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Exploring the functional roles of small-molecule metabolites in disease research: Recent advancements in metabolomics

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ABSTRACT

Metabolism encompasses a series of intricate biochemical processes that are vital for the sustenance of life in organisms. Metabolomics, an essential scientific discipline, is a field of study within the broader domain of systems biology that focuses on the comprehensive analysis of small molecules, known as metabolites including lipids, coenzymes, *etc*., which are synthesized during metabolism. With the continuous development of metabolomics, the multiple biological functions of metabolites are constantly being discovered, encompassing signal transduction and enzyme stimulation, while concurrently exhibiting associations with afflictions like cancer and diabetes. The comprehension of metabolite functionalities and their intricate interplay with disease conditions assumes paramount importance in both disease-focused research endeavors and the development of diagnostic tools. This scholarly exposition undertakes an extensive review of recent advancements in the investigation of functional roles assumed by metabolites, with specific emphasis on metabolites in lipid synthesis, glucose metabolism and exogenous metabolites.

1. Introduction

Metabolism generally refers to a series of orderly chemical reactions that take place in organisms to maintain life [1]. These reaction processes enable organisms to grow and reproduce, maintain their metabolism, and respond to the external environment. During metabolism, a series of intermediate or final products termed metabolites are produced, including endogenous small molecules with a molecular weight of <1000 Da, such as lipids, coenzymes, amino acids [2–4].

The continuous development of life sciences calls for in-depth analysis of metabolites and their functions at various omics technologies levels. Compared with genomics [5], transcriptomics [6] and proteomics, metabolomics is directly involved in biological activities, reflecting the biological events that have already occurred, and the turnover rates of metabolites are much higher than proteins and DNAs [7]. Metabolites also interact closely with genes/proteins and the external environment, and as a result they can be used to probe the physiological or pathological states of biological systems [8–10]. Meanwhile, changes in gene and protein expression can be amplified at the metabolite level [11]. The advancement of high-throughput and high-sensitivity analytical methodologies, such as mass spectrometry (MS) [12,13] and nuclear magnetic resonance (NMR) [14,15], has propelled the exploration of metabolite detection on a systems level, thereby emerging as a pivotal avenue in the field of metabolism research. In comparison, mass spectrometry has higher sensitivity and a wider dynamic range, while NMR can provide more comprehensive structural information of the analyte. Therefore, metabolomics, the study of all metabolites from a cell, tissue, or organ, has emerged to be an important research field indispensable for integrated omics researches.

While the detection and concurrent detection of metabolites at the large scale has seen tremendous progresses, hundreds of metabolites can now be routinely analyzed by liquid chromatography (LC)-MS-based metabolomics platform, the comprehensive structure characterization of metabolites is still technically challenging, although the estimated number of small molecule metabolites is much smaller than proteins and genes [16]. Besides, as one of the four categories of biomolecules, lipids are structurally diverse and their number is estimated to exceed 40,000, of which a significant portion is lipid isomers, such as lipid C=C isomers, fatty acyl isomers, and sn isomers. The advancement of lipid analysis tools utilizing mass spectrometry (MS) has been limited by their inability to precisely resolve complex lipid structures. Consequently, scholars have sought to explore alternative methods for the analysis of intricate lipid structures. Among the various techniques, photochemical reactions have garnered significant atten-

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tion due to their ease of operation and capacity to yield highly specific diagnostic outcomes. Notably, Xia *et al.* have successfully incorporated the Paternò–Büchi (PB) reaction, a UV-induced photocycloaddition reaction that selectively targets C=C bonds, with tandem MS [17]. This innovative approach has enabled the localization and quantification of lipid C=C location isomers and has facilitated the analysis of lipidomic samples including tissue, blood and plasma [18–20].

With the continuous development of metabolomics techniques, more biological functions of metabolites have been revealed in life activities, such as signal transduction [21], enzyme stimulation [22] and the impact on cell structure [23]. Besides, the relationship between metabolites and various diseases including cancer, diabetes, Alzheimer's disease, *etc.* [24–26] have attracted increased research efforts. For instance, metabolic reprogramming has been revealed in various cancers, and through such a reprogramming the metabolic intermediates and end products can affect the tumor immune microenvironment, immune cell functions, cytokines secretion, to promote cancer growth or metastasis [27]. Particular subsets of cancer cells are sensitive to fatty acid metabolism and, especially fatty acid desaturation, which suggests that cancer cells may rely on reprogrammed lipid metabolism for their own growth advantage [28]. Some metabolites can form covalent bonds with proteins, which can mediate their post-translational modifications *via* glycosylation, methylation and acetylation to modulate protein functions and regulate various cellular biological processes [29,30]. Metabolites can also be used as substrates or coenzyme factors of chromatin modifying enzymes to for epigenetic regulation of cells [31,32].

This paper reviews the recent progress on the functional studies of metabolites in their regulation of life activities, with a special focus on lipids, small-molecule metabolites and exogenous metabolites. Meanwhile, the relationship between metabolites and diseases, such as cancers and diabetes, are summarized. Such knowledge will underlie the fundamental studies of disease pathology as well as form the basis for developing disease diagnostic tools.

