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Cancer Chemoprevention and Nutri-Epigenetics: State of the Art and Future Challenges

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Abstract The term "epigenetics" refers to modifications in gene expression caused by heritable, but potentially reversible, changes in DNA methylation and chromatin structure. Epigenetic alterations have been identified as promising new targets for cancer prevention strategies as they occur early during carcinogenesis and represent potentially initiating events for cancer development. Over the past few years, nutri-epigenetics – the influence of dietary components on mechanisms influencing the epigenome – has emerged as an exciting new field in current epigenetic research. During carcinogenesis, major cellular functions and pathways, including drug metabolism, cell cycle regulation, potential to repair DNA damage or to induce apoptosis, response to inflammatory stimuli, cell signalling, and cell growth control and differentiation become deregulated. Recent evidence now indicates that epigenetic alterations contribute to these cellular defects, for example epigenetic silencing of detoxifying enzymes, tumor suppressor genes, cell cycle regulators, apoptosis-inducing and DNA repair genes, nuclear receptors, signal transducers and transcription factors by promoter methylation, and modifications of histones and non-histone proteins such as *p53*, *NF-κB*, and the chaperone *HSP90* by acetylation or methylation.

The present review will summarize the potential of natural chemopreventive agents to counteract these cancer-related epigenetic alterations by influencing the activity or expression of DNA methyltransferases and histone modifying enzymes. Chemopreventive agents that target the epigenome include micronutrients (folate, retinoic acid, and selenium compounds), butyrate, polyphenols from green tea, apples, coffee, black raspberries, and other dietary sources, genistein and soy isoflavones, curcumin, resveratrol, dihydrocoumarin, nordihydroguaiaretic acid (NDGA), lycopene, anacardic acid, garcinol, constituents of *Allium* species and cruciferous vegetables, including indol-3-carbinol (I3C), diindolylmethane (DIM),

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sulforaphane, phenylethyl isothiocyanate (PEITC), phenylhexyl isothiocyanate (PHI), diallyldisulfide (DADS) and its metabolite allyl mercaptan (AM), cambinol, and relatively unexplored modulators of histone lysine methylation (chaetocin, polyamine analogs). So far, data are still mainly derived from in vitro investigations, and results of animal models or human intervention studies are limited that demonstrate the functional relevance of epigenetic mechanisms for health promoting or cancer preventive efficacy of natural products. Also, most studies have focused on single candidate genes or mechanisms. With the emergence of novel technologies such as next-generation sequencing, future research has the potential to explore nutri-epigenomics at a genome-wide level to understand better the importance of epigenetic mechanisms for gene regulation in cancer chemoprevention.

Keywords Cancer chemoprevention • Dietary compounds • DNA methylation • Histone modifications • Nutri-epigenetics

Contents

1 Introduction 74
2 DNA Methylation 75
3 Histone Modifications 77
4 MicroRNAs 78
5 Interplay Between Chemopreventive and Epigenetic Mechanisms and Natural Products Effects 79
6 Detoxification 81
7 Cell Cycle Regulation 83
8 Apoptosis 89
9 DNA Repair 91
10 Inflammation and Regulation of NF-κB 92
11 Cell Signaling and Cell Growth 94
12 Cell Differentiation 98
13 Summary and Conclusions 100
References 116

1 Introduction

The term “epigenetics” refers to modifications in gene expression caused by heritable, but potentially reversible, changes in DNA methylation and chromatin structure [1]. Given the fact that epigenetic modifications are reversible and occur early during carcinogenesis as potentially initiating events for cancer development, they have been identified as promising new targets for cancer prevention strategies. Major epigenetic mechanisms of gene regulation include DNA methylation, modifications of the chromatin structure by histone tail acetylation and methylation, and small non-coding microRNAs, that affect gene expression by targeted degradation of mRNAs or inhibition of their translation (overview in Fig. 1) [3, 4].

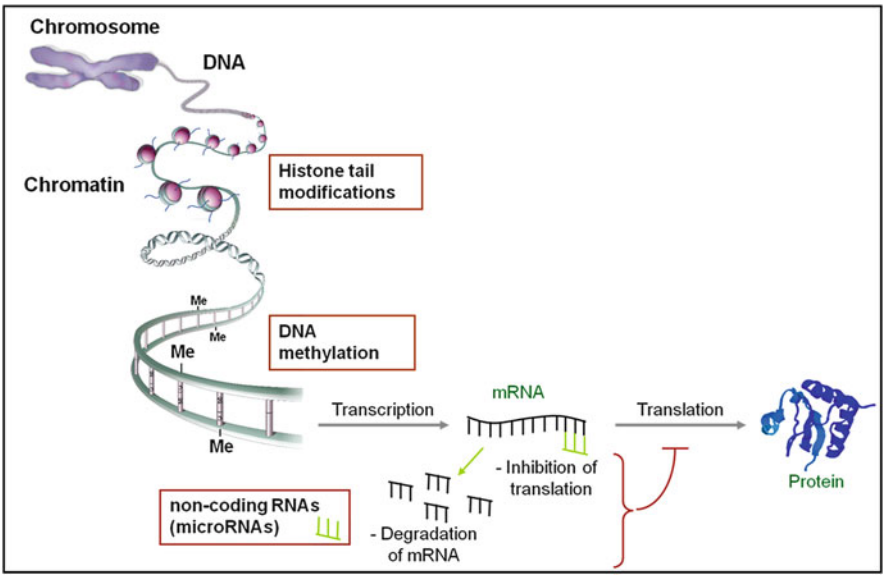

Fig. 1 Overview of epigenetic mechanisms including DNA methylation, histone tail modifications and non-coding (micro) RNAs, targeting DNA, N-terminal histone tails and mRNA (modified from [2], with permission of Nature Publishing Group)

Epigenetic mechanisms are essential to control normal cellular functions and they play an important role during development. Distinct patterns of DNA methylation regulate tissue specific gene expression and are involved in X-chromosome inactivation and genomic imprinting [5–7]. Histone modifications are critical for memory formation [4, 8]. Interestingly, epigenetic profiles can be modified to adapt to changes in the environment (e.g., nutrition, chemical exposure, smoking, radiation, etc.) [3, 9] as has been exemplified in studies with monozygotic twins and inbred animals [10, 11]. Consequently, alterations in DNA methylation and histone marks eventually contribute to the development of age-related and lifestyle-related diseases, such as metabolic syndrome, Alzheimer's disease, and cancer [8, 12, 13].

