

LETTER TO THE EDITOR

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The relationship between expression of PD-L1 and HIF-1 α in glioma cells under hypoxia

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Abstract

Hypoxia inducible factor-1 α (HIF-1 α) up-regulates the expression of programmed death ligand-1 (PD-L1) in some extracranial malignancies. However, whether it could increase PD-L1 expression in intracranial tumor is still unknown. Here, we explored the relationship between HIF-1 α and PD-L1 expression in glioma, and investigated their clinical significance. In glioma patients, HIF-1 α and PD-L1 were overexpressed in high grade glioma tissues and were significantly associated with poor survival. In glioma cells, PD-L1 expression was induced under hypoxia condition, and the enhanced PD-L1 expression was abrogated by either HIF-1 α knock-down or HIF-1 α inhibitor treatment. Furthermore, ChIP-qPCR analysis showed the direct binding of HIF-1 α to PD-L1 proximal promoter region, providing evidence that HIF-1 α up-regulates PD-L1 in glioma. In glioma murine model, the combination treatment with HIF-1 α inhibitor and anti-PD-L1 antibody caused a more pronounced suppressive effect on tumor growth compared to either monotherapy. Immunologically, the combination treatment improved both dendritic cell (DC) and CD8⁺ T cell activation. Overall, our results demonstrated that positive correlation between PD-L1 and HIF-1 α in glioma, and provide an alternative strategy, inhibiting HIF-1 α , as combination therapies with immunotherapies to advance glioma treatment.

Keywords: PD-L1, HIF-1 α , Glioma, Hypoxia, Immunotherapy

To the Editor

In clinical, the blockade of the PD-1/PD-L1 pathway hasn't been well-confirmed to prolong OS of glioma patients [1, 2]. With increasing malignancy, hypoxia as a major tumor microenvironment factor widely exhibit in glioma [3], however, the influence of hypoxia on tumor immune escape remains unclearly. Here, we aimed to

explore the relationship between the PD-L1 and HIF-1 α in glioma, and to investigate their prognostic values.

PD-L1 is widely used as a candidate biomarker for predicting patients that would respond to anti-PD-1/PD-L1 immunotherapy [4, 5], but not in glioma [6]. We analyzed RNA-seq data from a cohort (640 glioma patients) in CGGA dataset and found that PD-L1 is positively correlated with HIF-1 α (Additional file 1: Fig. S1, Additional file 2: S2). To determine this, the PD-L1 and HIF-1 α levels in 120 glioma patients' tissues were detected by immunohistochemical (Fig. 1a, Additional file 4: Table S1). Fifty patients (41.7%) were classified as PD-L1 positive ($\geq 5\%$). PD-L1 was positively associated with tumor grade (Fig. 1b, Additional file 5: Table S2). Moreover, our clinical data showed that high PD-L1 was significantly related to high HIF-1 α ($r = 0.412$, $P < 0.001$)

