The Change of Soluble Programmed Cell Death-Ligand 1 (sPD-L1) in Glioma Patients Receiving Radiotherapy and Its Impact on Clinical Outcome

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Abstract

Background: It has been proved that the levels of soluble programmed death-ligand 1 (sPD-L1) are associated with prognosis in extracranial malignancies. However, the expression of sPD-L1 in glioma patients receiving radiotherapy (RT) still remains unclear. The purpose of this study is to evaluate the concentration of sPD-L1 in the plasma of glioma patients before and after RT, and to explore its relationship with clinical outcomes.

Methods: Between October 2017 and September 2018, the glioma patients treated with RT (30 ± 10 Gy, 2 Gy/f) were enrolled and the blood samples were collected before and after RT. We quantified the sPD-L1 levels by enzyme-linked immunosorbent assay (ELISA). The isocitrate dehydrogenase-1 (IDH-1) promoter status and Ki-67 expression were evaluated by immunohistochemistry. The murine models of glioma were used to address whether circulating sPD-L1 molecules are directly targeted by the anti-PD-L1 antibody. The associations between sPD-L1 and clinical features were assessed with Pearson or Spearman correlation. The progression-free survival (PFS) and overall survival (OS) were determined by Kaplan-Meier method.

Results: Sixty glioma patients were included, with the median age 52-year-old. The proportion of grade I, II, III, IV were 6.7%, 23.3%, 28.4% and 41.6%, respectively. The baseline sPD-L1 levels were significantly associated with tumor grades, IDH-1 mutation status and Ki-67 expression. Using 14.35 pg/mL as the cutoff, significantly worse PFS and OS were both observed in patients with higher baseline level of sPD-L1 ($P = 0.027, 0.008$, respectively). RT significantly increased the mean level of sPD-L1 ($P < 0.001$). Further analysis showed that increased level of sPD-L1 in IDH-1 mutation patients was higher than that in wild-type ones. Furthermore, the murine models of glioma indicate that sPD-L1 can be blocked by anti-PD-L1 antibody.

Conclusion: This study reported that sPD-L1 might be a potential biomarker to predict the outcome in glioma receiving RT. The elevated level of sPD-L1 after RT suggested that the strategy of combination with immune checkpoint inhibitors and RT might be promising for glioma, especially for patients with IDH-1 mutation.

Introduction

Malignant gliomas are the most common type of primary brain tumors or central nervous system (CNS) tumors, with approximately 36% of 5-year over survival (OS) (1). Pathologically, glioma is categorized into grade I-IV according to World Health Organization (WHO) criteria. Grade IV glioblastoma (GBM), accounted for the majority of gliomas (56.6%) and it is the most aggressive advanced tumor with only 5.6% of 5-year relative survival estimates (1). Despite neurosurgical resection and adjuvant radio- and chemotherapy may prolong patients' survival times, most gliomas patients relapse and limit life expectancy. Over the past decades, there were few progresses in the treatment guidelines in gliomas. Considering the lack of effective treatments for gliomas, immunotherapy, especially block programmed death-1 (PD-1) / programmed death-ligand 1 (PD-L1) axis by antibodies, has brought a new hope in intracranial cancers (2–4).

Anti-PD-1/PD-L1 immunotherapy have shown clinical efficacies against many different solid tumor types and hematological malignancies (4–6). Clinical trials of anti-PD-1/PD-L1 immunotherapy for glioma are relatively delayed (7), largely remaining on phase II (e.g., NCT01952769, Pidilizumab) and phase III (e.g., NCT02017717, Nivolumab). Only one phase III clinical trial - Checkmate 143 has been completed, however, reporting that nivolumab (anti-PD-1 antibody) did not exhibit increased survival benefits over bevacizumab (8). It seems that the PD-1/PD-L1 axis only plays a role in the malignant biological behavior of glioma, while other molecular signaling networks may also play indispensable roles. Radiation, is commonly used to treat gliomas patients and has been identified to activate immune responses. Thus, researchers tried to explore the clinical efficacy of immunotherapy (nivolumab) + RT $\pm$ temozolomide (TMZ) in newly diagnosed glioblastoma patients in some ongoing phase III clinical trials, including Checkmate 498 (NCT02617589) and Checkmate 548 (NCT02667587). However, challenges remain to be addressed to maximize the efficacy of this promising

combination. One of them is identifying biomarkers to assess the dynamic changes in the immune system at the patient level undergoing RT.

