

Resisting fatal attraction: a glioma oncometabolite prevents CD8⁺ T cell recruitment

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Immunotherapy has emerged as a potent approach for treating aggressive cancers, such as non-small-cell lung tumors and metastatic melanoma. Clinical trials are now in progress for patients with malignant gliomas; however, a better understanding of how these tumors escape immune surveillance is required to enhance antitumor immune responses. With gliomas, the recruitment of CD8⁺ T cells to the tumor is impaired, in part preventing containment or elimination of the tumor. In this issue of the *JCI*, Kohanbash and colleagues present an elegant dissection of how gliomas exploit an enzymatic activity acquired through a common mutation to abrogate the migration of CD8⁺ T cells to the tumor. They show that the oncometabolite 2-hydroxyglutarate (2HG), generated by mutated forms of isocitrate dehydrogenase (IDH1 and IDH2), reduces the expression of STAT1, thereby limiting the production of the chemokines CXCL9 and CXCL10. As a result, IDH1-mutated tumors are less effectively infiltrated by CD8⁺ T cells, contributing to tumor escape. Finally, in mice harboring syngeneic gliomas, an inhibitor of 2HG synthesis complemented vaccination to ameliorate tumor control. Understanding how to increase immune infiltration of gliomas represents a key first step in achieving tumor destruction through immunotherapy.

Gliomas: aggressive tumors with poor prognosis

Gliomas are primary brain tumors derived from the malignant transformation of oligodendrocytes and astrocytes. A histological classification of gliomas based on the degree of anaplasia identifies four grades (1). While low-grade gliomas (grades I and II) have an initial benign prognosis, they invariably recur in a higher-grade form that is more invasive and undifferentiated. High-grade gliomas, whether primary or derived from low-grade gliomas, have a poor prognosis and a survival rate of approximately 15 months with the current standard of care, which includes resection and combinations

of chemotherapy and radiotherapy (2). Both de novo high-grade gliomas and those that develop following progression of low-grade gliomas are characterized by a profound immune dysfunction that disables antitumor immune responses (3). Understanding how this immune defect is initiated and reinforced during the transition from low- to high-grade gliomas is essential for the development and implementation of therapeutic strategies aimed at enhancing immune responses in the management of malignant gliomas. Indeed, immunotherapy through blockade of checkpoint receptors represents a novel approach in the treatment of advanced solid cancers.

Following initial breakthroughs in the treatment of melanoma (4) and non-small-cell lung cancer (5), this approach holds promise for other malignancies, including gliomas (6). Nevertheless, in order to achieve successful application of immunotherapy, a more comprehensive understanding of the tumor-immune system interactions in the CNS is required. For example, checkpoint immunotherapy is based on the assumption that an immune response is generated and attracted to the tumor site, where chronic antigenic stimulation and the local milieu trigger expression of checkpoint receptors on T cells, which deliver inhibitory signals to shut down proliferation, killing, and proinflammatory functions. Therefore, checkpoint immunotherapy for gliomas will prove effective only if tumor-specific immune responses can be induced and T cells are able to migrate to the tumor site. The goal of the study by Kohanbash and colleagues (7) was to understand whether targeted T cell migration is possible for gliomas, by deciphering how the tumor influences the recruitment of CD8⁺ T cells.

IDH mutation alters CD8⁺ T cell trafficking to gliomas

Kohanbash and colleagues were initially prompted by an observation they made by analyzing The Cancer Genome Atlas (TCGA) (<https://cancergenome.nih.gov/abouttcga>) database for differences in expression of immune-related genes between gliomas carrying mutations in the enzymes isocitrate dehydrogenase 1 and 2 (referred to by the authors as IDH-MUT) and WT tumors (IDH-WT). IDH mutations are of particular interest, because they are present in 70% to 80% of low-grade gliomas (8) and are maintained upon recurrence of the tumor, even when further transformation occurs. Kohanbash et al. observed that IDH-MUT tumors displayed reduced transcription of CD8⁺ T cell-associated genes, as well as lower expression of *IFNG* and IFN- γ -inducible genes, including the chemokines *CXCL9*

