miR-137 is a diagnostic tumor-suppressive miRNA that targets SPHK2 to promote M1-type tumor-associated macrophage polarization

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Received November 17, 2020; Accepted May 5, 2023

DOI: 10.3892/etm.2023.12096

Abstract. The present study investigated the expression level of microRNA (miR)-137 in glioma tissues and cell lines and explored its potential diagnostic significance as well as its function effects on glioma cells. miR-137 expression level was detected in glioma tissues using *in situ* hybridization, and in glioma cell lines using reverse transcription-quantitative PCR (RT-qPCR). The diagnostic significance of miR-137 in glioma was assessed using receiver operating characteristic curve analyses. Quantibody® Human Inflammation Array 1 was used to evaluate the impact of ectopic miR-137 expression on release of cytokines in glioma cell lines. IL-13, TNF-α and IFN-γ levels were detected using ELISA. To confirm that sphingosine kinase 2 (SPHK2) is a target of miR-137, RT-qPCR, western blot analysis and dual-luciferase assay were adopted. The results demonstrated that miR-137 expression was downregulated in both glioma tissues and cell lines. Downregulation of miR-137 was significantly associated with high grade gliomas. Additionally, it was found that overexpression of miR-137 reduced IL-13, but promoted TNFα and IFN-γ production. SPHK2 knockdown inhibited IL-13 release, promoted TNF-α and IFN-γ production. SPHK2 was a direct target of miR-137. Collectively, the results of the present study indicated that miR-137 expression plays a tumor-suppressive role in glioma. It is downregulated in glioma and may promote M1-type TAMs polarization, and may be a diagnostic biomarker and potential therapeutic strategy for glioma treatment in the future.

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Key words: microRNA-137, glioma, diagnosis, tumor associated macrophage

Introduction

Glioma is the most common primary brain tumor, among which World Health Organization (WHO) grade IV glioma, such as glioblastoma (GBM), is a highly aggressive and fatal malignant tumor (1). In recent years, numerous efforts have been made to improve the treatment strategy of glioma; however, the prognosis of patients with malignant glioma has not significantly improved (2). Therefore, it is necessary to investigate the genetic and epigenetic mechanisms of gliomas to identify objective diagnostic, classification and prognostic indicators and develop novel treatment strategies (3). Previous studies have preliminarily defined several molecular pathological subtypes of GBM and numerous molecular markers for prognosis (4). However, the pathogenesis and prognosis of gliomas are not yet fully understood. Therefore, malignant glioma has been the focus of tumor therapy research.

MicroRNAs (miRNAs/miRs) are a type of non-coding RNA with a length of 21-24 nucleotides, which are evolutionarily conserved (5). miRNAs mainly regulate the expression of target genes at the post-transcriptional level by binding to the 3'-untranslated region (UTR) complementary site of

target mRNAs (6). Therefore, miRNAs play an indispensable regulatory role in a number of important physiological processes, including embryonic development, cell differentiation, proliferation, apoptosis and metabolism (7). According to the association between miRNAs and the occurrence and development of malignant tumors, they can be divided into two categories: Oncogenic miRNAs (onco-miRNAs) and tumor-suppressive miRNAs (TS-miRNAs) (8). It is known that the abnormal increase in onco-miRNA expression and/or the abnormal decrease in TS-miRNA expression are important factors leading to the occurrence and development of a variety of malignant tumors (9). Previous studies have demonstrated that miR-137 is expressed at low levels in primary gliomas, and glioma cell proliferation and invasion are significantly inhibited following the overexpression of miR-137 (10-14). The tumor inhibitory effects of miR-137 have been confirmed in a variety of tumors, including pancreatic cancer, osteosarcoma, gastric, oral, ovarian, liver and lung cancer (15-21).

The authors have previously reported the association between sphingosine kinase (SPHK) 2 expression and glioma-associated macrophages and the Ki-67 proliferation index (22). It was demonstrated that SPHK2 was overexpressed in glioma and was positively associated with the Ki-67 index and M2-type tumor-associated macrophages (TAMs) (22). TAMs play an important role in the development, progression and invasion of gliomas. Activated macrophages include M1 and M2. M1-type macrophages can protect the organism from viruses and bacterial infection and eliminate tumor cells, while M2-type macrophages, in contrast to M1-type macrophages, exert immunosuppressive effects, which not only promote the growth and invasion of glioma, but also stimulate the formation of tumor-related blood vessels (23). However, the relevance of miR-137 to TAMs in gliomas remains unknown.

In the present study, it was confirmed that the downregulation of miR-137 resulted in the overexpression of SPHK2 in glioma, and that the upregulation of miR-137 may promote M1 TAMs by directly targeting SPHK2. The data presented herein suggested that miR-137 may be a novel diagnostic biomarker and a potential therapeutic target for human malignant gliomas.

