Trends in
Endocrinology & Metabolism

CellPress

Forum

Unconventional roles of lactate along the tumor and immune landscape

Luigi Ippolito,[1] Pierre Sonveaux,[2] and Paola Chiarugi[1,3,*]

The tumor ecosystem evolves with dynamic interactions between cancer and normal cells, and nutrients have emerged as new regulators of cancer hallmarks. Lactate has climbed the rankings as a multifunctional molecule orchestrating many aspects of the disease onset and progression. Here, we patchwork and discuss the main recent findings conferred during the EMBO workshop titled 'Lactate: Unconventional Roles of a Nutrient Along the Tumor Landscape.'

Although the repertoire is large, cancer metabolic plasticity is still limited

Tumors are often described to be highly plastic metabolically. However, flexibility is constrained by the nature of the cells (cancer versus host) and their location within the tumor that conditions their access to oxygen and nutrients, and it impacts metabolic waste removal, and cells' genetic/mutational background. If one focuses on malignant cancer cells, three main metabolic modes exist: oxidative, aerobically glycolytic, and anaerobically glycolytic. Within the same tumor, the distribution of these phenotypes varies with space and time.

Oxidative metabolism is permitted only in sufficiently oxygenated tumor areas, as oxygen is the final electron acceptor at complex IV of the electron transport chain (ETC). It requires coupling between the tricarboxylic acid (TCA) cycle that produces electron donors NADH and FADH2 in the mitochondrial matrix and oxidative phosphorylation at the ETC that generates ATP from ADP + Pi + H^+. Oxidative metabolism is by far the most flexible metabolic mode for cancer cells, as it can be alimented by a variety of substrates, including glucose, lipids, and glutamine, fueling glycolysis, lipolysis, and glutaminolysis, respectively, to quote only major substrates. Lactate was recently added to the list based on the discovery of a metabolic symbiosis between oxidative and glycolytic cancer cells [1], where oxidative cancer cells gain increased autophagy (to recycle damaged/oxidized proteins and organelles) when switching from glucose to lactate [2]. Lactate-fueled respiration depends on the expression and activity of inward lactate transporters of the monocarboxylate transporter (MCT) family and lactate dehydrogenase-1 (LDH-1). As products of LDH-1 activity, pyruvate and NADH fuel the TCA cycle, whereas protons participate in lysosomal acidification. Of note, the oxidative flexibility of cancer cells can be limited by rare mutations affecting mitochondrial enzymes.

Aerobic glycolysis is the preferred metabolic mode of proliferating cancer cells, which are a minority in slow-growing tumors (such as prostate cancer) but can be very abundant in fast-growing tumors (triple-negative breast cancer, for example). Functioning in the glycolytic mode depends on uncoupling glycolysis from the TCA cycle (which can then function in a biosynthetic mode using, e.g., reductive glutaminolysis) and coupling glycolysis to lactic fermentation. The latter is expected to fluctuate along the cell cycle to alternate periods of energy production (G_2-M-G_1 phases) and biosynthesis (S phase), with pyruvate kinase M2 (PKM2) acting as an oscillator under allosteric control. Aerobic glycolysis offers intermediate metabolic flexibility, as it can revert to an oxidative phenotype when cancer cells stop to proliferate. This change is under the control of growth factors and restricted by mutations that either activate growth factor pathways or repress cell division, such as those inhibiting DNA repair. It depends on the expression of outward lactate transporters of the MCT family that contribute to acidify the surrounding tumor microenvironment (TME).

Anaerobic glycolysis is the less plastic type of metabolism in cancer. Although lactic fermentation is shared by proliferating and anaerobic cells, mechanisms strongly differ. Anaerobic glycolysis primarily depends on the activation of hypoxia and redox-sensitive pathways eventually yielding to the activation of transcription factors [comprising hypoxia-inducible factors (HIFs) and c-Myc]. If glucose bioavailability decreases, hypoxic cancer cells die of necrosis.

Of note, hypoxia and/or nutrient restriction set(s) a pressure on cancer cells that are forced to adapt and to evolve. Adaptation comprises cooperative and competitive strategies between cancer and host cells (e.g., angiogenesis, metabolic exchanges, cellular cannibalism) and between cancer cells with different metabolic activities (e.g., exchanging lactate for glucose in metabolic symbionts). Selection rather refers to the acquisition of new traits supporting new capacities, such as metabolic hibernation (cancer stem cells) and escape (migration, invasion, metastasis). It involves the acquisition of passenger mutations and/or new epigenetic marks, which may well be under metabolic control. Conversely, both adaptation and selection impact the local TME, and lactate plays a major role.

