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Review Article

The Emerging Role of the Lactylation-autophagy Axis in Hepatocellular Carcinoma: A Metabolic-epigenetic Driver of Tumor Immune Remodeling

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Abstract

Hepatocellular carcinoma (HCC) exhibits profound metabolic reprogramming, with enhanced aerobic glycolysis and excess lactate production as defining features. Lactate, once regarded simply as a metabolic byproduct, is now recognized as a powerful epigenetic driver through lysine lactylation, while tumor cells co-opt autophagy to endure metabolic stress. Although traditionally investigated as separate hallmarks of cancer, growing evidence points to their functional intersection. Here, we propose the concept of a coordinated "lactylation-autophagy axis" and clarify its role in remodeling the tumor immune microenvironment. Within this integrated metabolic-epigenetic-proteostatic network, lactate-induced histone lactylation, such as H3K18la, exerts transcriptional control over tumor-associated macrophages, shifting them toward an immunosuppressive M2-like state. Excess extracellular lactate and robust autophagic adaptation cooperate to impair the effector functions of $CD8^+$ T cells and natural killer cells, while providing regulatory T cells with metabolic advantages. We further explore the bidirectional crosstalk within this axis: lactate modulates autophagic flux, potentially through mTOR signaling and non-histone lactylation of autophagy-related proteins (e.g., VPS34, TFEB), whereas autophagy in turn regulates intracellular metabolite pools and lactylation substrates. Notably, several nodes, particularly non-histone lactylation of VPS34, TFEB, and mTOR (K921), have been demonstrated mainly in non-HCC systems; we therefore grade each mechanism as confirmed in HCC, extrapolated from other systems, or hypothetical. This proposed self-reinforcing axis may sustain tumor plasticity, contribute to immune evasion and immunotherapy resistance, and we discuss rational combinatorial strategies—combined targeting of lactate metabolism, lactylation machinery, and autophagic pathways—to sensitize advanced HCC to immune checkpoint blockade.

Keywords: Hepatocellular carcinoma; Lactylation; Autophagy; Tumor immune microenvironment; Metabolic reprogramming; Immune evasion; Macrophage polarization.

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Introduction

Hepatocellular carcinoma (HCC) is the sixth most commonly diagnosed cancer and the third leading cause of cancer-related death worldwide.[1] Its incidence has increased alongside the increasing prevalence of chronic liver disease and metabolic dysfunction-associated steatotic liver disease (MASLD). Despite notable advancements in surgical resection, locoregional therapeutic approaches, tyrosine kinase inhibitors, and immune checkpoint blockade therapies, the overall prognosis for patients with advanced HCC remains unfavorable. This poor outcome is largely attributed to high rates of tumor recurrence, resistance to treatment, and the profound state of immunosuppression within the tumor microenvironment (TME).[1] A growing body of evidence suggests that metabolic reprogramming is not simply a secondary byproduct of malignant transformation; instead, it may contribute importantly to tumor progression and immune remodeling in HCC.[2] However, the precise molecular mechanisms by which these metabolic changes integrate with epigenetic regulation and intracellular quality-control systems to shape tumor evolution have yet to be fully elucidated.

One of the defining metabolic characteristics of HCC is the enhanced activation of aerobic glycolysis, a phenomenon known as the Warburg effect. This metabolic shift leads to increased lactate production even when oxygen levels are normal.[2] For many years, lactate was viewed merely as a waste product of metabolism, but it is now recognized as an important signaling metabolite that coordinates multiple aspects of tumor biology, including growth, angiogenesis, and immune evasion. Of particular importance, the landmark discovery of lysine lactylation has reframed lactate as an epigenetic substrate.[3] Through the direct modification of histone and non-histone proteins, lactylation functionally connects metabolic flux to the reprogramming of transcriptional

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activity. In HCC, histone lactylation, for instance, histone H3 lysine 18 lactylation (H3K18la), upregulates genes involved in hypoxic adaptation, the expression of immune checkpoints (such as CD274/PD-L1), and tumor plasticity.[3] Alongside this nuclear epigenetic reprogramming, tumor cells also co-opt cytoplasmic quality-control mechanisms, with autophagy being the most prominent, to survive the stresses associated with altered metabolism.

As a highly conserved lysosome-mediated degradation pathway, autophagy plays a context-dependent role in the development and progression of HCC. In established tumors, autophagic flux supports cancer cell survival by sustaining metabolic adaptation, eliminating damaged mitochondria (a process called mitophagy), and conferring resistance to various cell death pathways, including ferroptosis. Central metabolic sensors, most notably the mechanistic target of rapamycin complex 1 (mTORC1), regulate the delicate balance between glycolytic flux and autophagic activity. While lactylation and autophagy have been extensively studied as independent hallmarks of cancer, recent findings suggest that there is a mechanistic crosstalk between these two processes. Non-histone lactylation has been widely detected across complex metabolic and signaling networks.[4] Recent research has revealed that lactate can directly induce the lactylation of key autophagy-related proteins and their upstream regulators (e.g., PIK3C3/VPS34 and transcription factor EB [TFEB]), providing a non-transcriptional, post-translational pathway to rapidly modulate autophagic flux.[5,6] In turn, autophagy regulates the availability of intracellular metabolites, which can reciprocally influence lactate production and global lactylation dynamics.

This bidirectional interaction points to the existence of a tightly coordinated "lactylation-autophagy axis". Within the HCC microenvironment, the convergence of this axis may have important implications for tumor immune evasion. Accumulated lactate acidifies the TME, while lactate-induced lactylation directly promotes the expression of immunosuppressive gene programs. Together, the lactylation-autophagy axis may promote the polarization of tumor-associated macrophages (TAMs) toward an immunosuppressive M2-like phenotype—driven by elevated arginase 1 (Arg1) expression—impairs the effector functions of cytotoxic $CD8^+$ T cells and natural killer (NK) cells, enhances the metabolic flexibility of regulatory T cells (Tregs), and stabilizes the surface expression of PD-L1.[7,8] Currently, treating metabolic reprogramming and autophagy as isolated pathways limits our understanding of immune tolerance in HCC. There is an urgent need for a comprehensive synthesis of lactylation and autophagy as an integrated functional network. In this review, we propose a novel conceptual framework in which glycolysis-derived lactate induces histone and non-histone lactylation, and this process structurally and functionally intersects with the autophagic machinery (Fig. 1). By dissecting this unified metabolic-epigenetic-autophagic network, we aim to clarify its role in orchestrating the remodeling of the immune microenvironment and to highlight promising combinatorial therapeutic strategies that target this axis to overcome HCC progression.[9,10]

Metabolic reprogramming and lactate dynamics in HCC

Metabolic reprogramming is widely accepted as a fundamental hallmark of cancer, and it plays a central role in the pathogenesis and progression of HCC.[2,11] Under physiological circumstances, the liver acts as a metabolically specialized organ that regulates glucose homeostasis, lipid metabolism, amino acid turnover, and detoxification processes. However, during the development of hepatocarcinogenesis, this tightly controlled metabolic network undergoes profound rewiring. This rewiring is critical for sustaining rapid cellular proliferation, enabling adaptation to stress, and orchestrating immune evasion.[2] Among these diverse metabolic changes, enhanced aerobic glycolysis and the subsequent accumulation of lactate have emerged as key drivers, ones that fundamentally reshape both the intrinsic biology of tumor cells and the landscape of the tumor immune microenvironment (TIME).

Aerobic glycolysis and the Warburg effect in HCC

Nearly a century ago, Otto Warburg observed that cancer cells have a tendency to rely preferentially on glycolysis rather than oxidative phosphorylation for energy production, even when oxygen is abundant. This metabolic pattern is now commonly referred to as the Warburg effect.[11] In the context of HCC, this shift toward glycolysis is driven by the hyperactivation of oncogenic signaling pathways, including MYC and PI3K-AKT-mTOR. Additionally, the stabilization of hypoxia-inducible factor 1-alpha (HIF-1α) amid chronic inflammatory and hypoxic stress contributes to this glycolytic shift.[12] As a result, several key glycolytic enzymes are significantly upregulated in HCC, and their overexpression often correlates with poor clinical outcomes. Hexokinase 2 (HK2) is frequently overexpressed in HCC; it accelerates tumor growth by facilitating glucose phosphorylation and mitochondrial coupling. Pyruvate kinase M2 (PKM2)—the isoform associated with cancer—amplifies aerobic glycolysis and diverts biosynthetic flux toward the synthesis of essential nucleotides and lipids.[13] Furthermore, lactate dehydrogenase A (LDHA), the enzyme that converts pyruvate to lactate, is consistently upregulated in HCC and closely linked to aggressive tumor phenotypes.[14] The combined effect of these alterations is a pronounced shift toward increased glucose uptake, accelerated glycolytic flux, and increased lactate production. Crucially, this metabolic reprogramming allows glycolytic intermediates to be redirected into anabolic pathways. This redirection fulfills the dual bioenergetic and biosynthetic needs of rapidly expanding tumor biomass, supporting the unregulated growth of HCC cells.

Lactate production and export: beyond metabolic waste

For many years, lactate was dismissed as nothing more than a metabolic byproduct. However, it is now widely recognized as a critical signaling metabolite that coordinates tumor growth, angiogenesis, and immune evasion.[2] In HCC, elevated LDHA expression is directly associated with higher lactate concentrations in tumor tissues when compared to adjacent nontumorous liver parenchyma.[14] This accumulation of lactate is further worsened by hypoxia-induced HIF-1α stabilization, which transcriptionally upregulates both LDHA and monocarboxylate transporter 4 (MCT4).[12] In highly glycolytic tumor cells, lactate export is primarily mediated by MCT4, while monocarboxylate transporter 1 (MCT1) facilitates lactate uptake in oxidative cells.[15] This bidirectional transport creates a metabolic symbiosis within the tumor structure: hypoxic cells release lactate, which is then taken up by oxygenated cells to serve as an alternative carbon source.[16] Beyond maintaining intracellular pH and enabling metabolic exchange, extracellular lactate strongly acidifies the TME. This localized acidification creates a hostile environment that impairs the effector functions of cytotoxic T lymphocytes (CTLs), NK cells, and dendritic cells (DCs), making it an important contributor of tumor immune escape.[17]

