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The crosstalk between epigenetics and metabolism in the malignant cell

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Abstract

Metabolic-epigenetic coupling is recognized as a central hallmark of malignant transformation, characterized by dynamic interplay between cellular metabolism and epigenetic regulation. Cancer cells undergo metabolic reprogramming to meet new demands due to increased biosynthesis and shifted bioenergetics. These changes are responsible for the production of metabolites that are used as substrates or cofactors for key enzymes involved in epigenetic regulation. Thereby, the chromatin structure and gene expression suffer critical changes. Epigenetic modifications including DNA methylation, histone acetylation or non-coding RNA activity, are regulating the transcription of metabolic genes, reshaping the tumor metabolic phenotype. The bidirectional crosstalk between epigenetics and metabolism drives key cancer hallmarks such as proliferation, immune evasion, and drug resistance. Recent discoveries in multi-omics, single-cell sequencing and spatial transcriptomics have opened a new era of high-resolution mapping of metabolic and epigenetic alterations, revealing the high heterogeneity in tumor cells and highlight novel therapeutic targets. In this review we aim to summarize the latest approaches in technological advances in integrated metabolomics and epigenomics, to highlight the critical crosstalk between metabolism and epigenetic changes, and to underscore the need of a deep understanding of these interactions for integrating the multi-omics approach in precision oncology.

Keywords Metabolic reprogramming, Epigenetics, Methylation, Interdisciplinary approach, Precision oncology

1 The role of metabolism in cancer development—hallmarks and pathways

Cancer is the second leading cause of death in the United States of America and in Europe, with the Lung and Breast cancer having the highest incidence in population [1, 2]. For 2025, over 20 million new cases were estimated, with an increase compared to 2022 where around 20 million new cases of cancer were reported. However, there is a significant disparity between high-income countries and low-income countries, in terms of diagnosis in early and late stages of tumor development. Low-income countries are experiencing a diagnosis mostly in late stages of cancer [3]. According to Bizuayehu et

al., the projection for 2050, based on a cross-sectioned study on 36 cancer types and with data collected from 185 countries, is indicating that cancer cases are expected to rise by 77% and the number of deaths associated with cancer by 90% [4].

In order to mitigate the rising global burden of cancer predicted for 2050, the research focus should lean more towards a better understanding of the mechanisms involved in the initiation and progression of cancer, like the epigenetic (DNA methylation, histone acetylation, chromatin remodeling), and genetic (point mutations, copy number alterations, gene fusions) dysregulations of the tumor, and also the cross-talk with its metabolic profile. Certain genetic alterations disrupt oncogenes and tumor suppressor genes, while epigenetic events can influence gene silencing or activation, without altering the DNA sequence, affecting large chromatin domains and promoting resistance to therapy or enhance the metastatic potential [5–7].

Cancer cell metabolism has specific features, as tumor cells are set to adapt to different physiological conditions. Increased glycolysis—Warburg effect, altered amino acid and lipid metabolism, or the oncometabolite accumulation—are all linked to the genetic and epigenetic alterations, defining the complexity in cancer. The upregulation or downregulation of genes encoding metabolic enzymes promoting metabolic changes that may alter certain pathways leading to oncometabolite accumulation that will disrupt epigenome and gene expression [8].

The Warburg effect was established by Otto Warburg, and it defines the fact that cancer cells are using aerobic glycolysis instead of oxidative phosphorylation, thus the tumor cells are avoiding immune destruction and display a deregulated cellular metabolism, both being considered hallmarks of cancer and are related to Warburg effect [9]. Metabolic reprogramming is characterized by deregulated glucose and amino acids consumption, or glycolysis/ tricarboxylic acid cycle intermediates used in different biosynthetic pathways. Metabolic reprograming is essential for tumor cells, as they adapt for unfavorable conditions in tumor microenvironment (TME) such as low concentrations of oxygen, increased oxidative stress, low pH and lack of essential nutrients. Metabolic adaptation is a distinguishing characteristic of tumor cells, and it is considered as a hallmark of tumorigenesis and tumor progression, being influenced by multiple factors [10]. Tumorigenesis is largely driven by the shifting carbohydrate metabolism, characterized by three distinct metabolic phenotypes: aerobic glycolysis, oxidative phosphorylation (OXPHOS), and a hybrid version of the two [11]. The aerobic glycolysis is characterized by a high glucose uptake and conversion into lactate, to ensure rapid cell proliferation, while the OXPHOS phenotype is based on the optimization of energy efficiency via mitochondrial oxygen consumption. The third state includes features from both previously mentioned phenotypes, displaying the highest plasticity. The three subtypes are shifting according to external factors and cell states, ultimately affecting amino acid and lipid metabolism, by altering their physiological patterns [12].

The glycolysis and TCA cycle alterations are related, as in tumor cells the glycolysis is upregulated, and TCA cycle suffers alterations. During glycolysis the ATP is generated in high concentrations to maintain proliferation. Thus, pyruvate is converted into lactate, thus it is not entering the TCA cycle. Lactate is an important product for tumor invasion, metastasis and angiogenesis, has immunosuppressive functions and promotes tumor development and escape, as it stimulates the expression of HIF-1α and VEGF [13]. Furthermore, mitochondria play an important role in providing essential metabolites for

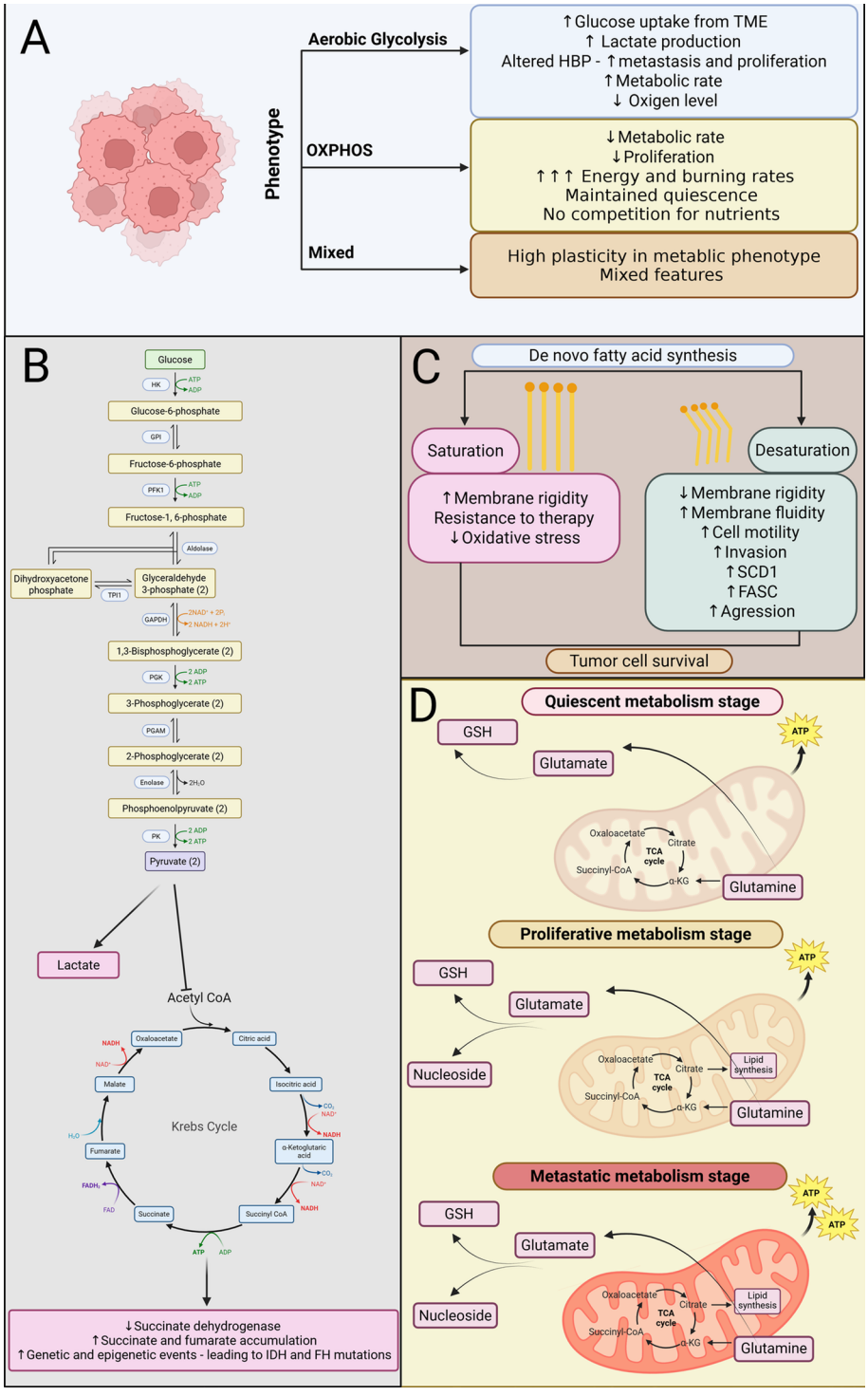

Fig. 1 (See legend on next page.)

generating oncometabolites. TCA cycle alterations are frequently observed in tumor cells. TCA intermediate metabolites can promote tumor cells growth, such as fumarate and citrate that can induce epigenetic or genetic alterations in enzymes that control TCA (e.g. isocitrate dehydrogenase -IDH or fumarate hydratase – FH).

(See figure on previous page.)

Fig. 1 Metabolic alterations in tumor cells. **A** Tumor cells phenotype in terms of glucose metabolism—the cells can undergo three pathways, the aerobic glycolysis with high glucose uptake from TME, high amount of lactate low levels of oxygen and high metabolic rates and increased proliferation; OXPHOS with low metabolic rate, low proliferative rates, high energy and high burning rates, with no competition for nutrients; and mixed phenotype with high plasticity in metabolic phenotype and mixed features from the previous two states. **B** The Krebs Cycle where the tumor cells are characterized by high levels of succinate and fumarate, multiple genetic and epigenetic events leading to IDH and FH mutations. **C** De novo fatty acid synthesis—the fatty acids becoming saturated leading to membrane rigidity, resistance to therapy, and oxidative stress; while the desaturation of the fatty acids leads to less rigid membranes, with high fluidity, increased cell motility and increased chances of invasion and more aggressive cells. **D** Mitochondrial dysfunctions–the cells can shift from quiescent metabolism with GSH production and ATP, to proliferative stage with increased glutamate production, GSH and nucleosides and ATP, and to a metastatic metabolic stage where high levels of ATP are produced, together with increased amounts of glutamate, GSH and nucleosides for accelerated cell division. Figure designed with Biorender

Lipid metabolism is another key player, as a key hallmark of cancer is represented by the de novo fatty acid synthesis, which serves as an important macromolecule provider for membrane stability, cell signaling molecules and energy supply. Membrane rigidity is critical in resistance to chemotherapy, protects the tumor cell from oxidative stress and promotes survival, all because of lipid saturation. On the other hand, lipid desaturation is used by the tumor cell to increase the membrane permeability, to decrease rigidity and to promote migration. Stearoyl CoA desaturase 1 (SCD1) is a key player in this balance, together with Fatty acid synthase (FASN), both being potential targets to inhibit fatty acid synthesis and membrane desaturation [14].

Amino acid metabolism alterations are crucial in cancer cells, as tumor cells relay on glutamine to maintain rapid growth and proliferation. Glutamine is the building block for proteins, nucleotides and other molecules needed for division. Also, it serves as a substrate in TCA for generating ATP. Tumor cells uptake high amounts of glutamine and metabolize it to α-Ketoglutarate influencing the TCA. Moreover, other amino acids are used by the tumor cells to promote survival and to evade immune surveillance. The altered tryptophane metabolism promotes immunosuppression and immune evasion. Leucine, isoleucine and valine support proliferation. All these metabolism alterations can be targeted, using inhibitors that target key enzymes in the amino acid pathways [15, 16].

All these alterations have been reported to be essential for tumor growth, proliferation and survival, and are depicted in Fig. 1.

The aim of the review is to present in a comprehensive manner the role of metabolism in cancer cell development, how metabolic changes can promote tumorigenesis and increase the risk of developing diverse malignancies, how metabolic reprograming can induce resistance to therapy, and how the metabolism could be targeted to change the biology of the cancer cell and for the development of novel therapeutical approaches.

