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Cancer cell metabolism, epigenetics and the potential influence of dietary components – A perspective

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Abstract

Cancer cells are characterized by alterations in cell metabolism including increased glucose consumption and aerobic glycolysis, commonly known as the "Warburg effect". Underlying mechanisms for this metabolic switch have been associated with genetic defects in pathways regulating metabolic processes. Recent research indicates that epigenetic alterations, e.g. in DNA methylation of promoter regions of glycolytic enzymes, might contribute to the deregulated expression of enzymes involved in cell metabolism. Alternatively, changes in cell metabolism could alter the availability of co-factors for epigenetic processes. This perspective will summarize current knowledge on interactions between cancer cell metabolism and epigenetic modulation of gene regulation, and how both processes can be affected by dietary components. Natural compounds with reported mechanisms targeting cancer cell metabolism include folate, flavonoids, green tea polyphenols, genistein from soy, silymarin from milk thistle, and the anthraquinone derivative shikonin. Reversible acetylation of histones and non-histone proteins has been shown to affect cell metabolism and other cellular processes, and can be targeted by inhibitors of histone deacetylases (HDACs) and histone acetyl transferases (HATs). Natural compounds with HDAC or HAT inhibitory activity include butyrate, sulforaphane from broccoli, diallyldisulfide (DADS) from garlic, cambinol and dihydrocoumarin, diindolylmethane, epigallocatechin gallate (EGCG), curcumin, anacardic acid and garcinol. Currently we can only speculate whether their influence on epigenetic mechanisms is of importance for normalization of the deregulated cancer cell metabolism. It can be anticipated that the emergence of sensitive metabonomics technologies and genome-wide detection of epigenetic alterations in DNA methylation, histone modifications, and miRNA expression, will improve our understanding of the relationship between epigenetics and cancer cell metabolism.

Keywords: Cancer cell metabolism, Warburg effect, epigenomics, DNA methylation, histone modifications, metabonomics, dietary compounds, cancer prevention.

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Cancer cell metabolism

Glucose is one of the most important energy sources of proliferating cells (schematic summary of cell metabolism in Fig. 1). In the first step of energy production, glucose is metabolized by glycolysis to pyruvate. Under aerobic conditions, pyruvate is oxidatively decarboxylated by the pyruvate decarboxylase complex inside the mitochondria to form acetyl Coenzyme A (acetyl-CoA). Acetyl-CoA is then completely oxidized to CO_2 by the tricarboxylic acid (TCA) cycle. This process provides NADH, which is oxidized during mitochondrial respiration to generate

high amounts of ATP through oxidative phosphorylation (OXPHOS). Under anaerobic conditions, pyruvate can be reduced ('fermented') to lactate by lactate dehydrogenase (*LDH*). This process of glucose fermentation is less efficient in generating ATP than the TCA cycle [1]. In the early 1920s, Warburg first noticed that cancer cells consume more glucose than normal cells leading to increased production of lactic acid even in the presence of sufficient amounts of oxygen (designated as 'aerobic glycolysis'). This phenomenon is known as the "Warburg effect" [2,3]. Higher glucose uptake in tumors is used diagnostically for cancer detection by monitoring the incorporation of [^{18}F]-labeled deoxyglucose (FDG) in FDG-positron emission tomography.

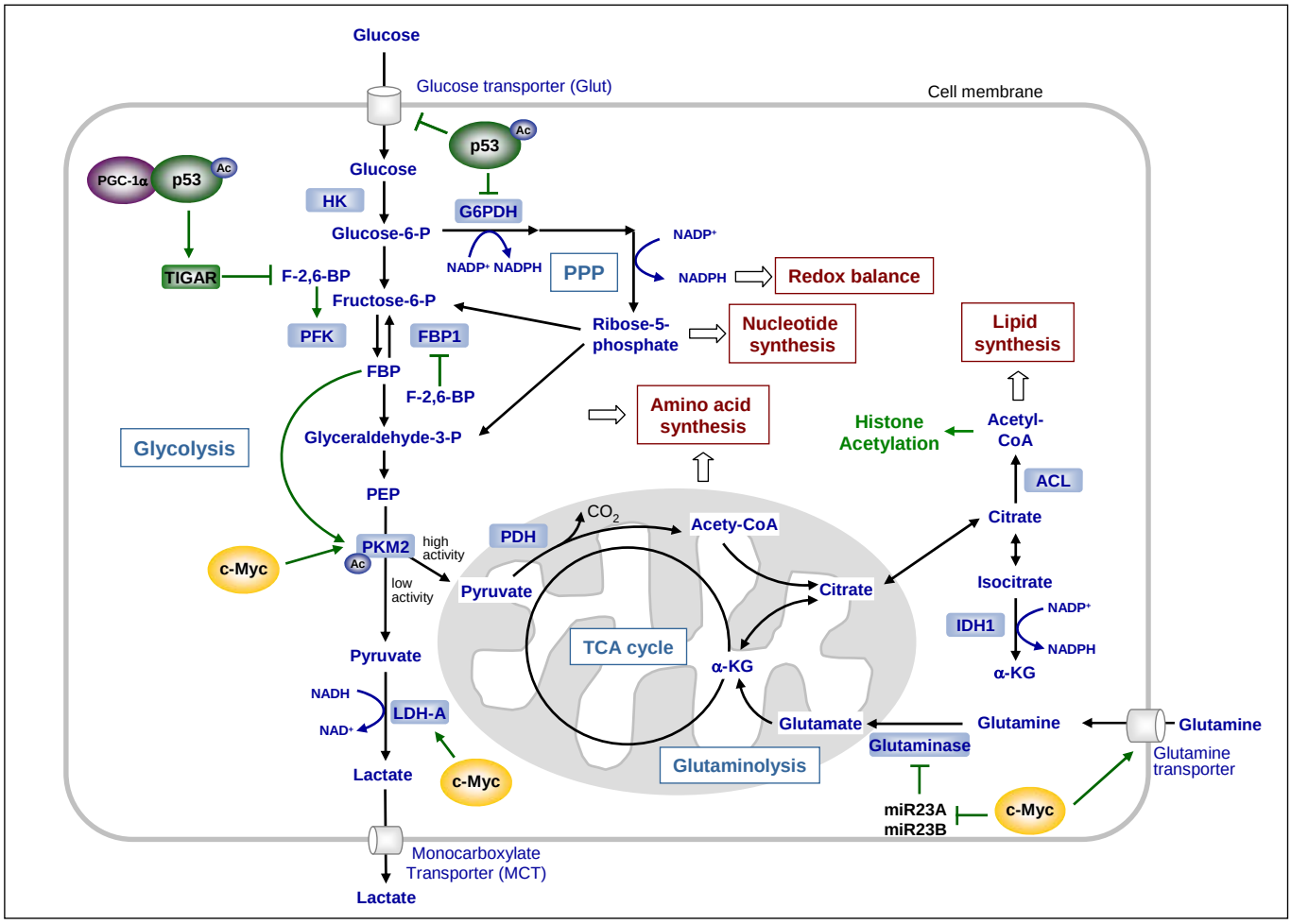

Figure 1. Interconnection of glycolysis, oxidative phosphorylation via the tricarboxylic (TCA) cycle, the pentose phosphate pathway (PPP) and glutaminolysis in proliferating cancer cells *(modified from [2,169,170]). Details are given in the text. α-KG, α-ketoglutarate; ACL, ATP citrate lyase; F-2,6-BP, fructose-2,6-bisphosphate; FBP, fructose-1,6-bisphosphate; FBP1, fructose-1,6-bisphosphatase; Fructose-6-P, fructose-6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; Glucose-6-P, glucose-6-phosphate; Glyceraldehyde-6-P, glyceraldehyde-6-phosphate; HK, hexokinase; IDH, isocitrate dehydrogenase; LDH-A, lactate dehydrogenase A-chain; NADPH, reduced nicotinamide adenine dinucleotide phosphate; p53, tumor suppressor protein p53; PDH, pyruvate dehydrogenase; PFK, 6-phosphofructo-1-kinase; PGC-1α, PPARγ-coactivator 1α; PKM2, pyruvate kinase isoform M2; PEP, phosphoenol pyruvate; TIGAR, TP53-inducible regulator of glycolysis and apoptosis protein*

So far, underlying mechanisms for the altered metabolic program in cancer cells are still not completely elucidated. Changes in the tumor's microenvironment, such as limited availability of oxygen and nutrients, may influence the metabolic switch from OXPHOS to aerobic glycolysis [4]. In addition, various genetic alterations in pathways regulating cell metabolism have been identified during the past years. These include for example the *PI3K* (phosphatidylinositol-3-kinase), *HIF* (hypoxia-inducible factor), *p53*, *c-Myc, Ras, AMPK* (AMP-activated protein kinase) and *LKB1* (liver kinase B1) pathways, as well as overexpression of glucose transporters (*Glut*), hexokinase

(*HK*) and other key enzymes involved in glycolysis [1,5-10]. Stimulated by the *c-Myc* oncogene, cancer cells exceedingly utilize glutamine as a carbon and nitrogen source as well as for the generation of ATP [1]. Eigenbrodt and his group identified that cancer cells adapt to the metabolic requirements of tumor growth by alterations in the activity of pyruvate kinase (*PK*) [11]. *PK* catalyses the rate limiting, ATP-generating step of glycolysis, in which phosphoenol-pyruvate (PEP) is converted to pyruvate. Surprisingly, cancer cells express high levels of the less efficient embryonic M2 isoform of *PK* (*PKM2*), resulting in the inhibition of glycolysis and reduced production of ATP. The advantage for cancer cells lies in the fact that intermediates of glycolysis accumulate and are available for alternative metabolic pathways. Rapidly dividing cells need biosynthetic intermediates for cell duplication during proliferation. Channeling glucose to aerobic glycolysis instead of OXPHOS enables the cell to export acetyl-CoA to the cytosol in form of citrate for the construction of fatty acids, to supply glycolytic intermediates for nonessential amino acid production, and to provide ribose-5-phosphate from the pentose phosphate pathway (PPP) for nucleotide synthesis. Additionally, the pentose phosphate pathway promotes the production of NADPH as an important cofactor that supplies reducing equivalents for many enzymatic reactions [9]. NADPH is also a key antioxidant required to control levels of reactive oxygen species (ROS) produced exceedingly during rapid cell proliferation [9]. Therefore, the altered cancer cell metabolism is likely to constitute a growth advantage to rapidly proliferating cancer cells [2].

