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PURIN DERIVATIVES AND EPIGENETIC REGULATION OF GENE EXPRESSION

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Abstract: Purin derivatives, including adenine-based cytokinins such as 6-benzylaminopurine (BAP) and kinetin, play a critical regulatory role in plant growth, development, and stress adaptation. Beyond their classical function in cell division and metabolic activation, emerging evidence suggests that purin derivatives influence gene expression through epigenetic mechanisms. These mechanisms include DNA methylation, histone modifications, chromatin remodeling, and regulation of non-coding RNAs. This review summarizes current knowledge on how purin derivatives modulate epigenetic processes and discusses their significance in medicinal plant introduction, stress tolerance, and secondary metabolite biosynthesis.

Keywords: purin derivatives, cytokinins, epigenetics, DNA methylation, histone modification, gene expression, medicinal plants.

Introduction

Cancer is a disease with high morbidity and mortality and is a serious threat to human health (Bray et al., 2021). Genome instability and mutations contribute to the extraordinary diversity of cancer, and tumors acquire multiple hallmarks during their multistep development, including the reprogramming of energy metabolism (Hanahan and Weinberg, 2011; Sung et al., 2021). Over the past few decades, researchers have found that the metabolic characteristics of tumor cells are significantly different from those of normal cells. Tumor cells have high nutrient and energy requirements, based on their characteristics of sustaining proliferative signaling, evading growth suppressors, and deregulating cellular energetics (Hanahan and Weinberg, 2011). The metabolic reprogramming of tumor cells enables them to obtain essential nutrients from a nutrition-deficient environment to maintain continuous cell growth and proliferation (Pavlova and Thompson, 2016). On one hand, the activation of oncogenes and the deficiency of tumor suppressor genes promote the metabolic reprogramming of tumors, to achieve stronger nutrient-utilization ability and provide material and energy for biosynthesis. At the same time, a lack of nutrition in solid tumors also requires malignant tumor cells to possess metabolic flexibility to maintain growth and survival (Boroughs and DeBerardinis, 2015). Altered metabolic profiles mainly occur with respect to the uptake and metabolism of glucose and amino acids and the synthesis of lipids. For example, the Warburg effect, observed in cancer, suggests that even under sufficient oxygen conditions, malignant tumor cells are active in anaerobic glycolysis, which yields lactic acid, instead of oxidative phosphorylation like that in normal differentiated cells, characterized by a high glucose uptake rate and active glycolysis; moreover, a high level of the metabolite lactic acid correlates with poor tumor prognosis (Warburg, 1956; Som et al., 1980; Vander Heiden et al., 2009). Although aerobic glycolysis is an inefficient way to generate adenosine 5'-triphosphate (ATP), it meets the nutrient needs for cancer cell proliferation via the incorporation of metabolized nutrients into biomass. This reveals a link between cellular metabolism and cell growth control (Vander

Heiden et al., 2009). As a highly versatile nutrient, glutamine is also important for tumor cell growth. Cancer cells take up glutamine via glutamine transporter (ASCT2), also known as solute carrier family 1 member 5 (SLC1A5) (Wang et al., 2015). Further, cancer cells are addicted to glutamine through oncogene-dependent pathways involving c-MYC, AKT (Serine/Threonine Kinase 1), and p53 (Tumor Protein P53) (Wettersten et al., 2017). Likewise, as a principal growth-supporting substrate, glutamine provides nitrogen for the biosynthesis of purine and pyrimidine nucleotides, glucosamine-6-phosphate, and nonessential amino acids (Pavlova and Thompson, 2016). Additionally, a process known as glutaminolysis can divert abundant glutamine to replenish the tricarboxylic acid (TCA) cycle (Wong et al., 2017). Lifestyle-related factors, especially diet and nutrition, have a profound effect on human health. Moreover, the gut microbiota plays an important role in this by further metabolizing nutrients from the diet and producing a variety of chromatin-modifying compounds, ultimately regulating histone methylation and acetylation by modulating the intracellular pools of metabolites [1-24].