Briefly, characteristics of the ideal biomarker should include: noninvasively accessible, stable *in vivo* and after collection, cost-effective and prognostic or predictive. To date, a number of candidate biomarkers of immune response during and after RT, both circulating (for example, circulating cytokines and other proteins associated with inflammatory and immune responses) and cellular (for example, circulating and tumor-infiltrating lymphocytes), have been reported (9–13). Among them, increasing evidence suggested that the expression of soluble PD-L1 (sPD-L1) in the blood was significantly associated with prognosis in glioma and several extracranial malignancies (14–16). A recent study showed that the elevated circulating and cerebrospinal fluid sPD-L1 levels are associated with aggressive biological activities in patients with gliomas (17). Zhang et al. reported that the levels of sPD-L1 were significantly correlated with abdominal organ metastasis in patients with advanced non-small cell lung cancer (NSCLC) ($P = 0.004$) (14). Similarly, a study about hepatocellular carcinoma (HCC) found that high serum sPD-L1 concentrations can increase mortality risk ($P < 0.001$) (16). Even the findings of Ugurel' study indicated sPD-1 and sPD-L1 could as useful biomarkers to predict the outcome of PD-1 inhibition therapy in melanoma patients (18). These preliminary results prompted us to further investigate the application of sPD-L1 in the treatment of tumors.

Given the limited evidence that sPD-L1 maybe a biomarker to predict response to immunotherapy in glioma. Our study was performed to evaluate plasma concentrations of sPD-L1 before and after radiotherapy (RT) in glioma patients, and to investigate its relationships with clinical outcomes. We hypothesized that circulating sPD-L1 molecules in the blood would deliver systemic inhibitory messages that could globally adversely impact anti-tumoral immune responses.

Materials And Methods

Patients

In this study, all patients had been histologically or cytologically diagnosed glioma or postsurgical recurrent glioma at Shandong Cancer Hospital Affiliated to Shandong University and Shandong Academy of Medical Sciences. Who treated with RT for glioma at our institution between October 2017 and September 2018 were prospectively recruited.

The study protocol was approved by the Ethics Committee of the Shandong Cancer Hospital and all patients gave their written informed consent prior to study inclusion. The optimal treatment option was determined through a multidisciplinary tumor board in accordance with our institution's treatment policy. RT was performed using conventional fractionated RT. It was considered in patients with an individual basis, with a total dose of 54 - 60 Gy in 30 fractions (f). Concurrent peroral chemotherapy with TMZ was administered at 75 mg/m² daily during the RT patients. Bevacizumab combine with CCRT was administered 10 mg/kg (2 weeks repeat). If the patient needs surgery firstly, RT should begin within 8 weeks after surgery. After the scheduled treatment was finished, regular follow-up was conducted every 3 months with imaging studies and tumor markers.

Blood sampling

Blood (5-10 mL) was collected from the patients before RT (0 Gy) and after RT (30 ± 10 Gy, 2 Gy/f), by use of aseptic tubes containing EDTA (5 mL). The blood samples were centrifuged at 2000 rpm for 10 minutes at 4°C to separate the plasma. Additional centrifugation for 10 minutes was performed to produce cell-free plasma, after which the plasma aliquots were immediately frozen at -80°C for the further analysis.

Soluble PD-L1 measurement

Patients' sPD-L1 level was measured using the human PD-L1 simple-step enzyme-linked immunosorbent assay kit (ab214565, Abcam, USA). In brief, 50µL of standards at different concentrations and patient plasma samples were added to the wells. Subsequently, 50µL of PD-L1 conjugated antibody was added, incubated for 1h at room temperature and then washed for 3 times. Next, substrate solution was used for the color reaction, which was stopped with stop solution, and the absorbance was immediately measured at 450nm using an enzyme-linked immunosorbent assay reader (VERSA max microplate reader; CA, USA). The sPD-L1 level was calculated according to standard curves. The minimum detectable concentration of sPD-L1 was 2.91pg/mL.

Immunohistochemistry and molecular pathology

The immunohistochemistry (IHC) sample slides were reviewed by two neuropathologists, and a systematic neuropathological review was based on the 2007 WHO classification of CNS tumors. Tumor tissue was formalin fixed and paraffin embedded according to standard laboratory practice. Specimens were stained with antibodies against isocitrate dehydrogenase-1 (IDH-1) R132H (clone H09, 1:50 dilution; Maxim, China) and Ki-67 (MIB1, Santa Cruz, Shanghai, China, 1:50 dilution), respectively. Cells with pale brown granular deposits were considered to be IDH-1 mutational status and Ki-67 positive. Ki-67 index is the percentage of positive cells in the densest visual field. IHC analyses were then performed with a quantitative approach under a light microscope.

