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REVIEW

Epigenetic mechanisms in anti-cancer actions of bioactive food components – the implications in cancer prevention

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The hallmarks of carcinogenesis are aberrations in gene expression and protein function caused by both genetic and epigenetic modifications. Epigenetics refers to the changes in gene expression programming that alter the phenotype in the absence of a change in DNA sequence. Epigenetic modifications, which include amongst others DNA methylation, covalent modifications of histone tails and regulation by non-coding RNAs, play a significant role in normal development and genome stability. The changes are dynamic and serve as an adaptation mechanism to a wide variety of environmental and social factors including diet. A number of studies have provided evidence that some natural bioactive compounds found in food and herbs can modulate gene expression by targeting different elements of the epigenetic machinery. Nutrients that are components of one-carbon metabolism, such as folate, riboflavin, pyridoxine, cobalamin, choline, betaine and methionine, affect DNA methylation by regulating the levels of S-adenosyl-L-methionine, a methyl group donor, and S-adenosyl-L-homocysteine, which is an inhibitor of enzymes catalyzing the DNA methylation reaction. Other natural compounds target histone modifications and levels of non-coding RNAs such as vitamin D, which recruits histone acetylases, or resveratrol, which activates the deacetylase sirtuin and regulates oncogenic and tumour suppressor micro-RNAs. As epigenetic abnormalities have been shown to be both causative and contributing factors in different health conditions including cancer, natural compounds that are direct or indirect regulators of the epigenome constitute an excellent approach in cancer prevention and potentially in anti-cancer therapy.

Abbreviations

DNMTs, DNA methyltransferases; EGCG, (–)-epigallocatechin-3-gallate; HATs, histone acetylases; HCC, hepatocellular carcinoma; HDACs, histone deacetylases; MBDs, methyl-CpG-binding domain proteins; miRNAs, micro-RNAs; MTHF, methylenetetrahydrofolate; RA, retinoic acid; RES, resveratrol; SAH, S-adenosyl-L-homocysteine; SAM, S-adenosyl-L-methionine; UHRF1, ubiquitin-like containing PHD ring finger 1; Vit.D3, vitamin D3

Introduction

Cancer initiation and progression are driven by the concurrent changes in the expression of multiple genes that occur via genetic and epigenetic alterations, leading to either activation of oncogenes and prometastatic genes, or silencing of tumour suppressor genes and to genome rearrangements and instability (Baylin *et al.*, 2001; Widschwendter and Jones,

2002; Szyf, 2005; Stefanska *et al.*, 2011a). Epigenetic modifications have attracted a significant amount of attention in the prevention and treatment of different illnesses, with cancer at the forefront, mainly due to their reversibility. Epigenetics refers to layers of information in addition to genetics and comprises several components such as DNA methylation, covalent histone modifications, particularly histone acetylation and methylation, and non-coding RNA-related mechanisms (Razin and Riggs, 1980; Strahl and Allis, 2000; Jenuwein and Allis, 2001; Bergmann and Lane, 2003). Recent findings have reported additional DNA modifications taking place on the methyl group such as hydroxy-methylcytosine and formyl- and carboxy-methylcytosine (Ito *et al.*, 2010), whose biological role remains to be determined. Although catalyzed by different enzymes and controlled by different protein complexes, all the elements of the epigenome influence each other at the level of the chromatin structure (Razin and Riggs, 1980; Strahl and Allis, 2000; Bergmann and Lane, 2003). DNA hypomethylation, histone acetylation and histone H3K4 methylation have been associated with active chromatin, whereas DNA hypermethylation, histone deacetylation, and histone H3K9 and K27 di- or trimethylation have been found in inactive chromatin regions (Strahl and Allis, 2000). Studies have shown that methylation of DNA results in the recruitment of histone deacetylases (HDACs), which changes chromatin configuration (Nan *et al.*, 1997; Eden *et al.*, 1998). However, alterations in histone marks can also trigger changes in DNA methylation patterns. For example, histone H3K27 methylation and EZH2 histone methyltranferase were required for methylation of EZH2 target genes (Vire *et al.*, 2006), whereas histone acetylation was followed by DNA demethylation (Selker, 1998; Cervoni and Szyf, 2001; Fahrner *et al.*, 2002). Furthermore, changes in chromatin modifications in patients with mutations in the *ATRX* gene encoding SWI/SNF-like chromatin modifying protein resulted in diverse alterations in DNA methylation patterns (Gibbons *et al.*, 2000). In addition, the third component of the epigenetic machinery, non-coding RNAs were shown to be required for *de novo* methylation of imprinted loci (Watanabe *et al.*, 2011) and target mRNA of DNA methyltransferases (DNMTs) and HDACs (Rajewsky, 2006; Tuddenham *et al.*, 2006; Zhou *et al.*, 2010). The trilateral relationship between epigenetic modifications (D'Alessio and Szyf, 2006; Iorio *et al.*, 2010) and their dynamic aspect, confirmed by a long list of studies (Weaver *et al.*, 2004; Levenson *et al.*, 2006; Miller and Sweatt, 2007; Feng *et al.*, 2010), can have important implications for human diseases, including cancer where epigenetic factors are known to have a causal and/or contributing role (Baylin *et al.*, 2001; Fahrner *et al.*, 2002; Widschwendter and Jones, 2002; Szyf *et al.*, 2004; Szyf, 2005; Kanwal and Gupta, 2011; Stefanska *et al.*, 2011a). More interestingly, it has been shown that these epigenetic modifications are regulated by a wide range of bioactive food components (Table 1). Compounds from one-carbon metabolism, such as folate, cobalamin, riboflavin, pirydoxine or methionine, are involved in the regulation of the DNA methylation reaction (Scott and Weir, 1998; Slattery *et al.*, 1999; Selhub, 2002). Their deficiency can lead to hepatocellular carcinoma (HCC) (Newberne and Rogers, 1986; Kanduc *et al.*, 1988; Singh *et al.*, 2003; Asada *et al.*, 2006; Ghoshal *et al.*, 2006; Calvisi *et al.*, 2007) and other types of cancer (Kraunz *et al.*, 2006; Bistulfi *et al.*, 2010; Duthie, 2011). Constituents of tea and soybean such as epigallocatechin and genistein, respectively, reverse hypermethylation and the silencing of tumour suppressor genes and inhibit cancer growth (Fang *et al.*, 2005; Lu *et al.*, 2006). Anthocyanins from black raspberries have been shown to suppress DNMT1 and reactivate tumour suppressor genes by demethylating their promoters (Wang *et al.*, 2011). A vegetarian diet has been found to be associated with promoter hypomethylation and the overexpression of a gene encoding the antioxidative enzyme mitochondrial superoxide dismutase, when compared with aged-matched omnivores (Thaler *et al.*, 2009). A lower incidence of sporadic colorectal cancer in the population of southern Italy in comparison with the rest of the Western world has been linked to the presence of Annurca apple in the diet (Fini *et al.*, 2011). Annurca apple contains polyphenols and is especially rich in chlorogenic acid, caffeic acid, catechin, epicatechin, rutin and phloridizin. Treatment of RKO, SW48 and SW480 colorectal cancer cell lines with an extract of Annurca apple polyphenols was shown to reduce DNA methylation in the promoters of *hMLH1*, *p14* and *p16* tumour suppressor genes and restore their expression with a concomitant decrease in DNMT1 and DNMT3B protein levels (Fini *et al.*, 2007). Using natural compounds to modulate the epigenome opens an emerging field of nutritional epigenetics and offers a new approach to cancer prevention and treatment. In this review, we summarize recent data suggesting that many bioactive dietary compounds exert anti-cancer effects through epigenetic mechanisms. Furthermore, we highlight the close relationship between all the components of the epigenome and its importance in the evaluation of the effects of a tested agent. We finally discuss the difficulty in assessing the efficacy of natural compounds due to poor bioavailability and their dose-dependent effects.

The role of epigenetic modifications in the regulation of gene transcription – relevance to carcinogenesis

DNA methylation

DNA methylation is a covalent modification of DNA that in mammalian cells takes place mainly at the fifth position of the cytosine pyrimidine ring located predominantly within CpG sequences (Hotchkiss, 1948; Wyatt, 1950; Gruenbaum *et al.*, 1981). CG-rich regions, called CpG islands, are mostly unmethylated in normal cells and located in regulatory regions of housekeeping genes, tissue-specific genes and tumour suppressors (Hermann *et al.*, 2004). CpG islands are also present in promoters of some oncogenes, where their methylated state is involved in the formation of an inactive chromatin structure leading to transcriptional silencing (Szyf *et al.*, 2004). Recent data indicate that methylation can also occur in cytosines within dinucleotide sequences other than CpGs, although the role of non-CpG methylation remains to be elucidated (Lister *et al.*, 2009).

DNA methylation in normal cells is implicated in oncogene repression, the control of expression of genes crucial for cell proliferation, differentiation and normal development as well as in parental imprinting, X chromosome inactivation,

Table 1

Source, pharmacological effects and epigenetic mechanisms of action of promising natural compounds

Natural compound	Natural sources	Pharmacological effects	Epigenetic mechanisms of action
Folate, cobalamin, riboflavin, pyridoxine, methionine	Folate and riboflavin: spinach, asparagus, beans, peas, lentils, sunflower seeds, almonds Cobalamin: fish, shellfish, poultry, milk, eggs Pyridoxine and methionine: grains, nuts, dragon fruit, sesame seeds	Anti-cancer, anti-proliferative, chemoprevention of malignant transformation	Regulation of one-carbon metabolism, SAM/SAH ratio, DNMT and MBD expression; regulation of miRNAs (tumour suppressor miR-122, miR-34a, miR-127, and oncogenic miR-21, miR-222)
Retinoic acid	Vietnamese gac, crude palm oil, yellow and orange fruits (mango, papaya), orange root vegetables (carrots), spinach, sweet potatoes	Anti-cancer, anti-proliferative, differentiating, pro-apoptotic	Regulation of DNMTs expression and enzyme activity by affecting p21, AP-1, PTEN and ERs; regulation of miRNAs targeting DNMTs; regulation of tumour suppressor miRNAs (miR-15, miR-16, let-7a, let-7c, miR-34a, miR-342) and oncogenic miRNAs (miR-10a); GNMT regulation; histone acetylation
Vitamin D3	Sun exposure, fish, fish liver oils	Anti-cancer, anti-proliferative, differentiating, pro-apoptotic	Regulation of DNMTs expression and enzyme activity by affecting p21, AP-1, PTEN and ERs; regulation of histone acetylation; regulation of oncogenic miRNAs (miR-181a, miR-181b)
Resveratrol	Roots of hellebore, grapes, mulberries, apricots, pineapples, peanuts	Anti-cancer, antioxidant, anti-proliferative, anti-angiogenesis, anti-inflammatory, pro-apoptotic, cardioprotective	Regulation of DNMTs expression and enzyme activity by affecting p21, AP-1 and PTEN; activation of deacetylase SIRT1 and p300 HAT; down-regulation of UHRF1; regulation of miRNAs
Genistein and daidzein	Soybeans, lupin, kudzu, psoralea, fava beans, coffee	Anti-cancer, antioxidant, antihelminthic, anti-metastatic, cancer protective	Regulation of DNMTs expression and enzyme activity by affecting p21, AP-1 and PTEN; increase in HAT activity; regulation of miRNAs (tumour suppressor miR-1296, miR-16, and oncogenic miR-27a)
EGCG	Green tea	Anti-cancer, antioxidant, anti-proliferative, anti-angiogenesis, anti-inflammatory, pro-apoptotic, cancer protective	Regulation of SAM/SAH ratio by COMT-mediated reactions; direct inhibition of DNMTs by binding to catalytic domain of the enzyme; regulation of tumour suppressor miRNAs (miR-16)
Curcumin	Spice turmeric	Anti-cancer, antioxidant, protects against heart failure	Direct inhibition of DNMTs by binding to catalytic domain of the enzyme; inhibition of HDACs and p300 HAT; regulation of miRNAs (tumour suppressor miR-22, miR-15a, miR-16, and oncogenic miR-21, miR-199a)

and preservation of chromosomal integrity by the silencing of transposons and repetitive elements (Hermann *et al.*, 2004; Szyf *et al.*, 2004). An inverse correlation between the extent of DNA methylation and gene expression was reported in many studies (Rauch *et al.*, 2009; Stefanska *et al.*, 2011a) and several mechanisms were proposed for DNA methylation-mediated transcriptional silencing (Stein *et al.*, 1982). Firstly, some transcription factors, such as CREB (cAMP response element binding protein), E2F (elongation 2 factor), NF-κB and AP-2 (activator protein-2), are unable to recognize specific sequences when they are methylated or methylation occurs in their proximity (Comb and Goodman, 1990; Inamdar

et al., 1991; Paluszczak and Baer-Dubowska, 2005). Secondly, the binding of transcription factors to regulatory elements within promoters and enhancers can be hindered by methyl-CpG-binding domain proteins (MBDs) that bind with high affinity to methylated DNA and cover recognition elements (Nan *et al.*, 1997; Reik and Dean, 2001). The third mechanism is associated with the MBD-mediated recruitment of HDACs and histone methyltransferases that set up a compacted inactive chromatin state around the gene (Nan *et al.*, 1997; Eden *et al.*, 1998; Das and Singal, 2004). Numerous data show that the hallmark of cancer is global hypomethylation and hypermethylation of specific regions, mainly within promoters of tumour suppressor genes. The increase in promoter DNA methylation was reported as a common mechanism of tumour suppressor gene silencing observed in many types of cancer (Baylin *et al.*, 2001; Szyf *et al.*, 2004; Hatziapostolou and Iliopoulos, 2011), and the reversal of the aberrant DNA hypermethylation reactivated gene transcription (Szyf, 2005; Stefanska *et al.*, 2010; 2011b). Recent whole-genome approaches demonstrate that loss of DNA methylation in gene promoters can have a detrimental effect in cancer as hypomethylation was linked to the activation of genes implicated in metastasis, invasion and other biological functions typical for cellular transformation (Stefanska *et al.*, 2011a).

DNA methylation is carried out by DNMTs, which catalyze the transfer of a methyl group from the ubiquitous methyl donor, S-adenosyl-L-methionine (SAM), to the fifth position of a cytosine pyrimidine ring (Gruenbaum *et al.*, 1981). The main enzyme responsible for the maintenance of DNA methylation patterns is DNMT1 (Chen *et al.*, 2007a), whereas *de novo* methylation activity is attributed to DNMT3A and DNMT3B (Okano *et al.*, 1999; Robertson *et al.*, 1999). Both DNMT3s play a role in genomic imprinting and silencing of transposons and repetitive elements during embryonic development (Okano *et al.*, 1998; 1999) and their substrate recognition is regulated by another member of DNMTs, DNMT3L (Bourc'his *et al.*, 2001; Bourc'his and Bestor, 2004).