2. Metabolites in lipid synthesis

Lipid metabolism is a critical physiological process, which is closely associated with the cell membrane remodeling, energy storage, hormone synthesis and signal transduction, *etc.* (Fig. 1a) [33,34]. Cholesterol, for example, is synthesized in liver and is an important component of cell membranes. It is also a precursor to a number of hormones, including testosterone, estrogen, and cortisol [35]. Other lipids such as phospholipids and sphingolipids also play critical roles, and dysregulation of lipid metabolism can have serious consequences for cellular health and can contribute to the development of a variety of diseases [36]. Lipid transport is also a crucial process that allows lipids to be transported throughout the body to the tissues and organs where they are needed. Lipids are transported in the bloodstream as lipoproteins, which are complexes of lipids and proteins, including chylomicrons, very low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs) (Fig. 1b) [37].

Imbalances in lipid metabolism can lead to a range of health problems, including dyslipidemia, obesity, type 2 diabetes, and cardiovascular disease. Diet is one of the key factors that can influence lipid metabolism and the risk of dyslipidemia. A diet high in saturated and trans fats can increase LDL cholesterol levels, which can contribute to the development of atherosclerosis and cardiovascular disease. On the other hand, a diet rich in unsaturated fats, such as those found in fish, nuts, and seeds, can help to lower LDL cholesterol levels and reduce the risk of cardiovascular disease [38–40].

2.1. Acetyl-CoA

Acetyl coenzyme A (Acetyl-CoA) is a molecule that plays a central role in metabolism that is involved in several metabolic pathways including the tricarboxylic acid cycle and fatty acid synthesis [41]. Acetyl-CoA can be formed through the breakdown of carbohydrates, fats, and proteins. For instance, glucose is broken down through glycolysis to form pyruvate, which is converted to Acetyl-CoA through a process called pyruvate oxidation [42]. Fatty acids are broken down through β-oxidation to form Acetyl-CoA [43]. In the breakdown of proteins, amino acids are converted to Acetyl-CoA through various metabolic pathways [44]. Once formed, Acetyl-CoA can enter the citric acid cycle, where it is used to generate energy in the form of ATP. It can also be used in fatty acid synthesis as the starting molecule. Overall, Acetyl-CoA is a critical molecule in metabolism, serving as a central hub for processing carbohydrates, fats, and proteins into energy and other important metabolic products [45].

Excessive Acetyl-CoA is often observed in cancer tissues, which indicates that its metabolic pathway is out of balance in cancer cells, thus promoting the proliferation of tumor cells [46]. Excessive Acetyl-CoA can be used for lipid synthesis to support cancer cell growth. In addition, Acetyl-CoA also participates in gene expression regulation through epigenetic modification (such as histone acetylation) [47]. Acetylation is a type of post-translational modification that involves acetyl addition to a lysine residue on proteins, to affect the function, stability, and localization of the protein modified [48,49]. Increased protein acetylation in cancer cells can have such effects as cell signaling, metabolism, and gene expression, which can promote tumor growth and survival. For example, protein acetylation can affect the activity of transcription factors that regulate gene expression, leading to the upregulation of genes that promote tumor growth. It can also affect the activity of enzymes involved in metabolism, leading to changes in the availability of nutrients and energy that can promote tumor growth [50]. As a result, the dysregulation of protein acetylation is associ-

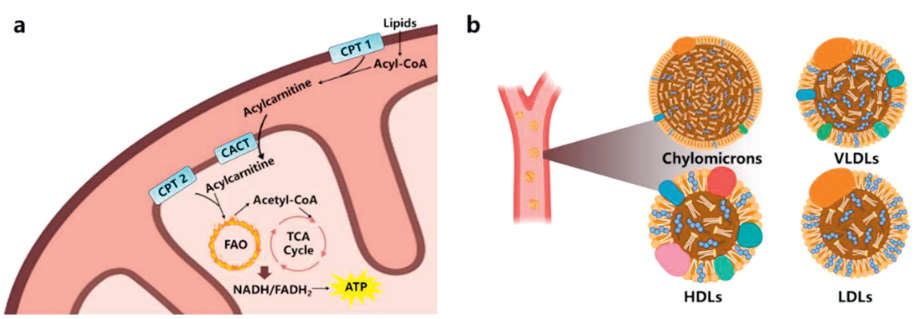

Fig. 1. (a) Lipids participate in the β-oxidation process and TCA cycle process, providing the energy required by the organism. (b) Lipids are transported in the form of lipoproteins (chylomicrons, VLDLs, HDLs, LDLs) in the blood. Created with BioRender.com.

ated with various types of cancer, including breast cancer, prostate cancer, and leukemia. Inhibition of protein acetylation can therefore a potential therapeutic strategy for cancer treatment [51,52]. HDAC inhibitors, such as Vorinostat and Romidepsin, are a class of drugs that have shown promise in cancer treatment by restoring proper acetylation levels and regulating gene expression [53].