2 DNA Methylation

DNA methylation is mediated by DNA methyltransferases (*DNMT*) that transfer methyl groups from *S*-adenosyl-L-methionine (SAM) to the 5′-position of cytosines. This reaction mainly takes place at cytosines when positioned next to a guanine (CpG dinucleotides) and creates 5-methylcytosine (5mC) and *S*-adenosyl-L-homocysteine (SAH). Three active mammalian DNMTs have been identified so far, i.e., *DNMT1*, *3a*, and *3b*. *DNMT1* is a maintenance methyltransferase that maintains DNA methylation during DNA replication. It preferentially methylates the newly synthesized,

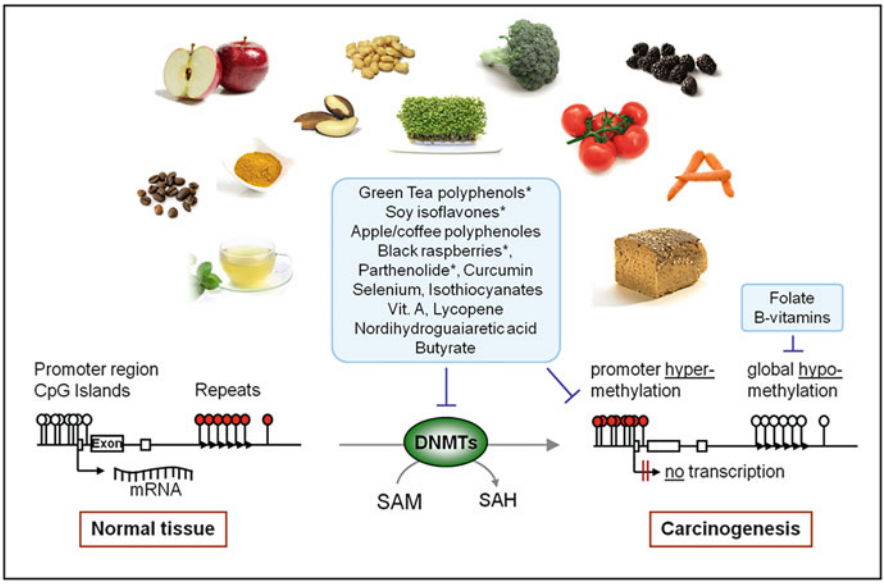

Fig. 2 Overview of DNA methylation changes during carcinogenesis and cancer chemopreventive agents inhibiting the activity of expression of DNMTs, thereby preventing aberrant (promoter) hypermethylation or genome wide hypomethylation. DNA methylation is catalyzed by DNA methyltransferases (DNMTs) using *S*-adenosylmethionine (SAM) as a substrate. See text and Table 1 (Appendix) for further details. *Asterisks* indicate epigenetic activity in vivo. Empty circles: unmethylated CpG dinucleotide; red circles: methylated CpG site

unmethylated DNA strand after replication and thus assures transmission of DNA methylation patterns to daughter cells. *DNMT3a* and *DNMT3b* are "de novo" methyltransferases that catalyze methylation of previously unmethylated sequences. *DNMT3b* is believed to play an important role during tumorigenesis [14, 15].

In normal cells, CpG-rich sequences (so-called CpG islands, CGIs) in gene promoter regions are generally unmethylated, with the exception of about 6–8% CGIs methylated in a tissue-specific manner [7]. Conversely, the majority of CpG sites in repetitive sequences such as ribosomal DNA repeats, satellite repeats, or centromeric repeats are often heavily methylated, thereby contributing to chromosomal stability by limiting accessibility to the transcription machinery [16]. This controlled pattern of DNA methylation is disrupted during ageing, carcinogenesis, or development of chronic diseases. Increased methylation (DNA hypermethylation) of promoter CGIs leads to transcriptional silencing of tumor suppressors and other genes with important biological functions [12, 16, 17]. In contrast, global loss of DNA methylation at repetitive genomic sequences (DNA hypomethylation) during carcinogenesis has been associated with genomic instability and chromosomal aberrations and was first described about 30 years ago [18, 19] (Fig. 2). Different from irreversible gene inactivation by genetic deletions or nonsense mutations, genes silenced by epigenetic modifications are still intact and can potentially be reactivated by small molecules acting as modifiers of epigenetic

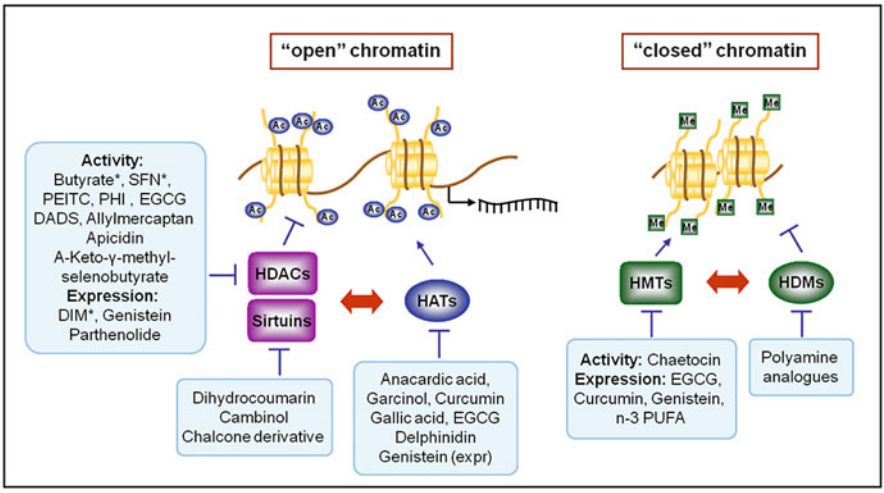

Fig. 3 Simplified overview of histone modifying enzymes with a focus on histone deacetylases (HDACs), histone acetyltransferases (HATs), histone methyl transferases (HTMs), and histone demethylases (HDM), and their influence on chromatin structure. Sirtuins represent a NAD^+-dependent subclass of HDACs (class III). Also indicated is the inhibitory potential of chemopreventive agents. See text and Tables 2 and 3 (Appendix) for further details. *Asterisks* indicate epigenetic activity in vivo

mechanisms. Consequently, development of agents or food components that prevent or reverse methylation-induced inactivation of gene expression is a new promising approach for cancer prevention [20].

3 Histone Modifications

Epigenetic regulation of gene expression is also mediated by post-translational modifications at the N-terminal tails of histones. These include acetylation, methylation, phosphorylation, ubiquitinylation, sumoylation, and ADP ribosylation and contribute to genomic stability, DNA damage response, and cell cycle checkpoint integrity [118–120]. Histones can be modified through sequence-specific transcription factors or on a more global scale through histone-modifying enzymes [120]. So far, histone acetylation and histone methylation have been investigated the most and disturbance of their balance has been associated with neoplastic transformation (Fig. 3).