2 DNA methylation and epigenetic regulation in cancer

The term of epigenetics describes the process by which cell's phenotype suffers modifications that can be passed down across generations without changes in the DNA sequence. Genetic information is decoded to enable cellular functions by a well-controlled multistep process that begins with the DNA-RNA transcription [17, 18]. Epigenetic modifications have critical role in fine-tuning gene expression, as the changes induced can modulate biological processes such as cell differentiation and embryogenesis. However, in cancer, the epigenetic changes drive transcriptomic heterogeneity and promote tumor cell growth, resistance to therapy and help tumor cells to evade immune surveillance

[19]. DNA methylation is the biological process characterized by the covalent addition of methyl group to the cytosine at carbon 5-atom position, process catalyzed by DNA methyltransferases (DNMT) [20, 21]. As depicted in Fig. 2, de novo DNA methylation is controlled by DNMT3A and DNMT3B, then, after methylation, DNMT1 maintains methylation status. However, the methylation is reversible, and demethylation can undergo passive process or active demethylation, via TET catalysis which converts 5-methylCytosine (5-mC) into 5-hydroxymethylcytosine (5-hmC), then 5-formylcytosine which can be next converted into 5-carboxylcytosine and then back to unmethylated cytosine, or the conversion into 5-carboxylcytosine (5-caC) can be skipped and 5-formylcytosine (5-fC) can be directly converted into unmethylated cytosine [21]. DNA methylation occurs in regions rich in Cytosine and Guanine, named CpG Islands (CGIs), sequences usually located in the promoter regions and display a hypomethylated status to maintain the chromatin available for transcription. In the case of tumor cells, the methylation patterns are changed, as hypermethylation is observed in promoter regions of specific genes, while the global DNA is hypomethylated. This leads to gene silencing, as hypermethylated tumor suppressor genes promoters trigger chromatin compaction, which can indirectly cause genetic instability. The methylation of specific CpG dinucleotides could provide a fine modulation of transcription and determine patterns specific to diseases, as it could silence specific genes or make others available for transcription [22, 23].

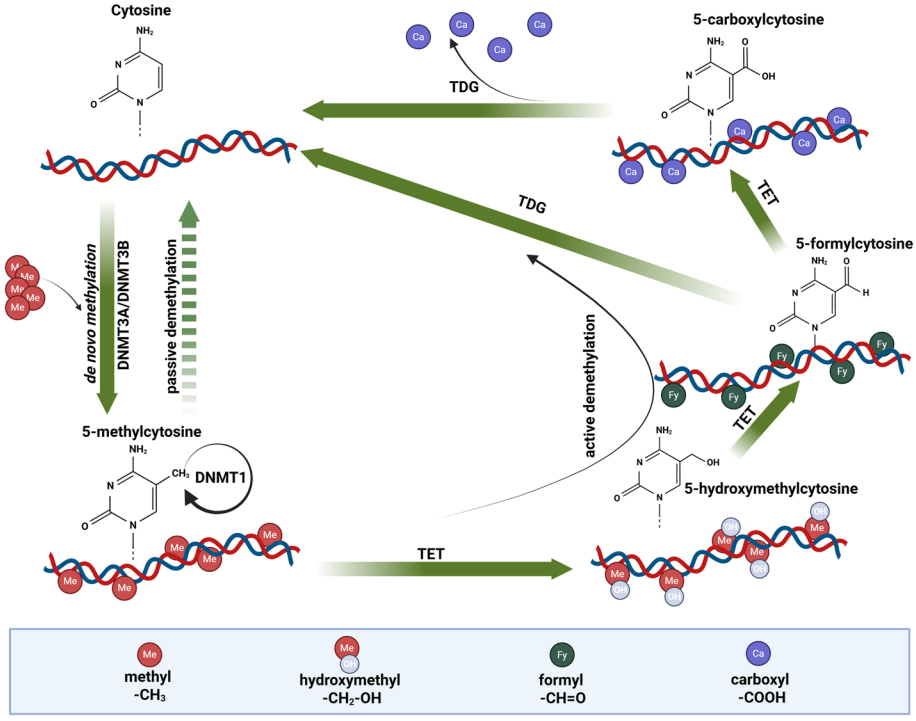

Fig. 2 DNA methylation pathway. The steps required for DNA methylation and demethylation require several enzymes to catalyze the bioprocesses. Cytosine is converted into 5-methylcytosine de novo methylation via methyl addition in carbon-5 position by DNMT3A and DNMT3B. The maintenance of the methylation status is controlled by DNMT1. A passive demethylation can occur at this stage, while active demethylation is controlled by TET enzymes that convert 5-methylcytosine into 5-hydroxymethylcytosine then into 5-formylcytosine. 5-formylcytosine can be directly converted into cytosine by TDG, or it can undergo an additional step controlled by TET and become 5-carboxylcytosine. Then catalyzed by TDG, 5-carboxylcytosine will be converted into cytosine and the methylation can start again. Figure designed with Biorender

Global hypomethylation and focal hypermethylation are two epigenetic alterations specific for tumor cells with distinct significance, particularly in the context of using methylation as a biomarker for cancer diagnosis and progression. DNMTs use S-adenosylmethionine (SAM) as substrate and due to their activity, they orchestrate the methylation pattern in the DNA. DNMT1, DNMT3A and DNMT3B are responsible for the methylation, DNMT3A and DNMT3B create new methyl marks and DNMT1 maintain the pattern during replication steps. S-adenosylhomocysteine is a result of this process. On the other hand, TET enzymes oxidize 5-mC and generate 5-hmC, 5-fC and 5-caC, with the final product being the unmodified cytosine, closing the methylation/demethylation loop [23, 24].

DNA methylation is one epigenetic marker that is of high importance in disease studies, as it has a high persistence and could be used as a predictor for disease progression, response to therapy and to discriminate between malignant and benign cells/tissues. Methylation affects CpG dinucleotides, and regions containing increased number of CpG are prone to modifications. The methylation in CpG can control the transcription of a gene or can determine if a biomarker can have clinical significance [25].

Widespread loss of DNA methylation across the genome, especially in the repetitive elements, is contributing to the genomic instability, chromosomal rearrangements, loss of imprinting and reactivation of transposable elements, all promoting tumor progression and tumor heterogeneity. Global hypomethylation can be used as a biomarker for specific diseases and aggressive tumor behavior, being detected even in cell-free DNA, feature that is exploited by different working groups to validate minimal invasive and cost-efficient methods for tumor detection [26–29].

On the other hand, focal hypermethylation, mostly at CpG islands in the promoter region of tumor suppressor genes, involves a site-specific gain-of-methylation, leading to transcriptional silencing of these genes. This feature is promoting malignant transformation and tumor progression. The focal hypermethylation can occur during early carcinogenesis, aspect that is making the research community study how these patterns can indicate early onset of the disease [30–34].

Methylation serves as a clinically relevant biomarker for cancer diagnosis and prognosis due to its role in gene regulation and its tumor-specific alterations during carcinogenesis. Aberrant methylation in the promoter regions leads to tumor suppressor gene silencing and together with global hypomethylation contributes to genomic instability. For diagnosis, methylation biomarkers are valued for their stability, frequency and detectability in tissues and biological fluids, enabling minimal invasive approaches such as liquid biopsies with different high-throughput methods such as sequencing [35–37]. Genome-wide methylation profiling, combined with machine learning, can increase the chances of a precise tumor classification and identify even a small number of tumor cells, making methylation profiling a good option for detecting MRD [38]. Cell-free DNA (cfDNA) released from cells that have abnormal methylation patterns could be detected in biological fluids in cancer patients, as high concentrations of cfDNA were detected in cancer patients, while in healthy donors the cfDNA was low [39–42]. Moreover, several methylation markers have been found to be related to a wide spectrum of tumors, as Rendek et al. highlighted in their publication: hypermethylated *APC, FOXA1* and *RASSF1A* in breast cancer, hypermethylated *TP53, CDKN2A, CTNNB1* and *APC* in esophageal cancer, hypomethylated *LINE1* and hypermethylated *p16, RUNX3, RASSF10*

and *APC* in gastric cancer, hypomethylated *ELMO3* and hypermethylated *DAL-1, EPHB6, HS3ST2* and *MGMT* in lung cancer, hypermethylated *HOXA1, EMX1, ECE1* and *PFKP* in hematic cancer, while colorectal cancer was characterized by hypermethylated *SEPT9, MGMT, SDC2, N-Myc* and *APC* [43].

Specific methylation patterns correlate with disease outcome and response to therapy. *PITX2* methylation can predict the prognosis in lymph node negative breast cancer [44, 45] and *MGMT* methylation is indicating the benefits from alkylating agents in glioblastoma [46]. Moreover, *SEPT9* methylation in colorectal cancer is strongly associated with poor outcomes [47], while *GSTP1* hypermethylation is a potential marker for diagnosis in prostate cancer, and is associated with poor outcomes in prostate and hepatocellular carcinoma [48, 49].

Methylation biomarkers could represent a superior diagnostic and prognostic tool, compared to traditional markers. Considering their novelty, and the potential interference with other processes that may modulate the epigenetic landscape, it is recommended to evaluate the methylation biomarkers with conventional approved diagnostic tools. The validation of such biomarkers requires comprehensive studies, involving large multicentric cohorts, with multiple diseases and their evaluation in dynamics.

3 Crosstalk between metabolism and epigenetics

Cancer cells exhibit metabolic adaptations that enable them to maintain growth and proliferation in response to various stress conditions, thus demonstrating metabolic plasticity. Alterations of metabolic processes are induced to satisfy the elevated bioenergetic and biosynthetic requirements necessary for proliferation. The epigenome is responsive to shifts in metabolic status. Metabolism generates a range of metabolites that serve as substrates, cofactors, or inhibitors for epigenetic enzymes. Changes in metabolic pathways and variations in intermediate metabolites will generate cross information about the intracellular metabolic condition to the nucleus by influencing the activity of epigenetic enzymes, thereby reshaping the epigenetic landscape and triggering transcriptional responses to diverse metabolic needs [50].

Epigenetic modifiers, chromatin remodelers, and non-coding RNAs are vital components of the regulatory systems that are involved in cancer biology, facilitating the process of malignant transformation. Nontheless, although the interplay metabolism and epigenetics in relationship to cancer has been extensively described, there are still ongoing research in need of further understanding of the metabolism-epigenetics cross-talk in cancer.Genetic and epigenetic changes in DNA are pivotal in influencing gene expression related to aging and cancer. In the context of human cancers, various epigenetic alterations such as DNA methylation, histone modifications, micro RNAs, and nucleosome remodeling are all instrumental in regulating gene expression [51].

Numerous metabolites influence the epigenetic modulators by serving as important substrates or cofactors, with their concentration being a main determinant of the chromatin-modifying enzyme activity. In addition to these functions, metabolites can also play a central role in orchestrating the epigenome by facilitating the establishment and preservation of epigenetic marks in reaction to variations in cellular metabolism, nutrient supply and environmental signals [52].

We've earlier discussed the Warburg effect, recognized as a fundamental example of metabolic reprogramming in cancer. Cancer cells are inclined to convert pyruvate, the

ultimate product of aerobic glycolysis, into lactate, instead of directing it towards mitochondrial metabolism, even when oxidative phosphorylation (OXPHOS) is operational. This tendency may stem from the increased requirements of cancer cells for macromolecule biosynthesis in contrast to energy production. The intermediate products generated through glycolysis can be shifted into biosynthetic routes, including serine metabolism, the hexosamine pathway, and the pentose phosphate pathway. These metabolic pathways, which are frequently disrupted, deliver reduced nicotinamide adenine dinucleotide phosphate (NADPH) necessary for reductive biosynthesis and for addressing oxidative stress, as well as S-adenosyl methionine (SAM) for methylation processes and the synthesis of proteins and nucleic acids. Moreover, cancer cells have the capability to use intermediates from the tricarboxylic acid (TCA) cycle for the synthesis of de novo fatty acids and non-essential amino acids. Studies have indicated that cancer cells may exhibit a dependency on glutamine and glucose. Glutamine plays a crucial role in the production of essential amino acids, purine bases, and pyrimidine bases. Additionally, glutamine can be converted into α-ketoglutarate (α-KG), which serves to replenish the TCA cycle within the mitochondria. Lipid metabolism is also subject to reprogramming in cancer. Cancer cells engage in active synthesis of fatty acids and cholesterol, which are essential to membrane composition and for the production of signaling molecules. In summary, metabolic reprogramming significantly influences various biological characteristics of cancer cells including the enhancement of proliferation and growth, as well as the facilitation of invasion and distant metastasis [53, 54].