► Related Article: p. 1425

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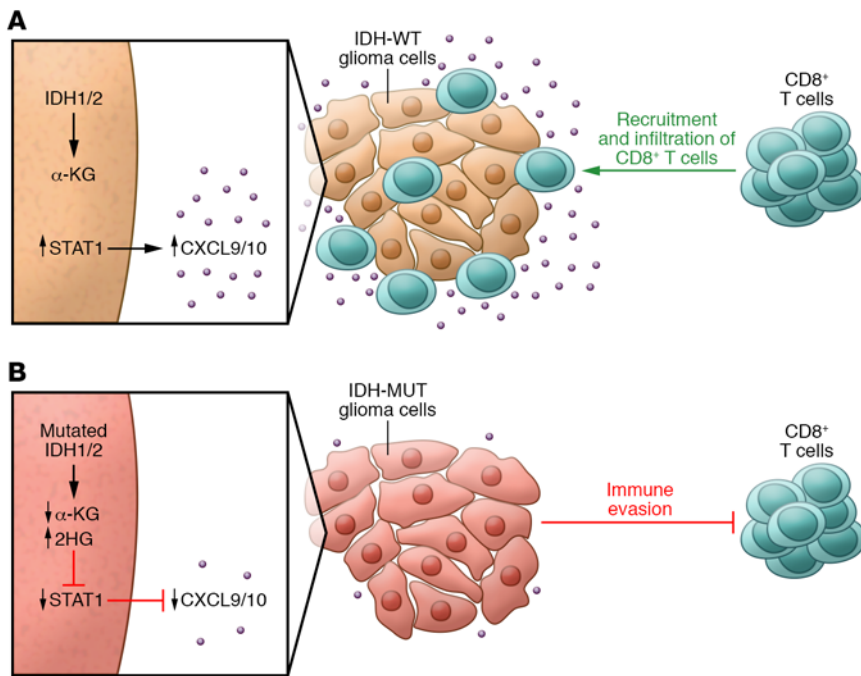


Figure 1. IDH mutations alter CD8⁺ T cell infiltration into gliomas. (A) In the presence of WT IDH1/2, STAT1 expression is intact and drives the production of chemokines that attract CD8⁺ T cells to the tumor. (B) IDH1/2 mutation results in the generation of the oncometabolite 2HG, which in turn represses STAT1, leading to tumor escape.

and *CXCL10*. Moreover, histological analysis of glioma resections revealed that fewer CD8⁺ T cells could be detected in IDH-MUT tumors compared with those detected in IDH-WT tumors.

The observed reduction of infiltrating CD8⁺ T cells was reproduced in a murine model, in which newly generated IDH-MUT glioma cell lines were implanted into syngeneic mice. Compared with those in animals with WT IDH glioma cells, the developing tumor in animals implanted with mutated IDH cells was less infiltrated by CD8⁺ T cells and expressed lower levels of *CXCL10*. Reduced chemokine production was associated with both a defect in STAT1 phosphorylation, the signal downstream of the receptor for IFN- γ , along with an overall reduction in the total amount of STAT1 protein. Moreover, a diminished density of STAT1⁺ cells was also observed by immunofluorescence microscopy in IDH-MUT human tumors, and gene knockdown experiments indicated that the abrogation of STAT1 signaling is sufficient to impair the production of *CXCL10*.

