Review Article
An overview of MCT1 and MCT4 in GBM: small molecule transporters with large implications

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Abstract: Monocarboxylate transporters (MCTs) represent a diverse group of transmembrane proteins encoded by the SLC16 gene family found ubiquitously across mammalian species. Two members of this family, MCT1 and MCT4, have been linked to key roles in the metabolic activity of tissues through the proton-coupled transport of monocarboxylates, most notably L-lactate, ketone bodies, and pyruvate. This review aims to provide an overview of MCT1 and MCT4, followed by the implications of their expression in a multitude of cancers and in glioblastoma (GBM) specifically. Further, the possible mechanisms underlying these effects will be discussed. Given the relationships between MCT1 and MCT4 and cancer, they offer a unique opportunity for novel treatment strategies. We aim to explore current therapies focused on MCT1 and MCT4 and propose future studies to better understand their role in GBM to optimize future treatment regimens.

Keywords: Monocarboxylate transporter, MCT1, MCT4, glioblastoma, cancer

Introduction

Glioblastoma (GBM) is one of the most difficult to treat and deadly cancers. In the era of radiation therapy plus temozolomide, the standard of care for GBM, the survival after diagnosis remains only 14.2 months [1]. Such a prognosis leaves much to be desired in the treatment of GBM. Further investigation of the molecular mechanisms involved in GBM tumorigenesis can provide novel targets for therapy.

The SLC16 gene family consists of fourteen members, each of which encodes 12-transmembrane domain transporters. Of the fourteen members, MCT1 and MCT4 are the only members that have been characterized to exhibit proton-coupled symport of monocarboxylic acids [2]. Encoded by the genes SLC16A1 (1p13.2) and SLC16A3 (17q25.3), MCT1 and MCT4 have been extensively studied and characterized (https://www.ncbi.nlm.nih.gov/gene/6566 https://www.ncbi.nlm.nih.gov/gene/9123). The expression of MCT1 has been found throughout nearly all tissues in the human body, with the most notable exception being the endocrine pancreatic beta cells, whereas MCT4 is typically associated with glycolysis dependent tissues [3-5]. With regards to cellular localization, both MCT1 and MCT4 are predominantly localized to the plasma membrane, however, MCT1 has also been shown to localize to the nuclear, sarcolemmal and mitochondrial membranes [6-8] (Table 1).

Characterization of MCT1 and MCT4 has demonstrated that both preferentially bind to l-lactate and ketone bodies, however, MCT1 has a higher affinity for pyruvate [9]. Additionally, MCT1 has generally higher affinities for its preferred substrates compared to MCT4 [6, 10]. Both MCT1 and MCT4 are capable of substrate import and export, however, MCT1 is typically involved in import and MCT4 in export [11, 12]. In fact, MCT4 was first identified as a key component of lactate efflux in highly glycolytic white fiber myocytes [13], and was later demonstrated to show a high affinity for lactate over other monocarboxylates, as referenced by its comparatively lower Km for lactate [11]. These findings suggest unique roles for MCT1 and MCT4 depending upon the needs of various tissues.

Table 1. Summary of MCT1 and MCT4 biochemistry

Transporter	Gene	Locus	Membrane Localization	Preferred Substrates	Typical Role
MCT1	*SLC16A1*	1p13.2	Plasma (mostly), Mitochondrial, Sarcolemmal, Nuclear	L-Lactate, Pyruvate, Ketone bodies	Lactate Import
MCT4	*SLC16A3*	17q25.3	Plasma	L-Lactate, Ketone bodies	Lactate Export

Table 2. Cancer associations with MCT1 and MCT4

Transporter	Overexpressed	Worsened Prognosis
MCT1	Breast, brain, colorectal, gynecological, head and neck, lung, prostate, pancreatic, stomach, bone, oral, renal cancers [24, 26, 27]	Bladder, endometrial cancer, clear cell renal cell carcinoma [24, 26, 27]
MCT4	Prostate, ovarian, cervical, lung, breast, colon, bone, oral, renal cancers [22-25, 28]	Oral, colorectal, prostate, lung cancer, clear cell renal cell carcinoma [24, 25, 29-32]

Both MCT1 and MCT4 exhibit two unique conformations in the unbound state, so-called "inside-open" and "outside-open" in reference to the orientation of the substrate binding site to the cytoplasm, i.e. "inside-open" has the substrate binding site open to the cytoplasm and may participate in export, and "outside-open" to the extracellular space and import [14]. The mechanism has been extensively studied in MCT1 and has been shown to be homologous to the action of MCT4 as well. The first step in the translocation cycle is the protonation of a lysine residue, followed by binding of the substrate. A translocation event then occurs through the transfer of proton and substrate to aspartate and arginine, respectively, followed by their release and subsequent return to the original transporter conformation [15].