SAM is a key metabolite produced via the methionine cycle, acting as the universal methyl donor for the methylation of DNA, RNA, and histones. SAM directly supports the function of DNA methyltransferases (DNMTs) and histone methyltransferases (HMTs), and it promotes posttranslational modifications (PTMs) including the methylation of lysine and arginine residues in nonhistone proteins. The synthesis of S-adenosylmethionine (SAM) is fundamentally connected to one-carbon metabolism, where nutrients such as methionine, serine, and folate influence its production through methionine adenosyltransferase (MAT) enzymes. After SAM donates a methyl group, it is converted into S-adenosylhomocysteine (SAH), which acts as a strong inhibitor of DNMTs and HMTs, thereby emphasizing the necessity of preserving the SAM/SAH ratio for effective epigenetic regulation. Elevated levels of SAM boost the activity of DNMT and HMT, thereby facilitating the methylation of both DNA and histones. In contrast, the buildup of SAH serves to inhibit these enzymes. Furthermore, the availability of SAM plays a crucial role in modulating the activity of demethylases, including ten-eleven translocation (TETs) family proteins and histone demethylases, which are influenced by the metabolic environment. As a result, SAM levels play a significant role as a metabolic checkpoint overseeing both methylation and demethylation activities [55].

SAM is synthesized from methionine and ATP during the methionine cycle, which is critical for one-carbon metabolism. Serine, along with other amino acids such as glycine and threonine, serves as the primary donors of one-carbon units in this metabolic process. Moreover, serine contributes to the production of SAM by supporting the synthesis of de novo ATP, which not only supplies adenosine but also provides one-carbon units for the remethylation of homocysteine.

In cancer, the one-carbon metabolic pathway is often upregulated to facilitate rapid cell division, with enzymes like phosphoglycerate dehydrogenase (PHGDH) boosting

serine biosynthesis, which increases SAM levels. This metabolic alteration provides the substrate for the hypermethylation of tumor-suppressor genes and modified histone structure, thereby advancing tumor progression [57].

DNA hypomethylation has the capacity to activate the transcription of oncogenes, consequently facilitating carcinogenesis and the progression of tumors. In types of cancer characterized by low levels of S-adenosylmethionine (SAM), replenishing this compound aids in restoring the balance of methylation rather than simply elevating methylation levels. SAM serves as a methyl donor in a variety of methylation reactions and suppresses the activity of intracellular demethylases, which leads to hypermethylation of DNA. A study focusing on gastric cancer revealed that treatment with SAM resulted in the hypermethylation of the promoter for vascular endothelial growth factor (VEGF)-C. The expression of VEGF-C, which is typically heigh in gastric cancer, was found to be downregulated following SAM treatment. The capacity of SAM to modulate VEGF-C methylation highlights its potential as a therapeutic agent for silencing oncogenes and impeding cancer progression, extending beyond its role in providing one-carbon units for the remethylation of homocysteine [58].

SAM has revealed anticancer capabilities across multiple malignancies. In the context of breast cancer, SAM has been shown to diminish tumor proliferation, migration, invasion, and metastasis in vitro, while also reducing tumor volume in vivo. In hepatocellular carcinoma (HCC), SAM effectively inhibits the growth, transformation, and invasiveness of cancer cells, without affecting normal liver cells. The potential of SAM in cancer therapy emphasizes its crucial role in bridging metabolism and epigenetics, with important consequences for the regulation of vital epigenetic transformations. The synthesis and functionality of SAM are affected by intracellular metabolic pathways and external factors, including nutrient availability, which directly influence SAM levels. By focusing on the one-carbon cycle or enhancing SAM production through dietary or pharmacological strategies, SAM can be effectively utilized for therapeutic purposes [57, 59, 60].

α-KG acts as a key intermediate within the TCA cycle, being synthesized from isocitrate by the enzyme isocitrate dehydrogenase (IDH). It also plays a role as a co-substrate for various dioxygenase enzymes, such as Jumonji C domain-containing histone demethylase (JHDMs), TETs, and prolyl hydroxylase. In human pluripotent stem cells, α-KG leads to the demethylation of histones and DNA, promoting the process of differentiation [61].

In pancreatic cancer (ductal adenocarcinoma), p53 inactivation results in a decrease of α-KG levels by modifying glucose and glutamine metabolism, which negatively affects TETs activity. Consequently, this process enables tumor cells to develop characteristics indicative of poor differentiation and increased aggressiveness [62].

In the presence of abundant exogenous serine, squamous cell carcinoma cells show an increase in mitochondrial pyruvate metabolism and impedes nicotinamide adenine dinucleotide (NAD +) regeneration by converting pyruvate to lactate. The scarcity of NAD + is not favorable for serine synthesis. Therefore, squamous cell carcinoma cells inhibit the de novo serine synthesis pathway, resulting in the accumulation of α-KG. The reduction of α-KG inhibits histone demethylases and the demethylation process, which inhibits the differentiation of cancer stem cells. This property maintains the characteristics of stem cells and encourages tumor initiation. The replenishment of the TCA cycle by glutamine is vital for the production of α-KG. When glutamine consumption

is increased, it can lead to a deficiency of glutamine in the tumor's core regions. The hypermethylation of histones, resulting from lower levels of glutamine and α-KG, is linked to the dedifferentiation of cancer cells and the development of resistance to BRAF inhibition. Increasing glutamine intake through oral supplementation raises the levels of α-KG. An increase in α-KG concentration could potentially inhibit the oncogenic pathways that are active in melanoma [63, 64].

Histone deacetylation serves as a vital mechanism for the regulation of chromatin structure and gene expression, facilitated by four distinct classes of histone deacetylases. Classes I, II, and IV are dependent on zinc. Class III, referred to as sirtuins, employs NAD + as a co-substrate. During the deacetylation process, NAD + is broken down into 2-O-acetyl-ADP-ribose and nicotinamide, with nicotinamide being recycled back into NAD + via the NAD + salvage pathway, which is an essential source of NAD + within the cell [65]. In cancer cells that are rapidly proliferating, the increased expression of nicotinamide phosphoribosyl transferase (NAMPT) guarantees an adequate supply of NAD + to meet metabolic requirements and facilitate sirtuin activity. In the salvage pathway, NAMPT facilitates the conversion of nicotinamide into nicotinamide mononucleotide (NMN), which is subsequently transformed into NAD + by NMN adenylyl transferases. As the rate-limiting enzyme in this pathway, NAMPT is often upregulated in various cancers, including colorectal tumors, which highlights its potential as a target for therapeutic intervention. Elevated NAMPT activity is critical for the maintenance of NAD + levels in rapidly growing cancer cells, aiding in metabolic reprogramming, such as increased glycolysis, a defining trait of cancer. This metabolic transition entails the transformation of NAD + into NADH, which lowers the NAD + /NADH ratio, consequently reducing sirtuin activity. The subsequent decline in sirtuin function results into histone hyperacetylation and modified gene expression patterns that can facilitate tumor formation. The metabolism of NAD + is significantly affected by nutrition patterns. For instance, calorie restriction results in an increased NAD + /NADH ratio and enhances sirtuin-mediated deacetylation, which has been linked to tumor suppression. Conversely, diets rich in fat, lower NAD + levels and sirtuin activity, contributing to a metabolic landscape that promotes cancer progression. Adjusting NAD + concentrations may alter the epigenetic environment, potentially facilitating or suppressing tumor development depending on the metabolic conditions of the cancer cells [66–68].

Another essential metabolite in cancer biology is 2-hydroxyglutarate (2-HG), which structurally resembles α-ketoglutarate (α-KG). The biochemical form of 2-HG is chiral, and it exists in two isoforms: D2-HG and L2-HG. This metabolite is of interest especially in tumors harboring isocitrate dehydrogenase (IDH1/2) mutations which tend to accumulate in environments characterized by hypoxia and acidity or through the action of enzymes such as phosphoglycerate dehydrogenase, malate dehydrogenase 1 and 2, and lactate dehydrogenase. The mutations in IDH1/2 facilitate the transformation of α-KG into D-2-HG, leading to its accumulation and the subsequent inhibition of α-KG-dependent dioxygenases, including TET2 and Jumonji-C histone demethylases. This process results in extensive DNA hypermethylation and histones transformations, which play a significant role in tumorigenesis. IDH mutations are capable of driving malignant transformation and resulting in the formation of poorly differentiated sarcomas. Similar observations have been made in gliomas and acute myeloid leukemia (AML), where IDH1/2 mutations cause defects in homologous recombination and increase the

sensitivity of tumor cells to polymerase inhibition. In addition, the mutant IDH synthesizes D2-HG and epigenetically suppresses the expression of interferon γ response genes, which impairs the immune response in cholangiocarcinoma [69–72].

DNA methylation-driven metabolic adaptation is a crucial mechanism of resistance to therapy in cancer cells, as it enables tumor cells to survive treatment through epigenetic silencing of genes involved in apoptosis or DNA repair mechanisms, can activate survival pathways, induce epithelial-to-mesenchymal transition and could reprogram metabolism to facilitate drug efflux and alter drug metabolism [73, 74]. However, several other mechanisms can contribute to the metabolic disfunctions, DNA methylation being just one critical player in the process.

Chromatin accessibility plays a critical role in genetic processes and hypoxia may alter chromatin accessibility through inhibition of oxygen-dependent histone demethylases, such as those containing Jumanji-C domains (e.g., KDM5A, KDM6A), leading to an increase in histone methylation and a more repressive chromatin state. Importantly, these effects occur independently of hypoxia-inducible factor (HIF) signaling, as a direct requirement for oxygen exists for histone demethylation. Consequently, hypoxia (i.e., a decrease in the amount of oxygen) reprograms chromatin and changes how genes respond to transcriptional signals. While hypoxia and chromatin were previously thought to be independent, recent data have shown a direct relationship between hypoxia and chromatin: studies by Batie et al. have shown that the inhibition of KDM5A, a demethylase, is responsible for the ability of chromatin to sense oxygen levels. [75, 76].

Another key process involved in epigenetic modifications is histone lactylation influenced by lactate [77, 78]. High levels of lactate under hypoxic or glycolytic conditions lead to lysine L-lactylation on histones, stimulating gene transcription and modulating cell fate, immune response and cancer cell response. The process is different from acetylation and is regulated by nuclear lactate dehydrogenase and L-lactyl-CoA-synthetase [79, 80].

Acetyl-coenzyme A levels can directly influence chromatin compaction as it servs as a substrate for histone acetyltransferases. Thus, increased levels of nuclear acetyl-CoA can promote histone deacetylation, promoting a more open chromatin state and transcriptional accessibility. The levels of acetyl-CoA are regulated by the different metabolic needs, with the fatty acid synthesis being critical, as it could influence chromatin regulation and histone acetylation [81, 82].

α-KG, succinate and fumarate, key metabolic intermediates, regulating TET enzymes and histone demethylases. TET requires α-KG as cofactor, and the accumulation of succinate and fumarate in different tumors may inhibit α-KG dependent dioxygenases resulting in hypermethylated DNA and increased histone methylation which can alter gene expression [83, 84]. Fe (II) and α-KG are required as cofactors to oxidize 5-mC to 5-hmC, 5-fC and 5-caC and therefore initiate DNA demethylation [85, 86]. Moreover, it was demonstrated that the Km value of TET1 and TET2 for oxygen is 30 μM which indicates a high activity under hypoxic conditions, but TET enzymes are still sensitive to metabolic perturbations affecting the α-KG availability [84, 87]. JmjC domain0containing histone demethylases (KDMs) also depend on α-KG and iron, to remove the methyl groups from histone lysine residues by an oxidative mechanism, the enzymes have substrate inhibition at high α-KG levels, with the optimal levels being like those observed in tumor cells [88]. α-KG availability is also orchestrated at nuclear level where α-KG

dehydrogenase can regulate histone demethylation through α-KG decarboxylation, limiting the substrate access to KDMs [89]. The battle for α -KG dependent dioxygenases involve succinate and fumarate which have similar structure to α-KG, with in vitro studies demonstrating that fumarate and succinate inhibit TET enzymes at micro molar dose, while in succinate dehydrogenase/fumarate hydratase mutant tumor may accumulate millimolar levels of the two compounds. These compete for α-KG binding sites on enzymes, preventing the cofactor from accessing the active site and blocking the catalytic activity of the enzyme. Crystallographic analysis confirms that succinate, fumarate and TCA cycle intermediates bind to α-KG binding pocket of KDMs [84, 90].

