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microRNA-106a induces the proliferation and apoptosis of glioma cells through regulating JNK/MAPK pathway

X.-H. CHEN, X.-M. LING, S. SHI

Department of Gynecology, Henan Provincial People's Hospital, Zhengzhou, [illegible]na

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Abstract. – OBJECTIVE: To investigate the expression of microRNA-106a (miRNA-106a) in the brain tissue and the plasma of glioma patients, and explore the mechanism underlying the effect of miRNA-106a on the proliferation and apoptosis of glioma cells.

PATIENTS AND METHODS: Brain tissues from 42 glioma patients admitted in our institution were included in study group, whereas normal brain tissues collected from 10 patients undergoing brain tissue resection due to decompression or exposure during cerebral surgery. Quantitative fluorescent RT-PCR (QF RT-PCR) was performed to measure miRNA-106a mRNA [illegible] in the brain tissue and peripheral bloo[illegible] tients in two groups. Human M059K glio[illegible]ells were transfected with miRNA-106a mimic a[illegible]hibitor, and the proliferation and apoptos[illegible] glioma cells were analyzed. In additi[illegible] JNK/MAPK in glioma cells was [illegible]ed at m[illegible]NA and protein levels.

RESULTS: Compared wi[illegible]ne nor[illegible] population, miRNA-106a expre[illegible] was s[illegible]ificantly increased in the brain tis[illegible] gl[illegible] ($p < 0.05$). Besides, [illegible]NA-1[illegible]ession level was significantly [illegible]ated in t[illegible]sma of peripheral blood of [illegible]a patients ([illegible]05). After the interferen[illegible]A-106a in M[illegible] glioma cells, the pro[illegible]ration [illegible]ioma cells was significantly re[illegible]ced. Howeve[illegible] apoptotic regulatory fac[illegible] Bcl-2, was sig[illegible]ntly increased, and J[illegible]MAPK protein leve[illegible]as significantly decr[illegible]sed. Ov[illegible]expression of miRNA-106a in gli[illegible]ells [illegible]ulted in significant increase in the p[illegible]n of gli[illegible]a cells and JNK/MAPK protein [illegible] but ob[illegible]s suppression in Bcl-2 [illegible] in lev[illegible]

[illegible]NCLUSI[illegible] [illegible]levated expression of miRNA[illegible]a plays a[illegible]rucial role in the development an[illegible]ogression of glioma, probably by promoti[illegible]ration and suppressing the apop[illegible]sis of g[illegible]ma cells through the JNK/MAPK sig[illegible]g pathway.

Key [illegible]ords:
Glioma, microRNA-106a, Proliferation, JNK, MAPK.

[illegible]troduc[illegible]

Glioma [illegible]t common p[illegible]ary intracranial tumo[illegible] that a[illegible] from neuroectoderm, and thereby is also know[illegible]euroectodermal tumors or [illegible]pithelial tum[illegible] which accounts for [illegible]% of primary intracranial tumors[1,2]. However, [illegible] key signaling [illegible]etworks regulating glioma cell p[illegible]iferation re[illegible] poorly defined[3]. To date, the e[illegible]y of gliom[illegible]emains elusive and is likely to be [illegible] asso[illegible]ed with multiple factors, including tu[illegible], infection, genetic factors, envi[illegible]nmental factors and N-nitroso compounds[3]. [illegible] infiltrate adjacent normal tissues and [illegible]ve[illegible] satellite lesions, which become the origin of tumor recurrence. Conventional treatment for gliomas includes surgery, chemotherapy and radiotherapy, which result in poor outcomes. Along with the advance in the research on the cellular immunology as well as the molecular etiology and pathology of gliomas, novel therapeutic strategies such as immunological therapy and genetic therapy has emerged[4]. MicroRNAs (miRNAs) are a class of 22 nucleotide (nt)-long, non-coding, single-stranded RNAs. A large body of evidence has shown that aberrant miRNA expression is critical for the development and progression of a variety of tumors[5-7]. miRNA-106a is a newly discovered miRNA that has been shown to be upregulated in patients with gastric cancer and the inhibition of its expression can suppress the proliferation, migration and invasiveness of gastric cancer cells[8]. In addition, miRNA-106a has been reported to be highly expressed in colorectal cancer cells[9], indicating that miRNA-106a, as an important tumor-promoting factor, involves in the migration of colorectal cancer cells. However, to date, few studies have been reported on miRNA-106a expression in glioma patients and its potential function and underlying mechanism in gliomas. In the present work, miRNA-106a ex-

Corresponding Author: Xiaohui Chen, MD; e-mail: chenxh1895@163.com

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pression in the brain tissues and serum of glioma patients were compared and further study was conducted on whether it regulates the proliferation and apoptosis of glioma cells through JNK/MAPK signaling pathway.

