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Review

Understanding lactate sensing and signalling

Michelangelo Certo, ^{1,3} Alba Llibre, ^{1,3} Wheeseong Lee, ^{2,4} and Claudio Mauro ^{1,4,*}

Metabolites generated from cellular and tissue metabolism have been rediscovered in recent years as signalling molecules. They may act as cofactor of enzymes or be linked to proteins as post-translational modifiers. They also act as ligands for specific receptors, highlighting that their neglected functions have, in fact, a long standing in evolution. Lactate is one such metabolite that has been considered for long time a waste product of metabolism devoid of any biological function. However, in the past 10 years, lactate has gained much attention in several physio-pathological processes. Mechanisms of sensing and signalling have been discovered and implicated in a broad range of diseases, from cancer to inflammation and fibrosis, providing opportunities for novel therapeutic avenues. Here, we review some of the most recently discovered mechanisms of lactate sensing and signalling.

Introduction

In the past few years, metabolites produced from the cellular metabolism have been rediscovered as signalling molecules, capable of orchestrating several biological processes, including the immune response, via specific transporters and receptors. Less than 10 years since this area of research began, metabolites are now seen as some of the most ancient signalling molecules on the evolutionary scale.

We and others focused on the signalling properties of lactate, previously considered a waste product devoid of any biological function, except in specific contexts, for example, in the Cori cycle [1–5]. Lactate is a main product of glycolytic metabolism that accumulates acutely upon exercise and chronically in cancer [tumour microenvironments (TMEs)] and inflammatory sites, for example, arthritic synovia, atherosclerotic arterial walls, and fibrotic lungs. Indeed, lactate is often the most concentrated metabolite in these sites when assessed by mass spectrometry methodologies [6,7].

Lactate is now recognized as a pleiotropic signalling molecule, capable of regulating the immuneinflammatory response, angiogenesis, and fibrosis. Its effects are context dependent and may be influenced by factors such as the concentration of lactate itself as well as the acidity and concentrations of other nutrients in the microenvironment where lactate is present [2]. Such factors could, for instance, determine the reported suppressive effects of lactate in the TME (30–40 mM lactate, pH ~6, nutrient-deprived) versus the proinflammatory effects in the inflammatory sites (10–15 mM lactate, pH ~6.9, presence of nutrients). The pKa of lactic acid is 3.86, meaning that at pH 3.86, 50% of lactic acid exists as the lactate anion. By contrast, H⁺ may also derive from other acids, for example, ketone bodies, sulfate, phosphate, and urate. This means that it is not appropriate to compare the acidity and lactate signalling pathways headto-head as it is done in some studies, particularly in the TME.

Excitingly, recent studies have established mechanisms of sensing of lactate, via transporters and, even more recently, receptors; they have also identified signalling pathways, including

Highlights

Lactate is a pleiotropic signalling molecule capable of regulating several biological processes, including immuneinflammatory responses, angiogenesis, and fibrosis.

Lactate is sensed via transporters of the families of monocarboxylate transporter (MCT) and sodium monocarboxylate transporter (SMCT) and the G protein-coupled receptors, GPR81 and GPR132.

Lactate is a high-energy carbon molecule actively taken up from the extracellular environment and preferentially used by cells to feed macromolecule biosynthesis.

Lactate carbon-dependent macromolecule biosynthesis is linked to metabolic reprogramming-dependent protein kinase activation and cytokine synthesis.

Lactylation is a newly discovered lactatedependent post-translational protein modification, and it impacts cell metabolism and function.

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metabolic reprogramming linked to kinase activation and cytokine production and protein modification, via a novel type of post-translational modification (PTM), that is, lactylation. These novel discoveries will be the focus of this review.

Lactate transporters and lactate signalling through G protein-coupled receptors (GPCRs)

Lactate is transported in a stereospecific manner across plasma membranes. Both proton (monocarboxylate transporters: SLC16A1, SLC16A7, SLC16A8, and SLC16A3, also known as MCT1–4) and sodium-dependent transporters (SLC5A8 and SLC5A12, also known as SMCT1–2) exist [8,9]. These transporters are promiscuous and can act on a variety of substrates, including pyruvate or ketone bodies [9].

Although each transporter primarily shuttles lactate in a specific direction (i.e., MCT4 efflux, MCT1 influx), this will ultimately depend on the lactate gradient. Therefore, when the extracellular milieu is lactate rich (e.g., TME, inflamed tissues), import of lactate into the cell prevails. Their tissue distribution is receptor specific, and while for some of them, it can be relatively broad [SLC16A7 (MCT2), SLC16A3 (MCT4)], and for others it is predominantly confined [i.e., SLC16A8 (MCT3) in the retinal pigment and choroid plexus epithelia]. Further details on lactate transporters can be found elsewhere [10,11].