The epigenetic consequences of fumarate hydratase and succinate dehydrogenase gene mutations can lead to loss of enzyme function and accumulation of their respective substrates- fumarate and succinate, causing TET inhibition—reducing 5hmC levels and promoting global DNA hypermethylation, histone demethylase inhibition—increasing histone methylation marks like H3K4me3, H3K9me3 or H3K36me3 and altering chromatin state, or it may induce a disruption into the DNA repair mechanisms [91, 92].The crosstalk between metabolism and epigenetics represents a bidirectional regulatory network, where metabolic pathways generate cofactors and substrates that directly modify chromatin structure and influence gene expression, while on the other hand, epigenetic mechanisms reciprocally control the expression of metabolic enzymes, creating a self-reinforcing loop that integrates cellular identity and functions with environmental stimuli. Chromatin-modifying enzymes are dependent on metabolites, cofactors or inhibitors, making the epigenome sensitive to metabolic variations [93–96].

Chromatin remodeling and metabolic enzyme expression are reciprocally regulated, ATP-dependent chromatin remodeling complexes modulate the transcription of genes encoding metabolic key enzymes in response to nutrient signals, while metabolic enzymes directly regulate chromatin structure through local metabolite production and nuclear translocations [97]. ATP-dependent chromatin remodeling enzymes use energy to alter histone-DNA interactions, evicting or repositioning nucleosomes to modulate transcription factors access to metabolic genes. SWI/SNF complex is a master metabolic regulator, as BAF60 subunit links the core SWI/SNF complexes to specific transcription factors and further modulate tissue specific metabolic processes and are frequently disrupted in glycolysis dependent cancers [98]. SWI/SNF control transcription of genes involved in TCA cycle, oxidative phosphorylation, glycolysis and lipid metabolism, and the loss of SNF subunit may cause disruptions in sulfur metabolic gene transcription and global distribution of Met4, fact that is highlighting the chromatin remodeling impact on entire metabolic pathways [99]. Same as SWI/SNF, INO80 complex is involved in this balance by organizing temporal expression of metabolic genes controlling cell division during metabolically favorable conditions, responding to nutrient variations [97].

Mutations in SWI/SNF subunits occur in one out of five cancers and drive metabolic reprogramming, loss of SWI/SNF act as a metabolic vulnerability that presents therapeutical opportunities, including dependencies on glycolysis or glutamine transport [100, 101].

Histone acetylation is catalyzed by histone acetyltransferases using acetyl-CoA as a substrate, and the availability of acetyl-CoA is directly dictating the histone acetylation status. Increased histone acetylation at promoters of metabolic genes increases the chromatin accessibility and promotes metabolic flux through glycolysis, lipid synthesis and

other key pathways. When a lack of nutrients is present, histone acetylation is focused on activating genes involved in gluconeogenesis and fat metabolism, supporting the cells while acetyl-CoA levels are low. The balance between acetylation and deacetylation can be maintained by acetyl-CoA recycling while modulating the chromatin accessibility [102, 103]. On the other hand, histone methylation is controlled by SAM availability, the universal methyl donor, which is linked to the one-carbon metabolism. Methylation of specific histones can activate or inactivate specific metabolic genes, modulating the metabolic flux. The activity of histone methyltransferases can influence SAM consumption and the availability of methyl groups, influence the global methylation and induce metabolic adaptation, thus both histone methylation and acetylation are influencing metabolism and are both influenced by the metabolic changes [104, 105].

Epigenetic changes are actively involved in the metabolic reprogramming associated with cancer. In contrast to genetic mutations, epigenetic regulations are both reversible and variable. Epigenetic modifiers influence metabolism by directly altering the transcriptional activities of metabolic enzymes or proteins involved in metabolism-related signaling pathways, in accordance with the demands of tumor cells. The functions of non-coding RNAs (ncRNAs) in the regulation of metabolic reprogramming are considerably more intricate, involving both transcriptional and post-transcriptional regulatory mechanisms. Delving into the epigenetic roles of ncRNAs in metabolism regulation will significantly broaden our understanding and knowledge base. Although there is a growing body of research, many significant questions remain unresolved. One major concern is how these epigenetic processes are orchestrated to enhance tumor development through metabolic regulation.

Metabolism and epigenetics are fundamentally intertwined and have profound implications for understanding homeostasis and diseases and could open new therapeutic paradigms that may target this integrated network.

4 Oncometabolites and epigenetic dysregulation

2-Hydroxiglutarate is an oncometabolite found in various types of cancer. It is produced by mutated versions of the isocitrate dehydrogenase enzyme (IDH). IDH consists of 3 isoforms, that are encoded by 5 genes. IDH3 is part of the TCA cycle where it acts by converting isocitrate to α-ketoglutarate (a-KG) and NAD + to NADH. The IDH1 and IDH2 isoforms act outside the TCA cycle, being located in the cytosol and mitochondria, where they generate NADPH from NADP + by catalyzing the oxidative decarboxylation of isocitrate to a-KG [127]. Therefore, IDH represents an important source of a-KG, which acts as a cofactor of many enzymes such as DNA and histone demethylases. Mutated IDH1 and IDH2 are involved in various types of cancer such as glioma. The mutations usually occur at Arginine 132 in IDH1 and Arginine 172 in IDH2, rendering the enzymes unable to bind isocitrate. Additionally, the mutations give IDH1 and IDH2 the neomorphic catalytic activity of converting a-KG to 2-HG [128].

2-HG can exist in healthy cells under physiological conditions at low concentrations, being a product of various enzymes: 3-phosphoglycerate dehydrogenase, hydroxiacid-oxoacid transhydrogenase, malate dehydrogenase and lactate dehydrogenase. 2-HG has two enantiomers, D-2-HG and L-2-HG, and in normal conditions it is degraded by 2-hydroxiglutaric acid dehydrogenases (2-HGDH) specific for each enantiomer [129].

2-HG acts as a competitive inhibitor of a-KG, inhibiting the activity of several DNA and histone demethylases, leading to hypermethylated epigenetic landscape [130]. It was shown that 2-HG has a much more increased inhibitory capacity in comparison with other oncometabolites such as fumarate or succinate, being a potent inhibitor of KDM4A, KDM4B, KDM6A. Additionally, the intracellular accumulation of 2-HG can lead to increased levels of H3K9me3 and H3K27me3 in breast cancer cell lines [131]. 2-HG was determined to be a potent inhibitor of TET2 leading to a hypermethylated epigenome in IDH-mutant brain tumors and leukemias. It was observed that IDH and TET2 mutations are mutually exclusive in AML, suggesting that the mutations are redundant, their effects converging on the same leukemogenic pathways [132]. Furthermore, 2-HG was found to disrupt different signaling pathways contributing to carcinogenesis. It was demonstrated that 2-HG can inhibit necroptosis, disrupt the architecture of the cytoskeleton and interfere with mTOR and HIF signaling. Moreover, studies have shown that 2-HG is an important oncometabolite promoting the carcinogenesis of various types of cancers like glioma, acute myeloid leukemia, breast, colorectal or lung cancer. Regarding the tumor microenvironment, it was demonstrated that 2-HG poses immunosuppressive effects by blocking the activity of NFATC1 and the expression of CD12 and CXCL10 [133]. Studies have shown that the oncometabolite 2-HG is also involved in DNA repair mechanisms. The AlkB family consists of a-KG/Fe(II) dependent dioxygenases involved in DNA repair mechanisms. Their function is to restore damaged DNA bases by oxidizing aberrant alkyl groups that occur in the DNA structure. The main substrates of this family are 3-methylcytosine, 1-methyladenine, 3-methylthymimne and 1-methylguanie. Additionally, AlkB enzymes can induce oxidative modifications of 5-methylcytosine to 5-hidroxymethylcytosine, 5-formylcytosine and 5-carboxycytosine, being involved in epigenetic regulation [134]. It was determined that 2-HG can inhibit the activity of AlkB family members, ALKBH2 and ALKBH3 in vitro, leading to impaired DNA repair and DNA damage accumulation under chemotherapy with alkylating agents. The same study showed that IDH mutant glioma producing high levels of 2-HG exhibit an increased sensitivity to chemotherapy with alkylating agents [135].

4.1 Oncometabolites – impact on cellular differentiation and stemness

The repressive hypermethylated epigenetic landscape induced by oncometabolites was shown to directly impair cellular differentiation programs, while also inducing cellular dedifferentiation and reprogramming the cells towards a stem cell-like phenotype.

A study demonstrated that the intracellular accumulation of 2-HG caused by mutant IDH impairs the differentiation mechanism of adipocytes in vitro. The increase of 2-HG was associated with an increase in repressive histone methylation marks, similar to the levels of repressive marks found in gliomas. The repressive effects were attributed to the inhibition of KDM4C, leading to increased H3K9 methylation levels. Moreover, the authors showed that the suppression of KDM4C was sufficient to inhibit differentiation, suggesting that 2-HG inhibition of histone demethylation can block cellular differentiation [71]. The inhibitory hypermethylation caused by mutant IDH was shown to be involved in the pathogenesis of liver cancers as well. Hepatoblasts expressing mutant IDH and producing high levels of 2-HG showed a decreased ability to differentiate. Mechanistically, this was caused by a decreased expression of HNF1a, a master

transcriptional regulator involved in hepatocyte differentiation [136]. Similar effects were obtained in the case of human mesenchymal stromal cells, where the accumulation of 2-HG and increased levels of methylation led to the inhibition of the Sonic Hedgehog pathway, impairing chondrogenic and osteogenic differentiation [137]. Additionally, high levels of 2-HG were shown to block the differentiation of renal proximal tubule cells, which are the precursors of clear cell renal carcinomas [138]. These studies show that 2-HG directly impairs the differentiation programs of various cancer precursor cells, contributing to cancer development.Stem cells are pluripotent self-renewing cells capable of both proliferation and differentiation into multiple lineages. Cancer stem cells (CSCs) share these characteristics, representing a minor tumor subpopulation with self-renewal capacity and unlimited but slow proliferation that maintains the CSC pool, while their differentiated progenitors exhibit the rapid proliferation typical of cancer cells [139]. Two theories explain CSC origin: the hierarchical model proposes that somatic mutations target stem/progenitor cells organized hierarchically, leading to asymmetric division that generates both CSCs and rapidly proliferating transient cells. The stochastic model suggests any cell can acquire self-renewal and differentiation capabilities through specific mutations, contributing to tumor heterogeneity [140, 141]. CSCs drive chemotherapy resistance and relapse, with high CSC levels associated with poor prognosis and aggressive disease. Chemotherapy can paradoxically induce dedifferentiation of cancer cells into CSCs [142]. CSC therapy resistance stems from multiple mechanisms: their slow/non-dividing nature makes them resistant to drugs targeting proliferative cells, allowing surviving CSCs to reinitiate tumors after initial treatment eliminates the bulk tumor mass. Additionally, CSCs overexpress ATP-binding cassette (ABC) transporters that actively pump chemotherapeutic drugs out of cells, conferring multidrug resistance.. Glutamine can act as an epigenetic regulator in CSCs, being converted into a-KG in the mitochondria by glutamate dehydrogenase 1, or transaminases like GPT2 or GOT2. Since several DNA and histone demethylases are dependent on a-KG, glutamine metabolism can indirectly contribute to the epigenetic dysregulation associated with CSCs [154].

Cancer cell metabolism and oncometabolites were shown to influence the chromatin structure and the epigenome of cancer cells, driving nuclear reprogramming and stemness. This creates a repressive epigenome that can facilitate cellular reprogramming from a differentiated state to an undifferentiated stem cell-like state. Using a computational model of metabolic nuclear reprogramming combined with in vitro experiments, Menendez et al. demonstrated that 2-HG can lower the epigenetic barriers of nuclear reprogramming enabled by stemness factors. Their results showed that IDH mutant MCF10A human mammary epithelial cells were characterized by the accumulation of 2-HG, leading to increased global levels of H3K4me3, H3K9me3, and H3K27me3. Further analyses confirmed that the IDH mutant cells had a repressed transcriptional differentiation program, while the baseline expression of stemness factors such as OCT4 and SOX2 remained unchanged. Moreover, the study demonstrated that 2-HG can effectively substitute for certain stemness factors like KLF4 and c-Myc, being able to reprogram MCF10A cells transduced with OCT4 and SOX2 into induced pluripotent stemlike cells (iPSLC). The same effects were observed in IDH wild-type MCF10A cells that were supplemented with 2-HG, further consolidating the fact that 2-HG is the major contributor to the cellular reprogramming towards a stem-like state. Therefore,

this study demonstrated that oncometabolic changes occurring in cancer cells can epigenetically regulate cells to induce nuclear reprogramming and driving stemness [155].

