Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research report

PTP4A3 is a target for inhibition of cell proliferatin, migration and invasion through Akt/mTOR signaling pathway in glioblastoma under the regulation of miR-137

Liling Wang[a], *Jianxun Liu*[a], *Zhiqiang Zhong*[a], *Xuhai Gong*[a], *Wei Liu*[b], *Lei Shi*[c], *Xuesong Li*[a,*]

[a]*Department of Neurology, Daqing Oilfield General Hospital, 9 Zhongkang Road, Daqing, Heilongjiang 163000, China*
[b]*Department of General Surgery, Daqing Oilfield General Hospital, 9 Zhongkang Road, Daqing, Heilongjiang 163000, China*
[c]*Department of Obstetrics and Gynecology, Daqing Oilfield General Hospital, 9 Zhongkang Road, Daqing, Heilongjiang 163000, China*

ARTICLE INFO

Article history:
Received 7 April 2016
Received in revised form
14 June 2016
Accepted 16 June 2016
Available online 18 June 2016

Keywords:
miR-137
PTP4A3
Akt
mTOR
Glioblastoma multiforme

ABSTRACT

Glioblastoma multiforme (GBM) is one of the most common primary malignant adult brain tumors. It is characterized by aggressive progression and poor prognosis. There is significant need to understand the mechanism of GBM malignancy and develop improved therapeutic options for GBM patients. We systematically studied the function of PTP4A3 in the malignancy of GBM. We found that PTP4A3 was upregulated in GBM tissues and cells. Knockdown of PTP4A3 expression in GBM cells inhibited cell proliferation, migration, and invasion. PTP4A3 knockdown modulated the activity of the Akt/mTOR signaling pathway by inducing de-phosphorylation of Akt and mTOR. We identified PTP4A3 as a direct target of miR-137. MiR-137 has been reported as a tumor suppressor in GBM development. In this study, overexpression of miR-137 in GBM cells also inhibited cell proliferation, migration, and invasion. Finally, restoration of PTP4A3 expression in miR-137 overexpressing cells partially reversed the inhibition of GBM cell malignancy, and the de-phosphorylation of Akt and mTOR. We identified that PTP4A3 regulated GBM via miR-137-mediated Akt/mTOR signaling pathway.

*Corresponding author.
E-mail address: 13329391666@163.com (X. Li).

http://dx.doi.org/10.1016/j.brainres.2016.06.026
0006-8993/

1. Introduction

Glioblastoma multiforme (GBM) is one of the most common primary malignant adult brain tumors (Louis et al., 2007). GBM is characterized by its aggressiveness and poor prognosis, with a median survival of only about 15 months (Stupp et al., 2005). Currently, the standard treatment regimen for GBM includes surgery removal, chemotherapy and radiotherapy (Stupp et al., 2005). Despite recent advancement in the therapeutic regimen, the successful rate for GBM treatment remains low. This situation is mainly due to the development of drug resistance by cancer cells, and the intrinsic ability of the cancer cells to migrate and invade through normal brain tissue, making complete surgical removal of cancer tissue nearly impossible. Therefore, there is a significant need to understand the mechanism of GBM malignancy and develop improved therapeutic options for GBM patients.

Protein tyrosine phosphatases (PTP) play important roles in modulating the levels of tyrosine phosphorylation. Increasing recent evidence has revealed that deregulation of protein tyrosine phosphatase activity is involved in a variety of diseases, including cancer (Ostman et al., 2006). One of the PTPs, PTP4A3, is characterized by the unique prenylation motif in its C-terminal end. Non-prenylated PTP4A3 is inactive and is usually located in the nucleus, and prenylated PTP4A3 is active and can be found in the membranes and intracellular structures (Zeng et al., 2000). PTP4A3 is known for its role in promoting cancer metastasis (Al-Aidaroos and Zeng, 2010). PTP4A3 upregulation was found in metastatic specimens from multiples cancer types (Polato et al., 2005; Radke et al., 2006; Saha et al., 2001; Zhou et al., 2009) including colorectal cancer (Saha et al., 2001), bladder cancer (Yeh et al., 2015), breast cancer (den Hollander et al., 2016) and glioma (Kong et al., 2007), and is associated with poor prognosis. Research has shown that the oncogenic function of PTP4A3 is accomplished via different cellular mechanisms. For example, PTP4A3 promotes cell proliferation, angiogenesis, and cell cycle progression (Basak et al., 2008; Guo et al., 2006; Polato et al., 2005). PTP4A3 also induces cell migration and invasion, and promotes metastasis through activation of the Rho GTPase and the PI3K/Akt pathway (Fiordalisi et al., 2006; Wang et al., 2007; Zeng et al., 2003). Recent studies demonstrated that autophagy could also play a critical role in PTP4A3-driven cancer progression (Huang et al., 2014).