Classical metabolites can signal directly through GPCRs [12]. Upon agonist binding, there is a GDP–GTP exchange in the GPCR alpha subunit. The downstream signalling events are driven by secondary messengers (e.g., cAMP, Ca²⁺) depending on the alpha subunit type (G α i, o, q, s, or 12) [13], although G α -independent signalling also exists. Specifically, lactate can signal though GPR81 (HCAR1) and GPR132 (G2A) [6], both being proton sensitive [14].

GPR81

Two homologs of GPR81 have been identified in zebra fish, allowing studies of conserved residues across species [15]. Arg71 in the transmembrane domain 2 and the C165-E166-S167-F168 motif and six conserved Cys in the extracellular domain are essential for GPR81 function [15].

Adipose tissue

GPR81 is mainly expressed in adipose tissue (mice, rats, humans), where signalling triggered by lactate results in inhibition of lipolysis [16,17], thus promoting lipid accumulation. On top of adipocytes, these effects have also been reported in skeletal muscle [18]. GPR81 primarily signals through Gai, inhibiting the cAMP-protein kinase A (PKA) pathway. Lactate signalling through GPR81 is responsible for insulin-driven prevention of lipolysis by inhibiting cAMP production [19]. In mice, the lactate-GPR81-p38 axis has been linked to adipose browning and thermogenesis [20].

Tumours

GPR81 is expressed in various tumours from patients and cancer cell lines [21]. *In vivo*, levels of GPR81 correlate with tumour growth and metastasis in pancreatic cancer, and both dramatically decline upon GPR81 silencing [21]. Mechanistically (Figure 1), lactate promotes (i) angiogenesis by signalling through the PI3K/Akt-cAMP-CREB (cAMP response element-binding protein) pathway leading to production of pro-angiogenic amphiregulin (AREG), as described in breast cancer [22] and (ii) lactate impairs antitumour immunity. It signals through GPR81 in tumour-infiltrating dendritic cells (DCs), compromising their ability to efficiently present antigen to T cells by downregulating MHC-II molecules [23]. Lactate limits type I interferon (IFN) production by intratumoural plasmacytoid DCs through G $\beta\gamma$ -Ca²⁺-calcineurin phosphatase (CALN) signalling [24]. Moreover, lactate–GPR81 induces programmed death-ligand 1 (PD-L1) expression in lung cancer cells via TAZ/TEAD





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Figure 1. Multiple effects of lactate–GPR81 signalling in different cell types. Lactate can signal through GPR81 and its alpha subunit, inhibiting the cAMP/PKA pathway and inducing lipid accumulation in adipocytes. The same pathway can be triggered in cancer cells resulting in TAZ/TEAD activation and PD-L1 transcription, inhibiting cytotoxic T cells. The alpha subunit in tumour cells can also increase chemoresistance, by upregulating the ABCB1 transporter through PKC/ERK. The beta/ gamma subunits can also transduce GPR81 signalling through PIK3/AKT, promoting amphiregulin transcription and, ultimately, its secretion, inducing angiogenesis, and favouring tumour growth. Lactate impacts immune cell behavior of multiple cell types, in and outside the tumour microenvironment. Lactate signalling through GPR81 reduces type I IFN production of pDCs, downregulates the antigen-presenting machinery of dendritic cells, and promotes S100A8/9 secretion of MDSCs, inhibiting T cells. In monocytes/macrophages, GPR81 can signal through the ARRB2 domain, inhibiting NLRP3 inflammasome and subsequent pro-IL1b processing by Caspase-1. Abbreviations: AC, adenylyl cyclase; AREG, amphiregulin; CALN, calcineurin; CASP1, caspase-1; DC, dendritic cell; HIF-1α, hypoxia-inducible factor 1; IFN, interferon; MDSCs, myeloid-derived suppressor cells; PKA, protein kinase A.

(encoded by the gene Wwtr1) transcription complex activation, which binds the PD-L1 promoter, resulting in impaired T cell cytotoxicity [25]. Dual blockade strategy of lactate/GPR81 and PD-1/PD-L1 potentiates metformin antitumour effects [26]. (iii) DNA repair is enhanced upon GPR81 signalling, which triggers expression of DNA repair proteins such as BRCA1, nibrin, DNA-PKs [27] and MLH1 (mutl homolog 1) [28], favouring chemoresistance. Chemoresistance is also increased by GPR81-dependent increased expression and activity of the drug-exporting transporter ABCB1, with the Gai-PKC-ERK (extracellular signal-regulated kinase) pathway being crucial [29]. Importantly, radiotherapy enhances the Warburg effect and lactate production in pancreatic cancer [30]. Lactate signalling through GPR81/mechanistic target of rapamycin (mTOR)/hypoxia-inducible factor (HIF)-1α/STAT3 activates myeloid-derived suppressor cells (MDSCs), favouring tumour growth [30].

