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Original article

MicroRNA-132 cause apoptosis of glioma cells through blockade of the SREBP-1c metabolic pathway related to SIRT1

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ABSTRACT

Background: The inhibition role of miRNA (microRNA or miR) on cancer signaling pathways has been used to prospective cancer treatment. SIRT1 might promote tumorigenesis in human glioma.
Methods: Here, we investigated whether miR-132 regulate the expression of SIRT1 and its downstream SREBP (Sterol regulatory element-binding protein)-lipogenesis-cholesterogenesis metabolic pathway in human glioma cells. Furthermore, we studied the effect on biology function of glioma cell induced by miR-132.
Results: MiR-132 inhibited SIRT1 and SREBP-1c expression and downregulated their targeted genes, including HMGCR and FASN. MiR-132 suppressed the cell growth, tumorigenicity, the invasion of glioma cells and migration as well as promoted their apoptosis. The pathways associated with cancer progression and tumorigenicity, and induce glioma cell apoptosis has been inhibited by miR-132 involving in a caspase-dependent apoptotic mechanism.
Conclusions: The recovery of miR-132 resulted in caspase-dependent apoptotic death in glioma cells. MiR-132 that was newly discovered represents a newly targeting mechanism in treatment for glioma.

1. Introduction

Glioma accounts for almost 80% of primary malignant brain tumors, leading to its survival less than any other tumors. With a median survival time of slightly over a year, the survival rate of glioma is very low. There has been few therapeutic advances over time for this highly fatal cancer [1]. It is extremely challenging for identifying the mechanism(s) of gliomagenesis [1–3].

The transcription or translation of mRNA is suppressed by the short 18–24 nt RNA of microRNAs [4–8]. MiRNA regulation of gene expression plays a key part in differentiation, proliferation and apoptosis [9–14]. In many kinds of cancers, expressions of miRNAs are abnormal and may play a critical role in tumorigenesis [15–22]. It has been identified that several miRNAs may act as tumor suppressor genes [23–28]. MicroRNA-132 (miR-132), an extremely stabile miRNA, is induced by BDNF in a CREB-dependent manner [29]. It has also been shown that miR-132 possibly regulated cellular excitability *via* regulation of ion channels in cultured cells [30]. The increase of neuronal activity and contextual fear conditioning attributed miR-132 to be rapidly transcribed was demonstrated by Lambert et al. in the hippocampus *in vivo* [31]. MiR132 is exposed to light still induce transcription in the suprachiasmatic nucleus *in vivo* [30]. MiR-132 could regulate the expression of SIRT1.

Under oxidative and genotoxic stress, SIRT1 called an NAD-dependent deacetylase will regulate apoptosis [32,33]. SIRT1 may function as an oncogene and is closely linked to tumorigenesis from recent data [34–37]. The transcription factor which controls the expression of genes, including triglyceride and fatty acid synthesis, is SREBP (Sterol regulatory element binding protein)-1c

Abbreviations: HMGCR, 3-hydroxy-3-methylglutaryl CoA reductase; FASN, fatty acid synthase; BDNF, brain derived neurotrophic factor; CREB, cAMP-response element binding protein.

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in high lipid synthesis rates of tissues such as liver and adipose tissue. *In vivo*, activation of SREBP-1c was inhibited by SIRT1 through deacetylation on Lys-289 and Lys-309, then lowering its stability and related to lipogenic target genes [38]. Comparing with normal brain tissues, we could observe over-expression of SREBP-1 in human glioma specimens [38]. A new therapy for glioma will be found by targeting the aberrant SREBP–lipogenesis–cholesterogenesis pathway.

Through deacetylating molecular targets including SREBP-1c, SIRT1 may regulate apoptotic thresholds. MiR-132 might regulate cellular apoptosis through a SIRT1-SREBP-1c pathway, implicating energy metabolism. Our study detailing the association between miR-132 and SIRT1-SREBP-1c will lay the groundwork for further study on the possible applications in clinical treatment for glioma.

2. Materials and methods

2.1. Culture cells

U251 and U87 cells were purchased from American type culture collection (ATCC), and then grown in DMEM medium with 10% FBS in humidified incubator containing 5% CO_2 at 37 °C.

2.2. Reagents

The mirVanaTM kit for isolation and analysis on miRNA isolation, miRNA precursors and inhibitors came from Life Technologies. The luciferase reporter gene detection kit of 3′UTR AKT (S900042) were purchased from SwitchGear. Cell Proliferation Assay kit was obtained from Promega (Madison, WI). The siRNA of AKT (sc-38910) and control siRNA (sc-37077) was purchased from Santa Cruz Company. Human miR-520a (KIT 001168, Applied Biosystems) precursors, anti-miR-520a (GS574467, QIAGEN) and NC (negative control; SI03650325) were used for trial. The miRNA inhibitors or precursors were transfected with Lipofectamine 2000 based on manufacturer's protocol.