Identified IDH1 and IDH2 mutations affect the catalytic site, conferring a gain-of-function enzymatic activity that converts α -ketoglutarate (α -KG) into 2-hydrox-

ylglutarate (2HG) (9); therefore, Kohanbash and colleagues addressed the role of this oncometabolite in regulating chemokine production by mutant tumor cells through STAT1. Strikingly, treatment of IDH-MUT cell lines with an inhibitor of 2HG synthesis (IDH-C35) restored the expression of STAT1, as well as *CXCL10*, to the levels observed in IDH-WT tumors. Conversely, exposure of IDH-WT cell lines to 2HG was sufficient to induce a loss of STAT1 expression in a dose-dependent manner. Finally, Kohanbash et al. explored the possibility that therapeutic application of IDH-C35 could ameliorate defects in CD8⁺ T cell infiltration into IDH-MUT tumors. Daily oral administration of IDH-C35 markedly improved the survival of IDH-MUT tumor-bearing mice that had received a prophylactic vaccination with glioma antigens. The observed clinical benefit was indeed associated with higher expression of *CXCL10* in the tumor and consequent restoration of CD8⁺ T cell recruitment.

Conclusions and future directions

This work by Kohanbash and colleagues provides an elegant elucidation of the mechanism that underlies reduced chemo-

taxis to IDH1-MUT/IDH2-MUT tumors and identifies the importance of the oncometabolite 2HG in suppressing STAT1 expression and signaling (Figure 1). While determining how 2HG functions was beyond the scope of this study, Kohanbash et al. suggest that, in this context, 2HG suppression might not involve epigenetic regulations through the well-described inhibition of DNA and histone demethylases (10). Indeed, analysis of data from TCGA database did not highlight any differences in methylation between IDH-WT and IDH-MUT tumors at the *STAT1* locus. It would be interesting to explore the hypothesis that disrupted metabolic pathways associated with IDH failure to generate α -KG might also contribute to STAT1 repression. These alterations include mitochondria compensation for diminished cytoplasmic levels of α -KG through the synthesis of this metabolite at the expense of fatty acid build-up (11). Moreover, the elevated consumption of NADPH by IDH-MUT cells exposes the cell to increased stress by ROS. Finally, 2HG can mimic the neurotransmitter activity of glutamate, a source of excitotoxicity. Of note, excitotoxicity has been associated with initiation of an inflammatory cascade in IDH-MUT low-grade gliomas (12), although inflammation is eventually overridden, as the tumor recurs as an IDH-MUT high-grade glioma that cannot be rejected by the immune system. One can thus speculate that difficulty in the recruitment of cytotoxic CD8⁺ T cells contributes to the failure to establish an effective antitumor response despite initial inflammation.

With regard to the effect of stabilization of STAT1 expression, in addition to CD8⁺ T cell chemotaxis, IFN- γ signaling on tumor cells through STAT1 has been implicated in tumorigenesis and induction of programmed death ligand 1 (PD-L1), which can suppress the proinflammatory function of PD-1-expressing T cells. Therefore, a broader analysis of the impact of STAT1 function on tumor cell biology and tumor-immune system interactions may be necessary to better achieve therapeutic translation of 2HG synthesis inhibition for the treatment of gliomas.

Finally, it is of interest that the effect of the 2HG synthesis inhibitor IDH-C35 was examined in the context of vaccination with glioma antigens. In fact, it has been argued that gliomas might not benefit from checkpoint immunotherapy because of the

low mutation load that characterizes these tumors (3). According to this hypothesis, a low mutation load represents an insufficient source of neoantigens to prime an endogenous immune response. Such tumors might benefit more from de novo induction of antitumor responses through vaccination. On the other hand, we and others have reported that the T cells that infiltrate high-grade gliomas express high levels of checkpoint receptors (13), suggesting that endogenous immune responses have been initiated but are repressed by triggering inhibitory signals. Phase III clinical trials with PD-1–targeting monoclonal antibodies for the treatment of high-grade gliomas are underway and will soon provide an answer to this question. Thus, both vaccination and checkpoint blockade strategies would benefit from this new understanding of how gliomas protect themselves from cytotoxic T cell infiltration. The elucidation of methods that increase CD8⁺ T cell infiltration into tumors opens a new therapeutic approach to the treatment of gliomas.

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