DNA methylation has been considered as a non-reversible reaction for decades. It was assumed that demethylation can only occur in a passive way by blocking the activity of DNMTs in dividing cells. However, evidence has been obtained showing that active demethylation does take place, such as demethylation of paternal genome, during embryogenesis or demethylation in post-mitotic neurons (Weaver *et al.*, 2004; Mastronardi *et al.*, 2007; Feng *et al.*, 2010). Szyf *et al.* (Bhattacharya *et al.*, 1999; Ramchandani *et al.*, 1999; Detich *et al.*, 2002) showed that one of the MBDs, MBD2, has demethylase activity and demethylates both fully methylated and hemimethylated DNA *in vitro*. One of the mechanisms of the active enzymatic removal of a methyl group from DNA can involve oxidation of 5-methylcytosine to 5-hydroxymethylcytosine, followed by the release of a methyl group in formaldehyde (Hamm *et al.*, 2008). This concept becomes more probable in the light of recent findings demonstrating the presence of 5-hydroxymethylcytosine in mammalian DNA (Kriaucionis and Heintz, 2009; Ndlovu *et al.*, 2011). Although the biological function of this modification still remains poorly understood, recent reports providing insights into 5-hydroxymethylcytosine genomic distribution suggest it is potentially highly relevant in gene regulation (Ndlovu *et al.*, 2011). The ten-eleven translocation (TET) family of enzymes has been demonstrated to catalyze the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine. In addition, TET1 was required for the maintainance of demethylation of *nanog* in embryonic stem cells (Ito *et al.*, 2010). This raises the possibility that this reaction might be an intermediate form of active DNA demethylation (Ndlovu *et al.*, 2011). An alternative mechanism suggested for DNA demethylation is based on the DNA repair system, where 5-methylcytosine is replaced with non-methylated cytosine (Razin *et al.*, 1986; Barreto *et al.*, 2007; Rai *et al.*, 2008). Deamination of 5-methylcytosine to thymine carried out by the activation-induced (cytosine) deaminase (Rai *et al.*, 2008) creates a G : T mismatch that is recognized by a specific thymine glycosylase, Mbd4, and the repair is mediated by the growth arrest and DNA-damage inducible (Gadd45) protein (Barreto *et al.*, 2007).

Histone modifications and non-coding RNAs

Chromatin consists of DNA, histones and non-histone proteins, and plays a role in compacting DNA, preventing DNA damage, controlling gene expression and DNA replication (Kouzarides, 2007; Cedar and Bergman, 2009). DNA is wrapped around a histone octamer that comprises two subunits of each of the core histones, H2A, H2B, H3 and H4 (Kouzarides, 2007; Kanwal and Gupta, 2011), containing amino acid residues with an amino group (Kouzarides, 2007; Bannister and Kouzarides, 2011; Fullgrabe *et al.*, 2011). The positively charged amino group interacts with the negatively charged phosphate groups of DNA, which results in the compaction of chromatin and the formation of transcriptionally inactive heterochromatin. It is well established that post-translational modifications of the histone tails regulate gene expression by determining chromatin structure; these modifications include methylation, acetylation, ubiquitination, phosphorylation, sumoylation, ADP ribosylation, biotinylation and proline isomerization (Jenuwein and Allis, 2001; Kouzarides, 2007; Bannister and Kouzarides, 2011). These modifications are dynamic and controlled by a variety of enzymes that add and remove specific groups. The most studied are histone acetylation mediated by histone acetyltransferases (HATs) and HDACs, and histone methylation controlled by methyltransferases (HMTs) and demethylases (HDMs) (Kouzarides, 2007; Yang and Seto, 2007; Bannister and Kouzarides, 2011). Actively transcribed genes are enriched with active histone marks such as acetylation of H3K9, H3K14 and H3K4, trimethylation of H3K4, dimethylation of H3K79 and monomethylation of H3K4, H3K9, H3K27, H3K79 and H2BK5, whereas regions transcriptionally inactive are characterized by dimethylation of H3K9 and H3K27, and trimethylation of H3K9, H3K27 and H2BK5 (Barski *et al.*, 2007; Koch *et al.*, 2007; Li *et al.*, 2007; Steger *et al.*, 2008; Rosenfeld *et al.*, 2009; Karlic *et al.*, 2010b). Recent studies with genome-wide approaches revealed that histone modifications have different functional outcomes on gene transcription and chromatin structure, depending on their positional context as well as the interplay among various histone modifications (Li *et al.*, 2007; Batta *et al.*, 2011).

Although still controversial, non-coding RNAs have been included as a component of the epigenome (Choud-

huri, 2011). Non-coding RNAs are encoded by a major part of our genome and regulate gene expression by controlling translation of mRNA into proteins. Without changing the DNA sequence, non-coding RNAs and more specifically micro-RNAs (miRNAs) are involved in heritable changes in gene expression. More interestingly, they are implicated in reciprocal interconnections between all the components of the epigenome. Aberrant miRNA regulation, especially that associated with cancer, can be influenced by DNA methylation and histone covalent modifications (Lu *et al.*, 2005; Iorio *et al.*, 2010). On the other hand, miRNA affects the epigenome by targeting enzymes of the epigenetic machinery (Iorio *et al.*, 2010; Zhou *et al.*, 2010). DNMT1 and DNMT3B are targets of miR-148a in cholangiocarcinoma and cervical cancer, respectively. HDAC1 is targeted by miR-449a and b in prostate cancer, and HDAC4 by miR-1 in HCC. miRNA genes are transcribed in the nucleus and the miRNA transcript is processed in the cytoplasm where it is cut by the endoribonuclease Dicer to approximately 22 nucleotide fragments. This short, double-stranded RNA is part of the RNA-induced silencing complex where one strand of miRNA is eliminated and the other strand is orientated by Argonaute protein, Ago2, for interaction with a target mRNA. Complementary binding to mRNA is followed by its cleavage, degradation and inhibition of translation (Fire *et al.*, 1998; Zhou *et al.*, 2010; Choudhuri, 2011).

Aberrations in both histone modifications and miRNA regulation have been shown to be associated with several diseases, including cancer (Kanwal and Gupta, 2010; Lovat *et al.*, 2011). Deregulation of miRNAs is linked to tumour initiation and progression in many human cancers (Lovat *et al.*, 2011).

The impact of natural compounds on DNA methylation

Accumulating evidence shows that natural compounds can alter DNA methylation patterns by directly interacting with enzymes responsible for epigenetic marks or indirect regulation of genes that encode proteins implicated in the epigenetic machinery.

Folate and other nutrients of one-carbon metabolism

A group of nutrients involved in one-carbon metabolism such as folate, cobalamin, riboflavin, pirydoxine and methionine have a direct effect on the level of a methyl donor, SAM (Figure 1). Folate is a water-soluble form of vitamin B9, which is important for DNA synthesis, repair and methylation. After dietary intake, folate is converted to tetrahydrofolate, which is involved in the remethylation of homocysteine to methionine (Scott and Weir, 1998; Selhub, 2002). Methionine is a precursor of SAM, which is the primary methyl group donor for most methylation reactions (Waterland, 2006). After transferring a methyl group, SAM is converted to S-adenosyl-L-homocysteine (SAH), an inhibitor of methylation reactions (Figure 1). Numerous studies have shown a causal role of a folate deficiency in cancer development (Duthie *et al.*, 2004), including amongst others colon (Kim, 2004; Duthie, 2011), prostate (Bistulfi *et al.*, 2010) and liver cancer (James *et al.*, 2003), as well as head and neck squamous cell carcinoma (Kraunz *et al.*, 2006). Malignant transformation was accompanied by SAM depletion, global DNA hypomethylation, gene-specific hypomethylation and

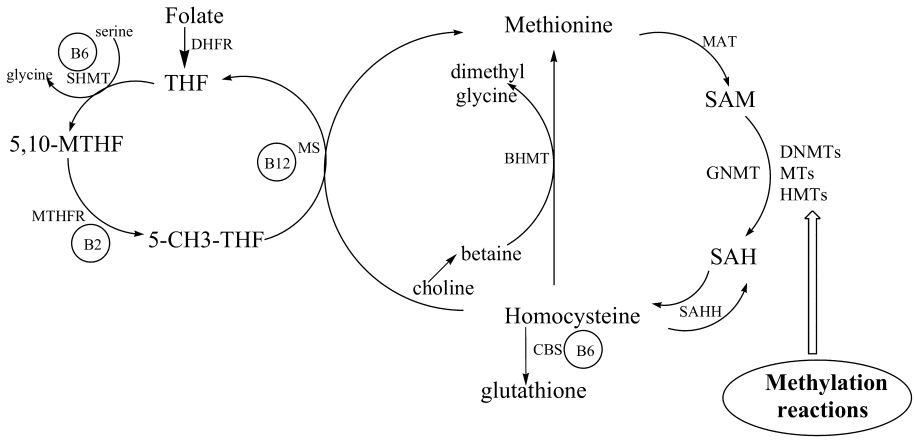

Figure 1

One-carbon metabolism. This network of biochemical reactions is essential for synthesis of S-adenosyl-L-methionine (SAM) that is the unique methyl donor for various methylation reactions including DNA and histone methylation. The crucial co-enzymes and intermediary compounds involved in the one-carbon metabolism comprise bioactive food components such as folate, pyridoxine (vitamin B6), riboflavin (vitamin B2) and cobalamin (vitamin B12), as well as methionine and choline. Abbreviations: 5,10-MTHF, 5,10-methylenetetrahydrofolate; 5-CH3-THF, 5-methyltetrahydrofolate; BHMT, betaine homocysteine methyltransferase; CBS, cystathionine-β-synthase; DHFR, dihydrofolate reductase; DNMTs, DNA methyltransferases; GNMT, glycine *N*-methyltransferase; HMTs, histone methyltransferases; MAT, methionine adenosyltransferase; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; MTs, methyltransferases; SAH, S-adenosyl-L-homocysteine; SAHH, S-adenosyl-L-homocysteine hydrolase; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate.

activation of oncogenes, and paradoxically in hypermethylation and silencing of tumour suppressor genes (James *et al.*, 2003; Davis and Uthus, 2004; Duthie *et al.*, 2004). Folate deficiency has been shown to alter components of the DNA methylation machinery with the increase in DNMT1, DNMT3B, MBD2 and MBD4 mRNA and protein levels during the early stages of hepatocarcinogenesis (Ghoshal *et al.*, 2006). The increase in DNMTs may explain the hypermethylation observed, whereas the stimulation of MBD2 and MBD4 may be responsible for hypomethylation of oncogenes and prometastatic genes, as both proteins have been implicated in active DNA demethylation (Bhattacharya *et al.*, 1999; Ramchandani *et al.*, 1999; Detich *et al.*, 2002; Rai *et al.*, 2008; Popp *et al.*, 2010). Hence, folic acid supplementation may have a chemopreventive effect; this has been demonstrated in a few cancer models, including hepatocarcinogenesis. In rats fed a folic acid-enriched diet followed by the chemical initiation of liver cancer, cell growth and the number of preneoplastic lesions as well as c-myc oncogene expression were diminished compared with a control group (Chagas *et al.*, 2011). Although no changes in global DNA methylation were detected in this study, the SAM/SAH ratio significantly increased, which has the potential to induce gene-specific alterations. Furthermore, folic acid supplementation has been found to reverse cervical dysplasia in patients using oral contraceptives and prevent cervical cancer (Butterworth *et al.*, 1982; Whitehead *et al.*, 1989). Recent prospective and epidemiological studies of 718 Chinese breast cancer cases, Shanghai Women's Health Study (1997–2008), indicated that high folate intake reduces breast cancer risk in premenopausal women and that low intakes of methionine, pyridoxine, cobalamin, niacin and riboflavin are associated with breast carcinogenesis (Shrubsole *et al.*, 2011). A protective role of folate in breast cancer, particularly in oestrogen receptor (ER)-negative cancer, was also shown in another prospective study (Maruti *et al.*, 2009).

Other B vitamins implicated in one-carbon metabolism include riboflavin (B2), pyridoxine (B6) and cobalamin (B12) (Figure 1). Riboflavin is a component of FAD (flavin adenine dinucleotide) that acts as a cofactor for methylenetetrahydrofolate (MTHF) reductase catalyzing the conversion of 5,10-MTHF to 5-MTHF, a substrate for homocysteine remethylation to methionine (Slattery *et al.*, 1999). As shown in Figure 1, blocking this enzyme activity will diminish methionine synthesis and DNA methylation reactions. It was reported that a reduced riboflavin intake can lead to changes in the methyl supply, followed by alterations in the DNA methylation state (Singh *et al.*, 2003). Pyridoxine acts not only as a cofactor for serine hydroxymethyltransferase in the synthesis of 5,10-MTHF from THF (Selhub, 2002) but also participates in the *trans*-sulfuration reactions, where glutathione is formed from homocysteine (Figure 1) (Maruti *et al.*, 2009). A low pyridoxine plasma concentration has been linked to an increased risk of colorectal cancer in a prospective nested case–control study of female participants (The Nurses' Health Study; Wei *et al.*, 2005). As a consequence of pyridoxine depletion, the SAH level increases, resulting in DNA hypomethylation through inhibition of methyltransferases (Maruti *et al.*, 2009). Cobalamin is another vitamin B that, by affecting one-carbon metabolism, is involved in the regulation of DNA methylation. As a cofactor for methionine synthase, cobalamin is important for the methylation of homocysteine to methionine (Figure 1) (Selhub, 2002). Indeed, in many studies, a diet deficient in major dietary sources of the methyl group, including folate and cobalamin, has been linked to the development of HCC (Newberne and Rogers, 1986; Kanduc *et al.*, 1988; Asada *et al.*, 2006; Calvisi *et al.*, 2007). Promoter hypomethylation and increased expression of oncogenes were detected in livers of rats fed methyl-deficient diets (Zapisek *et al.*, 1992). More interestingly, the effects of folate, pyridoxine and cobalamin supplementation on DNA methylation and cancer risk were dependent on MTHFR gene polymorphisms. Colon cancer risk was reduced by 30–40% in individuals with variant TT genotype of the MTHFR gene who consumed adequate amounts of the nutrients compared to those with CC genotype whose diet was low in nutrients (Slattery *et al.*, 1999; Davis and Uthus, 2004).

