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Research paper

HMGCR positively regulated the growth and migration of glioblastoma cells

Zhihua Qiu [a,1], Wen Yuan [a,1], Tao Chen [b,1], Chenzhi Zhou [b], Chao Liu [a], Yongkai Huang [a], Deqing Han [a,*], Qinghui Huang [b,c,**]

[a] Department of Neurosurgery, Central Hospital of Zhuzhou, Zhuzhou, Hunan Province 412000, PR China
[b] State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong Province 510120, PR China
[c] Department of Neurology, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong Province 510120, PR China

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ABSTRACT

The metabolic program of cancer cells is significant different from the normal cells, which makes it possible to develop novel strategies targeting cancer cells. Mevalonate pathway and its rate-limiting enzyme HMG-CoA reductase (HMGCR) have shown important roles in the progression of several cancer types. However, their roles in glioblastoma cells remain unknown. In this study, up-regulation of HMGCR in the clinical glioblastoma samples was observed. Forced expression of HMGCR promoted the growth and migration of U251 and U373 cells, while knocking down the expression of HMGCR inhibited the growth, migration and metastasis of glioblastoma cells. Molecular mechanism studies revealed that HMGCR positively regulated the expression of TAZ, an important mediator of Hippo pathway, and the downstream target gene connective tissue growth factor (CTGF), suggesting HMGCR might activate Hippo pathway in glioblastoma cells. Taken together, our study demonstrated the oncogenic roles of HMGCR in glioblastoma cells and HMGCR might be a promising therapeutic target.

1. Introduction

Glioblastoma (GBM) has been one of the leading causes of cancer-related death in the world (Siegel et al., 2014). The median survival for GBM is about 15 months (DeSantis et al., 2014). The drug resistance, high recurrence and poor prognosis promote us to identify novel biomarkers and explore novel therapeutic targets for the treatment of GBM.

The mevalonate-isoprenoid biosynthesis (MIB) pathway produced farnesyl pyrophosphate (FPP) and was critical for protein prenylation (Dricu et al., 1997; Lutz et al., 1992; Rilling et al., 1993). Hydroxymethylglutaryl coenzyme A reductase (HMGCR) was the rate-limiting enzyme of MIB pathway (Breitling and Krisans, 2002). Previous studies showed that HMGCR promoted the transformation of normal breast epithelial cells, highlighting the oncogenic roles of HMGCR in the tumorigenesis (Clendening et al., 2010). In addition, the functions of HMGCR in bladder cancer, ductal carcinoma-in-situ (DCIS) breast cancer were found (Butt et al., 2014). In glioblastoma cells, up-regulation of HMGCR and other cholesterol biosynthesis genes represented as a novel mechanism for the drug resistance, while inhibiting the HMGCR-mediated cholesterol-mevalonate pathway induced cell cycle arrest and autophagy in U87 glioblastoma cells (Hamm et al., 2014a, 2014b; Rios-Marco et al., 2013.). However, the expression pattern and the biological functions of HMGCR in glioblastoma remained largely unknown.

Through interacting with Yes-associated protein (YAP), transcriptional co-activator with PDZ-binding motif (TAZ) plays an important role in Hippo signal pathway and control the size of the organs (Piccolo et al., 2014). TAZ has been reported to regulate multiple physiological processes, including lung differentiation (Lin et al., 2015), adipogenesis and osteogenesis (He et al. 2012,; Tang et al., 2013). In the progression of tumorigenesis, TAZ was found to be up-regulated in various types of cancer and regulated the proliferation, migration as well as the epithelial–mesenchymal transition (EMT) (Liu et al., 2010; Pan, 2010). Dys-regulation of Hippo signaling was observed in glioblastoma cell. For example, proteolysis of MOB1 by the ubiquitin ligase praja2 activated YAP/TAZ signaling and supported the growth of glioblastoma cells (Lignitto et al., 2013). TAZ has been shown to mediate the biological functions of several oncogenes in the glioblastoma. CD44 was up-

Abbreviations: HMGCR, HMG-CoA reductase; CTGF, connective tissue growth factor; GBM, glioblastoma; MIB, mevalonate-isoprenoid biosynthesis; FPP, farnesyl pyrophosphate; DCIS, ductal carcinoma-in-situ; YAP, Yes-associated protein; TAZ, transcriptional co-activator with PDZ-binding motif; EMT, epithelial–mesenchymal transition; HDAC9, histone deacetylase 9; GFP, green fluorescence protein; PBS, phosphate-buffered saline.