Proper cellular localization of MCT1 and MCT4 is dependent upon an accessory protein, CD147 (also known as basigin, HT7, EMMPRIN, and OX-47), as first described by Kirk et al. 2000 [16]. The authors demonstrated that plasma membrane localization was dependent upon the CD147-MCT1/MCT4 interaction, and without functional CD147 both MCT1 and MCT4 remained in the golgi apparatus or endoplasmic reticulum. This interaction was further validated through CD147 knock-out mice, which demonstrated substantially limited plasma membrane localization of MCT1 and MCT4 [17]. Interestingly, CD147 is also a key for distributing MCT1 to the apical membrane in polarized cells, but MCT4 is able to distribute to the basolateral membrane independent of CD147 due to a C-terminal signaling sequence [18, 19].

MCTs and cancer

The significance of MCTs is known to extend beyond the regulation of normal physiology.

Much as MCTs are nearly ubiquitously found in the body, overexpression of MCT1 and MCT4 are found in many different cancer types [20-25]. Increased relative expression of MCT1 and MCT4 has also been linked to a worsened prognosis in several cancers (**Table 2**).

MCT1 and MCT4 in glioblastoma

GBM is a highly aggressive form of primary CNS malignancy that is largely unresponsive to current treatment modalities and corresponds to a very poor overall prognosis. Although the current understanding of the role MCTs play in GBM is limited, its many implications have warranted the necessity for further investigation. GBM exhibits increased expression levels of *SLC16A1* and *SLC16A3* when compared to normal brain parenchyma, as well as oligodendrogliomas and astrocytomas (**Figure 1A** and **1B**). Kaplan-Meier plots of their expression in a subset of gliomas (GBM, oligodendrogliomas, and astrocytomas) shows a significantly worsened prognosis for high *SLC16A1* and *SLC16A3* tumors, as defined by the median of the expression dataset of 275 total patient samples (**Figure 1C** and **1D**). Together, these suggest that *SLC16A1* and *SLC16A3* expression offers poor discriminatory prognostic value in GBM but is correlated with a far poorer prognosis amongst gliomas collectively.