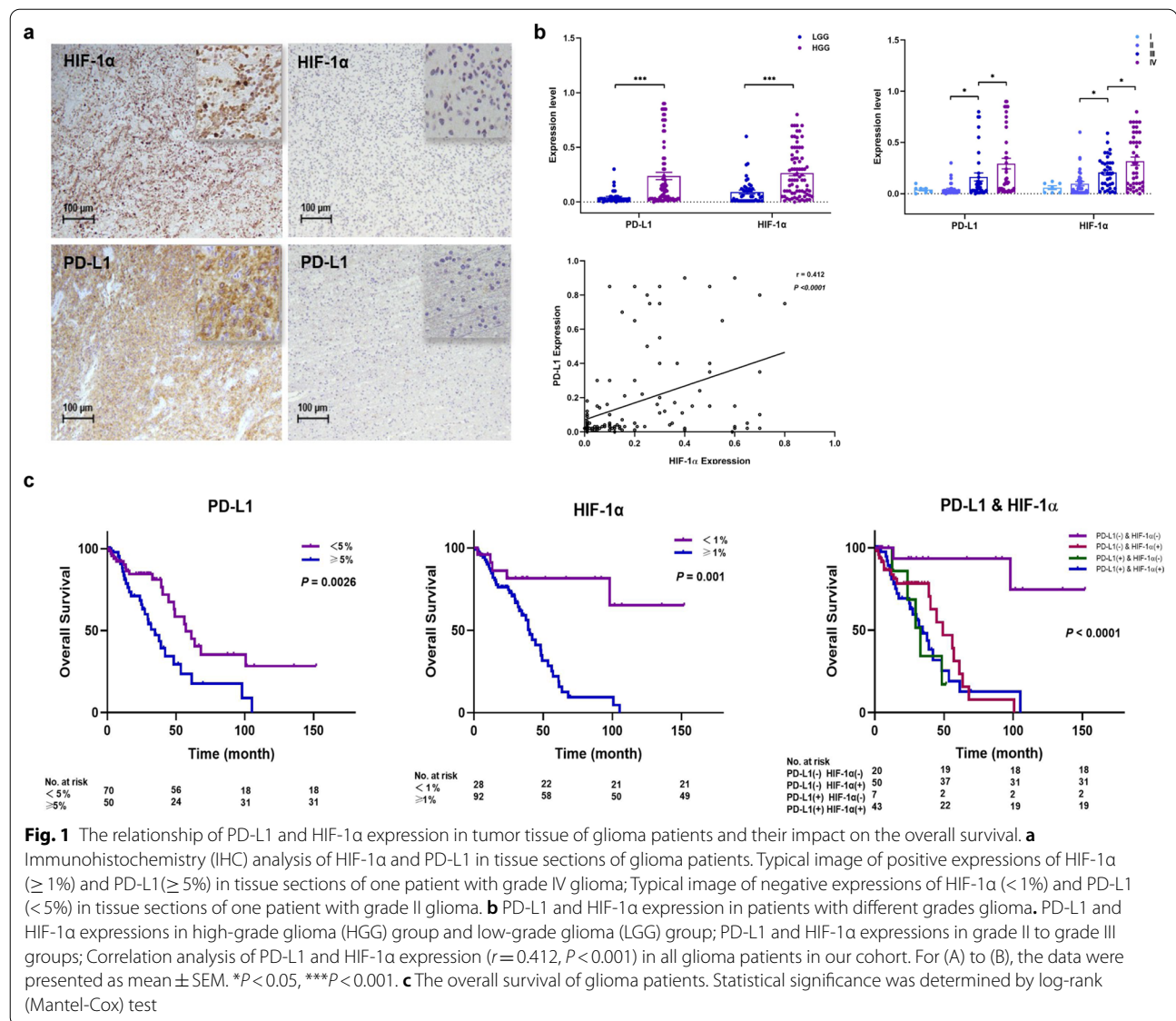
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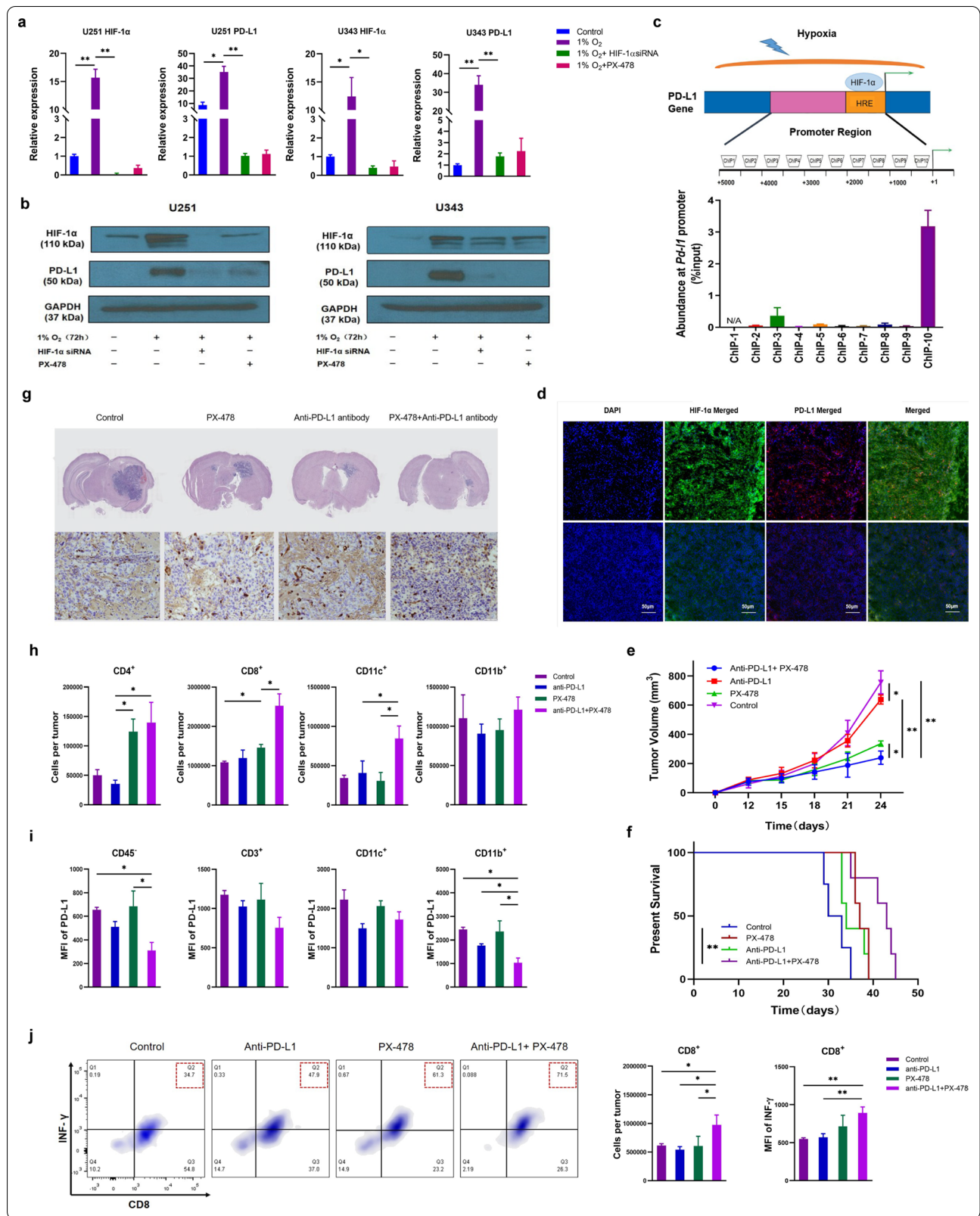
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Fig. 2 Hypoxia up-regulate PD-L1 expression via HIF-1 α in glioma cell lines and combination treatment with HIF-1 α inhibitor and anti-PD-L1 antibody can reduce tumor growth in murine model of glioma. **a** qPCR analysis of HIF-1 α and PD-L1 mRNA expression in U251 and U343 lines with different treatments as indicated. The qPCR data were normalized to *GAPDH*. The data were presented as mean \pm SEM. *P* values were calculated by unpaired two-tailed Student's *t* tests. $*P < 0.05$, $**P < 0.01$. **b** Western blot analysis of U251 and U343 cells with different treatments using indicated antibodies. **c** Chromatin immunoprecipitation (ChIP) analysis of the PD-L1 promoter in U251 cells using anti-HIF-1 α mAb. The experiments were performed in triplicates and repeated three times. **d** Immunofluorescence staining of HIF-1 α and PD-L1 expression in tumor cells analyzed by confocal microscopy. Representative images are shown. Scale bars, 50 μ m. **e** Mice bearing GL261 cells were divided into the indicated treatment groups. The tumor volumes of mice treated with control, anti-PD-L1 monoclonal antibody, HIF-1 α inhibitor (PX-478), or