Histone acetylation is maintained by the interplay of *histone acetyltransferases* (*HAT*s) and *histone deacetylases* (*HDAC*s). *HAT*s transfer acetyl groups from acetyl-CoA to the ε-amino group of lysine (K) residues in histone tails, whereas *HDAC*s remove histone acetyl groups by catalyzing their transfer to Coenzyme A (CoA). Acetylation of histone tails opens up the chromatin structure, allowing transcription factors to access the DNA. Consequently, proteins with *HAT* catalytic

activity are often transcriptional coactivators. So far at least 25 *HAT* proteins have been characterized. They are organized into four families based on structure homology [189] and often possess distinct histone specificity. Subgroups include the GNAT (*hGCN5*, *PCAF*), MYST (*MYST*, *Tip60*), p300/CBP (*p300*/*CBP*), SRC (*SRC-1*), and TAFII250 families (*TAFII250*) [119, 190]. In contrast to histone acetylation, histone deacetylation generally leads to chromatin condensation and transcriptional repression. So far, 18 proteins with *HDAC* activity have been classified [191, 192]. *HDAC*s 1–11 are subdivided into three classes – I, II, and IV – based on homology, size, sub-cellular expression, and number of enzymatic domains. Class III is comprised of *sirtuins* 1–7, which are structurally unrelated to class I and II HDACs and require NAD^+ as a cofactor for activity [191, 192]. Interestingly, *HDAC* substrates are not limited to histones. As further outlined below, several important regulatory proteins and transcription factors such as *p53*, *E2F*, and *nuclear factor-κB* (*NF-κB*) involved in stress response, inflammation, and apoptosis have been shown to be regulated by acetylation [193–195].

Histone methylation takes place at lysine and arginine residues. Histone lysine methylation has activating or repressive effects on gene expression. This is dependent on the lysine residue that is methylated (e.g., K4, K9, K27, K36, K79 in H3), the methylation status (mono-, di-, or tri-methylation), and the location (interaction with promoter vs gene coding regions) [118, 119, 196]. Methylation at H3K4, H3K36, and H3K79 is generally associated with transcriptional active chromatin (euchromatin), whereas methylation at H3K9, H3K27, and H4K20 is frequently associated with transcriptional inactive heterochromatin [190, 197]. Histone lysine methylation is mediated by *histone lysine methyltransferases* (*HMT*s) that transfer a methyl group from SAM to the lysine residue. *HMT*s can be classified as *Dot1* protein family and proteins containing a so-called SET domain, based on sequence similarity with Drosophila proteins suppressor of variegation (*SUV*), enhancer of zeste (*EZH*), and homeobox gene regulator *Trithorax* (*TRX*). So far, more than 50 SET domain family members have been identified in humans [197]. They are grouped into six subfamilies, SET1, SET2, SUV39, EZH, SMYD, and PRDM, and several SET-containing *HMT*s that do not fall into these groups [197].

Several types of *histone lysine demethylases* (*HDM*s) have been identified so far, for example *lysine specific demethylase 1* (*LSD1*) and the family of about 20 *Jumonji domain-containing* (*JmjC*) *histone demethylases* [118, 119, 197]. Similar to lysine acetylation, lysine methylation is not limited to histone proteins, and several non-histone protein substrates including *p53*, retinoblastoma protein (*RB*), the *NF-κB* subunit *RelA*, and estrogen receptor α (*ERα*) have been identified (summarized in [198–200]).

4 MicroRNAs

MicroRNAs (miRNAs) are small non-coding RNAs of 20–22 nucleotides that inhibit gene expression at the posttranscriptional level. MiRNAs are involved in the regulation of key biological processes, including development, differentiation,

apoptosis, and proliferation, and are known to be altered in a variety of chronic degenerative diseases including cancer [201]. MiRNAs are generated from RNA precursor structures by a protein complex system composed of members of the Argonaute protein family, polymerase II-dependent transcription, and the ribonucleases Drosha and Dicer [202]. MiRNAs regulate the transformation of mRNA into proteins, either by imperfect base-pairing to the mRNA 3′-untranslated regions to repress protein synthesis, or by affecting mRNA stability. Each miRNA is expected to control several hundred genes. They have been implicated in cancer initiation and progression, and their expression is often down-regulated during carcinogenesis. Major mechanisms of miRNA deregulation include genetic and epigenetic alterations as well as defects in the miRNA processing machinery [196].

5 Interplay Between Chemopreventive and Epigenetic Mechanisms and Natural Products Effects

Over the last few years, evidence has accumulated that natural products and dietary constituents with chemopreventive potential have an impact on DNA methylation (Fig. 2), histone modifications (Fig. 3), and miRNA expression. The available information on the topic has been summarized in several recent review articles [20–36, 121, 122, 203, 204].

As indicated in Fig. 2, *folate* and *B-vitamins* have a potential impact on DNA hypomethylation. They affect the so called "one-carbon metabolism" which provides methyl groups for methylation reactions. Folate is an important factor for the maintenance of DNA biosynthesis and DNA repair, and folate deficiency leads to global DNA hypomethylation, genomic instability, and chromosomal damage. As an essential micronutrient, folate needs to be taken up from dietary sources, such as citrus fruits, dark green vegetables, whole grains, and dried beans. Alcohol misuse is often associated with folate deficiency. Epidemiological studies have indicated that low folate levels are associated with an increased risk for colorectum, breast, ovary, pancreas, brain, lung, and cervix cancer [66, 76, 205]. Consequently, the relationship between folate status, DNA methylation, and cancer risk has been analyzed in numerous rodent carcinogenesis models and in human intervention studies. Overall, the results are inconclusive and depend on various parameters, for example dose and timing of the intervention, the severity of folate deficiency, and health status (reviewed in [23, 66–68, 76]). Excessive intake of synthetic folic acid (from high-dose supplements or fortified foods) may even increase human cancer risk by accelerating growth of precancerous lesions [66]. Therefore folate supplementation cannot be generally recommended, and deficiencies should be prevented by dietary intake. In a cohort-based observation study with 1,100 participants, Stidley et al. investigated the effect of various dietary factors on promoter methylation levels of eight genes commonly hypermethylated