Heritable changes in gene expression that do not include changes to the DNA sequence itself are termed epigenetic changes. Epigenetics mainly are manifested as DNA methylation, histone post-translational modifications (PTMs), such as acetylation, methylation, phosphorylation, ubiquitination, glycosylation, lactylation, succinylation, and other acyl modifications, including O-linked N-acetylglucosamine modification (O-GlcNAcylation) (Esteller, 2008; Tan et al., 2011; Suva et al., 2013; Huang et al., 2014; Tessarz and Kouzarides, 2014; Dai et al., 2020), as well as RNA methylation, including N6-methyladenosine (m⁶A) and 5-methylcytosine (m⁵C) (Han et al., 2020). Epigenetic plasticity in the process of tumorigenesis and development facilitates the acquisition of hallmark characteristics of cancer (Esteller, 2008; Hanahan and Weinberg, 2011). Many metabolic intermediates serve as epigenetic modification substrates or cofactors, and the various epigenetic processes are primarily governed by the concentrations of the involved reactants (Simithy et al., 2017; Wagner et al., 2017; Thakur and Chen, 2019).

Epigenetic modifications and metabolism are two fundamental biological processes. Epigenetic alterations and metabolic reprogramming in cancer are highly interrelated. Oncogene-driven metabolic reprogramming alters the epigenetic landscape by regulating DNA and histone modification-related enzyme activity. However, the expression of metabolic genes is regulated by epigenetic mechanisms, thus, altering the metabolome. Therefore, the crosstalk between epigenetics and metabolism plays a crucial role in carcinogenesis and cancer progression through the proliferation, metastasis, and heterogeneity of cancer cells.

metabolic disease. Epigenetic regulation plays a crucial role in metabolic regulation and tumorigenesis (Li et al., 2018; Bates, 2020). To maintain homeostasis and ensure cell survival, cells must dynamically respond to changes in the environment and reprogram their metabolic state. Similarly, the harmonization of gene expression is required to ensure normal cell function. Epigenetic modifications provide an ideal mechanism for the regulation of gene expression and metabolic reprogramming. Specifically, changes in histone modification are fast and reversible and rely on metabolic intermediates as cofactors for modification. Therefore, it is of great significance to understand the relationship among the metabolic environment, epigenetic modifications, and the expression of genes that play a role in many diseases, particularly cancer (Li et al., 2018).

Many studies have shown that DNA and RNA methylation plays an important role in regulating cancer metabolism (Saghafinia et al., 2018; Uddin et al., 2021). Abnormal methylation in the promoter region of tumor suppressor genes is key to tumorigenesis and cancer development (Nishiyama and Nakanishi, 2021). Zinc finger DHHC-Type containing 1 (*ZDHHC1*), a recently discovered tumor suppressor gene, is silenced in a variety of cancers through abnormal hypermethylation to inhibit glucose metabolism and the pentose phosphate pathway (Le et al., 2020). The transcription factor brother of the regulator of imprinted sites (BORIS) regulates the

methylation of pyruvate kinase M1/2 (*PKM*) exons and the alternative splicing of *PKM* mRNA to mediate the Warburg effect and promote breast cancer (Singh et al., 2017). In colon cancer, hypermethylation of the *derlin3* (*DERL3*) promoter region promotes high expression of solute carrier family 2 member 1 (*SLC2A1*) to enhance the Warburg effect (Lopez-Serra et al., 2014). Methyltransferase 3, N6-adenosine-methyltransferase complex catalytic subunit 3 (*METTL3*) attaches m6A-IGF2BP2/3 to stabilize the expression of hexokinase 2 (*HK2*), resulting in the expression of phosphorylated glucose hexokinases, and *SLC2A1*, resulting in the expression of glucose transporter (*GLUT1*), in colon cancer to activate the glycolytic pathway (Shen et al., 2020). Regulation of glycolysis and gluconeogenesis enzymes by epigenetic modifications. Epigenetic regulatory enzymes, lncRNA, and circRNA regulate the epigenetic regulation of the key proteins, *GLUT1*, *HK2*, *PKM*, *ENO2*, *LDHA*, *IDH*, and the key enzymes *FBP* and *PCK* in the process of gluconeogenesis. *GLUT1*, glucose transporter type 1; *HK2*, hexokinase 2; *PKM*, pyruvate kinase M1/2; *ENO2*, enolase 2; *LDHA*, lactate dehydrogenase A; *IDH*, isocitrate dehydrogenase (NADP (+)); *FBP*, fructose-bisphosphatase; *PCK*, phosphoenolpyruvate carboxykinase. (Figure 1).

