Biomedicine & Pharmacotherapy 99 (2018) 59–64

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Knockdown of TRIM37 suppresses the proliferation, migration and invasion of glioma cells through the inactivation of PI3K/Akt signaling pathway

Shi-lei Tang[a,1], Yuan-lin Gao[b,1], Wen-zhong Hu[a,*]

[a] Department of Neurosurgery, Huaihe Hospital of Henan University, Kaifeng 475000, Henan Province, China
[b] Department of Neurology, Kaifeng Central Hospital, Kaifeng 475000, Henan Province, China

ARTICLE INFO

Keywords:
TRIM37
Glioma
Invasion
EMT

ABSTRACT

Tripartite motif 37 (TRIM37), a member of the TRIM protein family, was involved in the tumorigenesis of several types of cancer. However, the expression pattern and role of TRIM37 in glioma remain unclear. Therefore, the aim of the present study was to investigate the role of TRIM37 in glioma, and to determine the molecular mechanisms. Our results demonstrated that TRIM37 was highly expressed in human glioma tissues and cell liens. Additionally, knockdown of TRIM37 dramatically inhibited the proliferation, migration/invasion, and the epithelial-mesenchymal transition (EMT) phenotype in glioma cells. Furthermore, knockdown of TRIM37 significantly reduced the levels of phosphorylated PI3K and Akt in U87MG cells, and an activator of PI3K/Akt signaling (SC79) partly reversed the inhibitory effects of si-TRIM37 on glioma cell proliferation and migration. Taken together, our results demonstrated that TRIM37 functions as an oncogene in the development and progression of glioma. TRIM37 knockdown inhibited the proliferation and invasion of human glioma cells at least in part through the inactivation of PI3K/Akt signaling pathway.

1. Introduction

Malignant glioma is one of the most common and devastating tumor in the brain. Its incidence has increased rapidly in the world [1]. Despite considerable progress in treatment strategies over the past decade, including surgery, radiotherapy and chemotherapy, the 5-year survival rate of glioma patients remains low due to the high recurrence and metastasis [2–4]. Therefore, there is an urgent need to further understand the molecular mechanisms underlying the process responsible for the development of glioma.

The tripartite motif (TRIM) family of proteins comprises over 70 members that are characterized by three zinc-binding domains, a RING, a B-box type 1 or a B-box type 2, and a coiled-coil region [5]. There is substantial evidence that TRIM proteins family plays multiple physiologic processes, including tumorigenesis, cell proliferation, inflammation, autophagy and innate immunity [6–8]. Several TRIM proteins, such as TRIM15, TRIM25, TRIM29, TRIM44, and TRIM59, have recently been verified to act as oncogenes or tumor suppressors that are involved in tumor progression [9–11]. Tripartite motif 37 (TRIM37), a member of the TRIM protein family, was involved in the tumorigenesis of several types of cancer, including breast cancer, pancreatic cancer, colorectal cancer, et al. [12–14]. In previous literature it has been reported that the expression of TRIM37 was significantly up-regulated in human hepatocellular carcinoma (HCC) tissues and was closely related with advanced stage; and overexpression of TRIM37 efficiently induced the migration and metastasis of HCC cells [15]. However, the expression pattern and role of TRIM37 in glioma remain unclear. Therefore, the aim of the present study was to investigate the role of TRIM37 in glioma, and to determine the molecular mechanisms. In the present study, we determined that TRIM37 was highly upregulated in glioma sample tissues and cell lines, and that knockdown of TRIM37 significantly suppressed the proliferation, migration and invasion of glioma cells.

2. Materials and methods

2.1. Tissue specimens

This study was approved by the Research Ethics Committee of Huaihe Hospital of Henan University (Certification No: 069; China). Fresh glioma and corresponding normal tissues were obtained from the patients with glioma in our hospital between May 2016 and July 2016. No patients had received chemotherapy or radiotherapy before sample collection. The specimens were quickly frozen in liquid nitrogen and

* Corresponding author at: Department of Neurosurgery, Huaihe Hospital of Henan University, No. 8 Baobei Road, Kaifeng 475000, Henan Province, China.
E-mail address: hu_wenzh@126.com (H. Wen-zhong).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.biopha.2018.01.054
Received 17 October 2017; Received in revised form 12 December 2017; Accepted 5 January 2018

