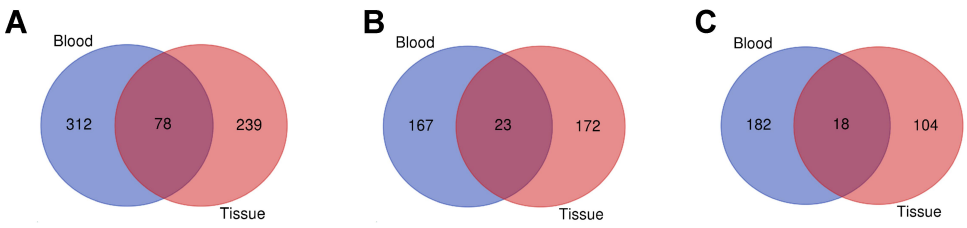

Figure 1 Venn diagram representing the number of DE-miRNAs in GBM compared with controls in blood and tissue. (**A**) All the DE-miRNAs found in blood and tissues; (**B**) the upregulated miRNAs found in blood and tissues; (**C**) the downregulated miRNAs found in blood and tissue.

Table 2 miRNAs Expressed Differentially in All Sample Types

UP	*hsa-miR-517c-3p* *hsa-miR-20b-3p* *hsa-miR-2114-5p* *hsa-miR-3667-5p* *hsa-miR-675-3p* *hsa-miR-195-5p* *hsa-miR-10b-5p* *hsa-miR-4763-5p*	*hsa-miR-4429* *hsa-miR-3646* *hsa-miR-4477a* *hsa-miR-15b-5p* *hsa-miR-4528* *hsa-miR-19a-3p* *hsa-miR-4753-3p* *hsa-miR-19b-3p*	*hsa-miR-320a* *hsa-miR-301b* *hsa-miR-891a* *hsa-miR-188-5p* *hsa-miR-20b-5p* *hsa-miR-106b-5p* *hsa-miR-21-3p*
DOWN	*hsa-miR-323b-5p* *hsa-miR-138-1-3p* *hsa-miR-425-3p* *hsa-miR-490-3p* *hsa-miR-330-5p* *hsa-miR-139-5p*	*hsa-miR-330-3p* *hsa-miR-138-5p* *hsa-miR-129-5p* *hsa-miR-4270* *hsa-miR-2116-5p* *hsa-miR-1225-5p*	*hsa-miR-338-5p* *hsa-miR-485-3p* *hsa-miR-338-3p* *hsa-miR-5589-5p* *hsa-miR-5187-5p* *hsa-miR-138-2-3p*

regulated both in blood and tissues of GBM, those miRNAs may regulate fatty acid metabolism by regulating its predicted or validated target genes (*ACSL1*, *ACSL3*, *ACSL4*, *ACSL6*, *FASN*, *OXSM*) (Figure 3B). GEPIA (http://gepia.cancer-pku.cn/detail.php) which can analyze RNAs from cancer genome atlas (TCGA) and genotype tissue expression project (GTEx) was used to analyze the differential expression of mRNA of *ACSL1*, *ACSL3*, *ACSL4*, *ACSL6*, *FASN* and *OXSM* in GBM and control tissues. *OXSM* is highly expressed in GBM (Figure 3C). High expression of OXSM in GBM suggests that OXSM may be involved in the mechanism of miRNAs regulating fatty acid metabolism.

Identification of OXSM as Direct Target of hsa-miR338-3p

In our study, OXSM is a possible target gene predicted by 3 miRNAs (*hsa-miR195-5p*, *hsa-miR15b-5p*, and *hsa-miR338-3p*, Figure 4B). In order to verify the regulation of *OXSM* by these three miRNAs, we overexpressed the corresponding miRNA expression in the U87 cell lines by transfecting miRNA mimics (Figure 4A). Using qRT-PCR to detect the expression of *OXSM*, we found that *hsa-miR338-3p* can significantly inhibit the expression of *OXSM* both in U87 cell lines (Figure 4B). Similarly, *hsa-miR338-3p* down-regulated the expression level of *OXSM* in U251 cell lines.

