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PERSPECTIVE

Targeting the lysine lactylome for the treatment of glioma

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The anomalous activation of glycolysis and subsequent accumulation of L-lactate under aerobic conditions, known as the Warburg effect, are prominent metabolic hallmarks of cancer[1]. Extensive research has revealed that lactate acts not only as an energy-supplying substrate but also as a signaling molecule in glioma[2]. In addition, lactate accumulation contributes to the formation of a suppressive immune microenvironment in glioma, owing to its acidifying effects. However, emerging findings regarding the lysine lactylome indicate that the roles of lactate extend beyond these functions[3].

In 2019, a study identified histone lactylation sites, including H3K23, H3K18, H4K8, and H4K12 in human and murine immune cells, that directly activate the transcription of inflammation-associated genes[4]. Subsequent studies have corroborated that histone lactylation is associated with regulation of the cell cycle, proliferation, invasion, and metastasis of glioma cells. Furthermore, histone lactylation in glioma cells modulates a tumor-promoting immune microenvironment *via* stimulating angiogenesis, enhancing the M2-type polarization of macrophages, and recruiting tumor-associated myeloid cells[5].

Recent advances in tandem mass spectrometry techniques have further facilitated the identification of non-histone lactylation[6]. To characterize the function of non-histone lactylation, a novel orthogonal translation system has been successfully developed as a precise tool to specifically enhance the lactylation levels of proteins at designated sites. These technical advances have substantially broadened understanding of the critical roles of non-histone lactylation in the pathological mechanisms of glioma.

The clinical potential of targeting the lysine lactylome for the treatment of glioma is becoming increasingly promising with ongoing research. Current clinical trials targeting HDACs, monocarboxylate transporter 1 (MCT1), and lactate dehydrogenase A (LDHA) are aimed at decreasing global lactylation in glioma cells and the accumulation of lactate in the immune microenvironment. Concurrently, recent preclinical studies have shifted their focus to specific lactylated proteins[7-21]. In the context of glioma diagnosis, antibodies that detect specific lactylated proteins have been shown to facilitate more rapid and precise prognosis determination. These antibodies also serve as biomarkers for predicting the sensitivity of chemoradiotherapy and immunotherapy; therefore, this clinical translational potential warrants extensive exploration of the mechanisms, functional validation, and intervention strategies targeting the lysine lactylome in glioma.

Histone lactylation and glioma

The critical roles of histone lactylation in cancer proliferation and evasion of apoptosis have been extensively studied. For example, H3K9 lactylation has been found to confer resistance to temozolomide treatment *via* a LUC7L2-mediated decrease in MLH1, a crucial component of the mismatch repair pathway in glioma cells. This impairment of the mismatch repair pathway increases genomic instability and

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resistance to chemotherapy[10]. In addition, GLUT1-expressing monocyte-derived macrophages suppress CD8+ T cell activity through the production of IL-10, as a result of enhanced PERK-driven glycolysis and histone lactylation. This immunosuppressive environment is further exacerbated by the accumulation of lactate and upregulated H3K18 lactylation in glioma-infiltrating CD4+ T cells and macrophages[8]. These modifications elevate the transcription of immunosuppressive molecules, such as CD39, CD73, and CCR8, thus contributing to the overall immunosuppressive microenvironment within gliomas. Oxamate, an inhibitor of lactate dehydrogenase (LDH), alleviates H3K18-induced immune suppression and enhances the efficacy of chimeric antigen receptor T-cell (CAR-T) immunotherapy. Oxamate decreases lactate production *via* inhibiting LDH, thereby decreasing histone lactylation and reversing the immunosuppressive effects[12,13].

These findings underscore the importance of histone lactylation in glioma pathogenesis and treatment resistance (**Table 1**), and highlight the need for additional research on targeted therapies aimed at modulating lactylation to improve clinical outcomes. Understanding the precise mechanisms through which histone lactylation influences glioma biology will be critical for the development of novel therapeutic strategies aimed at overcoming treatment resistance and enhancing the efficacy of existing therapies.