Murine models of glioma

C57BL/6 mice (6-8 weeks) maintained in the SPF level lab and were subcutaneously injected with 1×10^6 cells (GL261 cells) in the right flank of mice (day 0). When the tumor reaches a volume of approximately 100 mm³ (approximately 10 days), tumors received one dose of 20Gy ionizing radiation, and/or 200µg anti-PD-L1 (clone 10F.9G2) or isotype control antibody. The sPD-L1 level in the plasma was measured using the mouse PD-L1 DuoSet ELISA (DY1019-05, R&D Systems, USA) before RT and after RT. Tumor volume was measured twice weekly with calipers, and tumor volume was approximated using the equation for an ellipsoid: $abc/2$. Mice were sacrificed when tumors reach 2,000mm³. All experiments related to animals were strictly performed in accordance with guidelines approved by the Ethics Committee of the Shandong Cancer Hospital.

Statistical analysis

Continuous variables are shown as mean $\pm$ standard deviation and the minimum-maximum range. The differences between the two groups were calculated using the t-test or Mann-Whitney U-test according to the normality of the data. The Kruskal-Wallis test was used for comparison of three or more groups. The *post-hoc* Bonferroni test was used for multiple comparisons. Correlations between the sPD-L1 level and clinical factors were analyzed using the Pearson correlation analysis or using Spearman correlation analysis for continuous variables. Chi-squared test or Fisher's exact test were used for categorical variables. The receiver-operating characteristic (ROC) curve analysis was used to determine the optimal cut-off value of sPD-L1 and Ki-67 expression rates. The survival duration was calculated from the date of disease diagnosis (RT start) to the corresponding event. The Kaplan-Meier method with the log-rank test was used to compare survival between groups. Multivariable analysis was carried out by Cox regression hazard model. The dynamics of sPD-L1 in the serum were analyzed by the mixed-model approach. All statistical tests were two-sided, *P* values <0.05 were considered to be significant. All data were analyzed using IBM SPSS software version 22.0 (IBM, New York, USA). Figures were made by GraphPad Prism version 5.00 (San Diego, California, USA).

Results

Patient characteristics and survival outcome

From September 2017 to April 2019, 60 glioma patients who had measurable tumors and received RT in Shandong Cancer Hospital were enrolled. Out of them, 33 were female and 27 were male, with median age 52 years old (range, 18 - 75). Fifty-two out of 60 patients received pathologic diagnosis (20 with subtotal resections and 32 with tumor biopsies), the other 8 patients were diagnosed as glioblastoma by radiologic findings based on the current guideline. There were 25 patients (41.6%) with pathological grade IV, 17 patients (28.4%) with grade III, 14 patients (23.3%) with grade II and 4 patients (6.7%) with grade I. Of the 60 patients in our study, 42 (70%) patients received RT plus TMZ (CRT), 10 (16.7%) patients received RT plus both TMZ and bevacizumab (CRT+T) and the other 8 patients only received RT. The clinical baseline characteristics and outcomes of 60 gliomas patients were systematically reviewed and the results are summarized in Table 1.

Association between the baseline sPD-L1 levels and clinical factors

To investigate the association between the baseline sPD-L1 and clinical factors, we measured the sPD-L1 levels in plasma of 60 patients before radiation therapy and also detected the IDH-1 promoter status of 40 patients and the expression of Ki-67 of 44 patients by IHC. The mean levels of baseline sPD-L1 is 47.39 ± 59.01 pg/mL (range, 0 - 283.13 pg/mL). Spearman correlation analysis showed that the baseline sPD-L1 level was positively associated with tumor grade ($r = 0.495$, $P < 0.001$), IDH-1 mutational status ($r = 0.379$, $P = 0.016$) and Ki-67 expression rate ($r = 0.434$, $P = 0.003$). With the increase of glioma stage, the mean level of baseline sPD-L1 tended to increase (stage I: 8.18 ± 2.70 pg/mL; stage II: 10.52 ± 18.35 pg/mL; stage III: 29.65 ± 24.23 pg/mL and stage IV: 60.60 ± 65.95 pg/mL, Fig. 1A). Compared to patients with IDH-1 wild-type (WT) tumor, patients with IDH-1 mutation (MUT) tumor showed markedly lower levels of baseline sPD-L1 in plasma (17.28 ± 24.59 pg/mL vs. 61.18 ± 64.30 pg/mL, Fig.1B). In addition, we found that the sPD-L1 level was higher in patients with Ki-67 $> 27.5\%$ than those with Ki-67 $\leq 27.5\%$ (82.58 ± 70.77 pg/mL vs. 24.68 ± 27.89 pg/mL, Fig.1C). As expected, there were no significant associations between sPD-L1 levels and other factors *e.g.* sex, age, karnofsky performance score (KPS), Numerical Rating Scale (NRS), Nutritional Risk Screening 2002 (NRS 2002), location of the tumor.