Lactate as a signaling molecule in tumor progression

Independent of its role in bioenergetics, lactate exerts multi-

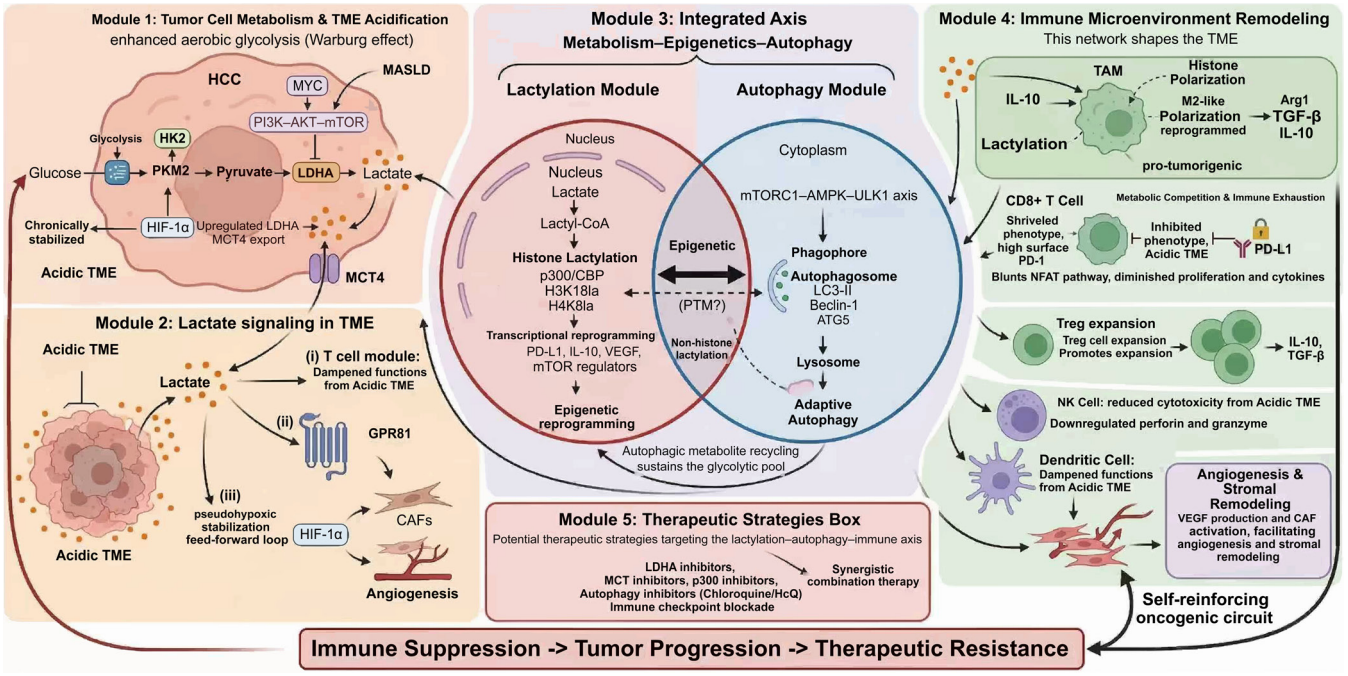


Fig. 1. Mechanistic model of the lactylation-autophagy-immune axis in HCC. Lactate dynamics play a central role in orchestrating immune microenvironment remodeling and driving self-reinforcing oncogenic circuits in HCC. This unified network is integrated across five functional modules. (Module 1) Tumor Metabolism and TME Acidification: Enhanced aerobic glycolysis (the Warburg effect) and upregulated glycolytic enzymes (HK2, PKM2, LDHA) lead to excessive intracellular lactate production. Chronic HIF-1α stabilization further drives LDHA expression and MCT4-mediated lactate export, generating an acidic TME, a process exacerbated by underlying metabolic conditions such as MASLD. (Module 2) Lactate Signaling in the TME: Extracellular lactate functions as a signaling molecule via the GPR81 receptor, sustaining a pseudohypoxic feed-forward loop that promotes CAF activation and angiogenesis, while simultaneously dampening localized immune responses (e.g., T cell functions). (Module 3) The Metabolism-Epigenetics-Autophagy Integrated Axis: This core network links metabolic and epigenetic adaptations with autophagic machinery. In the nucleus, lactate-derived lactyl-CoA facilitates p300/CBP-mediated histone lactylation (e.g., H3K18la, H4K8la). This epigenetic reprogramming drives the transcriptional upregulation of genes critical for immune evasion and metabolic adaptation (PD-L1, IL-10, VEGF, mTOR regulators). Concurrently, cytoplasmic autophagic flux operates via the mTORC1-AMPK-ULK1 axis, progressing from phagophore formation (involving LC3-II, Beclin-1, ATG5) to adaptive autolysosomes. Notably, autophagic metabolite recycling sustains the glycolytic pool, establishing a bidirectional crosstalk between metabolism and adaptive autophagy, potentially involving non-histone lactylation as a PTM. (Module 4) Immune Microenvironment Remodeling: The acidic and lactate-rich TME orchestrates widespread immunosuppression. TAMs are skewed toward a pro-tumorigenic M2-like phenotype driven by histone lactylation, secreting Arg1, TGF-β, and IL-10. $CD8^+$ T cells undergo immune exhaustion due to metabolic competition and high surface PD-1 expression, which engages tumor-derived PD-L1 to blunt the NFAT pathway and diminish proliferation and cytokine release. Furthermore, this milieu promotes Treg expansion while dampening the cytotoxicity of NK cells and the functions of dendritic cells. Concurrently, VEGF production and CAF activation facilitate robust angiogenesis and stromal remodeling. (Module 5) Therapeutic Strategies and Self-Reinforcing Oncogenic Circuit: The suppressed immune microenvironment feeds back into tumor metabolism, creating a vicious cycle of immune suppression, tumor progression, and therapeutic resistance. Disrupting this circuit requires targeting key nodes within the lactylation-autophagy-immune axis, utilizing agents such as LDHA/MCT inhibitors, p300/CBP inhibitors, autophagy inhibitors (e.g., chloroquine/HCQ), and immune checkpoint blockade, highlighting the potential for synergistic combination therapies. AMPK, AMP-activated protein kinase; Arg1, arginase 1; ATG5, autophagy related 5; CAFs, cancer-associated fibroblasts; GPR81, G-protein-coupled receptor 81; H3K18la, histone H3 lysine 18 lactylation; H4K8la, histone H4 lysine 8 lactylation; HCC, hepatocellular carcinoma; HCQ, hydroxychloroquine; HIF-1α, hypoxia-inducible factor 1-alpha; HK2, hexokinase 2; IL-10, interleukin-10; LC3-II, microtubule-associated protein 1 light chain 3 II; LDHA, lactate dehydrogenase A; MASLD, metabolic dysfunction-associated steatotic liver disease; MCT4, monocarboxylate transporter 4; mTORC1, mechanistic target of rapamycin complex 1; NFAT, nuclear factor of activated T cells; NK, natural killer; p300/CBP, p300/CREB-binding protein; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; PKM2, pyruvate kinase M2; PTM, post-translational modification; TAM, tumor-associated macrophage; TGF-β, transforming growth factor-beta; TME, tumor microenvironment; Treg, regulatory T cell; ULK1, unc-51 like autophagy activating kinase 1; VEGF, vascular endothelial growth factor.

ple signaling effects that promote tumor progression. These effects include the following:

- HIF-1α Stabilization: Even under normoxic conditions, lactate can stabilize HIF-1α by directly inhibiting prolyl hydroxylase activity, thereby inducing a "pseudohypoxic" state.[12] This feed-forward loop amplifies the transcription of glycolytic and angiogenic genes, further solidifying metabolic reprogramming and vascular remodeling.
- GPR81-Mediated Signaling: Extracellular lactate signals through the G-protein-coupled receptor GPR81, which is widely expressed across various types of malignancies.[2] Activation of GPR81 promotes tumor cell survival and angiogenesis. While detailed studies on GPR81 in HCC are still ongoing, GPR81-mediated signaling represents a crucial autocrine/paracrine mechanism through which lactate influences tumor behavior and recruits immunosuppressive cells.
- Angiogenesis and Stromal Remodeling: Lactate actively stimulates the production of vascular endothelial growth factor (VEGF), promoting angiogenesis.[2,17] Additionally, it modulates the activation of cancer-associated fibroblasts (CAFs) and promotes the remodeling of the extracellular matrix.[2,18]
- Beyond these classical stromal targets, recent evidence extends the lactylation-autophagy axis to the hepatic sinusoids: a lactylation- and autophagy-associated prognostic signature derived from HCC cohorts identified liver sinusoidal endothelial cells (LSEC)-secreted CLEC3B as a novel suppressor of HCC.[19] CLEC3B was markedly downregulated in HCC (The Cancer Genome Atlas [TCGA]-LI-Liver hepatocellular carcinoma vs. normal tissue, paired

clinical tissues, and serum samples), and conditioned medium from CLEC3B-overexpressing liver sinusoidal endothelial cells (LSECs) suppressed HCC cell proliferation, LDHA expression, global protein lactylation, and autophagic flux; mechanistic juxtaposition of lactate supplementation with rapamycin or trehalose treatment suggested that lactylation acts upstream of autophagy in this context.[19] Notably, CLEC3B ranks among the genes of a five-gene lactylation-autophagy prognostic signature with robust time-dependent AUC values (0.73–0.81 for 1–3 year OS), indicating that this LSEC-centric axis carries both mechanistic and prognostic weight.[19]

Lactate and immune suppression in HCC

The liver is inherently an immunologically unique organ, characterized by intrinsic tolerogenic properties. In the context of HCC, lactate accumulation pathologically enhances this immunosuppressive environment. Lactate derived from tumor cells significantly inhibits $CD8^+$ T cell proliferation and cytokine production by disrupting T-cell glycolysis and reducing nuclear factor of activated T cells (NFAT) pathway activation.[17] At the same time, elevated lactate impairs NK cell cytotoxicity and downregulates the expression of perforin and granzyme.[17] In the myeloid compartment, lactate plays an important role in skewing macrophages toward a pro-tumorigenic, M2-like phenotype, one characterized by the robust secretion of anti-inflammatory cytokines.[20] Importantly, recent breakthroughs have identified histone lactylation as the mechanistic link between lactate accumulation and the transcriptional reprogramming of these TAMs.[3,21] This finding highlights that lactate functions not only as an extracellular immunosuppressant but also as an intracellular epigenetic regulator that drives therapy resistance.[21] Thus, lactate serves as the central molecular link connecting tumor metabolic rewiring directly to immune remodeling.

Metabolic plasticity and adaptation under hypoxia

Rapid tumor growth and abnormal vascularization make hypoxia a common feature of the HCC microenvironment. Under these hypoxic conditions, HIF-1α activation further works in synergy with glycolytic gene expression to maximize lactate production.[12] To survive fluctuations in nutrient availability, HCC cells display remarkable metabolic plasticity, dynamically switching between glycolysis and oxidative phosphorylation. This adaptability is inherently supported by autophagy, a process of intracellular quality control that recycles substrates during periods of severe metabolic stress.[22] While the immunological implications of autophagy will be discussed in detail in subsequent sections, it is important to emphasize here that metabolic reprogramming and autophagic adaptation are functionally inseparable in HCC.[22] In advanced stages of the disease, persistent hypoxia and nutrient deprivation further increase lactate accumulation, thereby reinforcing a strong metabolic-epigenetic feedback loop.

Importantly, oxygen and nutrient gradients within the tumor generate pronounced regional differences: the hypoxic core is characterized by maximal HIF-1α stabilization, glycolytic flux, and lactate accumulation, whereas the invasive margin—more vascularized and metabolically permissive—exhibits lower lactate levels and distinct MCT isoform expression. Consequently, both lactylation intensity and autophagic activity are spatially non-uniform within a single tumor, and region-resolved profiling is required to map the axis onto distinct tumor compartments.

