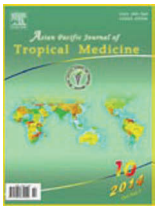
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

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



Document heading doi: 10.1016/S1995-7645(14)60135-8

MicroRNA-184 promotes proliferation ability of glioma cells by regulating FOXO3

Qing-Ke Cui[1,2], Wei-Dong Liu[2], Jian-Xin Zhu[2], Yun-Hua Wang[2], Zhi-Gang Wang[1*]

[1]*Department of Neurosurgery, The Qilu Hospital of Shandong University, Jinan, Shandong, 250012, P. R. China*

[2]*Liaocheng People's Hospital, Liaocheng, Shandong, 252000, P. R. China*

ARTICLE INFO

Article history:
Received 10 July 2014
Received in revised form 15 August 2014
Accepted 15 September 2014
Available online 20 October 2014

Keywords:
miR-184
Glioma
Cell proliferation
FOXO3

ABSTRACT

Objective: To investigate the effect of microRNA (miR-184) on regulating the genesis, development and proliferation of glioma cells. **Methods:** Lipidosome was used to transfect miR-184 mimic and inhibitor to glioma cell line, and the cell proliferation ability changes were determined by MTT and plate cloning experiment after the transfection. WB test was used to measure the levels of cyclinD1, p27 and FOXO3. Meanwhile, QPCR was used to detect miR-184 expression in glioma cell line, glioma tissues and adjacent tissues. Luciferase experiment was used to test *3'UTR* gene targeting regulation of miR-184 and FOXO3. **Results:** QPCR results showed a significant lower miR-184 expression level in glioma cell line and glioma tissues than that in juxtacancerous tissue. MTT and plate cloning experiments have shown that after over-expressing of miR-184, the cell proliferation capacity of glioma U87 and T98G was significantly increased, which was significantly inhibited after the inhibition of miR-184. WB results showed a lower expression level of p27 in U87 and T98G cells, and a higher expression level of cyclinD1 after over-expressing of miR-184 was observed. However, a lower expression level of cyclinD1 and a higher expression level of p27 after the inhibition of miR-184. The luciferase activity was inhibited after the over-expressing of miR-184. **Conclusions:** MiR-184 can affect the proliferation abilities of glioma cells and regulate the cell cycle related protein. It plays an important role in the occurrence and development of gliomas.

1. Introduction

Glioma is the most common and aggressive primary intracranial tumor and the prognosis of the disease is not ideal. Even if the surgery combined with radiation and chemotherapy, the average survival period can be only 14 months[1], and the median survival of malignant brain glioblastoma is less than 1 year[2]. Thus, the research of brain glioblastoma has become a hotspot and difficulty. As more and more attention has been paid to the biological target therapy, the current research is more focused on tumor molecular targeted therapy, but the current molecular treatment mechanism is unclear. MiRNA, as biomarkers of cancer, has tissue sensitivity. It plays a role in promoting the growth of oncogenes or inhibiting the growth of potential tumor suppressor genes, and affects the organism development, differentiation, proliferation, apoptosis, immune regulation and other physiological activities as well as the precise control of the occurrence of malignant tumor and many other diseases[3-10]. The miRNA expression changes can be found early, so this characteristics can be used for the early detection of cancer[11]. For example, studies have found that the different expression ratio of miR-221 to miR-375 can be used for differentiating tumor and normal tissue. Its sensitivity and speciality rate is 92% and 93%, respectively, which may be a diagnosis methods[12]. Other studies have found that microRNA-184 (miR-184) in liver cancer, gastric cancer, phosphorus tongue

*Corresponding author: Zhi-Gang Wang, Chief Physician, PhD, Department of Neurosurgery, The Qilu Hospital of Shandong University, Jinan Shandong, 250012, P. R. China.
Tel: 18660139199
E-mail: wangzgt1962@163.com
Fundation project: It is supported by National Science Foundation (Grant No. 81372721).

cancer and nerve cells involves in the regulation of cell proliferation, cell apoptosis, and cell migration and other important functions. What's more, it has close correlation with the prognosis[13-17]. However, there is no research on miR-184 in glioma reported, and the molecular mechanism is not clear. Our test showed a high expression level of miR-184 in glioma cell line and glioma tumor tissue samples, and it affected the proliferation ability of glioma cells, which suggests that miR-184 may be associated with the development of glioma, and affect the proliferation, invasion and metastasis ability of tumor. Therefore, this article mainly studied the higher expression of miR-184 can inhibit glioma cell proliferation and affect proliferation function of FOXO3 by regulating 3′UTR.