Brain

GPR81 is found in the brain, expressed in different cell types including neurons, astrocytes, and cells from the microvasculature. These cells can be activated at physiological concentrations of



lactate, linking neuronal activity and brain metabolism [31], potentially by optimising cAMP concentrations [32]. Using GPR81 knockout (KO) mice, GPR81 signalling was shown to reduce neuronal activity [33] through both Gai and G $\beta\gamma$ i subunits [34]. In rodents, lactate impacts learning and memory [35], and the effects are enantiomer specific [36]. Lactate has been linked to neuroplasticity [37,38], neurogenesis [39], and neuroprotection [40,41], potentially bridging intensive exercise (high lactate producing activity) with improved brain capacity.

Other tissues

The lactate–GPR81 axis is also involved in retinal function [42,43], osteoblast differentiation through GPR81-G $\beta\gamma$ -PLC-PKC-Akt signalling [44], inflammation regulation during labour [45], suppression of innate immunity [46–49], and regulation of intestinal [50,51], renal [52], cardiovascular [53], and atherosclerotic [54] homeostasis.

GPR132

GPR132 is expressed in several tissues such as the lung and the gastrointestinal tract and in immune cells, particularly macrophages (The Human Protein Atlas¹). It has abundant physiological ligands, including fatty acids and derivatives, such as oxylipins [55] and lipoamines [56], and presents G α promiscuity [57].

Lactate signalling through GPR132 has been limitedly studied so far. Most studies have been performed in mice, and the murine and human GPR132 forms present important differences in terms of sequence homology (67% shared amino acid identity) [58] and proton sensitivity [59].

L-lactate administration in high-fat diet (HFD)-fed mice mitigated fat accumulation, insulin resistance, and infiltration of proinflammatory macrophages in adipose tissue [16]. This was a result of lactate signalling through the GPR132-cAMP-PKA-AMPK α 1 (AMP-activated protein kinase α 1) pathway, with AMPK polarising macrophages toward an anti-inflammatory phenotype [60].

Lactate signalling through GPR132 in tumour-associated macrophages promoted a protumoural M2 (alternatively activated, anti-inflammatory) phenotype in models of breast cancer [26] and in Lewis lung carcinoma, here heterodimerising with Olfr78 [61]. In human breast cancer, GPR132 correlates with M2 macrophages and metastasis, and metastasis is absent in GPR132-KO animals [26].

Therefore, direct lactate signalling through GPCRs is arising as an essential mechanism by which this once thought metabolic waste product shapes key biological processes, in both health and disease.

Lactate-induced acidity

Extracellular acidosis is a hallmark of inflammatory processes. Tissue hypoxia caused by the damage of small blood vessels and the high glycolytic metabolism of resident or infiltrating cells leads to the accumulation of lactic acid in the extracellular space. The resulting acidosis, in turn, can alter the functions of cells of the immune system, including T cells, neutrophils, macrophages, and DCs [2]. The role of extracellular acidosis is not merely immunosuppressive but can have both supporting and suppressive effects on different immune cells (Figure 2). A clear example of a physiological and immune-modulating effect of low pH is the lymph node (LN) microenvironment. Although this low pH, resulting from activated T cell-generated lactic acid, does not inhibit T cell activation, it protects the LN from premature release of inflammatory cytokines [62]. MCTs, Na+/H+ exchanger 1, carbonic anhydrases (CAs), vacuolar ATPase, and protonsensing GPCRs can sense intra- and extracellular pH variations and modulate the immune system in inflammatory and immunoreactive processes.





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Figure 2. Differential immune cell-specific effects of acidity and lactate on the inflammatory microenvironment. Extracellular acidosis is a hallmark of inflammatory processes. Tissue hypoxia and the high glycolytic metabolism of infiltrating cells lead to the accumulation of lactic acid in the extracellular space. When dissociated, both H+ and La– can be sensed by immune cells including T cells, neutrophils, and macrophages, through specific receptors and transporters such as monocarboxylate transporters (MCT1–4), sodium monocarboxylate transporters (SMCT1–2), Na+/H+ exchanger 1, carbonic anhydrases (CAs), V-ATPase, and proton-sensing G protein receptors (GPRs), and alter their functions. These sometimes opposite effects can be both supportive and suppressive on different immune cells, thus leading to proinflammatory (red boxes) or anti-inflammatory (green boxes) phenotypes. Abbreviations: BM, bone marrow; G-CSF, granulocyte colony-stimulating factor; IL, interleukin; MCP1, monocyte chemoattractant protein-1; TNF-α, tumour necrosis factor alpha; V-ATPase, vacuolar ATPase.

