Targeting the lactic acid metabolic pathway for antitumor therapy

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Lactic acid is one of the most abundant products of cellular metabolism and has historically been considered a cell-damaging metabolic product. However, as research has deepened, the beneficial effects of lactic acid on tumor cells and the tumor microenvironment have received increasing attention from the oncology community. Lactic acid can not only provide tumor cells with energy but also act as a messenger molecule that promotes tumor growth and progression and protects tumor cells from immune cells and killing by radiation and chemotherapy. Thus, the inhibition of tumor cell lactic acid metabolism has emerged as a novel antitumor treatment strategy that can also effectively enhance the efficacy of conventional antitumor therapies. In this review, we classify the currently available therapies targeting lactic acid metabolism and examine their prospects for clinical application.

INTRODUCTION

Tumors have markedly different biological properties than normal human tissues, one being the Warburg effect.[1] The Warburg effect is that cancer cells use a fermentative rather than oxidative metabolism, even though the former is more inefficient in terms of energy production per molecule of glucose, which is called aerobic glycolysis, and then produces a large amount of lactic acid. Lactic acid concentrations in the tumor microenvironment (TME) can be as high as 10–30 mM, whereas its concentration under normal physiological conditions is ?1.5–3.0 mM,[2] and the pH can be as low as 6.0–6.5.[3] The acidic state of the TME is mainly caused by lactate, pyruvate, CO_2, and hydrogen ions transported out of cells, with lactate being considered an important contributor.[4] Lactic acid used to be considered a normal cellular metabolic waste product, but in recent years, studies have found that it is of great importance for tumor cells. Lactic acid can not only be used as the main energy source for the life activities of tumor cells but it can also activate the signal transduction pathways of tumors and other cells in the TME. Specifically, lactic acid induces the expression of programmed cell death 1 ligand 1 (PD-L1), which directly fosters immunosuppressive effects and stimulates endothelial cells to promote tumor angiogenesis, thereby facilitating tumor material exchange and distant metastasis.[5] At the same time, active lactic acid metabolism in tumors can also reduce tumor damage from external factors. Recent studies have shown that lactic acid, as a signaling molecule or chemical barrier factor, is almost entirely involved in the tolerability of antitumor treatments, such as chemotherapy, targeted therapy, immune checkpoint inhibitor therapy, and radiotherapy. In recent years, more attention has been paid to targeted therapeutic strategies for lactic acid metabolism. This paper summarizes recent research on lactic acid metabolism–targeted treatment and outlines current and future therapeutic strategies that aim to target this pathway.

LACTIC ACID METABOLISM REPROGRAMMING IN TUMOR CELLS HAS A SIGNIFICANT IMPACT ON TUMOR BIOLOGICAL BEHAVIOR

Lactic acid is an important source of energy for tumor cells

Lactic acid is both a glycolytic metabolite and a substrate for oxidative metabolism. Although aerobic glycolysis is considered one of the most important features of tumor metabolism, recent studies have shown that tumor cell growth is not only dependent on glycolysis but it is also extremely dependent on the tricarboxylic acid cycle.[6] Lactic acid can shuttle between glycolytic tumor cells and oxidative tumor cells, and is the metabolic link between their different metabolic types.[5] Glycolytic tumor cells degrade glucose to pyruvate by aerobic glycolysis, and then pyruvate is converted to lactic acid by lactic acid dehydrogenase A (LDHA), which is excreted via monocarboxylate transporter 4 (MCT4). This phenomenon is called metabolic symbiosis, which allows tumor cells to share lactic acid, decouples glycolysis and the tricarboxylic acid cycle, helps tumor cells distribute and use energy substances more efficiently, and ensures glycolysis. Lactic acid shuttling also occurs between tumor cells and carcinoma-associated fibroblasts. Hydrogen peroxide secreted by tumor cells can induce oxidative stress in fibroblasts, resulting in the upregulation of hypoxia-inducible factor 1α (HIF-1α) and glycolytic metabolism, thereby shuttling lactic acid to tumor cells, where it is used for oxidative metabolism.[7] This may be an important metabolic mechanism for lactic acid to support the rapid growth of cancer cells (Figure 1).