Retinoic acid and vitamin D3

Contrary to nutrients and vitamins such as folic acid or cobalamin, which act directly on the synthesis of a methyl group donor, the epigenetic activity of retinoic acid (RA) and vitamin D3 (Vit.D3) is mainly mediated by an interaction with their receptors. RA, which is generated via oxidative conversion from β-carotene absorbed from fruits and vegetables, binds to nuclear retinoic acid receptors (RARs) α, β and γ that heterodimerize with retinoid X receptors (RXRs). This complex further modulates transcription through cognate response elements in the promoters of target genes including *p21* and *AP-1* (Liu *et al.*, 1996; Wang *et al.*, 2001; Benkoussa *et al.*, 2002; Yu *et al.*, 2008). RA activated *p21* transcription in cervical squamous carcinoma (Wang *et al.*, 2001; Arany *et al.*, 2003), and inhibited AP-1 transcriptional activity in gastric cancer cells (Wu *et al.*, 2002). As p21 competes with DNMT1 for the same binding site on PCNA that is crucial for DNMT1 activity (Chuang *et al.*, 1997; Milutinovic *et al.*, 2000; Iida *et al.*, 2002), RA-mediated *p21* induction decreases DNA methylation efficiency (Figure 2B). On the other hand, AP-1 stimulates *DNMT1* transcription through binding sites located within the *DNMT*1 regulatory region (Bigey *et al.*, 2000). Therefore, inhibiton of AP-1 activity by RA will down-regulate AP-1 responsive genes, including *DNMT1* (Wu *et al.*, 2002) and decrease DNA methylation (Figure 2C). As changes in DNA methylation affect gene function and signalling pathway networks (Maradeo and Cairns, 2011), a modulation of DNA methylation patterns can contribute to the anti-proliferative, differentiating and pro-apoptotic actions of RA, which have been observed in a variety of cancers, such as leukaemia (Degos *et al.*, 1995), breast (Fujii *et al.*, 2008), ovarian (Zhang *et al.*, 2000), and head and neck cancer (Youssef *et al.*, 2004). Treatment of non-invasive MCF-7 cells with RA caused a reduction in promoter methylation and an increase in the expression of *RARβ2* and *PTEN* tumour suppressor genes, which was associated with inhibition of breast cancer cell growth (Stefanska *et al.*, 2010; 2011b). Although a decrease in *DNMT1* mRNA level was not observed in these studies, genome-wide analysis of all-*trans* RA on gene methylation and expression in neuroblastoma revealed the down-regulation of methyltransferases DNMT1 and DNMT3B, along with the up-regulation of endogenous miRNAs targeting these DNMTs

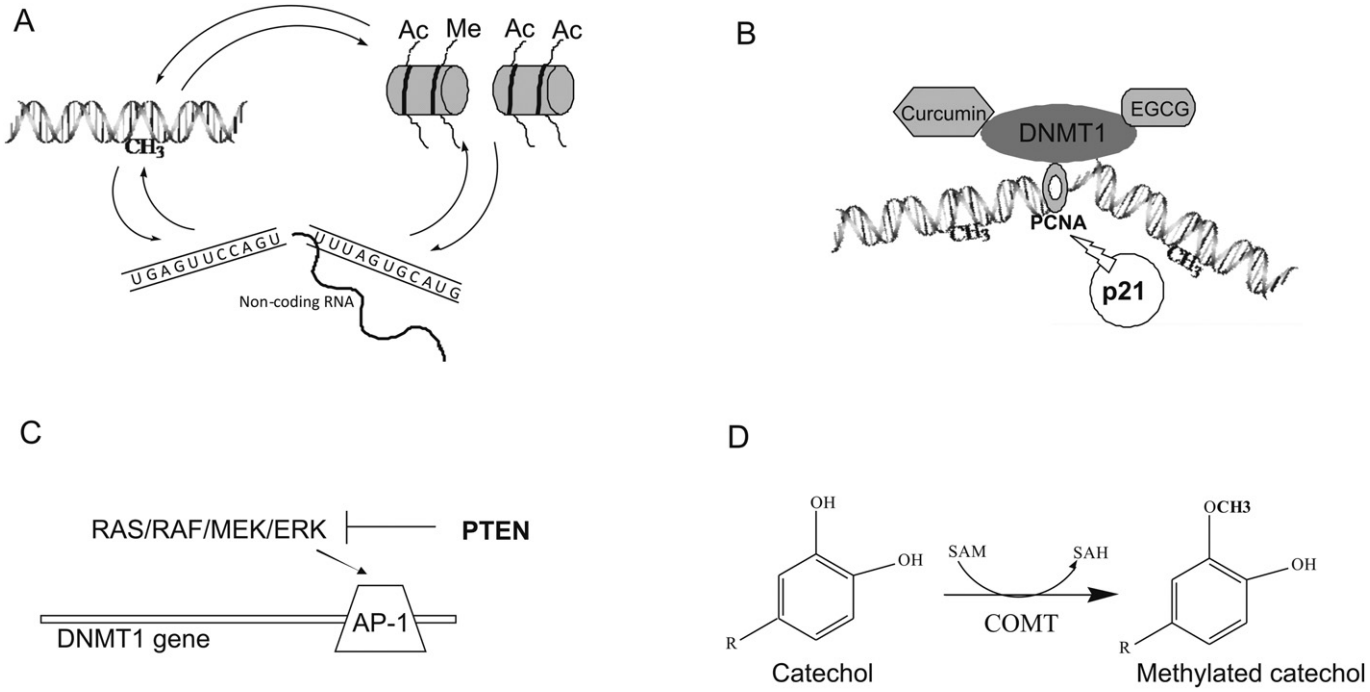

Figure 2

Scheme demonstrating mechanisms used by natural compounds to drive changes in the epigenome. (A) Reciprocal interconnections between all the components of the epigenome: DNA methylation, histone modifications and non-coding RNAs. Compounds driving changes in DNA methylation patterns may have indirect effects on other epigenetic components and *vice versa*. (B) PCNA is crucial for DNMT1 activity during replication when DNA methylation pattern is copied from a parental to a daughter DNA strand. p21 competes with DNMT1 for the same binding site on PCNA, which impairs DNMT1 activity. Compounds that lead to an increase in *p21* expression can affect DNA methylation. Several natural agents such as curcumin and EGCG bind to the DNMT1 catalytic domain and impair its enzymatic activity. (C) AP-1 binding sites have been identified within the *DNMT1* regulatory region. Compounds that inhibit the MAPK signalling pathway by, for example, up-regulating *PTEN* can attenuate *DNMT1* transcription. (D) Compounds with a catechol group are excellent substrates for catechol-*O*-methyltransferase (COMT) that catalyzes their methylation. The COMT-mediated methylation reaction results in depletion of the methyl donor SAM and formation of SAH, which is a potent feedback inhibitor of DNA methylation.

(Das *et al.*, 2010). These changes in DNMT expression were accompanied by the demethylation and overexpression of 82 genes, including *NOS1* that is required for neural cell differentiation. MiRNA-mediated attenuation of DNMTs after RA treatment constitutes another mechanism for the demethylating activity of this compound. Furthermore, a decrease in DNA promoter methylation has been linked to the ability of RA to up-regulate the activity of glycine *N*-methyltransferase (GNMT) that catalyzes the conversion of SAM to SAH (Rowling *et al.*, 2002). Activation of GNMT leads to the loss of methyl groups crucial for SAM-dependent methylation reactions (Figure 1).

Vit.D3, synthesized upon sun exposure and from diet, acts in a similar manner to RA by binding to vitamin D receptors (VDR) that can homodimerize or heterodimerize with RXR or RAR and affect gene transcription through VDR responsive elements (VDRE) in target genes including *p21* (Saramaki *et al.*, 2009) and *PTEN* (Pan *et al.*, 2010). Epigenetic effects of Vit.D3 that can be partially linked to its activity as a chemopreventive and anti-cancer agent (Dace *et al.*, 1997; Gurlek *et al.*, 2002; Lin *et al.*, 2002; Palmer *et al.*, 2003) were primarily observed as histone modifications, which will be discussed in the next chapter. However, demethylating effects were also reported, for instance in osteocalcin promoter in MG63 osteosarcoma cells (Haslberger *et al.*, 2006) and promoters of tumour suppressor genes in breast cancer cells (Stefanska *et al.*, 2010; 2011b). Exposure to Vit.D3 caused hypomethylation and induction of *PTEN* and *RARβ2* tumour suppressor genes in breast cancer cells (Stefanska *et al.*, 2010; 2011b). Vit.D3-mediated promoter demethylation was also observed in *CDH1* gene encoding for E-cadherin, which resulted in the activation of this gene and differentiation of breast cancer cells (Lopes *et al.*, 2012). Similar to RA, changes in DNA methylation patterns induced by Vit.D3 treatment may be attributed to the negative regulation of DNMT1 activity via stimulation of *p21* expression and inhibiton of AP-1 activity (Figure 2). In breast cancer, Vit.D3 increases *p21* expression (Wang *et al.*, 2001; Stefanska *et al.*, 2011b), whereas in breast cancer and leukaemic cells, Vit.D3 and its derivatives induce *PTEN* transcription (Hisatake *et al.*, 2001; Stefanska *et al.*, 2011b). Activation of *PTEN* that is another negative regulator of AP-1 (Chung *et al.*, 2006) may down-regulate AP-1 responsive genes, including *DNMT1* (Figure 2C) (Bigey *et al.*, 2000). More interestingly, the effects of Vit.D3 as well as RA seem to depend on cell invasiveness and ER status. Previous studies in human breast cancer cells indicated that activation of ERs stimulates the activity of c-Ha-ras, which leads to induction of the Ras/Raf/MAPK/

AP-1 signalling pathway (Pethe and Shekhar, 1999). As the AP-1 transcription complex activates the *DNMT1* promoter (Bigey *et al.*, 2000), inactivation of ERs, by causing inhibition of AP-1, can consequently reduce *DNMT1* expression. Vit.D3 as well as RA were shown to down-regulate ER abundance and function in MCF-7 cells (Pratt *et al.*, 1996; Swami *et al.*, 2000), which can explain their stronger epigenetic effects in this cell line compared with ER-negative MDA-MB-231 cells (Stefanska *et al.*, 2010; 2011b).

Phytoestrogens

Several polyphenols called phytoestrogens such as resveratrol (RES) and genistein interact with ERs and regulate oestrogen-responsive genes such as *p21* (Bowers *et al.*, 2000; Mandal and Davie, 2010). They can act as oestrogen agonists or antagonists depending on their concentrations, tissue type and genes tested on, as well as the presence of oestradiol (Pozo-Guisado *et al.*, 2002; 2004). RES (3,4′,5-trihydroxystilbene) is a dietary antioxidant found in a wide variety of plant species, including mulberries, peanuts, grapes, apricots and pineapples (Le Corre *et al.*, 2004). This polyphenol was shown to modulate the risk of cardiovascular disease (atherosclerosis) and breast cancer, inhibit chemical carcinogenesis in rodents and growth promotion of preneoplastic lesions by effects on multiple signalling pathways, inhibit cell proliferation, block cell cycle progression and induce apoptosis in numerous types of human cancer cell lines (Bowers *et al.*, 2000; Cornwell *et al.*, 2004; Whitsett and Lamartiniere, 2006). Genistein (4′,5,7-trihydroxyisoflavone) is a major isoflavone from soybean. This polyphenol has been shown to prevent carcinogenesis in animal models (Dixon and Ferreira, 2002), reduce mammary carcinogenesis in rats exposed to carcinogens (Cabanes *et al.*, 2004), inhibit growth of transplanted human prostate cancer cells in mice (Zhou *et al.*, 1999) and inhibit carcinogenesis and metastasis in various types of cancer (Arai *et al.*, 2000; Fang *et al.*, 2005). Moreover, it has been reported that genistein inhibits cancer cell proliferation and angiogenesis as well as induces apoptosis and cell cycle arrest (Li *et al.*, 1999; Fang *et al.*, 2005). Dietary intake of genistein decreases mammary tumour incidence and increases tumour latency in rats exposed to chemical carcinogens. These breast cancer protective effects were found to be associated with the down-regulation of the oncogenic Wnt-signalling pathway and the up-regulation of *PTEN* tumour suppressor gene in the mammary gland, which was coincident with increased apoptosis and enhanced differentiation (Dave *et al.*, 2005; Su *et al.*, 2007). Changes in the epigenome upon treatment with these phytoestrogens have been reported in numerous studies. In non-invasive MCF-7 breast cancer cells, RES exposure led to hypomethylation and reactivation of *RARβ2* and *PTEN* tumour suppressor genes with a concomitant decrease in *DNMT1* expression and up-regulation of *p21* (Stefanska *et al.*, 2011b). RES prevented *BRCA1* promoter hypermethylation and silencing induced by an aromatic hydrocarbon receptor ligand in MCF-7 cells (Papoutsis *et al.*, 2011). Treatment of KYSE510 oesophageal squamous cell carcinoma cells with genistein resulted in a partial reversal of *p16*, *RARβ* and *MGMT* (O^6-methylguanine methyltransferase) promoter hypermethylation and in reactivation of these genes, which was associated with the inhibition of DNMT activity (Fang *et al.*, 2005). Similarly, methylation-silenced *RARβ* was hypomethylated and reactivated in LNCaP and PC3 prostate cancer cells exposed to genistein (Fang *et al.*, 2007). Genistein significantly decreased promoter methylation and reactivated *BTG3* tumour suppressor gene in A498, ACHN and HEK-293 renal cell carcinoma cell lines, as well as LNCaP and PC3 prostate carcinoma cell lines (Majid *et al.*, 2009). Its effect was comparable with that of 5-aza-cytydine, which is a potent demethylating agent. Changes in DNA methylation were accompanied by increased histone acetylation and RNA polymerase II binding at the *BTG3* promoter, suggesting active chromatin formation (Majid *et al.*, 2009). Daidzein is another phytoestrogen found in soy (Barnes *et al.*, 1996). Multiple studies have indicated that daidzein may act as a growth modulator in various types of cancer, such as colon (Bielecki *et al.*, 2011), prostate (Vardi *et al.*, 2010), breast (Choi and Kim, 2008) and pancreatic cancer (Guo *et al.*, 2004). Treatment of human prostate cancer cells with daidzein led to demethylation of glutathione S-transferase P1 (*GSTP1*) and ephrin B2 (*EPHB2*) promoters and to an increase in their protein levels (Vardi *et al.*, 2010). Demethylation and activation of tumour suppressor gene *p16* was also detected in RKO colon cancer cells exposed to nordihydroguaiaretic acid found in the creosote bush (Cui *et al.*, 2008) and to quercetin, which is another flavonoid found in a wide variety of vegetables, fruits, leaves and grains (Tan *et al.*, 2009).

The epigenetic activity of phytoestrogens can be attributed to their ability to stimulate *p21* via ERE elements in its promoter or other indirect mechanisms (Le Corre *et al.*, 2004; Matsumura *et al.*, 2008; Mandal and Davie, 2010) as well as to suppress AP-1 transcriptional activity via antagonism of ERs (Pethe and Shekhar, 1999) or inducing *PTEN* expression (Chung *et al.*, 2006). As mentioned earlier, both p21 and AP-1 are involved in the regulation of DNMT1 expression/activity (Figure 2) (Chuang *et al.*, 1997; Bigey *et al.*, 2000; Iida *et al.*, 2002). RES-mediated *p21* up-regulation has been reported in many types of cancer, including prostate, bladder, colorectal and breast cancer (Tessitore *et al.*, 2000; Bai *et al.*, 2010; Stefanska *et al.*, 2010; 2011b; Hsieh *et al.*, 2011). Genistein treatment has been shown to induce an up-regulation of *p21* in mouse B16-F1 melanoma cells, mouse fibroblasts (Kuzumaki *et al.*, 1998) and breast cancer cells (Privat *et al.*, 2010). Also, exposure to both RES and genistein is associated with an up-regulation of *PTEN* tumour suppressor gene in breast and prostate cancer (Waite *et al.*, 2005; Kikuno *et al.*, 2008; Rahal and Simmen, 2010; Stefanska *et al.*, 2011b).