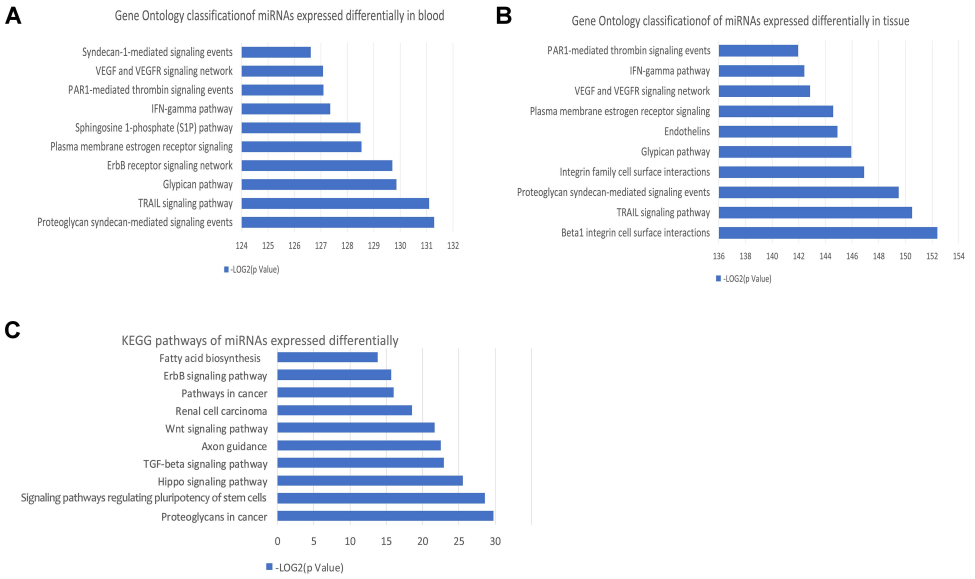

Figure 2 GO and KEGG pathway analysis of DE-miRNAs in GBM. (**A**) GO classification of genes targeted by 390 DE-miRNAs in blood. (**B**) GO classification of genes targeted by 317 DE-miRNAs in tissue. (**C**) KEGG pathways of 78 DE-miRNAs in all type of samples.