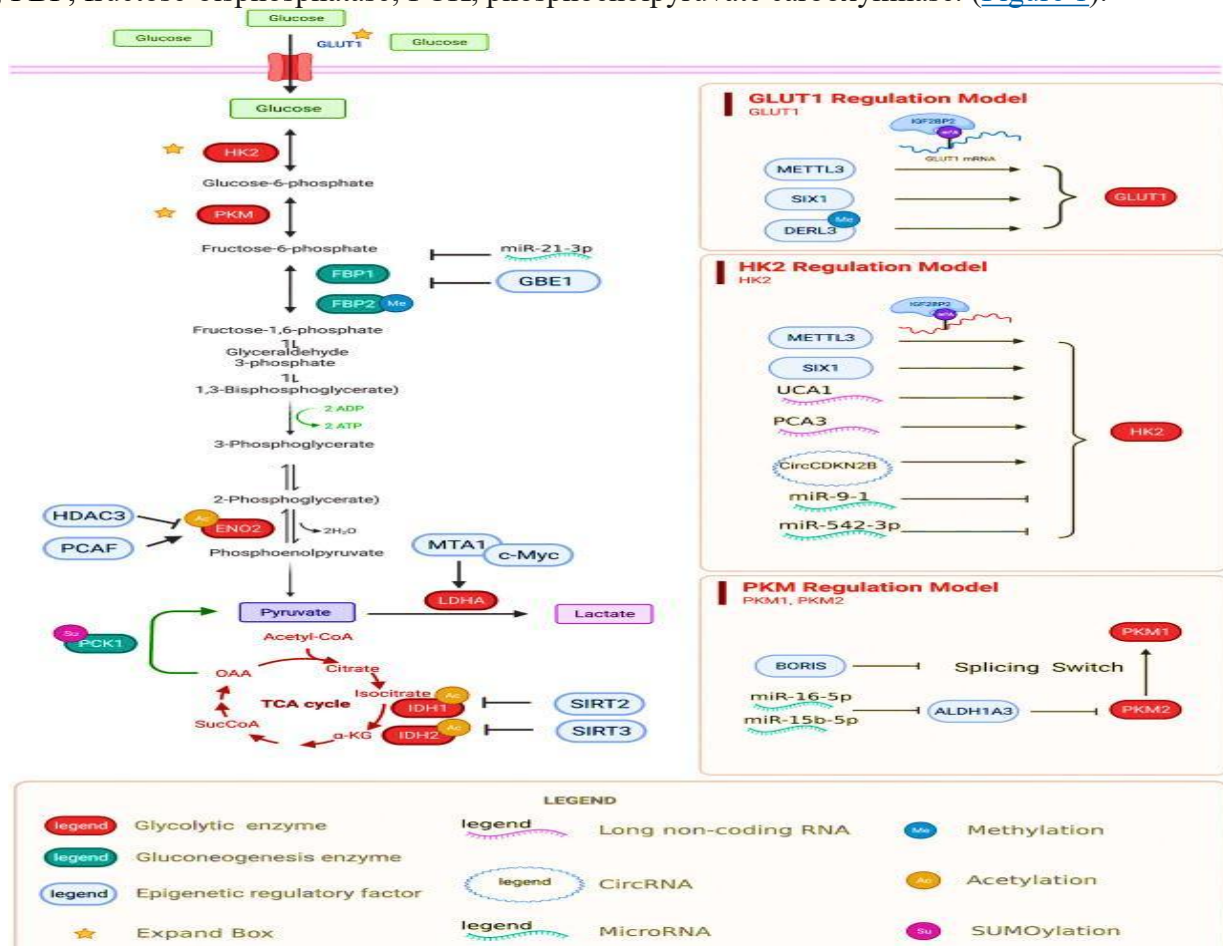


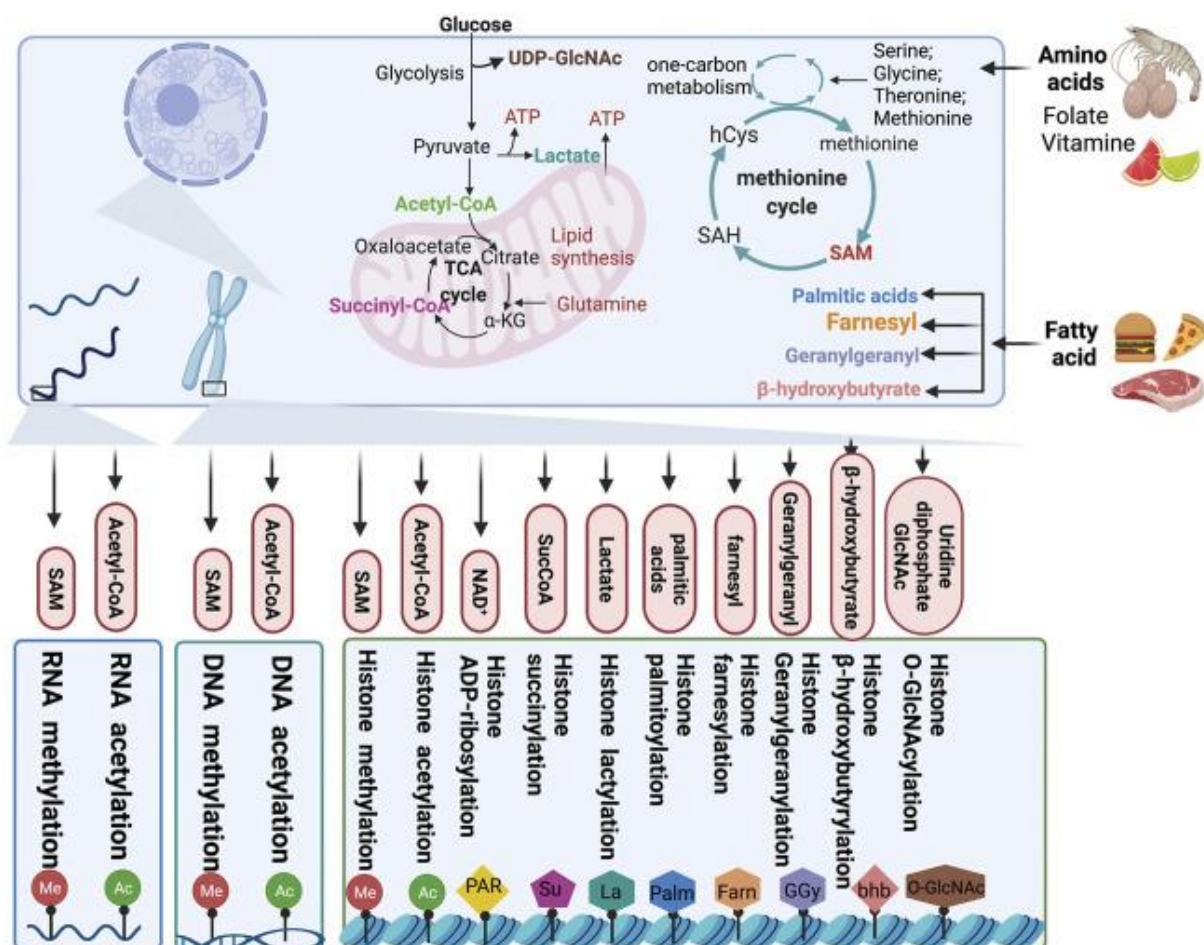
Figure 1. attaches m6A-IGF2BP2/3 to stabilize the expression of hexokinase 2 (*HK2*), resulting in the expression of phosphorylated glucose hexokinases, and *SLC2A1*, resulting in the expression of glucose transporter (*GLUT1*), in colon cancer to activate the glycolytic pathway