Lactate in intercellular metabolic relationships

Lactate has a dual role in metabolic pathways, as it is both a final product of glycolysis and an energy-rich oxidative

fuel. This two-faced characteristic places lactate at an important metabolic crossroads that supports intercellular cooperation, commensalism, and 'education' through epigenetic conditioning.

Metabolic cooperation is a system in which cells providing nutrients gain a reciprocal advantage. Lactate can be used for the purpose. In tissues such as muscles and tumors, lactate is produced and secreted together with a proton by glycolytic cells that convert pyruvate + NADH + H^+ in lactate + NAD^+ (the LDH-5 reaction). This reaction serves two objectives: (i) to covalently sequester protons in order to avoid cytoplasm acidification and (ii) to supplement NAD^+ production by hypoxic/failing mitochondria in order to maintain a high glycolytic flux. Cooperation occurs when oxidative cells take up extracellular lactate and use it as an oxidative fuel preferentially to glucose [3]. Of note, in these cells, NAD^+ is sourced at the ETC, transferred to the cytosol (malate-aspartate shuttle), and used to oxidize lactate to pyruvate (the LDH-1 reaction). Extracellular lactate in excess oozes out from tumors and reaches the systemic circulation. A mean clearance system is the Cori cycle, during which circulating lactate is taken up by the liver for gluconeogenesis at the expense of lipolysis that is used to provide energy for glucose production. Other tissues participate in lactate clearance. In the systemic context and in healthy individuals, lactate has even been described as the predominant source of circulating carbon that fuels tissue respiration.

Lactate exchange over glucose between glycolytic and oxidative cells has been reported in a wide variety of cancers. It is reminiscent of physiological lactate swapping between astrocytes and neurons (astrocyte–neuron shuttle), which plays a critical role in neuronal energetics, functions, and fate. In cancers and in the brain, it constitutes a clear cooperation leading to specific advantages for different cell populations. Cancer cells can also exploit stromal cells (including cancer-associated fibroblasts [CAFs] [4], stellate cells, endothelial cells [ECs], osteoclasts, macrophages, glial cells, and mesenchymal stem cells) in a form of commensalism (Box 1) [5]. The directionality of lactate exchange depends on the most convenient metabolic activity for cancer cells, which 'educate' the stroma accordingly. On the one hand, oxidative cancer cells usually abuse neighboring CAFs by forcing them to produce and release lactate and other nutrients, such as ketone bodies. This behavior can be achieved through mitochondrial poisoning following reactive oxygen species secretion. On the other hand, glycolytic cancer cells preferentially partner with more oxidative stromal cells (such as ECs and osteoblasts [6,7]), with the final objective to promote tumor development through processes including angiogenesis and osteolysis. To stimulate angiogenesis, lactate acts as a hypoxia mimetic that enters ECs and cancer cells through MCTs, is oxidized to pyruvate by LDH-1, and pyruvate acts as a competitor of 2-oxoglutarate to inactivate 2-oxoglutarate–dependent enzymes. These enzymes comprise prolyl-hydroxylases (PHDs), whose inhibition by lactate activates transcription factors HIFs (in ECs and cancer cells) and nuclear factor-κB (in ECs) independently of hypoxia. Consequently, lactate increases the production and secretion of vascular endothelial growth factor (VEGF) by cancer cells, which acts as a paracrine angiogenic factor on ECs; the expression of VEGF receptor-2 on ECs, which transduces the proangiogenic signal; and the production and secretion of basic fibroblast growth factor and interleukin-8 that both act in autocrine fashion on ECs. Lactate-sensitive enzymes also control collagen deposition, creating a track for EC migration [6]. Interestingly, proangiogenic hypoxia mimicry by lactate is shared by oncometabolites succinate, fumarate, and hydroxyglutarate that also compete with 2-oxoglutarate for specific enzymes [8].

Lactate further epigenetically affects several transcriptional responses. Two mechanisms have been reported so far: (i) a modulation of the activity of transcription factors, such as HIF-1 in ECs that activate their angiogenic program in response to lactate entry, and (ii) lactate-mediated histone modifications that include histone acetylation and lactylation. All of these events shape the plasticity of cancer cells during tumor progression and promote escape from the immune system.