Overview of epigenetic regulation

The term "epigenetics" refers to modifications in gene expression caused by heritable, but potentially reversible, alterations in chromatin structure and/or DNA methylation without changes in the DNA sequence [12]. Epigenetic mechanisms play an important role for diverse cellular processes, for example during development, tissue specific gene expression and memory formation [13,14]. However, epigenetic mechanisms are also involved in the development of age- and lifestyle-related diseases such as metabolic syndrome, cancer and Alzheimer's disease [14-16]. Best investigated epigenetic modifications comprise of DNA methylation, post-translational histone modifications including acetylation, methylation, phosphorylation, ubiquitination and sumoylation, and regulation of gene expression by non-coding (nc) RNA including microRNA [13,17]. These epigenetic modifications can be inherited to daughter cells. Thus epigenetic modifications have the potential to correct altered gene expression patterns that have been established as a consequence of environmental stimuli (e.g. nutrition, chemical exposure, radiation etc.) or as a result of signals in a cells microenvironment and therefore could act as a 'memory' for gene expression patterns [18].

DNA methylation

DNA methylation is considered as the best investigated epigenetic mechanisms. It is mediated by DNA methyltransferases *DNMT1*, *3a*, and *3b* that transfer methyl groups from S-adenosyl-*L*-methionine (SAM) to cytosines creating 5-methylcytosine (5mC) mainly when positioned next to a guanine (CpG dinucleotides). *DNMT1* is a maintenance methyltransferase that catalyze DNA methylation of the newly synthesized, unmethylated DNA strand after replication of a methylated sequence, thus assuring that DNA methylation patterns are transmitted to the next generation. On the other hand, *DNMT3a* and *DNMT3b* are 'de novo' methyltransferases that transfer methyl groups to DNA sequences not methylated before. Every normal tissue is characterized by a fine-tuned DNA methylation profile that represents the gene expression pattern within each cell type at a given developmental stage [19]. In general, CpG-rich sequences often located in gene promoter regions (CpG islands) are unmethylated, allowing the gene to be transcribed. In contrast, repetitive sequences such as ribosomal DNA repeats, satellite or centromeric repeats are often heavily methylated, thereby contributing to chromosomal stability by limiting their accessibility [20]. During ageing, carcinogenesis, or development of chronic diseases, this controlled pattern of DNA methylation is disrupted in two aspects: Global loss of 5-methylcytosine in tumor tissue (DNA hypomethylation) was first discovered almost 30 years ago [21]. This is mainly associated with loss of DNA methylation at repetitive genomic sequences, which is associated with chromatin changes that in turn cause genomic instability and chromosomal aberrations. On the other hand, increased methylation (DNA hypermethylation) of CpG islands in promoter regions is one of the most important epigenetic changes to occur in cancer cells, leading to transcriptional silencing of tumour suppressor and many other cancer relevant genes [15,20]. This list of epigenetically silenced genes includes genes with functions in cell cycle regulation (retinoblastoma protein *RB*, $p16^{INK4a}$, $p15^{INK4b}$, $p14^{ARF}$), DNA repair (*MGMT* and *hMLH1*), signal transduction (*RASSF1A, APC)*, apoptosis (*DAPK1, p53*, *caspase-8*), hormone response *(ER, PR, AR, RARβ*), carcinogen metabolism (*GSTP1*), angiogenesis (*maspin, thrombospondin 1*), and invasion or metastasis (*TIMP3, CDH1*) [20,22]. These changes are thought to be a key driving force in the development of cancer [15] and possibly of other chronic diseases. Earlier studies have been limited to investigations on the promoter methylation status of selected candidate genes. In recent years, DNA methylation profiling using gel-based techniques or DNA array hybridization have revealed that

the levels of epigenetically altered genes is in the order of thousands of genes in a cancer genome [19,20]. In contrast to genes irreversibly inactivated by genetic alterations, genes silenced by epigenetic modifications are still intact and can be reactivated by small molecules acting as modifiers of epigenetic mechanisms. Consequently, development of agents or food components that prevent or reverse the methylation-induced inactivation of gene expression is a new promising approach for cancer prevention [19].

Histone modifications

Post-translational modifications at the N-terminal histone tails of histones, including acetylation, methylation, phosphorylation, ubiquitinylation, sumoylation, and ADP ribosylation, contribute to epigenetic regulation of gene expression, genomic stability, DNA damage response, and cell cycle checkpoint integrity [23-25]. Histones are modified locally through sequence-specific transcription factors, or globally through multiple histone-modifying enzymes [25]. Two of the best investigated histone modifications include histone acetylation and histone methylation. Histone acetylation is mediated by the interplay of histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs add acetyl groups from acetyl-CoA as a cofactor to the ε-amino group of lysine residues in histone tails, whereas HDACs reverse histone acetylation and catalyze the transfer of acetyl groups to Coenzyme A. Perturbation of the balance between histone acetylation and deacetylation is considered as a key factor in neoplastic transformation. Acetylation of histone tails leads to an open chromatin structure allowing transcription factors to access DNA. Consequently, proteins with HAT catalytic activity are often transcriptional coactivators. Individual HATs possess distinct histone specificity. So far, at least 25 members have been characterized, organized in four families based on structure homology [26]. Subgroups include the GNAT (*hGCN5*, *PCAF*), MYST (*MYST*, *Tip60*), p300/CBP (*p300/CBP)*, SRC (*SRC-1),* and TAFII250 families *(TAFII250*) [24,27]. In contrast to histone acetylation, histone deacetylation generally leads to chromatin condensation and transcriptional repression. Eighteen proteins with HDAC activity have been classified so far [28,29]. *HDAC*s 1-11 are subdivided into three classes I, II and IV based on homology, size, sub-cellular expression and number of enzymatic domains. Class III comprises of *sirtuins 1-7*, which are structurally unrelated to class I and II HDACs and require NAD^+ as a cofactor for activity [28,29]. Recent research indicates that HDAC substrates are not limited to histones. As an example, important regulatory proteins and transcription factors such as *p53*, *E2F*, *NF-κB*, *FOXO*, *PGC-1α* involved in stress response, apoptosis and energy metabolism have been shown to be regulated by acetylation [28-31].

Histone methylation is the best characterized histone modification to date. It takes place at lysine and arginine residues. The influence on activation and repression of gene expression through lysine methylation is residue- (K4, K9, K27, K36, K79 in H3), status- (mono-, di-, and tri-methylation) and location-dependent (interaction with promoter *vs*. gene coding regions) [23,24,32]. Methylation at H3K4 and H3K36 is associated with transcriptional activation, whereas methylation at H3K9, H3K27, H3K79 and H4K20 is frequently associated with repressed genes [27]. Histone lysine methylation is performed by histone lysine methyltransferases (HMTs) containing a SET domain, that is **S**uppressor of Variegation (SUV) homologs, **E**nhancer of Zeste (EZH) proteins, and **T**rithorax group proteins, and the non-SET-domain *Dot1* protein family, using S-adenosyl-*L*-methionine (SAM) as the cofactor. In addition to HMTs, several types of histone demethylases (HDMs) have been identified, *i.e.* Lysine Specific Demethylase 1 (*LSD1*) and the Jumonji domain-containing (*JmjC*) histone demethylases [23,24]. Similar to lysine acetylation, lysine methylation is not limited to histone proteins, and several non-histone protein substrates including *p53*, retinoblastoma protein (*RB*), the *NF-κB* subunit *RelA*, estrogen receptor α (*ERα*), and *PGC-1α* have been identified (summarized in [33,34]).

MicroRNAs

MicroRNAs (miRNAs) are small non-coding RNAs of 20–22 nucleotides that inhibit gene expression at the posttranscriptional level. MiRNAs are involved in the regulation of key biological processes, including development, differentiation, apoptosis and proliferation, and are known to be altered in a variety of chronic degenerative diseases including cancer [35]. MiRNAs are generated from RNA precursor structures by a protein complex system composed of members of the Argonaute protein family, polymerase II-dependent transcription and the ribonucleases *Drosha* and *Dicer* [36]. MiRNAs regulate the transformation of mRNA into proteins, either by imperfect base-pairing to the mRNA 3'-untranslated regions to repress protein synthesis, or by affecting mRNA stability. Each miRNA is expected to control several hundred genes. They have been implicated in cancer initiation and progression, and their expression is often down-regulated during carcinogenesis. Major mechanisms of miRNA deregulation include genetic and epigenetic alterations as well as defects in the miRNA processing machinery [32].