Acidification can induce an anergic state in human and murine CD8⁺ T cells, characterized by reduced cytolytic activity and cytokine secretion, reduced expression of T cell receptors, and diminished activation of STAT5 and ERK [63,64]. Lactic acid inhibits the production of IFN- γ and interleukin (IL)-2 by activated CD8⁺ T cells and promotes cell death [65]. A possible mechanism by which extracellular acidosis alters T cell activity is the suppression of mTORC1 and its interaction with RHEB (Ras homolog enriched in brain) [66]. Low pH affects plasma membrane and microtubule mobility, thus decreasing the TCR-CD8 colocalization, with consequent T cell anergy [67]. Unlike the other T cell subsets, the activity and recruitment of Tregs are increased in the acidic microenvironment.



It has also been demonstrated that lactic acidosis induces the secretion of IL-23 by mononuclear phagocytes, thus stimulating T cell IL-17 secretion [68], and it induces the production of inflammatory cytokines, including IL-1β, IL-6, CXCL1, and CCL2, as well as the recruitment of neutrophils in murine models of pneumonia and peritonitis [69]. Low extracellular pH has also been shown to stimulate phagocytosis in macrophages, and one of the mechanisms underlying this effect may be the increased expression of the phagocytic receptor stabilin-1 [70]. Contrasting with these observations, other studies have shown that low extracellular pH inhibits tumour necrosis factor alpha (TNF- α) production by human monocytes [71]. Furthermore, acidosis limits the expression of proinflammatory genes such as inducible nitric oxide synthase (iNOS), monocyte chemoattractant protein-1 (MCP1), and IL-6 in M1 (classically activated, proinflammatory) macrophages, while it enhances the expression of M2 genes such as mannose receptor C-type 1 (MRC1), arginase 1 (ARG1), and chitinase-3-like protein [72]. Exposure of human macrophage foam cells to acidic conditions, such as those found in atherosclerotic lesions, reduces their intracellular pH with concomitant reduction of the cholesterol-esterifying enzyme acyl coenzyme A:cholesterol Oacyltransferase 1 (ACAT1) activity. This leads to a proatherogenic cascade affecting both cholesterol esterification and cholesterol efflux processes in macrophages.

Extracellular acidification also plays a key role in neutrophil function, leading to the upregulation of surface expression of CD18 and a decreased spontaneous apoptosis [73]. Extracellular acidosis induces neutrophil activation by a mechanism dependent on activation of phosphatidylinositol 3-kinase/Akt and ERK pathways. In the same study, it has been reported that extracellular acidification induces endocytosis of exogenous proteins, resulting in an increased ability to present antigens through the MHC class I-restricted pathway [74]. It has also been shown that acidic pH drives activation of integrin $\alpha\nu\beta3$, a receptor for vitronectin. This increase in integrin affinity and avidity facilitates cell–cell connections, limiting the migration of neutrophils [75]. However, other studies have shown that in the acidic environment, neutrophils can acquire an alternative phenotype, as is the case with macrophages, characterized by reduced phagocytic activity and reactive oxygen species (ROS) production, a very high expression of the $\beta2$ integrin CD11b/CD18, and an increased ability to inhibit T cell responses and to release the angiogenic factors IL-8, vascular endothelial growth factor (VEGF), and the matrix metallopeptidase 9 (MMP-9) [76].

Lactate-induced metabolic reprogramming in immune cells

To initiate and sustain an inflammatory response, immune cells need to activate metabolic pathways, and each population of immune cells relies on a different metabolism and nutrient utilization [77]. As a consequence of inflammatory activation and engagement in glycolysis, immune cells start to produce high amounts of lactate in the surrounding microenvironment. Lactate can be sensed by other immune cells via the expression of lactate receptors (discussed previously) or via MCTs on their cell surface and can affect their cellular metabolism, ultimately modulating their phenotype into pro- or anti-inflammatory [7,30,78] (Figure 2).