Meanwhile, studies have shown that Acetyl-CoA can act as the central metabolic regulator of autophagy, and maintaining a high level of Acetyl-CoA can inhibit autophagy. Chronic increase of Acetyl-CoA caused by excessive calorie intake may inhibit autophagy, thus accelerating the performance of age-related diseases [54]. By contrast, diet and drug manipulation leading to Acetyl-CoA reduction may reduce weight and inhibit aging by stimulating autophagy. This result reveals a series of metabolic and pharmacological operations that can improve our health by targeting Acetyl-CoA to induce or inhibit autophagy [55]. The development of drugs that affect the metabolism of Acetyl-CoA is now accelerating, such as inhibitors of Acetyl-CoA consuming enzymes such as ACACs and KATs are used to treat metabolic syndrome. Inhibitors of Acetyl-CoA producing enzymes such as ACL are used as weight loss promoters or anticancer agents [56,57].

2.2. Fatty acids and phospholipids

Fatty acids are important intermediates of lipid metabolism [58]. On one hand, it can be further used to provide energy needed for life activities, on the other hand, it can also be used as a component of cell membrane and affect its fluidity and the function of cell membrane channels. At the same time, fatty acids can also be used as signal molecules involved in regulating gene transcription [59,60].

Fatty acids can be divided into four general categories: saturated, monounsaturated, polyunsaturated, and *trans* fats [61]. In saturated fatty acids, the carbon chain has the maximum number of hydrogen atoms attached to every carbon atom. If pairs of hydrogen atoms on adjacent carbon atoms are lost to form double bonds, it is called unsaturated fatty acids.

Unsaturated fatty acids play a crucial role in maintaining the fluidity of cell membranes. This is because they have double bonds in their carbon chains, which create a kink in their shape and prevent them from packing closely together. This makes the membrane more fluid and flexible, allowing for better membrane dynamics and cell function [62]. In contrast, saturated fatty acids have no double bonds and have a straight shape, allowing them to pack tightly together. This makes the membrane less fluid and more rigid, which can impede cell function. The balance between unsaturated and saturated fatty acids in the membrane is important for maintaining proper membrane fluidity.

Moreover, some studies suggest that fatty acids (FA) itself can also play a regulatory role as a signal molecule. For example, certain FAs such as omega-3 and omega-6 polyunsaturated fatty acids (PUFAs), can act as precursors to signaling molecules called eicosanoids, which are involved in modulating the activity of ion channels and receptors in cell membranes, thus affect the release of neurotransmitters and other signaling molecules, regulating inflammation, immune function, and other processes [63–65]. In ovarian cancer stem cells, high unsaturated fatty acids can activate NF-κB signal path, while the activation of NF-κB signal pathway up-regulates the expression of desaturase, which forms a positive feedback regulation to maintain cell stemness and drive the occurrence of ovarian cancer [66].

In recent years, the combination of photochemical derivatization and tandem mass spectrometry has made the identification of double bond sites in unsaturated lipids a hot topic. With the PB-MS/MS tools established [17], scholars attempted to evaluate their performance in the analysis of biomedical samples by utilizing their structural characterization and lipid isomer quantitation capabilities.

Zhang *et al.* [20] found that at the lipid C=C location isomer level, variations in lipid isomeric ratios were significantly lower and there was a clear difference in PE 34:1 C=C location isomer composition between normal and T2D plasma samples (Fig. 2a). The isomer-resolving lipidomic analysis can also serve as an effective tool to study cell heterogeneity. The relative amounts of C18:1 (Δ11) PCs are highly correlated with breast cancer cell invasiveness. A principal component analysis (PCA) of the six pairs of normal and cancerous human lung tissue samples was then performed using lipid C=C location isomers or *sn*-isomers. Results shows that the high structural specificity to lipidomic may be used in cancer treatment and drug development (Figs. 2b and c) [18]. By analyzing glycerol phospholipid compositions in breast cancer cell lines with structural specification extended to the C=C location level, Cheng *et al.* [67] also found that the indicators of C16:1 (n-9) ratio can be used to infer FAO activity and cancer cell invasiveness (Fig. 2d).

PB-MS/MS technology has also been used in the field of single cell analysis [19], Li *et al.* using glutaraldehyde for cell fixation and perform a lipid derivatization with 2-acetylpyridine as the PB reagent. To analyze trace amounts of lipids in single cells, an electromigration was used to drive a single cell into the tip of a nano-ESI capillary, applied an assistant solvent, and then applied a high voltage to initiate an on-demand nano-ESI-MS analysis. The results showed that C=C location isomer level correctly discriminates gefitinib-sensitive from gefitinib-resistant cells (Fig. 2e).

3. Small-molecule metabolites in glucose metabolism pathway

Glucose, an essential monosaccharide, acts as the primary source of energy for the cells in the human body. The metabolic pathways that glucose can follow after its consumption or synthesis in the organism are multifaceted and can lead to the generation of energy or other biomolecules that play a critical role in cellular function [68,69]. The process of glucose metabolism encompasses a sequence of biochemical reactions that transpire within cells to convert glucose into usable energy, thus enabling cells to perform diverse biological activities. As a tightly regulated process, glucose metabolism plays a vital role in maintaining cellular homeostasis and supplying the body with energy. Disruptions to this intricate metabolic process can give rise to a spectrum of medical conditions, such as metabolic disorders, diabetes, and cancer, with significant implications for human health [70].

3.1. α-KG

Alpha-ketoglutarate (α-KG) is a key intermediate in the tricarboxylic acid cycle which contains a series of biochemical reactions that occur in the mitochondria of cells and is responsible for generating energy in the form of ATP [71]. It is a molecule that plays an important role in several metabolic pathways in the body.