2. Materials and methods

2.1. Cells and plasmid

Glioma cells U87, A-172, LN18, T98G and LN229, and normal control cells NHA and 293 FT were bought from Cell Bank of Chinese Academy of Sciences. *FOXO3* gene and 3′UTR plasmid were kept in Guangzhou Lang, Biological Technology Co., LTD. Tissue samples were collected from Qilu Hospital of Shandong University.

2.2. Main reagents

Fetal bovine serum, dulbecco's modified eagle medium (DMEM) and cell culture plate were from Gibco®. CyclinD1, p21cip1, p27kip1, FOXO3 primary antibody and goat anti-rabbit IgG (second antibody) were bought from Abcam Company. There were other reagents such as, Trizol (Invitrogen, Carlsbad, California), iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA) and SsoFast EvaGreen Supermix (Bio-Rad Laboratories, Hercules, CA).

2.3. Cell proliferation function experiment

2.3.1. Detection of cell proliferation ability by MTT method

Logarithmic growth cells were digested, and cell suspension was prepared and counted. Cells were inoculated in 96-well plates in the incubator and cell culture plate test was performed on Day 1, 2, 3, 4, 5, 6 by adding 20 μL of MTT (5 mg/mL) in each well. Cultured plate were packed by aluminum foil in moist environment at 37 ℃ for 4 h. And the culture medium and MTT (soft operation to avoid the loss of crystal) were abandoned and 150 μL DMSO was added to dissolve the residual of MTT-formazan crystallization. Then the absorbance value at 490 nm was determined via ELIASA and cell growth curve was recorded.

2.3.2. Tablet clone formation experiment

Logarithmic growth phase cells were collected, cell suspension concentration was adjusted to 500 cells/mL on 6-well plate with 2 mL per hole, namely, 1 000 cells/hole. After 7-10 d cultivation, the date of the appearance was observed and the growth condition of colony formed. And a colony with more than 50 cells made a clone and cell fixation started. 1×PBS was used to wash three times, plus 1 mL fixed liquid into each hole (fixed by methanol) and placed the plate on shaking bed for 10 min. Then 1 mL hematoxylin was added to each hole to dye and was put the plate on the shaker for 10 min. The hematoxylin was poured out and rinsed clean by tap water. Last, the number of clones in each hole was counted and photographed.

2.4. QPCR detection of miR-184 expression in cell and tissue samples

One mL Trizol was taken to digest cells, total RNA was extracted, and reverse transcripted into cDNA. miR-184 expression was detected, with housekeeping genes U6 as internal reference.

2.5. WB test for cell cycle of U87, T98G and the expression of protein involved in FOXO3 after cell infection

Logarithmic growth phase cells were taken into splitting decomposition, the protein concentration was tested by BCA protein kit. X film was used to test the protein expression levels of cyclinD1, p27 and FOXO3 after loading protein samples, transmembraning, sealing and incubating antibody.

2.6. Luciferase activity detection

Cell samples were collected and separately absorbed 20 μL supernatant. 100 μL luciferase detection reagent was added and the light values of luciferase was measured after mixing uniformity.

3. Results

3.1. miR-184 expression

With the normal NHA cells as control, QPCR detection results showed that the expression of miR-184 in glioma cells and glioma tissue samples were higher than that in NHA cells (Figure 1).

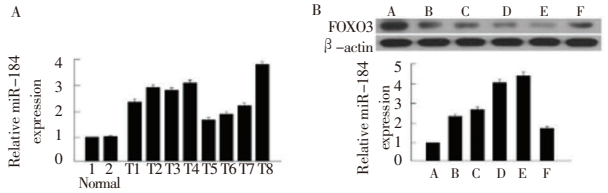


Figure 1. QPCR detection results of miR-184 expression in glioma cells and glioma tissue samples.
A: NHA; B: T98G; C: U87; D: A-172; E: LN-299; F: LN18.