Lactate reduces the extracellular acidification rate (ECAR) and increases the oxygen consumption rate (OCR) in macrophages treated with lipopolysaccharide (LPS), an this metabolic reprogramming is responsible for the phenotype shift of these cells from pro- to anti-inflammatory, as evidenced by the reduction of inflammasome assembly, LPS-induced cytokine secretion, and migration of macrophages and monocytes [79,80]. Lactate also reduces the induction of II1B, NIrp3, and Casp1, the activation of nuclear factor kappa B (NF- κ B), and the release of IL1 β in macrophages [47]. In addition, lactate injection reduces inflammation and organ injury in mice with immune hepatitis, acute pancreatitis, or acute liver injury [47]. Lactate also downregulates the activity of phosphofructokinase (PFK), the key regulatory glycolytic enzyme, favouring the



dissociation of enzyme active tetramers into less active dimers, thereby reducing the glycolysis flux and the proinflammatory phenotype of monocytes [81]. In addition, lactate can interact with the mitochondrial antiviral-signalling (MAVS) protein, inhibiting MAVS aggregation and therefore limiting type I IFN production [82]. However, other studies have found proinflammatory effects of lactate in macrophages. For instance, Samuvel *et al.* [83] showed an enhanced TLR4 signalling and increased NF-kB transcriptional activity and expression of inflammatory genes in human monocyte-derived macrophages. These effects are a result of lactate uptake through MCTs, as evidenced by the fact that they are reversed through their inhibition. Moreover, lactate has been shown to enhance prostaglandin E2 synthesis [84] and LPS-induced IL-23 production in monocytes, macrophages, and tumour-infiltrating immune cells [85]. The reason for these opposite effects may rely on the duration of lactate exposure and the cellular metabolic profile, and on the fact that both lactate and the related H⁺ ion can modulate macrophage function.

Neutrophils are commonly present in inflammatory sites that often have high lactate levels, but the role of lactate in neutrophil activity is less explored. These cells use different metabolic pathways to cope with energy demands during their proliferation and release of inflammatory mediators, including not only the pentose phosphate pathway, glutaminolysis, the mitochondrial oxidative metabolism, but also glycolysis which is crucial for phagocytosis, ROS, and networked protein family (NET) production [86,87]. In these cells, lactate may act as a signalling molecule as demonstrated by the fact that lactic dehydrogenase (LDH) inhibition reduces NET release, thereby indicating a key role of lactate-mediated metabolic pathways in NETosis [88]. It has also been reported that glycolytic lactate produced by neutrophils following stimulation with LPS promotes their mobilization acting on its receptor GPR81 functionally expressed by endothelial cells to locally increase vascular permeability. In addition, lactate induces neutrophil mobilization from the bone marrow through an increased release of CXCL1, CXCL2, and granulocyte colony-stimulating factor (G-CSF) [89].

Lactate suppresses T cell migration, contributing to the retention of these cells in the inflammatory site. In addition, lactate also induces the production of IL-17 in the CD4⁺ T cells and inhibits the cytolytic function of cytotoxic CD8⁺ T cells [64]. These effects are a consequence of the induced expression of the lactate transporters SLC5A12 and SLC16A1, which are present in CD4⁺ and CD8⁺ T cells, respectively. Inhibition of lactate transporters re-establishes T cell migration both in vitro and in a murine model of peritonitis [64]. On the contrary, it has been demonstrated that Tregs resist lactate-mediated suppression of T cell function and proliferation through Foxp3mediated repression of Myc and suppression of glycolysis [90]. Tregs metabolize lactate into pyruvate, citrate, and malate to fuel the tricarboxylic acid (TCA) cycle to sustain their proliferation. We have shown that the reduced T cell motility induced by lactate is associated with an inhibition of glycolysis [1]. T cells reduce NAD⁺ to NADH via glyceraldehyde-3-phosphate dehydrogenase and need NAD⁺ recycling through LDH to support glycolysis. In the presence of high lactate concentrations, this metabolite is reconverted to pyruvate with production of NADH and inhibition of glycolysis. A direct consequence of this dysregulation of the glycolytic pathway could be the diversion of glucose-derived carbons into the pentose phosphate pathway with subsequent generation of NADPH, which is the reduced cofactor in fatty acid synthesis. Furthermore, when exposed to high levels of lactate, such as those found in an inflamed tissue, activated CD4⁺ T cells take up lactate via the specific carrier SLC5A12 and its carbons are used as fuel for the TCA cycle. This leads to an increase in intracellular pool of citrate and acetyl-CoA and ultimately to an enhanced fatty acid synthesis, a metabolic pathway involved in the differentiation of the Th17 T cell subset [1,91]. Lactate can also modulate T cell cytokine production as a consequence of changes in cellular metabolism. For instance, we have found that sodium lactate can directly polarize CD4⁺ T cells toward a Th17 phenotype via PKM2 nuclear translocation and concomitant enhanced STAT3 phosphorylation, and



via enhanced fatty acid synthesis, which leads to the release of IL-17 in the inflamed tissue, thus sustaining the chronic inflammatory process [1]. Finally, lactate inhibits the cytolytic function of both human and mouse natural killer (NK) cells by lowering the expression of perforin and granzyme [92].