Role in tumor metabolism and metabolic symbiosis

As seen in many other cancers, glioblastoma often displays a glycolytic phenotype conferred by the upregulation of the Warburg effect, a phenomenon in which glycolysis is preferentially utilized even under aerobic conditions [33]. ATP production is less efficient through this metabolic behavior but is thought to result in a
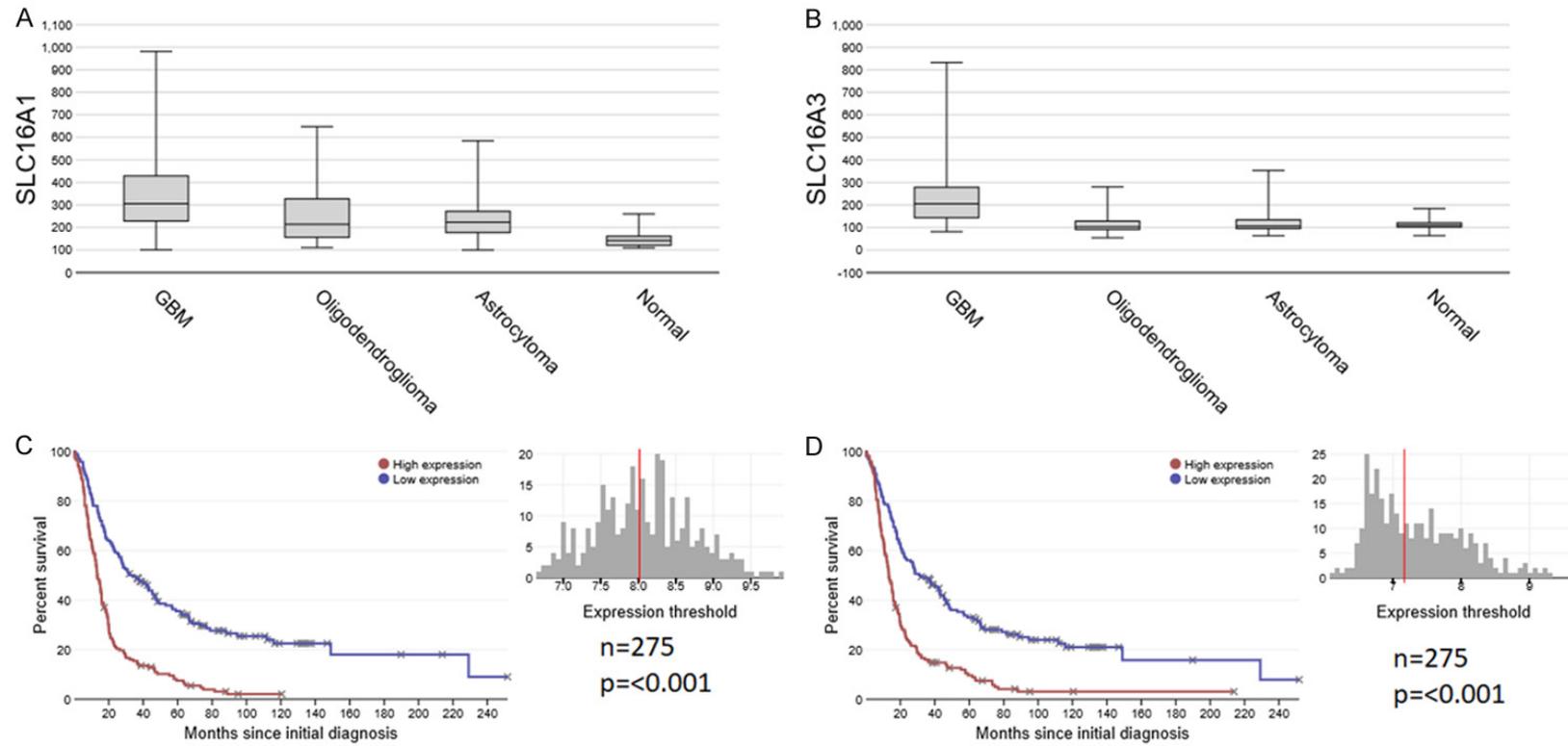
Figure 1. A, B. Boxplot representation of *SLC16A1* (MCT1) and *SLC16A3* (MCT4) gene expression in glioma and normal brain tissue (n=524). C, D. Kaplan-Meier curves for three glioma subtypes (GBM, oligodendroglioma, and astrocytoma; n=275) stratified into high and low expression of *SLC16A1* and *SLC16A3* relative to the median expression of the group. Generated by analysis of REpository for Molecular BRAin Neoplasia DaTa (REMBRANDT), accessed through betastasis.com.

greater production of substrates necessary to satisfy the high anabolic demand of cancer cells. A consequence of this metabolic reprogramming is the elevated fermentation of pyruvate into lactate [34]. This characteristic metabolism subsequently lowers the intracellular pH to conditions suboptimal to the function of intracellular machinery including glycolytic enzymes. As the maintenance of a slightly alkaline pH is essential to glioblastoma metabolism, the role MCTs serve in the maintenance of Warburg metabolism is substantial, irrespective to the availability of oxygen [28, 35]. Of the SLC16 family, MCT1 and MCT4 have been implicated in multiple facets of GBM pathogenesis including angiogenesis, cellular proliferation, and immune modulation [28, 36, 37]. Although the current understanding of the MCTs in glioblastoma remains limited, several pathophysiologic mechanisms have been proposed as to the function of MCT1 and MCT4 in the pathogenesis of GBM.