Besides being a product of the neomorphic activity of mutated IDH enzymes, 2-HG was found to be produced at high levels by IDH-WT cells under hypoxic conditions. It was shown that under hypoxic conditions, LDHA, MDH1 and MDH2 can mediate the oxidation of glutamine-derived a-KG to 2-HG [156]. Hypoxia induced 2-HG was shown to be involved in histone demethylation deregulation and stemness activation. Pancreatic cancer cells grown under hypoxic conditions exhibited increased levels of 2-HG due to increased LDH/MDH activity. This in turn led to repressive levels of histone methylation that suppressed pro-differentiation genetic programs and activated stemness-related genes. Moreover, the inhibition of LDH was shown to decrease histone methylation and tumor volume in vivo and modulate the immune microenvironment, thereby proving to be a potential therapeutic target [157]. Furthermore, HIF-1a and HIF-2a were shown to induce the over-expression of LDHA in cancer cells by binding to hypoxia response elements (HRE-D) in the LDHA promoter [158]. Due to 2-HG's ability to inhibit a-KG dioxygenases like PDH, its accumulation might stabilize HIF expression [159]. This in turn could create a possible feedback loop that leads to epigenetically induced transcriptional repression that blocks cell differentiation and reprograms cells towards a stem-like state. However, a series of studies reported contrary findings regarding the relation between 2-HG and HIF-1a, as it was shown that IDH1 mutant glioma samples can have reduced levels of HIF-1a [160] and that the L-2-HG enantiomer could increase the activity of the EglN prolyl hydroxylase, leading to decreased HIF-1a levels [161].

One of the most important characteristics of tumors is the variation between different cell populations within the same tumor, coined as intra-tumor heterogeneity. There are many factors contributing to tumor heterogeneity, with CSCs being an important contributor that in many cases leads to cancer progression and treatment failure [162]. Tumors consist of many different cell populations, such as CSCs, which proliferate slowly and are responsible for tumor renewal. These cells can differentiate giving rise to highly proliferative differentiated cancer cells (CDCs), which comprise the bulk of the tumor [139]. The metabolic reprogramming occurring in cancer cells is closely related to tumor heterogeneity and stemness. Regarding glucose metabolism, it was shown that differentiated cancer cells (CDCs) display increased levels of glycolysis and lactate production in comparison with CSCs, which exhibit higher lactate uptake. Lactate was shown to increase the CSC population within the same tumor, by inhibiting differentiation programs and by inducing the dedifferentiation of CDCs. Lactate acts as an epigenetic regulator of the MYC stemness factor, by increasing the levels of acetylation and chromatin accessibility at the *MYC* locus. Therefore, the intense glycolytic rates within a tumor could create a high lactate environment that drives the epigenetic reprogramming of the cancer cells towards a stem cell-like state, contributing to tumor heterogeneity [163].

One-carbon metabolism is a critical instance of how DNA and histone methylation are regulated through the activity providing methyl groups. The methionine cycle produces SAM, the universal methyl donor for every type of methyltransferase reaction, including DNA methyltransferases and histone methyltransferases. The availability of methionine (and therefore SAM) determines the extent to which DNA and histone proteins can be methylated, thus affecting the structure of chromatin and the expression of genes.

SAM levels are impacted by the nutrient status inside the cells, including influences from methionine, folate, and B vitamins, all of which are needed to allow for one-carbon metabolism. Limiting the intake of methionine, or interfering with the processes to synthesize SAM, will lead to a decrease in the extent to which histones like H3K4me3 and DNA are methylated, which alters the transcriptional profile and overall phenotype of a cell [164, 165].

The relationship between these metabolic/epigenetic pathways can be rapidly altered by changes in dietary methionine intake or metabolic fluxes. Any changes that take place due to those changes will be reversible once the diet returns to a normal state [166]. Methionine restriction has shown promise in preclinical models by depleting SAM pools, reducing H3K4me3 and other histone marks, and altering gene expression to suppress tumor growth [167].

The metabolites of glutamine include αKG, essential cofactor required for all TET DNA demethylase and Jumonji histone demethylase actions. Cancer cells can take advantage of this pathway to regulate the epigenetic landscape in their favor. Tumor areas which are deprived of glutamine produce less αKG than normal. This lack of αKG causes dramatic increases in histone methylation (mainly H3K27me3), which leads to dedifferentiation and provides a mechanism for developing resistance to treatment. For patients with melanoma who are deprived of glutamine, hypermethylation of H3K27 due to a reduction in KDM6B demethylase results in the development of resistance to BRAF inhibitors. Conversely, providing glutamine in the diet increases levels of intratumoral αKG which then leads to decreased histone methylation of H3K4me3, thus inhibiting oncogenic pathways that have been activated epigenetically by methylation, and resulting in decreased growth of melanoma cells and increased sensitivity to BRAF inhibitors. For glioblastoma, αKG derived from glutaminolysis stimulates KDM6A to demethylate H3K27, which activates the EGFR/PI3K/AKT pathway and reprograms the glycolytic metabolism of glioblastoma [64, 168]. Glutaminase (GLS1) inhibitors like CB-839 (telaglenastat) are in clinical trials for multiple cancers, particularly in combination therapies [169].

Lipid metabolism and the amount of acetyl-CoA are very important regulators of histone acetylation. In addition, fatty acid oxidation produces novel metabolic pathways (primarily through beta-hydroxybutyrate, an example of a fatty acid oxidation metabolite) that directly modify chromatin structure to create new types of histone modification. These metabolic and epigenetic connections create bidirectional regulatory circuits that are utilized by cancer cells for their growth, survival and resistance to cancer therapies. Acetyl-CoA is a universal acetyl donor for histone acetyltransferases (HAT) and is an important rate-limiting metabolite for histone acetylation. The Km for most of the HAT enzymes used by cells falls within the physiological range of acetyl-CoA concentrations found within the cell [170, 171].

Cancer cells produce nuclear and cytoplasmic acetyl-CoA through multiple different metabolic pathways with lipid metabolism being the most significant contributor. Fatty acid oxidation can account for approximately 90% of the acetylation that occurs at specific lysine on histones compared to an environment where there is sufficient glucose for cellular metabolism. This demonstrates how fatty acids can alter the metabolic programming of a cell so that lipid metabolism becomes the primary source of carbon for histone acetylation. Acetyl-CoA is primarily generated for use in the nucleus through

the ATP-citrate lyase (ACLY) pathway which converts citrate to acetyl-CoA. Oncogenic signals from Akt activate ACLY, increasing the availability of acetyl-CoA for creating histone acetylation changes in gliomas and prostate cancer prior to the development of tumors. It is important to note that cancer cells use metabolic pathways other than ACLY to create acetyl-CoA. For example, mitochondrial fatty acid oxidation and the carnitine acetyl transferase (CrAT) shuttle enable two-carbon units to be translated from the mitochondria to the nucleus providing access to acetyl-CoA to support histone acetylation and proliferation even when ACLY and ACSS2 are not present. These complex interactions between acetyl-CoA derived from lipid metabolism and histone acetylation result in the establishment of connections between lipid metabolism, histone acetylation and cancer cell growth, proliferation, metastasis and resistance to drugs [172, 173].

The lipid metabolism-epigenetics axis presents multiple therapeutic opportunities, like targeting acetyl CoA production to reduce oncogenic histone acetylation, with applications in hypoxic tumors [174]. Targeting fatty acid oxidation with inhibitors, could reduce histone acetylation, but it must be well applied as this process has dual role—in some cases is protecting against tumorigenesis, while in others it can support tumor growth [175]. Other approaches imply the use of histone acetyltransferases or the use of ketogenic diets or beta-Hydroxybutyrate, but it must be carefully monitored due to beta-Hydroxybutyrate roles in diverse tumor types [176, 177].

The spatial and temporal control of acetyl-CoA metabolism—including nuclear localization of metabolic enzymes like pyruvate dehydrogenase complex and 2-ketoacid dehydrogenases that produce acetyl-CoA locally at chromatin—adds another layer of specificity to how metabolic signals are translated into site-specific epigenetic changes.

5 Tumor microenvironment and epigenetic-metabolic changes

Tumor microenvironment (TME) and epigenetic-metabolic changes are tightly interconnected in cancer biology. TME is characterized by hypoxia, nutrient deprivation, low pH, and complex cellular interactions that drive metabolic reprogramming in immune and tumor cells. These alterations can influence the availability of key metabolites that serve as substrates or cofactors for chromatin modifying enzymes. The lack of these enzymes is responsible for DNA modulation and histone modifications, or even gene expression alterations, providing the tumor cells with a path for immune evasion [178, 179]

Malignant cells tend to use aerobic glycolysis rather than mitochondrial phosphorylation as a source of energy, process known as "the Warburg effect". The final product of glycolysis is the lactate, which is responsible for the low pH in TME. Lactate is used by tumor cells as fuel and promote M2 macrophage polarization and further inhibit the effector role of T helper cells Th1/2, Tc and Natural killer (NK) cells [180–183]. Another important effect of the high amount of lactate in TME is the metabolic support for tumor infiltrated Tregs, process that is suppressing the effector T cell function within TME [184, 185].

The strong proliferative environment in TME is leading to oxygen consumption and to dysfunctions in the vascular system, creating hypoxic conditions in TME. Low oxygen levels promote HIF-1α expression which is helping the tumor cells to adapt in the hypoxic conditions by promoting glycolysis and evasion of the immunosurveillance, while tempering the NK cells anti-tumor effects, reducing CD4 + effector T cell

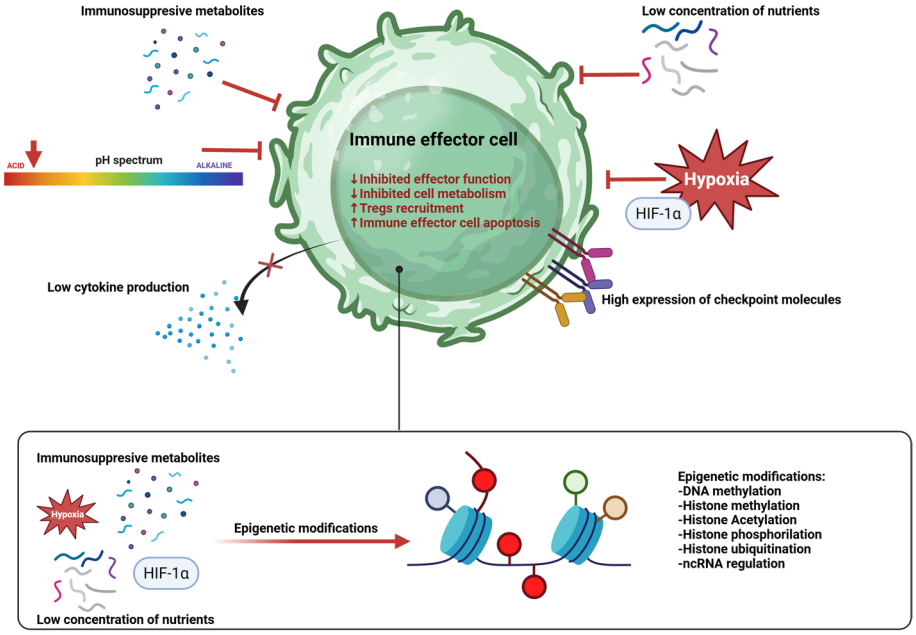

Fig. 3 TME immune inhibition and epigenetic changes induced by the TME. HIF-1α and hypoxic conditions specifically inhibit anti-tumor immunity, promoting tumor development and the escape from immune surveillance

differentiation, promoting Treg activity, enhancing checkpoint molecule expression and T cell apoptosis [186].

Hypoxic conditions in TME drive the accumulation of immunosuppressive metabolites such as lactate and adenosine, which promote tumor cells growth and inhibit immune cell functions. HIF-1α orchestrate these effects by stimulating glycolytic enzymes and adenosine-synthesis pathways, which in the end suppress CD8 + T cell cytotoxicity, impair CD4 + T cells effector functions and lower the NK cell activity. CD8 + and CD4 + T cells are affected by HIF-1α signaling as their metabolism is disturbed, leading to metabolic exhaustion, less cytokine production and increased apoptosis. HIF-1α are also responsible for the overexpression of several immune checkpoints such as PD-L1, thus facilitating the immune escape. As presented in Fig. 3, hypoxia and HIF-driven metabolic reprogramming create a TME that impairs effector lymphocytes and promotes Tregs accumulation, orchestrating a tumorigenic environment [187–189].

