Gene 658 (2018) 63–69

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

MicroRNA-128 inhibits proliferation and invasion of glioma cells by targeting COX-2

Yihai Lin, Zhangyi Wu*

Department of Neurosurgery, Zhejiang Tongde Hospital, Hangzhou, Zhejiang 310012, PR China

ARTICLE INFO

Keywords:
Glioma
miR-128
Proliferation
Invasion
COX-2

ABSTRACT

MicroRNAs (miRNA), a class of small noncoding RNAs, regulates message RNA (mRNA) by targeting the 3′-untranslated region (3′-UTR) resulting in suppression of gene expression. In this study, we identified the expression and function of miR-128, which was found to be downregulated in glioma tissues and glioma cells by real time PCR. Overexpression of miR-128 mimics into LN229 and U251 cells could inhibit proliferation and invasion of glioma cells. However, the inhibitory effects of miR-128 mimics on the invasion and proliferation of glioma cells were reversed by overexpression of cyclooxygenase-2 (COX-2). Our data showed that COX-2 was a candidate target of miR-128. Luciferase activity of 3′-UTR of COX-2 was reduced in the presence of miR-128. Additionally, miR-128 obviously decreased COX-2 mRNA stability determined by real time PCR. Contrarily, we found that miR-128 inhibitor significantly increased the COX-2 mRNA expression, and elevated the protein expression of MMP9 and ki67, and promoted the proliferation of glioma cells. Furthermore, luciferase activity of the 3′-UTR was upregulated by miR-128 inhibitor. All of these results supported that miR-128 was a direct regulator of COX-2. Further studies proved that COX-2 was elevated in glioma tissues and its expression was negatively correlated with the levels of miR-128. These findings may establish miR-128 as a new potential target for the treatment of patients with gliomas.

1. Introduction

Malignant gliomas, one of the most common tumor of the central nervous system, accounts for about 40–50% of the human adult brain tumors, are the most frequent and aggressive central nervous system tumors (Meyer, 2008; Jemal et al., 2009). The gliomas have been classified into grades I to IV by the World Health Organization (WHO) according to increasing levels of malignancy (Louis et al., 2007). Glioblastoma multiforme (GBM, WHO grade IV) has been the most common and serious malignant glioma and about 10,000 new cases of glioma are diagnosed each year (Jansen et al., 2010). Although the availability of multiple strategies such as novel surgical treatments and effective radiation or chemotherapy for the treatment of GBM in recent decades, the survival rate for five years of the patients still remains poor (Mittal et al., 2015). The metastasis of malignant glioma cells has been considered as the main obstacle for surgical resection and radiotherapy (Furnari et al., 2007). The following factors including unlimited proliferation, fast diffuse infiltration, and obvious apoptosis resistance afford the invasiveness characterization of glioma (Furnari et al., 2007; Zhuang et al., 2009). Thus, the molecular mechanism of glioma tumorigenesis and progression needs further studies and will develop novel treatments for gliomas.

MicroRNAs (miRNAs) are small endogenous noncoding RNAs, target the 3′-untranslated region (3′-UTR) of message RNA (mRNA) to repress translation or accelerate the degradation of the mRNA in regulating gene expression (Bartel, 2004; Bushati and Cohen, 2007). Functional studies have demonstrated the involvement of miRNAs in various biological processes, including cellular development, differentiation and proliferation (Bushati and Cohen, 2007; Krol et al., 2010). Several cancers exhibit imbalance of miRNA expression resulting in disease progression, including glioma (Singh et al., 2012). MiRNAs often serve as oncogenes or tumor suppressors by modulating the level of critical proteins (Esquela-Kerscher and Slack, 2006). As a tumor suppressor, miRNAs have been reported to regulate aberrant cyclooxygenase (COX) expression in several cancers (Strillacci et al., 2009; Chen et al., 2012; He et al., 2012; Pham et al., 2013). Till now, about 1527 human miRNAs are found, but only a few of them have been studied (Chen et al., 2012). It has been reported that miR-128 is an antioncogene which is expressed at low levels in cancers including gliomas (Lin et al., 2013; Hu et al., 2014). MiR-128 suppresses a lot of target