Many different tumor types are reported to maintain a phenotypically heterogeneous population of cancer cells [38]. As demonstrated by Soeda et al., GBM is no exception to this characteristic as the presence of GBM stem cells (GSCs) that proliferate indefinitely are thought to replenish a pool of indefinitely proliferating GSCs and also generate subpopulations of differentiated GBM cells [39]. Two notable subtypes of differentiated GBM cells include those that demonstrate a propensity towards either a glycolytic or oxidative metabolic phenotype. Adaptations to hypoxic conditions are associated with the glycolytic phenotype while GBM cells able to obtain adequate levels of oxygen are thought to participate in oxidative phosphorylation more readily [20]. As discussed previously, the glycolytic phenotype demonstrated by many GBM cells causes an accumulation of lactate problematic in the maintenance of GBM homeostasis. The current understanding of cancer tumor cells appears to suggest that MCT1 and MCT4 are responsible for the import and export of lactate, respectively. MCT4 has been described to hold a major role in the export of monocarboxylic acids such as lactate, whereas MCT1 has been reciprocally characterized to function in the intracellular influx of monocarboxylic acids [40]. Glycolytic cancer tumor cells have been described to upregulate export of lactate by increasing expression of MCT4 to better accommodate the lactate accumulation. Conversely, oxidative cancers tumor cells are reported to upregulate expression of MCT1 to mediate the uptake of lactate from the extracellular environment to fuel metabolism [20, 40]. A recent hypothesis suggests that this dynamic may create a metabolic symbiosis between the two GBM subpopulations that maintains a favorable environment for both subtypes [41]. Although MCT1 has generally been associated as the major isotype in many oxidative cancer cells due to its mediation of lactate import, there is also evidence that MCT1 may serve a role in the efflux of lactate as inhibition may also cause the accumulation of lactate within GBM cells [20, 42]. It appears that the functional activity of MCTs may vary based on the pH and monocarboxylate concentrations present in both the intracellular and extracellular environment [11, 40, 43]. GSCs also interact with stromal cells in an MCT dependent manner contributing to the pathophysiologic homeostasis of cancer cells. As such, GBM is known as a highly glycolytic cancer type that displays the capability to induce a favorable interaction with neighboring stromal cells [41]. Similar to oxidative cancer cells, vascular endothelial cells also express MCT1 and uptake lactate exported by glycolytic cancer cells [40]. Notably, the lactate shuttling occurring between the endothelial and GBM cells have been shown to upregulate signaling pathways responsible for the induction of angiogenesis [44]. The variable role of MCT1 and MCT4 seems to demonstrate a potential alteration of function based on environmental conditions and cellular interaction.

Role in hypoxia

A characteristic of GBM tumor growth is the development of necrosis within hypoxic regions of the tumor [45]. Hypoxic adaptation within these necrotic regions of GBM tumors has been associated with the activity of pseudo palisading GBM cells, a morphologically unique subset of cancer cells commonly found surrounding the periphery of tumor necrosis. The configuration of pseudo palisades and tumor necrosis has been termed pseudo palisading necrosis and is considered a hallmark response to hypoxic tumor necrosis observed in GBM. Although the exact mechanism underlying the formation of pseudo palisading necrosis remains unclear, it has been hypothesized that the pseudo palisading cells may represent tumor cells migrat-

ing away from the hypoxic region following a thrombotic event [46-48].

Adaptation to hypoxia is a well-established factor involved in the pathogenesis of solid tumors. Interestingly, both MCT1 and MCT4 exhibit increased expression under hypoxia in cancer, suggesting that they may play a role in this adaptation. The mechanisms underlying these changes in expression vary for MCT1 and MCT4. Ullah et al. first demonstrated that MCT4 upregulation under hypoxia was mediated by hypoxia-inducible factor-alpha (HIF-1α). The authors showed that during hypoxia, wild-type Chinese hamster ovary cells exhibited increased MCT4 expression, however, deletion of HIF-1α removed this effect [49]. This interaction was further validated in GBM, wherein knockdown of MCT4 inhibited the transcriptional response of HIF-1α regardless of lactate levels [36].

Rather than being regulated through an interaction with HIF-1alpha during hypoxia, MCT1 expression has been linked to the tumor suppressor protein p53. Wild-type p53 has been shown to act as a transcriptional repressor of MCT1 while also decreasing MCT1 mRNA stability in colon cancer cells [50]. This repression was eliminated in p53 null cells under hypoxia and further investigation identified nuclear factor kappa beta (NF-Kb), specifically the p65 subunit, as being associated with this change. This is an interesting finding considering that NF-Kb has previously been linked to increased MCT4 expression [51].

