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Original article

MicroRNA-539 inhibits glioma cell proliferation and invasion by targeting DIXDC1

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ABSTRACT

Dysregulation of microRNAs (miRNAs) has been suggested to contribute to malignant progression of glioma. Previous studies have demonstrated that miR-539 is dysregulated in malignant progression of cancers. However, the potential role and mechanism of miR-539 in the progression of glioma remains unclear. In this study, we aimed to investigate the expression status and functional significance of miR-539 in glioma. We found that miR-539 expression was significantly decreased in glioma cell lines and tissues. Overexpression of miR-539 markedly inhibited glioma cell proliferation and invasion, while miR-539 suppression exhibited the opposite effect. Bioinformatics analysis and dual-luciferase reporter assays showed that miR-539 directly targeted the 3′-untranslated region of Disheveled-axin domain containing 1 (DIXDC1). DIXDC1 expression was negatively regualted by miR-539 overexpression. An inverse correlation between DIXDC1 mRNA expression and miR-539 expression was found in glioma specimens. Furthermore, knockdown of DIXC1 significantly inhibited proliferation, invasion and Wnt signaling in glioma cells. Overexpression of DIXDC1 partially reversed the inhibitory effect of miR-539 on glioma cell proliferation and invasion. Overall, these findings demonstrate that miR-539 inhibits glioma cell proliferation and invasion by targeting DIXDC1. Our study suggests that the miR-539 may serve as a potential target for the clinical diagnosis and treatment of glioma.

1. Introduction

Glioma is one of the most common malignant tumors of the central nervous system causing substantial mortality worldwide [1,2]. Despite advances in treatment strategies including stereotactic radiotherapy, immunotherapy and new chemotherapy drugs, the prognosis of glioma has not been significantly improved [3–5]. The excessively proliferative and invasive characteristics of glioma cells contribute to the poor prognosis of glioma patients [6]. However, the underlying mechanisms of glioma malignancy remain largely unknown. Therefore, it is essential to reveal the molecular mechanisms of glioma tumorigenesis and to develop effective treatment methods.

In recent years, microRNAs (miRNAs), a subset of endogenous and small non-coding RNAs, have emerged as critical regulators of gene expression [7]. miRNAs are post-transcriptional regulators that binds to the 3′-untranslated region (UTR) of target mRNAs through base pairing, leading to translation inhibition [7]. An expanding body of evidences has documented that the dysregulation of miRNAs is involved in various physiological and pathological processes, such as tumorigenesis [8,9]. To date, several miRNAs have been found to be related to glioma development and progression, such as miR-637 [10] and miR-182-5p [11]. The dysregulated miRNAs have been suggested as novel biomarkers for diagnosis and prognosis, as well as being potential therapeutic targets for glioma [12–14]. Therefore, a better understanding of miRNAs in glioma tumorigenesis may help to provide novel strategies for diagnosis, prognosis and treatment.

Disheveled-axin (DIX) domain containing 1 (DIXDC1), containing a coiled-coil domain and a DIX domain, has emerged as a novel oncogene in several cancer types [15,16]. DIXDC1 is involved in interactions between Dishevelled, Axin, and β-catenin via the DIX domain, and functions as a positive regulator for the Wingless (Wnt) signaling pathway [17–19]. Several studies have shown that DIXDC1 is implicated in neuronal development [20–23]. In recent

Abbreviations: miRNAs, microRNAs; DIX, disheveled axin; DIXDC1, disheveled-axin domain containing; UTR, untranslated region; FBS, fetal bovine serum; CCK-8, cell counting Kit-8; PI3K, phosphatidyl inositol 3-kinase; Wnt, wingless.

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years, high expression of DIXDC1 has been found in many types of cancers including lung cancer [16], colon cancer [15], gastric cancer [24] and pancreatic cancer [25], and is associated with cell proliferation, migration and invasion. Moreover, DIXDC1 is found to be highly upregulated in glioma cancer tissues [26]. The silencing of DIXDC1 inhibits the proliferation and migration of glioma cells [26]. Therefore, DIXDC1 may serve as a potential therapeutic target for glioma.

miR-539 has been reported as a tumor suppressor in multiple cancers [27,28]. However, the expression and functional significance of miR-539 in glioma remains unclear. In this study, we performed gain- and loss-of-function analysis to investigate the function and molecular mechanism of miR-539 in glioma. Here, we found that miR-539 expression was significantly decreased in glioma cell lines and tissues. Biological experiments showed that miR-539 over expression inhibited the proliferation and invasion of glioma cells, while miR-539 suppression promoted the proliferation and invasion of glioma cells. We predicted that DIXDC1 was the target gene of miR-539 based on bioinformatics analysis and validated their relationship at the cell and tissue levels. Taken together, our study suggests that miR-539 functions as a novel tumor suppressor in glioma. Our results show that miR-539 inhibits glioma cell proliferation and invasion through down regulation of DIXDC1. This study thus provides novel insights into understanding the molecular pathogenesis of the malignancy and shows the potential of miR-539 as a therapeutic target for glioma.

