Review Article
Elucidating immunometabolic targets in glioblastoma

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Abstract: Immunometabolism has recently emerged on the forefront of cancer research as a new avenue to potentially develop more effective and targeted treatment options. Several pathologically altered metabolic targets across various cancer types have been identified, including lactate in aerobic glycolysis; tryptophan in amino acid metabolism; and arginine in the urea cycle. Numerous advancements have improved our understanding of the dual function of these targets in influencing immune functions as an auxiliary function to their well-established metabolic role. This paper provides a comprehensive overview of immunometabolism research and attempts to provide insight into potential immunometabolic targets in glioblastoma for the purpose of future development and study of targeted therapies.

Keywords: Immunometabolism, glioblastoma, lactate, lactic acid, LDHA, tryptophan, IDO, TDO, arginine, Arg1, iNOS

Introduction

Cancer metabolism is a long-studied topic of research, and as a result, the in numerous metabolic pathways and targets that develop altered signaling mechanisms have been well established over the decades. One such phenomenon of altered metabolism in cancer is known as the Warburg Effect, which describes an increased glucose uptake in tumor cells and shifts to a metabolism that relies primarily on anaerobic glycolysis, which was described as long ago as 1927 [1]. In the decades following Otto Warburg's discovery, several related metabolic changes involving the uptake of glucose and fate of glycolytic products as well as the dysregulation of other metabolic pathways including the amino acid and urea cycles have been identified and described throughout scientific literature. Interestingly, these metabolic changes often occur in tumors regardless of the presence of normoxic or hypoxic conditions, suggesting they serve a function beyond adapting to a low-oxygen microenvironment [2], as evidenced by the observation that increased levels of lactate are not always accompanied by hypoxic conditions [3].

In addition to an altered bioenergetic state, tumor cells have also developed unique mechanisms by which they evade the body's immune response. In contrast to aforementioned metabolic changes, immune evasion tends to occur through more variable pathways, many of which are still being elucidated. For example, some tumor cells reduce the expression of antigen-presenting proteins on their surface, rendering themselves "invisible" to T-cells [4]. However, this method of immune evasion occurs in a minority of tumor types. Most tumors evade the immune response by secreting various substances into the tumor microenvironment. Secreted lactate [5] has two main effects: 1) inhibition of the effector T-cell response and 2) upregulation of T-regulatory cells (Tregs) [6]. When thinking about tumors that are particularly resilient vis-à-vis metabolic and immunomodulatory changes, glioblastoma (GBM) is a prime example by which to further study such mechanisms of change.

GBM is the most common CNS tumor in adult patients, accounting for nearly 15% of primary brain tumors [7]. Patients diagnosed with GBM generally have a poor prognosis, with a median survival of 12-15 months after commencement

Table 1. Overview of metabolic targets in various cancer types

Metabolic target	Cancer type	Effect	Citation
Lactic Acid	Cervical cancer	↑	[13]
Lactic Acid	Pancreatic cancer	↑	[14]
Trp/IDO/TDO	Glioblastoma	↑	[20]
ARG1	Breast cancer	↑	[30]
ARG1	Hodgkin lymphoma	↑	[31]
iNOS	Ovarian cancer	↑	[26]

of combined chemotherapeutic and surgical treatment. GBM tumor cells develop variable signaling mechanisms that lead to chemoresistance, including a shift towards glycolysis and lactate production, partially independent of oxygen availability [8]. There have been some advances in the GBM treatment, but even after the adoption of temozolomide (TMZ) as a standard treatment for cancer in 2005 [9], improvements in patient outcomes have been decidedly unimpressive [10]. A new route for GBM treatment strategies has been furthered by recent advances in immunotherapy. For instance, pre-clinical trials in mouse models have shown promising results when a combinatorial blockade against IDO, CTLA-4 and PD-L1 is utilized. This combinatorial therapy was shown to substantially decrease the number of Tregs, which infiltrate GBM tumors and act as potent immunosuppressors.

The purpose of this paper is to provide a review of literature that explores the immuno-metabolic shifts that occur across many different cancer types, in order to better understand the interplay between the immunologic and metabolic changes demonstrated in tumor cells [Table 1]. Through investigation of literature and data analysis of glioblastoma (GBM), we hope to elucidate metabolic mechanisms by which GBM may evade immune recognition. Specifically, this review will highlight three promising immunometabolic targets: lactate, tryptophan, and arginine metabolism.

Elevated lactate levels influence immunosuppressive pathways

A 2016 case study presents a 37-year-old woman diagnosed with advanced stage glioblastoma whose lab results revealed lactic acidosis despite the absence of any apparent causes [11]. In parallel, an *in vitro* study of malignant glioma demonstrated upregulation of lactic acid transport to the tumor microenvironment, facilitated by monocarboxylate transporters (MCTs). Inhibiting MCTs led to a decrease in lactate efflux and anaerobic glycolysis, and impaired the characteristic invasiveness of the glioma [12]. The Warburg phenotype continues to shape our understanding of the underlying pathophysiology in GBM and suggests that GBM can be a potential contributing factor in the development of lactic acidosis seen in the aforementioned case study.