Patients and Methods

Patients

During May 2011 to May 2014, surgically resected specimens were harvested from 52 patients treated at the Department of Neurosurgery of our institution. Among these patients, 42 glioma patients were included in study group, which consisted of 22 males and 20 females with the age ranging from 27 to 68 years. According to WHO classification criteria, these gliomas were classified as 25 astrocytomas (12 fiber type, 8 protoplasmic, 5 anaplastic) and 17 glioblastomas. Control group comprised of pathologically confirmed normal brain tissues, which were collected from 10 patients undergoing brain tissue resection due to decompression or exposure during cerebral surgery. Among these patients, 4 were males and 6 we[illegible]males with the age ranging from 31 to 65 y[illegible].

Reagents

TRIzol RNA isolation kit was purchased f[illegible] Life Technologies (Carlsbad, CA, [illegible]A). Taka[illegible] [illegible]verse transcription kit was [illegible] from Y[illegible] Guangzhou-Dimensional [illegible]otech I[illegible]uments (Guangzhou, Guangdon[illegible] [illegible]R. Chin[illegible] PCR kit and DNA Marker wer[illegible] pur[illegible] [illegible] Biotechnology (Na[illegible]g, Jia[illegible] P.R. China). miRNA-106a mi[illegible] and inhibito[illegible] [illegible]ell as the primers for int[illegible]rence U6 w[illegible]esigned and synthesiz[illegible] by G[illegible]zhou RioBio Co. Ltd. (Guangzho[illegible] Guangdong, [illegible]a).

RNA [illegible]lation and Quant[illegible]tive Flu[illegible]scent [illegible]T-PCR (qf RT-PCR)

A [illegible] of [illegible] mL of f[illegible]sting peripheral venous blood sa[illegible] was coll[illegible]ed from each glioma pa[illegible] or no[illegible] sub[illegible]ct in the morning. Blood sa[illegible]es were [illegible]oagulated with EDTA fol-low[illegible] by centri[illegible]gation at 4000 g for 5 min at 4°[illegible] [illegible] plasma at upper layer was collected [illegible] st[illegible] -80°C.

A specimen of 150 mg brain tissue or 200 μL p[illegible] was homogenized in 1 mL Trizol reagent on [illegible]. Total RNA isolated from these samples was dissolved in 20 μL DEPC treated water, and reverse transcription was conducted using Femantes reverse transcription kit (MA, USA). The resulting cDNA products were stored at -20°C. PCR reaction conditions were as follows: denaturation at 95°C for 20s, followed by 40 cycles o[illegible] 20s and 70°C for 1s. The primers f[illegible] miR-106[illegible] were as follows: F: 5'-CGCA[illegible]AGTGCT-TACAGTGCA-3′ and R: 5'-GT[illegible]GGGTCC-GAGGT-3'. The primers for interna[illegible]nce U6 were F: 5′-CTCGCTTCGG[illegible]GCAC[illegible] R: 5′-AACGCT TCACGA[illegible]TGCGT-3′. [illegible] PCR was performed o[illegible] [illegible]I 7900 Real-Time [illegible] system and relative q[illegible]tive [illegible]alysis was performed using $2^{-\Delta Ct}$ metho[illegible].

Cell Transf[illegible]n

Transf[illegible] [illegible] human gl[illegible]a cell line M059K w[illegible]s per[illegible]d using Lipofectamine 2000 purchased from [illegible]trogen (Carlsbad, CA, US[illegible] [illegible]nterfere the [illegible]ssion of miRNA-1[illegible]a, and the interference efficiency was exam[illegible]d after 48h.

C[illegible] Counting [illegible]t-8 (CCK-8) Assay

T[illegible]cted h[illegible]an glioma M059K cells were seeded [illegible]oplate and incubated with CCK-8 [illegible]lution purchased from Wuhan Boster Bioengi[illegible] Inc. (Wuhan, Hubei, P.R. China) at a ra[illegible]0 (V/V) in serum-free Dulbecco's Modified Eagle Mediun (DMEM). Optical absorbance at 450nm was determined at 24, 48 and 72h after incubation, respectively; and cell growth curve was generated.