Lactic acid regulates signal transduction in cancer cells

Lactic acid in the immunological microenvironment of tumors can be used as an extracellular signaling molecule to bind to the membrane-bound receptor G protein-coupled receptor 81 (GPR81) and to mediate tumor cell growth through various signal transduction
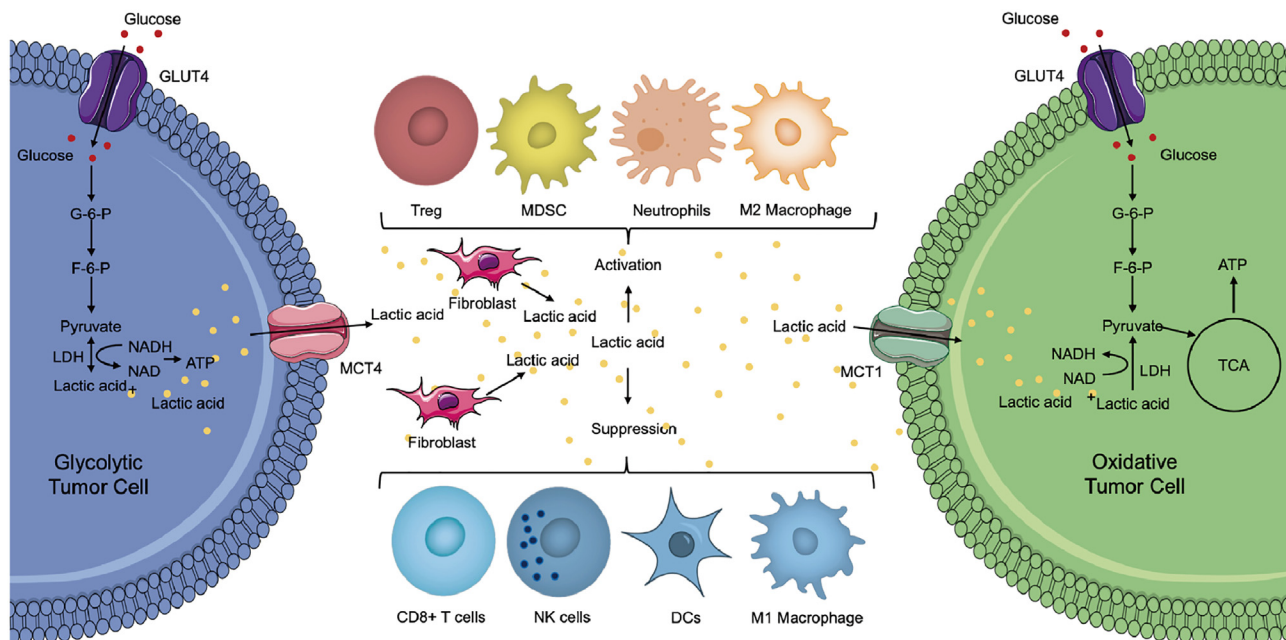
Figure 1. Lactic acid plays a dual role in the TME

Lactic acid serves as both the end product of tumor cell metabolism (in anaerobic tumors) and the energy source for tumor cells (in aerobic tumors). Moreover, it can have both inhibitory and activating effects on immune cell function. Overall, the activating effect of lactic acid on tumors is prominent, because it not only promotes tumor energy production but also facilitates immune the evasion of immune system surveillance by tumor cells. ATP, adenosine triphosphate; DCs, dendritic cells; F-6-P, fructose-6-phosphate; G-6-P, glucose-6-phosphate; GLUT4, glucose transporter 4; LDH, lactate dehydrogenase; MCT1, monocarboxylate transporter 1; MCT4, monocarboxylate transporter 4; MDSC, myeloid-derived suppressor cell; NAD+, nicotinamide-adenine dinucleotide; NADH, reduced form of nicotinamide-adenine dinucleotide; NK cells, natural killer cells; TCA, tricarboxylic acid cycle; Treg, regulatory T lymphocytes.

pathways. GPR81 is widely expressed in the global tumors studied so far, most of which are upregulated and correlate with tumor progression and prognosis. In breast cancer cells, the binding of extracellular lactic acid to GPR81 was found to activate the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway, which in turn activates the cyclic adenosine monophosphate (cAMP) response element-binding protein. This activation leads to the formation and release of amphiregulin, which promotes the angiogenesis of tumor endothelial cells, as well as tumor cell growth and proliferation.[8] Lactic acid may also be involved as a signaling molecule in the phospholipase D/mitogen-activated protein kinases/nuclear factor κB (NF-κB)[9] and the extracellular signal–regulated kinase (ERK)/90-kDa ribosomal s6 kinases[10] pathways, where it promotes basement membrane remodeling. It also functions in the transforming growth factor-β/Smad,[11] Wnt/β-catenin,[12] interleukin (IL)-6/signal transducer and activator of transcription 3,[13–15] and hepatocyte growth factor/mesenchymal epithelial transition factor[16–18] pathways, which promote epithelial-mesenchymal transition and increase tumor invasiveness.

Upon activation by lactic acid, GPR81 can decrease the level of cAMP and inhibit protein kinase A, ultimately resulting in the transcription and expression of PD-L1 on the surface of tumor cells and causing tumor cell immune escape.[19] In addition, the activation of GPR81 by tumor-derived lactic acid inhibited the expression of the major his-

tocompatibility complex class II on the cell surface, the process by which antigen-presenting cells present tumor cell antigens to T cells. Thus, the capacity of T cells was impaired, resulting in immunosuppression.[20] Activation of GPR81 by lactic acid can also activate human breast cancer susceptibility gene 1 and the ATP-binding cassette transporter subfamily B member 1 (ABCB1) via the protein kinase C/ERK signaling pathway, which increases tumor cell resistance to chemotherapeutic agents.[21] Moreover, GPR81 contributes to doxorubicin resistant in human cervical cancer (HeLa) cells by upregulation of the ABCB1 transporter.[22]