Taken together, these studies support a role for lactate in the modulation of immune cell functions through a reshaping of their metabolism. Targeting this metabolic switch may represent a valuable strategy for the development of new therapeutics against chronic inflammatory disorders [2].

Lactylation and other lactate-dependent PTMs

In 2019, lysine lactylation (Kla) was first identified by mass spectrometry (mass shifting) in both human and murine cells [93]. Protein lactylation is a highly regulated process. Existing evidence suggests a key role for p53 and p300 in lactylation of lysine residues [93-95], whereas HDAC1-3 and Sirtuin3 are enzymes with reported delactylase activity [96]. In this first study, 28 lactylation sites were described on core histones, with glycolysis being a key modulator. Post-translational histone modifications link the metabolic cellular state to gene expression, favouring cell adaptation to the new environment. In this seminal study, bone marrow-derived macrophages (BMDMs) treated with LPS + IFNy resulted in increased histone lactylation [93]. Kla associated with a shift in macrophage phenotype, in which glycolytic proinflammatory macrophages returned to homeostasis by lactylating specific genes (e.g., Arg1, Mmp9). Building on the role of lactate in inflammation resolution, a follow-up study identified the B cell adapter for PI3K (BCAP) as crucial for the reparative macrophage transition in a murine model of colitis [97]. Mechanistically, BCAP was necessary for optimal glycolysis, lactate production, and histone lactylation after microbial challenge. BMDMs of BCAP^{-/-} mice presented decreased histone lactylation and reduced expression of anti-inflammatory macrophage genes (i.e., Arg1, KLf4), which could be rescued by treatment with sodium lactate. However, the Kla-anti-inflammatory causal link was challenged by Dichtl et al. [98]. Kla and the anti-inflammatory gene expression program were shown to be uncoupled despite their concurrency. The authors identified the IL-6-Stat3 axis as essential in the expression of hallmark macrophage anti-inflammatory genes (i.e., Arg1) and proposed Kla as a consequence of macrophage activation with no direct impact on polarization [98]. Different models and methodologies used could account for these differences, and more mechanistic studies are required to elucidate the precise functional consequences of histone lactylation (e.g., mutation of specific histone lysines). Besides lactylation, lactate can influence the epigenetic landscape of the cells by inhibiting histone deacetylases [99], primarily increasing transcription. Specifically, lactate promotes high-mobility group box-1 (HMGB1) acetylation in macrophages in a mouse model of polymicrobial sepsis [100]. HMGB1 resides in the nucleus, and activated macrophages release it to orchestrate inflammatory responses. Lactate drives HMGB1 lysine acetylation by a dual action. On one hand, it suppresses SIRT1 deacetylase in a Hippo/YAP-dependent manner; on the other hand, lactate signals through GPR81 impairing nuclear recruitment of acetylases p300/CBP. This is the first study directly linking lactate-GPCR signalling with lactate-driven PTMs. Also, in the context of macrophages, mitochondrial lactate was shown to drive M2 polarisation through histone acetylation [101]. Adenosine triphosphate-citrate lyase (ACLY) was essential for this induction of antiinflammatory macrophages, and it aided tumour progression in a mouse tumour admixture model.

Histone lactylation is required for successful embryo development and implantation in mouse and sheep [95]. Kla controls mouse embryonic stem cells, expanding their transcriptional network [102]. H3K18la associates with increased pluripotency in mouse embryonic fibroblasts [103]. In this context, the transcription factor Glis1 was responsible for enhancing expression of glycolytic genes and consequent lactate production. Interestingly, increased glycolysis also resulted in enhanced acetyl-CoA levels, and histone acetylation (H3K27Ac) also promoted pluripotency [103].



Targets of protein lactylation extend beyond histones and have been investigated in different settings. Lactate is abundant in cancer because of abundant aerobic glycolysis (Warburg effect) [104]. In lung cancer, Kla sites were found in the promoters of the metabolic genes hexokinase-1 (HK-1; glycolysis) and isocitrate dehydrogenase [NAD] subunit gamma (IDH3G, Krebs cycle), down- and upregulating them, respectively [105]. In lung fibrosis, Kla sites are present in promoters of profibrotic genes [94]. Increased histone lactylation occurs in ocular melanoma, and preventing it suppresses tumour progression [106]. Mechanistically, lactylation enhances transcription of the YTH N6-methyladenosine RNA-binding protein 2 (YTHDF2), responsible for recognising the m6A RNA modification on PER1 and TP52 (two tumour suppressor genes), causing their degradation.