Links between cancer cell metabolism and epigenetics

Epigenetic mechanisms allow an organism to respond to changes in the environment [37]. Since these environmental changes might necessitate adaptations in cell metabolism, it is intriguing to search for potential links between cell metabolism and epigenetic regulation (perspectives in [33,38-40]). There are two major possibilities for interaction, *i.e.* the availability of essential co-factors for epigenetic enzymes, and alterations in the function of important regulatory proteins by genetic or epigenetic alterations.

Co-factors for epigenetic reactions

Epigenetic modifications of DNA and histones by methylation and acetylation reactions require co-factors that are derived from various metabolic pathways, including glycolysis, fatty acid oxidation, TCA cycle and OXPHOS (summary in Table 1) [33]. It has been speculated that alterations in the supply of these co-factors in cancer cells may affect DNA methylation, alter chromatin structure, and change posttranslational modifications of non-histone proteins that influence regulation of gene expression [33,38,39].

Table 1: *Link between metabolic co-factors and epigenetics*

Co-Factors	Epigenetic function	Reference
S-adenosyl-*L*-methionine (SAM)	co-factor for methylation reactions by DNA- and histone methyltransferases (DNMTs and HMTs)	[33,38,39]
Flavin adenine dinucleotide (FAD)	co-factor for lysine specific demethylase 1 (LSD1)	[33,44]
α-Ketoglutarate (α-KG)	electron donor for JmjC histone demethylases (HDM) co-factor for TET proteins	[33,38]
NAD^+/NADH	co-factor for sirtuins and poly(ADP-ribose)polymerase (PARP)	[28,31,38,47,48]
Acetyl Coenzyme A (acetyl-CoA)	co-factor for histone acetyl transferases (HATs) acetylation of non-histone proteins involved in cell metabolism	[30,39,53,54]

TET, ten-eleven translocation protein

S-Adenosyl-L-methionine (SAM)

S-Adenosyl-*L*-methionine (SAM) is one of the most important co-factors for methylation reactions. It is an essential methyl group donor for reactions catalyzed by DNMTs as well as HMTs. SAM is produced by SAM synthetases (methionine adenosyl transferase, *MAT*) by the addition of ATP to methionine [38,41]. High intracellular energy levels therefore could increase SAM levels and DNMT activity and affect global DNA methylation [33]. Transfer of a methyl group from SAM to DNA generates S-adenosyl homocysteine (SAH), which is then hydrolyzed to adenosine and homocysteine. Remethylation of homocysteine to methionine *via* methionine synthase requires 5-methyl tetrahydrofolate and vitamin B12; therefore, the formation of SAM is tightly liked to the folate status of a cell. Folate is an essential vitamin of the B-family (see below). Folate deficiency and mutations in the 5,10-methylene tetrahydrofolate reductase (*MTHFR*) gene have been shown to disrupt DNA methylation [19,38].

Rapid cancer cell proliferation often results in the overproduction of reactive oxygen species (ROS) [42]. Consequently, intracellular SAM levels may be depleted by increased oxidative stress-induced synthesis of antioxidants such as glutathione (GSH): In addition to *de novo* synthesis, GSH can be generated by cystathionine β-synthase *via* a transsulfuration reaction of homocysteine. This could diminish the levels of homocysteine for replenishment of the methionine pool (summarized in [38,43]).

Flavin adenine dinucleotide (FAD)

Flavin adenine dinucleotide (FAD) is another co-factor that might link metabolic processes to epigenetic regulation. FAD is synthesized from riboflavine *via* riboflavine kinase (*RFK*) and FAD synthase in an ATP-consuming reaction. Therefore, changes in ATP levels might affect FAD availability [33].

Beside its role as an electron acceptor in complex II of the mitochondrial respiratory chain, FAD is an important co-factor for the demethylation of histones by histone demethylase *LSD1*. *LSD1* is a flavin-dependent monamine oxidase which specifically removes methyl groups from mono- or dimethylated H3K4 or H3K9 trough a FAD-dependent oxidation reaction. This alters the chromatin structure and results in context-dependent activation or repression of transcription [33]. Interestingly, *LSD1* has also been shown to demethylate

di-methylated lysine 370 of tumor suppressor *p53* (p53K370me2), thereby reducing its activity [44].

α-Ketoglutarate (α-KG)

α-Ketoglutarate (α-KG) is used by *JmjC* histone demethylases that demethylate not only mono- and di-methylated, but also tri-methylated lysine residues of histones. α-KG stabilizes the enzyme–substrate complex in an α-KG/Fe^{2+}-dependent dioxygenase reaction [33]. In the TCA cycle, α-KG is converted to succinyl-Co A by α-KG dehydrogenase. This step is inhibited by high levels of ATP. α-KG is also generated from isocitrate *via* an interconversion reaction catalyzed by isocitrate dehydrogenase (*IDH*). In cancer cells, the pool of α-KG is elevated by the increased utilization of glutamine as an energy source. *c-Myc* has been shown to directly stimulate glutamate uptake by inducing the expression of glutamine transporters *SLC5A1* and *SLC7A1*. Glutamine is then converted to α-KG by glutaminolysis in two steps *via* glutaminase 1 (*GLS1*) and glutamate dehydrogenase. *c-Myc* has also been reported to influence glutamate synthesis indirectly by repressing the expression of microRNAs *miR-23A* and *miR-23B* that inhibit *GLS1* [9].

In addition to *JmjC* HDMs and prolyl hydroxylase (*PHD*, a repressor of pro-angiogenic hypoxia-inducible factor 1α (*HIF-1α*), not further highlighted here), α-KG is an essential co-factor for *TET* (ten-eleven translocation) proteins. Like *JmjC* HDMs, *TET* proteins belong to the group of α-KG and Fe^{2+}-dependent dioxygenases and use molecular oxygen to transfer a hydroxy group to 5mC to generate 5-hydroxy mC (5hmC). Increased 5mC hydroxylation can facilitate demethylation of 5mC through hydroxymethylcytosine glycosylase and might therefore contribute to active epigenetic regulation [33,45].

Nicotinamide adenine dinucleotide (NAD)

Nicotinamide adenine dinucleotide (NAD^+ oxidized form, NADH reduced form**)** is an important co-factor involved in energy metabolism, DNA repair and transcription [46]. NAD is synthesized *de novo* from tryptophan; on the other hand it is generated by recycling degraded NAD products such as nicotinamide [47]. During glycolysis and in the TCA cycle NAD^+ is converted to NADH; NADH is then re-oxidized mainly by NADH dehydroxygenase in complex I of the mitochondrial respiratory chain. In cancer cells, high lactate production has an important function to regenerate NAD^+ by conversion of pyruvate to lactate *via* lactate dehydrogenase (*LDH*) [1]. The NAD^+/NADH ratio (in mammals in the range of 3-10) is an important regulator of the intracellular redox state and many metabolic enzymes [46].

NAD not only serves as a coenzyme, but is also used as a substrate by enzymes such as **sirtuins** (NAD-dependent class III of HDACs) and **poly(ADP-ribose)polymerase (*PARP*)** that control gene expression. Since both sirtuins and PARPs require NAD, there might be a functional link and competition between both enzymes [48]. Sirtuins have been implied in longevity. Their activity is directly controlled by cellular NAD^+ levels. Reduced sirtuin activity by alterations in the NAD^+/NADH ratio in cancer cells might result in histone hyperacetylation, decondensed chromatin structure, stimulation of gene expression and consequent cell proliferation. *PARP* plays an important role in DNA-damage response (not further highlighted in this perspective). Recent research indicates that it is also involved in epigenetic regulation of chromatin structure and gene expression [48]. NAD^+ is utilized by *PARP* to transfer ADP-ribose moieties to proteins, including histones and *PARP* protein itself. *PARP* binding to nucleosomes can promote a compact and transcriptionally repressed chromatin structure. On the other hand, auto-poly(ADP-ribosyl)ation of *PARP* induces its dissociation from chromatin, resulting in open, transcriptionally active chromatin [49]. *PARP*-mediated poly(ADP-ribosyl)ation of histones and their subsequent stripping from chromatin also leads to chromatin decondensation [48]. Massive activation of *PARP* depletes NAD^+ levels. This will affect gene expression by reducing the activity of sirtuins, resulting in elevated levels of acetylation at histones and transcription factors [49].

Interestingly, poly(ADP-ribosyl)ation has been shown to influence DNA methylation: In experiments by Caiafa *et al.* blockage of poly(ADP-ribosyl)ation induced DNA hypermethylation, whereas hyperactive *PARP-1* and elevated levels of poly(ADP-ribosyl)ation resulted in DNA hypomethylation (summary in [50]). The authors suggest that poly(ADP-ribosyl)ated *PARP-1* binds to and consequently inactivates *DNMT1* and therefore prevents DNA methylation genome-wide and at specific CpG sites. Poly(ADP-ribosyl)ation may also control binding of the chromatin insulator protein CCCTC-binding factor (*CTCF*) to both imprinted and not-imprinted loci [48,50].