In tumor-associated endothelial cells, lactic acid can inhibit the activity of prolyl hydroxylase 2 (PHD2), and thereby increase the stability of HIF-1α,[23] thus activating NF-κB signaling from the inhibitor of NF-κBα, promotes the expression of IL-8, and stimulates tumor proliferation and the maturation of new blood vessels.[24] In addition, lactic acid can inhibit PHD2/Von Hippel-Lindau -dependent ubiquitination degradation of N-myc downstream regulated gene 3 through direct binding.[25]

In 2019, Zhang et al. found that hypoxia-triggered lactic acid production can act directly on lysine residues in cellular histones, leading to lactylation. Protein lactylation may directly activate transcription in a p53-dependent, p300-mediated manner.[26] It can also directly regulate gene expression by modifying histones, and affects the biological
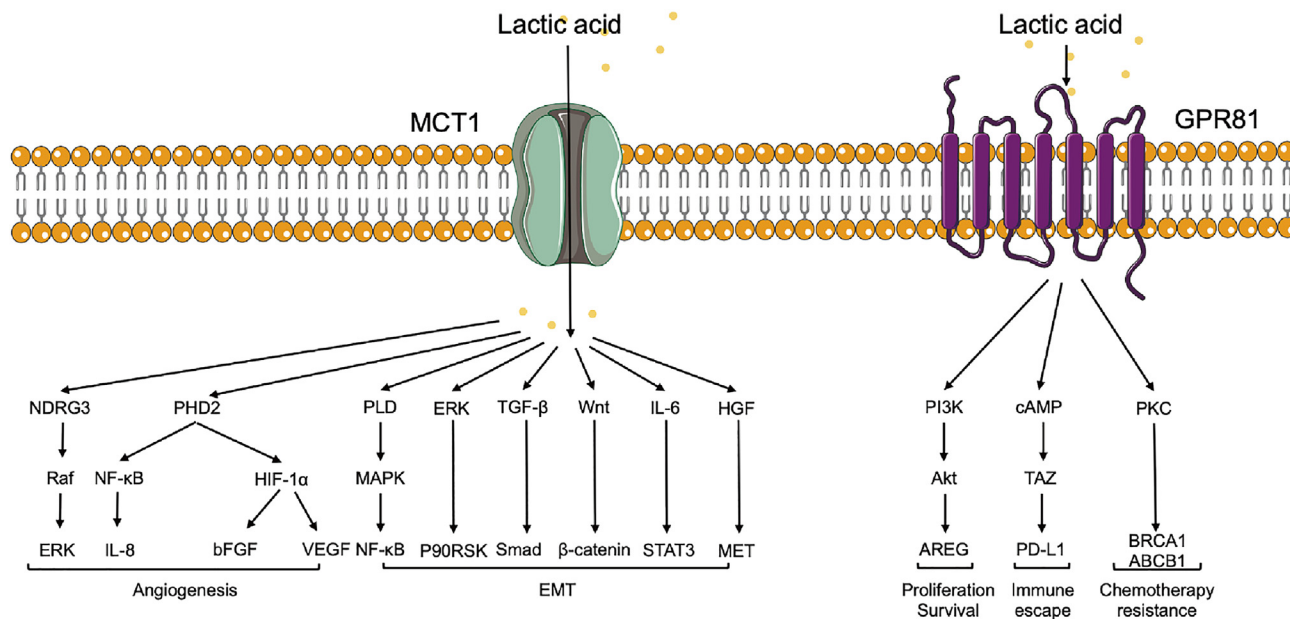
Figure 2. Lactic acid plays an important role as a signaling molecule in tumor cells

Lactic acid may trigger downstream signaling pathways via MCT1 or GPR81, resulting in several tumor-promoting effects, such as tumor cell growth and proliferation, epithelial-mesenchymal transformation, angiogenesis, and immune evasion and chemotherapy resistance. AREG, EGFR ligand amphiregulin, XX; bFGF, basic fibroblast growth factor;EMT, epithelial-mesenchymal transition; GPR81, G protein-coupled receptor 81; HGF, hepatocyte growth factor; MCT1, monocarboxylate transporter; MET, mesenchymal epithelial transitionfactor; STAT3, signal transducer and activator of transcription 3; TAZ, XX; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.

behavior of the tumor.[27] High amounts of lactic acid can also modify nonhistone proteins by lactation and then activate downstream pathways and perform their biological function.[28]

In summary, as a signaling component, lactic acid has far-reaching and important effects on tumor growth, proliferation, invasion, drug resistance, immune escape, angiogenesis, and posttranslational modification (Figure 2).

Lactic acid has an immunosuppressive effect on immune cells in the TME

Lactic acid is an important molecule in the extracellular matrix and is the main cause of TME acidosis.[29] Through the cooperation of MCT1/MCT4, lactic acid generates an internally directed proton gradient with a high intracellular pH and low extracellular pH, leading to the acidification and accumulation of lactic acid. Lactic acid also plays an important role in regulating the function of TME immune cells, which can inhibit the activity of anticancer immune cells—M1 macrophages,[30] cytotoxic T cells,[31] dendritic cells,[32] and natural killer cells[33]—while also enhancing immunosuppressive cells such as M2 macrophages, regulatory T lymphocytes,[34,35] myeloid-derived suppressor cells (MDSCs),[36,37] neutrophils[38] impeding antitumor immune responses, and mediating immune escape (Figures 1 and 2). Lactate induces MDSC differentiation, which in turn directly inhibits the antitumor response of immune cells and produces less interferon-γ.[36,37] However, it is important to note that the inhibitory effect of lactic acid on immune cells is reversible. By reducing the concentration of lactic acid or removing it from the TME, the inhibitory effect of immune cells on tumors can be restored.[32,39,40]

Lactic acid plays a key role in tumor development and progression, as well as tolerance to treatment. Thus, lactic acid metabolism is a promising antitumor therapeutic target that has gained growing attention. Current strategies targeting lactic acid metabolism focus on three aspects: (1) direct inhibitory effect on tumors, (2) combination with other antitumor therapies, and (3) as a drug delivery target.