Integration: lactate as a central metabolic hub in HCC

In summary, metabolic reprogramming in HCC leads to sustained glycolytic flux and substantial lactate production. Far from being a metabolic end product, lactate operates at four distinct levels: as a bioenergetic metabolite, a signaling molecule, an immune modulator, and an epigenetic substrate (through lactylation). This multifaceted role positions lactate as the central node connecting tumor metabolism with immune suppression and intracellular stress adaptation. Emerging evidence highlights that the biological impact of lactate extends far beyond extracellular acidification; it also acts directly as a substrate for post-translational modification (PTM). Therefore, we next explore the precise molecular mechanisms of histone and non-histone lactylation, as well as their significant immunometabolic implications in HCC.

Molecular mechanisms of histone and non-histone lactylation

The discovery of lysine lactylation has substantially expanded our understanding of how metabolic intermediates regulate gene expression and cellular function.[3] For many years, lactate was consigned to the role of a terminal glycolytic byproduct; today, however, it is clearly recognized as a powerful substrate for PTM. This direct link connects dynamic metabolic flux to both epigenetic and proteomic reprogramming.[3,23] In HCC, where glycolytic dominance stands as a defining metabolic hallmark, lactylation emerges as the critical molecular interface that bridges metabolic stress to transcriptional plasticity and immune modulation.

Discovery of histone lactylation: metabolism meets epigenetics

Zhang *et al.* made a report in 2019, identifying lysine lactylation—a novel lactate-derived modification—through the use of mass spectrometry-based proteomics.[3] Termed lysine lactylation (Kla), this modification occurs mainly on residues of histones H3 and H4 (for example, H3K18la) and functions to promote gene transcription.[3] Importantly, intracellular levels of histone lactylation dynamically reflect intracellular lactate concentrations. In lipopolysaccharide-stimulated macrophages, late-stage lactate accumulation leads to significant H3K18la enrichment at the promoters of genes associated with wound healing and M2-like functional polarization.[3] This landmark discovery established lactylation as a direct mechanism coupling metabolism and epigenetics. Unlike acetylation, which primarily acts as a sensor for acetyl-CoA availability, lactylation serves as a direct, specific epigenetic sensor of glycolytic flux and hypoxic stress.

Biochemical basis of lactylation: substrate origin and enzymatic catalysis

Lactyl-CoA as the primary donor: The biochemical mechanisms underlying lysine lactylation are being elucidated at a rapid pace. Similar to other forms of lysine acylation, lactylation requires an activated acyl-CoA donor, in this case, lactyl-CoA. While physiological intracellular lactyl-CoA pools are relatively lower than those of acetyl-CoA, robust enzymatic and non-enzymatic pathways support Kla formation. Recent research has shown that lactylation arises through both enzymatic catalysis and non-enzymatic acyl transfer, which originates from lactoyl-glutathione intermediates generated during methylglyoxal detoxification.[23] This finding indicates that multiple interconnected metabolic pathways converge to sustain Kla formation.

p300/CREB-binding protein (p300/CBP) as the primary lactyltransferase: Mechanistically, the canonical histone acetyltransferase p300/CBP possesses promiscuous acyltransferase activity and acts as the main "writer" of histone lactylation both *in vitro* and *in vivo*.[3,18] Given that p300/CBP activity is frequently dysregulated in malignant contexts, its ability to mediate both acetylation and lactylation positions it as a master integrator of metabolic and epigenetic signaling in HCC.

Beyond p300/CBP, several other acyltransferases have now been established as bona fide lactylation writers. General Control Non-derepressible protein 5 (GCN5), also known as K(lysine) acetyltransferase 2A (KAT2A), functions as a genuine lactyltransferase: Acyl-CoA Synthetase Short-Chain Family Member 2 (ACSS2) acts as a lactyl-CoA synthetase that couples with KAT2A in a nuclear complex (supported by co-crystal structures), enabling KAT2A-mediated histone lactylation that drives Wnt/β-catenin, NF-κB, and PD-L1 expression and tumor immune evasion.[24] Moreover, GCN5, rather than p300 or MOF, was identified as the principal lactyltransferase for histone H3 lysine 9 lactylation (H3K9la) during liver regeneration, where H3K9la upregulates Lipin1 to promote fatty acid oxidation,[25] and GCN5 also lactylates non-histone substrates such as ERK at K231, forming an oncogenic lactylation-phosphorylation feed-forward loop in KRAS-mutant cancers.[26] The MYST-family acetyltransferase KAT8/MOF likewise possesses pan-Kla writer activity across histone and non-histone substrates: it lactylates eEF1A2 at K408 to enhance protein synthesis and colorectal carcinogenesis,[27] catalyzes H4K16la to drive inflammatory gene expression in cardiomyocytes,[28] and notably, antagonizes the delactylase SIRT3 at the same non-histone residue (MDH2 K239) in renal cancer,[29] illustrating a bidirectional writer-eraser balance at individual Kla sites. By contrast, evidence for PCAF/KAT2B as a direct lactyltransferase remains circumstantial.[30] Beyond the KAT family, tRNA synthetases have emerged as non-canonical lactyltransferases: alanyl-tRNA synthetase 1 (AARS1) acts as a lactate sensor and lactyltransferase that lactylates p53 and other substrates to promote tumorigenesis,[31] while AARS2 mediates mitochondrial protein lactylation under hypoxia[32]; histone acetyltransferase 1 (HAT1) adds a further node by lactylating RPA1 to promote DNA repair in glioblastoma.[33] In HCC, direct evidence increasingly implicates p300 within this network: the histone chaperone ASF1B recruits p300 to raise H3K18la and establish an oncogenic feedback loop,[34] p300 lactylates Rab7A to promote lactate/MCT1-dependent exosome biogenesis and metastasis,[35] and the H3K18la reader BRD9 couples these marks to oncogenic chromatin remodeling in a p300/histone deacetylase (HDAC)-regulatable manner.[36] Collectively, these findings argue that hyper-lactylation in HCC reflects the activity of a multi-enzyme writer network rather than p300/CBP alone.

Histone lactylation in transcriptional regulation

Histone lactylation functions fundamentally as a permissive and activating epigenetic mark. Genome-wide profiling reveals a striking enrichment of H3K18la at the promoter regions of actively transcribed genes, which physically correlates with an open chromatin architecture.[3] In tumor contexts, histone lactylation is deeply involved in driving aggressive transcriptional programs. These programs include the upregulation of immune checkpoint molecules (e.g., PD-L1), the promotion of angiogenic factors (e.g., VEGF), and the induction of immunosuppressive cytokines (e.g., IL-10).[20,21] Recent evidence suggests that lactate-induced histone lactylation directly regulates the polarization of TAMs within the TME, thereby reinforcing local immune suppression.[21] Specifically in HCC, the sustained production of lactate creates a permissive epigenetic environment for histone lactylation, endowing both malignant and infiltrating immune cells with transcriptional plasticity.[18]

Crosstalk between lactylation and other histone modifications

Histone modifications function within a highly coordinated combinatorial code rather than acting in isolation. Since acetylation, crotonylation, and lactylation all target lysine residues, they inherently compete for shared genomic loci and available acyltransferase active sites.[18] Metabolic shifts that strongly favor lactate production, such as the Warburg effect in HCC, can fundamentally alter the epigenetic balance, shifting it from acetylation toward lactylation.[18,37] This dynamic epigenetic crosstalk directly modulates the recruitment of transcription factors, the binding of chromatin remodeling complexes, and the accessibility of RNA polymerase. Ultimately, this dictates tumor cell fate under conditions of hypoxic stress.

Non-histone lactylation: expanding the functional landscape

While early research focused solely on chromatin, advanced quantitative proteomics has revealed that lactylation is widely distributed across the non-histone proteome. Extensive lactylome mapping has identified hundreds of lactylated targets that regulate diverse cellular functions, including core metabolism, cytoskeletal dynamics, RNA processing, and intracellular signal transduction.[23] This finding expands the functional scope of lactylation, moving it beyond purely epigenetic control to direct enzymatic and structural regulation. Because numerous metabolic enzymes themselves contain lysine residues susceptible to acylation, non-histone lactylation establishes critical feedback loops that can rapidly fine-tune autophagic flux and metabolic throughput.[23]

Lactylation in cancer biology

Growing evidence links lactylation to the active progression of diverse malignancies. Within the TIME, lactylation drives TAMs to express Arg1 and other M2-associated markers, thereby disabling antitumor immunity.[3,20] In solid tumors like HCC, lactate-driven lactylation acts as an oncogenic driver that accelerates immune evasion, sustains angiogenesis, and promotes therapeutic resistance.[18,37] Mechanistically, lactylation serves to lock in oncogenic transcriptional programs and reinforce metabolic adaptation under severe nutrient deprivation, acting as a key mediator of tumor plasticity.

It should also be noted that lactylation in HCC does not operate in isolation: it intersects with essentially every hallmark of cancer—sustained proliferative signaling, metabolic plasticity, angiogenesis, invasion and metastasis, and, above all, therapeutic resistance—as comprehensively reviewed elsewhere.[18,38] Within this broader landscape, the present review deliberately focuses on the lactylation-autophagy axis as a unifying node that couples tumor metabolism, proteostasis, and immune remodeling, and we refer the reader to these companion reviews for lactylation across other HCC hallmarks.

Reversibility and emerging "erasers" of lactylation

A key characteristic of any dynamic epigenetic modification is reversibility. While initial studies failed to identify "erasers" of lactylation, recent breakthroughs have clearly demonstrated that lactylation is a reversible modification.[39] Emerging evidence has identified specific members of the HDAC fam-

ily, particularly HDAC1-3, and the NAD^+-dependent sirtuin (SIRT) family (e.g., SIRT1-3) as robust delactylases capable of removing lactyl groups from lysine residues.[39,40] The dysregulation of these specific erasers in HCC further amplifies the hyper-lactylation phenotype. This makes the targeted inhibition of lactylation writers (such as p300) or the modulation of its erasers potential therapeutic strategies.