Importantly, the transport of lactate across the plasma membrane is entrusted to MCTs (Box 2), which, together with LDHs,

Box 1. Monocarboxylate transporters

SLC16A1, *SLC16A3*, *SLC16A7*, and *SLC16A8* genes encode monocarboxylate transporters MCT1, MCT4, MCT2, and MCT3, respectively. MCTs are passive transporters that facilitate the proton-linked transport of lactate, short fatty acids, ketone bodies, and pyruvate along their concentration gradients. After dimerization, their activities on the plasma membrane require association with basigin or embigin glycoproteins. MCT1–4 differ in their substrate affinities, kinetics, tissue distribution, and histological localization. MCT1 is mainly expressed by respiratory tissues, including the heart, red skeletal muscle, and liver. MCT2 expression is restricted to the liver and kidney, where it modulates gluconeogenesis and lactate clearance, respectively. MCT3 is only expressed in retinal and choroid plexus epithelia, where it facilities lactate exchange. MCT4 has the lowest affinity among the group and is mainly involved in facilitating lactate efflux from glycolytic cells comprising white skeletal muscle fibers, astrocytes, and immune cells. MCT1, MCT2, and MCT4 play important roles in conveying lactate in cancer and ischemic diseases. Besides proton-coupled MCTs, other membrane transporters are able to escort lactate. Two members of a sodium-coupled MCT subfamily have been identified to date, *SLC5A8*/SMCT1 and *SLC5A12*/SMCT2, showing different affinity toward lactate but similarly expressed by epithelial cells. Due to their role in lactate shuttles, all MCTs are attractive pharmacological targets. In particular, MCT1 and MCT4 are druggable, with MCT1 inhibitors being currently tested in phase I/II clinical trials as antineoplastic and immunosuppressant drugs, whereas MCT4 inhibitors are still in the discovery phase.

are druggable targets. These passive symporters control lactic acid shuttling, depending on the gradient of lactate and protons across the plasma membrane. Accordingly, cells extruding lactic acid preferentially express MCT1 and/or (hypoxia-inducible and pyruvate-impermeable) MCT4 with a low affinity for lactate, whereas cells importing lactic acid preferentially express MCT1 and/or MCT2 with a higher affinity for lactate. In cancer cells, the upload of lactate/H^+ produces intracellular acidification, inhibiting glycolysis and shifting to the pentose phosphate pathway and NADPH production, favoring resistance to redox stress [9].

Lactate-based immunomodulation and signaling in cancer

Cancer cells have a deregulated metabolism that, together with a poorly functional vasculature, creates areas in the TME that are metabolite poor, hypoxic, and acidic. There, infiltrating cells enter in competition with cancer cells for appetizing metabolites (e.g., glucose) and alter their functions. Indeed, high levels of ambient lactate oppose lactate synthesis and release by T cells, which generates a low intracellular NAD^+/NADH ratio that is detrimental to NAD^+-dependent enzymatic reactions. Glyceraldehyde 3-phosphate dehydrogenase and 3-phosphoglycerate dehydrogenase are affected, depleting glycolytic intermediates and the serine pathway, thus specifically limiting effector T cell proliferation and function [10]. Conversely, regulatory T cells are less dependent on glucose/glycolysis and use 'alternative' metabolites to maintain their suppressive identity [11]. In malignant and nonmalignant ischemic tissues, environmental glucose limitation and lactate accumulation also corrupt the cytotoxic and antigen-presenting activities of natural killer and dendritic cells, respectively, by impairing their mitochondrial function and forcing them to undergo apoptosis. In this metabolic scenario, ECs release lactate, establishing a crosstalk with macrophages, where metabolic alterations are associated with transcriptional responses engaging an M0 ➔ M2 polarization, *de novo* angiogenesis, and the regeneration of ischemic tissues [12].

In diseases comprising cancers, lactate can further promote chronic inflammatory processes. In the arthritic synovium, for example, extracellular lactate that builds up following secretion by MCT4-expressing fibroblasts [13] acts as an entrapment signal for helper T cells via the Na^+-coupled lactate transporter SLC5A12. Upon activation, this system reduces the glycolytic flux and enhances fatty acid synthesis in T cells [14], resulting in their switch to a proinflammatory subset, such as Th1 cells and Th17 cells. Of note, bacteria-induced inflammation is further characterized by important lactate release from activated neutrophils, which enhances their mobilization by increasing bone marrow vascular permeability [15].