Materials and methods

Patient samples. A retrospective study was designed and included 60 patients with glioma (21 female patients, 39 male patients; age, 40.78±11.61 years, 18-64 years), who were admitted to The Shenzhen Second People's Hospital (Shenzhen, China) between January 2014 and December 2017. The following patients were excluded: i) Aged <18 years or ≥65 years; ii) those with other tumors or neurological diseases. After obtaining the written consent from all participants at The Second People's Hospital of Shenzhen (Shenzhen, China), formalin-fixed paraffin-embedded (FFPE) samples were collected from the Department of Pathology after diagnosis and treatment. The tissue was fixed in 10% formalin at room temperature for 12-24 h before embedding with paraffin. FFPE samples sections with a thickness of 5-μm were cut for hematoxylin and eosin (H&E) staining and miR-137 *in situ* hybridization (ISH). The pathological diagnosis was made independently by two neuropathologists according to the WHO classification of central nervous system tumors in 2016 (24). In a previous study, the authors summarized the WHO classification and histopathological subtypes of these gliomas (22). The present study followed the principles of The Declaration of Helsinki and was approved by the Ethics Committee of Shenzhen Second People's Hospital (approval no. XZ2019103101).

ISH. The deparaffinized tissue was partially hybridized with 50 nm LNA-modified and digoxin-labeled miR-137 oligonucleotide probes (Exiqon A/S) for 1 h at 55˚C, incubated with 5 μg/ml anti-digoxin-Rhodamine antibody (cat. no. 11093274910; Roche Applied Science) overnight at 4˚C, and stained with DAPI (cat. no. ZLI-9557; OriGene Technologies, Ins.) in the dark at room temperature for 15 min. The labeling index (LI) was expressed as the percentage of positive cells to the total number of cells.

Cell lines and culture. In the present study, the human GBM cell lines U251, U373, SK-MG3, U-343, A172, LNZ-308, U118 and U138, and normal human astrocytes (HAs) were used. These cell lines were purchased from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences and cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) (25,26). Authentications of U118MG and U373MG cell lines were detected with STR profiling. According to the authentication, U118MG and U373MG cell lines are from American Type Culture Collection. All cell lines were cultured in a humidified incubator at 37˚C in 5% CO_2/95% air.

Lentiviral vector transduction. The 2nd generation system was used for lentivirus transduction. 293T cells (The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences) were used as the interim cell line. Lentivirus packaging was performed by Hanbio Biotechnology Co., Ltd. in 293T cells using pSPAX2 (10 μg), pMD2G (5 μg), shuttle plasmids (10 μg) and Lipofiter (75 μl; cat. no. HB-LF-1000; Hanbio Biotechnology Co., Ltd.) for 16 h. A total of 48 and 72 h after transfection, lentiviruses were collected because more viruses are collected at two time points to ensure enough for the later experiments. Lentiviruses collected 48 and 72 h after transfection were both used in the subsequent experiments. Hblv-cmv-miR-137-GFP-puro, hblv-GFP-puro (control), hblv-cmv-SPHK2-GFP-puro [short hairpin RNA (sh)-SPHK2] and hblv-GFP-puro (NC) vectors (Hanbio Biotechnology Co., Ltd.) were used to transduce U251 and U373 cell lines at a multiplicity of infection of 3 at 37˚C for 24 h. A total of 72 h after transduction, puromycin (3 μg/ml; Gibco; Thermo Fisher Scientific, Inc.) was added to the culture medium of transduced cells to create stable cell lines for 7 days. The overexpression of miR-137 and knockdown of SPHK2 following transduction with lentiviral vector was detected according to the manufacturer's protocol.

Measurement of cytokine secretion. The culture supernatant was collected and the cytokines were analyzed. The quantity of cytokine secretion was quantified using the Quantibody® Human Inflammation Array 1 (RayBiotech, Inc.) to measure the levels of 10 types of human cytokines, including IFN-γ,

IL-1α, IL-1β, IL-10, IL-13, IL-4, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1) and TNFα. IL-13 was examined using an ELISA kit (cat. no. CHE0004; Beijing 4A Biotech Co., Ltd.). TNF-α and IFN-γ were detected with ELISA Kits purchased from PeproTech, Inc. (cat. nos. 900-M25 and 900-M27, respectively).

Luciferase plasmid construction. The candidate targets of miR-137 were predicted using TargetScan (http://www.targetscan.org/). The wild-type (p-WT) and mutant-type (p-MT) reporter vectors of the SPHK2 3'-UTR were constructed using the pEZX-MT01 Luciferase miRNA Expression Reporter Vector (GeneCopoeia, Inc.). The predicted target coding sequence of miR-137 was deleted from SPHK2 3'-UTR cDNA for p-MT construction by site-directed mutagenesis. The sequence and direction of the two vectors were verified with Sanger DNA sequencing by Genscript, Inc.

Transfection with miR-137 mimics and plasmids. dsRNA oligonucleotides of miR-137 mimics (cat. no. miR10000429) and scramble sequence (Scr; cat. no. miR1N0000001-1-5) were purchased from Guangzhou RiboBio Co., Ltd. dsRNA oligonucleotides (2,500 ng; 50 nM) were transfected either alone or with 0.75 μg/ml luciferase plasmids (p-WT and p-MT; GeneCopoeia, Inc.) using X-tremeGENE small interfering RNA (siRNA) Transfection reagent (cat. no. 4476093001; MilliporeSigma) for 48 h at 37˚C. A total of 48 h after transfection, subsequent experimentation was performed.

Dual-luciferase reporter assay. U373 and U251 cells (5,000 cells/well) inoculated into 96-well plates were transfected with 0.15 μg p-WT or p-MT and 0.08 μg miR-137 mimics or scrambled sequences using X-tremeGENE siRNA Transfection reagent for 48 h. Subsequently, the activities of *Renilla* and Firefly luciferase were detected on a Synergy 2 Microplate Reader Fluorometer (BioTek Instruments, Inc.) using the Dual-Luciferase Reporter Assay system (Promega Corporation). The results were normalized to the measured value of Firefly luciferase.