Acetyl Coenzyme A (Acetyl-CoA)

Acetyl-CoA is not only an important precursor for the construction of fatty acids, but also an essential co-factor for acetylation of histones and non-histone proteins. Acetyl-CoA can be synthesized by acetyl-CoA synthetase (*AceCS1*). Acetyl-CoA is also generated in the TCA cycle from pyruvate *via* pyruvate dehydrogenase (*PDH*). Increased glycolysis and pyruvate production allows the export of acetyl-CoA to the cyctosol in the form of citrate. Citrate is then converted back to acetyl-CoA by ATP citrate lyase (*ACL*) [39,51]. Recent research identified *ACL* as an important link between cell metabolism and histone acetylation in response to growth factor

stimulation [52]. Wellen *et al.* reported that acetyl-CoA generated by *ACL* specifically promoted histone acetylation, whereas acetylation of non-histone proteins was not affected by silencing of *ACL*. Interestingly, *ACL*-dependent acetylation was associated with increased expression of genes involved in glucose uptake (*Glut4*), glycolysis (*HK2* and *PFK1*) and lactate deydrogenase A-chain (*LDH-A*), linking nutrient uptake and metabolism to regulation of histone acetylation [52] and consequently gene expression and proliferation [39].

Although rather a posttranslational modification than an epigenetic mechanism, it should be mentioned that acetylation of non-histone proteins has recently been linked to the regulation of cellular metabolism. Based on a proteomics approach, several studies identified enzymes involved in cell glycolysis, gluconeogenesis, fatty acid metabolism, urea cycle and glycogen metabolism as preferentially acetylated [30,53]. Acetylation changed in response to nutrient availability and influenced protein activity or stability [53]. Choudhardy *et al*. demonstrated that non-histone protein acetylation also affected additional major cellular functions, including RNA splicing, DNA damage repair, cell cycle regulation, nuclear transport, actin cytoskeleton remodeling, chaperones and ribosomes [54].

Table 2: *Functional alterations of epigenetic and metabolic enzymes*

Protein	Alteration, consequence	Reference
Isocitrate dehydrogenase (*IDH1, IDH2*)	gain of function mutations convert the *IDH* product α-KG to 2-HG; 2-HG inhibits *TET2*, HDMs and *PHD*	[55]
Hexokinase II (*HK2*)	glycolytic enzyme activated by promoter demethylation in liver and brain tumors	[58,59]
Fructose-1,6-bisphosphatase 1 (*FBP1*)	key enzyme of gluconeogenesis silenced by promoter methylation in gastric, liver and colon cancer	[57,60]
Pyruvate kinase M2 *(PKM2)*	rate-limiting glycolytic enzyme inactivated through protein acetylation	[61]
p53 – TIGAR – PGC1α	posttranslational modifications through epigenetic enzymes may affect downstream targets	[34,62-64,66]

αKG, α-ketoglutarate; 2-HG, 2-hydroxyglutarate; TET2, ten-eleven translocation protein (a 5-hydroxymethylcytosine hydroxylases); HDM, histone demethylase; PHD, prolyl hydroxylase

Alterations in functions of epigenetic and metabolic enzymes

Interactions between cancer cell metabolism and epigenetic regulation have gained increasing interest in recent years (overview in **Table 2**). In addition to general effects on epigenetics brought about by changes in the availability of co-factors summarized above, mutations in metabolic enzymes such as *IDH1* and *IDH2* have been linked to alterations in DNA and histone methylation. On the other hand, hexokinase II (*HK2*) and fructose-1,6-bisphosphatase 1 (*FBP1*) with opposing roles in glycolysis have recently been identified as epigenetically regulated by promoter demethylation and methylation, respectively. Pyruvate kinase M2 (*PKM2)*, the rate-limiting glycolytic enzyme catalyzing the conversion of phosphoenol pyruvate (PEP) to pyruvate, was found to be targeted for degradation in a glucose-dependent manner by acetylation at lysine K305. In addition, posttranscriptional modifications of *p53* may affect cell metabolism through downstream targets such as *TIGAR* (TP53-induced glycolysis and apoptosis regulator) and interaction with *PGC-1α.*

Isocitrate dehydrogenase (IDH)

IDH1 (cytosolic expression) and *IDH2* (mitochondrial expression) are $NADP^+$-dependent enzymes that convert isocitrate to α-KG (**Fig. 2**). Both enzymes are frequently mutated in >75% of gliomas and >20% acute myeloid leukemia (AML) [55]. Mutations of *IDH1* and *IDH2* lead to a novel enzymatic activity and result in the accumulation of 2-hydroxyglutarate (2-HG) instead of α-KG. This not only reduces the pool of α-KG, but might effect the epigenome in two ways: 2-HG has been identified as a competitive inhibitor of α-KG/Fe^{2+}-dependent dioxygenases, such as *JmjC* HDMs, leading to an accumulation of methylated histones. 2-HG also inhibits the activity of *TET* (ten-eleven translocation) 5-hydroxymethylcytosine hydroxylases that transfer a hydroxy group to 5mC to generate 5-hydroxy mC (5hmC). *IDH* mutations in gliomas resulted in reduced levels of 5hmC accompanied by a significant increase in 5mC levels [55]. Since *IDH* mutations have been reported to occur very early during glioma and leukemia development, alterations in DNA and histone methylation that result from *IDH* mutations may have an impact on epigenetic gene regulation and thereby contribute to carcinogenesis [55].

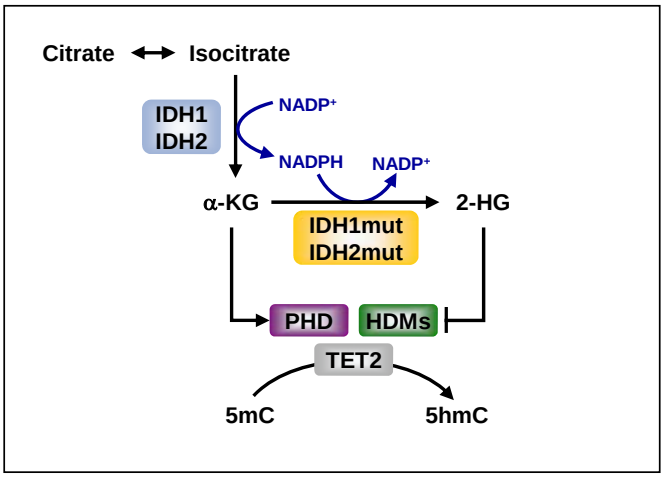

Figure 2. IDH1 and IDH2 mutations cause oncometabolite 2-HG gain of function *(modified from [9]). Mutations of isocitrate dehydrogenase 1 (IDH1) or IDH2 result in the conversion of α-ketoglutarate (α-KG) to 2-hydroxyglutarate (2-HG) with consumption of reduced nicotinamide adenine dinucleotide phosphate (NADPH). 2-HG inhibits the activity of Fe^{2+}/α-KG-dependent dioxygenases, including prolyl hydroxylase (PHD), which is involved in regulating the expression of hypoxia-inducible factor 1α (HIF-1α), histone demethylases (HDMs), and the ten eleven translocation protein 2 (TET2). TET2 is a 5-hydroxymethylcytosine hydroxylases and hydroxylates methylated cytosine residues. Hydroxylation of 5mC could be involved in demethylation of DNA.*

Epigenetic regulation of glycolytic enzymes - HK2 and FBP1

Hexokinase (*HK)* catalyses the first rate-limiting step of glucose metabolism in transferring a phosphate group from ATP to glucose to generate glucose-6-phosphate. Glucose-6-phosphate is then isomerized to fructose-6-phosphate, which is used by 6-phosphofructo-1-kinase (*PFK*) to generate fructose-1,6-bisphosphate (FBP). The reverse reaction form FBP to fructose-6-phosphate is catalyzed by fructose-1,6-bisphosphatase (*FBP1*), which is therefore antagonizing glycolysis. In cancer cells, activities of *HK* and *PFK* are high and facilitate high glycolytic capacity. In contrast, *FBP1* activity is often reduced in cancer cells, leading to accumulating levels of FBP. FBP levels control the activity of of *PKM2* and thereby ensure a high glycolytic rate [56].

Hexokinase isoform I (*HK1*) is expressed in all mammalian tissues, whereas *HK* isoform II (*HK2*) is the main regulated isoform and overexpressed in many cancer types [56]. Overexpression of *HK2* has been associated with poor prognosis [57]. Goel *et al.* addressed the question whether epigenetic mechanisms might contribute to the upregulation of *HK2* expression in hepatoma cells. They were first to describe that the *HK2* promoter harbors a CpG island which is generally methylated in normal liver cells. During liver carcinogenesis, methylation is reduced and *HK2* expression increased [58]. Similarly, Wolf *et al.* recently identified that overexpression of *HK2* in glioblastoma cells in relation to normal brain tissue was regulated at least in part *via* demethylation of its CpG island [59].

Fructose-1,6-bisphosphatase (*FBP1*) is an important regulatory enzyme in gluconeogenesis and is frequently downregulated in cancer cells. Liu *et al.* recently demonstrated that *FBP1* is silenced in gastric carcinogenesis by promoter methylation in an *NF-κB*-dependent manner. Depletion of *NF-κB* led to promoter demethylation and restored *FBP1* expression, which was associated with the induction of cell cycle arrest and cell growth inhibition. Interestingly, *FBP1* promoter methylation was identified as an independent biomarker for poor prognosis in gastric cancer [57]. Chen *et al*. recently demonstrated that epigenetic silencing of *FBP1* by promoter hypermethylation is also common in human liver and colon cancer [60].