TARGETING PRODUCTION: INHIBITION OF LACTIC ACID PRODUCTION IN TUMORS

Strategies for inhibiting LDHA

LDH is the key enzyme that catalyzes the conversion of the glycolysis products pyruvate to lactic acid.[41] In tumors, the high expression of LDH is closely related to the larger tumor volumes, the worse prognosis, and the resistance of various tumors to radiotherapy and chemotherapy.[42–45] It is surprising that a deficiency of the LDH protein has little impact on the human body, and patients with an LDH deficiency in their muscles mainly experience nonlethal symptoms, such as fatigue, muscle cramps, and myoglobinuria.[46] Therefore, the targeted use of LDH inhibitors is considered a potentially safe therapeutic strategy. LDH inhibitors reduce the lactic acid concentration in the TME by decreasing tumor lactic acid production, which leads to an enhanced killing ability of the immune cells. For example, oxamate can not only significantly increase the infiltration of activated CD8+ T cells in tumors[47] but it also inhibits the proliferation and growth of tumors

Table 1. LDHA inhibitors and MCT inhibitors used in tumor research

Compound	Mechanism of action	Cell lines/animal models	Research status
Inhibitors of LDHA			
Galloflavin	Nonselective LDH inhibition	Colorectal, endometrial, and breast cancer cells	Preclinical[53–55]
Oxamate	Nonselective LDH inhibition	Colorectal, pancreatic, gastric, and non-small cell lung cancer cells	Preclinical[56,57–59]
GNE-140	LDHA/LDHB inhibition	Breast, colorectaladenocarcinoma, and lung cancer cells; glioma xenografts	Preclinical[60,61,62]
AZ-33	LDHA inhibition	Breast cancer cells	Preclinical[63]
GSK 2837808A	LDHA inhibition	Nasopharyngeal carcinoma cells; pancreatic cancer xenografts	Preclinical[64,65]
Inhibitors of MCTs			
AZD3965	MCT1 inhibitor	Breast and colon cancer cells; lymphoma xenografts	Phase I[66]
AR-C155858	MCT1 inhibitor	Breast cancer tumor xenografts	Preclinical[67]
BAY-8002	MCT1 inhibitor	Diffuse large B cell lymphoma cells	Preclinical[68]
Syrosingopine	MCT1/MCT4 inhibitor	Breast, colon, cervical cancer, and leukemia cells	Preclinical[69]
α-Cyano-4-hydroxycinnamate	MCT1 inhibitor	Glioma, breast cancer, pancreatic ductal adenocarcinoma	Preclinical[70–72]
7ACC2	MCT1 inhibitor	Pancreatic adenocarcinoma cells	Preclinical[73]

LDHA, lactic acid dehydrogenase A; LDHB, lactic acid dehydrogenase B; MCT, monocarboxylate transporter.

caused by changes in glucose metabolism by tumor necrosis factor-α and IL-17 in the inflammatory microenvironment.[48]

Although LDH inhibitors have potential as a treatment for glycolytic tumors, their clinical use is still limited, probably because most are nonselective. LDH inhibitors (oxamate, aminooxyacetate, and dichloroacetate) can inhibit pyruvate kinase, enolase, and activite, inducing an increase in the cytosolic NAD(P)H, Fru-1,6-BP, and dihydroxyacetone phosphate levels, which can induce fatal toxicity in the human body.[49] FX-11 and hydroxyindole-2-carboxylates can inhibit NADH metabolism.[50] It can also inhibit pyruvate-driven OxPhos in heart mitochondria.[49] Moreover, gossypol has been shown to inhibit Mcl-1 and Bcl-2, which is thought to be the most important mechanism by which gossypol kills tumor cells, far exceeding the effect on LDH.[51] These findings suggest that the mechanism of LDH inhibition is not yet fully understood and raises concerns about potential off-target effects. Although LDH inhibitors may effectively kill glycolytic tumors, they may also promote the conversion of tumors to a more oxidative type and increase resistance to LDH inhibitors (Table 1).[52]