combined anti-PD-L1 antibody and PX-478 were measured and plotted ($n = 5$). Tumor volume was measured twice weekly. Data are presented as mean \pm SEM. and the statistical significance was determined by two-way ANOVA. **f** Survival from mice receiving the indicated treatments as described in **e**. Statistical significance was determined by log-rank (Mantel-Cox) test. For (e) to (f) $*P < 0.05$, $**P < 0.01$. **g** The HE staining of intracranial tumor and immunohistochemistry analysis of CD8 $^+$ T cells in intracranial tumor from mice receiving control, anti-PD-L1 antibody, PX-478, or anti-PD-L1 antibody and PX-478. **h** Representative flow cytometry analysis and quantification of CD4 $^+$ T, CD8 $^+$ T, CD11c $^+$ DC and CD11b $^+$ myeloid cells populations in GL261 tumors with the indicated treatments ($n = 5$). **i** Quantification flow cytometry analysis of the PD-L1 expression on CD45 $^-$, CD3 $^+$, CD11c $^+$ and CD11b $^+$ cells ($n = 5$). **j** Representative flow cytometry analysis and quantification of CD8 $^+$ INF- γ $^+$ T cells in GL261 tumors and the MFI of INF- γ in CD8 $^+$ T cells in U261 tumors at day 14 after treatment ($n = 5$). For (h) to (j), data are presented as means \pm SEM. *P* values were calculated by unpaired two-tailed Student's *t* tests. $*P < 0.05$, $**P < 0.01$