Acetylation of pyruvate kinase M2 (PKM2)

Pyruvate kinase (PK) is the rate-limiting enzyme of glycolysis. During tumorigenesis, there is a switch in expression from the more active isoform *PKM1* to the less active isoform *PKM2*, which is promoted by *c-Myc* though alternative exon splicing (cited in [9]). Recently, Lv *et al*. have identified *PKM2* as a target for protein acetylation. They describe that high glucose levels stimulate the acetylation of *PKM2* at lysine K305 through acetyltransferase *PCAF*. Acetylation decreased *PKM2* activity by reducing its affinity for phosphoenol pyruvate (PEP), but also lowered *PKM2* levels by increasing its interaction with the chaperone heat shock protein *Hsp70* that targets proteins to lysosomal degradation. The physiological significance of K305 acetylation was further tested using an acetylation mimetic mutant $PKM2^{K305Q}$, which harbors a glutamine residue instead of K305. Expression of the mutant $PKM2^{K305Q}$ lowered the production of pyruvate and lactate and lead to accumulation of glycolytic intermediates and NADPH. Most interestingly, $PKM2^{K305Q}$ promoted cell proliferation and tumor growth in a xenograft model, indicating that acetylation of *PKM2* under high glucose conditions provides a growth advantage for tumor cells [61]. Inhibition of *PCAF* might therefore directly influence cell metabolism by regulating activity and expression levels of *PKM2*.

p53, TIGAR and *PGC-1α*

It is well known that activity and stability of tumor suppressor *p53* is regulated by posttranslational

modifications, including lysine methylation and acetylation. Various histone-modifying enzymes known to modulate chromatin structure are involved in this process [34,62]. As an example, *p53* is acetylated by several HATs in response to stress, increasing its DNA binding capacity. On the other hand, deacetylation of *p53* by *HDAC1* and NAD^+-dependent *SIRT1* was shown to repress its transcriptional activity (review in [62]). Lysine methylation of *p53* at four specific lysine residues is dynamically regulated by HMTs and the α-KG-dependent demethylase *LSD1*. Similar to histone methylation, the degree and position of *p53* lysine methylation has distinct consequences on stability and activity (detailed overview in [34]). Demethylation of *p53* lysine 370 (p53K370me2) by *LSD1* reduced its activity [44]. So far it has not been systematically investigated how changes in the availability of co-factors for HMTs, HDMs, HDACs and HATs due to alterations in cancer cell metabolism would affect *p53* function.

On the other hand, several lines of evidence indicate that *p53*, in addition to its key functions in controlling cellular processes such as cell cycle progression, apoptosis and genomic stability, plays an important role in the regulation of cell metabolism (recent reviewed in [63]). In 2006, Bensaad *et al.* identified a novel *p53*-inducible protein ***TIGAR*** *(*TP53-induced glycolysis and apoptosis regulator) with structural similarity to 2,6-bisphosphatases. Fructose-2,6-bisphosphate (F-2,6-BP) stimulates glycolysis through activation of *PFK-1* and inhibition of *FBP1*. Overexpression of *TIGAR* mediated by *p53* led to degradation of F-2,6-BP, decreased the rate of glycolysis and increased flux of glycolysis intermediates into the PPP; this was linked to cell-type dependent sensitization or resistance to ROS-associated apoptosis [64]. In a study by Kawauchi *et al., p53* was shown to control glycolysis *via* the **expression of glucose transporters**. Loss of *p53* activated the *IKK–NF-κB* pathway and stimulated glycolysis by increasing the expression of glucose transporters, especially *Glut3*. The authors suggest a positive feedback loop between enhanced glycolysis and activation of *IKK–NF-κB*; this loop is hyperactivated by loss of *p53* and might contribute to cell transformation [65].

A recent report by Sen *et al.* has now established a link between *p53* and ***PGC-1α*** (peroxisomal proliferator-activated receptor (*PPAR*) gamma coactivator 1) [66]. *PGC-1α* belongs to a small family of transcriptional activators with key functions in the control of glucose, lipid and energy metabolism (review in [67]). Interestingly, *PGC-1* coactivators are also involved in the recruitment of chromatin-remodeling complexes such as *p300* and *GCN5* HATs to increase histone acetylation; in this process, *PGC-1α* itself is acetylated and inactivated. Conversely, *PGC-1α* is deacetylated and activated by the deacetylase *SIRT1*. *PGC-1α* also promotes histone H3 lysine 4 trimethylation (H3K4me3), a histone methylation mark associated with transcriptional activation, although the underlying mechanism is not known so far [67]. Sen *et al.* demonstrated that upon mild metabolic stress (short-term glucose starvation), *p53* is recruiting *PGC-1α* to pro-arrest and metabolic target genes such as *p21*, *GADD45*, *TIGAR* and *SCO2* [66]. Interaction of *p53* and *PGC-1α* prevented *p53* acetylation at K120 within the DNA binding domain. Prolonged metabolic stress however led to degradation of *PGC-1α via* the proteasome and consequent acetylation of *p53*, thereby promoting its transcriptional activation of pro-apoptotic genes. Thus, *PGC-1α* might play a key role in modulating the *p53*-mediated response to metabolic stress [66].

These examples indicate that modulators of *p53* expression, acetylation status or transactivating activity might directly influence cancer cell metabolism.

Influence of dietary factors on cancer cell metabolism and epigenetic mechanisms

It has been estimated that about 30% of all cancers in Western high-income societies a causally related to food and nutrition [68]. However, diet is also a source of bioactive food components with cancer preventive potential (extensive overview in [69]). So far, only few dietary agents have been described that are able to suppress tumor-specific metabolic pathways (review in [70]). The recent emergence of technologies that allow sensitive monitoring of cell metabolism *via* the detection of small molecule metabolites (metabonomics) will be instrumental for the identification and mechanistic investigations of bioactive food components with influence on cancer cell metabolism. On the other hand, accumulating evidence over the past few years indicates that natural products and dietary constituents have an impact on epigenetic mechanisms, including DNA and histone methylation, acetylation of histones and non-histone proteins, and miRNA expression. Food bioactive compounds targeting the epigenome include micronutrients (folate, selenium, retinoic acid, Vit. D and E), the carbohydrate fermentation product butyrate, polyphenols (from green tea, apples, coffee, and other dietary sources), genistein and soy isoflavones, curcumin found in curry, ellagitannin from berries, indol-3-carbinol and diindolylmethane derived from cruciferous vegetables, the lignan nordihydroguaiaretic acid, lycopene from tomatoes, sulfur-containing compounds from *Allium* and cruciferous vegetables (such as sulforaphane, phenylethyl isothiocyanate and diallyldisulfide), compounds affecting sirtuin activity (resveratrol, dihydrocoumarin, cambinol), inhibitors of histone acetyl transferases (anacardic acid, garcinol, ursodeoxycholic acid), and modulators of histone lysine methylation

(epigallocatechin gallate, chaetocin, n-3 polyunsaturated fatty acids) (reviews in [19,71-78]). By targeting epigenetic mechanisms they affect signal transduction pathways mediated by nuclear receptors and transcription factors such as *NF-κB*, cell cycle progression, cellular differentiation, induction of apoptosis, senescence and others. Investigations on whether their influence on epigenetic mechanisms might normalize alterations in cancer cell metabolism might be an interesting field of future research.

Here, selected examples of dietary factors will be given that have been shown to influence both cell metabolism and epigenetic mechanisms. Further studies will have to demonstrate whether both activities might be causally related.

Folate

Folate, a water-soluble vitamin of the B-family, plays an important role in one-carbon metabolism and synthesis of SAM. As an essential micronutrient, folate has to be provided with the diet. Important sources include citrus fruits, dark-green vegetables, whole grains, and dried beans, whereas alcohol misuse disturbs folate uptake. Folate deficiency has been associated with global loss of DNA methylation, genomic instability and chromosomal damage, and has been identified as a risk factor for several types of cancer [41,79,80]. Research over the past years failed to establish a conclusive link between folate status, DNA methylation and cancer risk. Overall, the results are inconclusive and are dependant on parameters such as the severity of folate deficiency, dose- and timing of the intervention, and health status (review in [41,73,80-82]). Recent studies even suggest that excessive uptake of synthetic folic acid in the form of high-dose supplements or fortified foods may increase human cancer risk by accelerating growth of precancerous lesions [80]. Therefore, folate supplementation can not be generally recommended.

As mentioned above, the proto-oncogene *c-Myc* promotes cancer cell metabolism by multiple mechanisms. It stimulates glutaminolysis through upregulation of glutamine transporters and stimulation of glutaminase, and contributes to the switch in expression of pyruvate kinase M1 to *PKM2*. In a mouse model for carcinogen-induced colorectal cancer, folate supplementation reduced tumor incidence by about 50%. In a subset of tumor samples, *c-Myc* was overexpressed, and overexpression was associated with promoter demethylation. Interestingly, tumors with *c-Myc* hypomethylation had lower serum folate levels than those with a methylated *c-Myc* promoter [83]. These data suggest that folate deficiency might contribute to carcinogenesis by promoter demethylation and consequent *c-Myc* overexpression.

Flavonoids and their influence on glucose transporters

Loss of *p53* has been associated with the upregulation of glucose transporters facilitating glucose uptake in cancer cells. Also, mutations in the *K-Ras* oncogene have recently been shown to enhance glucose uptake and glycolysis through upregulation of glucose transporter 1 (*Glut1*) [84]. Inhibition of expression or activity of glucose transporters has therefore been proposed as an interesting strategy to inhibit tumor proliferation [70]. Flavonoids, one of the largest groups of dietary polyphenols, are present in the diet as conjugates with glucose and other sugars. Studies on their cellular uptake with cultured cells have suggested that flavonoid glucosides are transported through the gut cell membrane *via* glucose transporters and act as competitive inhibitors of glucose uptake. Examples include myricetin, morin, rhamnetin, isorhamnetin, quercetin, and green tea polyphenols ([85,86] and citations therein). Future studies using metabolomics approaches might clarify whether dietary levels of flavonoids are sufficient to inhibit glucose uptake and cell metabolism *in vivo*.