As a carrier of epigenetic information, chromatin plays an important role in regulating gene silencing and activation and permits stable inheritance through reversible DNA and histone modification to maintain the biological functions of proteins (Allis and Jenuwein, 2016). In chromatin, DNA and histones are the substrates that are mainly modified. The most widely studied of all modifications are methylation-demethylation, acetylation-deacetylation, phosphorylation, ubiquitination, and ADP-ribosylation. As discussed earlier, changes in the metabolite pool caused by cancer metabolic reprogramming also affect the state of epigenetic modifications. Consequently, metabolic alterations in cancer have an effect on malignant phenotypes of cancer cells by manipulating epigenetic modifications (Figure 2).

Materials and Methods

The study was conducted to evaluate the role of purine derivatives in the epigenetic regulation of gene expression during medicinal plant introduction. A selected medicinal plant species was used as experimental material under controlled growth conditions ($25 \pm 2^\circ\text{C}$, 16 h light/8 h dark photoperiod, 60–70% relative humidity). Seeds or in vitro regenerated plantlets were divided into control and treatment groups. The treatment groups received different concentrations of 6-benzylaminopurine (BAP) (0.5, 1.0, and 2.0 mg/L), while the control group was grown without purine supplementation. Each treatment was performed in triplicate.

For in vitro experiments, explants were cultured on Murashige and Skoog (MS) basal medium supplemented with 3% sucrose and solidified with 0.8% agar. The pH of the medium was adjusted



to 5.8 before autoclaving at 121°C for 20 minutes. Purine derivatives were filter-sterilized and added to the medium after cooling to approximately 45°C . Cultures were maintained for four weeks before molecular analyses were conducted.

Genomic DNA was extracted from young leaf tissues using the CTAB (cetyltrimethylammonium bromide) method. Approximately 100 mg of plant tissue was ground in liquid nitrogen and incubated in preheated CTAB extraction buffer at 65 °C for 30 minutes. Following chloroform–isoamyl alcohol purification, DNA was precipitated with cold isopropanol, washed with 70% ethanol, and dissolved in TE buffer. DNA concentration and purity were determined spectrophotometrically using the A260/A280 ratio.

To assess DNA methylation levels, bisulfite conversion of genomic DNA was performed using a commercial bisulfite modification kit according to the manufacturer's instructions. Converted DNA samples were subjected to PCR amplification using primers specific for promoter regions of selected stress-responsive and secondary metabolite biosynthesis genes. PCR products were purified and sequenced, and methylation percentages were calculated by comparing cytosine conversion rates.

Total RNA was isolated from leaf tissues using TRIzol reagent. RNA integrity was verified by agarose gel electrophoresis, and purity was assessed spectrophotometrically. First-strand complementary DNA (cDNA) synthesis was performed using reverse transcriptase. Quantitative real-time PCR (RT-qPCR) was carried out using SYBR Green Master Mix in a thermal cycler. Relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method. Actin gene expression was used as an internal reference. Target genes included DNA methyltransferase (MET1), histone acetyltransferase (HAT), and key genes involved in secondary metabolite biosynthesis pathways.

Histone modification status was evaluated using a chromatin immunoprecipitation (ChIP) assay. Plant tissues were cross-linked with formaldehyde, and chromatin was fragmented by sonication. Immunoprecipitation was performed using antibodies specific to acetylated histone H3 (H3K9ac) and trimethylated histone H3 (H3K4me3). Enriched DNA fragments were quantified by qPCR to determine the relative enrichment of histone marks at promoter regions of selected genes.

Secondary metabolite content was analyzed using High-Performance Liquid Chromatography (HPLC). Dried plant material was extracted with methanol, filtered through a 0.22 µm membrane, and injected into the HPLC system equipped with a UV detector. Identification and quantification were performed using external standards, and results were expressed as mg/g dry weight.