Beyond their *in vitro* characterization,[39,40] delactylases have now been directly linked to HCC-relevant substrates. HDAC1 functions as a bona fide delactylase that erases AARS1-written lactylation on SPTAN1 (K1952/K1957) in hepatitis B virus-related HCC, thereby restraining NOTCH1/HES1 activation and immunosuppression[41]; SIRT3 delactylates cyclin E2 at K348 to suppress HCC growth, and pharmacologic SIRT3 activation recapitulates this effect.[42] The expression status of these erasers in HCC has likewise been characterized at scale. HDAC1 and HDAC2 are upregulated in most HCC tissues and independently predict cancer-specific mortality (n = 156 surgical cohort),[43] as confirmed by TCGA-based multi-omics analyses of the poor-prognosis association of HDAC2[44]; SIRT1 is elevated in HCC and its inhibition relieves hepatocarcinogenesis[45]; whereas low SIRT3 protein expression correlates with poor differentiation, advanced stage, and unfavorable overall survival (hazard ratio [HR]: 0.555, 95% confidence interval [CI]: 0.344–0.897, P = 0.016; n = 248).[46] HDAC3 exhibits context-dependent, bidirectional roles—hepatocyte-specific ablation paradoxically promotes HCC in females,[47] underscoring that eraser function cannot be equated with simple pro- or antitumor activity. At the systems level, a large multi-omics stratification (n > 1,700) identified a "low histone delactylase activity" phenotype characterized by elevated lactylation, upregulated PD-L1, and lenvatinib resistance, which could be reversed by restoring delactylase activity.[48] Clinically, elevated H4K5la/H3K18la/pan-Kla staining in HCC tissues predicts poor prognosis (H4K5la as an independent overall survival factor, P = 0.0064; n = 104) and associates with immunotherapy resistance.[49] Together, these data indicate that the hyper-lactylation phenotype of HCC arises from an imbalance between a hyperactive multi-enzyme writer network and available eraser capacity, with enzyme-specific and context-dependent dysregulation on both sides.[50]

Importantly, two intuitively appealing assumptions remain unverified in HCC and should be regarded as hypotheses: (i) that class I HDAC downregulation accounts for lactylation accumulation—in fact, HDAC1/2 tend to be upregulated in HCC,[43,44] and (ii) that SIRT1/SIRT2 act as delactylases within HCC cells, where their expression is often pro-tumorigenic rather than tumor-suppressive.[45] By contrast, a hyper-lactylation-driven suppression of eraser expression (H4K12la inhibiting HDAC3 in macrophages) has been demonstrated in another system and may represent a positive-feedback loop worth testing in HCC.[51]

Lactylation as a molecular bridge to autophagy and immune regulation

Given its broad substrate spectrum, including both genomic promoters and cytosolic signaling proteins, lactylation is mechanistically well-positioned to intersect with autophagic and immune signaling networks. Potential crosstalk nodes include the direct non-histone lactylation of mTOR pathway regulators, the transcriptional upregulation of core autophagy-related (ATG) genes, and the sustained modulation of immune checkpoint pathways.[18,23] These theoretical and emerging intersections form the fundamental mechanistic basis for the lactylation-autophagy axis, which will be explored in subsequent sections.

Autophagy in HCC: context-dependent roles in tumor progression and immunity

Autophagy is an evolutionarily conserved, lysosome-dependent degradation pathway. It plays an important role in maintaining cellular homeostasis by recycling cytoplasmic components. In HCC, autophagy exhibits complex, dual roles that are highly context-dependent. Specifically, it acts as a tumor suppressor during the early stages of hepatocarcinogenesis, while in established, advanced tumors, it promotes cancer cell survival and immune evasion.

Molecular machinery of autophagy

Macroautophagy occurs through several well-defined sequential steps. The initiation of autophagy is tightly regulated by the unc-51 like autophagy activating kinase 1 (ULK1) complex. Under nutrient-rich conditions, this complex is constitutively inhibited by mTORC1; however, during energy stress, it is strongly activated by AMP-activated protein kinase (AMPK).[52] Autophagic flux is closely controlled by metabolic signals, including amino acid availability, ATP levels, and the cellular redox state, positioning autophagy as a central sensor of metabolic status.

Tumor-suppressive role in early hepatocarcinogenesis

During the early phases of liver injury and tumor initiation, autophagy primarily functions as a tumor suppressor. Basal selective autophagy, particularly mitophagy, prevents liver tumor initiation by actively eliminating dysfunctional mitochondria that produce reactive oxygen species (ROS). This process helps maintain genomic stability and limit oxidative stress.[53] As a result, intact autophagic capacity effectively restrains malignant transformation during the early development of HCC.

Tumor-promoting role and metabolic adaptation in established HCC

In sharp contrast, once tumors are fully established, autophagy often supports cancer cell survival in a hostile metabolic environment. For example, sorafenib, a historically first-line targeted therapy for advanced HCC, induces autophagy. Recent comprehensive reviews have highlighted that this sorafenib-induced autophagy paradoxically acts as a protective mechanism against both apoptosis and ferroptosis, directly contributing to acquired multidrug resistance.[54] Inhibiting autophagic flux enhances sorafenib-induced cell death, supporting autophagy's tumor-promoting role in advanced HCC stages. Beyond bulk degradation, selective autophagy processes (such as mitophagy, lipophagy, and ferritinophagy) play critical roles in rewiring liver tumor metabolism.[53] Mitophagy effectively removes dysfunctional mitochondria, maintaining redox balance and metabolic flexibility, providing a direct functional connection to the glycolysis-driven lactate production discussed above.

Mechanistically, lactylation and autophagy intersect ferroptosis at the level of lipid peroxidation and iron metabolism. Autophagy can protect against ferroptosis by eliminating damaged mitochondria (mitophagy) and, paradoxically, promote it through ferritinophagy, which releases intracellular iron to fuel Fenton chemistry and lipid peroxidation; the net outcome depends on the cargo degraded and the cellular iron pool. Lactylation adds an epigenetic layer to this interplay: H3K18la-driven transcriptional programs can reduce ferroptosis sensitivity in HCC,[10] potentially by modulating the expression of ferroptosis-suppressive determinants such as SLC7A11/xCT and GPX4; in parallel, lactylation-mediated

stabilization of TFEB may indirectly reshape iron and redox homeostasis through lysosomal and autophagic machinery. This bidirectional lactylation-autophagy-ferroptosis interplay is clinically relevant because ferroptosis constitutes a key death mode induced by sorafenib and by emerging immunogenic cell-death strategies.[54]

Autophagy in tumor immune regulation

The massive accumulation of metabolic byproducts (such as lactate) driven by autophagy-mediated metabolic reprogramming not only sustains tumor survival but also profoundly reshapes the surrounding immune environment. Autophagy exerts significant pleiotropic effects on antitumor immunity, directly influencing antigen presentation, T cell homeostasis, macrophage polarization, and immune checkpoint regulation.

Antigen Presentation and PD-L1 Regulation: Autophagy is fundamental to major histocompatibility complex (MHC) presentation and is closely linked to immune checkpoint expression. Recent evidence points to a complex bidirectional regulatory relationship, where autophagic degradation pathways control the post-translational turnover of PD-L1.[55] Pharmacological inhibition of autophagy can alter PD-L1 surface expression and work synergistically to enhance the efficacy of antitumor immunotherapy.

Suppression of T and NK Cell Effector Functions: The hypoxic and acidic microenvironment within the TIME often exceeds the capacity of autophagy. More importantly, excessive lactate accumulation in this microenvironment directly inhibits both $CD8^+$ T cells and NK cells.[17] Elevated lactate impairs glycolytic flux, reduces NFAT activity, and subsequently downregulates the production of key cytotoxic molecules, including interferon-γ, perforin, and granzyme, executing a robust immune evasion program.

Macrophage Polarization and Epigenetic Remodeling: Tumor-derived lactic acid is the primary regulator of the functional polarization of TAMs.[20] Breakthrough studies have clearly identified intracellular lactate as an epigenetic substrate for histone lactylation.[3] Within TAMs, this lactate-induced epigenetic remodeling (e.g., H3K18la) upregulates pro-tumoral genes such as Arg1, solidifying an immunosuppressive M2-like phenotype.[21,56]

The lactate-autophagy axis: a non-canonical link

The shared reliance on metabolic stress signals suggests an integrated regulatory network between lactylation and autophagy in HCC. While the activation of AMPK by energy stress is a well-established canonical mechanism,[52] the direct non-canonical induction of autophagic flux via MCT-mediated lactate influx is currently a compelling hypothesis, largely derived from *in vitro* models. The active shuttling of lactate via proton-coupled monocarboxylate transporters (MCTs, particularly MCT1 and MCT4) establishes a sophisticated "lactate-autophagy axis".[57] Mechanistically, it is proposed that the influx of lactate and co-transported protons leads to intracellular acidification. This acidification then either alters the AMP/ATP ratio to activate the AMPK pathway or induces moderate ROS generation, both cascades act as powerful triggers to suppress mTORC1 signaling and initiate autophagosome formation.[58] Although this specific MCT-mediated lactate signaling requires further robust *in vivo* clinical validation in HCC, it theoretically serves as a potential adaptive mechanism that specifically confers ferroptosis resistance and multidrug resistance. Dual-targeting strategies that specifically uncouple this MCT-driven axis or inhibit histone lactylation hold potential for reversing immune suppression in HCC.

Mechanistic crosstalk between lactylation and autophagy in HCC

The convergence of metabolic reprogramming, epigenetic modification, and autophagic regulation serves as a central adaptive strategy in HCC. While lactylation and autophagy have each been independently linked to tumor progression, growing evidence suggests that these two processes functionally intersect through shared metabolic intermediates and signaling pathways.[38] In this section, we summarize current findings and propose a mechanistic framework that describes how lactate-driven lactylation and autophagy form a bidirectional regulatory axis in HCC (Table 1).[3,5,6,10,12,23,59–61]

Lactate-driven mTOR signaling as a convergence node

Normally, mTORC1 acts as a master regulator, integrating nutrient availability, energy status, and growth factor signaling to control the initiation of autophagy. Under nutrient-rich conditions, mTORC1 suppresses autophagy by inhibiting the ULK1 complex.[52] In HCC, aberrant activation of the PI3K/AKT/mTOR signaling axis is common and closely correlates with tumor progression. Lactate accumulation in the TME influences mTOR signaling through multiple mechanisms. Notably, studies in other tissue models have shown that lactate can regulate autophagy by promoting the lactylation of mTOR at the lysine 921 (K921) residue, which in turn inactivates mTORC1.[59] While the specific K921 lactylation event has not yet been experimentally confirmed *in vivo* in human HCC tissues, we hypothesize that similar lactylation events broadly modify mTOR-associated proteins or critical upstream regulators in the hepatic TME. Furthermore, quantitative lactylome analyses have identified widespread non-histone lactylation events across complex metabolic and signaling networks.[60] Given that PTMs dynamically regulate mTOR complex stability, it is plausible that lactylation broadly modifies mTOR-associated proteins or upstream regulators in HCC. Thus, lactate-driven modulation of mTOR represents a potential convergence node linking metabolic lactylation to autophagic regulation.

Histone lactylation-dependent transcriptional control of autophagy genes

The discovery of histone lactylation established lactate as an epigenetic substrate capable of regulating gene transcription.[3] In the context of cancer, histone lactylation (e.g., H3K18la) actively influences transcriptional programs that govern metabolic adaptation, macrophage polarization, and resistance to cell death.[62,63] ATGs are transcriptionally regulated by nutrient-sensitive factors such as HIF-1α and TFEB. Given that lactate stabilizes HIF-1α and increases histone lactylation at the promoter regions of hypoxia-responsive genes,[3] it is reasonable to propose that lactylation modulates the transcription of key autophagy machinery in HCC. We suggest that histone lactylation fine-tunes autophagic capacity at the transcriptional level, particularly under the hypoxic and highly glycolytic conditions that are characteristic of advanced HCC.