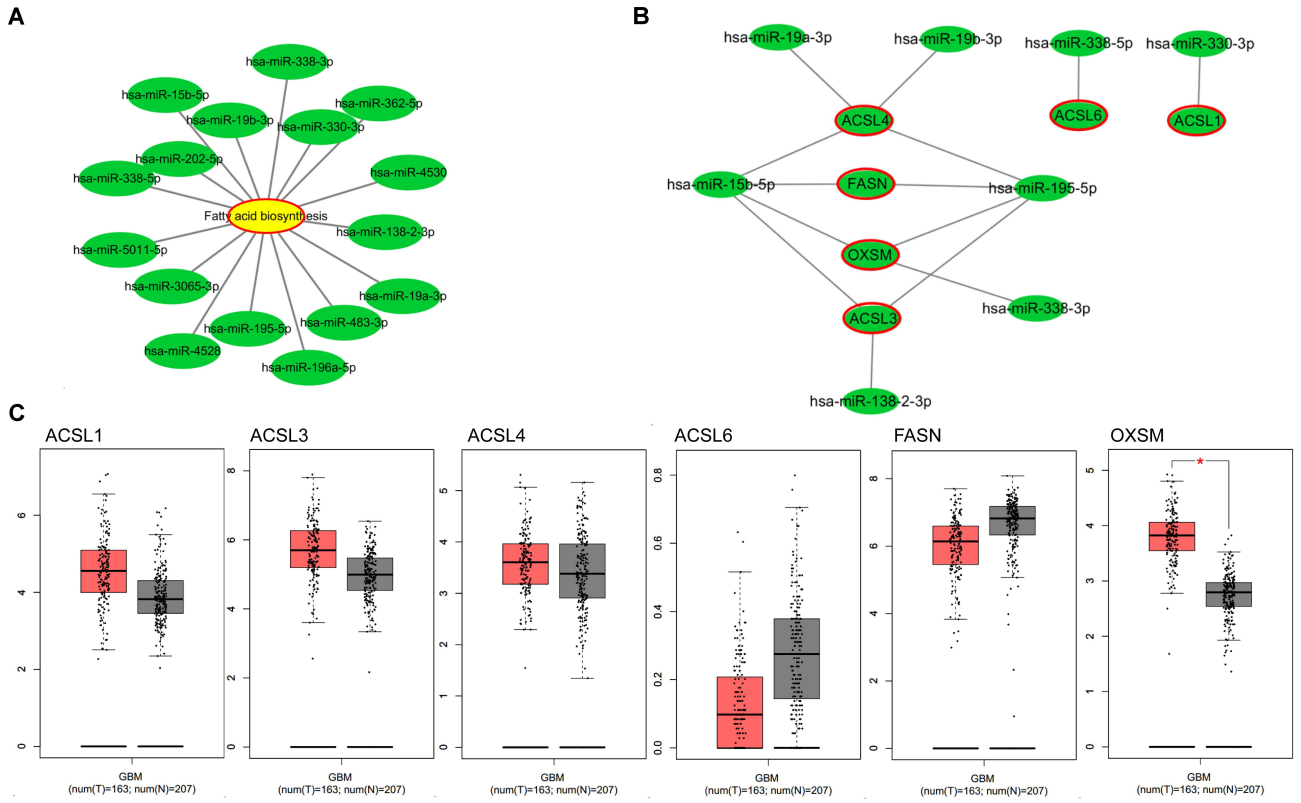

Figure 3 Fatty acid metabolism regulated by DE-miRNAs. (**A**) Network diagram of the DE-miRNAs and fatty acid biosynthesis produced by Cytoscape. (**B**) Network diagram of the upregulated miRNAs or downregulated miRNAs in all type of samples and their target genes in pathway of fatty acid biosynthesis produced by Cytoscape; (**C**) the expression levels of target genes in pathway of fatty acid biosynthesis from the GEPIA database. *$P < 0.05$.

We explored the potential of *hsa-miR338-3p* to directly target the 3′UTR of *OXSM*. The two putative binding sites between *OXSM* 3′UTR and *hsa-miR338-3p* are shown in Figure 4D. Luciferase report vectors carrying the full 3′UTR of wild-type (WT) *OXSM*, mutation site1 (MUT-1, 437–445), or mutation site 2 (MUT-2, 358–368) were constructed (Figure 4D). Reporters were transfected into U87 and U251 cells with *hsa-miR338-3p* mimics, luminescence in *hsa-miR338-3p*-treated cells was clearly less than in controls (Figure 4E). The results show that *hsa-miR338-3p* can directly target the 3′UTR of *OXSM* and affect subsequent transcription. Therefore, we also worked to identify the effective binding site between *OXSM* and *hsa-miR338-3p*. No substantial differences in the inhibition luciferase activity between the WT reporter and mut-2 were observed, while the luciferase activity after transfection of the mut-1 reporter was significantly higher than that of the WT reporter both in U87 and U251 cell lines (Figure 4F). We found that *hsa-miR338-3p* can directly target *OXSM* 3′UTR through bind site 1 (437–445).

Hsa-miR338-3p Contribute to the Development of Glioma Cells via Expression of OXSM

Omics studies have provided important information for understanding the pathogenesis of diseases. As an enzyme required for elongation of fatty acid chains in the mitochondria, we hypothesized that *OXSM* may contribute to the development of glioma cells by regulating the cell cycle and apoptosis. This was the first study addressing this question. Initially, we reduced the expression of *OXSM* in U87 and U251 cells by transfection with siRNA to investigate the effect of *OXSM* on cell cycle and apoptosis. After transfection (48 h), *OXSM* was low expressed (Figure 5A). We found that forced expression of *OXSM* significantly arrested U87 cell cycle (Figure 5B). Furthermore, the percentage of Annexin V^+ 7-AAD^- apoptotic cells was higher following the forced expression of *OXSM* (Figure 5C). These data indicate that *OXSM* promotes cell cycle and inhibits apoptosis of U87 and U251 cell lines.