Western Blot Assay

Transfected human glioma cells were lysed in 1×SDS. Cell proteins were collected, separated by SDS-PAGE and transferred to PVDF membrane at 120V for 100 mins. The membrane was blocked with 5% non-fat milk at 37°C for 80 mins followed by incubation with rabbit anti-human-JNK, -MAPK and –Bcl-2 antibodies (1:1000, Dakewe Biotech Company, Shanghai, P.R.China) at 4°C overnight and incubation with HRP-conjugated mouse anti-rabbit secondary antibodies (1;1000 dilution, Sunshine Biotechnology, Nanjing, Jiangsu, P.R. China) at 37°C for 30 min. The result was examined using ECL luminescence detection. Anti-human β-actin (1:3000 dilution, Cell Signaling Technology, Danvers, MA, USA) was used as internal reference.

Statistical Analysis

Statistical analysis was performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL,

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Table I. Expression of microRNA-106a mRNA in brain tissues.

Group	Number of cases (n)	microRNA-106a mRNA	t/p
Normal brain tissues	42	1.12 ± 0.41	[illegible]
Glioma brain tissues	10	3.48 ± 0.63*	[illegible]4.66/0.000

*vs. normal brain tissues, $t = 14.66$, $p < 0.05$.

USA). Differences between two groups were analyzed using Student's t-test, and $p < 0.05$ was considered as statistically significant.

Results

Expression of miRNA-106a mRNA in Two Groups

Compared to normal brain tissue, miRNA-106a mRNA expression was significantly increased in brain tissues of glioma patients ($t = 14.66$, $p < 0.05$), indicating that elevated miRNA-106a expression in glioma patients was closely associated with the development an[illegible]gression of glioma (Table I).

miRNA-106a mRNA Expression in the Plasma

Further analysis of miRN[illegible]NA expression in the plasma o[illegible]ioma p[illegible]ents revealed that miRNA-106[illegible]ession [illegible]s significantly higher in the [illegible]lasm[illegible] gl[illegible] than in that of normal [illegible]pulation ($t = 11.81$, [illegible] 0.05). This result fu[illegible] pro[illegible] that miRNA-106a might be cl[illegible]ely a[illegible]d with th[illegible]evelopment of glio[illegible]

Effect of [illegible]ression of [illegible]RNA-106a on the [illegible]olifera[illegible] and Apoptosis of Glioma Cells

T[illegible]ression of m[illegible]-106a mRNA was s[illegible]ificantly decreased in M059K glioma cells [illegible]nsfected with [illegible]iRNA-106a inhibitor (Figure [illegible] indicating [illegible] interference of miRNA-106a e[illegible]sion was a[illegible]eved. In addition, the proliferatio[illegible] the in[illegible]ered glioma cells was significantly [illegible] ($p < 0.05$) (Figure 1B). However, [illegible]he apoptotic regulatory factor, Bcl-2, was signifi[illegible]reased (Figure 1C) and JNK/MAPK pro[illegible] was significantly decreased (Figure 1D).

Effect of Elevated miRNA-106a Expression on the Proliferation and Apoptosis of Glioma Cells

miRNA-106a mRNA level was significantly increased in M059K cells transfected with miR-