Polyphenols with catechol group

Certain polyphenols such as bioflavonoids (quercetin, fisetin and myricetin), tea catechins [catechin, epicatechin, (–)-epigallocatechin-3-gallate (EGCG)] and coffee polyphenols (caffeic acid, chlorogenic acid) exert a profound inhibitory effect on DNA methylation and DNMT activities (Yang *et al.*, 2008). Treatment of MCF-7 and MDA-MB-231 breast cancer cells with caffeic acid or chlorogenic acid decreased *RARβ* promoter methylation (Lee and Zhu, 2006). Moreover, consumers of high doses of chlorogenic acid had an increased plasma concentration of total homocysteine, which is an inhibitor of methylation reactions (Olthof *et al.*, 2001). EGCG, a major polyphenol from green tea, inhibited UVB-

induced global hypomethylation and protected mice from photocarcinogenesis and transformation of papillomas to carcinomas (Mittal *et al.*, 2003). Exposure of human oesophageal cancer KYSE510 cells to EGCG resulted in demethylation and reactivation of *p16*, *RARβ*, *MGMT* and *hMLH1* tumour suppressor genes (Lu *et al.*, 2006). Promoter methylation-mediated silencing of *p16* and *RARβ* was also found to be reversed by EGCG in colon cancer HT-29, prostate cancer PC3, breast cancer MCF-7 and MDA-MB-231 cell lines (Lee *et al.*, 2005; Fang *et al.*, 2007).

The tea catechins and bioflavonoids have also been shown to inhibit the prokaryotic SssI methyltransferase-mediated DNA methylation as well as the human DNMT1-mediated DNA methylation reaction in a concentration-dependent manner with diverse potencies (Lee *et al.*, 2005). The presence of a catechol group in the structure of all these compounds can play a key role in inhibiting methyltransferase activity as compounds with a catechol group are excellent substrates for the methylation mediated by catechol-*O*-methyltransferase (COMT) (Figure 2D). COMT-mediated methylation results in the depletion of the methyl donor SAM and the formation of SAH, which is a potent feedback inhibitor of DNA methylation (Lee and Zhu, 2006). EGCG can exert a dual inhibitory effect on DNMT-mediated DNA methylation, which explains its high efficacy at inhibiting DNA methylation catalyzed by SssI methyltransferase and human DNMT1 compared with other compounds tested (Lee *et al.*, 2005). Like other catechol-containing polyphenols, EGCG inhibits methyltransferase activity by increase the level of SAH in the reaction catalyzed by COMT. In addition, this tea polyphenol was shown to interact directly with the catalytic site of human DNMT1. Molecular modelling studies indicate that EGCG is well accommodated in a hydrophilic pocket of DNMT1 and effectively tethered within the DNMT1 binding site by at least four hydrogen bonds (Fang *et al.*, 2007) and stabilized by magnesium ions (Figure 2B) (Lee *et al.*, 2005). EGCG analogues without the gallic acid moiety were found to be poor inhibitors of DNMTs, therefore, the galloyl moiety is assumed to be crucial for EGCG binding to DNMTs. Furthermore, myricetin, which has a pyrogallic acid moiety similar to the galloyl group of EGCG, is a more potent, direct inhibitor of DNMT activity than other bioflavonoids, such as quercetin or fisetin, in the presence of magnesium ions (Lee *et al.*, 2005).

Parthenolide and curcumin

Parthenolide was initially shown to have anti-cancer properties by inhibiting NF-κB activation through alkylation of Cys38 of p65 (Wiedhopf *et al.*, 1973; Woynarowski and Konopa, 1981; Garcia-Pineres *et al.*, 2004; Guzman *et al.*, 2005). This compound, with a sesquiterpene lactone structure, is naturally present in the plant feverfew. Recent findings indicate that parthenolide inhibits DNMT1 activity and decreases *DNMT1* expression; in K562, Kasumi-1, MV4-11 leukaemia cell lines and MCF-7 breast cancer cells, it decreased the global methylation and led to hypomethylation and activation of the *HIN-1* tumour suppressor gene (Liu *et al.*, 2009a). This inhibition of DNMT1 is probably linked to alkylation of the proximal thiolate of Cys1226 of the DNMT1 catalytic domain by parthenolide's γ-methylene lactone, as well as to interference with Sp1 binding to *DNMT1* promoter and down-regulation of *DNMT1* owing to the induction of subG1 cell cycle arrest (Liu *et al.*, 2009a). Another natural compound directly regulating activity of DNMTs is curcumin, which is present in the spice turmeric. This agent was found to induce global DNA methylation in MV4-11 leukaemia cells (Liu *et al.*, 2009b) and to hypomethylate and reactivate *RARβ2* and *WIF-1* tumour suppressor genes in human cervical and non-small cell lung cancer cells, respectively (Jha *et al.*, 2010; Liu *et al.*, 2011). Furthermore, exposure to curcumin led to promoter demethylation and re-expression of *NEUROG1* and *NRF1* in human prostate cancer cells (Khor *et al.*, 2011; Shu *et al.*, 2011). Curcumin can directly interact with SssI methyltransferase, a homologue of DNMT1, and covalently bind to the catalytic thiol group of Cys1226 present in DNMT1, which causes the inhibition of DNMT1 catalytic activity (Figure 2B) (Liu *et al.*, 2009b).

Compounds targeting UHRF1

Down-regulation of UHRF1 (ubiquitin-like containing PHD ring finger 1) may be one of the mechanisms by which natural compounds exert their effect on DNA methylation. Thymoquinone from black cumin oil (Alhosin *et al.*, 2011) and polyphenols from red wine such as RES, anthocyanins and procyanidins (Sharif *et al.*, 2010) have been shown to suppress UHRF. UHRF1 is a putative oncogene that was found to be overexpressed in multiple cancer types such as prostate, lung, cervical, pancreatic, breast, bladder and kidney (Bronner *et al.*, 2007; Unoki *et al.*, 2009). Through its SRA (set and ring associated) domain, UHRF1 recognizes hemimethylated DNA and interacts with DNMT1 in order to form a complex responsible for copying the DNA methylation pattern from a maternal to a daughter strand (Bostick *et al.*, 2007; Sharif *et al.*, 2007). The same domain plays an important role in the cooperation of UHRF1 with HDAC1 (Unoki *et al.*, 2004). UHRF1 up-regulation in cancer is associated with hypermethylation and inhibition of tumour suppressor genes (Daskalos *et al.*, 2011). DNA demethylation caused by natural compounds may be a consequence of UHRF1 inhibition and subsequent attenuation of DNMT activity. UHRF1 suppression may also result from up-regulation of negative regulators such as p53 and p73 that are activated by many natural compounds, leading to cell cycle arrest and apoptosis (Arima *et al.*, 2004; Alhosin *et al.*, 2011).

The impact of natural compounds on histone modifications

Numerous findings demonstrate interactions and feedback regulations between all the components of the epigenome, which should be considered when evaluating epigenetic effects of natural and synthetic agents (Figure 2A) (D'Alessio and Szyf, 2006; Iorio *et al.*, 2010). In addition, many compounds exert a direct effect on both DNA methylation and histone modifications, as well as on miRNAs. For instance, the complex of RA with receptors and coactivators from the p160 and p300 family contains HAT activity which changes chromatin structure, making it more easily accessible for proteins of the transcription complex (Chen *et al.*, 1997; Lamond and Earnshaw, 1998). Genistein-induced changes in DNA

methylation are also associated with a concomitant increase in histone acetylation and HAT activity (Majid *et al.*, 2009). Curcumin inhibits the activity of several HDACs, suppressing proliferation and inducing apoptosis of cancer cells (Liu *et al.*, 2005; Chen *et al.*, 2007b). More interestingly, other reports have revealed that curcumin binds p300 HAT, leading to a conformational change in the enzyme and its decreased affinity to histones H3 and H4 and acetyl CoA, which has therapeutic potential for cancer and heart failure (Reuter *et al.*, 2011). Curcumin-mediated hypoacetylation appears to be dependent on reactive oxygen species as antioxidant enzymes diminish its effect (Kang *et al.*, 2005). RES and quercetin activate the protein deacetylase SIRT1 (Borra *et al.*, 2005; Chung *et al.*, 2010), which targets different proteins including histones (Zhang and Kraus, 2010). After binding to SIRT1, RES induces a conformational change in the enzyme, which allows tighter fluorophore and peptide substrate binding to the enzyme (Borra *et al.*, 2005). SIRT1 activation leads to the formation of inactive chromatin and changes in gene transcription (Vaquero *et al.*, 2004). After the other hand, RES activates p300 HAT that participates in the formation of an active chromatin structure (Narayanan *et al.*, 2003). Further, recent studies have shown that RES also inhibits oncogenic miRNAs and induces tumour suppressor miRNAs (Tili *et al.*, 2010). The dual activity of RES on the regulation of histone modifications and targeting non-coding RNAs can partially explain the activation of tumour suppressor genes such as *PTEN* and *PDCD4* and attenuation of expression of prometastatic genes such as *TGFβ* (Tili *et al.*, 2010). This dual activity was also reported for Vit.D3. The VDR/RXR dimer interacts with HATs such as SRC-1 and CBP/p300 and participates in the activation of transcription (Chakravarti *et al.*, 1996; Voegel *et al.*, 1996). On the other hand, the VDR/RXR complex also binds to the so-called negative VDREs present, amongst others, within *CYP24* that recruits transcriptional co-repressors like NCoR and SMRT, leading to histone deacetylation and transcriptional silencing (Sanchez-Martinez *et al.*, 2008). More interestingly, the effects of Vit.D3 are modulated by epigenetic mechanisms that affect VDRs; polymorphic variations of VDRs have been associated variously with cancer development, aggressiveness and metastasis (Ingles *et al.*, 1997; Guy *et al.*, 2004; Khanim *et al.*, 2004; Karlic and Varga, 2011). Pan *et al.* (2010) found that the HDAC inhibitors, trichostatin A (TSA) and sodium butyrate, and the methylation inhibitor, 5-aza-2′-deoxycytidine (5-azaCdR), reactivate *VDR* expression and restore the sensitivity of cancer cells to Vit.D3-mediated apoptosis. Targeted co-treatments with Vit.D3 and HDAC inhibitors resulted in re-expression of antiproliferative target genes such as *GADD45α* and *CDKN1A*, and synergistic inhibition of proliferation. Microarray studies demonstrated that *VDR*-reactivation induced by TSA and followed by Vit.D3 treatment uniquely up-regulated a group of target genes associated with the control of proliferation and induction of apoptosis in prostate cancer cells (Khanim *et al.*, 2004). Silencing of *VDR* by promoter hypermethylation was reported in breast cancer cell lines and primary tumours that were resistant to Vit.D3 (Marik *et al.*, 2010). Treatment with 5-azaCdR restored *VDR* expression with concurrent reactivation of *VDR* target genes. Hence, it is increasingly clear that epigenetic mechanisms attenuate and selectively distort the transcriptional responsiveness of *VDR*, raising the possibility of reversal of Vit.D3 insensitivity by epigenetically active drugs (Karlic *et al.*, 2010a).

A set of natural compounds has been identified as new generation agents that mediate changes in histone, mainly through affecting the activities of histone modifying enzymes. Garlic organosulfur compounds, such as diallyl disulfide, as well as sulforaphane inhibit HDAC activity and affect gene transcription *in vitro* and *in vivo* coincident with tumour suppression (Nian *et al.*, 2009; Druesne-Pecollo and Latino-Martel, 2011). Diallyl disulfide can be metabolized to allyl mercaptan that is a competitive HDAC inhibitor. Its anti-proliferative activity was linked to the inhibition of HDAC, histone hyperacetylation and increase in *p21* expression in human colon cancer cells (Myzak and Dashwood, 2006; Druesne-Pecollo and Latino-Martel, 2011). Sulforaphane, found in broccoli and broccoli sprouts, induced global histone acetylation and increased histone acetylation associated with *p21* and *Bax* gene promoters in Apc^{min} mice and human colon and prostate cancer cells (Myzak and Dashwood, 2006; Dashwood and Ho, 2007). Another natural compound tha inhibits HDACs and increases histone acetylation within the *p21* promoter is sodium butyrate, a derivative of a short-chain fatty acid present in cheese and butter and produced from dietary fibre in the large intestine (Davie, 2003). Luteolin, a flavonoid found in high concentrations in parsley, thyme, peppermint, basil herb, celery and artichoke, acts as a HDAC inhibitor and is associated with the inhibition of cancer cell growth, survival and invasion, and also potentiated the cytotoxicity of cisplatin in lung LNM35 cells (Attoub *et al.*, 2011).

The impact of natural compounds on the regulation of miRNAs

Aberrant expression of miRNAs, driven by both genetic and epigenetic factors and involved in cancer initiation and progression, has been shown to be reversed by various dietary components (Davis and Ross, 2008; Saini *et al.*, 2010; Parasramka *et al.*, 2012). A recent study by Starlard-Davenport *et al.* (2010) demonstrated alterations in the expression of miRNAs, induced by methyl deficiency, as a prominent event during the early stages of liver carcinogenesis and suggests that the pattern of miRNAs expression may predict susceptibility to liver carcinogenesis. As mentioned before, rats fed a methyl-deficient diet deprived of folate, methionine and choline developed HCC (Asada *et al.*, 2006). More interestingly, this effect was associated not only with DNA hypomethylation but also with changes in miRNA expression (Davis and Ross, 2008; Saini *et al.*, 2010; Parasramka *et al.*, 2012). MiR-122, that acts as a tumour suppressor in liver, was down-regulated and the levels of miR-34a and miR-127 were also attenuated with concomitant alterations in the expression of *E2F3* and *Bcl-6* that are their predicted targets, respectively. Several miRNAs were increased including miR-21, which mediates tumour growth and is overexpressed in various types of cancer including breast, colon, lung and leukaemia (Davis and Ross, 2008; Parasramka *et al.*, 2012). Furthermore, changes in miRNAs detected upon culture of human lym-

phoblastoid cells in media containing low folate levels were reversed after replacement with a complete media. One of the up-regulated oncogenic miRNAs in this culture, miR-222, was found in the blood of individuals with low folate intake (Davis and Ross, 2008; Parasramka *et al.*, 2012), which supports the use of miRNAs as potential cancer biomarkers.