2.3. MiRNA transfection

In the light of the manufacturer's protocol, Lipofectamine 2000 was to performed Transient transfection of miRNA inhibitors or precursors. Human miR-132 (PM12827) precursors, a negative control (miR-NC; AM17110) and anti-miR-132 (MH12827) were conducted for experiments.

2.4. Quantitative RT-PCR

Total RNA of cells was extracted with Trizol method and then was reverse transcript. Fast Real-Time PCR System (ABI 7500) was used to perform RT-PCR assays. GAPDH and 18S rRNA was used to normalize the data. The expression of intrinsic miR-520a was detected with qRT-PCR. RNU6B was used to normalize the data.

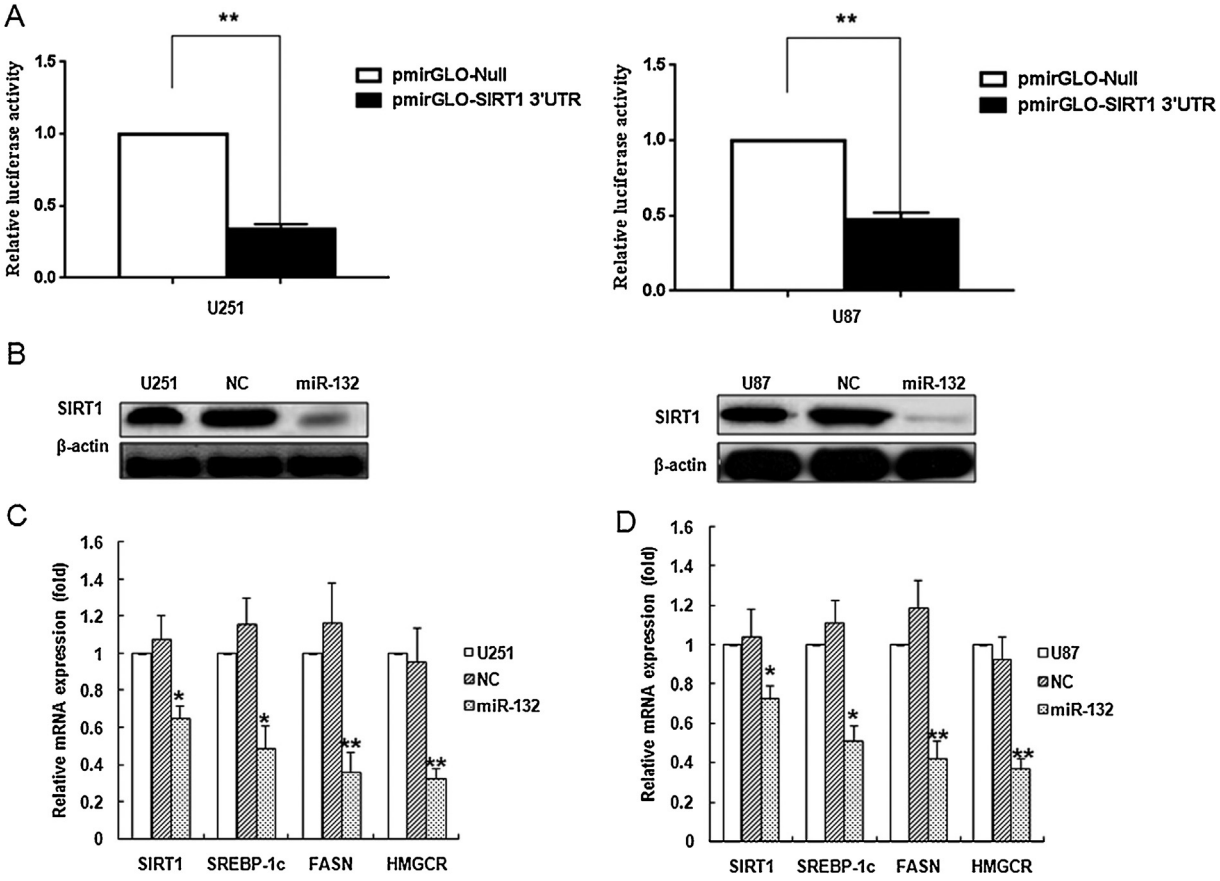

Fig. 1. MiR-132 inhibits expression of SIRT1, SREBP-1c, HMGCR and FASN in human glioma cell lines U251 and U87. To further explore if miRNAs modulate the SIRT1 and its downstream metabolic pathway SREBP–lipogenesis–cholesterogenesis in human glioma cell lines, at first DIANA microT v4.0 online software was used to predict if one or more miRNAs target SIRT1. The miRNA, miR-132, was considered to possibility target the 3′ UTRs of SIRT1 mRNAs. To identify whether miR-132 directly binds to 3′ UTRs of SIRT1, we performed a 3′ UTR luciferase reporter assay of SIRT1 in miR-132 transfected glioma cells (A). To value if the miRNAs modulate the metabolic pathway SREBP–lipogenesis–cholesterogenesis in human glioma cell lines U251 (C) and U87 (D), the Western blotting assay (B) and qRT-PCR quantification analyses was used. The data are presented as means ± SD from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$.