3.2. miR-184 impact on U87, T98G cell proliferation ability

The growth curve of the detection of cell proliferation ability through MTT showed that the mimic group transfected miR-184 cells had stronger proliferation ability than miR-184 inhibitor group. Results showed that miR-184 could promote the proliferation of glioma cells (Figure 2A and Figure 3A).

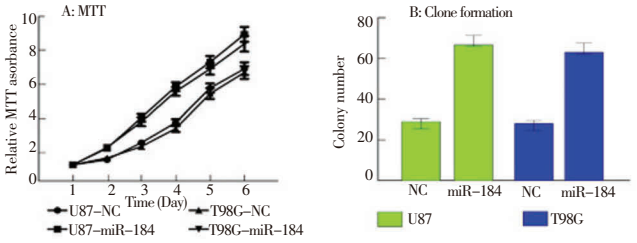


Figure 2. Promotion of clone formation by over-expressing miR-184 in U87 and T98G.

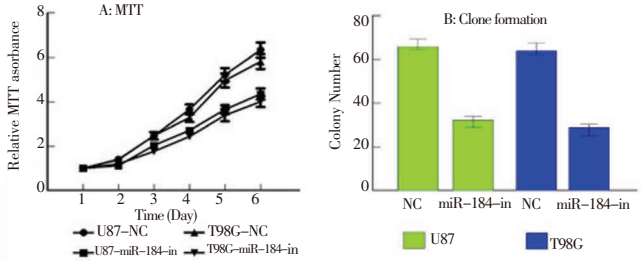


Figure 3. inhibition of clone formation by over-expressing miR-184 in U87 and T98G.

Plate detection of ability to promote clone formation by over-expressing miR-184 in U87 and T98G showed that miR-184 inhibitor group had a significant fewer colonies compared with NC control cells. (P<0.05) (Figure 2B and Figure 3B).

WB method showed that after the over-expressing of miR-184, D1 (CyclinD1) of U87 and T98G was significantly increased, while the cell cycle inhibitory protein p27 and FOXO3 expression was significantly lowered, which was opposite to the results after the inhibition of miR-184 (Figure 4B).

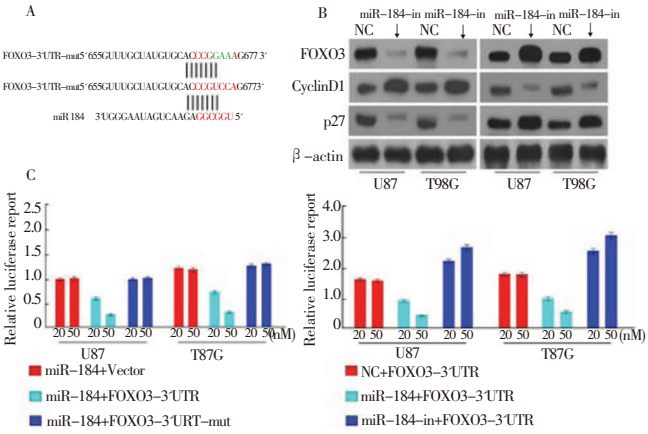


Figure 4. miR-184 targeted control on FOXO3 and detection of protein involved in cell proliferation.

miR-184 targeted combination with FOXO3 3′UTR (Figure 4A) was observed in TargetScan and Pictar and miRANDA software analysis, and a significant decreasing luciferase activity was found after the over-expression through luciferase activity test, while there was a significant increase in inhibitor group (Figure 4C).