Abbreviations: miRNA, MicroRNAs; 3′-UTR, 3′-untranslated region; mRNA, message RNA; WHO, World Health Organization; GBM, Glioblastoma multiforme; COX-2, cyclooxygenase-2; PGE2, prostaglandin E2; cDNA, complementary DNA; RT-qPCR, real time quantitative PCR detecting system; S.D., standard deviation; NC, negative control

* Corresponding author.
E-mail address: zhangyiwu004@163.com (Z. Wu).

https://doi.org/10.1016/j.gene.2018.03.020
Received 14 December 2017; Received in revised form 10 February 2018; Accepted 6 March 2018
Available online 07 March 2018
0378-1119/

genes associated with apoptosis and proliferation of tumor cells (Adlakha and Saini, 2011; Shi et al., 2012). Nevertheless, the role of miR-128 in human glioma development remains unknown and needs to be further elucidated.

COX exists as two isoforms, a constitutive Cox-1 and an inducible COX-2, and is critical in prostaglandin E2 (PGE2) synthesis (Rouzer and Marnett, 2009). COX-2 and PGE2 mediates acute inflammation and in excess, promotes growth and survival of cancer (Greenhough et al., 2009; Nakanishi and Rosenberg, 2013). Restoration of miRNA expression has been shown to abrogate COX-2 expression in tumors (Moore et al., 2012).

In this study, we identified that miR-128 was lowly expressed in glioma tissues. Moreover, miR-128 is involved in proliferation and invasion of gliomas. COX-2 has been studied in carcinogenesis and its mRNA 3′UTR provides binding sites for miR-128. Our data established COX-2 as the direct target of miR-128. The novel findings in this study indicated that abnormal expression of miR-128 is closely associated with invasion and proliferation of human glioma cells. Thus, miR-128 might be a promising target against gliomas.

2. Material and method

2.1. Clinical samples

19 clinical glioma specimens were obtained from Zhejiang Hospital (Hangzhou, Zhejiang, China) from January 2014 to December 2016, and 11 specimens were from male and 8 specimens were from female patients. Specimens were taken immediately after surgical resection and stored at −80 °C for further analysis. The detail clinicopathological parameters of patients were listed in Table 1, and the correlation of miR-128 expression and the patient parameters was analyzed by performing the Pearson χ^2 test. The study was approved by the Ethics Committee of our hospital. Informed and written consent were obtained from all patients or their advisors conforming to the ethics committee guidelines.

2.2. Cell culture

The U87, LN229, SNB19, U251 and LN308 glioblastoma cells and low grade glioma cell H4 in this study were obtained from the cell bank of the Shanghai Biology Institute, Chinese Academy of Science. These cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, CA, USA). The mediums contained 10% fetal bovine serum (FBS, Gibco) and 1% antibiotic (penicillin/streptomycin, Gibco). All the cells were incubated in 5% CO2 at 37 °C.

Table 1
The correlation between miR-128 expression and clinicopathological characteristics of 19 patients with gliomas.

Clinicopathologic feature	n (%)	Low expression (miR-128)	High expression (miR-128)	P-value
Age				P = 0.3215
≤40	8 (42.11%)	5	3	
≥40	11 (57.89%)	6	5	
Gender				P = 0.2634
Female	10 (52.63%)	6	4	
Male	9 (47.37%)	4	5	
WHO grade (%)				P < 0.0001
II	6 (31.58%)	3	2	
III	8 (42.11%)	6	2	
IV	5 (26.31%)	4	1	
Necrosis				P = 0.6829
Absence	9 (47.37%)	4	5	
Presence	10 (52.63%)	5	5	
Tumor size				P = 0.5542
< 4 cm	7 (36.84%)	3	4	
≥4 cm	12 (63.16%)	6	6	