Strategies for inhibiting lactic acid transporters

Targeting the MCTs has a significant effect on cell-to-cell lactic acid exchange. Specific inhibition of MCT1 prevents the uptake of lactic acid by tumor cells, thus preventing lactic acid from flowing into tumor cells. The inhibition of MCT1 can also block its downstream signaling pathway, thereby inhibiting tumor cell proliferation, invasion, and metastasis, and promoting tumor apoptosis. However, after MCT4 inhibition, tumor cells cannot remove lactic acid, which accumulates and ultimately leads to tumor cell death. AZD3965, an inhibitor of MCT1

and MCT2, has achieved some success in preclinical studies and has been shown to be effective in models of Burkitt's lymphoma, breast cancer, gastric cancer, and small cell lung cancer.[74] AZD3965 is currently in a phase I/II clinical trial. However, studies have shown that MCT4 can compensate for its lactic acid transporter function when MCT1 is inhibited, which may be a mechanism for resistance to AZD3965.[75] α-Cyano-4-hydroxycinnamate (CHC)[76] and BAY-8002 have also been developed as effective MCT1 inhibitors, but BAY-8002 has a higher (6-fold) selectivity for MCT1 over CHC and no off-target effects.[77] However, of the many MCT1 inhibitors, only AZD3965 was enrolled in a clinical trial (NCT01791595), but it was stopped early. According to the report from this trial, a 47-year-old male with metastatic melanoma died of rapid onset of hyperlactic acidemia, which strongly suggested an association with AZD3965.[78] This indicates that MCT1 inhibitor-induced hyperlactic acidemia carries a potential risk of death, creating a significant safety concern surrounding the use of MCT1 inhibitors as a primary anticancer treatment.

MCT4 mediates lactic acid efflux from glycolytic cancer cells.[79] Knockdown or silencing of MCT4 acidifies the cytoplasm of glycolytic cancer cells, leading to tumor cell death.[80] MCT4 is highly expressed in the hypoxic regions of rapidly growing tumors, and it may be an effective therapeutic target.[80] Although there are currently drugs that target MCT4, these drugs are not MCT4-specific inhibitors, and thus the feasibility of tumor treatment strategies to inhibit MCT4 with drugs remains to be explored.[81]

In addition, the localization and maintenance of MCT1 and MCT4 at the plasma membrane are influenced by CD147/Basigin, so targeting

CD147 may represent a novel strategy for inhibiting the activity of both transporters. AC-73 is a dimerized and humanized anti-CD147 antibody that targets CD147, and its antitumor activity has been demonstrated in preclinical studies.[82] In summary, inhibition of the lactic acid transporters MCT1, MCT4, and their chaperones has a significant killing effect on tumors, but there are no data from clinical practice.

Strategies for the neutralization of lactic acid

Neutralization of lactic acid *in vivo* with an alkaline buffer was the earliest therapeutic strategy to modulate lactic acid metabolism. Early studies found that neutralizing acidic pH in tumors by oral administration of weakly alkaline buffers increased the efficacy of weakly alkaline chemotherapeutic agents. The researchers found that a long-term intake of sodium bicarbonate (200 mM) in a mouse model of prostate cancer can increase the pH in the TME. The study also found that the use of buffer therapy at 4 weeks of age prevented the development of tumors. In addition, when buffer therapy was started after 10 weeks, it had no inhibitory effect on the primary tumor, but still had a controlling effect on distant metastases.[83] Other buffer systems, such as imidazole, free base lysine, hydroxymethylaminomethane, and Tris, have the same effect on reducing tumor hyperacidity and inhibiting tumor invasion and metastasis.[84–86] Despite the therapeutic advantages of buffer therapy, it is difficult to implement it in clinical practice. The phase I/II pancreatic ductal adenocarcinoma clinical trial of buffer therapy (NCT01198821) failed due to severe adverse effects and poor adherence. The HCl-absorbing nanoparticle TRC101 was shown to significantly increase bicarbonate in patients with chronic kidney disease in a recently completed phase III study. The incidence of adverse events was significantly reduced compared with the placebo group,[87] suggesting that TRC101 may be a novel and safe buffer treatment strategy. However, there is still no evidence of the success of this novel buffer in direct clinical practice in the treatment of tumors. Although alkaline buffers have not been widely used in the systemic treatment of tumors, they may play an important role in the treatment of local tumors. In recent years, the use of sodium bicarbonate as an embolic agent for interventional tumor therapy (targeting intratumoral lactic acidosis-transcatheter arterial-chemoembolization [TILA-TACE]) has achieved significant therapeutic effects in the treatment of liver cancer; however, following routine TACE treatment, the pH in the tumor can decrease further, making it harder to manage and increasing the risk of recurrence. However, Chao et al. conducted a clinical trial in which they added 5% sodium bicarbonate to chemotherapy drugs and performed chemoembolization, resulting in a 100% tumor remission rate.[88] Subsequently, Choi et al. used tanespimycin in combination with 5% sodium bicarbonate for TACE treatment of McA-RH7777 liver cancer transplanted mice and also achieved 100% tumor remission.[89]

Lactic acid depletion strategies in the TME driven by nanotechnology

Nanotechnology breaks through the above-mentioned limitations due to its selective targeting, increased drug payload, controlled release, and good biocompatibility, which can enhance carrier drug accumulation in the local TME, increase the tolerated dose, and promote efficacy. In addition, various metabolically targeted drugs/molecules encapsulated with nanoparticles have demonstrated significant targeting and anticancer capabilities due to the excellent permeability, retention, and precise targeting properties of nanotechnology.