Reverse transcription-quantitative PCR (RT-qPCR). TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) was used to extract total RNA from the two groups of cell lines. miR-137 was quantified using the Bulge-loop miRNA RT-qPCR Detection kit (Guangzhou RiboBio Co., Ltd.). The qPCR thermocycling conditions were as follows: Initial denaturation at 95˚C for 10 min, followed by 40 cycles of denaturation at 95˚C for 2 sec, annealing at 60˚C for 20 sec and extension at 70˚C for 10 sec; final dissociation was performed at 95˚C for 15 sec, 60˚C for 1 min and 95˚C for 15 sec. The expression of SPHK2 mRNA was detected using the Reverse Transcription System and GoTaq qPCR Master Mix kit (Promega Corporation) according to the manufacturer's instructions. U6 and GAPDH were used as internal controls for miR-137 and SPHK2 mRNA, respectively. All reactions were performed on the CFX Connect™ Real-Time PCR Detection system (Bio-Rad Laboratories, Inc.). The expression of miR-137 and SPHK2 were calculated using the $2^{-\Delta\Delta Cq}$ method (27). The miR-137-3P Primer Set (cat. no. MQPS0000619-1-100; Guangzhou RiboBio Co., Ltd.) and the U6 qPCR Primer Set (cat. no. MQPS0000002-1-100; Guangzhou RiboBio Co., Ltd.) were used in these experiments.

Western blot analysis. Western blot analysis was performed as previously described (28). The primary antibodies used were rabbit anti-SPHK2 (1:1,000; cat. no. ab264042; Abcam) and mouse anti-β-actin (1:1,000; cat. no. sc-81178; Santa Cruz Biotechnology, Inc.), used for incubation at 4˚C overnight. The secondary HRP-conjugated antibodies (used for incubation at room temperature for 1 h) were as follows: Anti-mouse IgG (1:2,000; cat. no. 7076) and Anti-rabbit IgG (1:2,000; cat. no. 7074) both purchased from Cell Signaling Technology, Inc.

Statistical analyses. All statistical analysis was performed using SPSS 24.0 software (IBM Corp.). The data are expressed as the mean ± SD. The normality of the distribution was estimated using the Kolmogorov-Smirnov test. The unpaired double-tailed Student's t-test was used to analyze the statistical difference between the two groups. The differences among the sample groups were analyzed by one-way ANOVA followed by multiple comparison Tukey's honestly significant difference post hoc test. In order to determine the optimal cut-off level of miR-137 expression for the diagnosis of gliomas, receiver operating characteristic (ROC) curve analysis was performed. The Youden index (J=sensitivity + specificity-1) was used to determine the optimal cutoff level. The optimal cutoff value was the cutoff value used to obtain the highest Youden index. $P<0.05$ was considered to indicate a statistically significant difference. All cell lines were tested at least three times and the samples were repeated three times.

Results

miR-137 is downregulated in human glioma and is associated with tumor grade. ISH with LNA-modified probes was applied to detect endogenous miR-137 expression in FFPE specimens of 60 gliomas and 11 non-tumorous brain tissues. It was found that the expression of miR-137 in gliomas was decreased compared with non-tumorous brain tissues, and that its expression was significantly decreased as the glioma grade increased; however, there was no significant difference between two high-grade gliomas (WHO III grade and WHO IV grade) (Fig. 1A and B). miR-137 expression was also detected in 8 glioma cell lines and normal HAs. The results revealed that the expression of miR-137 in all glioma cell lines was significantly lower compared with normal HAs (Fig. 1C). The expression curve of miR-137 between non-tumorous brain tissues (control group) and glioma tissues revealed that the area under the ROC curve was 0.7924 [95% Confidence Interval (CI), 0.6425-0.9423; $P<0.05$]. As demonstrated in Fig. 2A and B, the expression of miR-137 may prove helpful for the diagnosis of glioma. The optimal cutoff value of the miR-137 LI was 0.37% (sensitivity, 55%; specificity, 90.91%). The ROC curve of miR-137 between low-grade (WHO II) and high-grade (WHO III and V) gliomas revealed an area under the ROC curve of 0.6998 (95% CI, 0.5584-0.8411; $P<0.05$; Fig. 2C and D), indicating that the expression of miR-137 in gliomas contributes to the differentiation of glioma grades. The optimal cutoff value of the miR-137 LI in the diagnosis of glioma was 0.90% (sensitivity, 60%; specificity, 81.82%).