The treatment of acute promyelocytic cells with all-*trans* RA resulted in alterations in cellular levels of miRNAs implicated in haematopoietic differentiation and apoptosis (Davis and Ross, 2008; Saini *et al.*, 2010; Parasramka *et al.*, 2012). For instance, miR-15/miR-16 and let-7a were up-regulated, which correlated with decreased expression of their targets, *Bcl-2* and *Ras* respectively. Other non-coding RNAs regulated by RA include miR-10a, which promotes metastasis of pancreatic cancer cells, and miR-34a, which acts as a tumour suppressor in neuroblastoma cell lines. Studies have shown that the fusion protein PML/RARα binds to the regulatory regions of certain miRNAs, such as miR-342 and let-7c. Upon RA treatment, the fusion protein is released, leading to transcriptional activation (Saini *et al.*, 2010). This has been proposed to be one of the mechanisms of RA-mediated regulation of miRNAs. Another vitamin implicated in the regulation of miRNAs is Vit.D3. Vit.D3 induces differentiation of human myeloid leukaemia cells, which was associated with *p27* up-regulation, and a detailed analysis revealed the concomitant suppression of miR-181a and miR-181b that target *p27*. More interestingly, this effect of Vit.D3 appears to be interrupted by miR-125b that negatively regulates *VDR* expression through a potential miR-125b recognition element identified within 3′-UTR of human *VDR* mRNA (Parasramka *et al.*, 2012).

Exposure of BxPC-3 pancreatic cancer cells to curcumin changes the expression profiles of miRNAs, with a robust increase in the tumour suppressor miR-22 and a decrease in the oncogenic miR-199a (Davis and Ross, 2008; Saini *et al.*, 2010; Parasramka *et al.*, 2012). An up-regulation of miR-22 suppresses the expression of its target genes, *Sp1* and *ERα*. In contrast, miR-21 expression was attenuated by curcumin, which results in the induction of the tumour suppressor *PTEN* (Parasramka *et al.*, 2012). In another study, curcumin was found to increase the levels of pro-apoptotic miR-15a and miR-16 in MCF-7 breast cancer cells, which was accompanied by the inhibition of their target gene, *Bcl-2* (Saini *et al.*, 2010).

Aberrations in the expression of miRNAs and their targets were also observed after exposure to genistein and EGCG. Genistein caused a down-regulation of oncogenic miR-27a in unveal melanoma cells *in vitro* and *in vivo*, inhibiting their ability to proliferate (Saini *et al.*, 2010; Parasramka *et al.*, 2012). Furthermore, this isoflavone up-regulated the tumour suppressor miR-1296 in prostate cancer cells, which was accompanied by a decreased expression of its target gene, *MCM2*, involved in DNA replication and tumorigenesis (Parasramka *et al.*, 2012). Genistein and EGCG augment miR-16 levels in murine chronic lymphocytic leukaemia cells and HCC HepG2 cells, respectively, leading to *Bcl-2* inhibition and an increase in apoptosis (Saini *et al.*, 2010; Parasramka *et al.*, 2012).

Given that the expression of miRNAs is dysregulated in numerous types of cancer, a possibility of reversal of the changes by natural compounds may have tremendous potential for cancer prevention.

Bioavailability and dose-dependent effects of natural compounds

Numerous bioactive food components have been shown to possess diverse pharmacological effects, including anti-proliferative, anti-inflammatory, antioxidant, anti-angiogenic and anti-cancer. Clinical trials of natural compounds such as curcumin, RES, EGCG, lycopene, genistein, selenium and Vit.D3 as chemopreventive and therapeutic agents for cancer show poor bioavailability, which raises issues with extrapolation of *in vitro* results to physiological effects (Bemis *et al.*, 2006; Amin *et al.*, 2009). The main reasons for reduced bioavailability are high rates of metabolism and rapid elimination and clearance from the body. For example, rapid sulfate and glucuronide conjugation by the intestine and liver limits RES and curcumin bioavailability (Walle *et al.*, 2004; Anand *et al.*, 2007). Recent data indicate that administration of RES or curcumin with piperine, an alkaloid derived from black pepper, significantly improves their bioavailability through inhibition of glucuronidation, increasing the degree of exposure and the maximum plasma concentration (Shoba *et al.*, 1998; Johnson *et al.*, 2011). The absorption is even higher when the compounds are entrapped in nanoparticles (Shaikh *et al.*, 2009; Peng *et al.*, 2010). The development of different approaches aimed at increasing the bioavailability of natural agents is of great importance for their efficacy. For instance, stability and metabolism determine the effects of certain polyphenols, including EGCG on SIRT1 stimulation, as the metabolites are less active (de Boer *et al.*, 2006).

As the data from the literature indicate, the effects of several natural compounds depend on their concentrations. Folic acid is an excellent example; it was shown to reduce cancer cell growth, to demethylate and activate methylation-silenced tumour suppressor genes at 0.88 mg·L^{-1} of media in Caco-2 colon adenocarcinoma cells (Berner *et al.*, 2010). However, a 10-fold higher concentration led to enhanced cancer cell proliferation, with concomitant increase in methylation of tumour suppressor genes such as *ERα, p16* and *p15* (Berner *et al.*, 2010). Furthermore, oral treatment with folic acid and cobalamin at doses of 0.8 and 0.4 mg·day^{-1}, respectively, was associated with an increased risk of cancer incidence in patients with ischaemic heart disease (Ebbing *et al.*, 2009). Increasing concerns about food fortified with folic acid has resulted in large randomized, placebo-controlled trials, which have not confirmed a statistically significant effect of folic acid supplementation on increased cancer risk (Clarke *et al.*, 2010). Studies in healthy premenopausal women have demonstrated that the action of genistein depends on its circulating levels. Genistein decreases the methylation of *RARβ2* and *CCND2* at low concentrations, whereas high concentrations increase the methylation of these tumour suppressor genes (Qin *et al.*, 2009). These results should be taken seriously as *RARβ2* and *CCDN2* hypermethylation is observed at early stages of breast cancer development and is linked to poor prognosis. Other findings demonstrate that RES has no effect on promoter methylation of several tumour suppressor genes such as *RASSF1A*, *GSTP1* and *HIN-1* in MCF-7 cells, despite the fact that it attenuates DNMT activity (Paluszczak *et al.*, 2010). The possibility that

alterations in DNA methylation are located in regions other than those tested, or that different doses of the compound and/or chronic exposure are needed to observe the epigenetic effect cannot be ruled out.

In the last decade, the anti-cancer activity of multiple bioactive food components with a diverse range of molecular targets has been proven by intensive research but a major concern is their poor bioavailability, which remains a challenge. Attempts at improving their bioavailability are focused on the modulation of the route of administration, structural modifications, blocking metabolic pathways and combinations with other agents.

Future prospects

Numerous data have shown that alterations in epigenetic modifications produce various effects on the phenotype. Compounds that directly affect DNA methylation can induce changes in other components of the epigenome due to a trilateral relationship that exists between DNA methylation, histone covalent modifications and non-coding RNAs. Furthermore, cancer, like other multiple conditions such as diabetes, allergy, rheumatoid arthritis and cardiovascular disease, has been linked to chronic inflammation and metabolic stress. Studies have shown that epigenetics constitute the crossroads of cancer, inflammation and metabolic stress (vel Szic *et al.*, 2010). Signalling pathways responsive to chronic inflammation activate downstream mediators such as transcription factors that drive changes in the expression of target genes. However, binding of the transcription factors to DNA depends on the chromatin structure, which is regulated by epigenetic machinery. Thus, inflammatory gene expression is dependent on epigenetic mechanisms. Reciprocally, inflammatory genes, such as *IL-6*, have been reported to trigger epigenetic alterations, for instance *via* the regulation of DNMTs (vel Szic *et al.*, 2010). Dietary phytochemicals that modulate epigenetic components can thereby suppress the expression of inflammatory genes and inflammation-related disorders. Recent studies have added additional layers of complexity, showing that changes in DNA methylation can be transgenerationally transmitted to future generations (Szyf, 2012). Nutritional restriction during pregnancy (Heijmans *et al.*, 2008; Unterberger *et al.*, 2009) and low folic acid content before conception (Sinclair *et al.*, 2007) have been shown to permanently alter DNA methylation patterns, which are linked to chronic diseases in adulthood, such as obesity and cancer (vel Szic *et al.*, 2010). A methyl donor-rich diet or with a high genistein content during pregnancy in Agouti mice increased DNA methylation within a transposable element in the agouti gene, causing changes in fur colour and protecting the offspring from obesity (Waterland and Jirtle, 2003; Dolinoy *et al.*, 2006). Natural compounds are able to modulate our epigenome and probably the epigenome of future generations, which raises questions on phenotypic consequences and pharmacological applications. These important issues should be addressed in future nutritional research. From the experimental evidence discussed in the present review, epigenetic modifications are one of the mechanisms by which natural compounds inhibit cancer cell growth, invasive capacities and metastasis. Epigenetic aberrations lead to dysregulation of gene expression, with the activation of oncogenes and inhibition of tumour suppressor genes at initial and advanced stages of carcinogenesis (Chik *et al.*, 2011). Thus, natural chemicals can be efficacious in both cancer therapy and cancer prevention and are becoming potential targets of drug development. The main challenges in the investigation of phytochemicals as epigenetic agents are new strategies of increasing their bioavailability, assessing the efficacy of the metabolites and determining the role of natural compounds alone or in combination with existing drugs in improving cancer treatment.

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Conflict of interest

The authors have no conflicts of interest to declare.

References

Alhosin M, Sharif T, Mousli M, Etienne-Selloum N, Fuhrmann G, Schini-Kerth VB *et al.* (2011). Down-regulation of UHRF1, associated with re-expression of tumor suppressor genes, is a common feature of natural compounds exhibiting anti-cancer properties. J Exp Clin Cancer Res 30: 41.

Amin AR, Kucuk O, Khuri FR, Shin DM (2009). Perspectives for cancer prevention with natural compounds. J Clin Oncol 27: 2712–2725.

Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB (2007). Bioavailability of curcumin: problems and promises. Mol Pharm 4: 807–818.

Arai N, Strom A, Rafter JJ, Gustafsson JA (2000). Estrogen receptor beta mRNA in colon cancer cells: growth effects of estrogen and genistein. Biochem Biophys Res Commun 270: 425–431.

Arany I, Whitehead WE, Ember IA, Tyring SK (2003). Dose-dependent activation of p21WAF1 transcription by all-trans-acid in cervical squamous carcinoma cells. Anticancer Res 23: 495–497.

Arima Y, Hirota T, Bronner C, Mousli M, Fujiwara T, Niwa S *et al.* (2004). Down-regulation of nuclear protein ICBP90 by p53/p21Cip1/WAF1-dependent DNA-damage checkpoint signals contributes to cell cycle arrest at G1/S transition. Genes Cells 9: 131–142.

Asada K, Kotake Y, Asada R, Saunders D, Broyles RH, Towner RA *et al.* (2006). LINE-1 hypomethylation in a choline-deficiency-induced liver cancer in rats: dependence on feeding period. J Biomed Biotechnol 2006: 17142.

Attoub S, Hassan AH, Vanhoecke B, Iratni R, Takahashi T, Gaben AM *et al*. (2011). Inhibition of cell survival, invasion, tumor growth and histone deacetylase activity by the dietary flavonoid luteolin in human epithelioid cancer cells. Eur J Pharmacol 651: 18–25.

Bai Y, Mao QQ, Qin J, Zheng XY, Wang YB, Yang K *et al*. (2010). Resveratrol induces apoptosis and cell cycle arrest of human T24 bladder cancer cells *in vitro* and inhibits tumor growth *in vivo*. Cancer Sci 101: 488–493.

Bannister AJ, Kouzarides T (2011). Regulation of chromatin by histone modifications. Cell Res 21: 381–395.

Barnes S, Sfakianos J, Coward L, Kirk M (1996). Soy isoflavonoids and cancer prevention. Underlying biochemical and pharmacological issues. Adv Exp Med Biol 401: 87–100.

Barreto G, Schafer A, Marhold J, Stach D, Swaminathan SK, Handa V *et al*. (2007). Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. Nature 445: 671–675.

Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z *et al*. (2007). High-resolution profiling of histone methylations in the human genome. Cell 129: 823–837.

Batta K, Zhang Z, Yen K, Goffman DB, Pugh BF (2011). Genome-wide function of H2B ubiquitylation in promoter and genic regions. Genes Dev 25: 2254–2265.

Baylin SB, Esteller M, Rountree MR, Bachman KE, Schuebel K, Herman JG (2001). Aberrant patterns of DNA methylation, chromatin formation and gene expression in cancer. Hum Mol Genet 10: 687–692.

Bemis DL, Katz AE, Buttyan R (2006). Clinical trials of natural products as chemopreventive agents for prostate cancer. Expert Opin Investig Drugs 15: 1191–1200.

Benkoussa M, Brand C, Delmotte MH, Formstecher P, Lefebvre P (2002). Retinoic acid receptors inhibit AP1 activation by regulating extracellular signal-regulated kinase and CBP recruitment to an AP1-responsive promoter. Mol Cell Biol 22: 4522–4534.

Bergmann A, Lane ME (2003). HIDden targets of microRNAs for growth control. Trends Biochem Sci 28: 461–463.

Berner C, Aumuller E, Gnauck A, Nestelberger M, Just A, Haslberger AG (2010). Epigenetic control of estrogen receptor expression and tumor suppressor genes is modulated by bioactive food compounds. Ann Nutr Metab 57: 183–189.

Bhattacharya SK, Ramchandani S, Cervoni N, Szyf M (1999). A mammalian protein with specific demethylase activity for mCpG DNA. Nature 397: 579–583.

Bielecki A, Roberts J, Mehta R, Raju J (2011). Estrogen receptor-beta mediates the inhibition of DLD-1 human colon adenocarcinoma cells by soy isoflavones. Nutr Cancer 63: 139–150.

Bigey P, Ramchandani S, Theberge J, Araujo FD, Szyf M (2000). Transcriptional regulation of the human DNA methyltransferase (dnmt1) gene. Gene 242: 407–418.

Bistulfi G, Vandette E, Matsui S, Smiraglia DJ (2010). Mild folate deficiency induces genetic and epigenetic instability and phenotype changes in prostate cancer cells. BMC Biol 8: 6.

de Boer VC, de Goffau MC, Arts IC, Hollman PC, Keijer J (2006). SIRT1 stimulation by polyphenols is affected by their stability and metabolism. Mech Ageing Dev 127: 618–627.

Borra MT, Smith BC, Denu JM (2005). Mechanism of human SIRT1 activation by resveratrol. J Biol Chem 280: 17187–17195.

Bostick M, Kim JK, Esteve PO, Clark A, Pradhan S, Jacobsen SE (2007). UHRF1 plays a role in maintaining DNA methylation in mammalian cells. Science 317: 1760–1764.

Bourc'his D, Bestor TH (2004). Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. Nature 431: 96–99.