In addition to its role in energy metabolism, α-KG also serves as a co-substrate needed for the function of a family of enzymes called α-KG/Fe(II)-dependent dioxygenases. This enzyme family catalyzes a diverse range of oxidation reactions in proteins, DNA, RNA and lipids. In these reactions, α-KG binds to the active site of the enzyme to aid catalysis. One such enzyme is lysine histone demethylase (KDM), which modifies chromatin — the complex of DNA and proteins of which chromosomes are made. Thus, α-KG plays a key role in maintaining the stability of genome and signal pathway in normal tissues and cells [72]. However, 2-HG, succinate and fumarate can compete with α–KG for binding to this catalytic site and thus inhibit these enzymes [73,74].

In the metabolism of amino acids especially glutamine, α-KG also plays an important role. Glutamine is an important amino

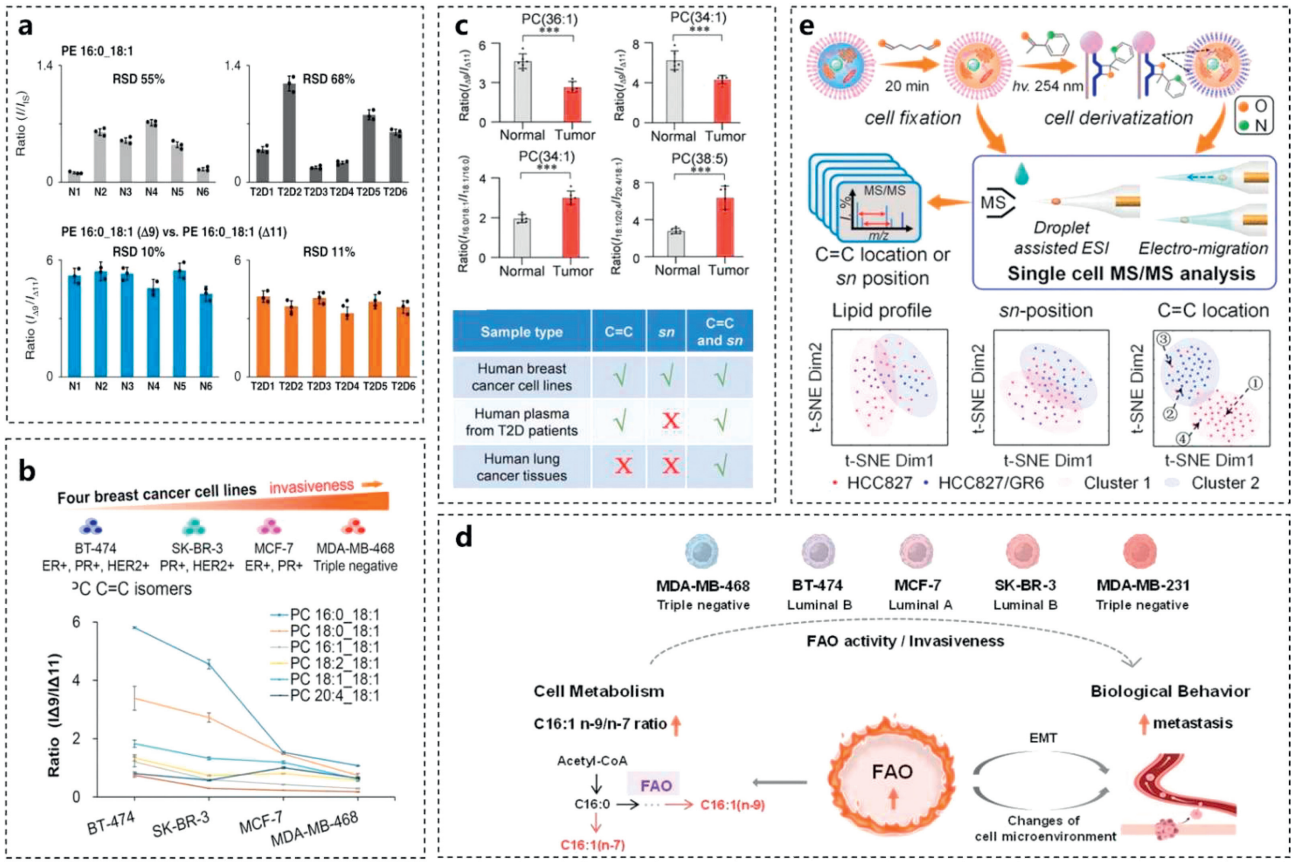

Fig. 2. PB-MS/MS technology has been used in the field of comprehensive mass spectrometric analysis of unsaturated phospholipid isomers in (a) plasma samples. Copied with permission [20]. Copyright 2019, Springer Nature Limited. (b, d) Breast cancer cell lines. Copied with permission [18]. Copyright 2020, Springer Nature Limited. Copied with permission [67]. Copyright 2023, The American Association for the Advancement of Science. (c) Lung cancer samples. Copied with permission [18]. Copyright 2020, Springer Nature Limited. (e) Single cell. Copied with permission [19]. Copyright 2021, Springer Nature Limited.