Non-histone lactylation and glioma

Despite extensive research elucidating the role of histone lactylation in glioma, the role of non-histone lactylation remains relatively unexplored. A recent groundbreaking study has shed light on this topic by identifying a novel mechanism involving ALDH1A3, a member of the aldehyde dehydrogenase family. ALDH1A3 promotes glycolytic activity and lactate accumulation by allosterically activating PKM2 in glioma stem cells, thus leading to the lactylation of XRCC1, a critical scaffold protein in the DNA SSBR pathway, at the K247 site, and significantly contributing to glioblastoma (GBM) cell resistance to chemoradiotherapy[7]. In agreement with previous findings linking metabolic reprogramming to therapeutic resistance, this study has provided novel insights into how non-histone lactylation directly influences DNA repair mechanisms in glioma. Additionally, the researchers developed a novel small-molecule drug, D34-919, that robustly decreases the lactylation of XRCC1 and sensitizes glioma cells to chemoradiotherapy, thus offering a promising therapeutic strategy for patients with glioma.

The clinical potential of non-histone lactylation in glioma has not been fully realized. However, emerging research in other cancer types is providing valuable insights. For example, in gastric cancer, lactylation of NBS1 at the K388 site is essential for the homologous recombination DNA repair pathway. This lactylation reaction is regulated by the balance between TIP60, a lactyltransferase, and HDAC3, a delactylase. Combining stiripentol, an LDHA inhibitor, with chemotherapy has been shown to improve the prognosis of patients with gastric cancer[14]. In addition, the lactylation of MRE11 at the K673 site facilitates DNA end resection and homologous recombination; this finding further underscores the crucial role of non-histone lactylation in DNA damage repair[15]. These results reveal the strong links between the Warburg effect and the DNA damage repair pathway.

Notably, the lactylation of cGAS, regulated by the lactate sensors AARS1 and AARS2, suppresses exogenous DNA sensing and innate immune activity in C57 mice. This novel mechanism suggests that non-histone lactylation has a critical role in the escape of cancer cells from innate immune surveillance[16]. Similarly, the lactylation of p53 hinders its DNA binding and transcriptional activation, thus contributing to tumorigenesis by coupling tumor cell metabolism to proteome alterations. In addition, the lactylation of MOESIN at the K72 site in Treg

Table 1 Influence of distinct histone lactylation sites on glioma progression

Histone lactylation site	Influence on glioma progression	Key findings
H3K9	Modulates immune suppression	H3K9 lactylation leads to upregulation of immunosuppressive cytokines and aids in immune evasion[10].
H3K18	Promotes immune evasion	H3K18 lactylation enhances the expression of genes involved in tumor proliferation and suppresses immune responses[8].
H3K27	Promotes tumor growth	H3K27 lactylation is associated with activation of oncogenes and suppression of tumor suppressor genes[9].
H4K12	Supports stemness and tumorigenicity	H4K12 lactylation maintains glioma stem cell properties, and contributes to tumor initiation and resistance to therapy[11].

cells increases its interaction with TGF-β receptor I and downstream SMAD3 signaling, thereby suggesting a robust potential for combined PD-1 antibody and LDH inhibitor therapy[17].

These findings highlight the essential regulatory roles of non-histone lactylation in DNA damage repair, immune evasion, and therapeutic resistance in cancer cells. Additional research on these mechanisms is necessary for the development of novel therapeutic strategies targeting non-histone lactylation, to improve clinical outcomes for patients with glioma and other cancers (**Figure 1**).

Targeting the lysine L-lactylome to improve outcomes of patients with glioma

Given the substantial potential of targeting glycolysis and the L-lactylome, numerous clinical trials have been designed to explore the therapeutic value of these targets (**Table 2**). GLUT1, a high-affinity glucose transporter, is closely associated with lactylation levels and is highly expressed in various cancer cells, including lung cancer, colon cancer, and glioma cells. The GLUT1 inhibitor BAY-876, when combined with the PD-1/PD-L1 blocker BMS-1 (Gel@B-B), has been found to significantly increase the infiltration of effector T cells into the glioma microenvironment and prolong survival in a GBM mouse model[18]. Other GLUT1 inhibitors, such as cytochalasin B, STF-31, WZB117, and CG-5, have also been shown to inhibit glucose uptake and suppress tumor growth in various cancer types. However, despite these promising preclinical results, only several of these inhibitors have demonstrated therapeutic efficacy in phase II/III clinical trials; therefore, further exploration and optimization are necessary to translate these findings into effective clinical treatments.