2.3. RNA isolation, complementary DNA (cDNA) synthesis, and real time quantitative PCR detecting system (RT-qPCR)

Total RNA was extracted from glioma tissues or glioma cells using Trizol reagent (Invitrogen, CA, USA) according to manufacturer's instructions, and this experiment was conducted as previously described (Sun et al., 2017). 2 μg of RNAs was used for cDNA synthesis using M-MLV reverse transcriptase (TAKARA, Dalian, China). Real time quantitative PCR was performed for the detection of COX-2 gene expression. The forward and reverse primers corresponding to human COX-2 were as follows: COX-2, sense, 5′-GCT TTA TGC TGA AGC CCT ATG A-3′, antisense, 5′-TCC AAC TCT GCA GAC ATT TCC-3′; GAPDH, sense, 5′-GCA GTG GCA AAG TGG AGA TT-3′, antisense, 5′-TGA AGT CGC AGG AGA CAA CC-3′. The real time PCR analysis was performed by Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, USA) using the SYBR1 Green PCR kits (Takara). The relative mRNA expression levels were analyzed using the $2^{-\Delta\Delta Ct}$ method as described previously (Rajeevan et al., 2001).

Next, to analyze miR-128 expression levels, RNAs were extracted from tissues. The stem-loop RT-PCR assay was employed to determine the miRNAs expression as described previously (Chen et al., 2005; Wang, 2009). The primers were used as below: reverse transcription primers of miR-128: 5′-CTC AAC TGG TGT CGT GGA GTC GGC AAT TCA GTT GAG AAA AGA GA-3′; PCR primers for miR-128: sense, 5′-ACA CTC CAG CTG GGT CAC AGT GAA CCG GTC-3′, antisense, 5′-TGG TGT CGT GGA GTC G-3′; reverse transcription primers for U6: 5′-TGG TGT CGT GGA GTC G. PCR primers for U6: Sense, 5′-CTC GCT TCG GCA GCA CA-3′, antisense, 5′-AAC GCT TCA CGA ATT TGC GT-3′. MiR-128 and U6 PCR primers were the same as described previously (Shi et al., 2012). RNA input was normalized to the levels of human U6 snRNAs. The real time PCR analysis was performed by Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, USA) using the SYBR1 Green PCR kits (Takara). The expression levels of miR-128 were normalized with reference to expression levels of U6 snRNA, and fold changes were calculated by relative quantification ($2^{-\Delta\Delta Ct}$) (Rajeevan et al., 2001).

2.4. Cell transfection

The U251 or LN229 cells (about 40% Confluence) were planted in plates about 16 h before transfection. Both has-miR-128 mimics and inhibitor were synthesized by Sangon Biotech (Shanghai, China), and the sequence of mature miR-128 was listed following, 5′-UCA CAG UGA ACC GGU CUC UUU U-3′. The miR-128 inhibitor was listed, 5′-UUU UGU GUC CGG UUC ACU GUG A-3′. Meanwhile, the plasmids for overexpressing COX-2 (NCBI Reference Sequence of COX-2: NM_000963.3) were cloned into pcDNA3.1 (+) by Sangon Biotech and the pcDNA3.1 vector was purchased from Invitrogen (Califorlia, USA). They were transfected into U251 or LN229 cells with lipofectamine 2000 (Invitrogen, CA, USA). The final concentration of miRNAs was 60 nM and the COX-2 expression vector was 0.5 μg/mL. The transfected cells were incubated for 4 to 6 h, and normal medium was added. The cells were harvested for further analysis after 48 h.

2.5. Proliferation assay

Cell proliferation was determined by Cell Counting Kit-8 (Keygentec, Nanjing, China) assay, as described previously (Mishra et al., 2015; Wang et al., 2016; Sun et al., 2017). The glioma cells were planted in 96-well plates about 2×10^3 cells per well cultured for 16 h. After transfection with miR-128 mimics for 24 h, the CCK-8 solution was added to each well another 2 h. The absorption at OD450 nm was measured by scanning with a microplate reader (Bio Rad, Hercules, CA, USA). Each experiment was repeated three times.