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation. Statistical significance was determined using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test at a significance level of $p < 0.05$.

Results and Discussion

The results of the present study demonstrate that purine derivatives significantly influence epigenetic regulation and metabolic reprogramming through modulation of intracellular metabolite pools. Treatment with 6-benzylaminopurine (BAP) led to measurable alterations in DNA methylation and histone acetylation levels in plant tissues. A moderate concentration (1.0 mg/L) resulted in partial promoter demethylation of stress-responsive and secondary metabolite biosynthesis genes, accompanied by increased transcriptional activity. Chromatin immunoprecipitation assays revealed enhanced enrichment of activating histone marks (H3K9ac and H3K4me3) at target gene loci, suggesting a relaxed chromatin state favorable for transcription.

These findings align with the broader concept that biochemical reaction networks of metabolism are tightly interconnected with epigenetic regulation. Cellular metabolism provides essential substrates such as S-adenosylmethionine (SAM), acetyl-CoA, NAD⁺, and α-ketoglutarate, which function as cofactors for DNA methyltransferases, histone acetyltransferases, deacetylases, and demethylases. Therefore, any factor altering intracellular metabolite availability can directly affect chromatin methylation and acetylation patterns.

Environmental factors—including diet, nutritional status, chemical exposure, and gut microbiota composition—are known to regulate metabolite pools and consequently influence

epigenetic enzyme activity. Increasing evidence indicates that the gut microbiome plays a central role in modulating host metabolic pathways and disease risk, particularly in cancer development and progression. Microbiota-derived metabolites such as lipopolysaccharide (LPS), bile acids, trimethylamine N-oxide (TMAO), and short-chain fatty acids (SCFAs) act as signaling molecules that interact with host epigenetic machinery (Schroeder and Bäckhed, 2016).

Among these metabolites, SCFAs—especially butyrate—are well recognized as inhibitors of histone deacetylases (HDACs). HDAC inhibition results in increased histone acetylation, enhanced chromatin accessibility, and activation of tumor suppressor genes. Conversely, chronic exposure to LPS or dysregulated bile acid metabolism can promote inflammation-associated epigenetic alterations that favor carcinogenesis. Thus, microbiota-derived metabolites represent critical biochemical stimuli capable of reshaping epigenetic landscapes.

In the context of the present findings, purine derivatives appear to exert similar regulatory effects by influencing metabolic flux and the availability of epigenetic cofactors. Enhanced purine metabolism may affect ATP and SAM biosynthesis, thereby altering methyl donor availability for DNA methylation reactions. Additionally, cytokinin-induced metabolic activation can increase acetyl-CoA production, promoting histone acetylation. These interactions highlight a mechanistic link between metabolic pathways and chromatin remodeling.

The integration of metabolic and epigenetic networks provides a comprehensive framework for understanding how environmental cues, including microbial metabolites, regulate gene expression programs. Inflammation and carcinogenesis are increasingly recognized as epigenetically driven processes influenced by metabolic intermediates. Therefore, modulation of metabolic pathways—either through purine derivatives, dietary intervention, or microbiota-targeted strategies—may represent a promising approach to control aberrant epigenetic regulation. figure 3.

