Biomedicine & Pharmacotherapy 104 (2018) 566–575

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Overexpression of STAT1 suppresses angiogenesis under hypoxia by regulating VEGF-A in human glioma cells

Yunsheng Zhang[1], Guishan Jin[1], Junwen Zhang, Ruifang Mi, Yiqiang Zhou, Wenhua Fan, Sen Cheng, Wenjie Song, Bo Zhang, Mengjiao Ma, Fusheng Liu*

Brain Tumor Research Center, Beijing Neurosurgical Institute, Department of Neurosurgery, Beijing Tiantan Hospital Affiliated to Capital Medical University, Beijing Laboratory of Biomedical Materials, Beijing 100050, PR China

ARTICLE INFO

Keywords:
Glioblastoma multiforme
Signal transducer and activator of transcription
Hypoxia-inducible factor-1α
Vascular endothelial growth factor A

ABSTRACT

Hypoxia is common in Glioblastoma (GBM). By regulating the 'hypoxia signaling cascade', hypoxia affects several processes including cell proliferation, invasion, and angiogenesis. Some studies have revealed that signal transducer and activator of transcription (STAT), including STAT1, is abnormal under hypoxia in several cancers. Here, we investigated the role of STAT1 under hypoxia in glioma progression. We found that STAT1 was downregulated under a hypoxic condition in U251 and U373. STAT1 overexpression can not only decrease proliferation, migration and invasion in U251 and U373 but also inhibit tube formation of HBMECs. Moreover, overexpression of STAT1 decreased tumor growth and prolonged the overall survival of xenograft mice. We also showed that STAT1 overexpression inhibited the expression of HIF-1α and VEGF-A. Our work suggests that STAT1 plays a pivotal role as a tumor suppressor in glioma under hypoxia, and it could be a potential new therapeutic target in glioma.

1. Introduction

Glioma, with high invasiveness and recurrence, is the most common and devastating primary brain tumor and accounts for 70% of malignant primary brain tumors in adults [1]. According to the 2016 World Health Organization (WHO) classification criteria, grades I and II are termed low-grade glioma, and grades III and IV are high-grade glioma [2]. Approximately half of gliomas in adults are Grade IV astrocytomas, which are also known as "glioblastomas multiforme" (GBM). Despite multiple therapeutic strategies including surgery, radiotherapy, and chemotherapy, GBM patients still cannot be cured, partly due to the histologic features of GBM. The histological features of GBM are necrosis and capillary endothelial proliferations, and hypoxia is common in GBM, especially in the areas of necrosis. By regulating the 'hypoxia signaling cascade', hypoxia affects several processes including cell proliferation, invasion, and angiogenesis [3,4]. Hence, identifying and elucidating the function of dysregulated genes under hypoxia is important for understanding various physiological and pathological processes.

Signal transducer and activator of transcription (STAT) proteins are a family of cytoplasmic transcription factors consisting of seven members, including STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. Multiple studies have demonstrated that this gene family controls many biological processes including immune responses, cell cycle, cell survival and angiogenesis [5,6]. STAT1 as a prototypical member plays an essential role throughout the development and progression of the tumor. Different expression of STAT1 has been demonstrated in some cells and clinical specimens, and there is accumulating evidence that changes in STAT1 can affect the biological characteristics of the tumor. Ramana [7] and Chin [8] reported that STAT1 can inhibit the proliferation and promote apoptosis in some mouse and human tumor cells. Ju et al. showed that STAT1 can inhibit human U87MG glioblastoma cell growth and induce low levels of STAT1 expression in human GBM tissue [9]. In contrast, constitutive activation of STAT1 has been reported in GBM tissues [10]. Other studies have reported that STAT1 can be activated by hypoxia-inducible factor 1 (HIF-1) under

Abbreviations: STAT, signal transducer and activator of transcription; IHC, immunohistochemistry; GBM, glioblastomas multiforme; HIF-1, hypoxia-inducible factor 1; VEGF, vascular endothelial growth factor; FBS, fetal bovine serum; ECM, endothelial cell medium; ECGS, endothelial cell growth supplement; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ECL, enhanced chemi-luminescence; CCK-8, Cell Counting Kit-8; MRI, magnetic resonance imaging; FACS, flow cytometry and cell sorting; MS, median survival; OS, overall survival

* Corresponding author at: Brain Tumor Research Center, Beijing Neurosurgical Institute, Department of Neurosurgery, Beijing Tiantan Hospital Affiliated to Capital Medical University, Tiantan Xili 6, Dongcheng District, Beijing 100050, PR China.

E-mail addresses: zys6237781@hotmail.com (Y. Zhang), liufushengs@hotmail.com (F. Liu).