Full lactylome studies (Table 1) have been performed in the plant fungal pathogen *Botrytis cinerea* [107], in rice (*Oryza sativa*) grains [108], and in the protozoan parasite *Trypanosoma brucei* [109]. These comprehensive analyses have revealed key targeted pathways and cellular compartments, as well as evolutionary conservation [108], suggesting a role for lactylation in regulating essential biological processes.

In the context of the brain and behavioural science, neural excitation and social defeat stress resulted in increased levels of lactate and lactylation in mouse brains [110]. Particular Kla sites correlated with reduced social behaviour and increased anxiety.

Refs	[107]	[108]	[109]
Species	Botrytis cinerea	Oryza sativa	Trypanosoma brucei
Lactate source	Glycolysis	Glycolysis	Glyoxalase pathway
Biological replicates, n	4	8	3
Lylated proteins, n	166	342	257
Kla sites, n	273	638	387
Kla sites – structure	Preference for alpha-helix	NA ^a	NA
Kla flanking region (enrichment)	A (+3 to +5)	A (-2, +1, +2)	A (-10, -4 to -3, +4, +6)
	G (-1 to +1)	E (-8, -3, -1)	G (–1, +1)
	K (-9 to -6 and +5 to +9)	G (-4, -1, +1, +2)	K (-9 to -8, -6, +2 to +10)
	R (+6 to +7)	K (-7, -9, +10)	R (-7)
Kla subcellular distribution (%)			
Nucleus	36	9.94	38.13
Cytoplasm	25	33.04	35.02
Mitochondria	27	9.06	11.28
Chloroplast	NA	38.3	NA
Top lactylated pathways	Ribosomal small subunit assembly	Central carbon metabolism	Translation, ribosomal structure, biogenesis
	Cytoplasmic translation	Protein biosynthesis	PTMs, protein turnover, chaperones
	Noncoding RNA (ncRNA) export from nucleus	Nutrition reservoir	Replication, recombination, repair
PTM overlap	Crotonylation	Acetylation	Succinylation
	2-hydroxyisobutyrylation	Succinylation	Crotonylation
		Malonylation	Acetylation
		2-hydroxyisobutyrylation	

Table 1. Summary of the three published proteome-wide lysine lactylation (Kla) analysis in Botrytis cinerea, Oryza sativa, and Trypanosoma brucei

^aAbbreviation: NA, not applicable.



Lactate levels can be used as a biomarker, as they often correlate with disease severity [111]. The potential role of Kla as a biomarker is being explored. Levels of H3K18la have been linked to infection and severity of critical illness [112]. A potential mechanism could be lactate-driven lactylation and acetylation of the HMGB1, which is secreted by macrophages via exosomes and increases endothelium permeability in polymicrobial sepsis [113].

Identification of KIa substrates and sites is key to understanding the mechanisms and regulatory roles of protein lactylation. A recent preprint proposes, instead of mass shifting, the use of cyclic immonium ion and liquid chromatography–tandem mass spectrometry for confident lactylation assignment. This approach allows interrogation of public datasets such as the human proteome [114] or the meltome atlas [115], which showed that lactylation impacts protein thermal stability, including enzymes involved in glycolysis, which is the most heavily lactylated pathwayⁱⁱ. Additional bioinformatic tools are being developed, based on protein sequence, structure, and physico-chemical properties, to predict KIa profiling using a few-shot learning (FSL) approaches [116], which is particularly useful to capitalise on limited datasets.

Although lactylation was only reported in 2019, abundant and exciting research is bringing new insights into the mechanisms behind biological contexts and functional consequences of this newly described PTM. A better understanding of the lactylation process may set the path for new therapeutic avenues, in cancer and other diseases.

Concluding remarks and implications for inflammatory conditions and fibrosis

The pathways for lactate sensing and signalling have been implicated in human disease. The immune suppressive role of lactate in TME has been largely covered elsewhere [117,118]. Here, we will refer to few examples of the implications for fibrosis and inflammation.

Myofibroblasts' glycolysis in fibrotic lungs enhanced lactate production, resulting in the promotion of profibrotic genes in alveolar macrophages through non-cell autonomous histone lactylation [94]. Furthermore, lactic acid is elevated in the lung tissue of patients with idiopathic pulmonary fibrosis (IPF), resulting in myofibroblast differentiation via a pH-dependent activation of latent TGF- β [119]. Also, lactate treatment induced stabilization of HIF-1 α , a master regulator of glycolysis, resulting in the priming of fibroblasts to a profibrotic phenotype via switching to a glycolytic metabolism with significantly increased PDK1 and PKM2 protein levels [120].

