Topotecan Decreases the Expression of Programmed Death-Ligand 1 in Glioblastoma Cell Lines; Implications for Immunotherapy

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Abstract
Glioblastoma (GBM) is the most aggressive primary brain tumor and thrives in a microenvironment of relative immunosuppression. The poor clinical outcome of these malignant tumors requires the development of novel treatment options/therapeutic regimens. Accordingly, numerous immunotherapies for GBM are currently being tested in ongoing clinical trials. Herein we have examined the ability of the FDA approved drug topotecan to suppress programmed death-ligand 1 (PD-L1) expression. Our results suggest a role for topotecan as an adjuvant therapy in treatment regimens targeting certain GBM patient subpopulations in whom the expression of PD-L1 has been confirmed.

Introduction
Glioblastoma (GBM) is the most common and aggressive primary tumor intrinsic to the central nervous system (CNS) being designated by the World Health Organization (WHO) as a grade IV glioma [1] [2]. The prognosis remains dismal despite a multimodal approach utilizing maximal surgical resection and adjuvant chemoradiation [3]. Therefore, novel therapies are urgently needed to improve outcomes for patients with this highly malignant neoplasm.

Evasion of the immune system is a hallmark of cancer, enabling tumor cells to escape immune surveillance [4]. Accordingly, therapeutic activation of the immune system through a variety of mechanisms is a rapidly expanding field in oncology, and numerous immune-activating agents have been approved by the FDA for cancer treatment over the last several years [5]. GBM tumors create an immunosuppressive microenvironment within the brain, resulting in both evasion of the body’s immune system and support of the growth of cancer [6].

Cancer cells often express immune inhibitory signaling proteins that cause immune cell dysfunction and/or apoptosis. One of these inhibitory molecules is programmed death-ligand-1 (PD-L1), which binds to programmed death-1 (PD-1) expressed on T-cells, B-cells, dendritic cells, and natural killer T-cells to suppress anti-cancer immunity [7]. PD-L1 expression has been confirmed in GBM cells [8] [9] and has been linked to poor outcomes for GBM patients [10] [11]. It is prudent to note that PD-L1 expression has also been reported on microglia/macrophages in GBM specimens [12] [13]. Immune checkpoint inhibition targeting PD-L1 or PD-1 has been found to increase response rates in multiple cancer types [14] [15] [16] [17]. Considering the increasing evidence that immune checkpoint blockade and other immunotherapy strategies lead to sustained anti-tumor responses in several cancer types [18], there are increasingly more immunotherapy trials evaluating checkpoint inhibitors in patients with glioblastoma [19].

Hypoxia, which induces the stability of the transcription factor hypoxia-inducible factor-1-alpha (HIF-1α) is a common feature of solid tumors [20] [21] [22]. Interestingly, recent reports have emerged demonstrating that HIF-1α can drive the expression...
of PD-L1 [23] [24], leading to immunesuppression and tumor evasion [7]. HIF-1α has also been shown to trigger angiogenesis in gliomas [25]. Moreover, activation of EGFR and downregulation of P53 and PTEN are seen in gliomas and have both been shown to affect HIF expression [26] [27] [28]. Of note, recent data show that chemotherapeutic agents used for GBM treatment, such as the topoisomerase I inhibitor topotecan, might have a suppressive effect on HIF-1α [29] [30].

Until recently, it was unclear as to whether PD-L1 on tumor cells was sufficient for tumor immune evasion or simply correlated with an inflamed/hypoxic tumor microenvironment. The elegant work of Juneja et al. has now established reduced CD8+ T-cell cytotoxicity as a key mechanism by which tumor PD-L1 suppresses antitumor immunity [31]. Accordingly, we sought to use the FDA approved small molecular topotecan to target the expression of PD-L1 in GBM in an effort to ultimately enhance the efficacy of targeted immunotherapies for this intractable CNS neoplasm.

**Objective**

To explore a role for topotecan as an adjuvant therapy in treatment regimens targeting certain GBM patient subpopulations in whom the expression of PD-L1 has been confirmed.
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Figure Legend
Topotecan inhibits hypoxia-inducible factor 1-alpha (HIF-1α) and programmed death-ligand-1 (PD-L1) in glioblastoma (GBM).

A. Treatment with 10 μM topotecan significantly decreases levels of HIF1A, CD274, and SLC2A1 mRNA transcripts in GBM cell line LN229. Expression fold change relative to the DMSO control was quantified using the $2^{-\Delta\Delta CT}$ method with ACTB as the endogenous control.

B. Similar effects are observed in the Mz18 cell line, with significant decreases in HIF1A, CD274, and SLC2A1 mRNA levels.

C. Immunoblot analysis confirms the decreased expression of HIF-1α and PD-L1 in response to topotecan treatment.

D. Western blot analysis also shows a notable decrease in HIF-1α and PD-L1 expression levels in Mz18 cells treated with 10 μM topotecan.

E. Diagram illustrating the mechanism of topotecan inhibiting hypoxia-inducible factor 1-alpha (HIF-1α) and programmed death-ligand-1 (PD-L1) expression in glioblastoma cells.