We subsequently assessed the role of *hsa-miR338-3p* in regulating the development of glioma cells by transfection

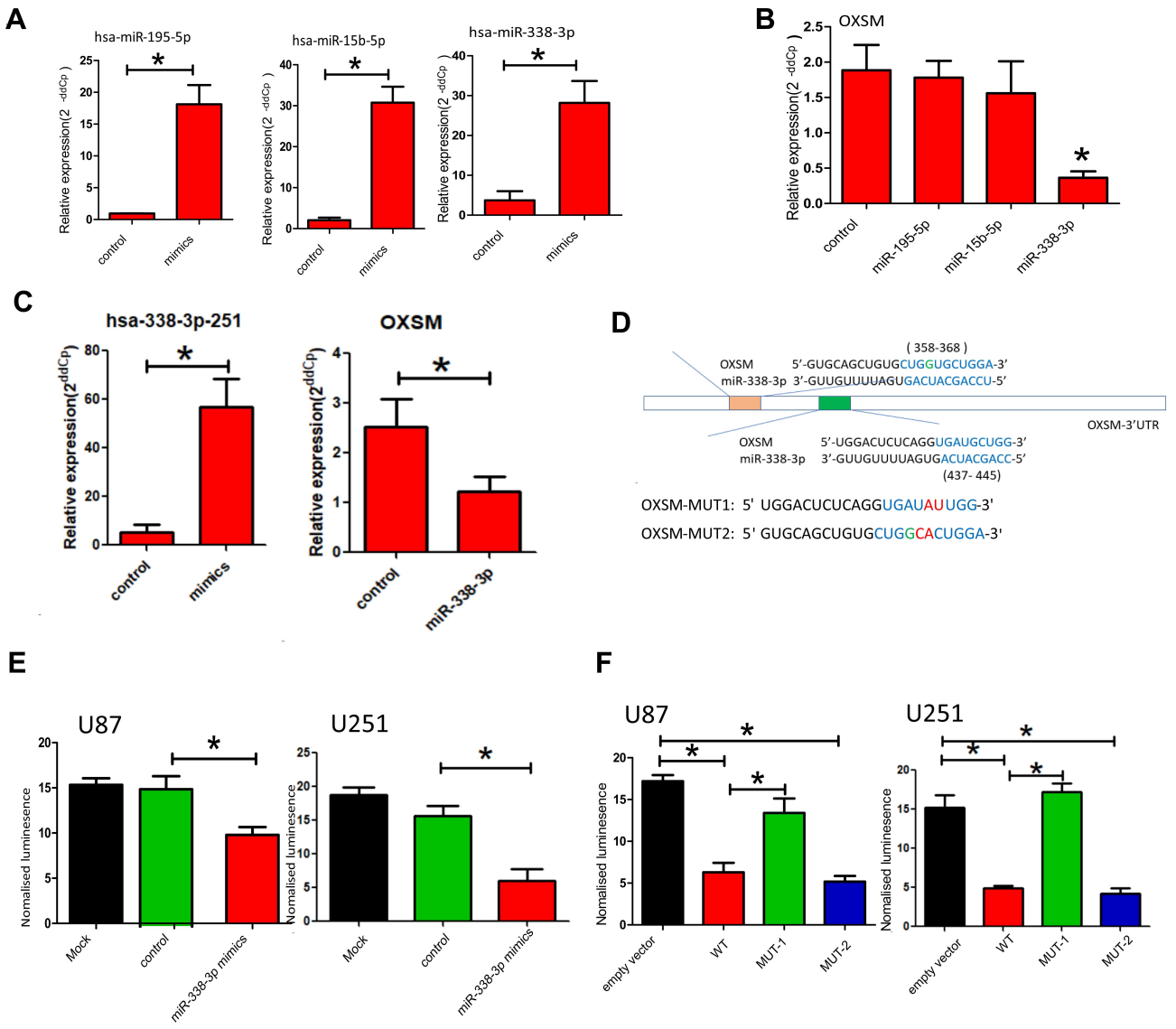

Figure 4 Identification of *OXSM* as direct target of *hsa-miR338-3p*. (**A**) *hsa-miR195-5p*, *hsa-miR15b-5p* and *hsa-miR338-3p* were overexpressed in the U87 cell lines by transfecting miRNA mimics; (**B**) the expression of *OXSM* in the U87 cell lines after transfecting of miRNA mimics; (**C**) *hsa-miR338-3p* were overexpressed in the U251 cell lines by transfecting miRNA mimics and the expression of *OXSM* in the U251 cell lines after transfecting of miRNA mimics; (**D**) the two putative binding sites between *OXSM* 3′UTR and *hsa-miR338-3p*; Luciferase report vectors carrying the full *OXSM* 3′UTR of mutation site1 (MUT-1, 437–445) and mutation site 2 (MUT-2, 358–368); (**E**) luminescence in *hsa-miR338-3p*-treated cells with wild-type reporters; (**F**) luminescence in *hsa-miR338-3p*-treated cells with MUT-1and MUT-2 reporters. *$P < 0.05$.

of *hsa-miR338-3p* inhibitor into U87 and U251 cells. Following the reduced expression of *hsa-miR338-3p* (Figure 5D), U87 and U251 cells showed a significant increase in cell cycle (Figure 5E), and apoptosis was significantly reduced in comparison with the controls (Figure 5F). Furthermore, siRNA was used to suppress the expression of *OXSM* in *hsa-miR338-3p* -low-expressing U87 or U251 cells to verify that *hsa-miR338* affects GBM cell development through regulation of *OXSM*. Following the downregulation of *OXSM*, there was no statistical difference detected in cell cycle or apoptosis compared with controls. These data suggest that *hsa-miR338-3p* inhibitors antagonize the effect of downregulation of *OXSM* on the function of GBM cells.

Discussion

It is believed that dysregulation of miRNAs is closely associated with the development of multiple human diseases in brain.[21–24] During the past few years, studies have intensively suggested that DE-miRNAs and their downstream target genes are closely associated with the development of GBM.[25–27] In this present study, we conducted a differential expression analysis using multiple public microarray datasets to investigate DE-miRNAs in blood