One therapeutic strategy using nanomedicines to target tumor lactic acid metabolism is to encapsulate lactic acid oxidase (LOX) and degrade lactic acid in the TME by catalyzing its oxidation. Cascade catalytic nanosystems deliver LOX and glycolysis inhibitors with hollow manganese dioxide (MnO_2) nanoparticles to achieve lactic acid depletion for synergistic metabolic therapy of tumors.[90] *Shewanella oneidensis* MR-1 is able to catabolize lactic acid anaerobically by respiration through electron transfer to metal minerals. The biohybrid form of lactic acid fuel (Bac@MnO_2$) was produced by *S. oneidensis* through a MnO_2 nanoflower on the surface of Streptococcus MR-1. Bac@MnO_2$ produced by biological hybridization uses the modified MnO_2 nanoflower as an electron acceptor and lactic acid as an electron donor, forming a complete bacterial respiratory pathway at the tumor site, leading to continuous degradation of lactic acid between cells. In addition, the modified MnO_2 nanoflower can catalyze the conversion of endogenous hydrogen peroxide to oxygen, preventing lactic acid production by downregulating the expression of HIF-1α. This can significantly inhibit tumor progression by coupling bacterial respiration with tumor metabolism.[91] He et al. developed a "nanofactory" that was nanopacked with cationic polyethylenimine, which is a cationic polymer, and supplemented with copper (Cu) ions.[92] The nanofactory system actively captured lactic acid and promoted its degradation with double efficiency. Hydrogen peroxide, a by-product of lactic acid degradation, was catalyzed by Cu ions to form antitumor reactive oxygen species (ROS) and mediate immunogenic cell death.[92] In another study, perfluorooctyl bromide (PFOB) nanodroplets coated with a tannic acid (TA)-iron(III) coordination complex and containing LOX (PFOB@TA-iron(III)-LOX) were used to simultaneously consume lactic acid and ATP while producing hydroxyl OH radicals.[93]

The removal of lactic acid in the TME by direct chemical degradation is a new treatment idea advanced by the development of nanotechnology. Since nanocarrier materials are relatively safe and targetable, the safety of the treatment is expected. However, whether this method is suitable for more complex *in vivo* systems and whether the drugs and their degradation products have adverse effects on normal tissues is unknown, and more *in vivo* experiments are needed before clinical application.

UNITED OFFENSIVE: COMBINATION THERAPY TARGETING LACTIC ACID METABOLISM

Lactic acid can provide not only energy and signals for tumor growth, proliferation, invasion, and metastasis but also a protective lactic acid environment from the human immune system, foreign drugs, and radiation. Inhibition of tumor lactic acid production or consumption and neutralization of lactic acid in the TME has been shown to have a significant killing effect on tumor cells and can restore immune

surveillance and attack on tumors by the human immune system. Taking advantage of targeted lactic acid therapy in combination with other drugs or technologies to enhance the effect of antitumor therapy and offset its disadvantages is an ideal therapeutic situation.

Several studies have shown that LDH inhibitors can reverse tumor cell resistance to various chemotherapeutic agents, such as paclitaxel, cisplatin, doxorubicin, and docetaxel,[42–44,94,95] and improve tumor sensitivity to radiotherapy.[45,60,96] These studies demonstrate the promise of a combined treatment regimen of targeted lactic acid therapy with antitumor agents. Such a regimen enhances the therapeutic effect of antitumor drugs and counteracts lactic acid metabolism–mediated resistance mechanisms while also allowing for a lower dose of lactic acid–targeted drugs to minimize side effects. In addition, antilactic acid therapy abolishes the inhibition of immune cells in the TME, which removes a significant obstacle to the potential use of immune checkpoint inhibitors and tumor immune cell therapy in the future.

LDH inhibitors can promote the development of drug resistance by causing tumor cells to become oxidized.[97] In contrast, drugs that target mitochondria can kill tumor cells by inhibiting the oxidative phosphorylation of glucose and increasing the production of ROS[98]; however, mitochondrial-targeted drugs produce large amounts of lactic acid, which can negatively affect normal cells.[99] Therefore, combining both types of drugs could provide an excellent balance of benefits. Studies have shown that oxamate, in combination with mitochondrial-targeted drugs, can improve therapeutic activity against tumors and reduce the risk of side effects.[99] When oxamate is used in combination with mitochondrial respiratory chain complex I inhibitors, it significantly inhibits LDH activity, resulting in a marked reduction in glucose uptake and lactic acid production, ultimately leading to the substantial depletion of cellular ATP and synergistic killing of cancer cells. In addition, *in vivo* studies have shown that oxamate is highly toxic to normal nasopharyngeal epithelial cells but is well tolerated in mice.[47] In ovarian cancer, LDH inhibitors, in combination with the oxidative phosphorylation inhibitor metformin, showed significant synergistic effects in inhibiting ovarian cancer cell growth by inhibiting glucose utilization.[52] LDH inhibitors plus phenformin can also significantly inhibit LDH activity and lactic acid production in cells and cause the rapid death of cancer cells by reducing ATP production and accelerating ROS production.[100] *In vitro* experiments have shown that phenformin is more effective at killing tumor cells when combined with oxamate than when combined with LDH silencing. In a homogeneous mouse model, phenformin and oxamate promoted apoptosis and tumor regression compared with the controls.[100]

Given the good efficacy and safety of LDH in combination with antitumor drugs or drugs targeting the mitochondria, the following question arises: Can all three of these therapies be employed together to treat tumors and achieve even more potent antitumor effects?