The correlation between malignant tumors and high lactate concentration is far more frequently studied in hematologic cancers compared to solid malignant neoplasms [11]. However, a preliminary study by Walenta et al. has demonstrated a correlation between metastasis and elevated lactate levels in the tumor core of human cervical cancer, and head and neck cancers. Additionally, a Kaplan-Meier survival curve of 34 cervical cancer patients revealed that patients who expressed low lactate concentrations were found to have a nearly double survival time when compared to high lactate counterparts [13]. Along the same lines, experiments conducted using a pancreatic cancer model demonstrated that when LDHA deficient cells were injected into mice, thereby producing low lactate conditions, the volume of the resulting tumor was significantly less compared to control mice [14].

In addition to lactate efflux, the tumor microenvironment and the tumor core are frequently infiltrated by a variety of immune cells with both suppressive and stimulatory functions to the immune response within and surrounding cancer. Immune molecules that function in an immunomodulatory manner are secreted from myeloid-derived suppressor cells (MDSC), Tregs, and include a number of pro-inflammatory cytokines, notably IL-23. Genetic models and transplanted pancreatic tumors in mice have shown the presence of these cells at the tumor and lesion sites before they even fully develop malignant properties [14]. This can be attributed to findings that suggest a correlation between sites of tumor development and sites of chronic inflammation, both infectious and non-infectious, within the body [15]. This inflammation serves as a stimulus for innate immune cells to secrete proinflammatory cytokines.

Typically, in response to infectious inflammation, immune cells secrete the proinflammatory cytokine IL-23. IL-23 is produced by antigen presenting cells (APCs) in response to toll-like receptors (TLR) (TLR2 in particular) and bacterial ligands including peptidoglycan and lipopolysaccharide. IL-23 is a well-recognized immune stimulating molecule that functions in maintaining helper T-cell populations, especially T-helper 17 (Th17) cells; in activating memory T-cells; and in producing other pro-inflammatory cytokines including IL-17 in what is known as the IL-23/IL-17 proinflammatory pathway [15]. Similarly, as a tumor proliferates, MDSCs simultaneously continue to infiltrate the tumor [14]. MDSCs are derived from myeloid cells and inhibit T-cell and Natural Killer (NK) cell activation and proliferation, among other anti-tumor effects [14].

In mice models, MDSC levels decreased when cultured with lactate dehydrogenase-A (LDHA)-deficient cells, and were less suppressive to NK cell activity. Accordingly, NK cell activity greatly increased in the presence of LDHA-deficient cells, suggesting a negative correlation between NK cell activity and tumor size modulated lactate in the tumor microenvironment [14]. Recent studies have found that IL-23 plays an important role in increased angiogenesis and decreased CD8+ T cell populations in tumor microenvironments, thus creating conditions in which tumorigenesis is favored. In fact, findings show that IL23-deficient mice exhibited a significantly decreased likelihood of developing chemically induced tumors, compared to their wild-type counterparts [15].

A study conducted by Shime et al. identified tumor secreted factors responsible for upregulation of IL-23 in the tumor microenvironment. Their findings led to the conclusion that lactic acid secreted by tumor cells is responsible for increased IL-23 levels in both human and mouse monocytes and macrophages. Specifically, lactic acid upregulates the TLR signal, leading to an increase in transcription of the p19 subunit of IL-23, and a consequent increase of IL-23 activity in the tumor microenvironment [15]. *In vitro* experiments demonstrate that mouse and human NK cells exhibit decreased cytotoxic activity when exposed to lactate. Furthermore, lactate-treated NK cells demonstrate significant underexpression of their

activation receptors, thereby inhibiting anti-tumor response through tumor-specific ligand recognition [14]. These collective findings support the hypothesis that lactate inhibits cytotoxic activity and anti-tumor response of tumor-specific immune cells. Existing research involving lactate levels in cervical and pancreatic cancers, and findings of elevated LDHA levels in GBM [16] provide a rationale for future investigation of the immunometabolic roles of lactate in GBM.

Tryptophan's role in immune function

Findings have demonstrated that the enzyme indoleamine-2, 3-dioxygenase (IDO) has an immunosuppressive function in both physiologic and pathologic conditions, including those of cancer [17]. IDO's physiologic role is to inhibit inflammatory reactions through the degradation of the amino acid tryptophan. The metabolites of this reaction have cytotoxic effects on CTL and Th1 cells, leading to inhibition of immune responses in the microenvironment where it is expressed [18]. These metabolites recruit regulatory T cells (Tregs) which mediate immunosuppression through the interaction of B7 on IDO-expressing dendritic cells and CTLA4 on Tregs, resulting in upregulated proliferation.