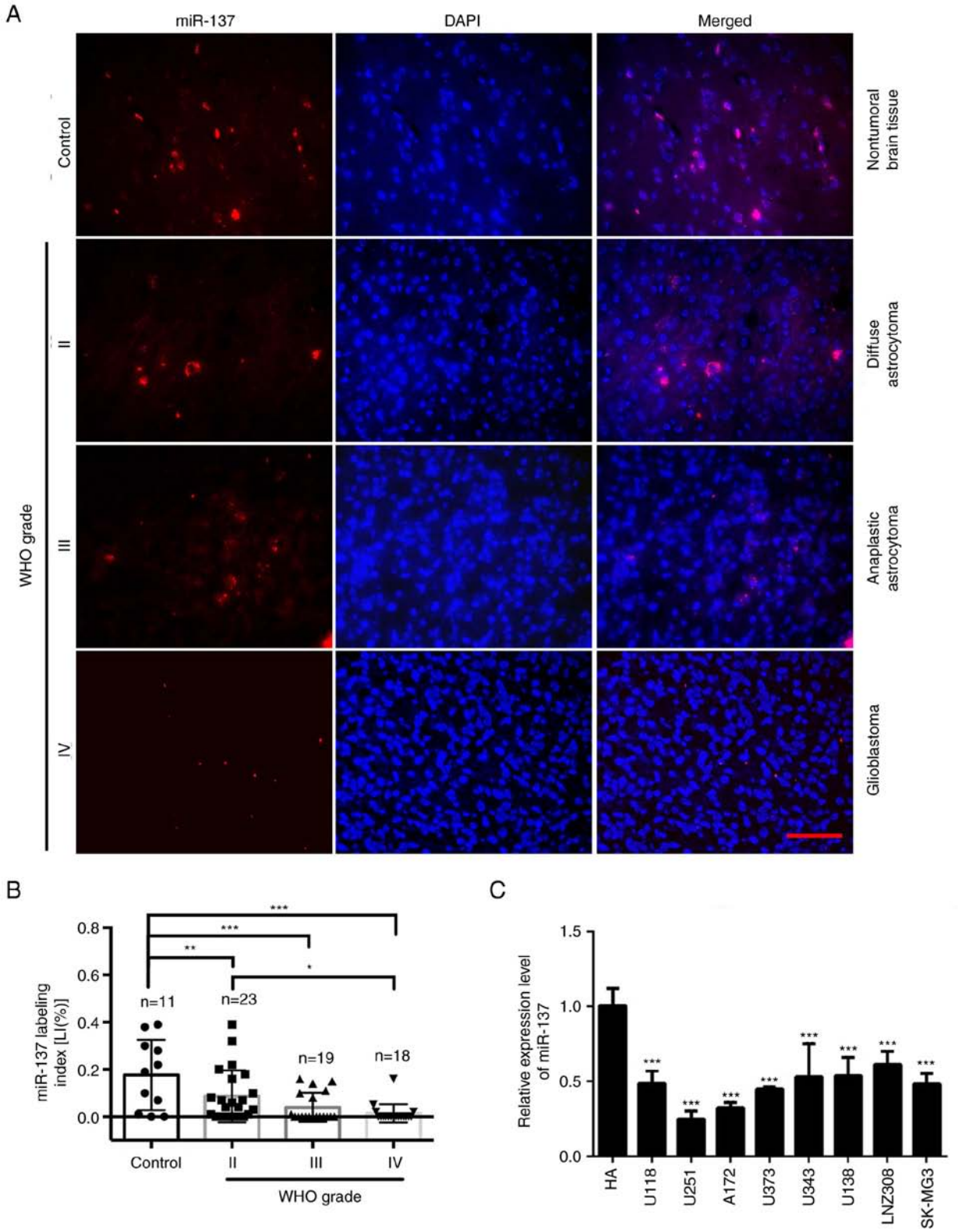

Figure 1. miR-137 expression is decreased in glioma tissues and cell lines. (A) Representative images of miR-137 in situ hybridization detection (scale bar, 50 μm). (B) Comparisons of miR-137 expression level among groups [(LI (%)] in the formalin-fixed paraffin-embedded samples of 60 gliomas and 11 non-tumorous brain tissues (control). The data in (B) are presented as the mean ± SD. *P<0.05, **P<0.01 and ***P<0.001. (C) Reverse transcription-quantitative PCR detection of miR-137 expression in 8 glioma cell lines and 1 normal HA cell line. ***P<0.001 vs. HA. LI, labelling index; miR, microRNA; HA, human astrocyte; WHO, World Health Organization.

These data indicated the inverse association of miR-137 expression with glioma and revealed that miR-137 may be a potential biomarker for the diagnosis of patients with glioma.

miR-137 inhibits the release of IL-13, and promotes the release of TNFα and IFNγ. To examine the effects of miR-137 on TAM-associated release of cytokines,