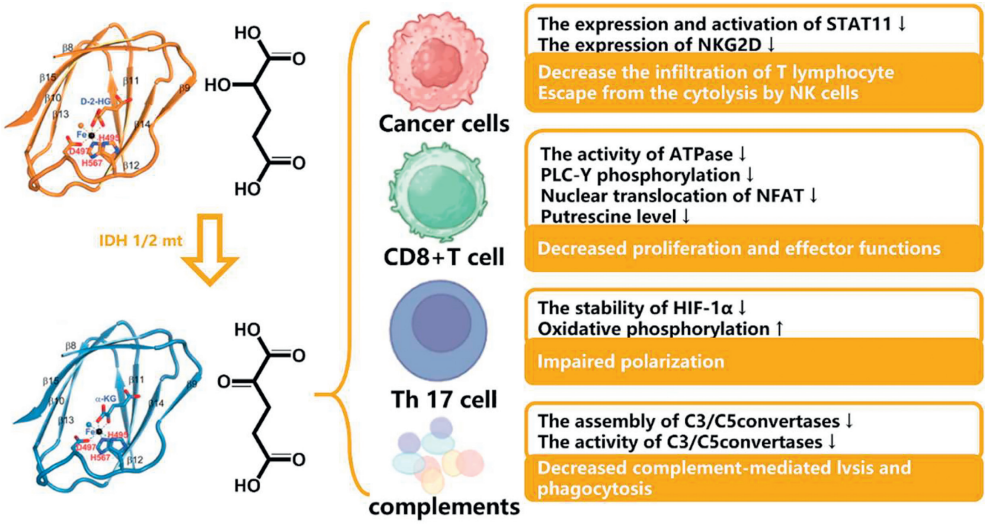

Fig. 3. D-2HG, derived from mutant IDH, serves as an immunosuppressive metabolite. Reproduced with permission [89]. Copyright 2011, Elsevier Inc. Reproduced with permission [91]. Copyright 2021, Frontiers Media S.A.

acid that is involved in several metabolic processes in the body, including protein synthesis, energy production, and immune function. In the metabolism of glutamine, α-KG acts as a receptor for the amino group of glutamines, forming glutamate in the process. Glutamate can then be further metabolized to produce α-KG again, completing the cycle [75]. This cycle is known as the glutamine-glutamate cycle and is important for maintaining the balance of nitrogen in the body [76,77]. In addition to its role in the metabolism of glutamine, α-KG is also involved in the metabolism of other amino acids, acting as a precursor for the synthesis of several amino acids, including proline and arginine, which are important for protein synthesis and other metabolic processes [78,79].

According to the above, α-KG is not only a simple metabolic intermediate, but has an important impact on the cell's epige-

netic level. It has been a subject of considerable interest due to its potential health benefits and is commonly employed as a dietary supplement and purported to exert a range of physiological effects, such as enhancing exercise performance, mitigating fatigue, and improving cognitive function [80,81]. Nevertheless, the mechanisms underlying these purported benefits remain poorly understood, and further investigation is needed to elucidate the full spectrum of α-KG's impact on human health.

3.2. 2-HG

2-Hydroxyglutarate (2-HG) is a molecule that shares structural similarities with α-Ketoglutarate (α-KG). In the tricarboxylic acid cycle, isocitrate dehydrogenase (IDH) is responsible for the oxidative decarboxylation of isocitrate to produce α-ketoglutaric acid. However, recent studies have demonstrated that mutations in the IDH gene are prevalent in certain clinical tumor samples [82]. These mutant IDH enzymes catalyze the production of a new metabolite, 2-HG. Accumulating evidence has revealed that 2-HG exerts profound effects on cellular differentiation and is implicated in promoting tumorigenesis, growth and invasion. While normal organisms can produce small amounts of 2-HG, they are efficiently metabolized, thus only trace amounts can be detected [83–86]. However, the production rate of 2-HG by mutant IDH exceeds the clearance rate, leading to a significant accumulation of 2-HG in IDH1 mutant glioma.

In certain types of cancer, including gliomas and leukemia, high levels of 2-HG can interfere with the normal function of enzymes and lead to changes in gene expression and cellular behavior, potentially contributing to the development and progression of cancer [87]. Notarangelo *et al.* demonstrated that elevated concentrations of D-2HG could directly inhibit lactate dehydrogenase in T cells of mice. The lactate dehydrogenase inhibition has been found to modulate glucose metabolism in T cells, thereby reducing their proliferative capacity, cytokine secretion, and cytotoxicity towards target cells. This observation by the authors posits that −2HG, a known inducer of cell-autonomous cancer progression, could potentially exhibit immunosuppressive features [88]. It has also been demonstrated that 2-HG is a competitive inhibitor of multiple α-KG-dependent dioxygenases, by occupying the same space as α-KG does in the active site of histone demethylases, thereby leading to alterations in genome-wide histone and DNA methylation [89]. Because of its role in cancer, 2-HG has become a potential target for cancer treatment [90,91], and the inhibition of the production or activity of 2-HG in cancer cells should slow down or prevent cancer growth (Fig. 3).