Lactate may be 'sensed' and signals at multiple subcellular localizations, including lysosomes, mitochondria, nucleus, and the plasma membrane. For example, lactate uptake activates mammalian target of rapamycin complex 1 in macrophages, resulting in a decreased lysosomal acidification associated with a decreased degradation of HIF-2α, leading to M2-like protumor gene activation [16]. Moreover, lactate drives Mg^{2+} mobilization into mitochondria, thereby supporting the phosphorylation and inactivation of pyruvate dehydrogenase, which represses the mitochondrial function [17]. Within the nucleus, lactate acts as an epigenetic modifier of histone lysines (histone lactylation), thus regulating a specific late-phase transcription of genes associated with wound healing and transforming growth factor-β–mediated pathways in M2-like macrophages [18].

Box 2. Cell populations within the tumor microenvironment

Cancer-associated fibroblasts (CAFs): These stromal cells contribute to cancer cell proliferation by providing nutrients and maintaining growth signals at primary and metastatic sites. They elicit a clear motility spur in cancer cells, activating the epithelial-to-mesenchymal transition and metabolic adaptations. CAFs also participate in therapy resistance and counter detachment-induced apoptosis in invading/metastatic cancer cells.

Cancer stem cells (CSCs): They support tumorigenesis through unique homing abilities to primary and metastatic sites, endowed with self-renewal and tumorigenic abilities when transplanted. CSCs are primary anticancer targets as they are resistant to chemotherapy and radiotherapy and are very likely at the origin of cancer metastasis and relapse.

Endothelial cells (ECs): Tumor-associated endothelial cells line the usually very leaky tumor vasculature, allowing transendothelial cancer cell migration at the onset of metastasis. For *de novo* angiogenesis, growing/hypoxic tumors are able to recruit ECs from the neighborhood or from circulating progenitor cells originating from the bone marrow. ECs are highly plastic. During tumor progression, they may undergo an endothelial-to-mesenchymal transition to transdifferentiate in CAFs.

M1 macrophages: They are activated/polarized by interferon-γ and lipopolysaccharide; produce proinflammatory cytokines, nitric oxide, and reactive oxygen species; phagocytize microbes; and initiate a proper immune response. They exert an antitumorigenic role.

M2 macrophages: They are activated/polarized by exposure to interleukin 4 or 10 and are associated with wound healing and tissue repair. They play immunosuppressive, proangiogenic, and protumorigenic functions.

Natural killer (NK) cells: These effector lymphocytes of the innate immune system mediate antiviral and antitumor responses. They are activated independently of antigen processing but are engaged in reciprocal relations with macrophages, T cells, and endothelial cells.

Tumor-infiltrating lymphocytes (TILs): These lymphocytes primarily comprise cytotoxic ($CD8^+$) and helper ($CD4^+$) T cells and a smaller proportion of B and NK cells. Their functions can dynamically change throughout tumor progression and in response to anticancer therapy. The presence of lymphocytes in tumors is often associated with a better clinical outcome, although this often depends on the particular cancer (sub)type.

Autocrine and paracrine extracellular lactate is also able to interact and activate intracellular signaling by triggering GPR81, a specific G protein–coupled receptor. Although downstream signaling events are currently poorly clarified, GPR81 activation has been reported in cancer, ECs, and immune cells and promotes cancer cell survival and proliferation, endothelial inflammation, and immune suppression [19].

General conclusions

Lactate is a multifaceted molecule acting as an energy source, modulating angiogenesis, the immune response, and signaling in disordered metabolic states associated with cancers and other pathologies (Figure 1). Although lactate-targeting therapies have been developed in oncology and for immune modulation, other approaches indirectly targeting lactate-dependent pathways are awaited to control the (un)conventional roles of lactate and overcome the 'fluctuating success' of pioneer drugs (i.e., MCT inhibitors). Given the role of lactate as a universal fuel, elucidating how this nutrient governs and is managed has the potential to reshape the current understanding of cell metabolism and disease treatment.

Acknowledgments

Related works at the authors' laboratories are supported by grants from Associazione Italiana Ricerca sul Cancro to P.C. (24731) and the French Community of Belgium (ARC 21/26-118), the Belgian National Fund for Scientific Research (F.R.S.-FNRS), the Fondation Belge contre le Cancer (Fundamental Research Grant FAF-F/2018/1282), the Télévie, and the Louvain Foundation (to P.S.). P.S. is an F.R.S.-FNRS Research Director. L.I. is Fondazione Umberto Veronesi fellow.

