

Cancer Immunoediting in Gliomas: Recent Advances and Implications for Immunotherapy

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Introduction

Gliomas are an aggressive class of primary brain tumors with high rates of recurrence and a dismal overall survival [1]. While existing therapeutic strategies provide some benefit, their effects are variable, and no curative modalities exist. The poor prognosis of these patients largely stems from the heterogenous molecular profile of these tumors and their tumor immune microenvironment (TIME) [2,3]. In addition to tumor expression of PD-L1, glioma cells release immunosuppressive cytokines and chemokines, like IL-10, TGF- β , and PGE₂ in the intercellular tumor milieu, which have been shown to reduce antigen presenting activity and downregulate the proliferation of natural killer (NK) and CD8⁺ cytotoxic T-cells [4-7]. These tumor-derived factors facilitate the increased presence of immunosuppressive cell populations like myeloid-derived suppressor cells (MDSC), tumor-associated macrophages (TAM), and regulatory T-cells (Tregs), which synergistically contribute to immune evasion and poor response to immunotherapies [4,8,9].

There is a growing body of evidence supporting the cancer immunoediting model as a critical mechanism underlying the immunosuppressive features acquired by cancers, and the ultimate failure of immunotherapies observed in aggressive tumors [10-12]. This process, as previously described in three dynamic phases, suggests that selective pressure from the body's immune system drives the evolution of cancer cell subclones that can eventually escape immune surveillance [10,13,14]. Elimination is a subclinical phase in which developing cancer cells are recognized and destroyed by immune defenses. Equilibrium is reached when cancer cells remain contained by the immune system, yet are not eliminated,

and fail to increase in number. Escape is the final phase in which tumor cells expand to a clinically appreciable disease and are able to evade immune response [13,14]. The reduced immunogenicity observed in "escaped" cancer cell populations has been previously shown to be associated with MHC-I and antigen presentation machinery (APM) defects [14,15]. Progression through these three states can be reversible and likely driven by a cumulative compounding of epigenetic and genetic alterations resulting from a dynamic tumor-immune interaction.

The cancer immunoediting process has been previously described in the context of aggressive tumors like melanoma, lung, and head and neck cancers, and there is a growing body of preclinical and clinical evidence supporting the role of this process in glioma development [14,16,17]. In this sense, the cancer immunoediting phenomena occurring in gliomas is highly relevant since understanding the mechanisms through which the immune system selects immuno-evasive tumor clones could help improve therapeutic strategies for these patients.

Evidence of Cancer Immunoediting in Gliomas

The factors contributing to gliomagenesis are likely multifactorial, and it has recently been suggested that advanced-age related immunosuppression may be associated with glioma onset and lack of response to immunotherapy [18,19]. A state of reduced immunity potentially serves to preclude glioma cell killing as a result of the reduced number of lymphocytes in the elimination phase. Although evidence of elimination is difficult to assess because of its subclinical phenotype, temporal

analysis of tumor progression demonstrates a CD4⁺ and CD8⁺ gene expression in the TIME in mouse glioma models even before onset of symptoms, followed by progressive decline [11,20]. Similarly, new clinical evidence suggests that the most robust selective pressures take place during the early stages of gliomagenesis, as demonstrated by the retention of the cell clonal architecture in newly diagnosed and recurrent gliomas and lack of significant recurrence-specific gene changes undergoing positive selection [21]. This is also manifested by the absence of a cancer immunoediting process between newly diagnosed and recurrent gliomas [21]. One possibility is that during the early stages of glioma development, CD8⁺ T-cells start to sculpt tumors by negatively selecting immunogenic cancer cell clones as shown in murine models of breast cancer, lymphoma, and fibrosarcoma [22]. Then, at the time

of clinical diagnosis, the acquired immunosuppressive phenotype of the tumor is able to effectively overcome anti-tumor immunity, preventing the further homing and survival of lymphocytes. If cancer immunoediting is occurring during glioma development, it is unclear until which point the lymphocytes exert a selective pressure to the tumor as low numbers of exhausted T-cells are commonly noted in glioma patients [23,24].