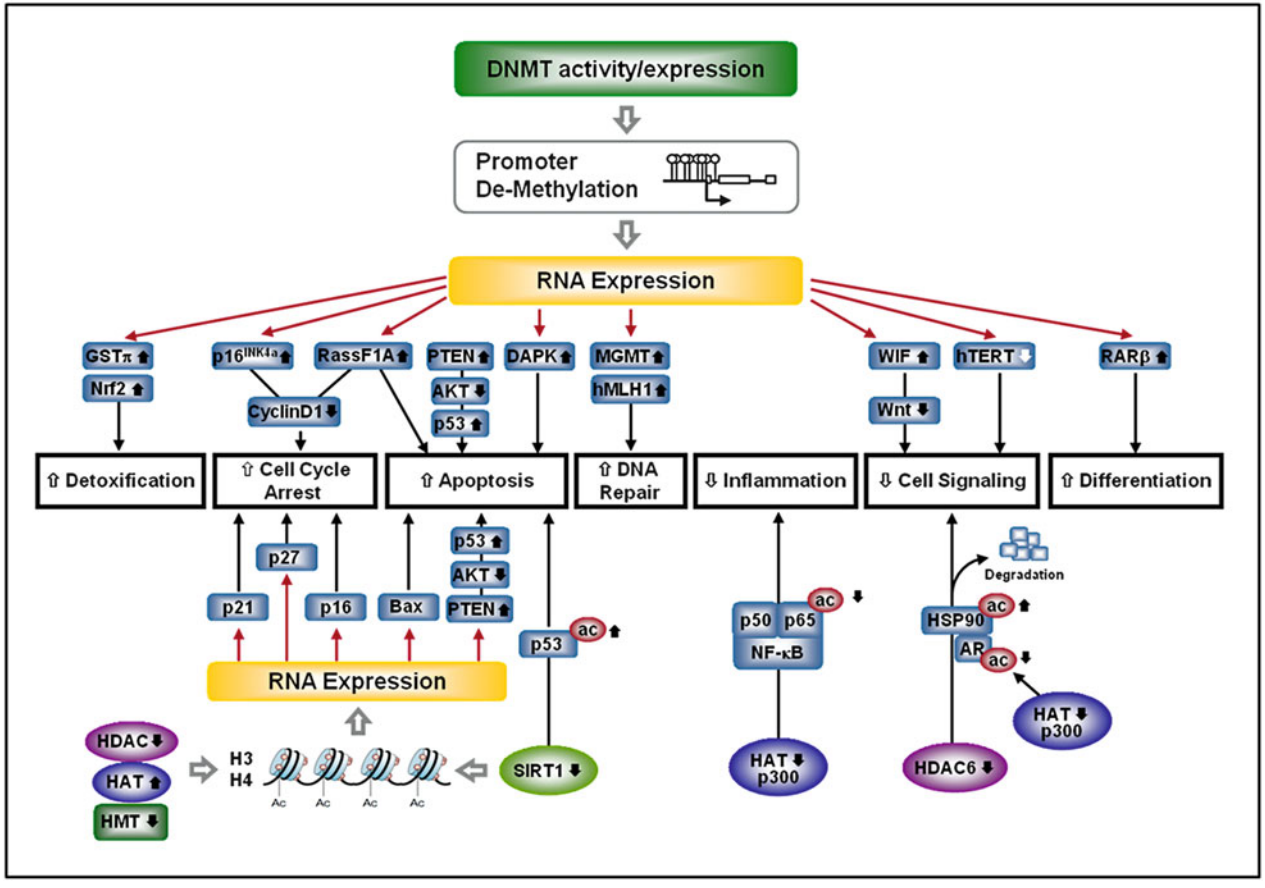

Fig. 4 Impact of DNA methylation and histone-modifying enzymes on the regulation of genes commonly deregulated during carcinogenesis. Inhibition/modulation of the activity or expression of DNMTs or histone-modifying proteins by chemopreventive agents can lead to reactivation of epigenetically silenced genes. See text for a detailed description of the indicated mechanisms and pathways and the influence of chemopreventive agents

in cancer, including *RassF1A*, *p16*, *MGMT*, *DAPK*, *GATA4*, *GATA5*, *PAX5α*, and *PAX5β* in exfoliated aerodigestive tract cells from sputum samples of current and former smokers. Significant protection from DNA methylation (less than two genes methylated) was observed for regular consumption of folate [OR (odds ratio) = 0.84 per 750 μg/day; CI (95% confidence interval), 0.72–0.99], leafy green vegetables (OR, 0.83 per 12 monthly servings; CI, 0.74–0.93), and multivitamin use (OR, 0.57; CI, 0.40–0.83) [77].

The following chapter will focus on pathways which are relevant for chemoprevention and are commonly deregulated by epigenetic mechanisms in cancer cells, including drug detoxification, cell cycle regulation, apoptosis induction, DNA repair, tumor-associated inflammation, cell signaling that promotes cell growth, and cell differentiation (overview in Fig. 4). It will present a summary of natural chemopreventive agents targeting these pathways by affecting DNA methylation and histone tail modifications. Their effect on miRNAs and subsequent gene expression will not be discussed.

Plant compounds which affect DNA methylation and inhibit DNMT enzymatic activity (DNMT inhibitors, DNMTi), revert aberrant DNA promoter methylation, or reactivate genes silenced by promoter hypermethylation, are listed in Table 1 (Appendix). Natural products with influence on histone acetylation and methylation that inhibit the activity or modulate the expression of histone-modifying enzymes including HDACs, SIRTs, HATs, and HMTs are summarized in Tables 2 and 3 (Appendix).

6 Detoxification

GSTP1 is a member of the glutathione *S*-transferase family of isoenzymes that conjugate reactive chemicals and carcinogens with the tripeptide glutathione (GSH) and thus enhance their excretion and detoxification [206]. Induction of GSTs and other enzymes involved in phase 2 of drug metabolism via the *Nrf2-Keap1* pathway is an important mechanism in cancer chemoprevention [207]. Recently, *GSTP1* activity has also been associated with cell-signaling functions critical for survival, for example the regulation of *c-Jun N-terminal kinase* (*JNK*) activity and modulation of protein functions by *S*-glutathionylation [208].

Loss of *GSTP1* expression by CGI hypermethylation is very common in prostate cancer [209]. *GSTP1* is expressed and unmethylated in normal prostate tissue. Hypermethylation increases with increasing prostate carcinogenesis and can be detected in up to 70–100% prostate adenocarcinoma [209]. *GSTP1* hypermethylation is also detectable in plasma, ejaculate, or urine, and is discussed as a promising prostate cancer biomarker. In addition to prostate cancer, *GSTP1* hypermethylation is frequent in ~30% and >80% of breast cancer and hepatocellular carcinoma, respectively [209]. Deletion of *GSTP1* in mice was shown to enhance susceptibility to chemically-induced skin and lung cancer, and to increase adenoma incidence and multiplicity when *mGstp1/p2* knockout mice were crossed

with *APC*$^{Min/+}$ mice [206]. Gene expression studies in these models indicate a protective role of *GSTP1* in inflammation and immune response.