3.3. Lactate

Lactate, a crucial metabolite, is synthesized by the body *via* anaerobic metabolism as a result of glucose degradation in the absence of oxygen [92]. Otto Warburg's research has demonstrated that neoplastic cells exhibit a heightened demand for glucose and produce significant quantities of lactate, even under aerobic conditions, which has been termed the "Warburg effect" or "aerobic glycolysis" [93]. As a result of this metabolic phenomenon, the extracellular pH in the tumor microenvironment is lowered to a range of 6.0–6.5 due to the accumulation of lactate [94]. According to recent research, lactic acid has become an important regulator of tumor and immune cells in microenvironment. Specifically, it is be-

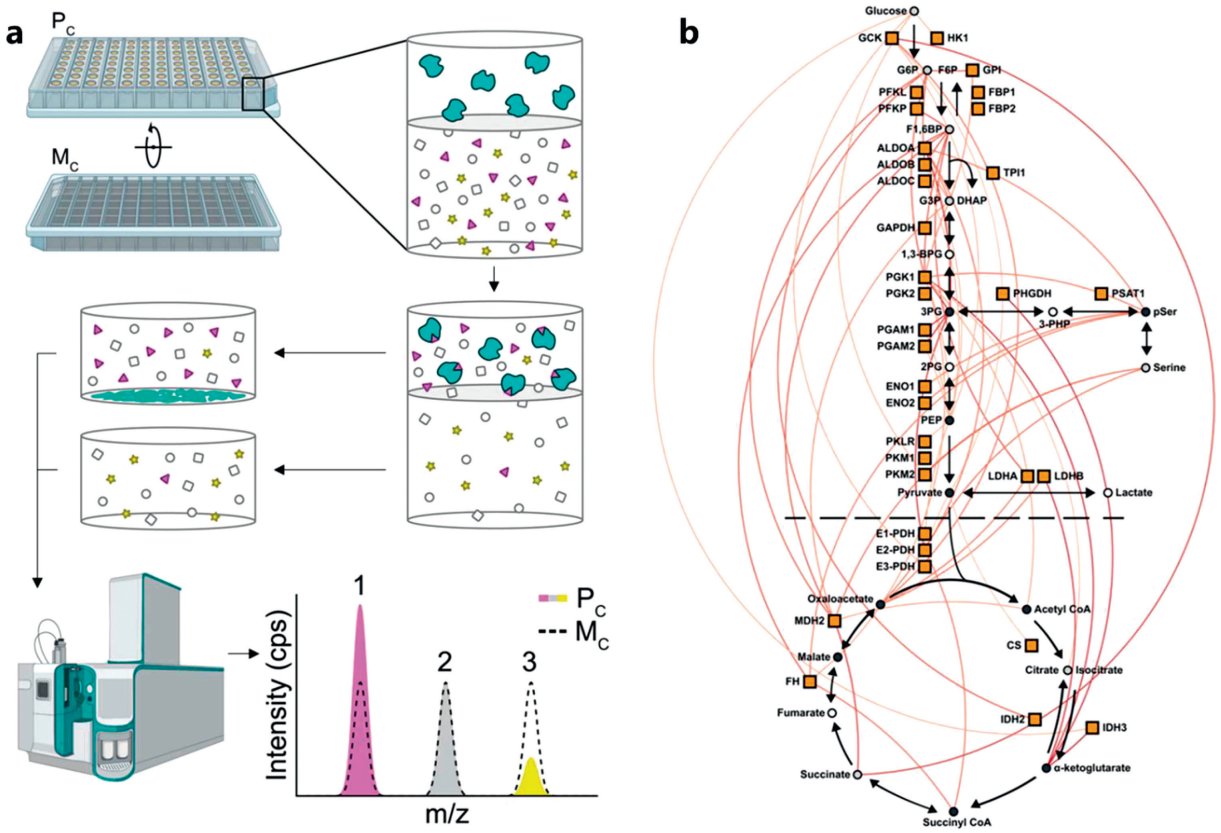

Fig. 4. (a) The MIDAS platform is an equilibrium dialysis tandem FIA-MS approach. Purified proteins (cyan) and defined pools of metabolites are loaded into separate chambers (Pc and Mc) divided by a dialysis membrane. After incubation to equilibrium, metabolite abundance is quantified using FIA-MS. Abundance differences in Pc and Mc indicate protein-metabolite interactions. (b) Significant interpathway interactions (colored lines) between metabolites (circles) and 33 enzymes in human carbohydrate metabolism (orange boxes). Reproduced with permission [99]. Copyright 2023, The American Association for the Advancement of Science.

lieved to promote the basic biological processes of tumor growth, including invasion, metastasis and angiogenesis [95]. This indicates that lactic acid is not only a by-product of tumor cell metabolic waste, but also a key molecule involved in carcinogenesis and tumor immune escape.

On one hand, lactate has been observed to function as a signaling molecule, which activates particular signaling pathways in cells and plays an immunosuppressive role by stimulating the production of immunosuppressive cells and molecules. For example, lactate has been shown to activate the G-protein-coupled receptor GPR81 in cancer cells, leading to the activation of the cAMP/PKA signaling pathway and increased cell proliferation [96,97]. Additionally, lactate has been found to activate the hypoxia-inducible factor 1 (HIF-1) pathway, which regulates the expression of genes involved in angiogenesis, glucose metabolism, and cell survival [98].