Bourc'his D, Xu GL, Lin CS, Bollman B, Bestor TH (2001). Dnmt3L and the establishment of maternal genomic imprints. Science 294: 2536–2539.

Bowers JL, Tyulmenkov VV, Jernigan SC, Klinge CM (2000). Resveratrol acts as a mixed agonist/antagonist for estrogen receptors alpha and beta. Endocrinology 141: 3657–3667.

Bronner C, Achour M, Arima Y, Chataigneau T, Saya H, Schini-Kerth VB (2007). The UHRF family: oncogenes that are drugable targets for cancer therapy in the near future? Pharmacol Ther 115: 419–434.

Butterworth CE, Jr, Hatch KD, Gore H, Mueller H, Krumdieck CL (1982). Improvement in cervical dysplasia associated with folic acid therapy in users of oral contraceptives. Am J Clin Nutr 35: 73–82.

Cabanes A, Wang M, Olivo S, DeAssis S, Gustafsson JA, Khan G *et al*. (2004). Prepubertal estradiol and genistein exposures up-regulate BRCA1 mRNA and reduce mammary tumorigenesis. Carcinogenesis 25: 741–748.

Calvisi DF, Simile MM, Ladu S, Pellegrino R, De Murtas V, Pinna F *et al*. (2007). Altered methionine metabolism and global DNA methylation in liver cancer: relationship with genomic instability and prognosis. Int J Cancer 121: 2410–2420.

Cedar H, Bergman Y (2009). Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet 10: 295–304.

Cervoni N, Szyf M (2001). Demethylase activity is directed by histone acetylation. J Biol Chem 276: 40778–40787.

Chagas CE, Bassoli BK, de Souza CA, Deminice R, Junior AA, Paiva SA *et al*. (2011). Folic acid supplementation during early hepatocarcinogenesis: cellular and molecular effects. Int J Cancer doi: 10.1002/ijc.25886 [Epub ahead of print].

Chakravarti D, LaMorte VJ, Nelson MC, Nakajima T, Schulman IG, Juguilon H *et al*. (1996). Role of CBP/P300 in nuclear receptor signalling. Nature 383: 99–103.

Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L *et al*. (1997). Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. Cell 90: 569–580.

Chen T, Hevi S, Gay F, Tsujimoto N, He T, Zhang B *et al*. (2007a). Complete inactivation of DNMT1 leads to mitotic catastrophe in human cancer cells. Nat Genet 39: 391–396.

Chen Y, Shu W, Chen W, Wu Q, Liu H, Cui G (2007b). Curcumin, both histone deacetylase and p300/CBP-specific inhibitor, represses the activity of nuclear factor kappa B and Notch 1 in Raji cells. Basic Clin Pharmacol Toxicol 101: 427–433.

Chik F, Szyf M, Rabbani SA (2011). Role of epigenetics in cancer initiation and progression. Adv Exp Med Biol 720: 91–104.

Choi EJ, Kim GH (2008). Daidzein causes cell cycle arrest at the G1 and G2/M phases in human breast cancer MCF-7 and MDA-MB-453 cells. Phytomedicine 15: 683–690.

Choudhuri S (2011). From Waddington's epigenetic landscape to small noncoding RNA: some important milestones in the history of epigenetics research. Toxicol Mech Methods 21: 252–274.

Chuang LS, Ian HI, Koh TW, Ng HH, Xu G, Li BF (1997). Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1. Science 277: 1996–2000.

Chung JH, Ostrowski MC, Romigh T, Minaguchi T, Waite KA, Eng C (2006). The ERK1/2 pathway modulates nuclear PTEN-mediated cell cycle arrest by cyclin D1 transcriptional regulation. Hum Mol Genet 15: 2553–2559.

Chung S, Yao H, Caito S, Hwang JW, Arunachalam G, Rahman I (2010). Regulation of SIRT1 in cellular functions: role of polyphenols. Arch Biochem Biophys 501: 79–90.

Clarke R, Halsey J, Lewington S, Lonn E, Armitage J, Manson JE *et al*. (2010). Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: meta-analysis of 8 randomized trials involving 37 485 individuals. Arch Intern Med 170: 1622–1631.

Comb M, Goodman HM (1990). CpG methylation inhibits proenkephalin gene expression and binding of the transcription factor AP-2. Nucleic Acids Res 18: 3975–3982.

Cornwell T, Cohick W, Raskin I (2004). Dietary phytoestrogens and health. Phytochemistry 65: 995–1016.

Cui Y, Lu C, Liu L, Sun D, Yao N, Tan S *et al*. (2008). Reactivation of methylation-silenced tumor suppressor gene p16INK4a by nordihydroguaiaretic acid and its implication in G1 cell cycle arrest. Life Sci 82: 247–255.

Dace A, Martin-el Yazidi C, Bonne J, Planells R, Torresani J (1997). Calcitriol is a positive effector of adipose differentiation in the OB 17 cell line: relationship with the adipogenic action of triiodothyronine. Biochem Biophys Res Commun 232: 771–776.

D'Alessio AC, Szyf M (2006). Epigenetic tete-a-tete: the bilateral relationship between chromatin modifications and DNA methylation. Biochem Cell Biol 84: 463–476.

Das PM, Singal R (2004). DNA methylation and cancer. J Clin Oncol 22: 4632–4642.

Das S, Foley N, Bryan K, Watters KM, Bray I, Murphy DM *et al*. (2010). MicroRNA mediates DNA demethylation events triggered by retinoic acid during neuroblastoma cell differentiation. Cancer Res 70: 7874–7881.

Dashwood RH, Ho E (2007). Dietary histone deacetylase inhibitors: from cells to mice to man. Semin Cancer Biol 17: 363–369.

Daskalos A, Oleksiewicz U, Filia A, Nikolaidis G, Xinarianos G, Gosney JR *et al*. (2011). UHRF1-mediated tumor suppressor gene inactivation in nonsmall cell lung cancer. Cancer 117: 1027–1037.

Dave B, Eason RR, Till SR, Geng Y, Velarde MC, Badger TM *et al*. (2005). The soy isoflavone genistein promotes apoptosis in mammary epithelial cells by inducing the tumor suppressor PTEN. Carcinogenesis 26: 1793–1803.

Davie JR (2003). Inhibition of histone deacetylase activity by butyrate. J Nutr 133: 2485S–2493S.

Davis CD, Ross SA (2008). Evidence for dietary regulation of microRNA expression in cancer cells. Nutr Rev 66: 477–482.

Davis CD, Uthus EO (2004). DNA methylation, cancer susceptibility, and nutrient interactions. Exp Biol Med (Maywood) 229: 988–995.

Degos L, Dombret H, Chomienne C, Daniel MT, Miclea JM, Chastang C *et al*. (1995). All-trans-retinoic acid as a differentiating agent in the treatment of acute promyelocytic leukemia. Blood 85: 2643–2653.

Detich N, Theberge J, Szyf M (2002). Promoter-specific activation and demethylation by MBD2/demethylase. J Biol Chem 277: 35791–35794.

Dixon RA, Ferreira D (2002). Genistein. Phytochemistry 60: 205–211.

Dolinoy DC, Weidman JR, Waterland RA, Jirtle RL (2006). Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. Environ Health Perspect 114: 567–572.

Druesne-Pecollo N, Latino-Martel P (2011). Modulation of histone acetylation by garlic sulfur compounds. Anticancer Agents Med Chem 11: 254–259.

Duthie SJ (2011). Folate and cancer: how DNA damage, repair and methylation impact on colon carcinogenesis. J Inherit Metab Dis 34: 101–109.

Duthie SJ, Narayanan S, Sharp L, Little J, Basten G, Powers H (2004). Folate, DNA stability and colo-rectal neoplasia. Proc Nutr Soc 63: 571–578.

Ebbing M, Bonaa KH, Nygard O, Arnesen E, Ueland PM, Nordrehaug JE *et al*. (2009). Cancer incidence and mortality after treatment with folic acid and vitamin B12. JAMA 302: 2119–2126.

Eden S, Hashimshony T, Keshet I, Cedar H, Thorne AW (1998). DNA methylation models histone acetylation. Nature 394: 842.

Fahrner JA, Eguchi S, Herman JG, Baylin SB (2002). Dependence of histone modifications and gene expression on DNA hypermethylation in cancer. Cancer Res 62: 7213–7218.

Fang M, Chen D, Yang CS (2007). Dietary polyphenols may affect DNA methylation. J Nutr 137: 223S–228S.

Fang MZ, Chen D, Sun Y, Jin Z, Christman JK, Yang CS (2005). Reversal of hypermethylation and reactivation of p16INK4a, RARbeta, and MGMT genes by genistein and other isoflavones from soy. Clin Cancer Res 11: 7033–7041.

Feng J, Zhou Y, Campbell SL, Le T, Li E, Sweatt JD *et al*. (2010). Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. Nat Neurosci 13: 423–430.

Fini L, Selgrad M, Fogliano V, Graziani G, Romano M, Hotchkiss E *et al*. (2007). Annurca apple polyphenols have potent demethylating activity and can reactivate silenced tumor suppressor genes in colorectal cancer cells. J Nutr 137: 2622–2628.

Fini L, Piazzi G, Daoud Y, Selgrad M, Maegawa S, Garcia M *et al*. (2011). Chemoprevention of intestinal polyps in ApcMin/+ mice fed with western or balanced diets by drinking annurca apple polyphenol extract. Cancer Prev Res (Phila) 4: 907–915.

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391: 806–811.

Fujii T, Yokoyama G, Takahashi H, Namoto R, Nakagawa S, Toh U *et al*. (2008). Preclinical studies of molecular-targeting diagnostic and therapeutic strategies against breast cancer. Breast Cancer 15: 73–78.

Fullgrabe J, Kavanagh E, Joseph B (2011). Histone onco-modifications. Oncogene 30: 3391–3403.

Garcia-Pineres AJ, Lindenmeyer MT, Merfort I (2004). Role of cysteine residues of p65/NF-kappaB on the inhibition by the sesquiterpene lactone parthenolide and N-ethyl maleimide, and on its transactivating potential. Life Sci 75: 841–856.

Ghoshal K, Li X, Datta J, Bai S, Pogribny I, Pogribny M *et al.* (2006). A folate- and methyl-deficient diet alters the expression of DNA methyltransferases and methyl CpG binding proteins involved in epigenetic gene silencing in livers of F344 rats. J Nutr 136: 1522–1527.

Gibbons RJ, McDowell TL, Raman S, O'Rourke DM, Garrick D, Ayyub H *et al.* (2000). Mutations in ATRX, encoding a SWI/SNF-like protein, cause diverse changes in the pattern of DNA methylation. Nat Genet 24: 368–371.

Gruenbaum Y, Naveh-Many T, Cedar H, Razin A (1981). Sequence specificity of methylation in higher plant DNA. Nature 292: 860–862.

Guo JM, Xiao BX, Dai DJ, Liu Q, Ma HH (2004). Effects of daidzein on estrogen-receptor-positive and negative pancreatic cancer cells *in vitro*. World J Gastroenterol 10: 860–863.

Gurlek A, Pittelkow MR, Kumar R (2002). Modulation of growth factor/cytokine synthesis and signaling by 1alpha,25-dihydroxyvitamin D(3): implications in cell growth and differentiation. Endocr Rev 23: 763–786.

Guy M, Lowe LC, Bretherton-Watt D, Mansi JL, Peckitt C, Bliss J *et al.* (2004). Vitamin D receptor gene polymorphisms and breast cancer risk. Clin Cancer Res 10: 5472–5481.

Guzman ML, Rossi RM, Karnischky L, Li X, Peterson DR, Howard DS *et al.* (2005). The sesquiterpene lactone parthenolide induces apoptosis of human acute myelogenous leukemia stem and progenitor cells. Blood 105: 4163–4169.

Hamm S, Just G, Lacoste N, Moitessier N, Szyf M, Mamer O (2008). On the mechanism of demethylation of 5-methylcytosine in DNA. Bioorg Med Chem Lett 18: 1046–1049.

Haslberger A, Varga F, Karlic H (2006). Recursive causality in evolution: a model for epigenetic mechanisms in cancer development. Med Hypotheses 67: 1448–1454.

Hatziapostolou M, Iliopoulos D (2011). Epigenetic aberrations during oncogenesis. Cell Mol Life Sci 68: 1681–1702.

Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES *et al.* (2008). Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A 105: 17046–17049.

Hermann A, Gowher H, Jeltsch A (2004). Biochemistry and biology of mammalian DNA methyltransferases. Cell Mol Life Sci 61: 2571–2587.

Hisatake J, O'Kelly J, Uskokovic MR, Tomoyasu S, Koeffler HP (2001). Novel vitamin D(3) analog, 21-(3-methyl-3-hydroxy-butyl)-19-nor D(3), that modulates cell growth, differentiation, apoptosis, cell cycle, and induction of PTEN in leukemic cells. Blood 97: 2427–2433.

Hotchkiss RD (1948). The quantitative separation of purines, pyrimidines, and nucleosides by paper chromatography. J Biol Chem 175: 315–332.

Hsieh TC, Huang YC, Wu JM (2011). Control of prostate cell growth, DNA damage and repair and gene expression by resveratrol analogues, *in vitro*. Carcinogenesis 32: 93–101.

Iida T, Suetake I, Tajima S, Morioka H, Ohta S, Obuse C *et al.* (2002). PCNA clamp facilitates action of DNA cytosine methyltransferase 1 on hemimethylated DNA. Genes Cells 7: 997–1007.

Inamdar NM, Ehrlich KC, Ehrlich M (1991). CpG methylation inhibits binding of several sequence-specific DNA-binding proteins from pea, wheat, soybean and cauliflower. Plant Mol Biol 17: 111–123.

Ingles SA, Ross RK, Yu MC, Irvine RA, La Pera G, Haile RW *et al.* (1997). Association of prostate cancer risk with genetic polymorphisms in vitamin D receptor and androgen receptor. J Natl Cancer Inst 89: 166–170.

Iorio MV, Piovan C, Croce CM (2010). Interplay between microRNAs and the epigenetic machinery: an intricate network. Biochim Biophys Acta 1799: 694–701.

Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y (2010). Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature 466: 1129–1133.

James SJ, Pogribny IP, Pogribna M, Miller BJ, Jernigan S, Melnyk S (2003). Mechanisms of DNA damage, DNA hypomethylation, and tumor progression in the folate/methyl-deficient rat model of hepatocarcinogenesis. J Nutr 133: 3740S–3747S.

Jenuwein T, Allis CD (2001). Translating the histone code. Science 293: 1074–1080.

Jha AK, Nikbakht M, Parashar G, Shrivastava A, Capalash N, Kaur J (2010). Reversal of hypermethylation and reactivation of the RARbeta2 gene by natural compounds in cervical cancer cell lines. Folia Biol (Praha) 56: 195–200.