Non-histone lactylation of autophagy machinery: emerging evidence and hypothesis

Beyond chromatin, lysine lactylation has been widely detected in numerous cytoplasmic and non-histone proteins, introducing a significant new layer of post-translational control.[60] The initiation and progression of autophagy are tightly regulated by PTMs of essential proteins, including ULK1, Beclin-1, and microtubule-associated protein 1 light chain 3 (LC3). Given the structural and functional similarities between acetylation and lactylation, it is hypothesized that

Table 1. Core molecules and functional nodes in the lactylation-autophagy crosstalk

Molecule/ target	Functional role in cell	Mechanism in the lactylation-autophagy axis	Validation context	Evidence grade	Ref
mTOR (K921)	Master metabolic sensor and autophagy suppressor	Direct non-histone lactylation at K921 inactivates mTORC1, initiating autophagic flux	*In vivo* and *in vitro* (Non-HCC models)	Moderate (extrapolated)	59
PIK3C3/VPS34	Core complex for autophagosome formation	Non-histone lactylation directly promotes autophagosome formation bypassing transcription	*In vitro* (Macrophage/non-HCC models)	Moderate (extrapolated)	5
TFEB	Master transcriptional regulator of lysosomal biogenesis	Lactylation stabilizes TFEB, preventing degradation and elevating lysosomal activity	*In vitro* (Neural cells/Macrophages)	Moderate (extrapolated)	6
Histone H3 (e.g., H3K18la)	Epigenetic activation mark on chromatin	Promotes transcription of immunosuppressive (M2-like) and metabolic genes; reduces ferroptosis susceptibility	*In vitro* & *In vivo* (HCC and TAMs)	High (confirmed in HCC)	3,10
HIF-1α	Hypoxia-responsive transcription factor	Stabilized by lactate; transcriptionally upregulates LDHA, MCT4, and ATGs	*In vitro* & *In vivo* (HCC and solid tumors)	High (confirmed in HCC)	12,61
ATG proteins (ULK1, Beclin-1, ATG5)	Core autophagy machinery	Direct non-histone lactylation of core ATG proteins (hypothesis, awaiting validation)	No HCC-specific evidence	Very low (hypothetical)	23,60

ATG, autophagy-related gene; H3K18la, lactylation of histone H3 lysine 18; HCC, hepatocellular carcinoma; HIF-1α, hypoxia-inducible factor 1-alpha; LDHA, lactate dehydrogenase A; MCT4, monocarboxylate transporter 4; mTOR, mechanistic target of rapamycin; mTORC1, mechanistic target of rapamycin complex 1; PIK3C3/VPS34, phosphatidylinositol 3-kinase catalytic subunit type 3/vacuolar protein sorting 34; TAMs, tumor-associated macrophages; TFEB, transcription factor EB; ULK1, unc-51 like autophagy activating kinase 1; Evidence level: High, direct experimental evidence confirmed in human HCC tissues or HCC-validated models; Moderate, direct evidence in non-HCC systems with plausible but unverified HCC relevance; Low, emerging in HCC, methodological caveats; Very low, mechanistically plausible, awaiting targeted validation.

lactylation directly occurs on lysine residues of autophagy-related proteins, modulating their enzymatic activity or protein-protein interactions. Large-scale lactylome studies have identified hundreds of lactylated non-histone proteins involved in diverse cellular processes.[60] If specific ATG proteins (such as PIK3C3/VPS34 and TFEB, as recently indicated in non-HCC models) are rigorously confirmed as direct lactylation substrates in HCC *in vivo*—a compelling hypothesis currently awaiting targeted validation, it would establish a mechanistic shortcut through which glycolytic flux directly rewires intracellular degradation pathways, entirely bypassing transcriptional intermediates.

Autophagy as a regulator of intracellular lactate and epigenetic plasticity

The crosstalk between lactylation and autophagy is bidirectional. Autophagy sustains HCC cell survival under severe metabolic stress by recycling intracellular components and preserving mitochondrial integrity.[22] Selective autophagy, such as mitophagy, limits the accumulation of ROS and actively shapes metabolic flux through glycolysis and the tricarboxylic acid cycle. By modulating the turnover of key glycolytic enzymes and maintaining mitochondrial quality, autophagy indirectly controls the rate of intracellular lactate production. Altered lactate availability, in turn, reshapes global lactylation patterns. Therefore, autophagic flux acts as an upstream regulator of the epigenetic landscape in HCC cells by controlling the availability of metabolic intermediates required for lactylation reactions.

A positive feedback model in advanced HCC

Integrating current evidence, we propose a conceptual model in which lactate-driven lactylation and autophagy form a self-reinforcing metabolic-epigenetic-proteostatic axis. In advanced HCC, lactylation-driven transcriptional reprogramming enhances metabolic adaptation and immunosuppression, while upregulated autophagy ensures cellular survival under microenvironmental stress. Notably, recent multi-omics integrations support this synergy; for example, lactylation of histone H3 (H3K18la) has been shown to reduce the susceptibility of HCC cells to ferroptosis, establishing a direct connection between lactylation and resistance to cell death.[10] This cooperative positive feedback loop creates a permissive environment for tumor growth, acquired multidrug resistance, and immune evasion.[1,10]

The tumor-cell-autonomous effects described above are not confined to the cancer-cell compartment. Lactylation of mTOR (inactivating mTORC1) and TFEB (enhancing lysosomal activity), together with H3K18la-dependent transcription, does not merely remodel intrinsic metabolism and autophagic flux; it also reshapes the secretory phenotype of tumor cells. Lactylation-driven transcriptional programs upregulate immunosuppressive cytokines and growth factors (e.g., IL-10, TGF-β, VEGF), stabilize surface PD-L1, and may reprogram exosome cargo—signals that act in a paracrine manner on neighboring immune cells.[18,20,21,63] Within the hepatic microenvironment, these secreted factors converge with the extracellular lactate pool to polarize TAMs toward an M2-like state, disable $CD8^+$ T-cell and NK-cell effector functions, and promote Treg expansion, thereby translating tumor-cell-autonomous lactylation into immune-cell-level modulation. This paracrine relay, from lactylation of mTOR/TFEB in tumor cells to secreted-factor-driven macrophage polarization, constitutes the mechanistic bridge between the molecular crosstalk described above and the immune-cell-specific effects detailed below (Fig. 1, Module 4).

The lactylation-autophagy axis in the HCC TIME

The HCC TIME is a highly specialized ecosystem. It is char-

acterized by intense metabolic competition, chronic inflammation, and multifaceted immune suppression. Growing evidence suggests that metabolic rewiring in tumor cells reshapes immune cell function not only through nutrient depletion and localized acidification, but also via metabolite-driven epigenetic reprogramming. In this context, lactate accumulation and autophagy activation act as coordinated regulators of immune remodeling. In the following sections, we discuss how the intersection of these two pathways—the lactylation-autophagy axis—influences major immune cell populations, ultimately fostering a permissive environment for HCC progression.

Tumor-associated macrophages: lactylation-driven polarization and autophagy modulation

TAMs are among the most abundant immune populations in the HCC niche, and their presence is consistently linked to a poor prognosis. TAMs primarily display an immunosuppressive M2-like phenotype, marked by high expression of IL-10, TGF-β, and Arg1, factors that collectively promote tumor growth and immune evasion.[63] Zhang *et al.*'s landmark study established lactate as a key epigenetic regulator of macrophage polarization, showing that tumor-derived lactate induces histone lactylation (e.g., H3K18la) to directly drive the transcription of M2-like genes.[3] Subsequent research has confirmed that elevated lactate levels in HCC correlate with dense infiltration of M2-like TAMs, amplifying immunosuppressive transcriptional programs through lactylation.[7,8] At the same time, autophagy plays a critical, context-dependent role in determining TAM fate. While autophagy deficiency often shifts macrophages toward a pro-inflammatory state, intact autophagic flux is necessary to support the survival and alternative activation of macrophages within hypoxic tumor niches.[64] Emerging mechanistic models point to a coordinated metabolic-epigenetic-autophagic program in TAMs: lactylation may transcriptionally regulate ATGs, while functional autophagy in turn modulates intracellular lactate pools, influencing global lactylation patterns. Additionally, lactate-activated mTOR signaling acts as a central hub, simultaneously suppressing autophagy and shaping macrophage differentiation.

$CD8^+$ T cells and NK cells: metabolic exhaustion and autophagic adaptation

Cytotoxic $CD8^+$ T cells and NK cells are the primary effectors of antitumor immunity; yet their functions are often impaired within the lactate-rich TIME. High extracellular lactate hinders the efflux of intracellular lactate via MCT1 in T and NK cells. This leads to severe intracellular acidification, which impairs glycolytic flux, interferes with NFAT signaling, and drastically reduces the secretion of key cytotoxic molecules, including interferon-γ, perforin, and granzyme.[17] To cope with this metabolic stress, autophagy serves as a vital adaptive mechanism. Autophagy is strictly necessary for T cell homeostasis, mitochondrial quality control, and the formation of immunological memory.[65] T cells deficient in autophagy show impaired survival and increased sensitivity to oxidative stress. Within lactate-rich HCC tumors, upregulated autophagy may enable $CD8^+$ T cells to partially resist metabolic exhaustion. On the other hand, the interplay between PD-L1 signaling and autophagic activity is context-dependent: autophagic pathways can regulate the stability and surface localization of PD-L1,[55] whereas tumor-intrinsic PD-L1 signals in turn modulate autophagy in tumor cells.[66] Thus, the lactylation-autophagy axis exerts significantly divergent, cell-type-specific effects that determine the balance of immune surveillance in HCC.

Tregs: metabolic advantage in a lactate-rich environment

Tregs are highly enriched in HCC and play a key role in enforcing immune tolerance. Unlike effector T cells, Tregs exhibit superior metabolic flexibility, relying heavily on oxidative phosphorylation. This metabolic feature allows them to efficiently metabolize lactate in acidic microenvironments.[67] This metabolic advantage also promotes Treg differentiation and maintains the stability of Foxp3, their master transcriptional regulator. Alongside this metabolic adaptation, functional autophagy is essential for Treg integrity; the loss of autophagic capacity impairs Foxp3 expression and disrupts their suppressive function.[68] Thus, the synergy between high lactate availability and intact autophagy preferentially sustains Treg populations, reinforcing the immunosuppressive architecture of HCC.

DCs and antigen presentation

Functional DCs are critical for initiating robust antitumor immunity. However, tumor-derived lactate impairs DC differentiation and reduces the expression of costimulatory molecules and IL-12. Furthermore, acidic microenvironments cripple their antigen-processing capacity. Autophagy is fundamentally required for effective antigen processing, MHC class II presentation, and cross-presentation.[64] The interaction between metabolic stress (lactate accumulation) and proteostatic pathways (autophagy) dictates DC functionality, suggesting that targeting this axis could restore DC-mediated immune recognition.

Immune checkpoint regulation and therapeutic implications

The expression and stability of immune checkpoints are closely regulated by metabolic and autophagic networks. Lactate-mediated stabilization of IF-1α directly induces PD-L1 transcription.[61] At the same time, autophagic degradation pathways regulate the post-translational turnover and surface localization of PD-L1 in tumor cells.[55] Given that the lactylation-autophagy axis orchestrates metabolic adaptation and immune suppression across multiple cell types, therapeutically targeting this integrated network, such as combining LDHA inhibitors, lactylation modulators (e.g., p300 inhibitors) and autophagy inhibitors, represents a rational strategy. This approach could sensitize HCC to immune checkpoint blockade and overcome current treatment resistance.