Recently, we interrogated a murine glioma model and directly demonstrated that cancer immunoediting by the CD8⁺ T-cell population plays a critical role in driving the dynamic and immunosuppressive nature of these tumors and their microenvironment (summarized by the schematic, Figure 1) [11]. These mice exhibit CD4⁺ and CD8⁺ T-cell infiltration at early stages of tumor development

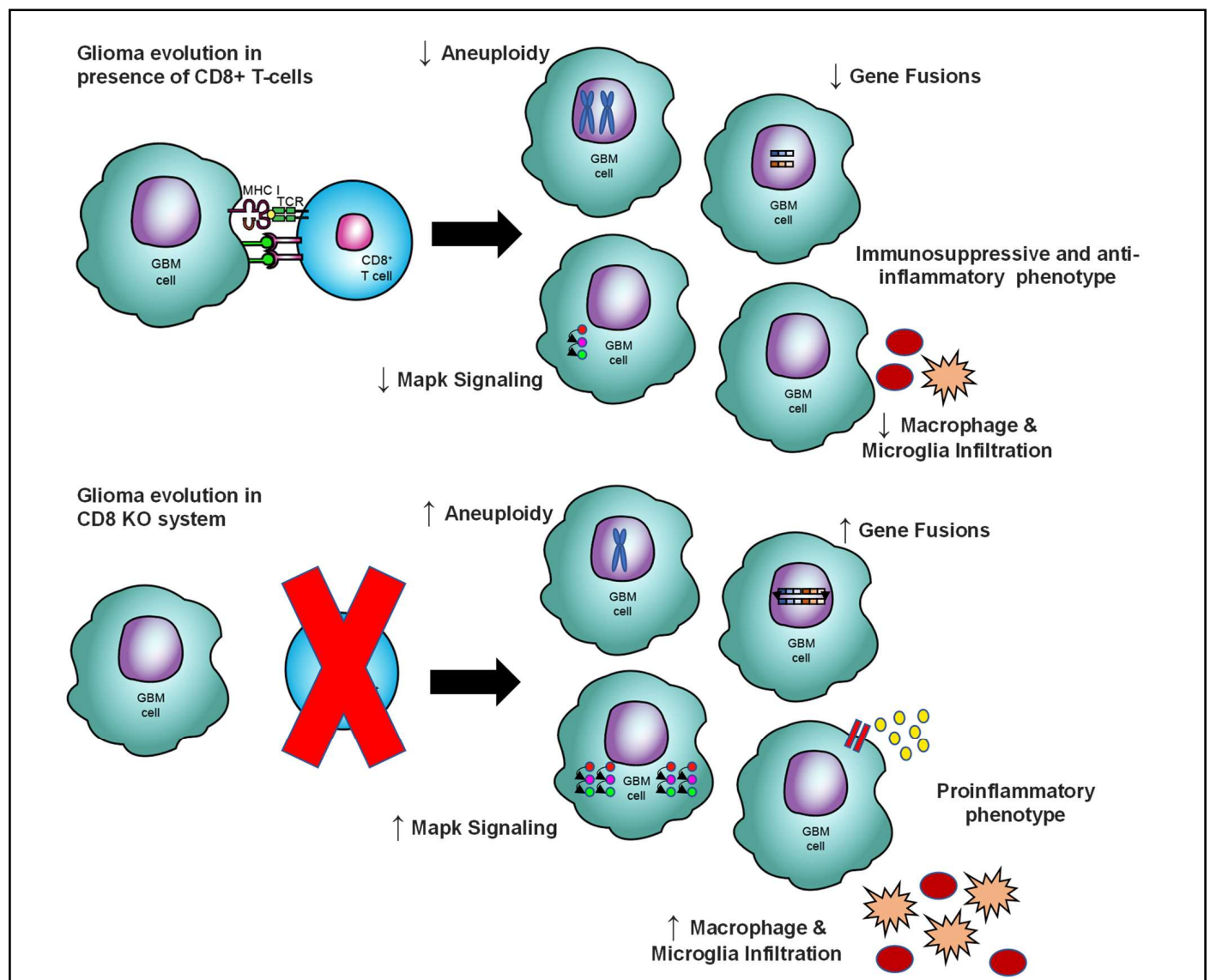


Figure 1: CD8⁺ T-cell mediated immunoediting drives the evolution of GBM to a phenotype and tumor immune microenvironment that is more immunosuppressive and anti-inflammatory.

followed by a decline in the number of lymphocytes at late stages of gliomagenesis, supporting the presence of early selection pressure in promoting the transition to an immunosuppressive phenotype [11]. Furthermore, murine gliomas grown in the absence of CD8⁺ T-cells exhibited unique genomic and TIME characteristics relative to those grown in immunocompetent mice; similar findings have been observed across both antibody-depleted and transgenic CD8⁺ T-cell KO models [11,25]. These changes in tumor characteristics can be appreciated at a macro-level by assessing grafting behavior: gliomas developed in CD8⁺ T-cell-depleted mice failed to grow when intracranially implanted in immunocompetent mice; whereas those from mice with intact CD8⁺ T-cells progressed after transplant in both immunocompromised and immunocompetent mice [11]. Interestingly, it has been noted that the presence or absence of CD8⁺ T-cells during gliomagenesis does not influence overall survival on these mouse glioma models [11,25]. While it appears that the immune pressure present during tumor progression contributes to a unique tumor phenotype, it does not promote the emergence of disproportionate pathogenicity/aggressiveness. The delicate and reciprocal cross-talk between tumor progression and immune response, and the magnitude of its effects, may provide insight to potential mechanisms underlying the Hellstrom Paradox of cancer biology; that is, the observation that despite an immunological response against cancer cells, this immune reaction is dysfunctional or attenuated to achieve tumor eradication [26,27].