Johnson JJ, Nihal M, Siddiqui IA, Scarlett CO, Bailey HH, Mukhtar H *et al.* (2011). Enhancing the bioavailability of resveratrol by combining it with piperine. Mol Nutr Food Res 55: 1169–1176.

Kanduc D, Ghoshal A, Quagliariello E, Farber E (1988). DNA hypomethylation in ethionine-induced rat preneoplastic hepatocyte nodules. Biochem Biophys Res Commun 150: 739–744.

Kang J, Chen J, Shi Y, Jia J, Zhang Y (2005). Curcumin-induced histone hypoacetylation: the role of reactive oxygen species. Biochem Pharmacol 69: 1205–1213.

Kanwal R, Gupta S (2010). Epigenetics and cancer. J Appl Physiol 109: 598–605.

Kanwal R, Gupta S (2011). Epigenetic modifications in cancer. Clin Genet 81: 303–311.

Karlic H, Varga F (2011). Impact of vitamin D metabolism on clinical epigenetics. Clin Epigenet 2: 55–61.

Karlic H, Thaler R, Varga J, Berger C, Spitzer S, Pfeilstöcker M *et al.* (2010a). Effects of epigenetic drugs (vorinostat, decitabine) on metabolism-related pathway factors in leukemic cells. Open Leuk J 3: 34–42.

Karlic R, Chung HR, Lasserre J, Vlahovicek K, Vingron M (2010b). Histone modification levels are predictive for gene expression. Proc Natl Acad Sci U S A 107: 2926–2931.

Khanim FL, Gommersall LM, Wood VH, Smith KL, Montalvo L, O'Neill LP *et al.* (2004). Altered SMRT levels disrupt vitamin D3 receptor signalling in prostate cancer cells. Oncogene 23: 6712–6725.

Khor TO, Huang Y, Wu TY, Shu L, Lee J, Kong AN (2011). Pharmacodynamics of curcumin as DNA hypomethylation agent in restoring the expression of Nrf2 via promoter CpGs demethylation. Biochem Pharmacol 82: 1073–1078.

Kikuno N, Shiina H, Urakami S, Kawamoto K, Hirata H, Tanaka Y *et al.* (2008). Genistein mediated histone acetylation and demethylation activates tumor suppressor genes in prostate cancer cells. Int J Cancer 123: 552–560.

Kim YI (2004). Folate and DNA methylation: a mechanistic link between folate deficiency and colorectal cancer? Cancer Epidemiol Biomarkers Prev 13: 511–519.

Koch CM, Andrews RM, Flicek P, Dillon SC, Karaoz U, Clelland GK *et al.* (2007). The landscape of histone modifications across 1% of the human genome in five human cell lines. Genome Res 17: 691–707.

Kouzarides T (2007). Chromatin modifications and their function. Cell 128: 693–705.

Kraunz KS, Nelson HH, Lemos M, Godleski JJ, Wiencke JK, Kelsey KT (2006). Homozygous deletion of p16INK4a and tobacco carcinogen exposure in nonsmall cell lung cancer. Int J Cancer 118: 1364–1369.

Kriaucionis S, Heintz N (2009). The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science 324: 929–930.

Kuzumaki T, Kobayashi T, Ishikawa K (1998). Genistein induces p21(Cip1/WAF1) expression and blocks the G1 to S phase transition in mouse fibroblast and melanoma cells. Biochem Biophys Res Commun 251: 291–295.

Lamond AI, Earnshaw WC (1998). Structure and function in the nucleus. Science 280: 547–553.

Le Corre L, Fustier P, Chalabi N, Bignon YJ, Bernard-Gallon D (2004). Effects of resveratrol on the expression of a panel of genes interacting with the BRCA1 oncosuppressor in human breast cell lines. Clin Chim Acta 344: 115–121.

Lee WJ, Zhu BT (2006). Inhibition of DNA methylation by caffeic acid and chlorogenic acid, two common catechol-containing coffee polyphenols. Carcinogenesis 27: 269–277.

Lee WJ, Shim JY, Zhu BT (2005). Mechanisms for the inhibition of DNA methyltransferases by tea catechins and bioflavonoids. Mol Pharmacol 68: 1018–1030.

Levenson JM, Roth TL, Lubin FD, Miller CA, Huang IC, Desai P *et al.* (2006). Evidence that DNA (cytosine-5) methyltransferase regulates synaptic plasticity in the hippocampus. J Biol Chem 281: 15763–15773.

Li B, Carey M, Workman JL (2007). The role of chromatin during transcription. Cell 128: 707–719.

Li Y, Upadhyay S, Bhuiyan M, Sarkar FH (1999). Induction of apoptosis in breast cancer cells MDA-MB-231 by genistein. Oncogene 18: 3166–3172.

Lin R, Nagai Y, Sladek R, Bastien Y, Ho J, Petrecca K *et al.* (2002). Expression profiling in squamous carcinoma cells reveals pleiotropic effects of vitamin D3 analog EB1089 signaling on cell proliferation, differentiation, and immune system regulation. Mol Endocrinol 16: 1243–1256.

Lister R, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J *et al.* (2009). Human DNA methylomes at base resolution show widespread epigenomic differences. Nature 462: 315–322.

Liu HL, Chen Y, Cui GH, Zhou JF (2005). Curcumin, a potent anti-tumor reagent, is a novel histone deacetylase inhibitor regulating B-NHL cell line Raji proliferation. Acta Pharmacol Sin 26: 603–609.

Liu M, Iavarone A, Freedman LP (1996). Transcriptional activation of the human p21(WAF1/CIP1) gene by retinoic acid receptor. Correlation with retinoid induction of U937 cell differentiation. J Biol Chem 271: 31723–31728.

Liu YL, Yang HP, Gong L, Tang CL, Wang HJ (2011). Hypomethylation effects of curcumin, demethoxycurcumin and bisdemethoxycurcumin on WIF-1 promoter in non-small cell lung cancer cell lines. Mol Med Report 4: 675–679.

Liu Z, Liu S, Xie Z, Pavlovicz RE, Wu J, Chen P *et al.* (2009a). Modulation of DNA methylation by a sesquiterpene lactone parthenolide. J Pharmacol Exp Ther 329: 505–514.

Liu Z, Xie Z, Jones W, Pavlovicz RE, Liu S, Yu J *et al.* (2009b). Curcumin is a potent DNA hypomethylation agent. Bioorg Med Chem Lett 19: 706–709.

Lopes N, Carvalho J, Duraes C, Sousa B, Gomes M, Costa JL *et al.* (2012). 1Alpha,25-dihydroxyvitamin D3 Induces de novo E-cadherin Expression in Triple-negative Breast Cancer Cells by CDH1-promoter Demethylation. Anticancer Res 32: 249–257.

Lovat F, Valeri N, Croce CM (2011). MicroRNAs in the pathogenesis of cancer. Semin Oncol 38: 724–733.

Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D *et al.* (2005). MicroRNA expression profiles classify human cancers. Nature 435: 834–838.

Lu Q, Qiu X, Hu N, Wen H, Su Y, Richardson BC (2006). Epigenetics, disease, and therapeutic interventions. Ageing Res Rev 5: 449–467.

Majid S, Dar AA, Ahmad AE, Hirata H, Kawakami K, Shahryari V *et al.* (2009). BTG3 tumor suppressor gene promoter demethylation, histone modification and cell cycle arrest by genistein in renal cancer. Carcinogenesis 30: 662–670.

Mandal S, Davie JR (2010). Estrogen regulated expression of the p21 Waf1/Cip1 gene in estrogen receptor positive human breast cancer cells. J Cell Physiol 224: 28–32.

Maradeo ME, Cairns P (2011). Translational application of epigenetic alterations: ovarian cancer as a model. FEBS Lett 585: 2112–2120.

Marik R, Fackler M, Gabrielson E, Zeiger MA, Sukumar S, Stearns V *et al.* (2010). DNA methylation-related vitamin D receptor insensitivity in breast cancer. Cancer Biol Ther 10: 44–53.

Maruti SS, Ulrich CM, White E (2009). Folate and one-carbon metabolism nutrients from supplements and diet in relation to breast cancer risk. Am J Clin Nutr 89: 624–633.

Mastronardi FG, Noor A, Wood DD, Paton T, Moscarello MA (2007). Peptidyl argininedeiminase 2 CpG island in multiple sclerosis white matter is hypomethylated. J Neurosci Res 85: 2006–2016.

Matsumura K, Tanaka T, Kawashima H, Nakatani T (2008). Involvement of the estrogen receptor beta in genistein-induced expression of p21(waf1/cip1) in PC-3 prostate cancer cells. Anticancer Res 28: 709–714.

Miller CA, Sweatt JD (2007). Covalent modification of DNA regulates memory formation. Neuron 53: 857–869.

Milutinovic S, Knox JD, Szyf M (2000). DNA methyltransferase inhibition induces the transcription of the tumor suppressor p21(WAF1/CIP1/sdi1). J Biol Chem 275: 6353–6359.

Mittal A, Piyathilake C, Hara Y, Katiyar SK (2003). Exceptionally high protection of photocarcinogenesis by topical application of (–)-epigallocatechin-3-gallate in hydrophilic cream in SKH-1 hairless mouse model: relationship to inhibition of UVB-induced global DNA hypomethylation. Neoplasia 5: 555–565.

Myzak MC, Dashwood RH (2006). Histone deacetylases as targets for dietary cancer preventive agents: lessons learned with butyrate, diallyl disulfide, and sulforaphane. Curr Drug Targets 7: 443–452.

Nan X, Campoy FJ, Bird A (1997). MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. Cell 88: 471–481.

Narayanan BA, Narayanan NK, Re GG, Nixon DW (2003). Differential expression of genes induced by resveratrol in LNCaP cells: P53-mediated molecular targets. Int J Cancer 104: 204–212.

Ndlovu MN, Denis H, Fuks F (2011). Exposing the DNA methylome iceberg. Trends Biochem Sci 36: 381–387.

Newberne PM, Rogers AE (1986). Labile methyl groups and the promotion of cancer. Annu Rev Nutr 6: 407–432.

Nian H, Delage B, Ho E, Dashwood RH (2009). Modulation of histone deacetylase activity by dietary isothiocyanates and allyl sulfides: studies with sulforaphane and garlic organosulfur compounds. Environ Mol Mutagen 50: 213–221.

Okano M, Xie S, Li E (1998). Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. Nat Genet 19: 219–220.

Okano M, Bell DW, Haber DA, Li E (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 99: 247–257.

Olthof MR, Hollman PC, Zock PL, Katan MB (2001). Consumption of high doses of chlorogenic acid, present in coffee, or of black tea increases plasma total homocysteine concentrations in humans. Am J Clin Nutr 73: 532–538.

Palmer HG, Sanchez-Carbayo M, Ordonez-Moran P, Larriba MJ, Cordon-Cardo C, Munoz A (2003). Genetic signatures of differentiation induced by 1alpha,25-dihydroxyvitamin D3 in human colon cancer cells. Cancer Res 63: 7799–7806.

Paluszczak J, Baer-Dubowska W (2005). Epigenome and cancer: new possibilities of cancer prevention and therapy? Postepy Biochem 51: 244–250.

Paluszczak J, Krajka-Kuzniak V, Baer-Dubowska W (2010). The effect of dietary polyphenols on the epigenetic regulation of gene expression in MCF7 breast cancer cells. Toxicol Lett 192: 119–125.

Pan L, Matloob AF, Du J, Pan H, Dong Z, Zhao J *et al.* (2010). Vitamin D stimulates apoptosis in gastric cancer cells in synergy with trichostatin A/sodium butyrate-induced and 5-aza-2′-deoxycytidine-induced PTEN upregulation. FEBS J 277: 989–999.

Papoutsis AJ, Borg JL, Selmin OI, Romagnolo DF (2011). BRCA-1 promoter hypermethylation and silencing induced by the aromatic hydrocarbon receptor-ligand TCDD are prevented by resveratrol in MCF-7 Cells. J Nutr Biochem doi: 10.1016/j.jnutbio.2011.08.001 [Epub ahead of print].

Parasramka MA, Ho E, Williams DE, Dashwood RH (2012). MicroRNAs, diet, and cancer: new mechanistic insights on the epigenetic actions of phytochemicals. Mol Carcinog 51: 213–230.

Peng H, Xiong H, Li J, Xie M, Liu Y, Bai C *et al.* (2010). Vanilin cross-linked chitosan microspheres for controlled release of resveratrol. Food Chem 121: 23–28.

Pethe V, Shekhar PV (1999). Estrogen inducibility of c-Ha-ras transcription in breast cancer cells. Identification of functional estrogen-responsive transcriptional regulatory elements in exon 1/intron 1 of the c-Ha-ras gene. J Biol Chem 274: 30969–30978.

Popp C, Dean W, Feng S, Cokus SJ, Andrews S, Pellegrini M *et al.* (2010). Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency. Nature 463: 1101–1105.

Pozo-Guisado E, Alvarez-Barrientos A, Mulero-Navarro S, Santiago-Josefat B, Fernandez-Salguero PM (2002). The antiproliferative activity of resveratrol results in apoptosis in MCF-7 but not in MDA-MB-231 human breast cancer cells: cell-specific alteration of the cell cycle. Biochem Pharmacol 64: 1375–1386.

Pozo-Guisado E, Lorenzo-Benayas MJ, Fernandez-Salguero PM (2004). Resveratrol modulates the phosphoinositide 3-kinase pathway through an estrogen receptor alpha-dependent mechanism: relevance in cell proliferation. Int J Cancer 109: 167–173.

Pratt MA, Deonarine D, Teixeira C, Novosad D, Tate BF, Grippo JF (1996). The AF-2 region of the retinoic acid receptor alpha mediates retinoic acid inhibition of estrogen receptor function in breast cancer cells. J Biol Chem 271: 20346–20352.

Privat M, Aubel C, Arnould S, Communal Y, Ferrara M, Bignon YJ (2010). AKT and p21 WAF1/CIP1 as potential genistein targets in BRCA1-mutant human breast cancer cell lines. Anticancer Res 30: 2049–2054.

Qin W, Zhu W, Shi H, Hewett JE, Ruhlen RL, MacDonald RS *et al.* (2009). Soy isoflavones have an antiestrogenic effect and alter mammary promoter hypermethylation in healthy premenopausal women. Nutr Cancer 61: 238–244.

Rahal OM, Simmen RC (2010). PTEN and p53 cross-regulation induced by soy isoflavone genistein promotes mammary epithelial cell cycle arrest and lobuloalveolar differentiation. Carcinogenesis 31: 1491–1500.

Rai K, Huggins IJ, James SR, Karpf AR, Jones DA, Cairns BR (2008). DNA demethylation in zebrafish involves the coupling of a deaminase, a glycosylase, and gadd45. Cell 135: 1201–1212.

Rajewsky N (2006). microRNA target predictions in animals. Nat Genet 38 (Suppl.): S8–S13.