Flavonoids and other dietary polyphenols from apples, coffee, green tea, citrus fruit, grapes etc. have been identified as inhibitors of DNMT activity *in vitro*. With few exceptions (isoflavones from soy, black raspberries, green tea polyphenols), DNA demethylating potential has however not been tested in cell culture or animal models (review in [19].

(-)-Epigallocatechin gallate-3-gallate (EGCG) and green tea polyphenols

Green tea polyphenols (GTP) are a mixture of catechins (flavan-3-ols), including (–)-epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC). EGCG and GTP have been shown to prevent tumor development in animal carcinogenesis models of all major organ sites (summarized in [87]). Results from human epidemiological studies are less conclusive; this was explained by low consumption of tea. EGCG acts as a pro- and antioxidant, triggers signal-transduction pathways, and inhibits enzyme activities, receptor-dependent signaling cascades, and angiogenesis. Concentrations required to demonstrate these activities are mostly higher than those usually detectable in human or in rodent models [19].

EGCG was the first polyphenol described to inhibit DNA methylation, postulated by direct binding of EGCG to the catalytic pocket of DNMT [88]. Later studies suggested that catechins, flavonoids and other polyphenols might inhibit *DNMT* activity also by an indirect mechanism, *i.e.* by depletion of SAM due to methylation of the compounds themselves, and through feedback inhibition

of DNMTs by accumulating SAH levels [19,89,90]. In addition to inhibition of *DNMT* activity *in vitro*, EGCG treatment in cell culture models led to reduced genomic 5meC levels and promoter hypermethylation of selected candidate genes (summarized in [19]). This was generally associate with mRNA re-expression of these genes. Genome-wide methylation studies with GTP in an animal model for prostate cancer have not been conclusive [91]. Therefore presently it is unknown whether EGCG or GTP might modulate the expression of glycolytic enzymes by changes in DNA methylation.

A recent study by Klaus *et al*. has indicated that GTP might be useful as anti-obesity compounds and modulate the expression of genes involved in glucose and fat metabolism. Chronic 4-week dietary supplementation of obese male New Zealand black mice with EGCG reduced body fat accumulation and resulted in a significant downregulation of hepatic glucokinase (a key enzyme of liver glycolysis) and non-significant reduction of pyruvate kinase expression. Malic enzyme (*ME*) and stearoyl-CoA desaturase 1 (*SCD1*), both involved in lipid synthesis, were also significantly downregulated in the liver. A reduced nocturnal respiratory quotient was indicated of reduced glucose oxidation [92].

Genistein from soy and effects on pentose phosphate pathway (PPP)

Genistein is a phytoestrogenic isoflavone derived from soy. Epidemiologic studies have established an inverse relationship between a traditional low-fat, soy-rich Asian diet and the risk to develop breast and prostate cancer [93,94]. Prevention of hormone-dependent tumors and a series of other cancer types has also been demonstrated in animal models [95]. This was mainly attributed to the hormonal activity of soy isoflavones mediated by estrogen receptor binding. Besides, genistein affects carcinogen bioactivation, cell-signaling, cell cycle regulation, angiogenesis, oxidative stress, and inflammation, and targets epigenetic mechanisms including DNA methylation, histone acetylation and miRNAs [19,75,96]. Recent breast cancer studies in rodent models have indicated that genistein might promote growth of hormone-dependent breast cancer; these data have raised concerns on the safety of genistein for human application [97]. Genistein is currently undergoing clinical testing for treatment of prostate, bladder, and kidney cancer, and for the prevention of breast and endometrial cancer [98].

Boros *et al.* proposed an alternative mechanism for the cancer inhibitory effects of genistein. By using a radiolabeled glucose tracer to monitor the accumulation of glucose metabolites, they demonstrated that genistein treatment of pancreatic cancer cells significantly reduced ribose synthesis through the non-oxidative branch of the PPP. Concomitantly, glucose oxidation to CO_2 was significantly inhibited, whereas lipid synthesis was not affected. Although the authors did not further investigate which enzymes were targeted by genistein, inhibition of nonoxidative ribose synthesis was hypothesized as an interesting target for the development of anticancer strategies [99,100]. In a thematically related study, fermented wheat germ extract was found to block ribose synthesis in Jurkat cells. The extract was shown to inhibit glucose-6-phosphate dehydrogenase (*G6PDH*), the key enzyme of the PPP cycle, transketolase as an indicator of non-oxidative glucose utilization, as well as two important mediators of glycolysis, *HK* and *LDH*, in a dose- and time-dependent manner. Inhibition of cell metabolism was associated with the induction of apoptosis; importantly, it was specific for the cancer cell line and not observed in peripheral blood lymphocytes [101]. Recent metabonomic studies on soy intake in healthy women indicate that soy intake might affect energy metabolism [102,103].

Milk thistle** (Silybum marianum) **flavolignans

Milk thistle (*Silybum marianum*) has a long traditional use as a liver tonic and protectant against various liver diseases. Milk thistle seeds are a rich source of flavolignans. A crude extract of milk thistle seeds is designated as silymarin that constitutes a complex of at least seven flavolignans and additional components. Silybinin (or silibinin), the most abundant component of silymarin, is still a mixture of two isomers, silybin A and B. In the literature silymarin and silybinin are often used synonymously, although in fact they may have very distinct biological properties (further information in [104]). Milk thistle compounds have been shown to prevent cancer in various experimental animal models, and preventive potential has been ascribed to their anti-inflammatory properties, cell growth inhibition by cell-cycle arrest and apoptosis induction, as well as inhibition of angiogenesis, tumor invasion and metastasis (review in [105,106]). Milk thistle extracts alone or in combination with other phytochemicals have been tested in several Phase I to III trials for colorectal and prostate cancer and were shown to delay progression of prostate specific antigen (PSA, a widely used marker for prostate cancer development) [107].

Several reports indicate that milk thistle components might affect cell metabolism. Silybin was shown to modulate glucose uptake in adipocytes by blocking *Glut4*. In rat hepatocytes, it inhibited pyruvate kinase activity and glycolytic flux, and blocked glucose-6-phosphate hydrolysis and glucose-6-phosphatase (review in [106]). Using ^{1}H-NMR spectroscopy, Raina *et al*. determined the effect of silybinin intervention on prostate cancer metabolic profiles in a transgenic mouse model. Beside other changes, tumors from the silybinin group had

significantly elevated glucose levels and reduced lactate content, indicating decreased glucose usage and reduced glycolytic activity. These interesting findings should stimulate future investigations on whether these observations are due to direct effects of silybinin on cancer cell metabolism or indirectly reflect reduced tumor progression and cancer preventive effects *via* other mechanisms [108]. Two recent report suggests that milk thistle compounds also target epigenetic mechanisms. Li *et al*. described silymarin as an activator of the deacetylase *SIRT1* in human melanoma cells [109]. On the other hand, Ciu *et al.* observed that silybinin increased H3 and H4 acetylation in human hepatocellular carcinoma xenografts [110]. So far it is unclear whether this silybinin-mediated effect is due to HDAC inhibition or activation of HATs, and whether modulation of histone acetylation is associated with additional preventive mechanisms.

Shikonin as a novel pyruvate kinase M2 (PKM2) inhibitor

Shikonin is a natural anthraquinone derivative isolated from the roots of the Chinese medicinal plant *Lithospermum erythrorhizon* (Zicao). Zicao has been traditionally used to treat various diseases including burns. Shikonin possesses anti-inflammatory and anti-tumor activity and inhibits tumor growth by induction of apoptosis, inhibition of DNA topoisomerase, and inhibition of angiogenesis. In a chemically-induced rat colon model, shikonin significantly reduced the incidence and numbers of intestinal tumors, indicating its cancer preventive potential [111].

A study by Chen *et al.* now described shikonin and structural analogs as inhibitors of cancer cell glycolysis by targeting *PKM2*. By using a proteomics approach with solid-phase shikonin bound to sepharose beads, the authors were able to show that *PKM2* was one of the molecular targets of shikonin. Shikonin selectively inhibited *PKM2* enzymatic activity at low µM concentrations and reduced glucose consumption and lactate production in various cancer cell lines, including multidrug-resistant ones [112]. Given the fact that *PKM2* is the rate limiting enzyme of glycolysis, shikonin and its analogs might be promising candidates to control cancer cell metabolism.