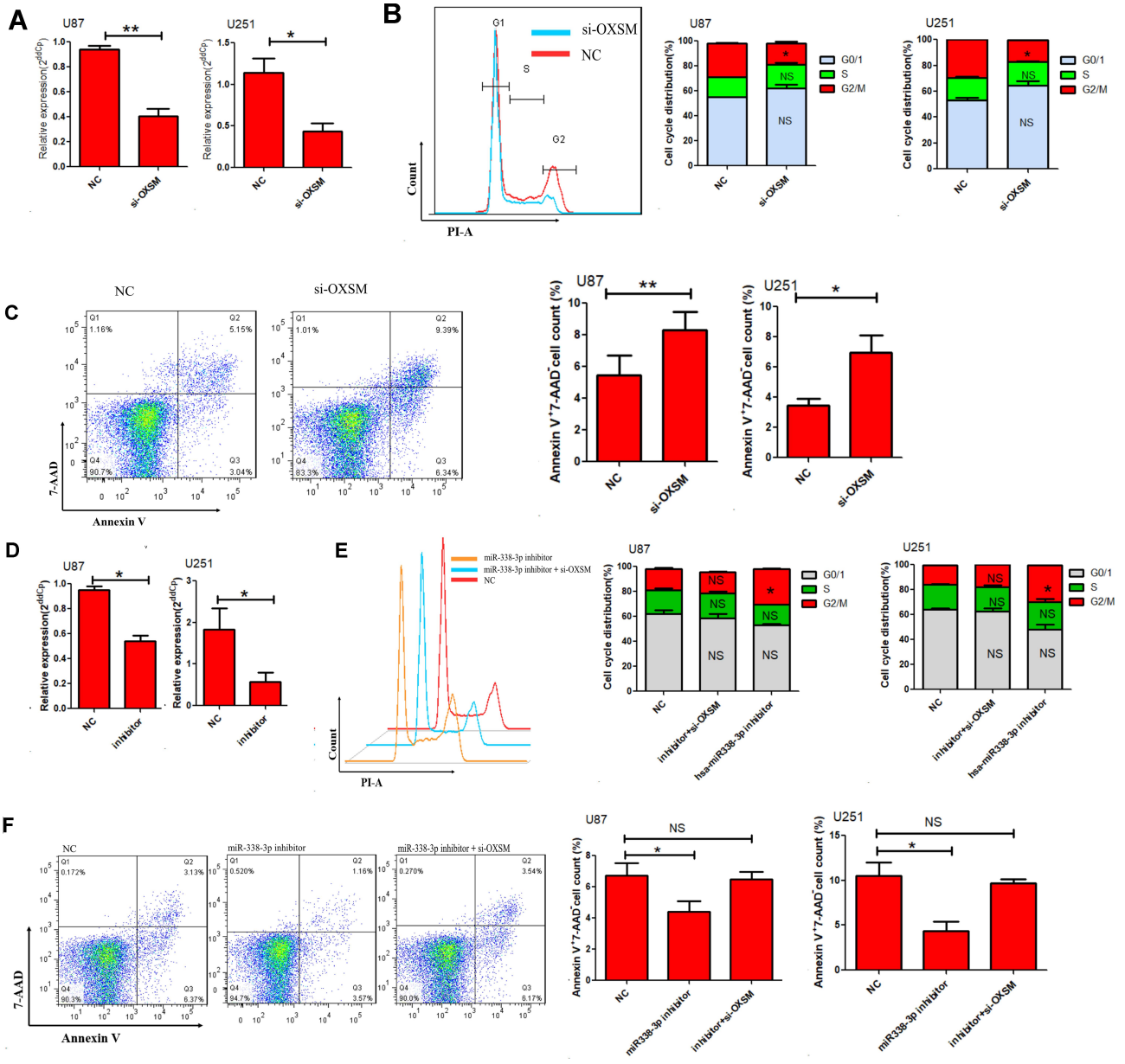

Figure 5 *Hsa-miR338-3p* regulate the cell cycle and apoptosis of glioma cells via *OXSM*. (**A**) The relative expression of *OXSM* was determined 48 h after transfection of 20 μM *OXSM* siRNA or controls through qRT-PCR. The cell cycle (**B**) and apoptosis (**C**) of GBM cells was determined after transfection of *OXSM* siRNA. (**D**) The expression of *hsa-miR338-3p* was inhibited by introducing 20 μM inhibitor to U87 and U251 cells. After transfection of *hsa-miR338-3p* inhibitor and *OXSM* siRNAs, the cell cycle (**E**) and apoptosis (**F**) of glioma cells were compared. *$P < 0.05$, **$P < 0.01$.

and brain tissues of GBM. Upregulated DE-miRNAs and downregulated DE-miRNAs were finally identified in blood and brain tissues. Most of the DE-miRNAs we screened are consistent with the analysis results of previous studies. For example, *hsa-miR-338-3p* found to be significantly downregulated in GBM, acted as a tumor-suppressing gene whose silencing can inhibit malignant biological behaviors of glioma cells, was an independent prognostic biomarker associated with poor prognosis in glioma patients;[28] *hsa-miR-139-5p* is lower in blood and brain tissues of GBM, was identified as a tumor suppressor by negatively targeting *Notch1*;[29] *hsa-miR-490-3p* expression was significantly downregulated in GBM, which can inhibit glioma cell proliferation and migration.[30]

GO and KEGG pathway analysis offered insight into the possible roles of DE-miRNAs in the pathogenesis of GBM. We found that the *IFN-gamma* pathway was involved in the most significantly enriched terms shared by all sample types, indicates that DE-miRNAs in GBM may affect the activation of immune system and is a key

factor affecting disease progression. Of note, in our study, "*TRAIL* signaling pathway" is one of 10 most significant pathways enriched for DE-miRNAs in blood and tissue. Type I interferon can activate DC cells to release tumor necrosis factor-related apoptosis-inducing ligand (*TRAIL*), thereby enhancing cytotoxicity of NK cells or directly killing tumor cells.[31,32] "*VEGF* and *VEGFR* signaling network" were also significantly enriched for DE-miRNAs in our study. Vascular endothelial growth factor (*VEGF*) is one of the core members of tumor angiogenic factors. GBM has an increasingly strong angiogenic effect, and it is more dependent on neovascularization.[33]