Studies have found that LDH inhibitors in combination with biguanide and doxorubicin drugs achieved a good therapeutic effect in the treatment of refractory tumors. The regimen is referred to as the Tt regimen. The combination of doxorubicin with oxamate and metformin can inhibit mammalian target of rapamycin phosphorylation and LDHA expression, promote apoptosis via the caspase-3 pathway, induce autophagy by accumulating LC3-II, and completely inhibit tumor growth in mice. The triple combination also showed significant tumor inhibition by a similar mechanism in colitis-associated colon cancer tumor models.[56,101] In addition, the combination of the novel glutaminase inhibitors CB-839 and oxamate caused significant cell killing in the breast cancer cell lines MDA-MB-231 and MCF-7 and improved the efficacy of doxorubicin in these cell lines.[102] Combining such therapies could have significant benefits for cancer treatment, such as improving tumor outcomes and reducing the amount of medication required to achieve those outcomes. This combined approach could also reduce the risk of side effects, such as cardiotoxicity.

Inhibition of TME lactic acid level may reduce lactic acid levels in the TME and restore the killing effect of the immune system on the tumor. Therefore, it is theorized that LDH inhibitors may promote the antitumor efficacy of immunotherapy. In a humanized mouse model of non-small cell lung cancer, the LDH inhibitor oxamate, in combination with the PD-1 inhibitor pembrolizumab, was more effective than immunotherapy alone.[103] The study found that treating tumors with oxamate significantly increased the infiltration of activated CD8+ T cells.[103] However, this synergistic effect disappeared in humanized mice treated with anti-CD8, indicating that oxamate works by activating CD8+ T cells. This is achieved by reducing lactic acid levels in the TME, thereby enhancing the efficacy of immune checkpoint inhibitors.[103] In addition, the use of MCT inhibitors to regulate the lactic acid concentration in the TME to regulate the immune status of the tumor and thus increase the efficacy of tumor immunotherapy is a new application of MCT inhibitors. One study found that patients with hepatocellular carcinoma with a high expression of MCT4 did not respond well to immune checkpoint inhibitor therapy.[104] It showed that treatment with the immune checkpoint inhibitor toripalimab and the presence of an increased number of CD8+ T cells led to increased tumor responsiveness.

LDH inhibitors may also reverse tumor resistance to some ERBB2 therapies. For example, a cetuximab-resistant head and neck squamous cell carcinoma cell line exhibited higher HIF-1α levels and a higher rate of glycolysis than the nonresistant parental cells under normoxic culture conditions.[105] However, a combination treatment with an LDHA inhibitor and cetuximab increased apoptosis in drug-resistant cells. In addition, amino acid salts have been shown to inhibit the HIF-1α-mediated glycolytic pathway and overcome cetuximab resistance in head and neck squamous cell carcinoma cells.[105,106] LDH inhibitors can also abolish MACC-1-induced HER-2+ resistance of gastric cancer cell lines to trastuzumab by inhibiting the PI3K/Akt pathway.[105,106]

In conclusion, targeting lactic acid metabolism as part of an antitumor combination therapy can enhance the benefits of different

treatment programs, leading to improved efficacy with reduced side effects and drug resistance for patients.

EXCELLENT FACILITATOR: DRUG DELIVERY BY EXPLOITING THE HIGH LACTIC ACID TME

Lactic acid metabolism can be exploited not only as a target for the treatment of tumors but also as a natural condition for the introduction of carriers into tumors. Recently, researchers have developed a new method to enhance low-dose radiotherapy in tumors by exploiting the high levels of lactic acid in the TME. They accomplished this using platinum nanoparticles, which were modified with LOX, to create a multifunctional sensitizer that demonstrated lactic acid–driven directional chemotaxis, migrating specifically toward the lactic acid–rich region of the TME. Once there, the sensitizer facilitated cascade catalytic oxygen production, which further enhanced the sensitization effect of radiotherapy.[107] This is of great significance for the development of directional chemotaxis of nanopreparations guided by enzyme substrate concentration gradients and sensitization strategies for low-dose radiotherapy based on TME characteristics. Studies have shown that the incorporation of AZD3965 into pH-hypersensitive nanoparticles may reduce drug distribution and toxicity in cardiac and hepatic tissues,[108] creating new opportunities for the use of AZD3965 in the clinical treatment of tumors. These nanoparticles are designed to rapidly disintegrate and release the payload when exposed to acidic pH levels, which is a promising basis for precise and safe drug delivery.[78] Nanotechnology has multiple advantages in antitumor therapy. However, several limitations, including nonselective uptake of nanoparticles by normal healthy cells and potential toxicity of the nanoparticle component, also exist. This is an important issue that must be addressed immediately. For example, reducing toxicity of nanodelivery by direct nanodrug synthesis using active drug molecules as nanodrug carriers and using targeted nanoparticles by modifying the surface to contain targeting ligands may be a potential solution.