Epigenetic modifications can regulate the expression of genes encoding critical players in metabolism, enabling tumor cells to adapt to hostile TME and sustain proliferation, invasion and resistance to therapy [179]. The crosstalk between TME, metabolism and epigenetic changes underlie tumor heterogeneity, plasticity and therapeutic resistance, representing a promising target for novel cancer therapies [190].

Hypoxia could interfere with DNA methylation during all stages of the biological process. The expression of DNMTs is influenced by hypoxia, as increased HIF-1α activity is correlated with enhanced binding to *DNMT1* and *DNMT3B* promoters, promoting methylation and silencing of tumor suppressor gene expression. DNMTs can suppress *HIF* expression in a negative feedback loop [191–193]. Renal cell carcinoma and glioblastoma cells showed low *DNMT3A* expression associated with low *HIF* promoter methylation and increased *HIF* expression, sustaining the fact that there might be a link between methylation of *HIF* promotor and methylation process.

The stimuli from and through TME are affected by different hypoxia related mechanisms. Hypoxia-driven HIF-1α mediated metabolic rewiring is strongly related to histone methylation through interconnected mechanisms such as HIF-1α transcriptional reprograming of cellular metabolism shifting to a more intense glycolysis rather than oxidative phosphorylation and altering the metabolite availability for histone-modifying enzymes. Another mechanism is involving a direct inhibition of oxygen-dependent histone demethylases, causing a global increase in histone methylation which is finally translated into gene expression and chromatin reprogramming [76, 194, 195].

HIF-1α is a master regulator of cellular metabolic response during hypoxia, controlling the shift from oxidative to glycolytic metabolism. HIF-1α transactivates genes encoding glycolytic enzymes including hexokinase, phosphofructokinase (PFKL), aldolase A (ALDA), enolase 1 (ENO1), phosphoglycerate kinase 1 (PGK1), pyruvate kinase M and LDH-A [196]. LDH-A is converting pyruvate into lactate, the terminal product of anaerobic glycolysis, while GLUT1 and GLUT3 increased expression are enhancing glucose uptake [197, 198].

TCA cycle can be influenced as PDK1 phosphorylates and inactivates pyruvate dehydrogenase, preventing pyruvate conversion to acetyl-CoA. The reduced TCA cycle activity is influencing the α-KG which is inhibiting mitochondrial activity [199].

HIF-1α induces expression of certain histone methyltransferases like SET1B, which is recruited to target genes to deposit H3Kme3 marks and activate the transcription of the gene. On the other hand, KDMs expression can be increased by HIF-1α generating a context-dependent effects on histone methylation and in some case, HIF-1α can induce a site-specific demethylation of target genes [195, 200].

Epigenetic mechanisms can regulate HIF expression and activity, as intermittent hypoxia increases KDM4 activity, inducing H3K9 demethylation and increased HIF-1α mRNA expression, while chronic hypoxia has the opposite effects. Moreover, chromatin architecture can be changed due to epigenetic events, which can modulate HIF expression, stability and activity [201].

The effects of hypoxia are visible in the downstream of metabolic pathways. Lactate directly impairs T-cell metabolism through redox disruption, changing the NAD + / NADH ration inducing a metabolic block at GAPDH, serine depletion which impairs T cell proliferation and modulates the signaling mechanisms in T cells. CD40LG is downregulated, JAK1/STAT3 pathways suppressed, both reducing T cell activity in cancer [202, 203]. Histone lactylation is a novel epigenetic modification directly involved in cellular metabolism and gene transcription, process that stimulates gene transcription from chromatin. Hypoxia is inducing lactate production and promotes histone lactylation, thus modulating the process controlled by histone acetyltransferases that act as writers, histone deacetylases which act as erasers of the lactyl groups, and readers which interpret lactylation marks to translate them into functional outputs [112, 204].

Histone lactylation drives CD8 + T cell dysfunction through pathways including H3K9la-IL-11 axis in Head and neck squamous cell carcinoma, METTL3-CCT2 pathway in gastric cancer and indirect through dendritic cells [205, 206]. Regulatory T cells thrive in lactate abundant environment due to Foxp3 mediated metabolic reprogramming into oxidative phosphorylation and lactylation of MOESIN enhances TGF-β/SMAD pathway, finally enhancing Tregs stability [184, 207]. Luckily, lactylation levels can serve as prognostic and predictive pattern, as Histone lysine lactylation can be a biomarker across

many tumors, MOESIN lactylation in Tregs can predict anti-PD-1 response in hepatic cancers and H3K9la is positively correlated with IL-11 in unfavorable immunotherapy response [207, 208].

Nutrient competition in the TME arises due to a high proliferation rate of tumor cells, tumor-infiltrating immune cells and stromal cells, as all require the same nutrients for survival, creating a metabolic battleground where cancer cells often overcome immune cells, resulting in tumor progression due to immunosuppression [209].

Previous studies have established that tumor cells avidly consume methionine and outcompete T cells, as tumor cells overexpress methionine transporter SLC43A2. Thus, tumor cells disrupt methionine metabolism in T cells, reducing intracellular levels of methionine and SAM. Because of SAM depletion, a loss of H3K79me2 is observed, which reduces STAT5 expression and impairs T cell immunity [210]. Methionine cycle is the primary substrate for SAM biosynthesis which serves as universal methyl donor for DNA and histone methylation. A recent study highlighted that methionine availability during the first 30 min of T-cell receptor engagement determines subsequent T cell fate—0as limiting methionine during the initial steps of TCR engagement increases calcium influx and NFAT1 activation, which will further lead to T cell exhaustion [211].

Serine is another key molecule for T cell expansion and epigenetic programming. Upon activation T cell upregulates enzymes from specific pathways including serine, glycine, and one-carbon network. Extracellular serine may be required for optimal T cell expansion when glucose is absent, and a serine restriction impairs expansion of T cells [212]. Serine availability is influencing Tregs differentiation through epigenetic mechanisms, as serine limits Treg polarization, and one-carbon metabolism inhibition increases Tregs polarization and suppressive functions [213].

TME is influenced by Glutamine and arginine availability, as glutamine deprivation affects T-cell differentiation through α-KG, glutamine dependent α-KG production influences the balance between Th 1 and Tregs, as low levels of glutamine promote differentiation into Foxp3 + Tregs. Local glutamine deficiency in tumor can lead to hypermethylation due to decreased α-KG levels and could induce H3K27 hypermethylation which is responsible for resistance to therapy [168, 214].

TME creates a metabolic-epigenetic trap for TILs as tumor cells outcompete T cells in competition for nutrients, reduced SAM and α-KG disrupt enzyme function, loss of activating marks and gain of repressive marks alter genes through chromatin remodeling and the decreased expression of STAT5, IFN-γ and effector molecules induce functional impairment in the immune cells. A metabolic-epigenetic combination therapeutic approach can target tumor nutrient consumption and support T-cell function and inhibit tumor cells proliferation [215, 216].

The α-KG/Succinate ratio determines dioxygenase activity as the ratio serves as metabolic sensor that is modulating the epigenetic enzyme activity. A high ratio promotes demethylation and active chromatin, while decreased ratio inhibits demethylation and promotes repressive chromatin. The α-KG/Succinate ratio is influencing TCA cycle, glutamine metabolism and mitochondrial function [217, 218].

Oncometabolites reshape tumor cells and TME through epigenetic reprogramming, blocked differentiation and immune suppression via macrophage polarization and T cell differentiation. All these mechanisms are making the process targetable by different therapeutical approaches. Nonetheless, α-KG dependent dioxygenases represent

a fundamental mechanism used by cells to integrate metabolic status with epigenetic regulation, enabling adaptive response to nutrient availability, oxygen levels and cellular stress, and it also serves as critical node for oncogenic transformation, making it a good target for cancer therapy [219, 220].

6 Metabolite-driven epigenetic reprogramming in cancer

Metabolic reprogramming in tumor cells alters the availability of essential metabolites that serve as substrates or cofactors for chromatin-modifying enzymes (SAM, a-KG, acetyl-CoA), directly influencing the DNA and histone methylation patterns. This interplay is finally modulating gene expression and reprogram critical pathways involved in proliferation, survival and cellular adaptation [106]. Several metabolites are able to directly bind epigenetic modulators and regulate their activity, while others can indirectly modulate the availability and functionality of these enzymes. Thus, metabolites can also orchestrate the epigenome by coordinating the maintenance and availability of epigenetic marks as a response to metabolic changes, nutrient availability or external factors [107].

SAM is the main metabolite synthetized through methionine cycle and acts as a donor for methyl groups for DNA, RNA and histone methylation. It works as a fuel for DNMTs and Histone methyltransferases, facilitating post-transcriptional modifications. SAM formation is regulated by one-carbon metabolism linking the methionine and folate cycles. Methionine originates from dietary sources or is regenerated from homocysteine and transformed into SAM by methionine adenosyl transferase (MAT), mainly by MATA2 in tumor cells. Serine supplies one-carbon units by the folate cycle which regulates the plasma levels of methionine and SAM. One-carbon metabolism is upregulated in tumor cells; thus, the nucleotide formation is increased, and SAM production is promoting epigenetic reprogramming. Increased serine and methionine levels result in increased SAM formation, which stimulates EZH2 activity and deposition of H3K27me3 [108, 109].

DNA hypomethylation results in activation of oncogenes that promote tumor formation. In tumor cells with low SAM, the methylation balance can be restored if SAM levels are replenished, as SAM is a methyl donor and could inhibit DNMTs thus promoting DNA hypermethylation. In gastric cancer, SAM induces hypermethylation of VEFG-C promoter, downregulates VEGF-C expression and leads to reduced tumor formation. Moreover, the dietary restriction of methionine or threonine reduces SAM levels and disturbs proliferation and histone methylation. SAM can inhibit oncogenic pathways in breast, liver and bone tumors, by hypermethylating genes that control metastasis [110]. Thus, metabolism and epigenetic regulation can be synergistically related and targeted by therapies, inhibiting tumor progression.

Another important mechanism of histone modification is represented by histone L-lactylation, which consists of the addition of lactyl groups to the ε amino group of a lysine residue from the structure of histones. The mechanism involves the production of lactyl-CoA from lactate and the transfer of the lactyl residues is most likely catalyzed by p300 [112]. Histone lactylation is directly stimulated in cancer cells due to the Warburg effect and the accumulation of intracellular lactate, leading to altered epigenetic landscapes that influence cancer progression [113]. Therefore, histone lactylation was investigated in the context of different types of cancer. It was found that histone lactylation

leading to high levels of H3K18la increases the proliferation and migration of pancreatic ductal adenocarcinoma cells by overexpressing *BUB1B* and *TTK* [114]. Elevated histone lactylation induced bevacizumab resistance in colorectal cancer patients, by upregulating *RUBCNL* [115]. Moreover, high levels of histone lactylation promoted breast cancer survival and proliferation, by upregulating the c-Myc-SRSF10 axis and driving MDM4 and Bcl-x splicing [116]. Additionally, histone lactylation contributes to the immunosuppressive tumor microenvironment by upregulating Il-10 expression of monocyte derived macrophages in glioblastoma [117]. Furthermore, all of these studies revealed that the direct or indirect impairment of glucose metabolism could diminish the effects of lactylation, providing anti-cancer effects [116].

The tricarboxylic (TCA) cycle represents a converging point of anabolic and catabolic metabolism, being involved in macromolecule synthesis, energy production and redox balance. The TCA cycle is composed of a series of enzymatic reactions taking place in the mitochondrial matrix and it is the main pathway for the generation of reducing agents like NADH and FADH2, that take part in the oxidative phosphorylation process [120]. It was shown that several enzymes involved in the TCA cycle can become mutated in cancer, leading to an altered metabolism and accumulation of TCA specific metabolites that shape the molecular landscape of the disease [121].