At the molecular level, gliomas developed in CD8⁺ T-cell-depleted systems have been shown to uniquely exhibit increased immunogenicity. Although the increase in aneuploidy and number of gene fusions observed in these murine tumors are observed less frequently in human gliomas, it has been demonstrated in several other cancers that high aneuploidy is associated with reduced expression of immune-response genes [28]. Interestingly, of the 12 tumor types analyzed by Davoli et al., only aneuploid gliomas (LGG and GBM) failed to exhibit this differential expression signature. These findings lend further support that aneuploidy in gliomas may contribute to immunogenicity and thus drive subsequent loss of this characteristic via negative selection by CD8⁺ T-cells, as is often observed in the clinical setting.

Additionally, gene fusions are known to generate neoantigens which demonstrate a high affinity for MHC-I [29]. An analysis of *IDH* wild-type high grade gliomas (HGG) showed that chromosomal instability through whole genome duplication was noted to be an early event in tumorigenesis and was associated with increased gene fusions and tumor progression [30]. Similarly, we found that gliomas that did not undergo CD8⁺ T-cell pressure develop more gene fusions than tumors developed with

this immune pressure [11]. These findings support that negative selection against cancer cells with gene fusions may be occurring early in tumorigenesis. Aneuploid gliomas are also associated with a more aggressive clinical course and shorter overall survival [21]. At the same time, chromosomal instability has been shown to activate the cGAS-STING pathway which subsequently leads to a memory phenotype of CD8⁺ T-cells, further supporting negative selection of these features by the immune system [31,32]. In a murine model, it has been shown that tumors evolved to possess high chromosomal instability are initially immunogenic through cGAS-STING, contributing to increased interferon and APM activation. The presence of immune pressure, however, leads to eventual suppression of anti-tumor mechanisms and the emergence of immune evasive mechanism, notably by hypermethylation of genes associated to the MHC-I and APM genes [33]. Previously, we performed a comprehensive analysis of the genes that are part of the APM machinery in GBM and LGG from TCGA. In this analysis, we showed that almost a half of GBM patients and a third of LGG patients acquired defects in the genes coding for the MHC-I and APM pathway [14]. Moreover, these findings further support an immune editing-driven mechanism to explain the paradoxically aggressive clinical course of gliomas which exhibit aneuploidy and how tumors that may retain chromosomal instability may evade immune response.