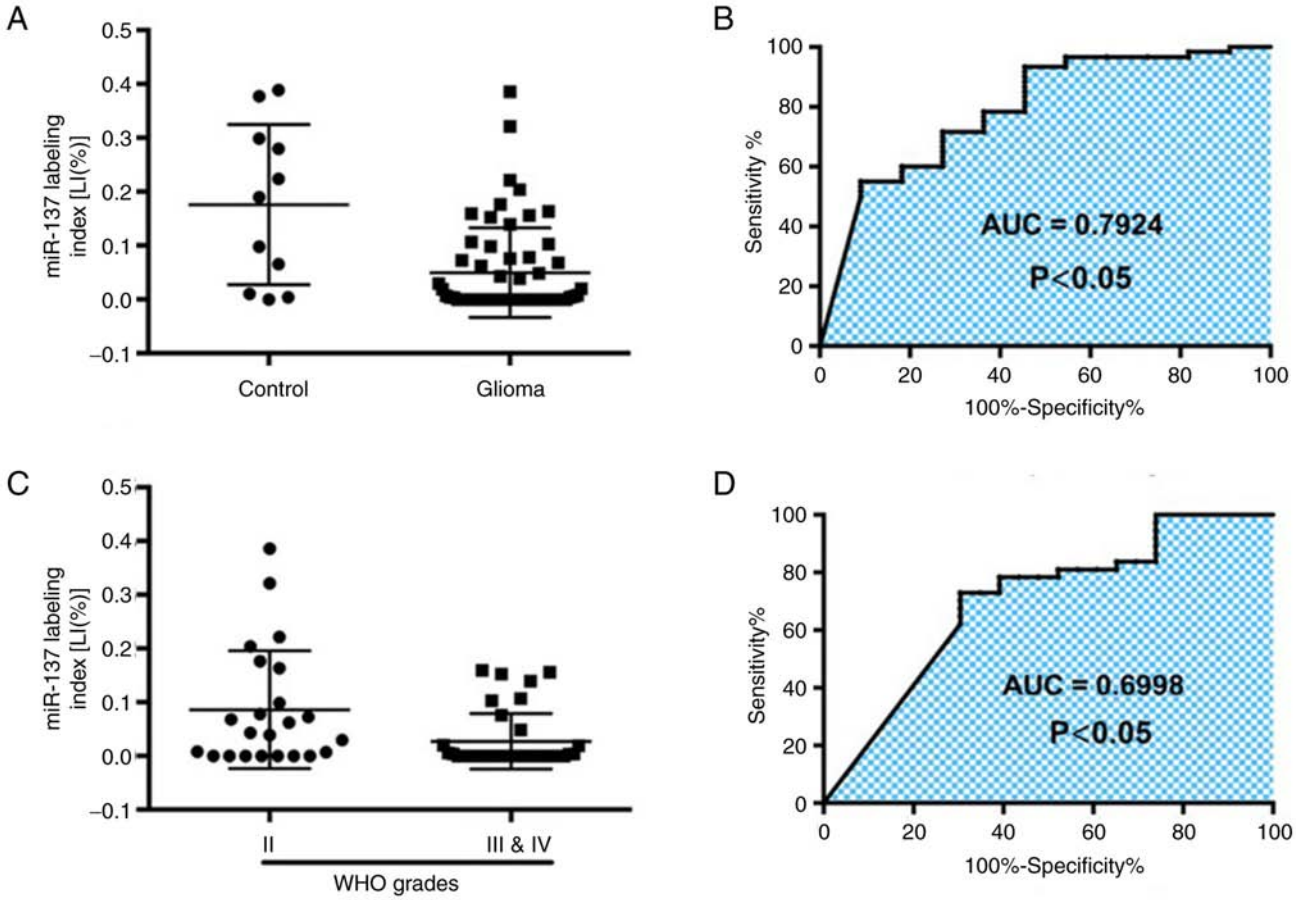

Figure 2. ROC curve was used to determine the specificity and sensitivity of miR-137 for the diagnosis of glioma. (A) Expression of miR-137 between glioma and non-tumorous brain tissues (control). (B) The area under the ROC curve was 0.7924 (95% confidence interval, 0.6425-0.9423; P<0.05). The optimal cutoff value for glioma diagnosis using the miR-137 LI was ≤0.37% (sensitivity, 55%; specificity, 90.91%). (C) The expression of miR-137 between low-grade glioma (WHO II grade) and high-grade gliomas (WHO III vs. WHO IV grade). (D) The area under the ROC curve was 0.6998 (95% confidence interval, 0.5584-0.8411; P<0.05). The optimal cutoff value for glioma diagnosis using the miR-137 LI was ≤0.90% (sensitivity, 60%; specificity, 81.82%). ROC, receiver operating characteristic; miR, microRNA; WHO, World Health Organization.

miR-137-overexpression cells were constructed by transducing miR-137 overexpression lentiviral vector (empty lentiviral vector transduced cells as control). The Quantibody® Human Inflammation Array 1 was used to detect the 10 human cytokines, including IFN-γ, IL-1α, IL-1β, IL-10, IL-13, IL-4, IL-6, IL-8, MCP-1 and TNFα. The results revealed that miR-137 overexpression reduced the IL-13 levels, whereas it promoted TNFα and IFNγ production (Fig. 3A-C). As previously reported, IL-13 can promote M2 polarization, and TNFα and IFNγ can promote M1 polarization (29,30). Hence, it was hypothesized that miR-137 may promote M1 TAM polarization by inhibiting M2-associated cytokine and promoting M1-associated cytokine release. There were no differences in the levels of IL-1α, IL-1β, IL-10, IL-4, IL-6, IL-8 and MCP-1 between NC and miR-137 overexpression (data not shown). To analyze the effect of SPHK2 on TAMs associated release of cytokines, knockdown of SPHK2 was carried out in U251 and U373 cells at mRNA and protein levels (Fig. 3D-F). Two cell lines were transduced with lentivirus containing knockdown shRNA sequence (HK as control group, and SH1, SH2, SH3 as knockdown groups). After confirming SPHK2 knockdown, IL-13, TNFα and IFNγ released by NC and sh-SPHK2 cells were examined using ELISA. It was found that SPHK2 knockdown reduced IL-13 concentration in U373 and U251 cells, promoted TNFα release in U373 cells, and increased IFNγ release both in U373 and U251 cells (Fig. 3G-I).