Ramchandani S, Bhattacharya SK, Cervoni N, Szyf M (1999). DNA methylation is a reversible biological signal. Proc Natl Acad Sci U S A 96: 6107–6112.

Rauch TA, Wu X, Zhong X, Riggs AD, Pfeifer GP (2009). A human B cell methylome at 100-base pair resolution. Proc Natl Acad Sci U S A 106: 671–678.

Razin A, Riggs AD (1980). DNA methylation and gene function. Science 210: 604–610.

Razin A, Szyf M, Kafri T, Roll M, Giloh H, Scarpa S *et al.* (1986). Replacement of 5-methylcytosine by cytosine: a possible mechanism for transient DNA demethylation during differentiation. Proc Natl Acad Sci U S A 83: 2827–2831.

Reik W, Dean W (2001). DNA methylation and mammalian epigenetics. Electrophoresis 22: 2838–2843.

Reuter S, Gupta SC, Park B, Goel A, Aggarwal BB (2011). Epigenetic changes induced by curcumin and other natural compounds. Genes Nutr 6: 93–108.

Robertson KD, Uzvolgyi E, Liang G, Talmadge C, Sumegi J, Gonzales FA *et al.* (1999). The human DNA methyltransferases (DNMTs) 1, 3a and 3b: coordinate mRNA expression in normal tissues and overexpression in tumors. Nucleic Acids Res 27: 2291–2298.

Rosenfeld JA, Wang Z, Schones DE, Zhao K, DeSalle R, Zhang MQ (2009). Determination of enriched histone modifications in non-genic portions of the human genome. BMC Genomics 10: 143.

Rowling MJ, McMullen MH, Schalinske KL (2002). Vitamin A and its derivatives induce hepatic glycine N-methyltransferase and hypomethylation of DNA in rats. J Nutr 132: 365–369.

Saini S, Majid S, Dahiya R (2010). Diet, microRNAs and prostate cancer. Pharm Res 27: 1014–1026.

Sanchez-Martinez R, Zambrano A, Castillo AI, Aranda A (2008). Vitamin D-dependent recruitment of corepressors to vitamin D/retinoid X receptor heterodimers. Mol Cell Biol 28: 3817–3829.

Saramaki A, Diermeier S, Kellner R, Laitinen H, Vaisanen S, Carlberg C (2009). Cyclical chromatin looping and transcription factor association on the regulatory regions of the p21 (CDKN1A) gene in response to 1alpha,25-dihydroxyvitamin D3. J Biol Chem 284: 8073–8082.

Scott JM, Weir DG (1998). Folic acid, homocysteine and one-carbon metabolism: a review of the essential biochemistry. J Cardiovasc Risk 5: 223–227.

Selhub J (2002). Folate, vitamin B12 and vitamin B6 and one carbon metabolism. J Nutr Health Aging 6: 39–42.

Selker EU (1998). Trichostatin A causes selective loss of DNA methylation in Neurospora. Proc Natl Acad Sci U S A 95: 9430–9435.

Shaikh J, Ankola DD, Beniwal V, Singh D, Kumar MN (2009). Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. Eur J Pharm Sci 37: 223–230.

Sharif J, Muto M, Takebayashi S, Suetake I, Iwamatsu A, Endo TA *et al.* (2007). The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. Nature 450: 908–912.

Sharif T, Auger C, Alhosin M, Ebel C, Achour M, Etienne-Selloum N *et al.* (2010). Red wine polyphenols cause growth inhibition and apoptosis in acute lymphoblastic leukaemia cells by inducing a redox-sensitive up-regulation of p73 and down-regulation of UHRF1. Eur J Cancer 46: 983–994.

Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PS (1998). Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta Med 64: 353–356.

Shrubsole MJ, Shu XO, Li HL, Cai H, Yang G, Gao YT *et al.* (2011). Dietary B vitamin and methionine intakes and breast cancer risk among Chinese women. Am J Epidemiol 173: 1171–1182.

Shu L, Khor TO, Lee JH, Boyanapalli SS, Huang Y, Wu TY *et al.* (2011). Epigenetic CpG demethylation of the promoter and reactivation of the expression of Neurog1 by curcumin in prostate LNCaP cells. AAPS J 13: 606–614.

Sinclair KD, Allegrucci C, Singh R, Gardner DS, Sebastian S, Bispham J *et al.* (2007). DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. Proc Natl Acad Sci U S A 104: 19351–19356.

Singh SM, Murphy B, O'Reilly RL (2003). Involvement of gene-diet/drug interaction in DNA methylation and its contribution to complex diseases: from cancer to schizophrenia. Clin Genet 64: 451–460.

Slattery ML, Potter JD, Samowitz W, Schaffer D, Leppert M (1999). Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. Cancer Epidemiol Biomarkers Prev 8: 513–518.

Starlard-Davenport A, Tryndyak V, Kosyk O, Ross SR, Rusyn I, Beland FA *et al.* (2010). Dietary methyl deficiency, microRNA expression and susceptibility to liver carcinogenesis. World Rev Nutr Diet 101: 123–130.

Stefanska B, Rudnicka K, Bednarek A, Fabianowska-Majewska K (2010). Hypomethylation and induction of retinoic acid receptor beta 2 by concurrent action of adenosine analogues and natural compounds in breast cancer cells. Eur J Pharmacol 638: 47–53.

Stefanska B, Huang J, Bhattacharyya B, Suderman M, Hallett M, Han ZG *et al.* (2011a). Definition of the landscape of promoter DNA hypomethylation in liver cancer. Cancer Res 71: 5891–5903.

Stefanska B, Salame P, Bednarek A, Fabianowska-Majewska K (2011b). Comparative effects of retinoic acid, vitamin D and resveratrol alone and in combination with adenosine analogues on methylation and expression of phosphatase and tensin homologue tumour suppressor gene in breast cancer cells. Br J Nutr 107: 781–790.

Steger DJ, Lefterova MI, Ying L, Stonestrom AJ, Schupp M, Zhuo D *et al.* (2008). DOT1L/KMT4 recruitment and H3K79 methylation are ubiquitously coupled with gene transcription in mammalian cells. Mol Cell Biol 28: 2825–2839.

Stein R, Razin A, Cedar H (1982). *In vitro* methylation of the hamster adenine phosphoribosyltransferase gene inhibits its expression in mouse L cells. Proc Natl Acad Sci U S A 79: 3418–3422.

Strahl BD, Allis CD (2000). The language of covalent histone modifications. Nature 403: 41–45.

Su Y, Simmen FA, Xiao R, Simmen RC (2007). Expression profiling of rat mammary epithelial cells reveals candidate signaling pathways in dietary protection from mammary tumors. Physiol Genomics 30: 8–16.

Swami S, Krishnan AV, Feldman D (2000). 1alpha,25-Dihydroxyvitamin D3 down-regulates estrogen receptor abundance and suppresses estrogen actions in MCF-7 human breast cancer cells. Clin Cancer Res 6: 3371–3379.

vel Szic KS, Ndlovu MN, Haegeman G, Vanden Berghe W (2010). Nature or nurture: let food be your epigenetic medicine in chronic inflammatory disorders. Biochem Pharmacol 80: 1816–1832.

Szyf M (2005). DNA methylation and demethylation as targets for anticancer therapy. Biochemistry (Mosc) 70: 533–549.

Szyf M (2012). The early life social environment and DNA methylation. Clin Genet 81: 341–349.

Szyf M, Pakneshan P, Rabbani SA (2004). DNA methylation and breast cancer. Biochem Pharmacol 68: 1187–1197.

Tan S, Wang C, Lu C, Zhao B, Cui Y, Shi X *et al.* (2009). Quercetin is able to demethylate the p16INK4a gene promoter. Chemotherapy 55: 6–10.

Tessitore L, Davit A, Sarotto I, Caderni G (2000). Resveratrol depresses the growth of colorectal aberrant crypt foci by affecting bax and p21(CIP) expression. Carcinogenesis 21: 1619–1622.

Thaler R, Karlic H, Rust P, Haslberger AG (2009). Epigenetic regulation of human buccal mucosa mitochondrial superoxide dismutase gene expression by diet. Br J Nutr 101: 743–749.

Tili E, Michaille JJ, Alder H, Volinia S, Delmas D, Latruffe N *et al.* (2010). Resveratrol modulates the levels of microRNAs targeting genes encoding tumor-suppressors and effectors of TGFbeta signaling pathway in SW480 cells. Biochem Pharmacol 80: 2057–2065.

Tuddenham L, Wheeler G, Ntounia-Fousara S, Waters J, Hajihosseini MK, Clark I *et al.* (2006). The cartilage specific microRNA-140 targets histone deacetylase 4 in mouse cells. FEBS Lett 580: 4214–4217.

Unoki M, Nishidate T, Nakamura Y (2004). ICBP90, an E2F-1 target, recruits HDAC1 and binds to methyl-CpG through its SRA domain. Oncogene 23: 7601–7610.

Unoki M, Brunet J, Mousli M (2009). Drug discovery targeting epigenetic codes: the great potential of UHRF1, which links DNA methylation and histone modifications, as a drug target in cancers and toxoplasmosis. Biochem Pharmacol 78: 1279–1288.

Unterberger A, Szyf M, Nathanielsz PW, Cox LA (2009). Organ and gestational age effects of maternal nutrient restriction on global methylation in fetal baboons. J Med Primatol 38: 219–227.

Vaquero A, Scher M, Lee D, Erdjument-Bromage H, Tempst P, Reinberg D (2004). Human SirT1 interacts with histone H1 and promotes formation of facultative heterochromatin. Mol Cell 16: 93–105.

Vardi A, Bosviel R, Rabiau N, Adjakly M, Satih S, Dechelotte P *et al.* (2010). Soy phytoestrogens modify DNA methylation of GSTP1, RASSF1A, EPH2 and BRCA1 promoter in prostate cancer cells. In Vivo 24: 393–400.

Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C *et al.* (2006). The Polycomb group protein EZH2 directly controls DNA methylation. Nature 439: 871–874.

Voegel JJ, Heine MJ, Zechel C, Chambon P, Gronemeyer H (1996). TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. EMBO J 15: 3667–3675.

Waite KA, Sinden MR, Eng C (2005). Phytoestrogen exposure elevates PTEN levels. Hum Mol Genet 14: 1457–1463.

Walle T, Hsieh F, DeLegge MH, Oatis JE, Jr, Walle UK (2004). High absorption but very low bioavailability of oral resveratrol in humans. Drug Metab Dispos 32: 1377–1382.

Wang LS, Arnold M, Huang YW, Sardo C, Seguin C, Martin E *et al.* (2011). Modulation of genetic and epigenetic biomarkers of colorectal cancer in humans by black raspberries: a phase I pilot study. Clin Cancer Res 17: 598–610.

Wang Q, Lee D, Sysounthone V, Chandraratna RAS, Christakos S, Korah R *et al.* (2001). 1,25-dihydroxyvitamin D3 and retonic acid analogues induce differentiation in breast cancer cells with function- and cell-specific additive effects. Breast Cancer Res Treat 67: 157–168.

Watanabe T, Tomizawa S, Mitsuya K, Totoki Y, Yamamoto Y, Kuramochi-Miyagawa S *et al.* (2011). Role for piRNAs and noncoding RNA in de novo DNA methylation of the imprinted mouse Rasgrf1 locus. Science 332: 848–852.

Waterland RA (2006). Assessing the effects of high methionine intake on DNA methylation. J Nutr 136: 1706S–1710S.

Waterland RA, Jirtle RL (2003). Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 23: 5293–5300.

Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR *et al.* (2004). Epigenetic programming by maternal behavior. Nat Neurosci 7: 847–854.

Wei EK, Giovannucci E, Selhub J, Fuchs CS, Hankinson SE, Ma J (2005). Plasma vitamin B6 and the risk of colorectal cancer and adenoma in women. J Natl Cancer Inst 97: 684–692.

Whitehead N, Reyner F, Lindenbaum J (1989). The Journal of the American Medical Association, Volume 226, 1973: megaloblastic changes in cervical epithelium. Association with oral contraceptive therapy and reversal with folic acid. Nutr Rev 47: 318–321.

Whitsett TG, Jr, Lamartiniere CA (2006). Genistein and resveratrol: mammary cancer chemoprevention and mechanisms of action in the rat. Expert Rev Anticancer Ther 6: 1699–1706.

Widschwendter M, Jones PA (2002). DNA methylation and breast carcinogenesis. Oncogene 21: 5462–5482.

Wiedhopf RM, Young M, Bianchi E, Cole JR (1973). Tumor inhibitory agent from Magnolia grandiflora (Magnoliaceae). I. Parthenolide. J Pharm Sci 62: 345.

Woynarowski JM, Konopa J (1981). Inhibition of DNA biosynthesis in HeLa cells by cytotoxic and antitumor sesquiterpene lactones. Mol Pharmacol 19: 97–102.

Wu Q, Zhang M, Liu S, Chen Y, Su W (2002). Retinoic acid receptor beta is required for anti-activator protein-1 activity by retinoic acid in gastric cancer cells. Chin Med J (Engl) 115: 810–814.

Wyatt GR (1950). Occurrence of 5-methylcytosine in nucleic acids. Nature 166: 237–238.

Yang CS, Fang M, Lambert JD, Yan P, Huang TH (2008). Reversal of hypermethylation and reactivation of genes by dietary polyphenolic compounds. Nutr Rev 66 (Suppl. 1): S18–S20.

Yang XJ, Seto E (2007). HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. Oncogene 26: 5310–5318.

Youssef EM, Lotan D, Issa JP, Wakasa K, Fan YH, Mao L *et al.* (2004). Hypermethylation of the retinoic acid receptor-beta(2) gene in head and neck carcinogenesis. Clin Cancer Res 10: 1733–1742.

Yu Z, Li W, Lu Q, Wang L, Zhang X, Han P *et al.* (2008). p21 is required for atRA-mediated growth inhibition of MEPM cells, which involves RAR. J Cell Biochem 104: 2185–2192.

Zapisek WF, Cronin GM, Lyn-Cook BD, Poirier LA (1992). The onset of oncogene hypomethylation in the livers of rats fed methyl-deficient, amino acid-defined diets. Carcinogenesis 13: 1869–1872.

Zhang D, Holmes WF, Wu S, Soprano DR, Soprano KJ (2000). Retinoids and ovarian cancer. J Cell Physiol 185: 1–20.

Zhang T, Kraus WL (2010). SIRT1-dependent regulation of chromatin and transcription: linking NAD(+) metabolism and signaling to the control of cellular functions. Biochim Biophys Acta 1804: 1666–1675.

Zhou H, Hu H, Lai M (2010). Non-coding RNAs and their epigenetic regulatory mechanisms. Biol Cell 102: 645–655.

Zhou JR, Gugger ET, Tanaka T, Guo Y, Blackburn GL, Clinton SK (1999). Soybean phytochemicals inhibit the growth of transplantable human prostate carcinoma and tumor angiogenesis in mice. J Nutr 129: 1628–1635.