* Correspondence to: D. Han, 116 South Changjiang Road, Central Hospital of Zhuzhou, Zhuzhou, Hunan Province 412000, PR China.
** Correspondence to: Q. Huang, 151 West Yanjiang Road, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong Province 510120, PR China.
E-mail addresses: hdqmail@163.com (D. Han), qinghui@gird.cn (Q. Huang).
[1] Authors contributed equally to this work.

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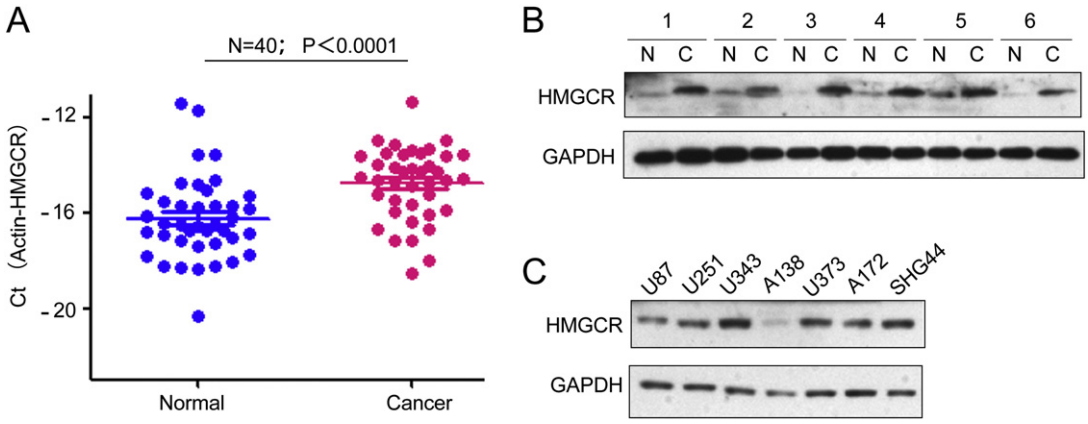

Fig. 1. Expression of HMGCR was increased in the clinical glioblastoma samples. (A) The mRNA level of HMGCR in 40 glioblastoma samples and paired adjacent non-cancerous tissues was examined using Real-time PCR. HMGCR expression level was normalized according to the expression of beta-actin. The C_T of the beta-actin subtracted from the C_T of HMGCR was plotted for each sample. Data was calculated from triplicates. $P < 0.0001$. (B) Protein level of HMGCR in 6 pairs of glioblastoma tissues and matched normal tissues was examined by Western blotting. N, normal tissues; C, cancer tissues. (C) The protein level of HMGCR in a panel of glioblastoma cell lines was examined by Western blotting.

regulated in glioblastoma and its depletion blocked the growth and tumorigenesis of glioblastoma by inhibiting TAZ/YAP signaling (Xu et al., 2010). Also, TAZ was reported to interact with histone deacetylase 9 (HDAC9) and knocking down of TAZ attenuated the oncogenic effects of HDAC9 in glioblastoma cells (Yang et al., 2015). However, how metabolic clues regulated the YAP/TAZ signaling remains largely unknown.

In this study, we examined the expression of HMGCR in the clinical GBM samples and studied the biological functions of HMGCR in glioblastoma through a series of in vitro and in vivo assays.

2. Materials and methods

2.1. Cell culture

Human glioblastoma cell lines U87, U251, U343, U373, A138, A172 and SHG44 were maintained in DMEM medium (Invitrogen) supplemented with 10% fetal bovine serum, 100 units/ml penicillin and 100 mg/ml streptomycin. Cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2.