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B. Treatment with 10 μM topotecan significantly decreases levels of HIF1A, CD274, and SLC2A1 mRNA transcripts in the GBM cell line Mz18. Expression fold change relative to the DMSO control was quantified using the 2ΔΔCT method with ACTB as the endogenous control. Means +/- SD from N ≥ 3 independent experiments. **p<0.01, ****p<0.001.

C. Treatment with topotecan significantly decreases levels of HIF-1α and PD-L1 protein in GBM cell line LN229 at doses of 1 μM and 10 μM. Representative immunoblots are shown. The bands corresponding to HIF-1α (~116-132 kDa) and PD-L1 (~40-50 kDa) were cropped in each lane and the total intensities were measured in ImageJ, normalized to the β-actin loading control, and expressed as a fold difference relative to the DMSO control. Means +/- SD from N ≥ 3 independent experiments. ***p<0.005, ****p<0.001.

D. Treatment with topotecan significantly decreases levels of HIF-1α and PD-L1 protein in GBM patient cell line Mz18 at doses of 1 μM and 10 μM. Representative immunoblots are shown. The bands corresponding to HIF-1α (~116-132 kDa) and PD-L1 (~40-50 kDa) were cropped in each lane and the total intensities were measured in ImageJ, normalized to the β-actin loading control, and expressed as a fold difference relative to the DMSO control. Means +/- SD from N ≥ 3 independent experiments. **p<0.01, ***p<0.005.

E. The binding of PD-L1 to the cell surface receptor programmed cell death protein 1 (PD-1) suppresses CD8+ T-cell cytotoxicity and permits immune evasion in tumor cells; topotecan inhibits PD-L1 expression.

Results & Discussion

Herein we confirm that topotecan is an inhibitor of HIF-1α in two human GBM cell lines, concordant with previous reports [29] [30]. Data obtained using topoisomerase I-resistant cell lines and inactive camptothecin analogs suggest that the targeting of topoisomerase I is critical to the inhibition of HIF-1α [32]. Since this effect was abrogated via the interruption of RNA transcription [33], topotecan-induced perturbations of HIF-1α may be occurring at the transcriptional level.

We therefore employed qPCR to determine whether the effects of topotecan occurred at the transcriptional level with regard to the reported decreases in HIF-1α, PD-L1, and GLUT1 (a canonical target of HIF-1α) [34]. We show that topotecan significantly decreased the expression of HIF1A, CD274, and SLC2A1 at a dose of 10 μM in both the LN229 and Mz18 GBM cell lines (Figure A and B). To further investigate this effect, we conducted a dose-response study on HIF-1α and PD-L1 protein expression. We demonstrated that topotecan significantly decreases the expression of both HIF-1α and PD-L1 proteins at doses of 1 μM and 10 μM in both the LN229 and Mz18 GBM cell lines (Figure C and D). Of note, such findings are in line with previous reports showing that HIF-1α is capable of driving PD-L1 expression via binding to the hypoxia-response element (HRE) located within the promoter of CD274, the gene encoding PD-L1 [7] [24] (Figure E).

HIF-1α activates the transcription of myriad genes that are involved in fundamental aspects of cancer biology (e.g. angiogenesis, cell survival, glucose metabolism, and/or invasion) [34]. Intratumoral hypoxia and genetic alterations can lead to HIF-1 overexpression, which has been associated with increased patient mortality in several cancer types [34]. This is becoming particularly apparent in GBM, a tumor characterized by pronounced areas of necrosis and hypoxia [20] [35]. Critically, our findings provide insight into a novel function of the camptothecin derivative topotecan (i.e. an inhibitor of HIF-1α and PD-L1) in glioblastoma cells. Further studies investigating the effects of topotecan-mediated downregulation of PD-L1 (with or without a combination of checkpoint inhibitors) are needed to ultimately elucidate the clinical benefit of such a novel observation in GBM and other cancers.

Conclusions

Our findings suggest a role for topotecan as an adjuvant small molecule in immunotherapy regimens in GBM (and/or other cancers in which HIF-1α and/or PD-L1 is expressed by the tumor) as an agent that may enhance the effect of immunomodulators such as
checkpoint inhibitors. The authors therefore contend that the data presented suggest that repositioning topotecan may ultimately improve outcomes for GBM patients undergoing immunotherapy. Accordingly, we will seek to further this work in relevant animal models of GBM/immunotherapy.

Limitations
We understand and acknowledge that our work has limitations (i.e. we present solely in vitro data). Further, we rely on the literature with regard to the translational value which we assign our findings and remain uncertain if HIF-1α is the sole driver of PD-L1 expression within our GBM cell lines. However, we are confident in the observations being put forward (i.e. topotecan decreased the levels of HIF-1α and PD-L1) and feel they may ultimately be of translational value.

Our findings suggest a therapeutic role for the drug in GBM and other cancers, which express PD-L1 (i.e. with the understanding that topotecan has FDA approval for the treatment ovarian, cervical, and small cell lung cancers). It is therefore the authors’ contention that the data presented suggest that adjuvant therapy with topotecan (i.e. drug repositioning) may ultimately improve outcomes for GBM patients undergoing immunotherapy. Accordingly, we will seek to further this work in relevant animal models of GBM/immunotherapy.

Additional Information

Methods and Supplementary Material
Please see https://sciencematters.io/articles/201709000008.

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Ethics Statement
Not Applicable.

Citations