Declaration of interests

P.S. is inventor of patents/patent application families WO2016/075161, WO2020/221899, and EP21154636 related to therapeutic and diagnostic agents exploiting/targeting the oxidative pathway of lactate in cancer. He

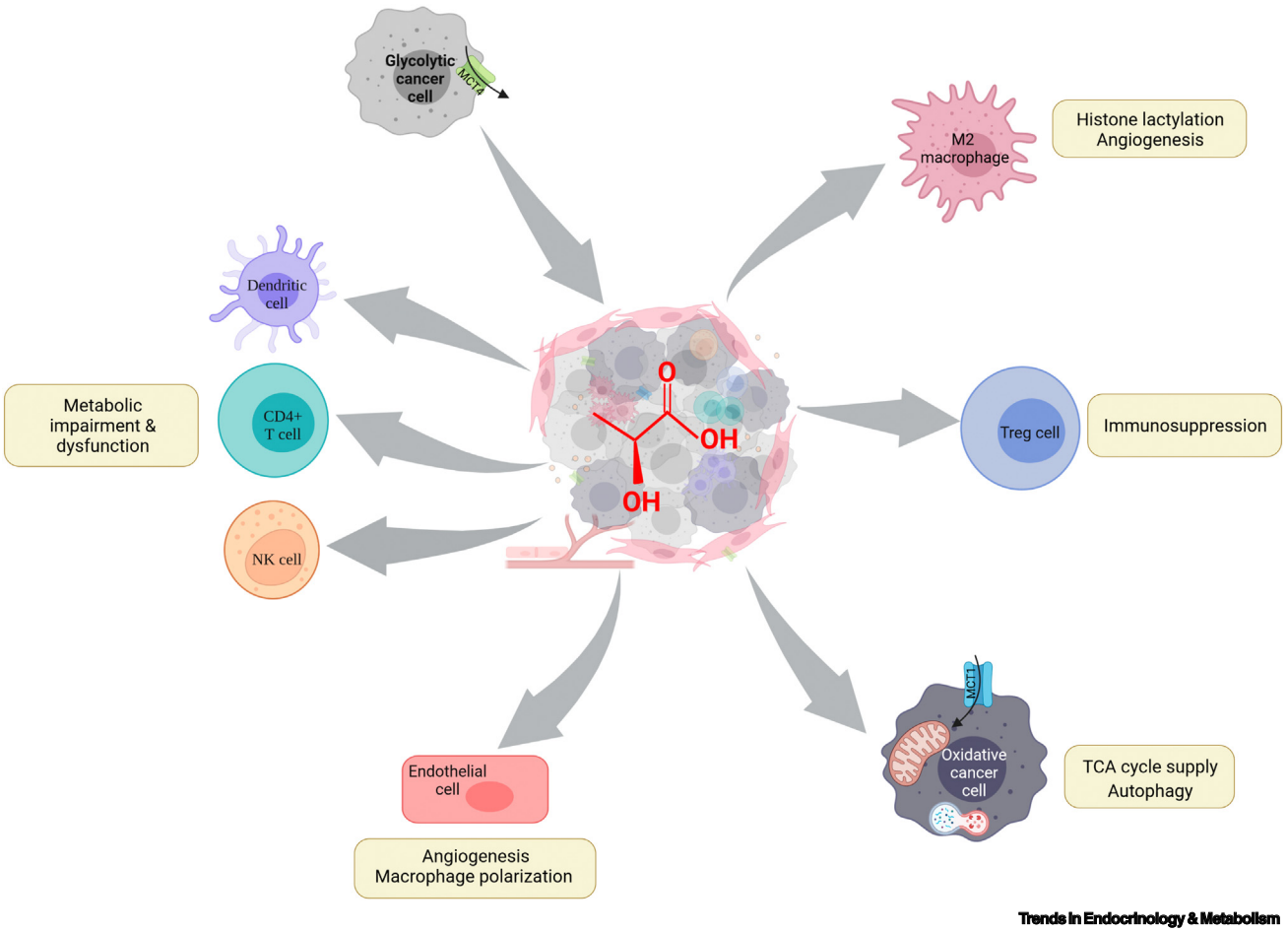

Figure 1. The impact of lactate on the tumor microenvironment. Lactate, highly abundant in the tumor environment due to the massive fermentation of glucose by cancer or cancer-forced stromal populations, has a multifunctional role in macrophages, endothelial cells, tumor-infiltrating lymphocytes, and cancer cells as a driver of metabolism, signaling, and differentiation. The main effects are included in the yellow boxes, and the *arrows* indicate the directionality of the lactate-induced action. Abbreviations: NK, natural killer; TCA, tricarboxylic acid; Treg, regulatory T cell. The figure was created using BioRender.com.