4. Discussion

Cell proliferation is a complex process, the main factors of this process is the growth factors in the environment around the cells and the intracellular signaling transduction inside the cells. Cell is a unit controlled by comprehensive network and affected by various of cellular functions. And the cells could proliferate or even transform viciously when an exception occurs during the control of the signal transduction[18]. Now it is very clear that PI3K/AKT signal plays a very important role in cell proliferation. FOXO (including FOXO1, FOXO3a and FOXO4) is the main target protein of AKT kinase, which shuttles between the inside and outside of nucleus and plays an important role in cell proliferation, apoptosis, differentiation and resisting oxidative stress in the body[19]. FOXO1/3 a can be transferred to the cytoplasm for ubiquitin after AKT phosphorylation, then degraded through the proteasome pathway, resulting in the decrease of its protein levels and transcriptional activity[20,21]. Activated AKT can phosphorylate its downstream transcription factors, which leads to FOXO proteins move out of nucleus and transcription activity loss. FOXO transcription molecules can directly increase p21cip1 and p27kip1 transcription and decrease CyclinD1 transcription which can inhibit the cell proliferation[22-24].

MiRNAs is the small molecules widely distributed in the body. It plays an important role in multiple genes and a variety of biological signaling pathways, and it widely exists in eukaryotic organisms with high conservation, scheduling and tissue specificity. The birth of the miRNA research suggests that it has a profound significance and value in the regulation of life activities, including the regulation of gene expression, the growth and development of the body and all kinds of behaviors. In recent years, a lot of literatures reported the abnormal expression of miRNAs has close correlation with tumor, some miRNAs play the role of promoting cancer gene, while others played the role of tumor suppressor genes[25,26]. QPCR results showed a significant lower miR-184 expression level in glioma cell line and glioma tissues than in adjacent tissues; MTT and plate cloning experiments have shown that after the over-expressing of miR-184, the cell proliferation capacity of glioma U87 and T98G was significantly increased. WB results showed a lower expression level of p27 in U87 and T98G, and a higher expression level of cyclinD1 after the over-expressing of miR-184. A higher expression level of p27 in U87 and T98G, and a lower expression level of

cyclinD1 after the inhibition of miR-184. The luciferase activity was inhibited after the over-expressing of miR-184. According to the results, we can make a hypothesis that miR-184 raises the proliferation of U87 and T98G in glioma cells through targeted adjustment of FOXO3 3′UTR.

Above all, raising miR-184 promote the ability of the glioma cell proliferation means targeted regulating FOXO3, which can affect the related proteins of the cell cycle to influence the ability of cell proliferation. The result showed that miR-184 might played a role of a cancer gene in tongue cancer. miR-184 is expected to become the new novel target of glioma occurrence and development, and prognosis biomarkers and treatment.

Conflict of interest statement

We declare that we have no conflict of interest.

Reference

[1] Baranwal S, Alahari SK. miRNA control of tumor cell invasion and metastasis. *Int J Cancer* 2010; **126**(6): 1283-1290.

[2] Liu X, Jiang L, Wang A, Yu J, Shi F, Zhou X. MicroRNA-138 suppresses invasion and promotes apoptosis in head and neck squamous cell carcinoma cell lines. *Cancer Lett* 2009; **286**(2): 217-222.

[3] Xu Y, Ma H, Yu H, Liu Z, Wang LE, Tan D, et al. The miR-184 binding-site rs8126 T>C polymorphism in *TNFAIP2* is associated with risk of gastric cancer. *PLoS One* 2013; doi: 10.1371/journal.pone.0064973.

[4] Foley NH, Bray IM, Tivnan A, Bryan K, Murphy DM, Buckley PG, et al. MicroRNA-184 inhibits neuroblastoma cell survival through targeting the serine/threonine kinase AKT2. *Mol Cancer* 2010; **9**: 83.

[5] Tivnan A, Foley NH, Tracey L, Davidoff AM, Stallings RL. MicroRNA-184-mediated inhibition of tumour growth in an orthotopic murine model of neuroblastoma. *Anticancer Res* 2010; **30**(11): 4391-4395.

[6] Gao B, Gao K, Li L, Huang Z, Lin L. miR-184 functions as an oncogenic regulator in hepatocellular carcinoma (HCC). *Biomed Pharmacother* 2014; **68**(2): 143-148.

[7] Tzivion G, Dobson M,Ramakrishnan G. FoxO transcription factors; regulation by AKT and 14-3-3 proteins. *Biochim Biophys Acta* 2011; **1813**(11): 1938-1945.