(Fig. 1b). These findings were consistent with the external central nervous system tumors [7, 8].

Next, we investigated the correlation of PD-L1/HIF-1 α expression and the OS of these glioma patients. The OS in either PD-L1 or HIF-1 α positive group was significantly poorer than that in negative group (Fig. 1c). Subsequently, we classified all the patients with combining PD-L1 and HIF-1 α expression into four subgroups. The Kaplan–Meier curves indicated that the patients in PD-L1(+) HIF-1 α (+) group had worse OS than those in PD-L1(–) HIF-1 α (–) group ($P < 0.0001$) (Fig. 1c). Univariate analysis identified PD-L1 $\geq 5\%$, HIF-1 α $\geq 1\%$, HGG, and older age as unfavorable prognostic predictors (Additional file 6: Table S3). Multivariate analysis was also performed and indicated that both PD-L1 and HIF-1 α expression were independent poor prognostic factors (Additional file 6: Table S3). We also found the consistent results in primary glioma patients in CGGA dataset (Additional file 2: Fig. S2).

To further verify the relationship between PD-L1 and HIF-1 α , we cultured U251 and U343 glioma cell lines under hypoxic condition for different time and detected the PD-L1 expression. The western blot results showed higher HIF-1 α and PD-L1 levels in hypoxia condition (1% O₂) for either 24-, 48-, or 72-h culturing than those in control condition (21% O₂) (Additional file 3: Fig. S3). Given these data, we used 1% O₂ (72 h) as the hypoxia condition for further experiments. Similarly, we observed that PD-L1 expression was also increased with the hypoxia mimic CoCl₂ treatment (Additional file 3: Fig. S3).

To further dissect the roles of the HIF-1 α in PD-L1 up-regulation under hypoxia, we first knocked down HIF-1 α using siRNA or inhibited HIF-1 α activity using HIF-1 α inhibitor (PX-478) and then detected PD-L1 expression. The results showed that either HIF-1 α knockdown or PX-478 treatment can significantly decrease PD-L1 expression in glioma cells under hypoxia (Fig. 2a, b, Additional file 7: Table S4 and Additional file 8: Table S5). Given that HIF-1 α protein can activate its target genes via directly binding to their promoter [8, 9], we verified whether PD-L1 is a direct target of HIF-1 α in glioma cells using ChIP-qPCR assay. The results showed that HIF-1 α directly interacts with the PD-L1 promoter region (~0.5 kb proximal to the transcription start site) (Fig. 2c and Additional file 9: Table S6). Furthermore, the co-staining PD-L1 and HIF-1 α in glioma murine model showed that PD-L1 was highly express in hypoxic regions of tumors (Fig. 2d). These suggest that hypoxia upregulated PD-L1 via increasing HIF-1 α in glioma cells.

We hypothesized that combining anti-PD-L1 and HIF-1 α inhibitor would trigger an antitumor effect. Thus, we inoculated GL261 cells into wild type mice

and treated the mice with anti-PD-L1 antibody and/or HIF-1 α inhibitor. The combination treatment exerts a more pronounced antitumor effect, assessed in terms of both tumor growth and survival, than each monotherapy (Fig. 2e, f). Of interest was that, in situ glioma model (Luci⁺GL261), PX-478 can also enhance the intracranial efficacy of anti-PD-L1 antibody (Fig. 2g). Immunologically, our FACS results showed that the combination treatment significantly increased the percentage of tumor-infiltrated CD4⁺ T, CD8⁺ T, CD11c⁺ DC (Fig. 2h) and also decreased PD-L1 expression (Fig. 2i). Moreover, we also found the increased numbers of cytotoxic CD8⁺ T cells (IFN γ ⁺CD8⁺) (Fig. 2j). Collectively, these indicate that combination treatment can reverse the immunosuppression microenvironment in glioma.

Our study demonstrated that positive relationship between HIF-1 α and PD-L1 in glioma and provide the evidence that targeting HIF-1 α can boost anti-PD-1/PD-L1 efficacy for glioma treatment.

Abbreviations

HIF-1 α : Hypoxia inducible factor-1 α ; PD-L1: Programmed death ligand-1; PD-1: Programmed death-1; LGG: Low grade glioma; HGG: High grade glioma; OS: Overall survival; IHC: Immunohistochemical; CGGA: Chinese Glioma Genome Atlas; ChIP-qPCR: Chromatin immunoprecipitation coupled with quantitative PCR; DC: Dendritic cell; FACS: Fluorescence activated Cell Sorting.

Supplementary Information

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Additional file 1: Fig. S1. The relationship of PD-L1 and HIF-1 α mRNA expression in glioma samples from the Chinese Glioma Genome Atlas (CGGA) dataset. **a.** The relationship of PD-L1 expression and different clinical factors, including grade, IDH-1 mutant, MGMT methylated status and 1p19q deletion status. **b.** The relationship of HIF-1 α expression and different clinical factors, including grade, IDH-1 mutant, MGMT methylated status and 1p19q deletion status. **c.** Correlation analysis of PD-L1 and HIF-1 α expression in glioma patients. **d.** Correlation analysis of PD-L1, HIF-1 α expression and different clinical factors in glioma patients.