SPHK2 is a direct target of miR-137 in human glioma cells. Using TargetScan, it was found that SPHK2 was one of the potential targets of miR-137. miR-137 only had one putative binding site in the SPHK2 3'-UTR (Fig. 4A). In order to obtain direct evidence of miR-137 targeting SPHK2, two recombinant luciferase reporter gene vectors of SPHK2 3'-UTR were constructed: p-WT and p-MT. The recombinant luciferase mRNA transcribed by p-WT carried the predicted miR-137 target (SPHK2-3'-UTR-WT) in SPHK2-3'-UTR, while the mRNA transcribed by p-MT lacked the predicted target (SPHK2-3'-UTR-MT) (Fig. 4A). The results of the dual-luciferase reporter assay revealed that the relative luciferase activity significantly decreased following p-WT co-transfection with the miR-137 mimic (the scramble sequence was used in the control group); however, no change was observed following p-MT transfection (P<0.05; Fig. 4D and E). To further verify whether miR-137 directly targeted SPHK2, RT-qPCR and western blot analysis were performed to detect the changes in SPHK2 expression in miR-137-transfected cell lines. As revealed in Fig. 4B and C, SPHK2 protein expression in the

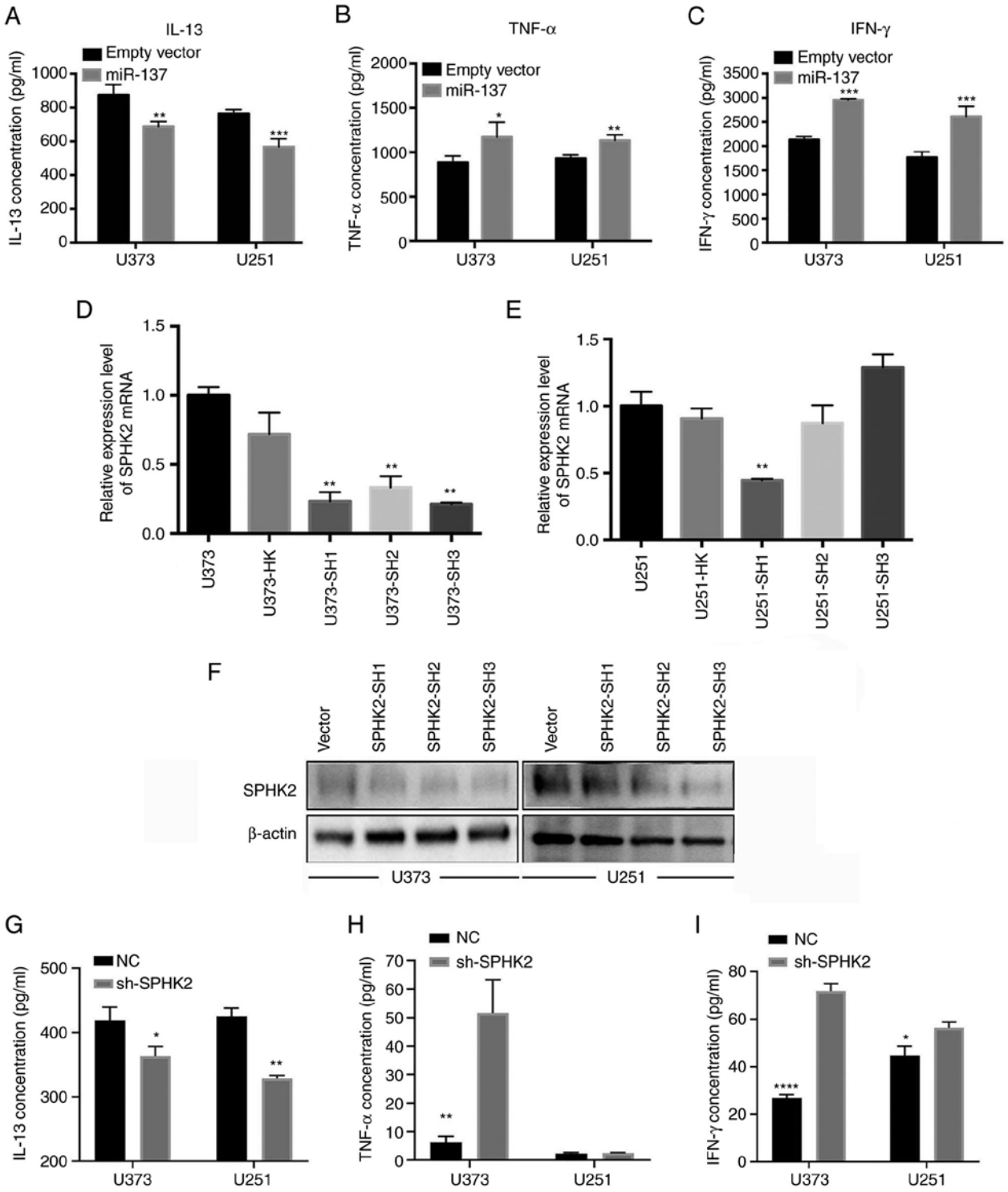

Figure 3. miR-137 inhibits the release of IL-13, and promotes the release of TNFα and IFNγ. HK was used as control group, and SH1, SH2, SH3 as knockdown groups. (A) miR-137 overexpression in glioma cells reduced IL-13 production. (B) miR-137 overexpression in glioma cells promoted TNFα production. (C) miR-137 overexpression in glioma cells promoted IFNγ production.*P<0.05, **P<0.01 and ***P<0.001 vs. empty vector. (D and E) After transduction with lentiviral vectors, SPHK2 mRNA was detected using reverse transcription-quantitative PCR. **P<0.01 vs. U373-HK. (F) SPHK2 protein level was assessed using western blot analysis. (G-I) The supernatant from NC and sh-SPHK2 was collected, and IL-13, TNF-α and IFN-γ were detected using ELISA. *P<0.05, **P<0.01, and ****P<0.0001 vs. NC. miR, microRNA; SPHK2, sphingosine kinase 2; sh-, short hairpin; NC, negative control.

miR-137-transfected cell lines was significantly decreased compared with the scramble control-transfected cell lines (Fig. 4C); however, the SPHK2 mRNA level was not altered (Fig. 4B). These results suggested that miR-137 may directly target SPHK2 in glioma cells and downregulate SPHK2 protein expression by inhibiting its mRNA translation.