The protein SARM1, which is the enzyme responsible for axon degeneration, is activated in an acidic pH [121]. Recent evidence showed that H4K12 histone lactylation activates the transcription of glycolytic genes through a PKM2 positive feedback loop in microglia in Alzheimer's disease [122]. Also, host-derived lactate promotes intestinal epithelial cell migration, resulting in the mitigation of colitis through the promotion of intestinal wound healing [123].

In this review, we discussed the mechanisms underpinning lactate shaping of cell behaviour and function, including acidosis, GPCR signalling, lactylation, and lactate as a fuel. These mechanisms do not occur in isolation, and the precise contribution of each of them to the overall cell outcome is a matter of current investigations. For instance, we know that lactate signalling through GPR81 prevents acetylation in macrophages [100] and that acidosis directly impacts protein-sensing receptors, including MCTs and GPCRs. Also, constant research is being done to elucidate new lactate signalling pathways, and novel players have emerged in recent years. Lactate-driven stabilization of HIF-1 α in macrophages has been linked to tumour angiogenesis and growth [124,125]. Lactate can also regulate hypoxic responses in a HIF-1 α -independent manner. An elegant study showed that lactate accumulated under hypoxic conditions stabilizes N-myc

Outstanding questions

What are the precise mechanisms of direct lactate signalling through GPCRs, how do they impact human diseases, and can GPR81 and GPR132 be pharmacologically targeted for human health benefit?

How do lactate and lactate-dependent acidosis impact foam cell formation in the atherosclerotic vessel wall?

What are the biochemical pathways activated by cells consuming lactate from the extracellular space and how do they drive specialised functions, such as cytokine synthesis or cell migration?

What are the precise functional consequences of protein lactylation (e.g., mutation of specific histone lysines) for physiology and pathology, and can this process be targeted for therapeutic benefit?



downstream-regulated gene 3 (NDRG3), which can then bind to c-Raf enabling Raf-ERK signalling [126]. Therefore, lactate arises as a master regulator of hypoxia responses, promoting cell growth and angiogenesis. Lactate also contributes to increasing acetyl-CoA and fatty acid (e.g., palmitate) intracellular pools in CD4⁺ T cells [1]. These lactate-derived metabolites have the potential to post-translationally modify histone and nonhistone proteins (e.g., palmitoylation), broadening the potential impact of lactate at the cytoplasmic and epigenetic levels.

Targeting lactate transporters is gaining attention as a pharmacological approach in inflammatory disorders. Inhibition of these transporters can modulate the effector functions of fibroblasts and T cells in rheumatoid arthritis and can reduce the severity of disease in mouse models of arthritis [1,127]. It was also shown that alpha-cyano-4-hydroxycinnamic acid, an inhibitor for MCTs, inhibits lactate-induced inflammatory gene expression and nuclear NF-kB activity [84]. Inhibition of SLC5A12 has been successful in promoting CD4⁺ T cell egress from the inflamed site in a murine model of zymosan-induced peritonitis, while having no effect on CD8+ T cells, and thus suggesting an interesting specificity of these lactate transporter inhibitors [64].

Targeting specific metabolic pathways induced by lactate is also becoming a useful and promising therapeutic strategy in inflammatory conditions. We have shown that T cells in a lactate-rich microenvironment can utilize this metabolite as a source of carbons in fatty acid synthase (FAS) and that the internalization of lactate in T cells leads to the translocation of PKM2 from the cytosol into the nucleus, resulting in the induction of proinflammatory genes [1]. Intriguingly, the inhibition of FAS can reduce the proinflammatory behaviour of T cells [91], while cytosolic PKM2 stabilization with TEPP-46 inhibits the proliferation of Th1 and Th17 cells and ameliorates experimental autoimmune encephalomyelitis (EAE) *in vivo* [128].

Overall, the effects of lactate are pleiotropic and context dependent. Understanding the key molecules to its sensing and signalling promises to open avenues for pharmacological approaches in a broad spectrum of human diseases (see Outstanding questions).

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Declaration of interests

W.L. is CEO of Lmito Therapeutics. C.M. is SAB member of Lmito Therapeutics.

Resources

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ⁱⁱhttps://assets.researchsquare.com/files/rs-916390/v1/ad6b5380-9ba0-4a71-b291-9929f3763e95.pdf?c=1632495885

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