Succinate is metabolite produced in the TCA cycle in a reversible three-step reaction that is catalyzed by the enzyme succinyl-CoA synthetase. Succinate is then oxidized to fumarate by the enzymatic complex succinate dehydrogenase. Mutations in genes encoding this complex lead to a pathological accumulation of succinate in mitochondria. Succinate is a regulator of various enzymes involved in DNA and histone demethylation such as the Jumonji C-domain containing histone demethylases (JMJDs) and the Ten eleven translocation (TET) family of enzymes. The accumulation of succinate blocks the activity of these classes of DNA demethylases by product inhibition which can directly impact gene expression. Besides inhibiting DNA and histone demethylases, succinate can also influence the epigenome via its interaction with the transcription factor HIF-1a [122]. HIF-1a is a transcription factor that initiates the metabolic reprogramming of cells under hypoxic conditions. Structurally, it consists of two subunits—an oxygen sensitive alpha subunit and an oxygen insensitive beta subunit. Under normoxic conditions HIF-1a is degraded in a mechanism that involves the prolyl hydroxylase domain protein 2 (PHD2), an a-KG dependent enzyme, which catalyzes the hydroxylation of two proline residues from the ODD domain of HIF-1a. Upon hydroxylation, HIF-1a is recognized by the von Hippel Lindau (VHL) E3 ubiquitin ligase and undergoes ubiquitination and subsequent proteasomal degradation [123]. Studies have shown that there is a direct link between HIF-1a and histone modifications. It was reported that HIF-1a is involved in HDAC recruitment and regulation and that it can bind to specific promoter sites on histone 3 lysine 9 demethylases, inducing the expression of enzymes like JMJD1a and JMJD2A, JMJD2B and JMJD2C [124]. High levels of succinate can inhibit the catalytic activity of PHDs via product inhibition, leading to the stabilization of HIF-1a at normal oxygen levels and its subsequent metabolic and epigenetic reprogramming [122].

Fumarate is another TCA cycle metabolite that is produced from succinate in an enzymatic reaction catalyzed by SDH. Fumarate is then hydrated by fumarate hydratase (FH) and turned into malate, reaction that can take place in the mitochondrial matrix and cytosol. Under pathological conditions, FH can become deficient due to mutations,

leading to an increase in fumarate levels. It was determined that the accumulation of fumarate in FH deficient cells can contribute to the epithelial to mesenchymal transition (EMT) in cancer cells. Various genes that contribute to EMT, such as *Snai2* and *Zeb1*/2 can be repressed by the antimetastatic miR-200 family members. High levels of fumarate can inhibit the activity of DNA demethylases like Tets, leading to the hypermethylation and repression of *miR-200* and the expression of EMT markers [125]. Furthermore, fumarate is associated with cell growth under nutrient deficient conditions. In normal cells, during nutrient deficiency, AMPK phosphorylates FH and the transcription factor ATF2, followed by their subsequent binding to promoter regions of genes involved in cell growth arrest. Here, the catalytic activity of FH increases the levels of fumarate, leading to the inhibition of the histone demethylase KDM2A and stabilizing H3K36me2, which eventually triggers the transcription of genes involved in cell cycle arrest. In cancer cells, this mechanism is disrupted by the post-translational modification O-GlcNAcylation of the FH-AMPK phosphorylation sites, leading to the impairment of FH-ATF2 signaling and abnormal cell growth under nutrient deficient conditions [126].

7 Technological advances in integrated metabolomics and epigenomics

Recent technological advances in integrated metabolomics and epigenomics are characterized by three main developments: high-throughput methylation sequencing, stable isotope-resolved metabolomics (SIRM) and complex multi-omics data integration approaches.

7.1 High-throughput methylation sequencing

High-throughput methylation sequencing has undergone significant technological advances, particularly relevant for integrated metabolomics and epigenomics. This field has shifted from traditional bisulfite-based methods, which are prone to DNA degradation and sequence bias, towards enzymatic conversion techniques such as Enzymatic Methyl-Seq (EM-seq) which utilizes TET2, T4-BGT and APOBEC enzymes to produce and convert cytosines, enabling accurate and degradation free methylation profiling with low DNA input and high library yields. This feature is critical for multi-omics studies where sample quantity is usually limited [221]. Recent optimizations have been made for targeted methylation sequencing (TMS) for cost efficient process and scalability, allowing population-scale studies and multispecies applications, maintaining the high concordance with gold-standard methods and making them suitable for integration with other omics data (e.g. transcriptomics, metabolomics) to investigate regulatory networks and specific disease mechanisms [222, 223].

Long-read sequencing (LRS) technologies (e.g. Oxford Nanopore, PacBio platforms) enable direct detection of methylated bases and simultaneous assessment of genetic and epigenetic information at single-molecule resolution, facilitating comprehensive epigenomic profiling, including chromatin state and transcriptional dynamics. LRS is increasingly used in multi-omics integration to link methylation patterns with metabolic and transcriptional changes [224, 225].

Single-cell methylation sequencing methods further expand the ability to resolve cellular heterogeneity and rare cell populations, which is critical for integrated analysis in complex tissues and diseases states [190]. The single-cell methylation sequencing methods started with in 2008 with the RRBS-based single cell DNA methylation methods,

which measure enrich CpG dense regions [226], followed by Whole-Genome Bisulfite Sequencing in 2009 [227], restriction enzyme-based methods in 2011 [228], and continued from 2012 the PBAT with post-bisulfite adapter tagging, scRRBS–single tube reaction, scBS-seq with adapter ligation prior to bisulfite treatment, combined multiplex qPCR (SCRAM), scPBAT with amplification free, snmC-seq–with application of adaptase, Sci-MET–with random hexamers, MscRRBS–streamlined library preparation, MID-RRBS–using microfluidics, scXRBS–using unphosphorylated adapter and random hexamer to add the second adapter, scTAM-seq using Tapestri platform, sciMET with linear amplification or splint ligation [229–239].

Conventional sequencing requires a large number of cells and offers an average genetic, epigenetic and transcriptional landscape. Bulk sequencing cannot highlight the events in rare cells or specific subpopulations. Thus, single cell sequencing technologies provide tools that offer a precise profile of the DNA methylation of individual cells, with applications focused on cancer evaluation and specific disease development [240].

Of course, several downsides and limitations are associated with drugs or reagents used in high-throughput methylation sequencing studies, particularly in the context of integrated metabolomics and epigenomics research, including DNA degradation, sequence bias or incomplete conversion. Bisulfite-based methods, which use sodium bisulfite to convert unmethylated cytosines to uracil, are prone to substantial DNA fragmentation and loss, especially with low-input samples, leading to reduced library complexity and potential bias in methylation calls [241–243]. This degradation can negatively impact downstream multimodal omics integration, especially when the amount of sample is limited. Enzymatic conversion strategies (e.g., EM-seq) reduce damage to DNA but require prolonged laboratory processing time and may impact batch variability based on enzyme efficiencies and reaction conditions. Affinity methods (e.g., MeDIP-seq) and restriction methods (e.g., MRE-seq) can be subject to incomplete enrichment or digestion, thereby establishing unequal CpG coverage and quantitative inaccuracy, possibly undermining the reliability of integrated analyses, respectively [244, 245].

High-throughput methylation sequencing can reveal metabolic-epigenetic mechanisms by enabling single-base resolution mapping of DNA methylation and its oxidation products – 5mC, 5-hmC, 5-fC and 5-caC across the whole genome. The technique offers a perspective to directly link changes in methylation patterns to metabolic pathways that control the availability of methyl donors and other cofactors for key epigenetic enzymes, connecting cellular metabolism to epigenetic regulation of various genes [246]. Oxidative bisulfite sequencing, bisulfite sequencing and TET-assisted bisulfite sequencing provides detailed mapping of cytosine modifications demonstrating that variations in the metabolic state can alter DNA methylation dynamics, which affect chromatin structure and gene expression. By profiling these modifications at a single-base resolution, it could be clarified how metabolic fluxes can modulate epigenetic marks, highlighting the potential direct mechanistic link between the two processes [247]. Thus, the integration of methylome data with chromatin accessibility profiles and transcriptomics could reveal that metabolic perturbations can promote epigenetic changes and impact cells and disease development. The insights obtained about these mechanisms are possible due to the precise and accurate high-throughput methylation sequencing technologies.

7.2 Stable isotope-resolved metabolomics (SIRM)

SIRM is an analytical approach that uses isotope-labeled substrates such as 13C-glucose, 15N-glutamine or 2H-water, that are administered to biological systems and incorporate these isotopes into downstream metabolites which can be tracked using high-resolution mass spectrometry or nuclear magnetic resonance (NMR) spectroscopy. This approach enables the direct measurement of metabolites fluxes indicating the dynamic activity of specific metabolic pathways, but not directly the static metabolite concentrations [248–250].

Studies that combine metabolomics and epigenomics use Stable Isotope Resolved Metabolomics (SIRM) to better understand the link between metabolic pathway activity and epigenetic modification. By measuring the flux of carbon or nitrogen through key metabolic pathways, such as glycolysis, the TCA cycle, and amino acid metabolism, SIRM can link these fluxes to the substrate availability of metabolites such as S-adenosylmethionine and acetyl-CoA that are used in the biological methylation or acetylation of DNA or histones. This work serves to elucidate mechanistic connections between cellular metabolism and epigenetic regulation, thereby linking changes in nutrient usage in conditions such as cancer or metabolic diseases with changes in DNA methylation patterns. SIRM is also used in conjunction with pharmacological controls and time-course labeling to improve the interpretation of metabolic fluxes and their impact on epigenomic states, supporting robust multi-omics integration and systems-level understanding of disease mechanisms [251–254].

Stable isotope tracers used in SIRM are normally considered safe and pose no negative effects in the doses used in clinical and research applications. This is well-supported by the considerable amount of data available from many human and animal studies, including pediatric studies, for the most used tracers, such as 13C, 15N, 18O. The only exception is in the case of deuterium, or 2H (heavy water), which may be toxic at very high concentrations. However, the threshold of toxic effects is well above the doses used in metabolic research. For all tracers, assurance should be made that chemical purity, stability and, if given intravenously, sterility and freedom from pyrogens are assured to prevent non-specific reactions. Limitations of SIRM tracers include the requirement to establish both metabolic and isotopic steady-state for accurate measurements of flux, the possibility of secondary labeling (e.g. labeled lactate from labeled glucose), and the potential for isotopic exchange phenomena which may confound the accurate evaluations of net metabolic flux. If these technical limitations are not rigorously considered in experimental design and data analysis, artifacts or errors in the interpretation of pathway activity may result [255–258]. Non-specific effects produced by stable isotope tracers are exceedingly rare in standard research doses and the limitation of the technique is due much more to technical and interpretation than to clinical limitations.

SIRM could reveal metabolic-epigenetic mechanisms by enabling direct tracing of nutrient-derived carbons into metabolites that are used as substrates for epigenetic modifications. Using stable isotope-labeled precursors like 13C glucose, 13C serine or 13C glutamine to track the incorporation into key epigenetic donors like SAM or acetyl CoA, both required for histone and DNA methylation or acetylation [259, 260].

Traditional metabolomics used for clinical purposes differ in that SIRM provides dynamic, mechanistic understanding of the interaction between epigenetic factors and metabolic pathways as opposed to simply measuring the static concentration of

metabolites. For example, through the measurement of metabolite abundance using traditional metabolomics, a biological marker can be identified or a change in metabolic state may occur. However, traditional metabolomics do not allow the identification of the source of metabolite and cannot provide the measure of metabolic fluxes. Using SIRM, manufacturers use isotopically labeled molecules to track how nutrients that are labeled are incorporated into metabolic products. This enables the measurement of the effect of metabolism on genes and histones through various modifications, including DNA and histone acetylation or methylation. One sounding example is the use of stable isotope tracing to show that increased serine flux can enhance SAM synthesis and methylation, and glutamine metabolism can alter the acetyl-CoA pools, influencing histone acetylation [259, 261].

7.3 Multi-omics data integration approaches

Approaches for integrating multi-omics data in studies of stable isotope-resolved metabolomics (SIRM) and epigenomics can be classified into high, mid, and low-level integration of omics data through concatenation (low-level), transformation (mid-level), and model-based (high-level) approaches, in addition to methods such as machine learning and network approaches [262–264].

Concatenation methods involve integrating raw data matrices or normalized matrices from the different omics levels into a common data set for combined analysis of the omics data. This method is easy to apply and allows for combined statistical modeling, but it is subject to problems such as data scale differences, missing data, and high dimensionality, which limit the ability to detect biological signals. Transformation methods include transforming each omic layer into major features, such as principal components or latent variables, and then integrating the transformed data. This method improves the interpretability of the data and the amount of noise but can lose information about small interactions of the omic layers during integration [265, 266].

There is an increased use of Machine-Learning-based techniques (both supervised and unsupervised) to discover complex, non-linear relationships and predictive biomarkers in large molecular datasets, such as SIRM and epigenomics. While these techniques can enhance classification and prediction accuracy, they require a large sample size and rigorous validation to ensure generalizability. Each method has its own specific advantages with respect to interpretability, scalability and biological insight, though there is a problem with data heterogeneity, batch effects and the overall requirements of a strong computational infrastructure and specific expertise [267, 268].