Correlation between baseline sPD-L1 level and clinical outcomes

The median follow-up duration was 28.7 (range, 5.4 - 38.7) months. Twenty-three/60 (38.3%) patients underwent disease progression and 22/60 (36.7%) patients died within the observation time. The median OS and progression-free survival (PFS) were 28.7 months and 23.2 months, respectively. In order to evaluate the predictive value of the baseline sPD-L1 level for survival, a cut-off value of 14.35 pg/mL was obtained using receiver-operating characteristic ROC curve analysis (AUC = 0.73; $P = 0.003$). Thirty-three patients (55%) had high sPD-L1 levels more than 14.35 pg/mL, and the other 27 patients had low sPD-L1 level (< 14.35 pg/mL). Significantly worse median OS was noted in patients with higher baseline sPD-L1 level than those with lower ones (23.1 vs. 28.7 months, $P = 0.008$; Fig.2A). Additionally, patients with decreased sPD-L1 after RT had significantly worse median OS (20.8 vs. 29.5 months, $P = 0.040$) (Fig. 2B). Some other factors including IDH-1 WT ($P = 0.036$), GBM ($P = 0.010$), tumor locate in brainstem ($P = 0.001$) and Ki-67 expression rate $\geq 27.5\%$ ($P = 0.001$) can also affect the OS of patients (Fig. 2C-F). In this study, the change of sPD-L1 levels, IDH-1 promoter status and tumor position were the independent prognostic factors ($P = 0.003$, HR = 0.019, 95% CI: 0.001 - 0.268; $P = 0.011$, HR = 0.029, 95% CI: 0.002 - 0.448; $P = 0.002$, HR = 26.302, 95% CI: 3.239 - 213.550) (Table 2). However, the baseline sPD-L1 level was not an independent prognostic factor for glioma patients ($P = 0.516$, HR = 2.231, 95% CI: 0.198 - 25.126). For PFS, patients with sPD-L1 concentrations above 14.35 pg/mL had a worse PFS than those with low sPD-L1 levels (20.4 vs. 26.7 months, $P = 0.027$; Fig. 3A), whereas it wasn't proved to be an independent prognostic factor for glioma patients in the multivariate analysis. High tumor grade was a poor prognostic factor for PFS both in univariate analysis ($P < 0.001$) (Fig. 3D) and multivariate analysis. ($P = 0.001$, HR = 3.091, 95% CI: 1.592 - 6.002) (Table 2).

Radiation induces sPD-L1 levels in gliomas patients

To explore whether radiation can induce sPD-L1 levels, we also measured the sPD-L1 levels in plasma sample of 51 out of 60 patients after RT (30 ± 10 Gy, 2 Gy/f), and found that the mean sPD-L1 levels after RT were significantly higher than their baseline sPD-L1 levels before RT (57.21 ± 60.95 pg/mL vs. 36.65 ± 49.77 pg/mL, $P < 0.001$) (Fig.4A). In details, sPD-L1 levels were increased in 31 patients and were reduced in 20 patients (Fig.4B).