Hexokinase is the first rate-limiting enzyme in the glycolytic pathway. The HK2 isoform has been found to upregulate

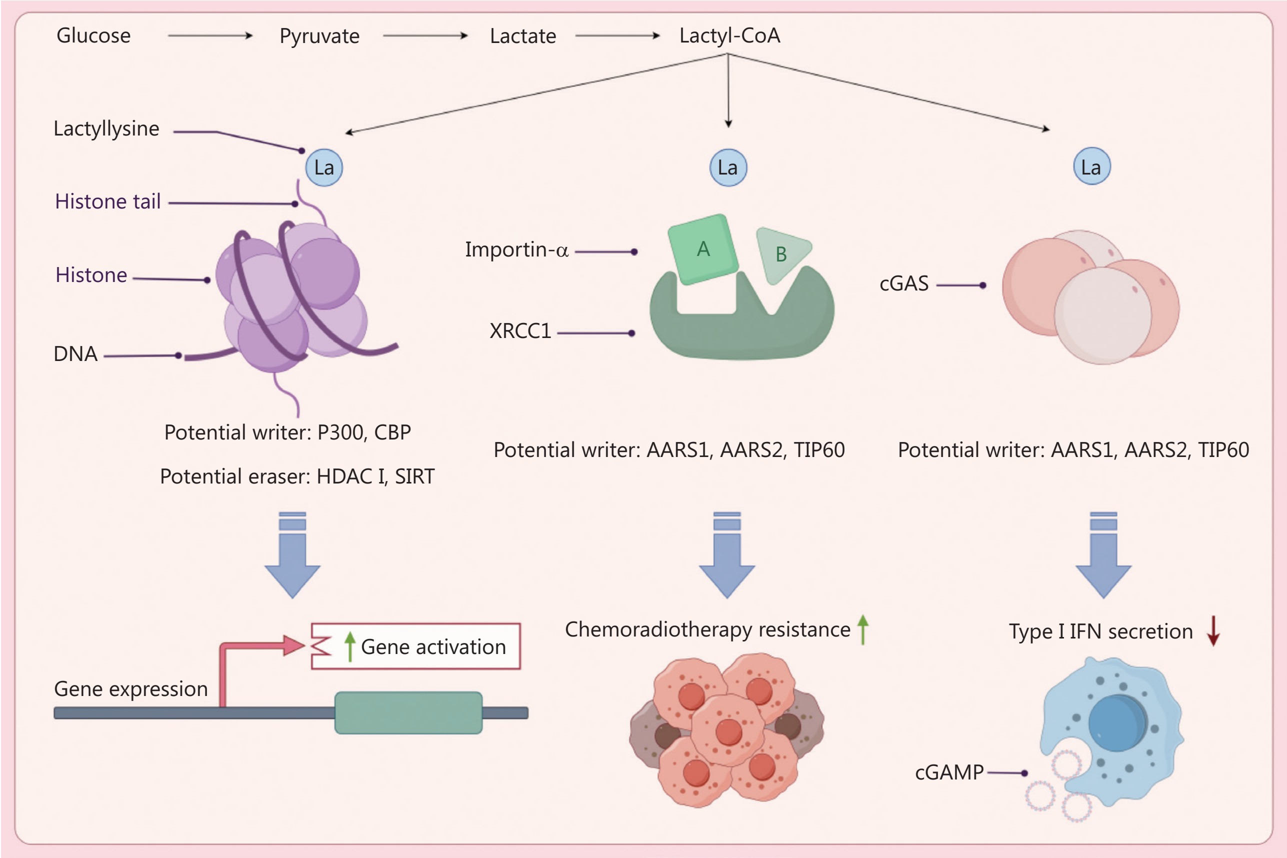

Figure 1 Mechanisms through which lactylation promotes tumorigenesis and chemotherapy resistance in glioma. Left, mechanism of regulation of gene expression through histone lactylation. Middle, XRCC1 lactylation enhances chemoradiotherapy resistance. Right, cGAS lactylation supports immune invasion in glioma.