Currently, model-based and network-based approaches in the integration of multi-omics data can yield the most biologically-relevant and clinically-translatable insights in studies that relate the metabolic fluxes, as measured by stable isotope resolved metabolomics (SIRM), with epigenomic changes in human disease. Model-based strategies, particularly those which employ constraint-based stoichiometric metabolic flux models, allow for integration of SIRM data with transcriptomics and epigenomics and the modelling of regulatory hierarchies of metabolic flux. Such approaches can provide insight into whether variations in metabolism are due to gene expression changes, differences in substrate availability or post translational modifications, thus providing key insights into metabolic processes that are necessary for the identification of therapeutic targets for the disease and their treatment in a personalized manner [265, 269]. Network-based

integration approaches on the other hand are the trans-omic and pathway-based approaches. These allow detailed bioinformatic network modelling of biological networks portraying the interactions of metabolites, epigenetic markers, and a variety of macromolecular species both directly and indirectly. Such analyses allow the identification of key clocks and functional modules, enhancing the identification of novel and translatable markers and pathways in complex diseases. Notably, the use of pathway-based integration substantially improves interpretation and statistical power thus making these methods particularly beneficial for translation of multi-omic discovery into clinical applications [270].

The use of statistical methods to integrate various forms of omics (genomic, transcriptomic, epigenomic, proteomic, and metabolomic) can provide insight into complicated biological processes. Different ways that these multi-omics data integration approaches utilize these different types of omics datasets include early integration (combining raw data sets), intermediate integration (combining processed omics data features), and late integration (combining multiple analysis results). Other methods provide hierarchical or mixed modes of omics data integration and/or utilize network approaches to model interactions across the different types of omics datasets. Advanced computational protocols leverage mathematical modeling and network analysis to identify cross-pathway regulatory links, such as those connecting transcription factors, signaling proteins, and metabolic enzymes [263, 271].

Multi-omics studies demonstrate that metabolic molecules impact chromatin structure and gene expression through a metabolic-epigenetic relationship. For instance, specific metabolic molecules can serve as substrates and/or cofactors for enzymes that modify chromatin, thus connecting cellular metabolism with chromatin structure, as seen in their effects on gene expression. Importantly, integrative analyses suggest that metabolic enzyme activity directly influence chromatin dynamics after which these dynamic chromatin states in turn affect metabolic enzyme activity, establishing multiple points of interdependence between metabolic enzymes and the activity of all other components of a metabolic network; metabolic enzyme activity influences, through PTMs, the activity of other enzymes; and other enzyme activities influence the production of, and the activities of, PTMs. These results highlight the significant and critical function of integration of metabolic and epigenetic data through multi -omics in understanding how metabolism and epigenetics cooperate to facilitate adaptation of cells, define mechanisms of disease, and identify potential therapeutic targets [272].

Abnormal metabolism affects the amount of glucose that goes through the glycolytic pathway. Because of this, there will be altered levels of metabolites replaced by acetyl-CoA or S-adenosylmethionine. These two metabolites serve as substrates for the addition of acetyl groups to histones or the addition of methyl groups to DNA, which can change chromatin structure and gene expression. These types of changes in metabolism can promote cancer development and increase the rate of tumor growth. Combining metabolic and epigenetic therapies has shown potential in treating cancer because metabolic inhibitors may change the way that epigenetic drugs work on a tumor and make tumors more responsive to epigenetic therapy [273].

8 Future perspectives and challenges

Personalized medicine is a revolutionary approach in oncology, providing important advancements in cancer therapy. This concept refers to developing medical approaches that are based on the unique molecular and genetic profile of each patient's tumor, yielding better therapy outcomes. The basic tools of cancer biomarker detection are represented by immunohistochemistry (IHC), polymerase chain reaction (PCR), or next-generation sequencing (NGS) approaches. Recent technological advances like whole exome sequencing (WES), whole genome sequencing (WGS) and RNA sequencing (RNA seq) led to more complex molecular analyses, providing more specific data about a patient's tumor. Some of the best-known examples of cancer biomarkers consist of the EGFR mutation in lung cancer, BRAF mutations in melanoma, HER2 amplifications in breast cancer or cKIT mutations in gastrointestinal stromal tumors [274].

Next-generation sequencing (NGS) is a novel technology capable of massive simultaneous sequencing of millions of DNA or RNA sequences. NGS comes with a series of advantages over traditional sequencing methods, like higher throughput, sample multiplexing, higher sensitivity for detecting low-frequency variants and reduced costs. NGS has various uses in oncology, being able to detect SNVs, small indels, CNVs and fusion genes in solid or hematological tumor samples. Targeted gene sequencing using predetermined panels is the standard practice in clinical laboratories for cancer diagnosis. Small NGS panels, including less than 50 genes, can be applied for specific cancers such as acute myeloid leukemia or breast cancer. The Thermo Fisher Oncomine Dx Target Test was approved by the FDA in June 2017. The test can evaluate mutated variants in a series of genes associated with non-small cell lung cancer, including *BRAF* V600E, *EGFR* L858R, *EGER* exon 19 deletions and *ROS1* fusions. The Illumina TruSight Oncology 500 test is another NGS panel approved by the FDA. The test targets 523 genes for SNV and indel detection and 55 genes for fusion and splice variant detection [275].

8.1 Liquid biopsies—ctDNA, cfDNA and CTCs

The liquid biopsy technology allows the uncovering of molecular characteristics of a patient's tumor by analyzing blood or other liquid samples from a patient. The technique is usually based on analyzing circulating extracellular nucleic acid such as cell free DNA (cfDNA) and circulating tumor DNA (ctDNA) or circulating tumor cells (CTCs) which are cells shed from the tumor site. Moreover, CTCs can undergo different genomic, transcriptomic or proteomic analyses [276].

Cell-free DNA is DNA released from healthy or diseased cells from the body, that is freely circulating in the blood. Cell-free DNA is constantly being cleared from the bloodstream and comes with the advantage of providing information about the molecular characteristics of dying cells throughout the body, acting as a great biomarker [277].

Circulating tumor DNA is a subset of cfDNA that is of tumor cell origin. Different studies have shown that the concentration of cfDNA, including ctDNA, is higher in cancer patients compared with healthy individuals. Moreover, it was shown that ctDNA enables the detection of different cancer associated mutations such as point mutations in the N-Ras or K-Ras genes [278]. The concentration of ctDNA in the blood was shown to be correlated with disease stage and prognosis, late-stage patients showing a 100-fold increase compared with early-stage patients, high ctDNA concentrations being associated with worse survival outcomes. In addition, ctDNA analysis could provide an earlier

detection of disease and tumor localization, as it was shown that cancer associated mutations can be detected in the plasma years before diagnosis and that ctDNA possesses tissue and cell specific methylation patterns [279]. Therefore, the use of ctDNAs to guide clinical decision making regarding oncological patients is increasing, improving diagnosis and disease management [278].

NGS is not limited to mutation detection, as in different studies it is used for methylation profiling, chromatin accessibility assays, mapping histone modifications or for transcriptome analysis. The methylation profiling uses NGS in methods such as bisulfite sequencing, technique that enables a single-base resolution mapping of DNA methylation pattern across the whole genome. This is the golden standard for DNA methylation analysis and is a useful tool both for research and clinical approaches [225, 280]. Chromatin accessibility can be assessed by NGS using assay for transposase-accessible chromatin sequencing (ATAC-seq) to identify open chromatin regions and active regulatory elements, and complex protocols such as methyl-ATAC-seq integrate methylation evaluation with accessibility measurement, in a single assay [281]. ChIP-seq is used for mapping histone modifications, using NGS to identify genome-wide binding sites of histone marks and chromatin associated proteins [282]. On the other hand, RNA seq is using NGS to quantify gene expression and define transcriptional reprogramming, offering insights into transcriptome dynamics and information about the alternative splicing [283]. NGS platforms enable comprehensive multi-omic profiling by integrating transcriptomic information with epigenetic and genetic data, for advanced molecular characterization, being the foundation in modern genomics and epigenomics research [224].

In oncology, methylation profiling is key for early detection of cancer cells, risk stratification and for monitoring therapy. Methylation patterns and detected aberrations serve as biomarkers in multiple tumors and are validated for clinical use, as the assays can detect cancer-associated epigenetic changes in tissue or cfDNA supporting personalized treatment strategies and non-invasive diagnostics [284, 285]. Chromatin accessibility assays and histone modification mapping are frequently applied to understand disease-specific regulatory landscapes, to identify epigenetic alterations that may trigger diseases, and to stratify patients on molecular subtypes. These approaches are compatible with clinical sample preservation and single cell analysis, thus even heterogenous samples can be tested for biomarker discovery and precise disease stratification [286]. The metabolic crosstalk with epigenetic changes can be assessed by NGS-based profiling, which reveals how metabolic gene mutations such as IDH1/2 disrupt DNA methylation patterns and chromatin architecture, leading to oncogenesis. Integrating methylation and chromatin accessibility perspectives with metabolic pathway analysis could lead to a better understanding of the disease mechanisms and the molecular triggers, supporting the therapeutical approaches targeting both metabolic pathways and epigenetic mechanisms [287, 288].

Collectively, these technologies are revolutionizing clinical diagnostic and precision medicine by offering accurate and comprehensive molecular insights for disease classification, prognosis and therapeutic targeting.

9 Conclusion

Cancer cells undergo metabolic reprogramming to meet increased bioenergetic and biosynthetic demands, producing metabolites that serve as substrates or cofactors for epigenetic enzymes, influencing the chromatin structure and gene expressions. Conversely, epigenetic modifications can regulate the transcription of metabolic genes, shaping the metabolic phenotype of tumor cells. Recent advances in multi-omics technologies and single-cell profiling have enabled detailed mapping of these metabolic and epigenetic interactions, revealing spatial and temporal dynamics that contribute to tumor heterogeneity, immune evasion, or resistance to therapy. The metabolic-epigenetic axis impacts TME, immune cell function and promotes a tumor-permissive milieu.

Understanding metabolic and epigenetic interaction with one another in cancer has undergone substantial gains, however, there remain many issues that are of great importance. An example incident of this can be found from the group of bulk metabolome work, which yields important information; however, misses out on finding the differing types of metabolic processes occurring in the presence of different types of environmental influences relating to trans-generational change. New technologies are emerging that help identify the different metabolic processes occurring in an environment, using new types of multiplexes, imaging mass spectrometer techniques and modalities, however, due to a lack of sensitivity in their measurements, the process of determining the association between metabolomics and epigenomics will be extremely difficult.

A major limitation when examining clinical samples, is that metabolites are often unstable in their biological sample. Several biological samples contain metabolites with considerable instability and will degrade rapidly once the samples are removed from the tissue, subjected to an ischial event, or exposed to an extensive delay before processing out. This means that the metabolic data generated in a single biological sample will differ significantly based upon how well the sample was pre-analytically handled (including temperature fluctuations, freeze–thaw cycles, and duration of storage). Therefore, standardization of how tissues are collected, cryopreserved and then stored are critical for the metabolic profiles to have integrity when used for subsequent analysis with any retrospective data.

While there is substantial progress towards first establishing the relationship between metabolic pathways and epigenetic changes, it is a challenging process, particularly in establishing causality. The metabolic profile(s) that are generated in biological samples often change frequently or rapidly, based upon the environment, whereas the epigenetic profiles experienced in a biological sample are the result of cumulative and delayed effects in the biological sample. For example, isolating individual metabolic regulation vs. secondary responses requires the use of isotope tracing techniques, time resolved studies with multi-omic technologies at a single-cell specificity using different forms of hybrid follow-up studies. In addition, computational models for building nonlinear networks of association between the metabolic and epigenetic systems continue to be developed.

As single-cell multi-omic technology, stable isotope resolved metabolomic techniques, and high-resolution spatial analysis improve, they will provide an important set of tools in identifying how these variables are coupled in terms of providing insight into the complex relationships between the two systems.

Therapeutically, targeting the metabolic-epigenetic circuit offers promising avenues for oncological treatments, including the development of agents that may disrupt critical metabolic pathways or epigenetic regulators, and combinatorial strategies that integrate metabolic, epigenetic and immunotherapies in a complex therapeutical approach. The coupling of metabolism and epigenetics is a central driver of malignant transformation, and its elucidation can offer novel insights and opportunities for precision oncology.

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Author contributions
All authors contributed to the conceptualization and design of the study., AI, CSC, OG, DK, AB, MN, AT, VT, SB and CC have prepared the materials collected the data and drafted the manuscript. ABT designed the figures in text. CT revised the manuscript and prepared the final version. All authors read and approved of the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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