[1] These authors contributed equally to the work.

https://doi.org/10.1016/j.biopha.2018.05.079
Received 7 February 2018; Received in revised form 16 May 2018; Accepted 17 May 2018

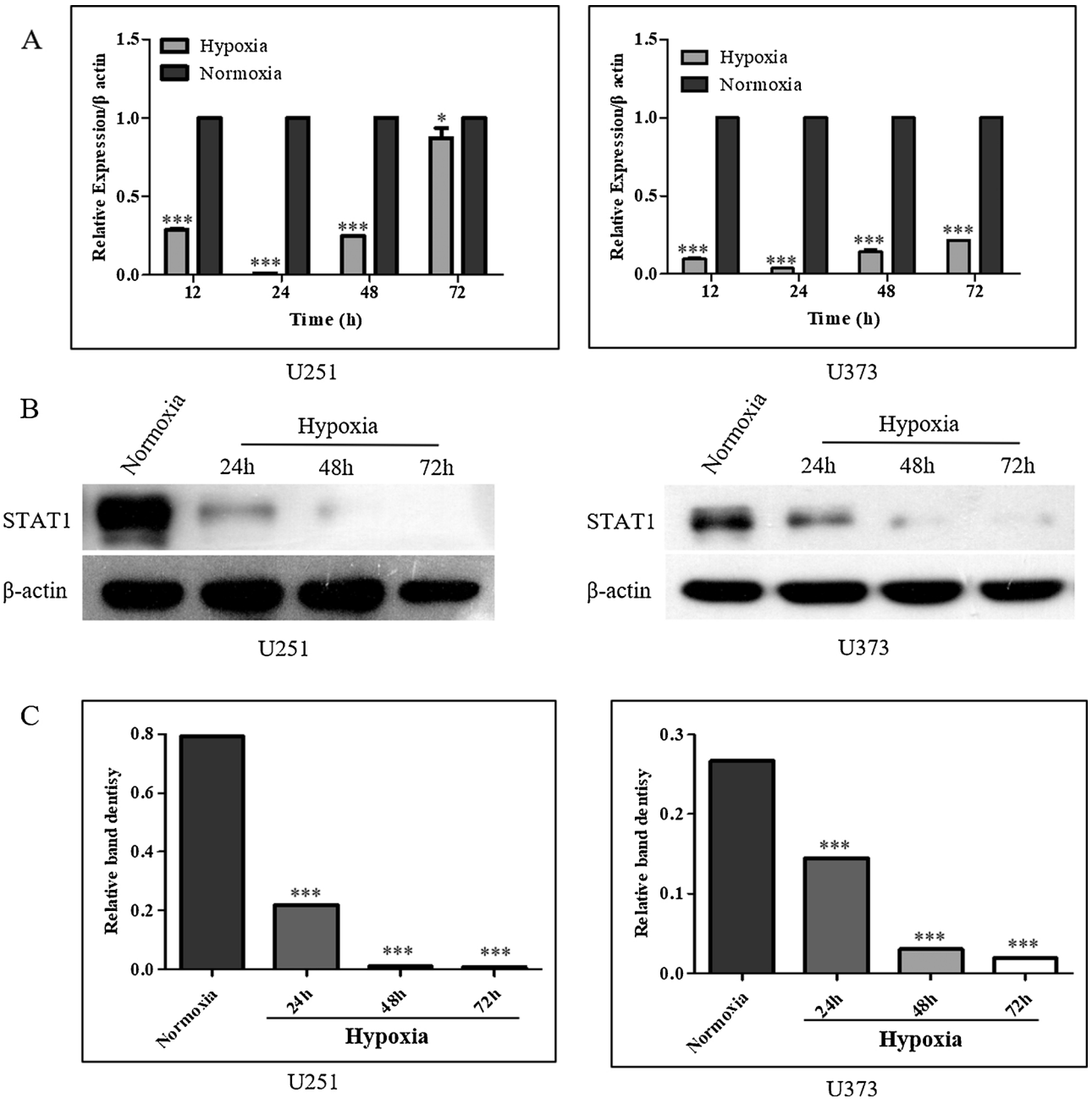

Fig. 1. The expression levels of STAT1 in different oxygen concentrations. (A). Quantitative real-time PCR of STAT1 mRNA expression level of U251 and U373 under hypoxia compared with normoxia. (B–C). Western blot assays of STAT1 protein expression level of U251 and U373 under hypoxia compared with normoxia. Representative blot pictures are shown in B, and quantitative graphs for relative protein expression levels are shown in C (*P < 0.05; ***P < 0.001).

normoxia [11]. These studies showed that STAT1 might play a biological function in the tumor. However, whether and by what mechanism STAT1 functions in tumor progression under hypoxic conditions is still not known.

Here, we detected the expression of STAT1 and found a significant fold change in GBM cell lines under different oxygen microenvironments. We also elucidated the role of STAT1 in glioma progression and angiogenesis under a hypoxic condition and found that STAT1 can abolish hypoxia induced HIF-1α activation and reduce the expression of vascular endothelial growth factor (VEGF), which is associated with tumor cell ability to attenuate tube formation of endothelial cells in vitro. Note that STAT1 may be an anti-angiogenic factor in hypoxic glioblastoma.

2. Materials and methods

2.1. Culture of cell lines

U251 and U373 human glioblastoma multiforme cell lines were used in this study. The cells were obtained from the Chinese Academy of Sciences Cell Bank (Shanghai, China) and cultured in DMEM (Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA). HBMECs were purchased from Sciencell (USA). The cells were cultured in endothelial cell medium (ECM, Sciencell, USA) supplemented with endothelial cell growth supplement (ECGS, Sciencell, USA) and 5% FBS in 37 °C with 5% CO_2. To create a hypoxic microenvironment, an N_2-O_2 incubator (Thermo, USA) was used to maintain hypoxia with 1% O_2.