Next, we undertook further analysis to measure other potential factors might influence the sPD-L1 levels. We first assessed the IDH-1 mutational status by pathology analysis. In IDH-1 MUT group, the mean sPD-L1 levels were 17.52 ± 25.50 pg/mL before RT, 36.60 ± 39.66 pg/mL after RT, while they were 66.40 ± 66.55 pg/mL before RT, 70.32 ± 68.96 pg/mL after RT in IDH-1 WT group. The baseline sPD-L1 level was significantly higher in IDH-1 WT group compare with IDH-1 MUT group ($P = 0.016$), however there was no statistical significance after RT between two groups ($P = 0.107$) (Fig.1A). These results showed that sPD-L1 levels tended to increase in these two groups after RT, whereas the fold-change in IDH-1 MUT group seems more prominent. Conversely, in treatment scheme subgroup analysis, the results showed that there were no significant differences in sPD-L1 levels among RT, CRT and CRT+T groups either before or after treatment (41.17 ± 43.85 pg/mL, 27.04 ± 30.96 pg/mL vs. 50.73 ± 81.71 pg/mL, $P = 0.332$; 56.69 ± 47.52 pg/mL, 42.18 ± 53.46 pg/mL vs. 71.56 ± 95.54 pg/mL, $P = 0.432$. Fig.1D). Thus, the addition of chemotherapy and bevacizumab with RT seems no further influence the levels of sPD-L1 in this study.

Anti-PD-L1 antibody could reduce the expression of sPD-L1

To address whether circulating sPD-L1 molecules are directly targeted by the anti-PD-L1 antibody, we performed in vivo studies using the murine model of glioma treated with RT (20 Gy) alone, anti-PD-L1 alone and RT plus anti-PD-L1 groups. We found that there was no difference in baseline sPD-L1 expression levels in different groups (1.58 ± 0.315 pg/mL, 1.69 ± 0.24 pg/mL vs. 1.18 ± 0.51 pg/mL, $P = 0.227$, Fig. 5B). In line with the clinical data, an increase in the expression of sPD-L1 was observed after radiation compared with expression levels in the nonirradiated control group (16.68 ± 11.22 pg/mL vs. 28.50 ± 11.18 pg/mL, $P = 0.031$, Fig. 5B). Notably, the concentration of sPD-L1 can't be detected in both anti-PD-L1 alone group and RT plus anti-PD-L1 group (Fig. 5B) suggesting that sPD-L1 can be blocked by PD-L1 antibody, and led to significantly downregulation of sPD-L1. Although either RT or anti-PD-L1 alone can affect the tumor growth, the combination of RT plus anti-PD-L1 more effectively controlled tumor growth (anti-PD-L1 vs. RT plus anti-PD-L1: 789.67 ± 55.86 mm³ vs. 292.16 ± 102.98 mm³ on day 31, $P < 0.001$; RT vs. RT plus anti-PD-L1 = 697.02 ± 12.98 mm³ vs. 292.16 ± 102.98 mm³ on day 31, $P < 0.001$) (Fig. 5C).

Discussion

More recently, circulating sPD-L1 in the blood have been discovered in various malignancies. However, few studies have been reported sPD-L1 expression in patient with glioma until now (17). To further explore the existence of sPD-L1 and evaluate the pathological significance of this circulating factor in human cancer, we developed this study for the detection and quantification of sPD-L1 in glioma patients receiving RT. In the present study by using ELISA formats, we detected about 90% of glioma patients expressed sPD-L1 in the plasma before RT. Further, we determined that RT could up-regulate the sPD-L1 levels compare with their baseline levels. In addition, the high level of sPD-L1 and some other clinical factors, such as IDH-1 WT, GBM, tumor locate in brainstems or Ki-67 expression rate $\geq 27.5\%$, were demonstrated to be related to poor prognosis in glioma patients. Using the murine models of glioma, our data showed that sPD-L1 level could be significantly declined by the anti-PD-L1 antibody.

PD1/PD-L1 axis is associated with tumor microenvironment as a regulator of inhibitory signals, and its expression could be a candidate biomarker for patient selection for anti-PD1/PD-L1 monoclonal. Aberrant PD-L1 expression has been already reported to occur in glioma tumor tissues basing on IHC (19). Consider that the sPD-L1 level may associate with the tumor burden and the aggressive biological activities of tumors, we investigated whether there are associations between the baseline level of sPD-L1 and the tumor grade or the Ki-67 expression, and finally demonstrated that the