2. Materials and methods

2.1. Patient specimens

Fifteen glioma tissue samples were obtained from glioma patients with surgical resection at the Second Affiliated Hospital of Xi'an Jiaotong University. All patients were first diagnosed with glioma and had received no chemotherapy, radiotherapy, or hormone therapy before tissue collection. Brain tissue samples from internal decompression patients with surgical operation were used as controls. Clinical tissue donation was conducted with patients' informed consent according to the Declaration of Helsinki following an Institutional Human Experiment and Ethics Committee-approved protocol by the Second Affiliated Hospital of Xi'an Jiaotong University.

2.2. Cell culture

Human glioma cell lines including SHG44, A172, U87MG and U251MG and normal human astrocytes were purchased from the Cell Library of the Chinese Academy of Sciences (Shanghai, China). Glioma cells lines were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS), 100 U/ml Penicillin and 0.1 mg/ml Streptomycin (Sigma, St. Louis, MO, USA). Normal human astrocytes were cultured in DMEM containing 15% FBS, 4 mM L-glutamine, 1.5 g/L sodium bicarbonate and 1 g/L glucose. Cells were maintained at 37 °C in a humidified incubator of 95% air/5% CO_2. The fourth passage was used in the this study. Cells were subjected to the experiments when reaching 80% confluence.

2.3. Quantitative real-time PCR

For quantitative miRNA detection, total RNA was harvested using the mirVana miRNA isolation kit (Ambion, Carlsbad, CA, USA) and quantitative real-time PCR was performed using the TaqMan microRNA assays kit (Ambion) according to the manufacturer's protocols. U6 was used as an endogenous control for the normalization of miR-539 [28]. For quantitative mRNA detection, total RNA was harvested using TRIzol (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed into cDNA using M-MLV reverse transcriptase (BioTeke, Beijing, China). Quantitative real-time PCR was performed using the SYBR Green system (Applied Biosystems, Carlsbad, CA, USA). GAPDH was used as the endogenous control for normalization of DIXDC1 [26]. The primer sequences for quantitative real-time PCR were as follows: miR-539 forwards: 5′-ACACTCCAGCTGGGGGAGAAATTATCCTTG-3′ and reverse: 5′-TGGTGTCGTGGAGTCG-3′; U6 forward: 5′-CTCGCTTCGGCAGCACA-3′ and reverse: 5′-AACGCTTCACGAATTTGCGT-3′; DIXDC1 forward: 5′-TGCATGTTATGGAGACGCAGAAG-3′ and reverse: 5′-AGGTGCTGCTGACAGTTGGAGA-3′; GAPDH forward: 5′-TGGCAAAGTGGAGATTGTTG-3′ and reverse: 5′-CTTCTGGGTGGCAGTGATG-3′. For analysis of miR-539 expression in glioma specimens, gene expression was obtained by absolute quantification and value of miR-539 expression was normalized against U6 expression. Otherwise, relative gene expression was determined using the $2^{-\Delta\Delta Ct}$ method.

2.4. Cell transfection

The miR-539 mimics, miR-539 inhibitor, negative control (NC)-miRNA, DIXDC1 siRNA (sense, 5′-r(AUGCCUUGCAGCAGAGAU)dTdT-3′ and antisense, 5′-r(AUCUCUGCUGCAAGGCAU)dCdC-3′ and NC-siRNA were purchased from Shanghai GenePharma Co., Ltd (Shanghai, China). DIXDC1 cDNA without 3′-UTR was cloned into the pcDNA3.0 plasmid. Transfections were performed using Lipofectamine 2000 reagent (Invitrogen) as per the manufacturer's protocols.

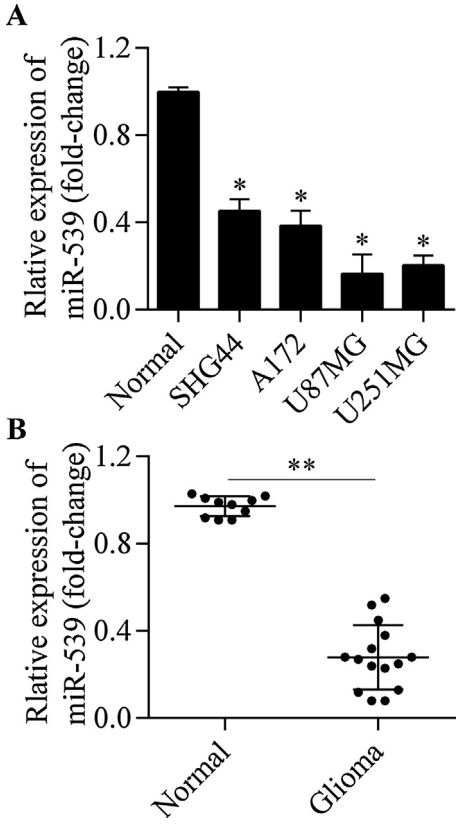

Fig. 1. Expression of miR-539 in glioma. (A) Relative expression of miR-539 in SHG44, A172, U87MG, U251MG and normal human astrocytes (Normal) was detected by quantitative real-time PCR. *$p < 0.05$ vs. normal. (B) Quantitative real-time PCR analysis of miR-539 expression in glioma tissues and normal brain tissues. **$p < 0.01$.