Reexpression of *GSTP1* after treatment with natural products has been tested in prostate and breast cancer cell lines. Ramachandran et al. was unable to detect demethylation and reexpression of *GSTP1* in LNCaP and PC-3 prostate cancer cells after treatment with *seleno-DL-methionine*. More recently, reactivation of *GSTP1* by *sodium selenite* in LNCaP cells was shown to involve a dual effect on both DNA methylation and histone modifications. Incubation with low dose sodium selenite lowered *DNMT1* mRNA and protein expression, reduced global DNA methylation, and led to the reexpression of *GSTP1* associated with reduced *GSTP1* promoter methylation [115]. An earlier study identified sodium selenite and organic seleno-compounds as inhibitors of DNMT activity in vitro [112]. Therefore, direct inhibition of DNMT enzyme activity might contribute to the demethylating potential of sodium selenite. *Phenethylisothiocyanate* (*PEITC*) derived from the glucosinolate gluconasturtiin from watercress was able to revert epigenetic silencing of *GSTP1* in LNCaP cells. Reduced DNA methylation at specific CpG sites was associated with enhanced protein expression and increased *GSTP1* enzymatic activity [100]. *Green tea polyphenols* (*GTP*) and *epigallocatechin gallate* (*EGCG*) inhibited DNMT enzyme activity and DNMT protein expression in LNCaP cells. DNMT inhibition was associated with reduced methylation of the *GSTP1* proximal promoter and reactivation of *GSTP1* expression. Transcription was facilitated by enhanced binding of transcription factor Sp1 to the *GSTP1* promoter [45]. Intervention of prostate cancer cell lines with the *soy phytoestrogens genistein and daidzein* significantly reduced *GSTP1* promoter methylation and resulted in reexpression of *GSTP1* protein, determined by immunocytochemistry and western blotting [83, 84]. The mechanism of inhibition was not further analyzed. King-Batoon et al. investigated the effects of genistein and the tomato-derived carotenoid *lycopene* on DNA methylation in breast cancer cells. A single application of lycopene reactivated *GSTP1* mRNA expression within 1 week, associated with reduced promoter methylation in MDA-MB-468 cells, whereas genistein was weakly effective only after repetitive treatments. Both compounds were ineffective in the MCF7 cell line, and also did not reduced *RARβ* and *HIN1* promoter methylation in both cancer cell lines [79]. Similarly, treatment of MCF7 cells with a series of dietary polyphenols, including *ellagic acid*, *protocatechuic acid*, *sinapic acid*, *syringic acid*, *rosmarinic acid*, *betanin*, and *phloretin* did not lead to demethylation and reexpression of *GSTP1*, *RASSF1A*, and *HIN1*, although all of these compounds at the same concentrations inhibited DNMT activity in vitro by 20–88% [40]. Lack of demethylating activity in cell culture might indicate an unspecific enzyme inhibitory effect.

As mentioned above, transcription factor *Nrf2* (nuclear factor-erythroid 2 p45-related factor 2) plays an important role in phase 2 enzyme induction [207]. Recently, *Nrf2* was shown to be epigenetically silenced by promoter methylation at specific CpG sites during prostate carcinogenesis in tumors of transgenic adenocarcinoma of mouse prostate (TRAMP) mice and tumorigenic TRAMP C1 cells. In contrast, the *Nrf2* promoter CGI was unmethylated in normal prostate tissue and non-tumorigenic TRAMP C3 cells. Methylation led to transcriptional repression by

increased binding of methyl binding protein 2 (MBD2) and H3K9me3, and reduced interaction with RNA polymerase II and the activating histone mark acetylated histone 3 (ac-H3) [210]. Treatment of TRAMP C1 cells with *curcumin* significantly reduced *Nrf2* promoter methylation at five specific CpG sites and led to mRNA reexpression of *Nrf2* and NAD(P)H:quinone reductase (*NQO1*) as a downstream target [49]. Curcumin (diferuloyl methane) is a well characterized cancer chemopreventive agent derived from turmeric (*Curcuma longa*) [211].

7 Cell Cycle Regulation

One of the hallmarks of cancer cells is their ability to evade growth-suppressing signals. Various genes affecting cell cycle progression have been identified as tumor suppressor genes, first of all *p53* and *pRB* [212]. Progression through the cell cycle is regulated through activation and inactivation of cyclin-dependent kinase (Cdks) that form sequential complexes with cyclins A–E during the different phases G_1, S, G_2, and M of the cell cycle. During G_1 phase, Cdk2–cyclin E and Cdk4/6–cyclin D1 complexes promote entry into S-phase by phosphorylation of *pRB*, thereby releasing the transcription factor *E2F* [213]. The activity of Cdks is controlled by binding of Cdk inhibitors (CKIs) to Cdk–cyclin complexes. CKIs *p21*, *p27*, and *p57* preferentially interact with Cdk2– and Cdk4–cyclin complexes, whereas CKIs $p15^{INK4B}$ and $p16^{INK4A}$ are more specific for Cdk4– and Cdk6–cyclin complexes and block their interaction with cyclin D [213].

Interestingly, both DNA methylation and histone acetylation are involved in the regulation of CKI expression, as exemplified with $p16^{INK4A}$ and $p21^{CIP1/WAF1}$. $p16^{INK4A}$ (inhibitor of Cdk4, also known as *CDKN2*, CDK inhibitor 2) is genetically inactivated by point mutations, deletion, or DNA methylation in about 50% of all human cancers [214]. Hypermethylation of the *p16* promoter is frequently observed in all major human malignancies, including hepatocellular carcinoma, primary gastric carcinoma, Barrett's esophagus and esophageal adenocarcinoma [214], breast cancer [215], squamous cell carcinoma of the lung [216], colorectal cancer [217], lymphoma [218], as well as tumors of the ovary, uterus, head and neck, brain, kidney, bladder, and pancreas [219]. Murine *p16* knockout strains are more prone to spontaneous tumorigenesis than wildtype littermates, whereas overexpression of *p16* led to a threefold reduction of spontaneous cancers [220].