Lactate can also regulate the activity of specific enzymes involved in cellular signaling or directly stimulate gene transcription

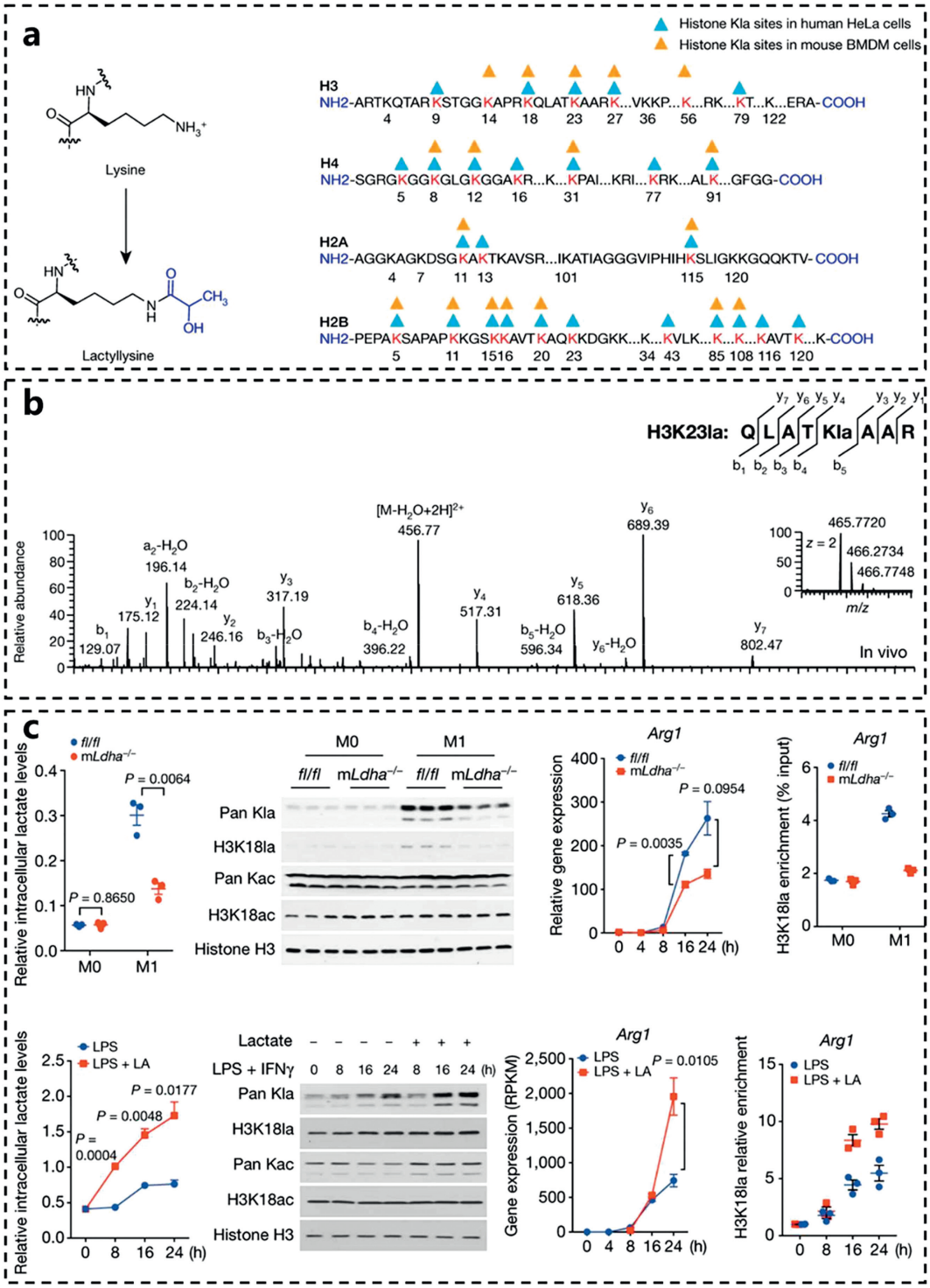

Fig. 5. (a) Illustration of histone Kla sites identified in human and mouse cells. (b) MS/MS spectra of a lactylated histone peptide (H3K23la). (c) Lactate activates M2-like gene expression through histone Kla. Reproduced with permission [100]. Copyright 2019, Springer Nature Limited.

from chromatin through lactate-derived lysine lactylation (Kla). To gain insights into the impact of metabolic state on cellular processes, a comprehensive examination of the low-affinity interactions between metabolites and proteins is imperative. Hicks *et al.* [99] introduced a rigorous approach known as MIDAS (mass spectrometry integrated with equilibrium dialysis for the discovery of allostery systematically) to enable investigation of such interactions across 33 enzymes associated with human carbohydrate metabolism and over 400 metabolites, revealing the regulation of lactate dehydrogenase (Fig. 4).

Zhao *et al.* [100] identified 28 lactylation sites on core histones in human and mouse cells and found that histone lactylation has different temporal dynamics from acetylation. For instance, In the late phase of M1 macrophage polarization, increased histone lactylation induces homeostatic genes that are involved in wound healing, including Arg1, thus representing an opportunity to improve our understanding of the functions of lactate and its role in diverse pathophysiological conditions, including infection and cancer (Fig. 5).