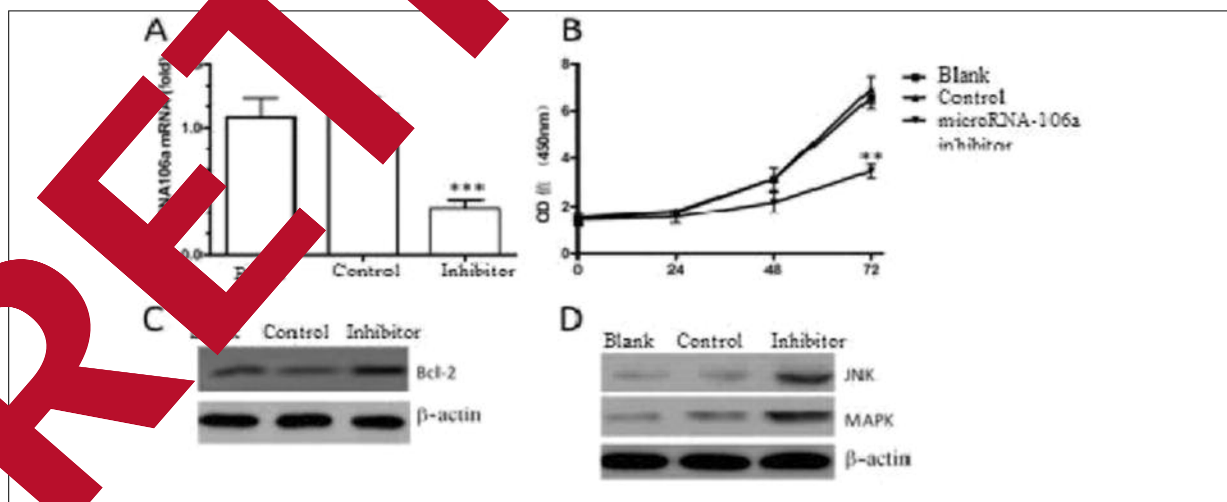

Figure 1. Effect of low expression of miRNA-106a on the proliferation and apoptosis of glioma cells as well as on JNK/MAPK pathway (**vs. control group, $p < 0.01$; ***vs. normal group, $p < 0.001$).

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Table II. Expression of microRNA-106a mRNA in the plasma.

Group	Number of cases (n)	microRNA-106a mRNA	t/p
Normal brain tissue	42	1.31 ± 0.39	
Glioma brain tissues	10	3.14 ± 0.62*	[illegible].81/0.000

*vs. normal brain tissues, $t = 11.81$, $p < 0.05$.

NA-106a mimic (Figure 2A), and miRNA-106a overexpression resulted in significantly increased proliferation of glioma cells (Figure 2B). However, Bcl-2 protein level was significantly decreased (Figure 2C) and JNK/MAPK protein level were significantly decreased ($p < 0.05$) (Figure 2D). These results suggested that miRNA-106a promoted the proliferation but suppressed the apoptosis of glioma cells, which might be closely associated with JNK/MAPK signaling pathway.

Discussion

Glioma is a common malignant tumor [illegible] nated from neuroectoderm. The diagno[illegible] treatment of the disease remains a big ch[illegible]ge in clinical practice. Gliomas comprise of se[illegible]l subtypes, including astrocytoma (inclu[illegible] glioblastoma multiforme), olig[illegible]droglion[illegible] medulloblastoma and colloid [illegible]nting fo[illegible] 35-45% of total inciden[illegible]f intra[illegible]nial tumors[11]. The onset of mo[illegible]mas is [illegible]w, which usually lasts for seve[illegible] weeks [illegible] months, [illegible] even for several year[illegible]me [illegible]s. The clinical manifestation of [illegible]oma [illegible]ied into [illegible] major categories. [illegible] categor[illegible]mpt[illegible]s resulted from incr[illegible] intracranial [illegible], which includes hea[illegible]miting and ps[illegible]iatric symptoms. The [illegible]ther o[illegible]ocal symptoms caused by tumor suppression, [illegible]ion and brain tissue da[illegible]lioma is diffi[illegible] be completely re[illegible]ed surgically due to its invasive nature. To [illegible] end, increasing attention has been drawn to t[illegible] research on [illegible] cellular and molecular mech[illegible] underlyi[illegible] pathogenesis of glioma, in an eff[illegible] disco[illegible] a more rational and effective treatm[illegible]ular or molecular level.

microRNAs, as a group of small RNAs regu[illegible]e expression at transcriptional level, are [illegible]istributed in the tissue, the plasma and the serum of human body. microRNAs have multiple functions. Besides, aberrant expression of microRNAs in many cancer patients have been reported in recent years and these molecules are believed to play a crucial role in the initiation