Additional file 2: Fig. S2. The impact of PD-L1 and HIF-1 α mRNA expression on the overall survival (OS) in primary or recurrent glioma patients in CGGA dataset. **a-b.** the OS of patients with primary or recurrent glioma in CGGA dataset that was stratified by high versus low PD-L1 (a) or HIF-1 α level (b). **c.** Correlation analysis of PD-L1 and HIF-1 α expression in primary and recurrent glioma patients.

Additional file 3: Fig. S3. Western blot analysis and quantification of PD-L1 and HIF-1 α expression in glioma cell lines. **a.** Western blot analysis of U251 cell line under 21% O₂ (72 h), 1% O₂ (24 h), 1% O₂ (48 h), 1% O₂ (72 h) and hypoxia mimic CoCl₂ (24 h), respectively using anti-PD-L1 antibody and HIF-1 α inhibitor. **b.** Quantification analysis of PD-L1 and HIF-1 α expression in U251 cells (as a). **c.** Western blot analysis of U343 cell line with indicated treatments using anti-PD-L1 and anti-HIF-1 α antibodies. **d.** Quantification analysis of PD-L1 and HIF-1 α expression in U343 cells (as c). The data were presented as mean \pm SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Additional file 4: Table S1. Patient and tumor characteristics.

Additional file 5: Table S2. The expression of PD-L1 and HIF-1 α in different grades of glioma patients.

Additional file 6: Table S3. Univariate and multivariate Cox regression of glioma patients.

Additional file 7: Table S4. Primers used in qRT-PCR.

Additional file 8: Table S5. siRNA Target Sequences.

Additional file 9: Table S6. Primers used in ChIP-qPCR.

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Authors' contributions

MH, JY and RRW designed of the work and provided the financial support. XD performed all experiments and data analysis with help from LW, PL, LX, ZZ and JX for doing experiments and from JY and XZ for analyzing the patient data. XD was a major contributor in writing the manuscript. LW and HL revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All supporting data are included in the manuscript and supplemental files. Additional data are available upon reasonable request to corresponding authors.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Shandong Cancer Hospital. All patients who provided clinical specimens signed the written informed consent form. All the mice were used in accordance with the animal experimental guidelines set by the Institute of Animal Care and Use Committee of the Shandong Cancer Hospital.

Consent for publication

All the authors have signed the form of consent to publication.

Competing interests

The authors declare that there is no competing interests.

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References

1. Filley AC, Henriquez M, Dey M. Recurrent glioma clinical trial, Check-Mate-143: the game is not over yet. *Oncotarget*. 2017;8(53):91779–94.
2. Kurz SC, Wen PY. Quo vadis-do immunotherapies have a role in glioblastoma? *Curr Treat Options Neurol*. 2018;20(5):14.
3. Hu M, Zhu Y, Mu D, Fan B, Zhao S, Yang G, et al. Correlation of hypoxia as measured by fluorine-18 fluoroerythronitroimidazole (18F-FETNIM) PET/CT and overall survival in glioma patients. *Eur J Nucl Med Mol Imaging*. 2020;47(6):1427–34.
4. Akinleye A, Rasool Z. Immune checkpoint inhibitors of PD-L1 as cancer therapeutics. *J Hematol Oncol*. 2019;12(1):92.
5. Yi M, Niu M, Xu L, Luo S, Wu K. Regulation of PD-L1 expression in the tumor microenvironment. *J Hematol Oncol*. 2021;14(1):10.
6. Xue S, Hu M, Iyer V, Yu J. Blocking the PD-1/PD-L1 pathway in glioma: a potential new treatment strategy. *J Hematol Oncol*. 2017;10(1):81.
7. Barsoum IB, Smallwood CA, Siemens DR, Graham CH. A mechanism of hypoxia-mediated escape from adaptive immunity in cancer cells. *Can Res*. 2014;74(3):665–74.
8. Noman MZ, Desantis G, Janji B, Hasmim M, Karray S, Dessen P, et al. PD-L1 is a novel direct target of HIF-1 α , and its blockade under hypoxia enhanced MDSC-mediated T cell activation. *J Exp Med*. 2014;211(5):781–90.
9. Masoud GN, Li W. HIF-1 α pathway: role, regulation and intervention for cancer therapy. *Acta Pharm Sin B*. 2015;5(5):378–89.

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