baseline level of sPD-L1 was significantly elevated in patients with advanced brain tumors or patients with Ki-67 > 27.5%. Ki-67 is one of the most widely used markers for proliferation in clinical practice and has been validated as a marker of in the initial phase of adult neurogenesis (20). Although the mechanistic remains unclear, we speculated that mostly sPD-L1 may shed from surface of cells in tumor by the cleavage of membrane-bound proteins, and are found free in the plasma. In addition, the circulating sPD-L1 could led to the immune tolerance, consequently, neoplastic cells would have no limits to proliferation. Therefore, sPD-L1 may be considered to exist from the early stage during glioma progression. In recent years, distinct molecular classes of gliomas have been identified. It is well established that IDH-1 MUT and IDH-1 WT gliomas have distinct tumor behavior driven by different oncogenic signals and respond differently to current treatment paradigms. Notably, by comparing the immune responses between IDH-1 MUT and IDH-1 WT patients, some studies have been identified that a marked reduction in expression of immune related genes, including the *CD274* (PD-L1 coding gene) gene, in IDH-1MUT gliomas(21–23). The results were in line with ours, the downregulated sPD-L1 levels tended to occur in the glioma patients with IDH-1 MUT. Collectively, these findings may suggest that immunological tumor microenvironment may differ according to IDH mutational status in glioma.

In addition to the association with some clinical factors, sPD-L1 levels might predict the survival outcomes in cancer patients, however its prognostic relevance was contradictory in different cancers. In gastric adenocarcinoma, elevated levels of sPD-L1 were associated with a favorable prognosis (65.6% vs. 44.7%, $P = 0.028$) (24). Inversely, studies in natural killer/T-cell lymphoma (NKTCL), aggressive renal cell carcinoma (RCC), NSCLC, large B-Cell lymphoma, HCC and nasopharynx cancer (NPC) demonstrated that patients with high concentration of sPD-L1 exhibited markedly worse survival than patients with lower concentration (16, 25–28). In current study, we observed that high baseline levels of sPD-L1 (> 14.35 pg/mL) in glioma patients were correlate with not only poorer OS (23.1 vs. 28.7 months, $P = 0.008$) but also PFS (20.4 vs. 26.7 months, $P = 0.027$) in univariate analysis. The biological reason why sPD-L1 is more strongly associated with survival outcomes has to be further elucidated. It is very possible that as sPD-L1 spreads throughout the body via the blood and lymphatic circulation, it exerts a widespread inhibitory effect to the T cells by interacting with cell surface receptors such as membrane-bound PD-1(29–31). In addition, we found that sPD-L1 molecules might represent a direct target of the therapeutic PD-L1 antibodies. The described functions might work as escape mechanisms from immune-surveillance and/or result in an impairment of anti-PD-1/PD-L1-directed antibody therapy, and thus translate into prognostic and/or predictive factors in cancer patients. Altogether, the quantification of circulating sPD-L1 may also be of use as a predictive marker of anti-PD-1 treatment outcome, helping the clinician to select patients for PD-1/PD-L1-based therapy strategies. Unfortunately, since the sample size of the present study was limited, the multivariate analysis in this study didn't reveal that sPD-L1 was an independent risk factor of the survival for glioma patients ($P = 0.283$). Thus, further studies with a large number of patients are required to clarify our finding.

Next, we attempted to uncover the dynamics of circulating sPD-L1 levels in glioma patients undergoing RT. It is known that RT cloud complicate the interpretation of the immune landscape in patients. The ultimate goal of combining immunotherapy with RT is to achieve a long-lasting, therapy-induced immune response at all sites of disease, assessment of the dynamic changes in the immune system at the patient level is essential. Our group analyzed the changes in the sPD-L1 level before and following RT, and found that RT significantly increased the sPD-L1 expression in both patients with glioma and murine models of glioma. Similarly, Hyun et al. has reported that RT significantly increased the sPD-L1 expression in patients with HCC (32). However, most investigations have only focused on the PD-L1 level at baseline, and data on changes in PD-L1 expression after RT are still extremely limited.

In sub-analyzed of this study, we also noticed that sPD-L1 levels were increased significantly in IDH-1 MUT patients compare with IDH-1 WT patients. To our best knowledge, it is the first prospective study to evaluate the sPD-L1 levels following RT in glioma patients with different IDH mutational status. It can be explained by that IDH-1 mutation could apparently improve the tumors' sensibility to radiotherapy, then cells in tumor which killed by RT could release a mass of sPD-L1 in the blood. However, we also found that adding chemotherapy or/and bevacizumab to RT didn't further

upregulate the increase of sPD-L1 compared with RT only. We suspect that RT as a local therapy have been provide sufficient damage to the tumor target, chemotherapy or/and bevacizumab probably were no room to further improve the efficacy with the combined modality of RT in the primary tumor. Taken together, the sPD-L1 level increased after RT suggested that RT combined with immune checkpoint inhibitors might be better than use RT alone for glioma patients, especially for patients with IDH-1 MUT. Further well-designed studies are needed to clarify the optimal RT scheme, dose, and time for combination.