Table 2 Summary of crucial studies on lactylation targeting therapy in glioma

Target	Study	STAGE	Key findings
GLUT1	Li et al.[18]	Pre-clinical	Dual-regulation thermogels that inhibit lactate excretion and block PD-1/PD-L1 significantly enhance the efficacy of immunotherapy for glioblastoma.
HK2	Agnihotri et al.[19]	Phase 0/1 trial (NCT03763396)	Ketoconazole and posaconazole suppress GBM cell proliferation by decreasing glycolysis activity.
PKM2	Li et al.[7]	Pre-clinical	D34-919, a novel inhibitor of PKM2, reverses glycolysis reprogramming, decreases XRCC1 lactylation levels, and sensitizes glioblastoma to chemo-radiotherapy.
LDHA	Sun et al.[12]	Pre-clinical	Inhibition of LDHA by oxamate diminishes the immunosuppressive tumor microenvironment and enhances CAR-T cell therapy efficacy against glioblastoma.

PD-L1 expression through activation of the NF-κB pathway. Therefore, HK2 is a promising therapeutic target for glioma treatment. Several hexokinase inhibitors have been developed, including 2-deoxy-D-glucose, lonidamine, and 3-bromopyruvate[19].

PKM2, an isoform of pyruvate kinase, the last rate-limiting enzyme in glycolysis, has been identified as a crucial target in glioma. In healthy tissues, PKM2 exists predominantly in tetrameric form and exhibits high pyruvate kinase activity. However, in malignant cells, PKM2 exists primarily in dimeric form, which has low pyruvate kinase activity and is conducive to tumor growth. Current PKM2 inhibitors, such as shikonin, metformin, and vitamin K, have shown potential in treating liver, renal, and prostate cancers; however, these inhibitors face challenges such as off-target effects and difficulties in delivery.

LDHA is crucial in glycolysis, by converting pyruvate to lactate. Inhibiting LDHA markedly decreases pan-lactylation in glioma cells. Sun et al.[12,13] have found that oxamate, an LDHA inhibitor, diminishes the immunosuppressive tumor microenvironment and enhances the efficacy of CAR-T therapy against glioblastoma. These findings suggest that combining oxamate with CAR-T therapy might improve glioblastoma treatment outcomes.

Future perspectives

Emerging evidence highlights the critical roles of both histone and non-histone lactylation in the treatment of glioma[20]. Lactylation promotes malignant cell growth, evasion, and recurrence, and contributes to the formation of an inhibitory immune microenvironment. However, current research focuses predominantly on upstream regulatory enzymes in glycolysis, thereby presenting several challenges.

In the future, a focus on more precise and direct targets is expected. For example, inhibiting MCTs, particularly MCT1 and MCT4, has shown promising synergistic effects with temozolomide treatment in GBM[21]. Targeting specific molecular mechanisms of lactylation in glioma, for example, by using the novel inhibitor D34-919, is another promising strategy. D34-919 effectively inhibits the allosteric activation of PKM2 by ALDH1A3, and has shown good blood–brain barrier penetration and robust glioma inhibition in combination with radiochemotherapy[7].

In addition, the discovery of delactylases and lactyltransferases offers new, precise targets for the treatment of glioma. These enzymes play crucial roles in regulating lactylation; therefore, targeting these enzymes might pave the way to the development of novel therapeutic approaches. Continued research in this area has the potential to usher in a new era of glioma treatment with improved patient outcomes and more effective therapeutic options.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Conceived and designed the analysis: Wei Zhang, Tao Jiang.
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