Immune modulation and evasion

Lactate presence in the extracellular space, which is mediated by MCTs, has strong immunosuppressive effect in addition to inducing various other pathways [52]. A primary method through which cancer cells exert their immunosuppressive effect is through the lactate shuttle. The movement of lactate extends to stromal cells which are programmed and recruited to help create the tumor microenvironment [46, 53]. MCT4 couples the export of lactate with H+, causing the region to become highly acidic. Cytotoxic (CD8+) T cells primarily rely on glycolysis for energy production and as such produce lactate. CD8+ T cells would have their lactate export halted by this disruption in the pH gradient and would lead to subsequent intracellular acidification. This acidity leads to the reduced

cytotoxicity and cytokine production of CD8+ T cells, and eventually apoptosis of the cell [54]. Using a buffer to bring the pH levels surrounding tumors back to a physiological range rescues the cytotoxic T cell activity [46]. The presence of lactate additionally induces macrophages into a M-2 like state that suppresses the inflammatory response [53], inhibits monocyte differentiation, and prevents mature dendritic cells from releasing cytokines [46]. Immunosuppressive cells are recruited by the presence of lactate rather than inhibited. MCT1 additionally facilitates the transport of branched-chain keto acids (BCKAs) which are a byproduct of branched-chain amino acid (BCAA) metabolism taking place in glioblastoma cells. These BCKAs are taken up by phagocytes and as a result have their phagocytic activity inhibited [55]. Regulatory T cells are unaffected by the increase in acidity and lactate presence adding to the immunosuppressive effect of MCTs and lactate [54]. It should be noted that contrary to most cancers, GBM has reversed roles of MCT1 (export) and MCT4 (import). Together, these effects allow GBM to evade the immune system and continue to proliferate unopposed.

Cellular proliferation

MCT1 and MCT4 serve an integral role in the propagation of GBM pathogenesis [28]. As discussed above, monocarboxylate transporters are essential in the maintenance of the GBM intracellular environment. By providing homeostatic stability, MCTs are thought to help perpetuate conditions favorable to the induction of cellular proliferation of glioma tumor cells [28]. The valuable role monocarboxylate transporters hold in the viability of GBM has been demonstrated in multiple studies. A past study conducted by Mathupala et al. demonstrated that the siRNA mediated silencing of MCT1 and MCT2 induced cell death in the form of both increased necrosis and apoptosis in the GBM cell line U87 [42]. MCT1 seems to be especially significant in high grade astrocytoma cells displaying the glycolytic phenotype such as GBM. When compared to a phenotypically oxidative form of high grade astrocytoma, the inhibition of MCT1 in glycolytic GBM appears to be much more detrimental. Miranda-Gonçalves et al. demonstrated this finding when comparing the effects of the MCT1 inhibitor 2-Cyano-3-(4-hydroxyphenyl)-2-propenoic acid (CHC) on high

Table 3. Summary of targets and corresponding inhibitors

Target	Non-specific	MCT1/MCT2	MCT1	MCT4-CD147	MCT1/MCT4-CD147
Inhibitors	CHC	AR-C177977	AZD3965	ACF	pCMBS
	DIDS	AR-C122982			
	DBDS	AR-C155858			

grade, oxidative and glycolytic astrocytoma cell lines. The study demonstrated that the CHC treatment caused decreased glycolytic activity in the glycolytic GBM cell line U251 in addition to an increase in cell death when treated with CHC. In comparison, the effects of CHC administration to the oxidative cell line SW-1088 appeared be much less profound, only causing a reduction in cellular proliferation [28].

Small molecule inhibitors

Since their initial characterization, there have been a multitude of compounds reported which are capable of MCT inhibition (**Table 3**). Some of the earliest inhibitors identified were α-cyano-4-hydroxycinnamate (CHC) and the stilbene disulfonates, which include 4,4'-di-isothiocyanostilbene-2,2'-disulfonate (DIDS) and 4,4'-dibenzamidostilbene-2,2'-disulfonate (DBDS). While effective at inhibiting lactate transport *in vitro*, both inhibitors demonstrated substantial off-target effects, with CHC more potently inhibiting mitochondrial pyruvate transport and DBDS the erythrocyte chloride/bicarbonate exchanger AE1, thereby necessitating the development of more specific inhibitors [14, 56, 57]. Two such compounds, AR-C177977 and AR-C122982, were developed as highly specific MCT1 and MCT2 inhibitors that act through direct binding to transmembrane helices 7-10 intracellularly [58]. These compounds were quite efficacious in the prevention of allograft rejection and disruption of the graft-versus-host in rats and mice and AR-C177977 for glioblastoma cancer stem cells with high MCT1 expression [35]. Both, however, exhibited low oral bioavailability with short plasma half-lives, limiting their clinical value [59-61]. Several novel compounds based on the two predecessors demonstrated substantially increased oral bioavailability and PK half-lives, however, they all remained outside the range of clinical practicality [61]. Within this group of compounds, AR-C155858 has been shown to be effective at reducing lactate export in Ras-transformed fibroblast and reducing tumorige-

nicity both in-vitro and in-vivo. These effects, though, were reversed upon exogenous or increased endogenous MCT4 expression [62].