Regarding the limitation of this study: First, this study analyzed a limited sample size. Second, the timing of blood sampling was only on 30 ± 10 Gy after RT, and we could hence not determine whether the sPD-L1 level will change after more dose RT. Third, some patients underwent partial excision and the others only underwent biopsy, which might affect the sPD-L1 level.

Conclusion

In conclusion, this study reported that sPD-L1 can be assayed in the plasma of glioma patients. It maybe means that compensating for potential tie-up of antibody needs to be considered in optimizing PD-L1 blockade therapies. Because, not all administered anti-PD-L1 immunotherapeutic antibody may reach the surface of tumor cells, with a potentially appreciable proportion being sequestered by sPD-L1 within the circulation. In addition, the baseline level of sPD-L1 might be a potential marker to predict the outcome in glioma. Which is really remarkable, after all predictive biomarkers discriminating responders from non-responders already at therapy baseline are scarce. Finally, the elevated level of sPD-L1 after RT suggests that the strategy of combination with immune checkpoint inhibitors and RT is may be a promising therapeutic strategy in glioma, especially for patients with IDH-1 MUT.

Abbreviations

RT: radiotherapy

CNS: central nervous system

OS: over survival

WHO: World Health Organization

GBM: glioblastoma

PD-1: programmed death-1

PD-L1: programmed death-ligand 1

TMZ: temozolomide

sPD-L1: soluble programmed death-ligand

NSCLC: non-small cell lung cancer

HCC: hepatocellular carcinoma

IHC: immunohistochemistry

ROC: receiver-operating characteristic

CRT: radiotherapy add temozolomide

CRT+T: radiotherapy add temozolomide and bevacizumab

IDH-1: isocitrate dehydrogenase-1

WT: wide type

MUT: mutation

KPS: karnofsky performance score

PFS: progression-free survival

NPC: nasopharynx cancer

NKTCL: natural killer/T-cell lymphoma

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the Shandong Cancer Hospital

Consent for publication

All patients gave their written informed consent prior to study inclusion.

Availability of data and materials

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Xing-chen Ding was a major contributor in writing the manuscript. Liang-liang Wang and Yu-fang Zhu analyzed and interpreted the patient and animal data. Xing-chen Ding, Xin-bin Bai and Yang Jia do the experiments in the manuscript. Jin-ming Yu and Man Hu designed of the work and provided the financial support. All authors read and approved the final manuscript.

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Tables

Table 1
Patient characteristics and outcomes

Parameter	Patients
Epidemiology	
Patients, n	60
Gender, m/f (%)	27/33 (45/55)
Age, median, range	52, 18–75
Karnofsky performance score, median, range	90, 60–90
Numerical Rating Scale (pain measurement), median, range	0, 0–6
Nutritional Risk Screening 2002, median, range	1, 0–3
Pathological grading	
I n (%)	4 (6.7)
II n (%)	14 (23.3)
III n (%)	17 (28.4)
IV n (%)	25 (41.6)
Tumor position	
Left brain, n (%)	27 (45)
Right brain, n (%)	11 (18.3)
Brain stem, n (%)	9 (15)
Other, n (%)	13 (21.7)
IDH-1 status	
Mutation, n (%)	15 (25)
Wild type, n (%)	25 (41.7)
Unknown, n (%)	20 (33.3)
Ki-67 expression (cut off rate)	
≤ 27.5%, n (%)	28 (46.6)
>27.5%, n (%)	16 (26.7)
Unknown, n (%)	16 (26.7)
Diagnostic style	
Subtotal resection, n (%)	20 (33.3)
Tumor biopsy, n (%)	32 (53.4)
No biopsy, n (%)	8 (13.3)
Type of therapy	
Radiotherapy + TMZ, n (%)	42 (70)

Parameter	Patients
Radiotherapy + TMZ + bevacizumab, n (%)	10 (16.7)
Radiotherapy only, n (%)	8 (13.3)
Follow-up time, median, range	28.7 (5.4–38.7)
Recurrence and/or metastasis at last follow-up	
Yes, n (%)	23 (38.3)
No, n (%)	28 (46.7)
Unknown, n (%)	9 (15)
Alive at last follow-up	
Yes, n (%)	38 (63.3)
No, n (%)	22 (36.7)
Unknown, n (%)	0 (0)