[8] Zhang Y, Gan B, Liu D, Paik JH. FoxO family members in cancer. *Cancer Biol Ther* 2011; **12**(4): 253-259.

[9] Kloet DE, Burgering BM. The PKB/FOXO switch in aging and cancer. *Biochim Biophys Acta* 2011; **1813**(11): 1926-1937.

[10] Gabriely G, Yi M, Narayan RS, Niers JM, Wurdinger T, Imitola J, et al. Human glioma growth is controlled by microRNA-10b. *Cancer Res* 2011; **71**(10): 3563-3572.

[11] Nan Y, Han L, Zhang A, Wang G, Jia Z, Yang Y, et al. MiRNA-451 plays a role as tumor suppressor in human glioma cells. *Brain Res* 2010; **1359**: 14-21.

[12] Zhang C, Han L, Zhang A, Yang W, Zhou X, Pu P, et al. Global changes of mRNA expression reveals anincreased activity of the interferon-induced signal transducer and activator of transcription (STAT) pathway by repression of miR-221/222 in glioblastoma U251 cells. *Int J Oncol* 2010; **36**(6): 1503-1512.

[13] Bu XY, Guo XH, Ding YC, Chen PX, Yan ZY, Zhou W, et al. Clinical research of reoperation in the treatment of patients with recurrent brain maliganat gliomas after initial surgery and chemoradiotherapy. *Chin J Clinicians (Electronic Version)* 2012; **6**(5): 1135-1139.

[14] Liu BL, Cheng JX, Zhang X, Zhang W. Controversies concerning the application of brachytherapy in central nervous system tumors. *J Cancer Res Clin Oncol* 2010; **136**(2): 173-185.

[15] Lu Z, Guo Q, Shi A, Xie F, Lu Q. Downregulation of NIN/RPN12 binding protein inhibit the growth of human hepatocellular carcinoma cells. *Mol Biol Rep* 2012; **39**(1): 501- 507.

[16] Lin Y, Peng S, Yu H, Teng H, Cui M. RNAi-mediated downregulation of NOB1 suppresses the growth and colony-formation ability of human ovarian cancer cells. *Med Oncol* 2012; **29**(1): 311-317.

[17] Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 2001; **411**: 494-498.

[18] Pecot CV, Calin GA, Coleman RL, Lopez-Berestein G, Sood AK. RNA interference in the clinic: challenges and future directions. *Nat Rev Cancer* 2011; **11**(1): 59- 67.

[19] Guo D, Wang B, Han F, Lei T. RNA interference therapy for glioblastoma. *Expert Opin Biol Ther* 2010; **10**(6): 927-936.

[20] Zhou Y, Su Z, Huang Y, Sun T, Chen S, Wu T, et al. The Zfx gene is expressed in human gliomas and is important in the proliferation and apoptosis of the human malignant glioma cell line U251. *J Exp Clin Cancer Res* 2011; **30**: 114.

[21] Ma Y, Wang B, Li W, Liu X, Wang J, Ding T, et al. Intersectin1-s is involved in migration and invasion of human glioma cells. *J Neurosci Res* 2011; **89**(7): 1079-1090.

[22] Wang BB, Niu RF, Ma YJ. Effect of ITSN1-S on apoptosis of glioma cells. *Chin J Clin Oncol* 2010; **37**(11): 608-610.

[23] Tsyba L, Nikolaienko O, Dergai O, Dergai M, Novokhatska O, Skrypkina I, et al. Intersectin multidomain adaptor proteins: regulation of functional diversity. *Gene* 2011; **473**(2): 67-75.

[24] Li ZH, Gu F, Ma YJ. Research progress of the effect of intersectin protein in cell signaling pathways. *Chin J Exper Surg* 2012; **29**(1): 148-149.

[25] Wang L, Liu XL, Li ZH, Gu F, Ma YJ. ITSN1-S SH3 domains regulate glioblastoma U87 cell proliferation. *Chin J Clin Oncol* 2013; **40**(18): 1089-1093.

[26] Wang HL, Li P, Zhao B. Effect of NOB1 on the proliferation and apoptosis of guman glioma cells. *Chin J Clinicians (Electronic Version)* 2013; **7**(4): 1609-1613.