MicroRNAs (miRNAs) are a group of short, non-coding RNAs that modulate protein expression by complementing with the 3′-UTR of mRNAs (Bartel, 2004). MiRNAs have been shown to induce the translational silence or cleavage of target genes and thereby participate in a variety of key cellular progresses that are associated with the onset and progression of cancer development (Gurtan and Sharp, 2013). Increasing recent evidences demonstrated aberrant regulation of miRNAs in a number of cancers such as gastric cancer, breast cancer, and gliomas (Zhu et al., 2014). Of particular interest to this study is miR-137. MiR-137 is located on chromosome 1p22, and has been found to be downregulated in many cancers including lung cancer (Zhu et al., 2013), colorectal cancer (Balaguer et al., 2010), gastric cancer (Chen et al., 2011), melanoma (Deng et al., 2011), oral cancer (Wiklund et al., 2011), and breast cancer (Zhao et al., 2012).

In this study, we for the first time systematically studied the function of PTP4A3 and miR-137 in the malignancy of GBM. We found that PTP4A3 was upregulated in GBM tissues and cells. Knockdown of PTP4A3 expression in GBM cells inhibited cell proliferation, migration, and invasion. Mechanistically, we found that PTP4A3 was able to modulate of activity of the Akt/mTOR signaling pathway. We also identified PTP4A3 as a direct target of miR-137. Finally, we demonstrated that miR-137 regulated GBM cell malignancy, and this regulation was realized partially through PTP4A3-mediated Akt/mTOR signaling pathway.

2. Results

2.1. *PTP4A3 is upregulated in GBM*

PTP4A3 has been shown to function as an oncogene in several types of cancers (Sharlow et al., 2014). Overexpression of PTP4A3 was reported in glioma (Kong et al., 2007). However, the roles of PTP4A3 in glioblastoma multiforme (GBM) development have not been studied before. In order to reveal the possible regulatory mechanism of PTP4A3 in GBM, we first examined the expression levels of PTP4A3 in 20 GBM samples by qRT-PCR. We found that the endogenous PTP4A3 levels were significantly higher in GBM tissues compared to those in non-tumor tissues ($P<0.001$) (Fig. 1A). This finding was corroborated by immunohistochemistry results, which revealed that PTP4A3 staining was much stronger in GBM tissues than that in non-tumor brain tissues (Fig. 1B). We further confirmed elevated PTP4A3 protein levels in GBM by performing Western blot analysis on four randomly selected GBM samples (Fig. 1C). In addition, upregulated levels of PTP4A3 were also observed in U87, U251 and LN229 GBM cell lines (Fig. 1D). These results collectively indicated that PTP4A3 was upregulated in GBM tissues and cells.

2.2. *Knockdown of PTP4A3 inhibits GBM cell proliferation, migration and invasion*

To study the biological roles of PTP4A3 in GBM, we transfected siRNA against PTP4A3 into LN229 and U87 cells to knock down endogenous PTP4A3 expression. Both mRNA (Fig. 2A) and protein (Fig. 2B) levels of PTP4A3 were decreased by this treatment. With these cell lines, we then evaluated the effects of PTP4A3 downregulation on a series of behaviors of GBM cells. We first performed MTT cell proliferation and colony formation assays. We found that knockdown of PTP4A3 significantly inhibited cell proliferation (Fig. 2C) and colony formation (Fig. 2D) of both LN229 and U87 cells. We also examined the effects of PTP4A3 knockdown in GBM cell migration and invasion. With wound healing assay, we were able to determine that PTP4A3 knockdown substantially suppressed the migration of both LN229 and U87 cells. The migration distances of PTP4A3 knockdown LN229 and U87 cells were reduced by approximately 52.3% and 58.4%, respectively (Fig. 3A). Consistently, in transwell invasion assay, we found that the invasive abilities of LN229 and U87 cells were