In the past several years, a wealth of evidence has emerged on how metabolism affects aspects of biology. Considerable progress has been made in the field of cell metabolism, becoming one of the hottest areas in research.[34,35] Metabolomics methods can establish a direct correlation between changes in metabolite content and changes in biological phenotypes.[36,37] Similar to other types of cancers, fatty acid uptake and lipid metabolism are deregulated in malignant glioma.[38,39] Among 10 most significantly KEGG pathways in our study, "Fatty acid biosynthesis" was identified for DE-miRNAs both in blood and tissue. The network relationship between DE-miRNA and fatty acid biosynthesis was further analyzed in this study and our results revealed that *hsa-miR-338-3p* may regulate fatty acid metabolism by directly targeting *OXSM*, an enzyme required for elongation of fatty acid chains in the mitochondria.

Emerging evidences suggest that cancer cells frequently reprogram metabolic pathways to meet their high demands of biogenesis and rapid proliferation. Dysregulation of metabolism ultimately influences cancer cell fate decision. Thus, understanding how key metabolic pathways, such as lipid metabolism, are aberrantly regulated, and what advantages these metabolic changes confer to cancer cells are of great interest and may benefit the follow-up research and therapeutic targeting. As an enzyme required for elongation of fatty acid chains, we assessed whether *OXSM* contributed to fate of GBM by regulating the function of glioma cells. In this study, the data revealed an accelerated cell cycle and an anti-apoptotic role for *OXSM* in U87 and U251 cells, which has not been reported. Our experiments show that *OXSM*, an enzyme required for elongation of fatty acid chains in the mitochondria, which is abnormally expressed in GBM, can not only participate in the regulation of fatty acid biosynthesis but also affect the biogenesis and rapid proliferation of glioma cells. *hsa-miR-338-3p* is abnormally expressed in various malignant tumors and participates in the proliferation, differentiation and invasion of tumor cells.[40] It is evident that *has-miR-338-3p* is downregulated in metastatic tumor tissues of neuroblastoma compared to primary tumors, and that has-*miR-338-3p* can inhibit cell proliferation by inducing cell cycle arrest, as well as restrain cell migration and invasion.[41] Similarly, our data revealed the arrest-cell cycle and pro-apoptosis role of *hsa-miR-338-3p* in glioma cells. Considering that *hsa-miR-338-3p* can down-regulate the expression of the target gene *OXSM*, we also investigated whether *hsa-miR-338-3p* is involved in the regulation of U87 cell function by *OXSM*. This study confirmed that *hsa-miR-338-3p* inhibitor antagonized the effect of downregulation of *OXSM* on the function of U87 cells. Our results indicate that *hsa-miR-338-3p* may affect the biogenesis and rapid proliferation of glioma cells by regulating the level of *OXSM*. Current data have highlighted the importance of *hsa-miR-338-3p* and *OXSM* in GBM, which provide potential targets for improved immune intervention.

In summary, our analysis of microarray studies will facilitate the understanding of DE-miRNAs in GBM. The investigation identified *hsa-miR-338-3p*, down-regulated in GBM, plays an important metabolic regulatory role in GBM and may also affect the biogenesis and rapid proliferation of tumor cells by regulation the level of *OXSM*. This study provides useful information for the exploration of new intervention paths in GBM.

Abbreviations

OXSM, *mitochondrial 3-oxoacyl-ACP synthase*; GBM, glioblastoma; DE-miRNAs, miRNAs differentially expressed; OS, overall survival; miRNAs, microRNAs; TCGA, cancer genome atlas; GTEx, genotype tissue expression project; WT, wild-type; *TRAIL*, *tumor necrosis factor-related apoptosis-inducing ligand*; *VEGF*, *vascular endothelial growth factor*; *EGFR*, *a receptor for members of the epidermal growth factor*.

Data Sharing Statement

The data that support the findings of this study are available from University of California Santa Cruz Genome Browser and GEO database.

Ethics Approval

This work was approved by the Ethical Board of China Medical University.

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Disclosure

The authors report no conflicts of interest in this work.

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