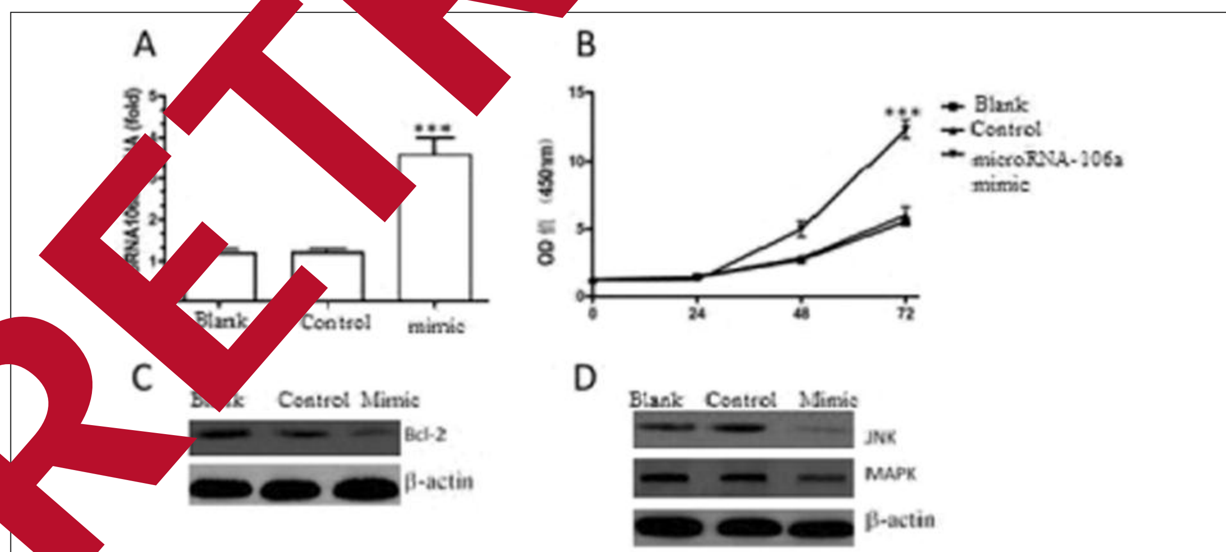

Figure 2. Effect of high expression of microRNA-106a on the proliferation and apoptosis of glioma cells as well as on JNK/MAPK pathway (***vs. normal group, $p < 0.001$).

and development of tumors. Currently, miRNA has been shown to have both carcinogenic and tumor suppressive function[12-16]. Numerous studies have focused on the relationship between miRNA-106a and tumors, and aberrant expression of miRNA-106a has been mainly reported in patients with gastric cancer and colorectal cancer[8,9]. However, few studies are done on miRNA-106a expression in glioma patients.

In the present work, miRNA-106a expression in the brain tissues and the plasma of glioma patients was investigated with an effort to explore the function and the implication of miRNA-106a in glioma patients. The results showed that miRNA-106a expression was significantly increased in the brain tissues and the plasmas of glioma patients than in those of normal population, indicating that aberrant expression of miRNA-106a in glioma patients might be closely associated with the initiation and development of glioma. Zhu et al[17] have reported that miRNA-106a expression is up-regulated in gastric cancer tissue compared to normal tissue, suggesting that miRNA-106a as a potential target for the diagnosis of gastric cancer, which supports the results of the pr[illegible] study.

In addition, miRNA-106a express[illegible] in glioma cells was interfered with an effort [illegible]vestigate the effect of miRNA-106a on the pr[illegible]eration and apoptosis of glioma [illegible] The resu[illegible] demonstrated that miRNA-[illegible] [illegible]oted th[illegible] proliferation of glioma ce[illegible] Beside[illegible] miRNA-106a was involved in the [illegible]lation o[illegible]mor cell apoptosis by suppressing B[illegible]

Previous studies [illegible]ve sug[illegible] that JNK/MAPK signaling [illegible]hway influe[illegible] biological activities of ce[illegible] as prolifera[illegible], differentiation, co[illegible]rsion [illegible] apoptosis) by affecting gene t[illegible]nscription a[illegible]gulation in animal cells. T[illegible]refore, furthe[illegible]lysis was performe[illegible] observe the relevan[illegible] mechanisms by whi[illegible] miRN[illegible]06a affects the proliferation and [illegible] of glio[illegible]a cells. To this end, JNK/M[illegible] expre[illegible]on was examined in [illegible]ma ce[illegible] an[illegible]cted with miRNA-106a m[illegible]/inhibi[illegible]e results showed that JNK/MA[illegible]K expression was decreased in glioma ce[illegible] low expression of miRNA-106a but i[illegible] the cells with high expression of [illegible]RNA-106a. These results suggested that [illegible]A-106a promoted the proliferation of gli[illegible]a cells through stimulating JNK/MAPK pathway and suppressed the apoptosis of cancer cells via Bcl-2.

Conclusions

The up-regulation of miRNA-106a in glioma patients may play an important role in [illegible] the development and progression of [illegible]oma, a[illegible] miRNA-106a is involved in the p[illegible]eration and apoptosis of glioma cells mainly [illegible]NK/MAPK pathway. Hence, detection of mi[illegible]106a in glioma patients may have a [illegible]tential [illegible] the diagnosis of glioma.

Conflict of Inter[illegible]

The Authors decla[illegible]at there are [illegible]flicts [illegible]terest.

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