Therapeutic implications: targeting the lactylation-autophagy axis in HCC

The identification of a lactylation-autophagy-immune regulatory axis provides a conceptual framework for rational combinatorial therapeutic strategies in HCC. Metabolic reprogramming, epigenetic remodeling, and autophagic adaptation together sustain immune suppression and tumor progression. As such, targeting this integrated network has the potential to enhance the efficacy of existing immunotherapies (Fig. 2). In this section, we discuss emerging therapeutic approaches focused on lactate metabolism, lactylation machinery, autophagic pathways, and immune checkpoint modulation (Table 2).[3,14,17,61,64,66,69–80]

Targeting lactate metabolism

LDHA Inhibition: LDHA is often overexpressed in HCC. It drives the conversion of pyruvate to lactate and is associated with poor clinical outcomes. Pharmacological blockade of LDHA not only reduces tumor-intrinsic glycolysis but also

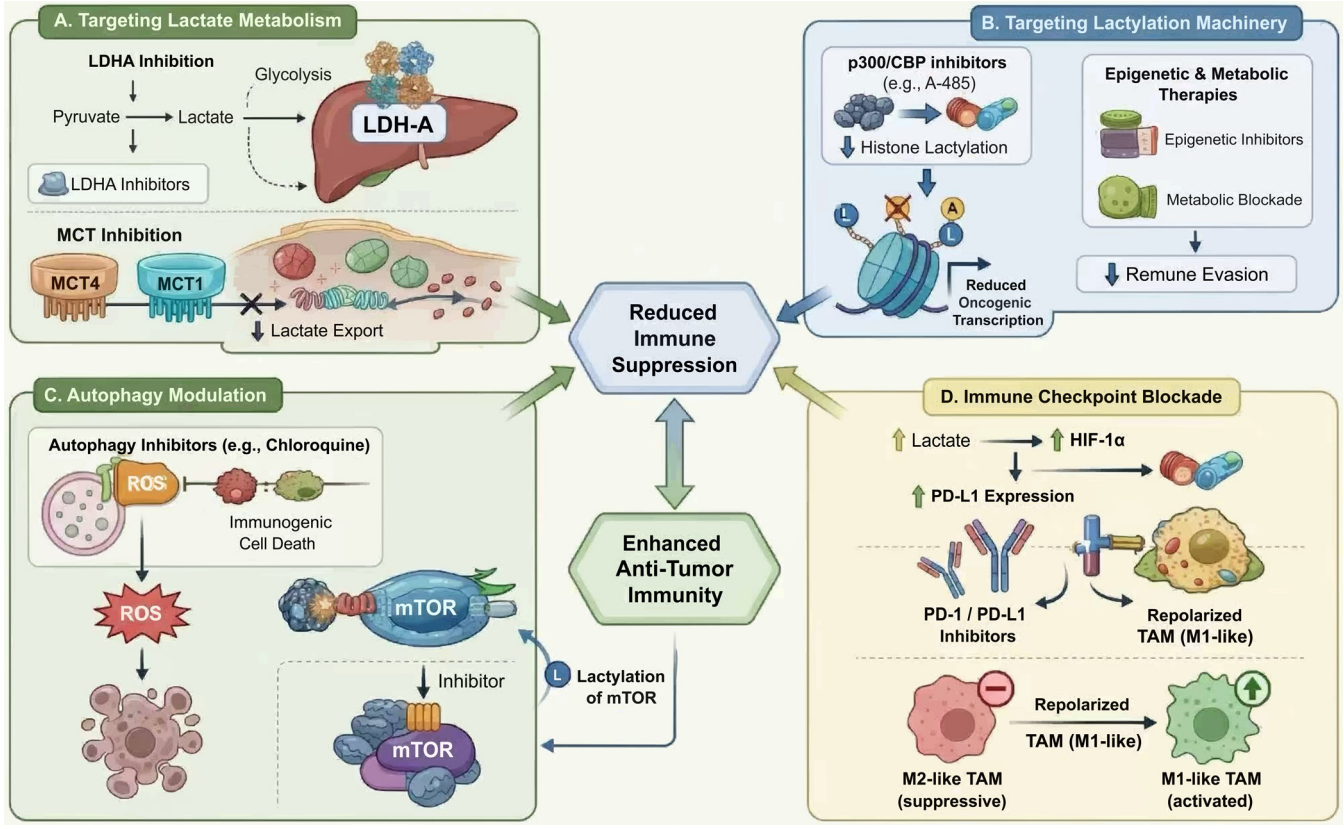


Fig. 2. Therapeutic implications of targeting the lactylation-autophagy axis in HCC. This mechanistic diagram outlines four primary combinatorial strategies to overcome the immunosuppressive TIME by targeting the interconnected lactylation-autophagy network. (A) Targeting Lactate Metabolism: Blockade of LDHA inhibits the conversion of pyruvate to lactate. Concurrently, MCT1/4 restricts lactate efflux, thereby preventing extracellular acidification. (B) Targeting Lactylation Machinery: p300/CBP inhibitors (e.g., A-485) suppress histone lactylation, reducing oncogenic transcription and mitigating immune evasion when combined with metabolic blockade. (C) Autophagy Modulation: Autophagy inhibitors such as chloroquine disrupt late-stage autophagic degradation, leading to the accumulation of ROS and subsequent immunogenic cell death. Additionally, targeting the mTOR pathway disrupts the feedback loop where mTOR lactylation suppresses its own activity. (D) Immune Checkpoint Blockade: Extracellular lactate stabilizes HIF-1α, driving PD-L1 expression. Combining PD-1/PD-L1 inhibitors with lactylation-autophagy uncoupling strategies may help repolarize TAMs from an immunosuppressive M2-like state to an activated M1-like state. HCC, hepatocellular carcinoma; HIF-1α, hypoxia-inducible factor 1-alpha; LDHA, lactate dehydrogenase A; MCT, monocarboxylate transporter; mTOR, mechanistic target of rapamycin; p300/CBP, p300/CREB-binding protein; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; ROS, reactive oxygen species; TAM, tumor-associated macrophage; TIME, tumor immune microenvironment.

weakens lactate-driven immune suppression within the TIME. Preclinical models show that LDHA inhibition may restore the metabolic fitness of $CD8^+$ T cells and enhance overall antitumor immunity.[17]

MCT Blockade: The transport of lactate across cell membranes is mainly mediated by MCTs. Tumor cells primarily use MCT4 for lactate efflux, while immune cells rely on MCT1 for lactate influx. Inhibiting MCT1 (e.g., the selective inhibitor AZD3965) or MCT4 prevents extracellular acidification and disrupts the lactate shuttle.[69] From a clinical translation standpoint, the selective MCT1 inhibitor AZD3965 has completed a phase I study in 40 patients with advanced solid tumors and lymphomas (NCT01791595; recommended phase II dose 10 mg twice daily), although it has not yet been evaluated in HCC.[70] Early clinical data suggest that uncoupling this metabolic symbiosis is pharmacologically feasible and could sensitize cold tumors to immunotherapy. By limiting extracellular lactate availability, MCT inhibitors can indirectly abolish lactylation-dependent transcriptional programs in macrophages[8] and restore the cytolytic capacity of effector T cells.[17]

An important nuance must be considered when targeting this axis: intracellular and extracellular lactate represent two functionally distinct yet mechanistically coupled pools, and transporter inhibition does not modulate them concordantly. Blocking MCT4-mediated efflux reduces extracellular lactate accumulation, thereby alleviating acidification and GPR81-dependent immunosuppression but simultaneously raises intracellular lactate, paradoxically sustaining or even augmenting the substrate pool available for lactylation. Conversely, MCT1 inhibition (e.g., AZD3965) primarily restricts lactate uptake by oxidative tumor cells and effector immune cells, relieving MCT1-dependent metabolic suppression, while secondarily reducing the lactate supply available to lactylation-dependent transcriptional programs within cells that rely on import. These opposing effects imply an inherent trade-off: MCT-targeted monotherapy may relieve extracellular immunosuppression while leaving or even reinforcing intracellular lactylation-driven immune evasion. Rational combination design therefore requires either concurrent blockade of lactylation machinery, sequence-optimized regimens, or pharmacodynamic monitoring of both extracellular lactate and tissue Kla marks to guide dosing.

Targeting the lactylation machinery

p300/CBP Inhibition: The acetyltransferase p300 has been

Table 2. Potential therapeutic agents targeting the lactylation-autophagy-immune axis

Therapeutic target	Drug class/ representative agents	Mechanism of action within the axis	Current clinical/development status	Potency/ specificity	PK/PD limitations · HCC-relevant clinical stage	Ref
Lactate Production	LDHA Inhibitors (e.g., Oxamate, FX11)	Block the conversion of pyruvate to lactate; diminish intracellular lactate pool for lactylation	Preclinical evaluation	Oxamate: Ki ≈ 136.3 μM, non-selective (all LDH isoforms); FX11: Ki ≈ 8 μM (NADH site), relative LDHA selectivity	High polarity/ poor permeability (oxamate); low solubility, metabolic instability (FX11); no clinical trial; no HCC trial[73–76]	14,17
Lactate Transport	MCT1/MCT4 Inhibitors (e.g., AZD3965)	Prevent extracellular lactate efflux/influx; uncouple metabolic symbiosis; reverse T cell acidification	Phase I completed (advanced solid tumors/ lymphomas, NCT01791595); not yet tested in HCC	MCT1 binding Ki = 1.6 nM; ~6× vs MCT2; no MCT4 inhibition	Oral; RP2D 10 mg BID; retinal/ cardiac DLTs; Phase I completed (NCT01791595); no HCC trial[70,77]	69
Epigenetic Writers	p300/CBP Catalytic Inhibitors (e.g., A-485)	Directly suppress acyltransferase activity, reducing global histone lactylation and oncogenic transcription	Preclinical evaluation	p300/CBP IC50 9.8/2.6 nM; no other KAT inhibited at 10 μM	No CNS penetration; BID dosing; preclinical only; no HCC trial[71,78]	3,71
Autophagic Flux	Lysosomotropic Agents (e.g., Chloroquine, HCQ)	Impair lysosomal acidification; block late-stage autophagy; accumulate ROS to induce immunogenic cell death	FDA-approved for other indications; HCC: phase II (HCQ + sorafenib) completed; preliminary positive signal reported	No classical enzyme IC50; HCC cells 2.5–40 μM	Very long half-life; cumulative retinopathy; HCC: NCT03037437 Phase II completed (positive)[72,79,80]	64
Immune Checkpoints	Anti-PD-1/Anti-PD-L1 Antibodies	Counteract lactate/ HIF-1α/autophagy-mediated PD-L1 upregulation; repolarize TAMs	FDA-approved agents are used as standard components of care in advanced HCC	–	–	61,66

CBP, CREB-binding protein; FDA, Food and Drug Administration; HCC, hepatocellular carcinoma; HCQ, hydroxychloroquine; LDHA, lactate dehydrogenase A; MCT, monocarboxylate transporter; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; ROS, reactive oxygen species; TAMs, tumor-associated macrophages; BID, bis in die (twice daily); DLTs, dose-limiting toxicities; RP2D, recommended phase 2 dose, KAT, k(lysine) acetyltransferase; CNS, central nervous system. Clinical stages and potency values were verified against the cited primary studies and ClinicalTrials.gov as of manuscript revision.