Analysis of the cellular composition and dynamics in the glioma tumor microenvironment provide further evidence of immune system-mediated evolution. Immunosuppressive macrophages infiltrate gliomas and other tumors during progression to suppress the immune system and promote tumor growth [34,35]. Uniquely, gliomas developed in the absence of CD8⁺ T-cells exhibited a robust macrophage and microglial infiltration; however, their phenotype is changed to one that is proinflammatory, rather than tumor-supportive [11,25]. There is a strong correlation between these proinflammatory cell populations and MAPK signaling, suggesting a potential mechanistic link for activating CD8⁺ T-cell activity [11,36]. Interestingly, we found that gliomas arising in CD8⁺ T-cell-depleted mice similarly exhibited phosphorylation/activation of Erk and p38, relative to gliomas in immunocompetent mice, suggesting the attenuation of this cascade is facilitated by selective pressure from CD8⁺ T-cell-mediated immune response [11,25].

The use of anti-PD-1 therapy against gliomas in a *CD8* KO mouse model similarly demonstrates increased infiltration of proinflammatory CD11b⁺ and Iba1⁺ monocytes and macrophages which are associated with reduced tumor proliferation [25,37]. In addition, there is an anti-PD-1 mediated reduction of macrophages and microglia from the TIME, suggesting an alternative therapeutic mechanism of

action from previously explored CD8⁺ T-cell activation [25]. Nonetheless, it appears that CD8⁺ T-cell selection pressure against proinflammatory cellular populations in the TIME may serve to mitigate this phenotype. Moreover, tumor subclones which are able to shift the TIME phenotype to one that is more immunosuppressive, as often observed in clinical stage gliomas, will be able to escape elimination by CD8⁺ T-cells.

Transcriptomic analysis of glioma cells at different stages of progression in another preclinical model showed LGG were not able to graft in immunocompetent mice with greater abundance of infiltrating lymphocytes and immunostimulatory transcript profile, suggestive of a TIME associate with the elimination stage [38]. Similar to CD8⁺ T-cell-depleted systems, temporal analysis demonstrated that these genes are downregulated during glioma progression and immune infiltrate shifts to a more pro-tumorigenic phenotype. Gliomas eliciting neurological symptoms at an earlier time period in mice are noted to be less malignant and more immunogenic than those that manifest clinically at a later time point [38]. This is consistent with the cancer immunoediting model, as tumors early in development may have been exposed to milder selection pressures and thus may not have developed as robust of an immune-evasive arsenal.

A recent preclinical study employing GL261 glioma model in *Pfp^{-/-} Rag2^{-/-}* and wild-type murine hosts showed differences in the diversity of the tumor clonality [39]. By using a multicolor cell tracking system, it was shown that tumors injected into the brains of immunodeficient mice displayed a highly clonal composition compared to tumors grown in immunocompetent mouse hosts. Although some of the findings of this study are different to ours, likely due to the glioma models employed, our results were consistent in showing that the immune system shapes the tumor clonal architecture. These findings also further highlight the complexity of the interaction between the immune system and cancer cells at different time points of gliomagenesis.