Discussion

Over the years, research focusing on the characteristics of glioma cells has made certain progress in elucidating the molecular mechanisms responsible for the occurrence, progress and prognosis of gliomas; however, the treatment efficacy

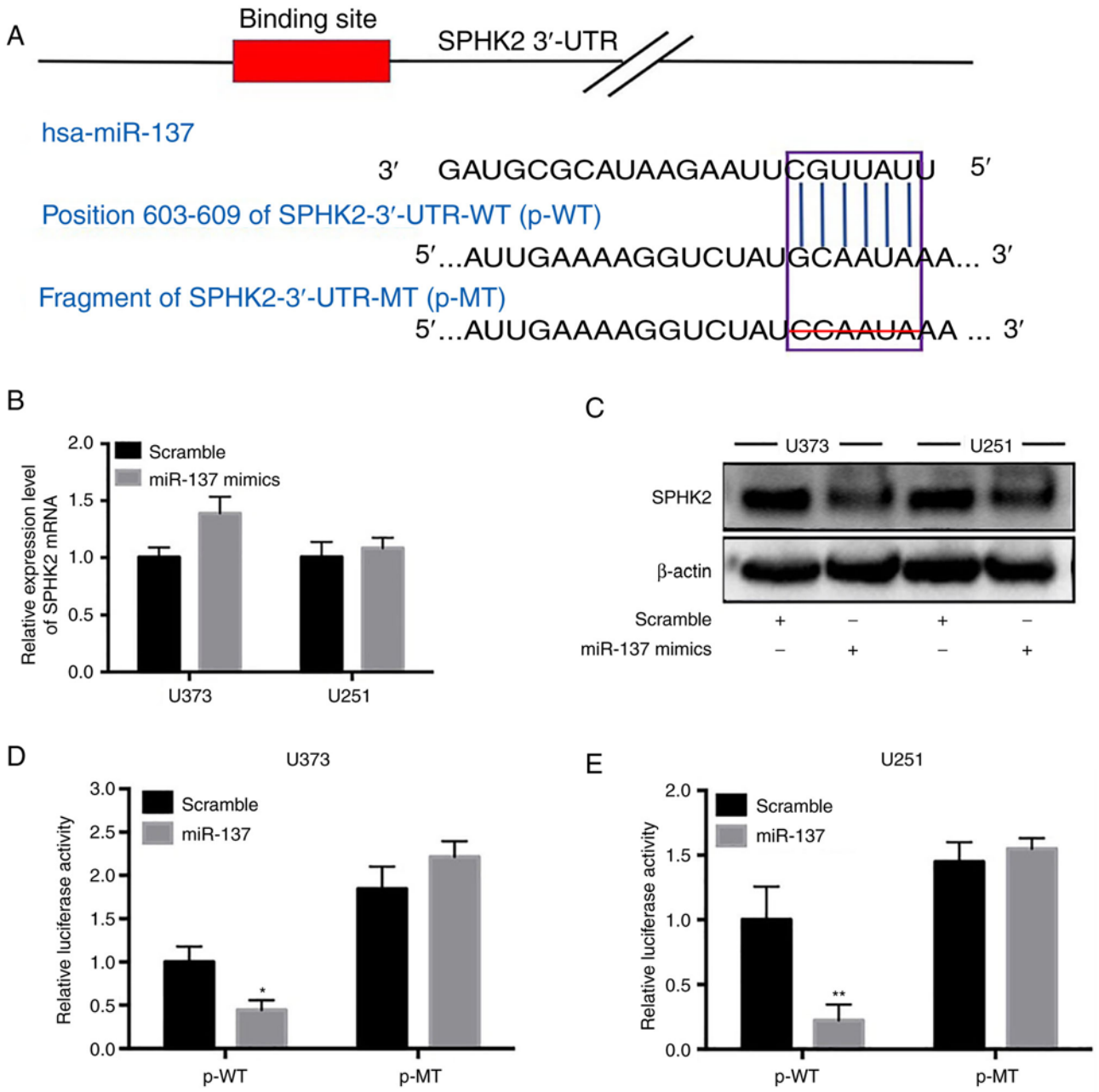

Figure 4. SPHK2 is a direct target of miR-137. (A) miR-137 binding site in SPHK2 3'-UTR was predicted using TargetScan. SPHK2-3'-UTR-WT and SPHK2-3'-UTR-MT carried in recombinant luciferase mRNAs transcribed by p-WT and p-MT. (B) Reverse transcription-quantitative PCR analysis of SPHK2 mRNA expression in the cells as indicated. Their relative expression levels were normalized against GAPDH. The ratios of SPHK2/GAPDH in cells transfected with the scramble sequence were set to 1.0. (C) Western blot analysis of SPHK2 protein expression in the cells as indicated. (D and E) Dual-luciferase reporter assays were performed using (D) U373 and (E) U251 cells co-transfected with p-WT or p-MT and scramble sequence or miR-137 mimics. All experiments were performed at least in triplicate and the data in (B-E) are presented as the mean ± SD. *P<0.05 and **P<0.01 vs. Scramble. SPHK2, sphingosine kinase 2; miR, microRNA; UTR, untranslated region; WT, wild-type; MUT, mutant.

