Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Up-regulation of MSH6 is associated with temozolomide resistance in human glioblastoma

Quanye Sun [a, b], Chunying Pei [a, b], Qiuyuan Li [c], Tianxiu Dong [d], Yucui Dong [a, b], Wenjing Xing [a, b], Peng Zhou [e], Yujiao Gong [a, b], Ziqi Zhen [a, b], Yifan Gao [a, b], Yun Xiao [f, ***], Jun Su [g, **], Huan Ren [a, b, *]

[a] Department of Immunology, Harbin Medical University, Harbin 150081, China
[b] Immunity & Infection Key Laboratory of Heilongjiang Province, Harbin 150081, China
[c] Fundamental Medicine Institute, Heilongjiang University of Chinese Medicine, Harbin 150040, China
[d] Department of Abdominal Ultrasound, The 1st Affiliated Hospital to Harbin Medical University, Harbin 150081, China
[e] Department of Neurosurgery, The 4th Hospital Affiliated to Harbin Medical University, Harbin 150081, China
[f] Department of Bioinformatics, College of Bioinformatics and Technology, Harbin Medical University, Harbin 150081, China
[g] Department of Neurosurgery, The 3rd Hospital Affiliated to Harbin Medical University, Harbin 150086, China

ARTICLE INFO

Article history:
Received 5 January 2018
Accepted 13 January 2018
Available online xxx

Keywords:
Glioblastoma
Temozolomide resistance
MSH6
DNA mismatch repair
O^6-methylguanine-DNA methyltransferase

ABSTRACT

The impact of DNA mismatch repair (MMR) on resistance to temozolomide (TMZ) therapy in patients with glioblastoma (GBM) is recently reported but the mechanisms are not understood. We aim to analyze the correlation between MMR function and the acquired TMZ resistance in GBM using both relevant clinical samples and TMZ resistant cells. First we found increased expression of MSH6, one of key components of MMR, in recurrent GBM patients' samples who underwent TMZ chemotherapy, comparing with those matched samples collected at the time of diagnosis. Using the cellular models of acquired resistance to TMZ, we further confirmed the up-regulation of MSH6 in TMZ resistant cells. Moreover, a TCGA dataset contains a large cohort of GBM clinical samples with or without TMZ treatment reinforced the increased expression of MSH6 and other MMR genes after long-term TMZ chemotherapy, which may resulted in MMR dysfunction and acquired TMZ resistance. Our results suggest that increased expression of MSH6, or other MMR, may be a new mechanism contributing to the acquired resistance during TMZ therapy; and may serve as an indicator to the resistance in GBM.

1. Introduction

Glioblastoma (GBM), the highest grade in the World Health Organization (WHO) classification of the central nervous system tumor (WHO IV), is the most common and aggressive primary brain tumor in adults [1–3]. Current standard therapies include maximal surgery and chemoradiotherapy known as Stupp scheme, daily concomitant temozolomide (TMZ) at 75 mg/m^2 as an adjunct to radiotherapy followed by 6 cycles of TMZ at 150–200 mg/m^2 on 5 of out 28 days [4,5]. Over the past decade, as compared to radiotherapy alone, radiotherapy plus concomitant and adjuvant TMZ chemotherapy after surgery has significantly improved the survival rates in patients with GBM, increasing 5-year survival rates from 1.9 to 9.8% [6]. Nevertheless, virtually all GBM patients experience tumor regrowth with recurrent tumor showing a strong chemoresistant phenotype [7]. Therefore, identifying mechanisms of TMZ resistance and developing effective strategies are of clinical significance in GBM.

TMZ is an oral alkylating agent producing a variety of DNA base lesions, such as N^3-methyladenine (N^3-meA) and N^7-methylguanine (N^7-meG), which are primarily repaired by base excision repair (BER), and O^6-methylguanine (O^6-meG) which is the most cytotoxic lesion [8]. O^6-methylguanine-DNA methyltransferase (MGMT) directly repairs TMZ induced O^6-meG lesion; and thus, high levels of MGMT are thought to contribute to resistance to the

* Corresponding author. Department of Immunology, Harbin Medical University, 157 Baojian Road, Harbin, 150081, China.
** Corresponding author. Department of Neurosurgery, The 3rd Hospital Affiliated to Harbin Medical University, 150 Haping Road, Harbin, 150086, China.
*** Corresponding author. Department of Bioinformatics, College of Bioinformatics and Technology, Harbin Medical University, Harbin 150081, China.
E-mail addresses: xiaoyun@ems.hrbmu.edu.cn (Y. Xiao), sujun6686@126.com (J. Su), renhuan@ems.hrbmu.edu.cn (H. Ren).

https://doi.org/10.1016/j.bbrc.2018.01.093
0006-291X/

treatment with TMZ [9,10]. Hypermethylation of the MGMT promoter results in gene silencing and predicts benefit from TMZ treatment in GBM [11,12]. Therefore, enzyme activity of MGMT is one of the key mechanisms underlying TMZ resistance.