identified as a primary "writer" of histone lactylation.[3] Small-molecule inhibitors of p300/CBP (e.g., A-485) have shown strong antitumor activity by suppressing oncogenic transcription.[71] Although originally developed to target acetylation, these inhibitors also reduce histone lactylation, thereby disrupting lactate-driven epigenetic remodeling.[3] Theoretically, p300 blockade could suppress M2-like macrophage polarization and downregulate PD-L1 transcription, but the development of highly selective lactylation inhibitors remains an urgent unmet clinical need. A major safety consideration is the inherent lack of selectivity of p300/CBP inhibition. Because p300/CBP catalyze both acetylation and lactylation, systemic blockade yields broad epigenetic consequences beyond lactylation, including genome-wide changes in H3K27ac, pleiotropic transcriptional repression, interference with acetyl-CoA-sensing programs, and dose-limiting toxicity. Whether lactylation-selective inhibition is theoretically achievable depends on discriminating the acyl-CoA donor (lactyl-CoA vs. acetyl-CoA) at the catalytic center, or on exploiting allosteric sites, substrate-induced conformational differences, and p300-vs-CBP isoform selectivity; targeted protein degradation (PROTACs) offers a complementary route. To date, no validated compound achieves such discrimination, and a "lactylation-preferring" inhibitor remains an unmet need. Interim strategies include dose-titrated combination regimens and parallel pharmacodynamic monitoring of both acetyl- and lactyl-marks to avoid unintended epigenetic perturbation.

Epigenetic-Metabolic Combinatorial Approaches: Given the close interdependence of metabolic flux and epigenetic modification, dual targeting of glycolysis and chromatin remodeling offers a synergistic therapeutic opportunity.[63] Blocking lactate production while simultaneously inhibiting epigenetic writers may prevent the compensatory transcriptional adaptations that often lead to therapeutic resistance.

Autophagy modulation

Autophagy Inhibition: Inhibitors such as chloroquine and hydroxychloroquine (HCQ) impair lysosomal acidification, effectively blocking late-stage autophagic degradation. In preclinical HCC models, disrupting autophagy increases metabolic stress and enhances sensitivity to targeted therapies. Within the context of the lactylation-autophagy axis, autophagy inhibition impairs the tumor's ability to recycle damaged mitochondria. This leads to the accumulation of toxic ROS and

Table 3. Predictive biomarkers of the lactylation-autophagy axis: clinical feasibility

Biomarker	Source & assay	Clinical feasibility (reported values)	Evidence level	Ref
Serum LDH	Blood; enzymatic assay	Routine, inexpensive; meta-analysis (10 studies): OS HR 2.07 (1.63–2.62), $P < 0.001$; on-treatment LDH dynamics predict PFS ($P = 0.0029$); CLEAR score (LDH + CRP) validated in ATZ/BEV cohorts	Moderate (correlative meta-analysis)	82,83,84
Tumoral LDHA/MCT4	Tissue; IHC/qPCR	Routine pathology; indicates glycolytic output; prognosis-associated	Moderate (expression-prognosis)	14,17,82
H3K18la (tissue Kla)	Tissue; WB/MS/ChIP-seq/IHC (pan-Kla antibody)	Research-grade; pan-Kla antibody cross-reactivity; H3K18la-TNM stage correlation (89 pairs) and anti-PD-1 resistance ($n = 46$); H4K5la independent OS factor ($P = 0.0064$; $n = 104$)	Low (emerging in HCC; method caveats)	49,81
Lactylation-ferroptosis gene signature	Tumor RNA-seq; transcriptomic score	Independent multivariate HR 2.41 (1.87–3.11); AUC > 0.65; not yet prospectively validated	Moderate (prognostic; not prospectively validated)	10
Autophagic flux (LC3-II/p62)	Tissue/blood; WB/ELISA	Unstable, sampling-dependent; no standardized cutoffs	Very low (hypothetical)	52,54

ATZ/BEV, atezolizumab plus bevacizumab; AUC, area under the curve; CI, confidence interval; HR, hazard ratio; LDH, lactate dehydrogenase; OS, overall survival; PFS, progression-free survival; Kla, lysine lactylation; CRP, C-reactive protein; MCT4, monocarboxylate transporter 4; IHC, immunohistochemistry; qPCR, Quantitative polymerase chain reaction; WB, western blot; MS, mass spectrometry; ChIP-Seq, Chromatin immunoprecipitation sequencing; TNM, Tumor-Node-Metastasis; H3K18la, histone H3 lysine 18 lactylation; H4K5la, histone H4 lysine 5 lactylation; LC3-II, microtubule-associated protein 1 light chain 3-II; ELISA, enzyme-linked immunosorbent assay. Evidence level: High/Moderate/Low/Very low (definitions as in Table 1).

immunogenic cell death.[64]

Targeting the mTOR Pathway: As a central metabolic sensor, mTOR integrates lactate signaling and autophagic flux. Recent findings that identify mTOR lactylation as a critical inhibitor of its activity highlight this complex feedback loop.[59] While mTOR inhibitors have shown limited efficacy as monotherapies in HCC, using them in rational combinations to disrupt the lactate-mTOR-autophagy axis holds significant translational potential.[59]

Integration with immune checkpoint blockade

Immune checkpoint inhibitors have transformed HCC treatment; however, the highly immunosuppressive, lactate-rich TIME limits patient response rates. Lactate accumulation directly stabilizes HIF-1α, leading to upregulated PD-L1 expression,[61] while simultaneously impairing T cell effector functions.[17] Furthermore, intact autophagic flux regulates the post-translational stability and membrane localization of PD-L1.[66] Therefore, therapeutically uncoupling the lactylation-autophagy axis via lactate dehydrogenase (LDH)/MCT inhibitors or autophagy modulators can rationally sensitize HCC tumors to anti-PD-1/PD-L1 therapies by relieving metabolic exhaustion in T cells and repolarizing TAMs.

Precision medicine and biomarker development

Translating these network-targeted strategies into clinical practice requires reliable predictive biomarkers. Potential candidates for patient stratification include serum LDH levels, tumoral MCT4/LDHA expression, global histone lactylation marks (e.g., H3K18la), and indicators of autophagic flux (LC3-II/p62 ratios). Integrating spatial metabolomics with immune profiling will be important for developing personalized therapeutic regimens in HCC.

Beyond the candidates summarized in Table 3,[10,14,17,49,52,54,81–84] we propose an integrated stratification principle: a metabolic axis (serum LDH × tumoral LDHA/MCT4), an epigenetic axis (tissue H3K18la or global Kla), and a proteostatic axis (autophagic flux markers) should be measured in combination, since each captures a different layer of the same axis and they are not interchangeable.

In the context of targeted therapy, lactylation-mediated therapeutic resistance is now mechanistically anchored: AARS1-mediated lactylation of AKR1B10 at K173 stabilizes an aerobic-glycolysis positive-feedback loop, AKR1B10 is elevated in lenvatinib-resistant HCC tissues, and the AKR1B10 inhibitor epalrestat restores lenvatinib sensitivity in patient-derived xenografts.[85]

Future directions

While targeting the lactylation-autophagy-immune axis represents a potential strategy to overcome therapeutic resistance in HCC, several important knowledge gaps remain. Future research should prioritize the development of lactylation-specific inhibitors that do not cross-react with acetylation pathways, as well as explore nanoparticle-based targeted delivery systems to minimize systemic metabolic toxicity. Specifically, no LDHA-, MCT-, or p300/CBP-targeting agent has yet entered HCC-specific clinical trials, and MCT blockade entails an inherent intra-/extracellular lactate-pool trade-off; future studies should therefore combine these agents with autophagic-flux or immune-checkpoint modulation and monitor both serum lactate and tissue Kla as pharmacodynamic endpoints. Ultimately, refining our mechanistic understanding of this bidirectional axis will open the door for next-generation combinatorial immunotherapies.

Future perspectives and outstanding questions: toward mechanistic and translational dissection of the lactylation-autophagy-immune axis

Emerging evidence supports the existence of a lactylation-autophagy regulatory axis in HCC, but the field is still in its early conceptual stages. Current knowledge is mostly derived from parallel studies of metabolic reprogramming, histone lactylation, and autophagy, with limited direct mechanistic integration. To move beyond correlative observations, future research must focus on defining causal relationships, cell-type specificity, spatial organization, and therapeutic vulnerabilities. In the following sections, we outline major unresolved questions and propose experimentally feasible

directions.

We emphasize that the lactylation-autophagy axis should currently be regarded as an integrative conceptual framework rather than a fully validated functional entity in HCC. Direct causal evidence is available for only a subset of its nodes, most notably H3K18la-driven macrophage polarization; the non-histone lactylation events central to our model (VPS34, TFEB, mTOR K921) were identified in macrophages, neural cells, and skeletal muscle, and must be formally validated in HCC. To ensure transparency, each node is graded in Table 1 as "High (confirmed in HCC)," "Moderate (extrapolated)," or "Very low (hypothetical)." Encouragingly, HCC-relevant evidence for the coupling of the two processes is beginning to emerge: LSEC-derived CLEC3B suppressed both global protein lactylation and autophagic flux in HCC cells, with pharmacological rescue experiments positioning lactylation upstream of autophagy in this HCC context.[19]

Direct lactylation of the autophagy machinery

Non-histone lactylation has been identified across hundreds of proteins,[3,4] yet the precise lactylation landscape of the autophagy machinery in HCC remains incompletely mapped. Core autophagy proteins (e.g., Beclin-1, ULK1, and ATG5) are tightly regulated by PTMs such as acetylation and phosphorylation.[52] Given this, it is mechanistically plausible that lactylation modifies analogous lysine residues on these proteins. Encouragingly, recent studies have shown that lactate directly mediates the lactylation of PIK3C3/VPS34 to promote autophagosome formation,[5] and lactylates the transcription factor TFEB to enhance lysosomal activity.[6] Future research must use quantitative lactylome profiling and site-directed mutagenesis to systematically validate whether specific ATG proteins undergo functional lactylation in the hypoxic HCC microenvironment. This will establish a direct molecular linkage, rather than merely an inferred pathway overlap. For non-histone Kla sites whose functional validation has not yet been performed, publicly available bioinformatics resources—such as KlaDB (https://www.nmqczl.com/)[86]—can prioritize candidate lactylation targets and guide site-directed mutagenesis and functional studies.

Spatiotemporal dynamics and cellular heterogeneity

Most lactylation studies have used bulk tumor analyses; however, the HCC microenvironment exhibits significant cellular heterogeneity and metabolic zonation. Beyond the regional gradients discussed above, a critical unresolved question is how the cell-type-specific lactylome varies between malignant hepatocytes and infiltrating immune subsets (e.g., TAMs, Tregs, and exhausted $CD8^+$ T cells). Integrating spatial metabolomics with single-cell multi-omics (e.g., paired scRNA-seq and CUT&Tag) will be important to unraveling whether lactylation drives distinct transcriptional programs in different cellular compartments. Furthermore, longitudinal analyses in murine models are needed to determine whether the lactylation-autophagy axis shifts dynamically from a protective, tumor-suppressive role in early hepatocarcinogenesis to a contributor of metabolic adaptation and immune evasion in advanced stages.