A variant of AR-C155858, AZD3965 was developed as a better candidate for clinical use. In addition to having a greater specificity for MCT1 over MCT2 (6-fold greater specificity), AZD3965 was shown to be effective against diffuse large B-cell lymphoma, non-Hodgkin lymphoma, and Burkitt's lymphoma in-vitro [63]. Based on this, AZD3965 was selected for clinical review, currently undergoing phase I clinical trials in the United Kingdom in patients with advanced cancers, with an expected primary completion date of August 2019. Started in February 2013, this study represents the first instance of a targeted MCT inhibitor therapy in clinical trials and remains the only of its kind as of August 2018. Aside from hematological malignancies, AZD-3965 has shown variable efficacy against small cell lung cancer in-vitro and in-vivo, with it being most effective against cancer cells expressing high levels of MCT1 in hypoxia with concurrent low expression of MCT4; high levels of MCT4 expression correlated with increased rates of resistance to the drug [64].

While there have been a variety of targeted inhibitors identified towards MCT1, few direct inhibitors exist for MCT4 despite it having been shown to be an effective target through gene silencing in a variety of cancers [65]. One such inhibitor, acriflavine (ACF), inhibits the function of MCT4 through disruption of the MCT4-CD147 interaction necessary for the functional localization of MCT4 to the plasma membrane. ACF was validated in glioblastoma stem cells, in-vitro and in-vivo, where it significantly reduced angiogenesis and tumor progression, most effectively under hypoxia [66]. p-chloromercuribenzene sulfonate (pCMBS), an organomercurial reagent, is capable of inhibiting the interaction between MCT4 and CD147, but it also inhibits the MCT1-CD147 interaction owing to its direct targeting of basigin [67]. This has yet to be utilized in any cancer studies.

Future directions/conclusion

GBM has lesions in multiple cancer related pathways substantiating the need for therapies targeting various points within these pathways. Despite our imperfect understanding of MCT1 and 4 in GBM and the lack of therapeutic options targeting these transporters, current research indicates MCT1 and MCT4 as promising targets for GBM treatment. Understanding MCT1 and MCT4 will shed light on the development of GBM and provides an attractive therapeutic option for GBM patients. Research into potential cooperative effects of MCT1 and MCT4 are warranted to understand how they work together to promote tumorigenesis. Understanding the mechanisms behind the interaction between MCT1 and MCT4 and stromal cells could illuminate potential targets of study. Additionally, the full effects that the tumor microenvironment, created by metabolic symbiosis, has on MCT1 and MCT4 function are unknown. The role of p53 in managing MCT1 and MCT4 during hypoxia is not fully understood, and further research would be of great benefit.

Novel, more efficacious inhibitors targeting these transporters can be developed once the relationship between MCT1 and MCT4 and the development of GBM is better understood. As previously mentioned, there are many in vitro inhibitors available for MCT1 and MCT4 but few have made it to human trials and few are specific to MCT1 and MCT4 [58, 64, 66]. The majority of the specific inhibitors aren't feasible for GBM trials in humans [59-61]. ACF is particularly promising as a therapeutic agent for GBM. Treatment with ACF in temozolomide resistant mouse models inhibited tumor growth. Additionally, treatment with ACF did not promote resistance. What is more, only one clinical trial for an MCT inhibitor is currently ongoing with no trials for MCTs in glioblastoma and no MCT inhibitors approved for the treatment of glioblastoma.

In conclusion, MCT1 and MCT4 play integral roles in cancer development and metabolism. The current literature indicates MCT1 and MCT4 to be equally significant in GBM. This, however, gives us just a glimpse of their roles. Gaining a better understanding of MCT1 and MCT4 in GBM is critical to the development of novel inhibitors. As discussed, GBM displays the Warburg effect and must maintain an alkaline intracellular pH in order to continue undergoing glycolysis. Inhibiting MCT and MCT4, which play a key role in maintaining this balance would prevent glucose metabolism through glycolysis. While multiple inhibitors currently exist for MCTs generally, research for potential therapeutic drugs specific to GBM is absent.

Disclosure of conflict of interest

None.

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