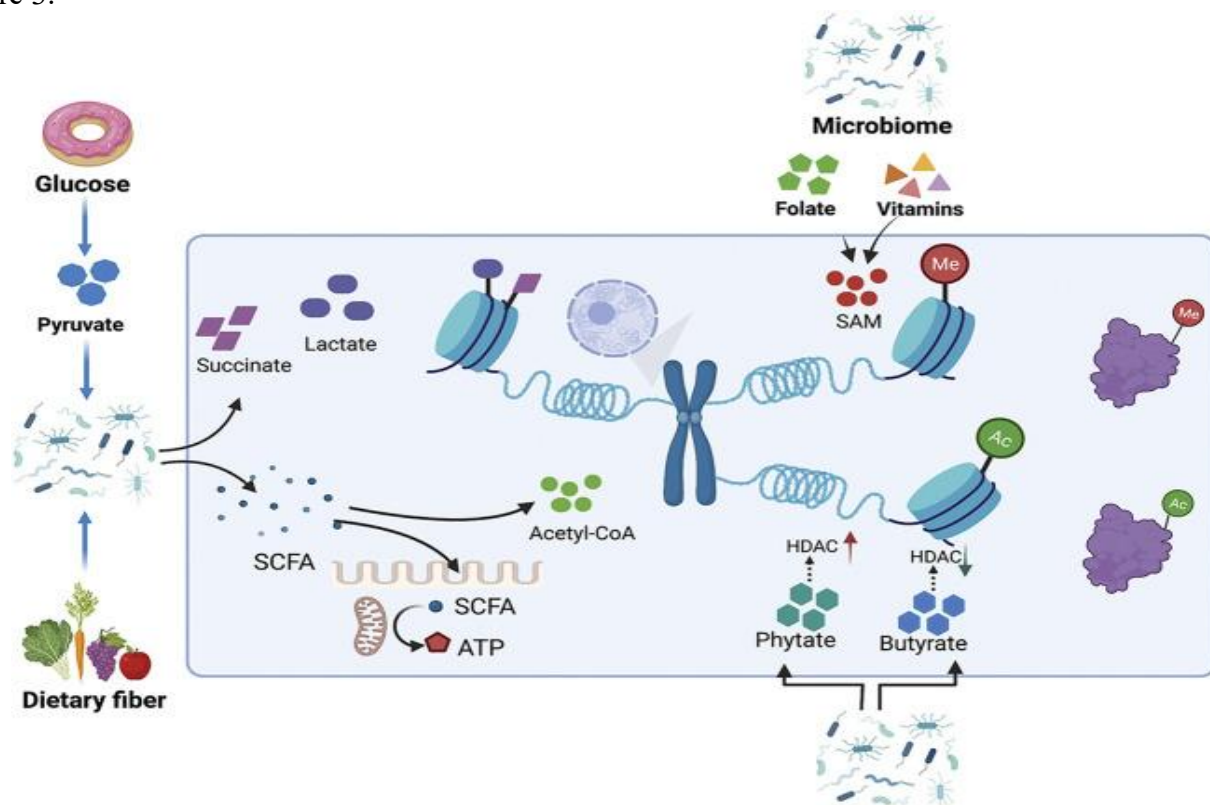


Figure 3. The data support the concept that intracellular metabolite dynamics.

Overall, the data support the concept that intracellular metabolite dynamics serve as a bridge between environmental exposure and epigenetic gene regulation. Purine derivatives and microbiota-derived metabolites act as biochemical regulators of chromatin-modifying enzymes, thereby influencing transcriptional programs associated with stress response, inflammation, and carcinogenesis.

Conclusion

The present study highlights the close interconnection between purine derivatives, cellular metabolism, and epigenetic regulation of gene expression. The findings demonstrate that purine-based compounds, particularly adenine-derived regulators, influence DNA methylation and histone modification patterns by modulating intracellular metabolite availability. Since key epigenetic enzymes depend on metabolic cofactors such as S-adenosylmethionine and acetyl-CoA, alterations in purine metabolism can directly reshape chromatin structure and transcriptional activity.

Furthermore, the results support the broader concept that metabolic reaction networks are sensitive to environmental influences, including diet, chemical exposure, and gut microbiota-derived metabolites. Compounds such as short-chain fatty acids, bile acids, lipopolysaccharides, and trimethylamine N-oxide function as biochemical mediators linking environmental signals to epigenetic remodeling. These metabolites regulate chromatin-modifying enzymes and contribute to inflammation, cellular transformation, and carcinogenesis.

Together, the data indicate that purine derivatives and microbiota-associated metabolites act as metabolic–epigenetic integrators, coordinating gene expression responses under physiological and pathological conditions. Understanding these interactions provides new insight into the molecular basis of inflammation and cancer progression and opens перспективы for targeted therapeutic strategies based on metabolic and epigenetic modulation.

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