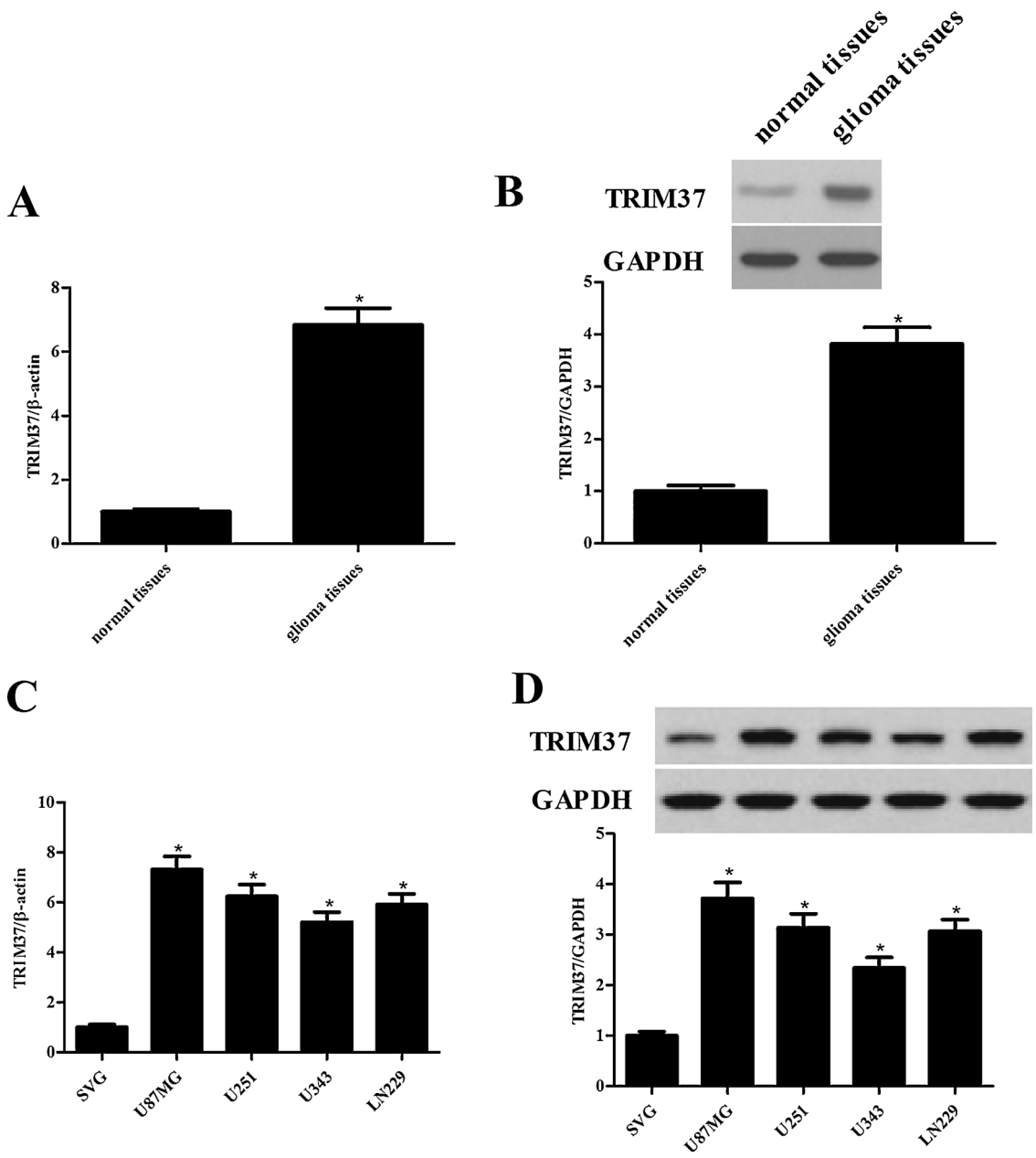

Fig. 1. TRIM37 is highly expressed in human glioma tissues and cell lines. The mRNA expression of TRIM37 in human glioma tissues (A) and cell lines (C) was analyzed by RT-qPCR. The protein expression of TRIM37 in human glioma tissues (B) and cell lines (D) was determined by western blot analysis. GAPDH was used as a loading control. These data are from three independent experiments and presented as the mean ± SD. *$P < 0.05$ *vs.* normal or SVG group.

stored at −80 °C until use. The informed consents from all of the patients involved were obtained prior to the initiation of this study.

2.2. Cell culture

Four human glioma cell lines (U87MG, U251, U343, LN229) and the normal human astrocytes SVG were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Sigma, St Louis, MO, USA) and 1% penicillin/streptomycin (Sigma) in a humidified 5% CO_2 atmosphere at 37°C.

2.3. Quantitative real-time PCR

Total RNA was isolated from human glioma tissues or cells using the RNA plus kit (Invitrogen,). cDNA was amplified with 1 μg of total RNA using a Primer Script Kit (TaKaRa, Dalian, China). Real-time quantitative PCR was performed using SYBR Green PCR kit (Takara) on CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The specific primers for TRIM37 were sense, 5′-TCAGCTGTATTAGGCGC TGG-3′, and antisense, 5′-ACTTCTTCTGCCCAACGACA-3′; and for β-actin were sense, 5′-AAATCGTGCGTGACATCAAAGA-3′ and antisense, 5′-GGCCATCTCCTGCTCGAA-3′. β-actin was used as a control for normalizing gene expression levels. The data obtained were analyzed using the $2^{-\Delta\Delta Ct}$ method.

2.4. Western blot

Glioma tissues or cells were homogenized and lysed with radioimmunoprecipitation assay lysis buffer supplemented with 1 mmol/l phenylmethylsulfonyl fluoride and phosphatase inhibitor (Pierce;