Numerous unanswered queries necessitate further investigation in the subject of lactate [101]. By delving into these questions and exploring other avenues related to lactic acid metabolism, it is possible to obtain a more comprehensive understanding of the fundamental physiological mechanisms that govern energy production and metabolism within the human body.

4. Exogenous metabolites

Exogenous metabolites mainly refer to external molecules obtained from sources such as food, medication or gut microbiota, which play a crucial role in the body's metabolism and have a profound impact on overall health [102]. Among them the metabolites produced by gut microbiota have recently gained significant attention [103]. Intestinal microbiota refers to a diverse population of microorganisms that reside in the human digestive tract, including bacteria, viruses, fungi, and other single-celled organisms, which are essential in maintaining human health. Metabolites produced by gut microbiota are small molecules formed through the metabolism of nutrients and other substances in the small intestine. These metabolites consist of short chain fatty acids (SCFA) [104], bile acids [105], indole derivatives [106] and other compounds (Fig. 6).

Recent years have seen a surge in interest in intestinal microbiota and its metabolites due to the increasing awareness of their role in human health. Studies have revealed that imbalances in the intestinal microbiota and changes in its metabolites are linked to various health conditions, including obesity, type 2 diabetes, inflammatory bowel disease and other mental health disorders [107,108].

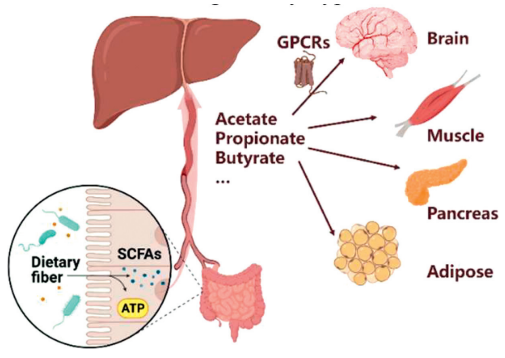

Fig. 6. The short chain fatty acids (acetate, propionate and butyrate) produced by anaerobic fermentation of dietary fiber in the colon play a crucial role in maintaining intestinal health and life activities.

4.1. SCFA

SCFA, in particular, has gained widespread recognition as one of the most prominent metabolites. It is mainly produced through the fermentation of dietary fiber and resistant starch by specific colonic anaerobes, primarily comprising acetic acid, propionic acid, and butyric acid. SCFAs play a pivotal role in maintaining intestinal health and participate in numerous physiological processes throughout the body. The majority of SCFAs produced in the gut are absorbed into the bloodstream and transported to the liver, where they are metabolized and used for energy production [109]. In addition to their role in energy production, SCFAs have been shown to regulate numerous physiological processes, including immune function, inflammation, and metabolism [110].

G-protein-coupled receptors (GPCRs) constitute a substantial family of membrane-bound receptors that are fundamental in mediating signal transduction. These receptors are present on the surface of a diverse range of cell types and are involved in various physiological processes, including vision, olfaction, gustation, hormonal regulation, synaptic transmission, and immunological response [111]. Research indicates that short-chain fatty acids (SCFAs) can activate three distinct GPCRs, namely GPR 41, GPR 43 and GPCR 109A, each of which can elicit disparate effects contingent on the cell type and the specific receptor activated. For instance, SCFA-induced activation of GPR 41 and GPR 43 can prompt the secretion of hormones that modulate energy balance and appetite [112], while activation of GPCR 109A by butyrate can exhibit anticancer properties [113].

4.2. Other exogenous metabolites

In addition to SCFAs, bile acids produced by lactobacillus can Absorb dietary fats and lipid-soluble vitamins, facilitate lipid absorption, signal systemic endocrine functions to regulate triglycerides, cholesterol, glucose and energy homeostasis [114]. *E. coli* can increase expression of anti-inflammatory genes and strengthen epithelial cell barrier properties by Indole derivatives [115]. Intestinal microorganisms are diverse and complex. They form a balanced micro-ecosystem with host and environmental factors. Understanding the complex interactions between the gut microbiota and their effects on host physiology, is an important area of research that has the potential to inform the development of new therapeutic strategies for a wide range of diseases.

5. Conclusion

Metabolites, as products of metabolic activities, have been demonstrated to be fundamental to the proper functioning of organisms, impacting a broad range of biological processes in diverse and complex ways. Metabolites are not only by-products, but also have important effects on various physiological systems [116]. Specifically, metabolites act as basic precursors for the synthesis of many biological macromolecules, including proteins and nucleic acids, which are vital for numerous cellular functions [117]. Moreover, metabolites function as signaling molecules, facilitating the transmission of information within and between cells, thereby regulating cellular activities such as metabolism, growth, and differentiation [118]. Metabolites also play a significant role in the regulation of gene expression through epigenetic modifications that modify the structure and function of DNA and associated proteins [119]. In addition to their signaling and structural functions, metabolites are critical for regulating enzyme activity by serving as cofactors or inhibitors, thereby modulating the rates of metabolic reactions.

Overall, the importance of metabolites in maintaining biological processes and proper functioning of organisms cannot be over-

stated. Without metabolites, the body would not be able to generate energy, synthesize important molecules, or regulate metabolic pathways effectively. Therefore, understanding the role of metabolites in biological processes is essential for advancing our knowledge of physiology and developing effective treatments for diseases.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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