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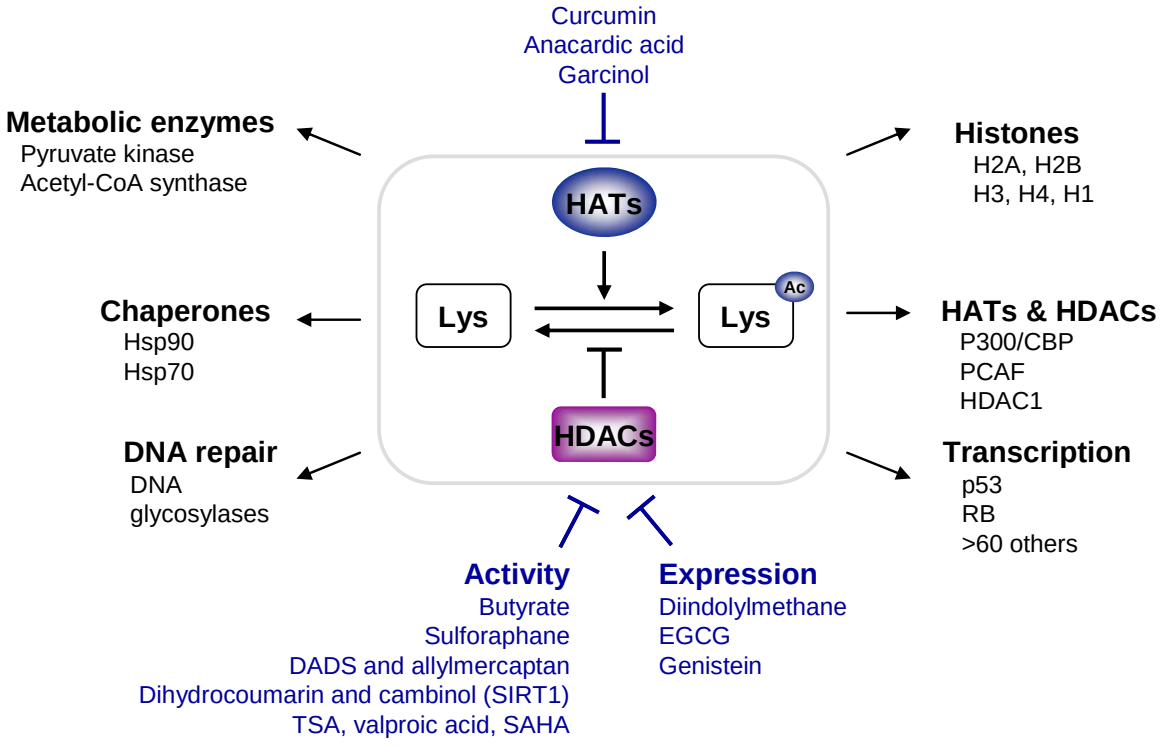

Figure 3. Prevalence of reversible protein lysine (Lys) acetylation (Ac) in various cellular processes and overview of natural product inhibitors of histone deacetylases (HDACs) and histone acetyltransferases (HATs) *(modified from [171]). DADS, diallyldisulfide; EGCG, epigallocatechin gallate; Hsp, heat shock protein; PCAF, p300/CBP-associated factor; SAHA, suberoylanilid hydroxamic acid; SIRT1, sirtuin 1; TSA, trichostatin A.*

Compounds affecting acetylation of histones and non-histone proteins

As mentioned above, chromatin structure is dynamically regulated by the interplay between histone acetylation and deacetylation mediated by HDACs and HATs. However, HDACs and HATs also regulate multiple non-histone proteins and thereby control many important biological functions, including cell proliferation and growth arrest, DNA repair, cell death pathways such as apoptosis and autophagy, mitosis, generation of reactive oxygen species, senescence, angiogenesis and cellular bioenergetics [113]. Beside affecting HDACs and HATs activity directly by autoacetylation, protein acetylation at lysine residues has been shown to influence various transcription factors, chaperones, DNA repair enzymes and metabolic enzymes including pyruvate kinase and acetyl-CoA synthase (overview in **Fig. 3**). In addition to effects on glycolytic enzymes, HDAC inhibitors might affect cancer cell metabolism also *via* modulating the acetylation of *p53*. This might then change the expression of its target proteins such as *TIGAR* that influence glycolytic enzymes.

During the past decade, several dietary agents have been identified as modulators of histone and non-histone acetylation. They inhibit HDAC enzyme activity (butyrate, sulforaphane, diallyldisulfide and allylmercaptan inhibiting HDACs; dihydrocoumarin and cambinol inhibiting *SIRT1* activity) as well as expression (curcumin, diindolylmethane, EGCG, genistein). In addition, several natural compounds inhibiting HAT activity have been identified, such as curcumin, anacardic acid and garcinol (Fig. 3). So far, cancer preventive activity of these compounds has not been associated with inhibitory effects on cancer cell metabolism. Therefore, only a brief overview of their preventive efficacy and epigenetic mechanisms related to histone and non-histone acetylation will be given. A comprehensive overview of their chemopreventive potential and other epigenetic mechanisms has been compiled recently [19].

Butyrate

Butyrate is a major short chain fatty acid produced by colonic fermentation of resistant starch and dietary fiber by the gut microbiota. More than 30 years ago, butyrate was first described to inhibit *HDAC* activity and to cause rapid histone hyperacetylation in cell culture [114-116]. First studies were focused on leukemia cells, induction of cell cycle arrest and cell differentiation. Recently, potential of butyrate to prevent colon carcinogenesis was attributed to the induction of differentiation, cell cycle arrest and apoptosis in transformed colonocytes [117,118]. Many of these effects on gene expression and anti-proliferative activity are likely related to changes in chromatin structure and acetylation of non-histone proteins. Interestingly, butyrate was shown to reduce the expression of *c-Myc* mRNA and protein expression in leukemia, prostate and colon cancer cell lines during the course of cell differentiation [119-121]. Given the role of *c-Myc* in promoting glutaminolysis in cancer cells and inducing the expression of glycolytic enzymes, it is tempting to speculate that reduced *c-Myc* expression by butyrate treatment might contribute to normalization of cancer cell metabolism.

Along these lines, several recent studies have indicated that *HDAC* inhibitors can affect cell proliferation by targeting cell metabolism (overview in [113]). As an example, treatment of colon cancer cells with butyrate inhibited glucose uptake and oxidation, and reduced ribose and fatty acid synthesis. Similar effects were observed with trichostatin A, a fungal metabolite HDAC inhibitor. In lung cancer cells, butyrate and TSA treatment reduced glycolytic flux indicated by a dose-dependent decrease in lactate release. Butyrate inhibited *Glut1* expression, whereas *Glut3* mRNA levels and *G6PDH* activity were increased. Also, butyrate treatment altered the glycolytic metabolite profile, detected by ^{13}C-NMR spectroscopy [122]. Two synthetic HDAC inhibitors, valproic acid and suberoylanilide hydroxamic acid (SAHA) decreased glucose uptake in myeloma cells, reduced *Glut1* and *HK* expression, and induced apoptosis (overview in [113]). These studies indicate that HDAC inhibitors in general might be able to affect tumor cell homeostasis.

Sulforaphane from broccoli

Sulforaphane is a dietary isothiocyanate derived from broccoli and other *Cruciferous* vegetables [123]. Sulforaphane belongs to the best investigated cancer chemopreventive agents (reviewed in [19,124-129]). It potently modulates carcinogen metabolism by inhibition of Phase I and induction of Phase II enzymes, blocks *NF-κB* and hormone receptor signaling, inhibits cell proliferation by induction of apoptosis and cell cycle arrest, induces autophagy [19,130-133] and inhibits angiogenesis [134]. Sulforaphane has been shown to prevent or inhibit carcinogenesis in various animal models [125,127], and first clinical trials have been initialized [135].

The *HDAC* inhibitory potential of sulforaphane was first described in 2004. *In silico* modeling predicted that sulforaphane-cysteine, a sulforaphane metabolite, might fit into the catalytic pocket of *HDAC*s [136]. Subsequent studies confirmed the *HDAC* inhibitory activity of sulforaphane in various human cancer cell lines [136-138] and in an animal model for colon cancer [139]. In a pilot study with healthy volunteers, ingestion of broccoli sprouts as a source of sulforaphane resulted in *HDAC* inhibition and transient induction of histone H3 and H4 acetylation in peripheral blood cells [140]. These data indicate that *HDAC* inhibition is a relevant mechanism of dietary components such as sulforaphane and can be achieved in humans. Future studies will have to demonstrate the relevance of *HDAC* inhibition by

sulforaphane for modulation of the deregulated cancer cell metabolism.

Diallyldisulfide (DADS) and its metabolite allylmercaptan (AM)

Diallyl disulfide (DADS) is an organosulfur compound found in garlic and other *Allium* species. Regular consumption of *Allium* vegetables has been associated with a reduced risk to develop stomach and colon cancer [141]. DADS has a broad spectrum of chemopreventive mechanisms, including induction of carcinogen detoxification, inhibition of DNA adduct formation, free radical scavenging, inhibition of tumor cell proliferation, induction of cell cycle arrest and apoptosis, inhibition of angiogenesis, and suppression of metastasis. Consequently, DADS was shown to prevent carcinogenesis in various chemically-induced tumor models and inhibit the growth of cancer cells in xenograft models (review in [142]). Allyl mercaptan (AM) is a metabolite of DADS [143]. Induction of histone acetylation by DADS was first described in murine erythroleukemia cells [144]. It should be noted that *HDAC* inhibitory concentrations of DADS exceeded those that might be achievable by dietary consumption of *Allium* vegetables. With respect to direct inhibition of *HDAC* activity *in vitro*, AM was identified as the more potent *HDAC* inhibitor than DADS [143].

Cambinol and dihydrocoumarin

Dihydrocoumarin and cambinol have been identified as *SIRT* inhibitors, but they have not been tested for chemopreventive potential so far. Among other substrates, *SIRT1* has been shown to deacetylate the transcription factor *p53*. Given the regulatory functions of *p53* in cell metabolism, inhibition of *SIRT1* might contribute to inhibition of glycolysis and inhibit cell proliferation.