CellPress

is an independent consultant for Telix Pharmaceuticals Ltd. The other authors have no interests to declare.

[1]Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy
[2]Pole of Pharmacology and Therapeutics, Institut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain (UCLouvain), Brussels, Belgium
[3]Tuscany Tumor Institute (ITT) and Excellence Centre for Research, Transfer and High Education DenoTHE, Florence, Italy

*Correspondence:
paola.chiarugi@unifi.it (P. Chiarugi).

https://doi.org/10.1016/j.tem.2022.01.005

References

1. Sonveaux, P. *et al.* (2008) Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. *J. Clin. Invest.* 118, 3930–3942
2. Brisson, L. *et al.* (2016) Lactate dehydrogenase B controls lysosome activity and autophagy in cancer. *Cancer Cell* 30, 418–431
3. Hui, S. *et al.* (2017) Glucose feeds the TCA cycle via circulating lactate. *Nature* 551, 115–118
4. Ippolito, L. *et al.* (2019) Cancer-associated fibroblasts promote prostate cancer malignancy via metabolic rewiring and mitochondrial transfer. *Oncogene* 38, 5339–5355
5. Ippolito, L. *et al.* (2019) Lactate: a metabolic driver in the tumour landscape. *Trends Biochem. Sci.* 44, 153–166
6. Payen, V.L. *et al.* (2015) Common responses of tumors and wounds to hypoxia. *Cancer J.* 21, 75–87
7. Lemma, S. *et al.* (2017) MDA-MB-231 breast cancer cells fuel osteoclast metabolism and activity: A new rationale for the pathogenesis of osteolytic bone metastases. *Biochim. Biophys. Acta Mol. basis Dis.* 1863, 3254–3264
8. Beyoğlu, D. and Idle, J.R. (2021) Metabolic rewiring and the characterization of oncometabolites. *Cancers (Basel)* 13, 2900
9. Tasdogan, A. *et al.* (2020) Metabolic heterogeneity confers differences in melanoma metastatic potential. *Nature* 577, 115–120
10. Quinn, W.J. *et al.* (2020) Lactate limits T cell proliferation via the NAD(H) redox state. *Cell Rep.* 33, 108500
11. Watson, M.J. *et al.* (2021) Metabolic support of tumour-infiltrating regulatory T cells by lactic acid. *Nature* 591, 645–651
12. Zhang, J. *et al.* (2020) Endothelial lactate controls muscle regeneration from ischemia by inducing M2-like macrophage polarization. *Cell Metab.* 31, 1136–1153.e7
13. Fujii, W. *et al.* (2015) Monocarboxylate transporter 4, associated with the acidification of synovial fluid, is a novel therapeutic target for inflammatory arthritis. *Arthritis Rheumatol.* 67, 2888–2896
14. Pucino, V. *et al.* (2019) Lactate buildup at the site of chronic inflammation promotes disease by inducing $CD4^+$ T cell metabolic rewiring. *Cell Metab.* 30, 1055–1074.e8
15. Khatib-Massalha, E. *et al.* (2020) Lactate released by inflammatory bone marrow neutrophils induces their mobilization via endothelial GPR81 signaling. *Nat. Commun.* 11, 3547
16. Liu, N. *et al.* (2019) Lactate inhibits ATP6V0d2 expression in tumor-associated macrophages to promote HIF-2α-mediated tumor progression. *J. Clin. Invest.* 129, 631–646
17. Daw, C.C. *et al.* (2020) Lactate elicits ER-mitochondrial Mg^{2+} dynamics to integrate cellular metabolism. *Cell* 183, 474–489.e17
18. Zhang, D. *et al.* (2019) Metabolic regulation of gene expression by histone lactylation. *Nature* 574, 575–580
19. Brown, T.P. *et al.* (2020) The lactate receptor GPR81 promotes breast cancer growth via a paracrine mechanism involving antigen-presenting cells in the tumor microenvironment. *Oncogene* 39, 3292–3304