Several studies have investigated whether natural products were able to demethylate and reactivate *p16* in a wide variety of cancer cell lines. Fang et al. reported demethylation and re-expression of *p16* in KYSE510 esophageal cancer cells and HCT116 colon cancer cells after treatment with *EGCG* [37, 55]. These results could not be confirmed in a subsequent study by Chuang et al. [56] using T24 bladder cancer cells, HT 29 colon cancer cells, and PC3 prostate cancer cells. In A431 epidermoid carcinoma cells, EGCG decreased global methylation and inhibited DNMT activity as well as expression of *DNMT1*, *3a*, and *3b*, which led to the reexpression of *p16* mRNA and protein [61]. *Genistein* treatment of KYSE510

esophageal cancer cells resulted in dose-dependent and time-dependent demethylation and re-expression of *p16* [78]. In a study by Fini et al., intervention of RKO, SW48, and SW480 colon cancer cells with an *apple polyphenol extract* also resulted in *p16* promoter demethylation and mRNA or protein reexpression. This was explained by downregulation of *DNMT 1* and *DNMT 3b* protein expression in RKO and SW480 cells [38]. *Nordihydroguaiaretic acid* (*NDGA*) was investigated in RKO and T47D breast cancer cell lines. *p16* promoter demethylation and reactivation was associated with reduced *cyclin D1* expression and *RB* phosphorylation, G_1 cell cycle arrest, and increased senescence [96]. *Phenylhexyl isothiocyanate* (*PHI*) was initially identified as an HDAC inhibitor, as described below. Lu et al. were able to demonstrate that intervention in RPMI8226 myeloma cells reduced *p16* promoter methylation and induced cell cycle arrest in G_1 phase [102].

p21, also known as CDK-interacting protein 1 (Cip1) or wild-type p53-activated fragment 1 (WAF1), is encoded by the cyclin-dependent kinase inhibitor 1 *CDKN1A* gene locus [221–223]. *p21* directly inhibits the activity of Cdk2/cyclin E and functions as an adaptor protein for Cdk4/6/cyclin D complexes, thereby modulating cell cycle progression at S-phase [224]. Overexpression of *p21* can lead to G_1-phase, G_2-phase, or S-phase arrest, whereas *p21*-deficient cells fail to undergo cell cycle arrest in response to p53 activation after DNA damage [225]. In addition to cell cycle regulation, *p21* is involved in regulation of cell differentiation, senescence, gene transcription, apoptosis, and DNA repair (review in [223]). *p21* knockout mice are prone to development of spontaneous tumors [223]. In contrast to *p16* or *p53*, mutations in *p21* are extremely rare (summarized in [225]). In comparison to other tumor suppressor genes, methylation at the *p21* promoter was not frequently observed in hematological malignancies [226]. *p21* was overexpressed after downregulation of DNMTs, but the mechanism of induction might be independent of changes in promoter methylation and rather involve competing interactions of DNMTs and *p21* with *PCNA* and enhanced stability [224, 227]. *p21* expression is more commonly regulated at the transcriptional level, and chromatin structure controlled by histone acetylation seems to play an important role. The *p21* promoter region contains binding sites for *p53* and Sp1/3, several E-boxes, and can be repressed by the oncogene *c-Myc* [224]. Inhibition of *HDAC* activity, in addition to opening the chromatin structure, has been suggested to lead to a release of *HDAC1* from the *p21* promoter, thereby facilitating binding of Sp1/3 and HATs *p300* or *PCAF*. Indirectly, hyperacetylation of *p53* through HDAC inhibition may promote *p21* transcription by enhancing the affinity of *p53* to the *p21* promoter (summarized in [224]). Alternatively, *p21* expression can be transcriptionally silenced through recruitment of *CTIP2* (*COUP-TF-interacting protein 2*) and interactions with HDACs and histone methyltransferases (HMTs) [180].

Butyric acid (its sodium salt being referred to as "butyrate") is a major short-chain fatty acid produced by colonic fermentation of resistant starch and dietary fiber. Butyrate was first described to inhibit *HDAC* activity in vitro and in cell culture models more than 30 years ago. Initial work focused on its anti-proliferative and differentiation-inducing effects in leukemia cell lines [228–230]. Since dietary fiber consumption has been associated with colon cancer prevention [231], Archer

et al. established a link between butyrate-mediated *HDAC* inhibition, *p21* induction, and cell growth inhibition in colon cancer cell lines [130]. Induction of *p21* mRNA and protein expression was also associated with histone hyperacetylation and colon cancer prevention in 1,2-dimethylhydrazine-induced tumorigenesis in a mouse model of colorectal cancer [133].

Dietary sources of selenium, such as Se-methyl-Se-cysteine (SMC) and Se-methionine (SM), can be metabolized to *α-methylselenopyruvate* (*MSP*) and *α-keto-γ-methylselenobutyrate* (*KMSB*) with structural similarity to butyrate [156]. Consequently Nian et al. investigate *HDAC*-inhibitory potential of these α-keto acid metabolites. MSP and KMSB caused a dose-dependent inhibition of human *HDAC1* and *HDAC8* activities in vitro. Enzymatic kinetic studies and computational molecular modeling identified MSP as a competitive inhibitor of *HDAC8*, based on reversible interaction with the active site zinc atom. In human colon cancer cells, MSP and KMSB dose-dependently inhibited *HDAC* activity and increased global H3 acetylation and *p21* expression levels, which led to G_2/M cell cycle arrest and apoptosis induction [156]. In a seminal study published in 2004, Myzak et al. first suggested that *sulforaphane* (*SFN*) might possess *HDAC*-inhibitory activity, based on the observation that SFN treatment caused *p21* upregulation and cell cycle arrest, similar to the activities of butyrate. SFN failed to inhibit directly *HDAC* activity in cell-free systems in vitro. Rather, in silico modeling indicated that SFN-Cys, an SFN metabolite, might possess *HDAC* inhibitory potential. Consistently, cell culture media after incubation with SFN contained a metabolite able to inhibit *HDAC* enzymatic activity [169]. Further studies confirmed the *HDAC* inhibitory activity of SFN intervention in various human cancer cell lines [169, 170, 174]. In human prostate cancer cells, SFN treatment increased global histone acetylation, accompanied by locus-specific hyperacetylation of H3, H4, or both at the *p21* promoter [170]. A study of SFN intervention in $APC^{Min/+}$ mice underlined the relevance of HDAC inhibition for chemopreventive activity of SFN. A single dose of SFN lowered *HDAC* activity and transiently increased ac-H3 and ac-H4 levels in colonic mucosa of wild-type mice [176]. Long-term application for 10 weeks produced similar effects in ileum, colon, prostate, and peripheral blood mononuclear cells (PBMC). In $APC^{Min/+}$ mice, SFN treatment reduced tumor multiplicity, increased ac-H3 levels, and ac-H3 occupancy at the *p21* and *Bax* promoter in tumor samples, and induced expression of pro-apoptotic *Bax* [176]. *Bax* is a member of the Bcl-2 protein family of apoptosis regulators which play an important role in mediating the intrinsic, mitochondrial pathway of apoptosis induction [232, 233]. SFN reduced growth of androgen-independent human prostate cancer cells in a xenograft model, and increased global histone acetylation in prostate tissue and in xenografts [177]. In a human pilot study, three healthy volunteers ingested 68 g of broccoli sprouts as a source of SFN. After 3 h and 6 h the intervention transiently induced strong hyperacetylation of H3 and H4 in PBMCs, concomitant with HDAC inhibition. Both acetylation and enzyme activity returned to normal levels by 24 and 48 h [178]. These findings support a role for SFN as an HDAC inhibitor in vivo, with evidence for decreased HDAC activity in various tissues, increased global histone acetylation, as well as enhanced

localization of acetylated histones at specific promoters. These findings may also be relevant for human cancer prevention.