Immunotherapies as Drivers of Cancer Immunoediting

The implications of cancer immunoediting in glioma will be important to consider when making treatment decisions for patients and should help further guide the development of therapeutic strategies. Current immunotherapies, which rely on augmenting immunogenicity directly through activation of the immune response and/or indirectly through the TIME, may effectively drive the cancer immunoediting process in eliminating tumor cells initially, and subsequently contribute to the positive selection of resistant cancer clones leading to tumor escape [40]. The

failure of many existing immunotherapies for gliomas may in part be attributable to the editing-out of neoantigens that many of these treatments target [10,14]. Previous studies have demonstrated the reduced expression of therapeutic targets at glioma recurrence, notably following EGFRvIII peptide vaccination and CAR-T targeting of IL13Ra2 [41,42].

Although successful for melanoma and non-small cell lung cancer, immunotherapies have shown limited effects in glioma, largely due to the scarcity of CD8⁺ T-cells and predominance of immunosuppressive macrophages and Tregs in the TIME (43, 44). Our group has previously demonstrated that recurrent GBM treated with anti-PD-1 therapy demonstrate progressive loss of non-silent mutations predicted to be antigenic, and supporting the case for therapy-driven immunoediting [12]. Recurrent GBM tumors that have been shown to be more susceptible to anti-PD-1 immunotherapy share important characteristics with tumors grown in a CD8⁺ T-cell-depleted environment, notably MAPK pathway alterations and a more immunogenic TIME [12]. Thus, this may contribute to greater potential for evolutionary sculpting by the activated immune response and may lead to the development of resistance and recurrence in the future.

Other approaches, like IL-12-based therapies, have attempted to regulate the tumor microenvironment to induce greater differentiation into Th1 cells, effectively promoting IFN γ release and thus indirectly increasing CD8⁺ T-cell activation [40]. It will be important to further assess the potency and effectiveness of such TIME-augmenting strategies given that they may also indirectly accelerate cancer immunoediting via CD8⁺ T-cell activation, and subsequently drive tumor escape.

Moving forward, therapies may aim to target other cellular populations (e.g. NK cells, MDSC, B cells, etc.), for direct or indirect manipulation [45,46]. It has recently been shown that anti-PD-1 therapy may also mediate its effects through an innate immune response, in addition to CD8⁺ T-cell activation, providing an alternative mechanism to augment therapy [25]. The impact of other cell populations on cancer immunoediting will need to be assessed in the future to better understand the extent of selective pressure they may exert on glioma evolution.

Treatment strategies will also benefit from combination therapies based on the unique profile of an individual's tumor to effectively limit tumor immune escape. By determining the optimal therapeutic regimen at diagnosis, it may be possible to overcome the immune-evasive mechanisms of tumor cells. These findings also highlight the importance of exploring alternative immunotherapeutic innovations to effectively mitigate

escape and avoid priming tumors for recurrence with reduced immunogenicity. A better understanding of the cancer immunoediting dynamics may enable future therapies to manipulate tumor evolution towards a more immunogenic path or surpass CD8⁺ T-cell-mediated immune evasion. Furthermore, because it appears that much of cancer immunoediting is subclinical, it will also be important to investigate potential strategies for early detection of gliomas early in their progression (i.e. while they are still in the elimination or equilibriums stages). In doing so, it may be possible to treat glioma patients with immunotherapies while tumor cells remain in these immunogenic states.

Overall, it is evident that the increase in understanding of glioma biology, its immune system interactions, and the tumor microenvironment support the critical role of cancer immunoediting in sculpting these tumors. The heterogenous and immunosuppressive manifestations of clinical gliomas which contribute to their resistance to immunotherapy appear to be a direct result of this process. Moving forward, it will also be critical to further interrogate the dynamics between different immune system components and glioma tumorigenesis to better understand tumor progression during elimination, equilibrium, and escape and potential avenues for intervention. Notably, the effects of immunotherapies in driving cancer immunoediting, and subsequently contributing to glioma evasion and recurrence, must also be considered as use of this class of therapeutics increases in the clinical setting. In taking to account the mechanisms and implications of cancer immunoediting in gliomas, it may be possible to develop new therapeutic platforms for more effective and absolute treatment for this group of cancers.

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