2.2. GBM clinical samples

This study was approved by the ethics commitment of Central Hospital, Zhuzhou, Hunan Province, China. Clinical GBM tissues and paired non-cancerous tissues were collected at Zhuzhou Central Hospital after obtaining the agreement of all the patients. Tissues were stored at −80 °C in the freezer.

2.3. Plasmid construction and transfection

The full-length HMGCR coding sequence was cloned into the expression vector pcDNA3.1-myc. The plasmids were transfected into U251

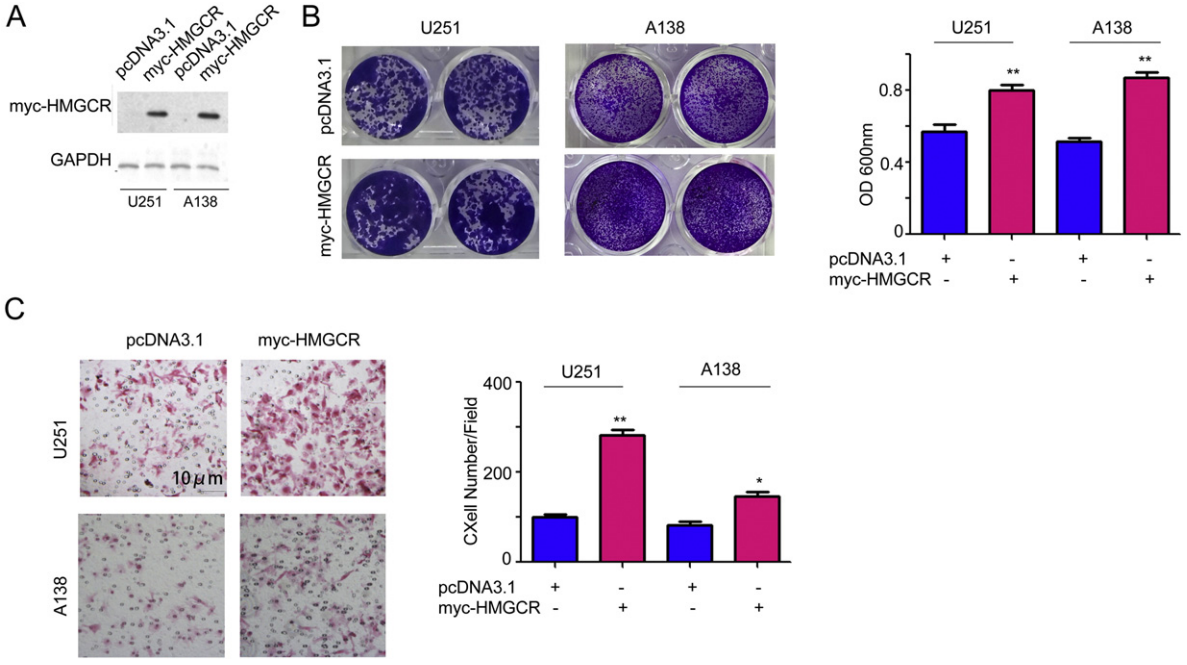

Fig. 2. HMGCR promoted the growth and migration of U251 and A138 cells. (A) Forced expression of HMGCR in U251 and A138 cells through lipofectamine-mediated transfection. (B) Stable transfection of U251 and A138 cells with myc-HMGCR plasmids promoted cell growth. Equal number of cells was seeded in 12-well plates. Ten days later, cells were stained with crystal violet for 5 min and then resolved with 1% SDS solution. OD 600 nm was measured. (C) Forced expression of myc-HMGCR in U251 and A138 cells promoted cell migration. Cell migration was measured using a modified Boyden chamber. 1×10^5 cells suspended in DMED medium containing 0.1% FBS were put on the upper of chamber. The lower chamber was loaded with DMED medium containing 10% FBS. The migrated cells were stained with HE staining. *, $P < 0.05$; **, $P < 0.01$.