Cambinol is a β-naphthol compound that inhibits *SIRT1* and *SIRT2*, whereas class I and II *HDAC*s are not inhibited. Treatment of cancer cell lines with cambinol led to hyperacetylation of *p53* [145]. *Dihydrocoumarin* (DHC) is a dietary compound found in sweet clover *(Melilotus officinalis)*. It is utilized as a flavoring agent in food supplements and in cosmetics. DHC inhibited the deacetylase activities of yeast *Sir2p* and human *SIRT1*. Exposure of human lymphoblastoid cells to DHC led to dose-dependent increases of *ac-p53*, cytotoxicity, and apoptosis [146].

3,3'-Diindolylmethane (DIM)

Vegetables of the *Cruciferae* family are a rich source of glucosinolates (review in [128]). Upon physical damage of the plant cell (e.g. during cutting or chewing) the enzyme myrosinase is released and catalyses the conversion of glucosinolates to the corresponding isothiocyanates. The main hydrolysis product of the glucosinolate glucobrassicin is indole-3-carbinol. Under low pH conditions as in the stomach indole-3-carbinol is condensed to lager compounds, resulting in the formation of 3,3'-diindolylmethane (DIM) as the major condensation product. DIM has been shown to suppress cell proliferation and induce apoptosis in various cancer cell lines, by modulation of nuclear receptor- and kinase-mediated signaling pathways and induction of ER stress. DIM also possesses anti-angiogenic activities [147,148]. Recently, DIM was reported to selectively induce proteasome-mediated degradation of class I HDACs (*HDAC 1, 2, 3* and *8*) *in vitro* and in tumor xenografts, whereas class II HDACs were not affected [149]. In addition to *HDAC* inhibition, regulating the expression of HDACs might constitute an alternative mechanism to target cancer cell metabolism.

EGCG

As mentioned above, EGCG has so far mainly been investigated with respect to its effects on DNA methylation (summary in [19]). Recent data has now provided first evidence that EGCG also influences histone acetylation, at least in cell culture. In a study of Nandakumar *et al.,* EGCG decreased global DNA methylation in skin cancer cells and reduced expression of DNMTs. This was accompanied by a decrease in *HDAC* activity and consequent increase in histone H3 and H4 acetylation [150]. In a study analyzing the influence of EGCG on expression of polycomb group proteins involved in histone methylation in skin cancer, Choudhury *et al.* observed an increase in H3 acetylation. This was explained by reduced expression levels of *HDAC1* [151]. Changes in histone acetylation might also be brought about by upregulation or activation of HATs. Accordingly, Li *et al.* reported that treatment of breast cancer cells with EGCG significantly increased *HAT* activity, whereas *HDAC1* protein levels were reduced [152]. These data indicate that EGCG targets the epigenome by multiple mechanisms including histone acetylation. These activities can act coordinately to modulate chromatin structure and gene expression.

Genistein

There is convincing evidence from several studies that genistein modulates histone and non-histone protein acetylation though alterations in the expression of histone modifying enzymes. Genistein caused upregulation of several HAT proteins in prostate cancer cell lines. This resulted in hyperacetylation of histones H3 and H4, increased association of acetylated H3K4 with the transcription start sites of cell-cycle regulators *p21* and *p16,* and subsequent re-expression [153]. Kikuno *et al.* were interested whether the suppressing effects of genistein on *AKT* signaling in prostate cancer might be mediated *via* epigenetic mechanisms. Genistein reduced the expression and nuclear localization of the class III

histone deacetylase *SIRT1*, and increased H3K9 acetylation in the promoter region of *PTEN, CYLD, p53,* and *FOXO3a* [154]. Treatment of androgen-responsive prostate cancer cells with genistein lowered *HDAC6* expression. Since *HDAC6* is estrogen responsive, this was assigned to phytoestrogenic properties of genistein. *HDAC6* is a cytosolic protein and deacetylates α-tubulin and the androgen receptor (*AR*) chaperone *heat shock protein 90* (*Hsp90*). Consequently, reduced *HDAC6* expression by genistein increased *Hsp90* acetylation, dissociation of *AR* and enhanced *AR* poteasomal degradation, and ultimately reduced *AR*-mediated cell signaling [155].

Curcumin

Curcumin (diferuloyl methane) is a yellow pigment and the major active ingredient of turmeric (*Curcuma longa)*. Curcumin is traditionally used in India and South-East Asia to treat wounds, inflammation and tumors. Curcumin is well tolerated and non-toxic, and has been shown to suppress tumor growth through multiple signaling pathways, particularly *NF-κB* signaling. It has demonstrated cancer preventive efficacy in various *in vivo* carcinogenesis models. Activity was attributed to its effects on cell proliferation, invasion, metastasis, and angiogenesis [156-158]. Several clinical trials in patients with inflammatory diseases and cancer have been initiated and show promising first results [157].

Recently, curcumin was identified as a specific inhibitor of the histone acetyl transferase *p300/CBP in vitro* and in cell culture, whereas other histone modifying enzymes, including *PCAF*, *HDAC* and *HTM* activities were not inhibited by curcumin [159,160]. *HAT* inhibition was attributed to a structural modification of *p300*, thereby preventing binding of histones or cofactor acetyl-CoA. Curcumin also inhibited acetylation of *p53* as a non-histone target of *p300/CBP*. In Raji cells, curcumin treatment led to a significant and dose-dependent decreases in *HDAC1* and *p300* protein and mRNA levels [161]. *p300/CBP* can enhance *NF-κB* transcriptional activity as co-activator by acetylating both *NF-κB/p65* and surrounding histones. Direct inhibition and down-regulation of *p300/CBP* could therefore contribute to the well-known inhibition of *NF-κB* by curcumin [162]. Since *NF-κB* was involved in the upregulation of glucose transporters, there might be an interesting link between the activities of curcumin and cancer cell metabolism which needs to be further explored.

Sun *et al*. investigated the effect of curcumin on expression profiles of miRNAs in BxPC-3 human pancreatic cancer cells using a custom miRNA microarray. Treatment with curcumin resulted in the significant upregulation of 11 miRNAs and downregulation of 18 miRNAs. Interestingly, *miRNA23A* and *miRNA23B* were among the miRNAs upregulated by curcumin treatment [163]. These array-based results were not confirmed by alternative methods yet and should not be overinterpreted. However, by increasing the expression *miR-23A* and *miR-23B*, curcumin might affect cell metabolism by enhancing the inhibitory activity of these miRNAs on glutaminase, thereby decreasing the utilization of glutamine for cell metabolism in cancer cells.

Anacardic acid

Anacardic acid (6-nonadecyl salicylic acid) is a component of cashew nut shell liquid. Interestingly, anacardic acid was identified as the first natural product inhibitor of *HAT* activity. In *in vitro* assays it was found to inhibit *p300*, *PCAF* and *Tip60* HAT activities with halfmaximal inhibitory concentrations below 10 μM [164,165]. Similar to the activities of curcumin, anacardic acid was identified to interfere with *NF-κB* signaling through its HAT inhibitory activity. It inhibited the activation of *IκB*α, and reduced acetylation and nuclear translocation of *NF-κB* subunit *p65*. These findings suggest that anacardic acid might be an interesting lead compound for further development in cancer prevention [166].

Garcinol

Garcinol is a polyisoprenylated benzophenone isolated from the Mangosteen tree *Garcinia indica* Choisy (*Clusiaceae*). Garcinol demonstrated chemopreventive properties by anti-oxidant activity, induction of phase II detoxifying enzymes, anti-inflammatory effects, inhibition of cell proliferation, and induction of apoptosis, and prevented colon and tongue cancer *in vivo* (review in [167]). Garcinol was identified as a cell-permeable *HAT* inhibitor which inhibits *PCAF* and *p300* HAT activity with IC_{50} values of 5 μM and 7 μM, respectively. In HeLa cells, garcinol repressed general histone acetylation and induced apoptosis [168].

Summary and outlook

This perspective has summarized our current knowledge on the interrelationship between cancer cell metabolism and epigenetic modulation of gene regulation, and how both processes can be affected by dietary components. As outlined above, the interaction might be in two directions: metabolic processes are involved in providing essential co-factors for epigenetic mechanisms such as DNA methylation and acetylation reactions. Alterations in cancer cell metabolism might therefore shift the availability of co-factors and influence epigenetic gene regulation. On the other hand, expression of selected metabolic genes has been shown to be epigenetically

controlled. So far, only few candidate genes have been described, such as hexokinase II (*HK2*) and fructose-1,6-bisphosphatase 1 (*FBP1*). Systematic analysis of genome-wide alterations in DNA methylation during carcinogenesis by array-based approaches or next-generation sequencing might provide a better overview of metabolic genes aberrantly regulated for example by changes in methylation in their promoter regions.

Over the last few years, evidence has accumulated that dietary agents can influence the epigenome by multiple and interacting pathways. Their effects on global DNA methylation and genes silenced by promoter methylation, histone modifications, and miRNAs deregulated during carcinogenesis might contribute to their cancer preventive potential. Future investigations in animal models will have to demonstrate that these observations are functionally linked. Currently, we can only speculate whether their influence on epigenetic mechanisms is of importance for normalization of the deregulated cancer cell metabolism. It can be anticipated that the emergence of sensitive metabonomics technologies based on nuclear magnetic resonance (NMR) and mass spectrometry (MS) will improve our understanding of natural products' effects on cell metabolism. In combination with genome-wide detection methods for epigenetic alterations and bioinformatic tools to systematically integrate available information, systems are available to address open questions in future studies to better define their impact on epigenetics and cancer cell metabolism and understand mechanistic links.

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