In the context of glioblastoma, IDO showed some promise as a target for the development of new therapies. IDO is not typically expressed in healthy brain tissue [19]; however, it is upregulated in 90% of glioblastoma cases [20]. Findings also note that levels of IDO expression are higher in GBM relative to low-grade gliomas, and increased IDO expression is inversely correlated with survival [21]. Experiments have shown that silencing IDO in glioma cells allows for T cell-mediated rejection of the tumor [22]. Subsequent studies found that the interaction of IDO, CTLA-4, and PD-L1 are dominant participants in immunosuppression [23]. As mentioned previously, immunotherapy that utilized a combinatorial blockade of these three targets yielded promising results in mouse models. Given the success observed in treating melanoma patients with CTLA-4 and PD-L1 antibodies, researchers attempted to use the same treatment with gliomas. Ninety percent of mice treated with a combination of the two antibodies were alive after 90 days (compared to all mice dying after 29 days without

treatment). The addition of the IDO inhibitor 1-methyl-tryptophan (1-MT) increased 90-day survival to 100% (compared to 20% with 1-MT alone). For confirmation that the outcomes were truly T cell mediated, anti-CD4 and CD8 antibodies were administered, which eliminated all treatment benefits [23]. The proposed mechanism for the treatment outcome appears to be a reduction in immunosuppressive Tregs, which decreased dramatically in the brain parenchyma following the administration of the combinatorial treatment [23].

The rationale for utilizing a combinatorial approach emerged from previous experiments, in which gliomas were treated with the IDO inhibitor 1-MT in combination with temozolomide. This treatment did not lead to significant increases in survival in the mice [23]. It was later hypothesized that, given the role of IDO2 and tryptophan 2,3-dioxygenase (TDO) in tumor immunity, other tryptophan catabolic pathways may be present in gliomas [23].

Arginine metabolism and immune function

L-arginine has long been recognized as a key player in both the urea cycle and immune function. Immunologically, L-arginine has a prominent role in promoting T-cell activation, proliferation, and survival [24]. In metabolism, two enzymes utilize L-arginine as substrates. Arginase 1 (ARG1) converts L-arginine into urea and regenerates the initial metabolic substrate ornithine to be re-used in the urea cycle. Cytokine-inducible nitric oxide synthase (iNOS) utilizes arginine to form citrulline and nitric oxide (NO), which is used by macrophages, in cytotoxic immune defense [25]. The method of arginine metabolism has a direct effect on the phenotypic variation of macrophages, and their ability to respond to the presence of tumors. M1 macrophages are tumor-suppressive and are NO-dependent for their cytotoxic targeting of tumor cells. Conversely, M2 macrophages are recognized as tumor-promoters, and favor ARG1 to generate urea and ornithine, resulting in local anti-inflammatory effects and inhibition of CD4+ T-cell activation and proliferation [25].

iNOS has been identified as an important pathophysiologic agent in ovarian cancer. A new study has outlined the mechanism by which iNOS expression, and therefore nitric oxide levels, influence the metabolic shift favoring or inhibiting aerobic glycolysis [26]. In regions of mild or moderate inflammation, low nitric oxide favors glycolysis. Conversely, hyper-inflammatory conditions result in excess a nitric oxide, favoring inhibition of pyruvate kinase M2 translocation. Interestingly, researchers found that promotion of PKM2 expression was induced via the EGFR/ERK2 signaling pathway [26]. This finding has important implications for GBM, as over-expression of the EGFR/ERK2/mTOR signaling pathway has been widely recognized as a vital contributor to GBM survival and proliferation [27].

In addition to arginine depletion affecting immune response, recent studies indicate that ornithine metabolites may also play individual roles in promoting immune suppression. Specifically, these metabolites activate IDO1 and TGF-β1, which result in anti-inflammatory and immunosuppressive effects, as discussed previously [28]. These findings suggest an important correlation between tryptophan and arginine metabolism, which should be taken into account for future research endeavor.

As with any cancer therapy, selective cytotoxicity to GBM remains a major challenge for new therapeutic strategies. As a method of treatment in GBM, arginine depletion therapy is still under investigation but has shown promise. A recent study using pegylated recombinant human Arginase 1 cobalt to induce arginine deprivation in GBM cells was successful in promoting selective cytotoxicity through caspase-independent, non-apoptotic cell death [29]. This finding, when taken together with the increased expression of arginase 1 in GBM, underscores the importance of ARG1 in promoting tumor survival through immunosuppression and maintenance of glutamine and arginine metabolism.

Conclusion

This paper demonstrates the interconnected metabolic and immune functions of many altered pathways and targets present in tumor cells, with a particular focus on the Warburg Effect, products of aerobic glycolysis, metabolites involved in the amino acids cycle, and enzymes of the urea cycle. The targets investigated have consistently been over-expressed across different cancer types including pan-

creatic, cervical, ovarian, and breast cancer. Positive outcomes using these targets as therapeutic agents in other cancers highlight their ongoing importance moving forward. While current therapeutic options for GBM may be limited, we anticipate that the existing research involving immunometabolism in various cancer types can be used as a potential guide when developing future research directives for GBM.

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