Two additional isothiocyanates (ITCs), *PEITC* found in water cress [234, 235], as well as the synthetic *PHI* were also confirmed as inhibitors of HDACs, suggesting that this might be a more common mechanism of ITCs. Exposure of prostate cancer cells to PEITC significantly enhanced histone acetylation, cell cycle arrest, and *p53*-independent up-regulation of CKIs, including *p21* and *p27* [158]. Similar to SFN and PEITC, PHI was first identified as an HDAC inhibitor and inducer of cell cycle arrest, but was also shown to reduce *p16* promoter methylation in myeloma cells [102]. HDAC inhibitory potential and chromatin modifications were confirmed in human prostate and liver cancer, and leukemia and myeloma cells. PHI affected both the expression as well as the activity of *HDAC1* in LNCaP and HL-60 cells [159, 160]. In leukemia cells, PHI treatment increased expression of the HAT *p300/CBP* [161]. Increased levels of ac-H3 and ac-H4 were commonly detected in all cell lines, as well as in bone marrow of AML patients [163]. This was further associated with increased interaction of acetylated histones with the *p21* promoter, *p21* induction, G_0/G_1 cell cycle arrest, and apoptosis induction [160–162].

In addition to sulfur-containing ITCs, dietary organosulfur compounds found in garlic and other *Allium* species such as *diallyldisulfide* (*DADS*) have been shown to inhibit HDAC activity. After consumption, DADS is converted to the active metabolite *S*-allylmercaptocysteine (SAMC). Both compounds are further metabolized to *allyl mercaptan* (*AM*) and other metabolites (reviewed in [121]). Induction of histone acetylation by DADS and SAMC was first described in murine erythroleukemia cells [236]. Interestingly, when testing *HDAC* inhibitory potential in vitro, AM was more potent than the precursor compounds DADS and SAMC. Nian et al. predicted direct binding of AM to the *HDAC* active site by in silico docking studies and confirmed inhibitory potential in vitro and in cell culture. *HDAC* inhibition by AM led to hyperacetylation of H3 and H4, enhanced ac-H3 association with the *p21* promoter, upregulation of *p21*, and cell cycle arrest [123]. DADS treatment induced transient histone hyperacetylation followed by *p21* induction, cell-cycle arrest, and induction of differentiation and apoptosis in various cancer cell lines (reviewed in [141]). Intracecal perfusion or intraperitoneal injection of DADS (200 mg/kg b.w.) to male rats also resulted in histone hyperacetylation in normal hepatocytes and colonocytes [142]. These data indicate that effects on histone acetylation and downstream mechanisms induced by organosulfur compounds may be relevant for preventive efficacy, although the described effects observed both in vitro as well as in vivo require doses that might not be reached by dietary consumption of *Allium* vegetables. Also, inhibition of *HDAC* activity and histone hyperacetylation are transient effects. This may suggest that the compounds or dietary sources have to be consumed regularly to achieve long-term effects in vivo. *Apicidin*, a fungal metabolite, is a cyclic tetrapeptide antibiotic with broad spectrum antiparasitic, antiprotozoal, and potential antimalarial properties [127]. Apicidin treatment at low microgram per milliliter concentrations inhibited cell proliferation in a series of cancer cell lines. Apicidin

induced morphological changes, accumulation of ac-H4, and G_1 cell cycle arrest in human cervical cancer cells. This led to induction of *p21* and *gelsolin* involved in cell cycle control and cell morphology, respectively. Decreased phosphorylation of *Rb* protein was indicative of Cdk inhibition. Interestingly, in contrast to the dietary HDAC inhibitors described above, the effects of apicidin on cell morphology, expression of *gelsolin*, and *HDAC1* activity appeared to be irreversible [127]. So far, apicidin has not been tested in animal models for chemopreventive activity.

In addition to these direct effects on HDAC activity, several chemopreventive agents, including the soy isoflavone *genistein*, *3,3'-diindolylmethane* (*DIM*) derived from cruciferous vegetables, *parthenolide*, a sesquiterpene lactone from feverfew, the fungal metabolite *chaetocin*, and *EGCG* have been described to modulate histone acetylation by changing the expression of histone modifying enzymes.

In prostate cancer cell lines, *genistein* treatment caused an upregulation of histone acetyl transferases (HATs) *CREB-binding protein* (*CREBBP*), *p300*, *PCAF*, and *HAT1*. This resulted in hyperacetylation of histones H3 and H4, increased association of acetylated H3K4 with the transcription start sites of *p16* and *p21*, re-expression of *p16* and *p21*, and cell cycle arrest [153]. Indole-3-carbinol (IC3) is the main hydrolysis product of the glucosinolate glucobrassicin [234]. Under low gastric pH conditions I3C is condensed to polycyclic compounds such as *DIM* as the major condensation product [237]. In a study by Li et al., DIM selectively induced proteasomal degradation of the class I histone deacetylases *HDAC1*, *2*, *3*, and *8* in human colon cancer cells in vitro and in tumor xenografts, without affecting class II HDACs. HDAC depletion resulted in re-expression of *p21* and *p27* and triggered cell cycle arrest in G_2/M phase. Additionally, HDAC depletion was associated with DNA damage and apoptosis induction [144]. *Parthenolide* was described as an HDACi-like compound with ability to induce transient and selective ubiquitination and proteasomal degradation of *HDAC1* in breast cancer and other cancer cell lines, whereas other classes I and II HDACs were not affected. Downstream effects were similar to those of HDACi, with *p53*-independent upregulation of *p21* and global histone hyperacetylation. Downregulation of *HDAC1* involved the phosphoinositide-3-kinase-like kinase *ATM* (ataxia telangiectasia), as siRNA-mediated knockdown of *ATM* severely affected parthenolide-induced degradation of *HDAC1*. However, the exact mechanism how parthenolide induces *HDAC1* degradation via *ATM* is presently unknown [157].

In addition to increased histone acetylation through various mechanisms, inhibition of repressive histone methylation marks also results in upregulation of *p21*. *Chaetocin*, a fungal metabolite, was one of the first identified selective inhibitors for the *SUV39* class of HMTs targeting H3K9 (overview in [238]). H3K9 trimethylation is generally associated with repressed chromatin. Chaetocin treatment of microglial cells transfected with a *p21*-promoter reporter construct repressed H3K9 trimethylation at the *p21* promoter, stimulated *p21* expression, and induced cell cycle arrest [180].