Table 2
Univariable and multivariable analyses of OS and PFS in the patients

Parameter	OS				PFS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	<i>P</i>	<i>P</i>	HR	95%CI	<i>P</i>	<i>P</i>	HR	95%CI
Gender (male vs. female)	0.642				0.656			
Age (≤ 52-yr vs. >52-yr)	0.783				0.952			
Grade (I, II, III vs. IV)	0.010*	0.855	1.158	0.242–5.534	< 0.001*	0.001*	3.091	1.592–6.002
IDH-1 (WT vs. MUT)	0.036*	0.011*	0.029	0.002–0.448	0.393			
sPD-L1 (≤ 14.35 pg/mL vs. >14.35 pg/mL)	0.008*	0.516	2.231	0.198–25.126	0.027*	0.617	0.737	0.223–2.435
Ki-67 (≤ 27.5% vs. >27.5%)	0.001*	0.846	0.824	0.117–5.818	0.066	0.635	1.278	0.464–3.524
Position (Brain hemispheres vs. Brainstem)	0.001*	0.002*	26.302	3.239–213.550	0.876			
Treatment (RT vs. RT + chemotherapy ± target)	0.708				0.715			
Change of sPD-L1 (up vs. down)	0.040*	0.003*	0.019	0.001–0.268	0.770			
Factors with $P \leq 0.1$ in univariate analysis can be included in the multivariate analysis.								
Abbreviations: OS = Overall survival; PFS = Progression-free survival; HR = Hazard ratio; CI = Confidence interval								
* means $P \leq 0.05$								

Figures

Figure 1

Correlations of soluble PD-L1 (sPD-L1) levels with: A. Grade (I, II, III and IV), B. IDH-1 mutational status (mutation and wide type), C. Ki-67 ($\leq 14.35\text{ng/mL}$ and $> 14.35\text{ng/mL}$) and D. Treatment method (Radiotherapy (RT), radiotherapy plus chemotherapy (CRT), CRT and beacizumab (CRT+T)) before and after RT. ns, non-significant. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 2

Overall survival (OS) of patients according to: A. The baseline level of soluble PD-L1 1 (sPD-L1) ($\leq 14.35\text{ng/mL}$ vs. $> 14.35\text{ng/mL}$, P = 0.008), B. Changes of sPD-L1 after radiotherapy (up vs. down, P = 0.04), C. IDH-1 mutational status (mutation vs. wide type, P = 0.036), D. Grade (I, II, III vs. IV, P = 0.01), E. Tumor position (brain hemispheres vs. brainstems, P = 0.001) and F. Ki-67 ($\leq 27.5\%$ vs. $> 27.5\%$, P = 0.001).

Figure 3

Progression-free survival (PFS) of patients according to: A. The baseline level of soluble programmed death-ligand 1 (sPD-L1) ($\leq 14.35\text{ng/mL}$ vs. $> 14.35\text{ng/mL}$, $P = 0.027$), B. Changes of sPD-L1 after radiotherapy (up vs. down, $P = 0.770$), C. IDH-1 mutational status (mutation vs. wide type, $P = 0.393$), D. Grade (I, II, III vs. IV, $P < 0.001$), E. Tumor position (brain hemispheres vs. brainstems, $P = 0.876$) and F. Ki-67 ($\leq 27.5\%$ vs. $> 27.5\%$, $P = 0.066$).

Figure 4

Changes in the soluble PD-L1 (sPD-L1) levels after radiotherapy (RT). A. Overall change of sPD-L1 levels in patients (before RT vs. after RT: 36.65 ± 49.77 pg/mL vs. 57.21 ± 60.95 pg/mL, $P < 0.001$); individual change of sPD-L1 levels in the B (increased in 31 patients and reduced in 20 patients). *** $P < 0.001$. Data are presented as mean $\pm$ SEM

Figure 5

The murine models of glioma were divided into radiotherapy (RT) alone (20Gy), anti-PD-L1 alone, RT plus anti-PD-L1 and control groups ($n = 20$). A. The scheme of experiment. B. The soluble PD-L1 (sPD-L1) were measured before and after RT respectively, there was no difference in baseline expression levels in different groups, however RT can upregulate the expression of sPD-L1 and anti-PD-L1 can effectively reduce the expression of sPD-L1. C. Treated tumor was measured every 3-4 days for 21 days starting from the day of IR. ns, non-significant. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Each experiment was repeated 3 times. Data are presented as mean $\pm$ SEM