Provided that TMZ-induced O^6-meG are not repaired by MGMT, DNA polymerase will insert thymine opposite O^6-meG resulted in mispair bases between O^6-meG: T during the next round of DNA replication. O^6-meG:T could be fixed by the DNA mismatch repair (MMR) system, MSH2-MSH6 heterodimer (MutSα) recognizes and binds to single base-base mismatches, recruiting MLH1-PMS2 heterodimer (MutLα) and activates a large number of downstream proteins to complete the repair process. At the end of repair, DNA polymerase fills the gap prior to DNA ligation which inserts thymine opposite O^6-meG once again, and another round of MMR is activated. The futile repair MMR cycling finally results in DNA double strand breaks and cell death [13–15]. Disturbance on MMR function may be another important mechanism mediating TMZ resistance in malignant tumors. A recent study indicated that acquired TMZ resistance was caused by MMR deficiency in GBM [16]. However, Maxwell et al. [17] showed that MMR deficiency did not mediate clinical resistance to TMZ in malignant glioma. These data imply that the role of MMR in TMZ resistance is complicated.

In this study, we found greatly increased MSH6 expression in recurrent GBM samples post TMZ treatment in both collected biopsies and relevant TCGA datasets. Then we used GBM cell lines with acquired TMZ resistance in vitro to confirm the related MMR changes and analyzed mechanisms for MSH6 up-regulation during TMZ treatment in GBM cells. Our data suggest that, whereas the mechanisms are far complex, the treatment-induced up-regulation of MMR including MSH6 may cooperate in alternative cellular signaling pathways leading to TMZ resistance in patients with GBM.

2. Materials and methods

2.1. Cell culture and GBM tumor tissues

Human GBM cell line LN18 was originally obtained from ATCC (American Type Culture Collection); RG cells were obtained through immortalization by serial passage of a resected human GBM specimen [18]. Cells were cultured in DMEM supplemented with 10% FBS in a standard humidified incubator at 37 °C and 5% CO_2. The matched pre- and post-TMZ treatment GBM samples were obtained from Department of Neurosurgery, The 4th Hospital Affiliated to Harbin Medical University, Harbin, China.

2.2. Immunohistochemistry staining

Paraffin-embedded slides were deparaffinized. Antigen retrieval was performed in antigen retrieval buffer (1.8 mM citric acid, 8.2 mM sodium citrate, 0.05% Tween 20, pH 6.0) in microwave oven. Endogenous peroxidase activity was blocked by incubation in 3% H_2O_2, and non-specific binding was blocked by incubation with 10% goat serum. Slides were incubated with the primary antibody in PBS containing 1% BSA and 10% goat serum. Biotinylated secondary anti-rabbit antibody (Santa Cruz) were added an incubated at 37 °C for 30 min. Streptavidin-HRP (Santa Cruz) was added and after 30 min the sections were stained with DAB substrate and counter-stained with hematoxylin.

2.3. Generation of TMZ-resistant GBM cell lines

LN18 and RG cells were treated with 0.1 or 0.05 mM of TMZ as the starting concentration for 3 passages and then exposed to gradually increased concentrations up to 0.4 or 0.2 mM for 3 months, respectively. The control cells were treated with 0.1%–0.4% DMSO instead. During the total 3 months treatment, the medium was replaced with freshly prepared TMZ or DMSO every 2 days. LN18 and RG cells were respectively grown to stable TMZ-resistant cell lines LN18-TR and RG-TRand were harvested at varied time points for subsequent study.

2.4. Cell viability assays

Standard microplate MTT assays were employed to examine the growth rate in both parental GBM cells and TMZ-resistant cells under the drug treatment. Briefly, tumor cells were plated at a density of 2×10^3 cells per well in 96-well plates with varied concentrations of TMZ. TMZ was replenished after 48 h. At the end of 96 h of incubation, cells were stained by 0.5 mg/ml MTT (Sigma-Aldrich) for further 4 h at 37 °C in an incubator. The medium was removed afterwards and the intracellular formazan crystals were dissolved by adding 100 μl DMSO/well. Absorbance was read at 570 nm on a microplate reader (EL × 800, Biotek Instruments, Heidelberg, Germany).

2.5. Western blotting analysis

GBM tissues or GBM cells lysates were prepared in a 1 × sodium dodecyl sulfate buffer. Equal quantities of proteins were separated

Table 1
Clinical data of the patients with recurrent glioblastoma pre- and post-TMZ treatment.

sample ID	diagnosis	sex	age (y)	PFS (m)	OS (m)	MGMT expression	treatment
pre 624	GBM	F	49	5	12	positive	Non-treated
post 624-A*			50				XRT + TMZ (4 cycles)
pre 318	GBM	M	45	10	21	positive	Non-treated
post 318-A			46				XRT + TMZ (2 cycles)
pre 874	GBM	F	35	34	36	positive	Non-treated
post 874-A			37				XRT + TMZ (4 cycles)
pre 215	GBM	M	45	17	22	positive	Non-treated
post 215-A			46				XRT + TMZ (2 cycles)
pre 116	GBM	M	21	49	57	negative	Non-treated
post 116-A			25				XRT + TMZ (4 cycles)
pre 408	GBM	M	62	4	14	positive	Non-treated
post 408-A			63				XRT + TMZ (2 cycles)
pre 646	GBM	M	50	24	29	positive	Non-treated
post 646-A			52				XRT + TMZ (5 cycles)
pre 577	GBM	F	56	5	13	positive	Non-treated
post 577-A			56				XRT + TMZ (2 cycles)

Note: *-A indicates post-TMZ treated samples. Abbreviations: GBM, glioblastoma; PFS, progression-free survival; OS, overall survival; MGMT, O^6-methylgunine DNA methyltransferase; XRT, X-ray therapy; TMZ, temozolomide; y, year; m, month.

Please cite this article in press as: Q. Sun, et al., Up-regulation of MSH6 is associated with temozolomide resistance in human glioblastoma, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.01.093