OUTLOOK

The most fundamental principles of clinical medicine are to prevent harm and promote patient well-being. LDH inhibitors and MCT inhibitors have shown promising tumor-inhibitory effects in preclinical research; however, their use has been limited, and fatal side effects have been reported. The outlook for these traditional therapeutic targets of lactic acid metabolism for single-agent treatment of tumors is bleak. The main reason is that LDH and MCTs have important life-preserving functions in both healthy people and patients with tumors. The inhibition of their functions poses a great threat to survival. Thus, the primary strategy for existing targeted lactic acid metabolism therapies should focus on maximizing the antitumor effects of drug combinations while minimizing the risks of adverse effects. This can be achieved by leveraging the strengths of each drug and avoiding their weaknesses. In addition, it is essential to ensure that the drug doses are within a safe range. Although it is true that reducing the dose of these drugs may attenuate their killing effect on tumors, the drugs can be used as adjuvants to increase the effectiveness of other antitumor drugs. Although LDH inhibitors have been shown to improve the treatment efficacy of many chemotherapeutic agents, the current research on this topic is insufficient and somewhat outdated. Recently, small molecule targeted therapy and immune checkpoint inhibitors have emerged as the core of antitumor drug treatment due to their superior efficacy and minimal side effects. An interesting area of research is whether low-dose inhibitors of lactic acid metabolism can increase the sensitivity of tumors to immune checkpoint inhibitors by reducing lactic acid levels in the TME. In addition, it is worth exploring whether lactic acid is involved in the resistance mechanism of targeted small molecule inhibitors other than ERBB2 inhibitors, which could improve drug efficacy or even overcome resistance.

Lactic acid in the TME may be more valuable in antitumor therapy than key proteins in lactic acid metabolism. In the local treatment of tumors, the good efficacy and safety in neutralizing lactic acid therapy is clearly understood. This provides a new therapeutic approach and idea for early tumor treatment. In addition, high lactic acid concentration, as a hallmark feature of TME, is one of the best targets for drug delivery. Combining antitumor drug delivery with lactate neutralization therapy using tumor lactate metabolic characteristics may become a new idea for tumor treatment because of its precision, efficiency, and safety. Finally, the effect of lactic acid on tumors has not been given adequate attention, and the focus of many targeted drug studies has been the direct killing of tumors rather than on targeting factors that can affect lactic acid metabolism, such as MYC and ERBB2, and potentially affect tumor growth. Furthermore, the formation of an acidic environment in the TME is a combination of the production of numerous acidic metabolites, of which lactate is one of the important factors, and the aberrant regulation of the expression and activity of the hydrogen ion transporter, which leads to excessive transport of hydrogen ions into the TME leading to TME acidification. However, none of the quantitative studies on TME lactate are available to illustrate this important information. Numerous studies have shown that different tumor cells cause different pH changes in the culture medium in *in vitro* studies. Lactate dehydrogenase has been widely used as an important tumor-associated marker in the clinical prediction of tumorigenesis and tumor load. Because the quantitative study of lactate concentration in tumor TME is still unclear, however, there are no research results to prove the direct relationship between lactate concentration in TME and the degree of tumor development. It is believed that after this technique is overcome, the data on lactate concentration in TME obtained through pathological sampling will better guide us in individualized analysis and treatment of tumor malignancy, prognosis, and drug resistance.

Thus, if we consider lactic acid metabolism to be at the core of combined therapy, then combining antitumor drugs that inhibit lactic acid production with therapeutic regimens that cause treatment resistance due to lactic acid may lead to more effective antitumor treatments in the future.

SUMMARY

The large amount of lactic acid produced by tumors through the Warburg effect is one of the important metabolic features of tumors

that differ from normal cells in the human body. Lactic acid metabolism is closely related to tumor occurrence and development; thus, targeting this process has become an important strategy for therapeutic interventions against tumors. At present, LDH inhibitors, MCT inhibitors, and lactic acid buffer/consumables have shown some therapeutic efficacy, but their use is still restricted by certain factors. They have limited selectivity for targeting tumors and therefore have unwanted side effects, which makes them difficult to examine in human clinical trials. Thus, to improve the effectiveness of antitumor therapy and minimize the potential side effects of lactic acid metabolism inhibitors, it may be beneficial to explore combinations of these inhibitors with other treatments while also reducing their dosage. Moreover, we need to identify new therapeutic targets and drugs that can efficiently target lactic acid metabolism pathways, thereby improving the safety and effectiveness of tumor treatments. An approach to consider is using the high lactic acid concentrations found in tumors as a target for drug identification, while also developing a carrier that can safely and accurately deliver antitumor drugs. In short, tumor lactic acid metabolism is a valuable target for antitumor therapy, and inhibiting the lactic acid produced by tumors or exploiting the properties of tumor hyperlactic acid can greatly improve antitumor therapy.

AUTHOR CONTRIBUTIONS

Z.L. cowrote the article and created the figures. J.C. cowrote and reviewed the article.

DECLARATION OF INTERESTS

Both of the authors report no conflicts of interest.

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