Shaping immune checkpoint responsiveness

Metabolic constraints are increasingly recognized as key determinants of resistance to immune checkpoint inhibitors in HCC.[87] How the lactylation-autophagy axis governs the expression and membrane stability of immune checkpoints remains incompletely elucidated. Recent evidence indicates that lysine lactylation rewires metabolic pathways to promote HCC immune escape, and targeting these lactate-dependent pathways can sensitize tumors to immune checkpoint inhibitor therapy.[9] Future mechanistic studies should investigate whether histone lactylation (e.g., H3K18la) directly regulates the CD274 (PD-L1) promoter, and how autophagic flux modulates the turnover of these checkpoint molecules. Testing rational combinations of LDHA or MCT inhibitors with PD-1 blockade in syngeneic models will clarify the translational potential of uncoupling this axis.

Precision targeting and translational implementation

A major pharmacological challenge lies in the selective targeting of lactylation. Current metabolic inhibitors (e.g., p300 inhibitors) exhibit cross-reactivity with acetylation pathways, as both processes share acyltransferases.[71] Furthermore, the field is currently hampered by significant detection limitations. Researchers rely heavily on pan-lactyl antibodies, which often show problematic cross-reactivity with structurally similar acetylation marks, leading to false-positive signal amplification.

Additionally, the sensitivity limits of high-resolution mass spectrometry remain a technical bottleneck for capturing low-abundance non-histone lactylation events within highly heterogeneous clinical HCC samples. The identification of lactylation-specific "writers," "readers," and particularly "erasers" (putative delactylases analogous to HDACs) is a critical research priority. For successful clinical translation, these targeted approaches must be integrated with robust systems biology frameworks and biomarker development, including serum lactate profiling, H3K18la quantification, and the use of lactylation-related gene signatures for accurate patient stratification.[10]

Methodological advances are beginning to close this gap: genetic code expansion now permits site-specific installation of lactyl-lysine at defined lysine residues of target proteins in living cells, enabling direct dissection of the structural and functional consequences of individual Kla events in mammalian systems.[88] Using an engineered pyrrolysyl-tRNA synthetase/tRNA pair, Shao *et al.* introduced lactylation at a single non-histone site—aldolase A at lysine 147—in living cells and demonstrated that this modification abolished the catalytic activity of the enzyme, increased its thermal and proteolytic stability, triggered nuclear translocation, and reshaped cell adhesion-related transcription and the protein interactome.[88] This technology thus establishes causal, site-specific Kla-function relationships that cannot be reliably inferred from K-to-Q/E mimetic mutations, providing a generalizable platform for validating the non-histone lactylation events hypothesized in this review.

Strengths and limitations of the evidence

The strength of the lactylation-autophagy axis as a framework lies in the convergence of complementary evidence layers: clinical cohorts and meta-analyses anchoring the glycolytic/lactate arm (HK2, serum LDH; Table 4),[10,19,43,46,48,49,70,72,81–84,89–92] tissue-level quantification linking histone lactylation to TAM polarization, immune evasion, and prognosis (n = 89–104 paired samples;[49,81]), large multi-omics stratifications connecting delactylase activity to therapeutic response (n > 1,700;[48]), and early-phase clinical data testing the translational consequences of autophagy modulation.[70,72] Several limitations, however, temper direct translation into clinical practice. First, the evidence hierarchy is uneven: only a subset of nodes, most robustly the H3K18la-driven macrophage and PD-L1 programs,[81,89] has been confirmed in HCC, whereas non-histone lactylation events central to the

Table 4. Quantitative evidence summary (all values compiled from the published literature; no original bioinformatics analysis was performed)

Key conclusion	Supporting study (design)	N	Effect size (95% CI)	*P* value	Ref
HK2 overexpression and HCC survival	Liu Y 2016 (21 studies, solid tumors)	2,532	HCC OS pooled HR 2.06 (1.67–2.54)	<0.001	91
HK2 overexpression and HCC survival	Wu J 2017 (15 studies, digestive tumors)	1,932	HCC HR 1.87 (1.58–2.21)	<0.001	90
HK2 positivity and post-resection mortality	Zhang 2016 (IHC cohort)	155	HK2(+) 42.6%; univariate HR 2.70 (1.76–4.15)	<0.0001	92
Serum LDH and HCC survival	Kong W 2018 (meta-analysis, 10 studies)	10 studies	OS HR 2.07 (1.63–2.62); RFS 1.62 (1.37–1.90)	<0.001	82
Baseline LDH predicts outcomes on ATZ/BEV	Tanaka K 2025 (CLEAR score)	Two cohorts	LDH > ULN HR 1.97 (1.48–2.64); PFS 11.1/9.1/3.3 months	<0.001	84
On-treatment LDH dynamics predict PFS	Toshida K 2025 (ATZ/BEV)	66	Worse PFS in LDH-increase group; independent multivariate factor	0.0029/0.0045	83
H3K18la and anti-PD-1 resistance	Chen S 2026 (IHC pairs + immunotherapy cohort)	89 pairs + 46	H3K18la-TNM association; high H3K18la associated with anti-PD-1 resistance	–	81
Histone lactylation predicts poor prognosis	Yamane M 2026 (H4K5la-ENO2 loop)	104	H4K5la independent OS factor	0.0064	49
PFKFB4–lactate-H3K-18la-M2 TAM axis	Gan C 2026 (IHC/flow + *in vivo*)	30 pairs	PFKFB4 positivity correlated with M2-TAM infiltration; poor prognosis	–	89
Lactylation-ferroptosis signature	Meng J 2025 (TCGA-LIHC complete + internal validation)	TCGA-LIHC	Multivariate HR 2.41 (1.87–3.11); AUC > 0.65	<0.001	10
Low delactylase activity and stratification	Wu Z 2026 (multi-omics stratification)	> 1,700	Low-delactylase phenotype: high lactylation, PD-L1 up, lenvatinib resistance	–	48
Lactylation-autophagy signature (CLEC3B etc.)	Song Y 2026 (TCGA + ICGC + clinical samples)	TCGA *n* = 369–371	1/3-yr AUC 0.81/0.78 (TCGA), 0.73/0.71 (ICGC); cluster OS	0.00016	19
HDAC1/2 independent predictors of mortality	Ler SY 2015 (competing-risk regression)	156	Independent prediction of cancer-specific mortality	–	43
SIRT3 low expression and prognosis	Zhang CZ 2012 (IHC cohort)	248	Multivariate HR 0.555 (0.344–0.897)	0.016	46
Sorafenib + HCQ in advanced HCC	Arora SP 2025 (Phase II, NCT03037437)	Phase II cohort	mTTP 8.0 vs. 5.5 months (historical); ORR 27%; mOS 10.0 months	0.03	72
MCT1 inhibitor AZD3965, Phase I	Halford S 2023 (NCT01791595)	40	RP2D 10 mg BID; retinal/cardiac/acidotic DLTs	–	70

ATZ/BEV, atezolizumab plus bevacizumab; AUC, area under the curve; CI, confidence interval; HR, hazard ratio; OS, overall survival; TGI, tumor growth inhibition; HK2, hexokinase 2; HCC, hepatocellular carcinoma; IHC, immunohistochemistry; LDH, lactate dehydrogenase; ULN, upper limit of normal; PFS, progression-free survival; H3K18la, histone H3 lysine 18 lactylation; PD-1, programmed cell death 1; TNM, tumor-node-metastasis; H4K5la, histone H4 lysine 5 lactylation; ENO2, enolase 2; TAM, tumor-associated macrophage; TCGA, The Cancer Genome Atlas;LIHC, liver hepatocellular carcinoma; ICGC, International Cancer Genome Consortium; HDAC1/2, histone deacetylase 1/2; SIRT3, sirtuin 3; HCQ, hydroxychloroquine; mTTP, median time to progression; mOS, median overall survival; MCT1, monocarboxylate transporter 1; RP2D, recommended phase 2 dose; BID, bis in die (twice daily); DLTs, dose-limiting toxicities; ORR, objective response rate; RFS, recurrence-free survival.

axis (VPS34, TFEB, mTOR K921) remain extrapolated from non-HCC systems. Second, the clinical data are dominated by small, single-center, retrospective cohorts (*n* = 30–155) and computational TCGA-based analyses, and even the available meta-analyses are constrained by heterogeneity and publication bias (e.g., publication bias detected among the overall-survival studies in the LDH meta-analysis, mitigated only by trim-and-fill).[82,90–92] Third, methodological caveats apply: pan-Kla antibodies cross-react with acetylation marks; protein-level immunohistochemistry and transcriptomic markers can diverge (e.g., SIRT3 protein vs. mRNA); and bulk analyses lack spatial resolution, so the intratumoral heterogeneity discussed above cannot yet be resolved in clinical samples. Fourth, the causal ordering within the axis (e.g., lactylation upstream of autophagy) rests largely on pharmacological comparison rather than definitive *in vivo* rescue, as acknowledged by the original studies.[19] Finally, translational evidence is thin: no HCC trial has yet tested LDHA-, MCT-, or lactylation-specific agents, and the only positive HCC-specific clinical signal arises from a small phase II study of hydroxychloroquine plus sorafenib against historical controls.[72] For clinical practice, the most immediately actionable, evidence-backed readouts remain serologic—serum LDH dynamics predict outcomes in real-world immune-oncology cohorts,[83,84] whereas tissue-level Kla quantification remains a research tool pending antibody standardization

and prospective validation. Accordingly, the lactylation-autophagy axis is best regarded at present as a mechanistic hypothesis that generates testable trial designs (combination strategies; pharmacodynamic monitoring of both serum lactate and tissue Kla) rather than an established basis for routine treatment decisions.

Conclusions

The lactylation-autophagy axis exemplifies a broader paradigm: metabolites function not only as bioenergetic substrates but also as potent epigenetic and immunologic regulators. In HCC, the quantitative evidence compiled in this review, such as clinical cohorts, meta-analyses, tissue-level lactylation profiling, and early-phase combination trials (Table 4), supports an emerging self-reinforcing circuit linking glycolytic flux, lactylation, and autophagic adaptation. At the same time, as detailed above, the axis remains, at present, a mechanistic hypothesis whose nodes are only partially confirmed in HCC and whose clinical value awaits prospective validation. Its translation will therefore depend on targeted validation studies (site-specific lactylation technologies such as genetic code expansion, spatial and single-cell multi-omics), on robust biomarker development (serum lactate/LDH, tissue Kla), and on combination trials designed with pharmacodynamic endpoints. Decoding this circuit by these means holds the potential to redefine future immunometabolic strategies and to open the door for next-generation combinatorial therapies in HCC.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization (YN); literature search and data curation (YS); original draft preparation (YN); manuscript review and editing (LX); supervision (YY); funding acquisition (CZ). All authors have read and approved the final manuscript.

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