for gliomas has not improved. In recent years, an increasing number of studies have demonstrated that tumor resistance to cancer treatment is not necessarily an internal problem, and the interaction between glioma and the tumor microenvironment will also render the tumor resistant to drugs (31-33). It has been previously reported that during the process of tumor development, a large number of immune cells accumulate around the tumor, most of which are TAMs. TAMs are a 'double-edged sword', which can play two different roles, either promoting inflammation and exerting antitumor effects, or inhibiting inflammation and exerting tumor-promoting effects (34). Therefore, it is of utmost importance to guide the research and development of antitumor drugs by inducing and amplifying the pro-inflammatory and antitumor effects of TAMs.

Ceramide (Cer), sphingosine (Sph) and sphingosine-1-phosphate (S1P), the metabolites of sphingomyelin, play an important role in the development of a number of tumors (35) by regulating cell proliferation, survival and apoptosis. SPHK is the key enzyme which maintains the metabolic balance between Cer, Sph and S1P (36). SPHK consists of two isomers, SPHK1 and SPHK2. SPHK1 has been proven to be a type of oncogenic kinase, which is highly expressed in a number of

types of tumors, and is closely related to the occurrence and development of tumors (35,37,38). SPHK2 is highly expressed in colon, lung, liver and breast cancer, and the inhibition of its expression can effectively suppress the proliferation of tumor cells and promote cell apoptosis (39-42). In papillary thyroid carcinoma (PTC), miR-613 can target SPHK2, thus inhibiting the proliferation, migration and invasion of PTC cells (43). In breast cancer, the stimulation of EGF can promote the phosphorylation and activation of SPHK2, thus increasing the production of S1P and promoting the migration of breast cancer cells (44). In addition, as previously demonstrated, in a mouse xenograft model of breast cancer following SPHK2 knockdown, TAMs presented an antitumor phenotype and expressed pro-inflammatory markers, indicating that SPHK2 was closely related to the phenotype of TAMs (36). In a previous study, it was revealed that SPHK2 protein was highly expressed in gliomas, and it was positively associated with the infiltration of M2-type TAMs and the proliferation index of Ki-67 (23). Therefore, the abnormal increase in SPHK2 expression may be an important reason for TAM infiltration and M2 polarization in glioma, and thus an important factor for the progression of glioma.

An increasing number of studies have demonstrated that miRNA plays an important role in determining the functional state and differentiation performance of TAMs. Numerous miRNAs in macrophages can increase pro-inflammatory signaling (including miR-155 and miR-125a/b) or weaken the function of M2 macrophages (including miR-511-3p, miR-378 and miR-155) to maintain the M1 TAM phenotype, while others (such as miR-146a, miR-187, miR-21 and miR-147) can inhibit the function of positive regulatory factors in the macrophage pro-inflammatory signaling pathway, thus promoting the M2 polarization of TAMs (45-47). Previous studies have demonstrated that miR-137 expression is downregulated in gliomas, and glioma cell proliferation and invasion are significantly inhibited following the overexpression of miR-137 (11,12). The tumor-suppressive effects of miR-137 have been confirmed in a variety of tumors, including pancreatic cancer, osteosarcoma, gastric, oral, ovarian, liver and lung cancer (13-17). The findings of the present study demonstrated that the expression levels of miR-137 were downregulated in glioma cell lines and tissues, which further suggests the antitumor role of miR-137 in glioma. Bioinformatics analysis predicted that SPHK2 was a potential target gene of miR-137 and the experimental results also confirmed that prediction. Moreover, miR-137 inhibited the release of IL-13, and promoted the release of TNFα and IFNγ, and subsequently promoted M1 TAM polarization. Research has indicated that the intra-nuclear SPHK2-S1P axis facilitates the M1-to-M2 shift of the microglia by suppressing histone deacetylase 1-mediated Kruppel-like factor 4 deacetylation (48). The aforementioned study also demonstrated the positive association between SPHK2 and M2-type TAM infiltration. Thus, it was hypothesized that miR-137 may promote M1 TAM polarization by inhibiting M2-associated cytokine release and promoting M1-associated cytokine release by targeting SPHK2.

In conclusion, the present study demonstrated that miR-137 expression was downregulated in glioma and that its overexpression promoted M1-type TAM polarization by targeting SPHK2. miR-137 functions as a tumor suppressor and may thus be used as a diagnostic biomarker and therapeutic target for glioma.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Shenzhen Science and Technology Innovation Commission (grant nos. JCYJ20170306090714854 and JCYJ20200109120205924), and the University of South China Innovation Foundation for Postgraduate (grant no. 203YXC038).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

JL, YX and HT performed the experiments and acquired data. JL and YX confirm the authenticity of all the raw data. YZ and GH contributed to conception, design and revision. XL, YS, TW, MG, PC and HH were involved in acquisition and analysis of data and drafting the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Shenzhen Second People's Hospital. Signed informed consents were obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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