Recent research indicates that *EGCG* may regulate expression of cell cycle regulators *p21* and *p27* and apoptotic proteins by influencing *polycomb group* (*PcG*)-mediated histone modifications [184]. *PcG* proteins, including *BMI-1* and

EZH2, are HMTs that increase H3K27 methylation leading to a repressed chromatin conformation and enhanced cell survival. In skin cancer cells EGCG treatment reduced levels of *BMI-1* and *EZH2*, lowered H3K27me3 levels, and reduced cell survival. This was associated with induction of cell cycle regulators and activation of caspases and *Bcl-2* family proteins. The inhibitory effects of EGCG on *BMI-1* expression were corroborated by overexpression of *BMI-1* [184]. EGCG treatment of human epidermoid carcinoma cells reduced H3K9 methylation and concomitantly increased H3 and H4 acetylation by HDAC inhibition. This was associated with an upregulation of *p16* and *p21* mRNA and protein levels [61].

RassF1A (Ras Association Domain family 1, isoform A) is a candidate tumor suppressor gene located on the chromosome 3p21.3 locus that is frequently inactivated in cancer by loss of heterozygosity. *RassF1A* promoter methylation and silencing have been described as the most frequent epigenetic change observed in human cancers, including lung, breast, pancreas, kidney, liver, cervix, nasopharyngeal, prostate, thyroid, and other cancers [239, 240]. Loss of *RassF1A* is associated with advanced tumor stage and poor prognosis. Since *RassF1A* hypermethylation is detectable in various body fluids including blood, urine, nipple aspirates, sputum, and bronchial alveolar lavages, it may serve as a valuable diagnostic or prognostic marker [239]. *RassF1A* knockout mice are viable and fertile, but prone to spontaneous tumorigenesis [241]. *RassF1A* is involved in two pathways commonly deregulated in cancer – cell cycle regulation and apoptosis [239, 240]. Overexpression of *RassF1A* in vitro was found to inhibit accumulation of *cyclin D1*, thereby blocking G_1/S cell cycle progression [242].

Numerous studies have attempted to demethylate and reexpress *RassF1A* by chemopreventive agents in vitro or dietary intervention in vivo. Most of these studies have reported negative results. As summarized in Table 1 (Appendix), *genistein* and *seleno-D,L-methionine* did not influence the methylation status of *RassF1A* in prostate cancer cell lines in vitro [83, 111]. In a randomized 4-week human intervention study with *cruciferous vegetables* or *soy products* in combination with *green tea*, neither treatments influenced methylation of *RassF1A* and a series of other candidate genes in PMBCs of heavy smokers, whereas methylation of the repetitive element *Line1* (long interspersed nuclear element) was slightly but significantly increased [47]. Also, 4-week dietary intervention in 34 healthy premenopausal women with daily doses of 40 or 140 mg *isoflavones* did not influence *RassF1A* methylation in intraductal specimens [92]. Jagadeesh et al. tested the effect of *mahanine*, a carbazole alkaloid found in some Asian vegetables, in a series of prostate cancer and several other human cancer cell lines. Mahanine treatment at low microgram per milliliter concentrations led to reexpression of *RassF1A,* reduced expression of *cyclin D1* and inhibition of cell proliferation. The authors did not investigate changes in *RassF1A* promoter methylation, but *DNMT* activity in mahanine-treated prostate cancer cell lines was significantly reduced. In a subsequent study, a synthesized mahanine derivative was equally or even more effective as mahanine with respect to inhibition of PC-3 cell proliferation, DNA synthesis, and *DNMT* activity, reactivation of *RassF1A* mRNA expression, and downregulation of *cyclin D1* [94]. The derivative was shown to act by sequestering

DNMT3b, but not *DNMT3a* in the cytoplasm. Consistently, depletion of *DNMT3b* was shown previously to cause *RASSF1A* reactivation, cell growth inhibition, and apoptosis induction in cancer cell lines, but not in normal cells [14]. In Balb/c nude mice, the mahanine derivative was not toxic after oral application at concentrations up to 550 mg/kg. It reduced growth of PC-3 xenografts by 40% when applied at 10 mg/kg body weight every other day for 4 weeks. The influence of epigenetic mechanisms for tumor growth inhibition was however not investigated [94].

8 Apoptosis

Tissue homeostasis is balanced by cell proliferation and cell death. Evading apoptosis (programmed cell death) has been recognized as one of the hallmarks of cancer cells [243]. Apoptosis can be triggered when cells sense abnormalities such as DNA damage, imbalance in signaling by aberrant activation of oncogenes, lack of survival factors, or hypoxia [243]. *p53* is one of the most important pro-apoptotic mediators involved in sensing DNA damage. It is lost or functionally inactivated in more than 50% of all human tumors [243]. p53 activity is also epigenetically controlled: deacetylation of *p53* through *SIRT1* (silent information regulator 1), a member of the sirtuin HDAC class III family, prevents *p53*-mediated transactivation of cell cycle inhibitor *p21* and pro-apoptotic *Bax*, allowing promotion of cell survival after DNA damage and ultimately tumorigenesis [193]. Inhibition of *SIRT1* should therefore lead to induction of apoptosis by counteracting the deacetylation of *p53* and other key factors such as *FOXO3a*. However, despite the fact that SIRT1 can inactivate *p53* and is upregulated in several human cancer types, recent data suggest that SIRT1 is a tumor suppressor in vivo [244].

Two natural products, *cambinol* and *dihydrocoumarin* (*DHC*) have been identified as *SIRT* inhibitors. The β-naphthol compound *cambinol* was identified in a chemical screen and inhibits both *SIRT1* and *SIRT2*, whereas class I and II *HDAC*s were not affected [134]. Cambinol acts as a competitive inhibitor with respect to the histone H4 peptide and as a non-competitive inhibitor with respect to the co-substrate NAD^+. In lung cancer cells, cambinol treatment in combination with etoposide to induce DNA damage led to hyperacetylation of *SIRT* target proteins such as *p53, FOXO3a* and *Ku70*. Deacetylation of these later proteins promoted cell survival under stress, which was abrogated by inhibition of *SIRT* with cambinol. *BCL6* is a transcriptional repressor that is also deacetylated by *SIRT*. In *BCL6*-expressing Burkitt lymphoma cells, treatment with cambinol induced apoptosis, accompanied by hyperacetylation of *BCL6* and *p53*. In vivo, cambinol intervention at a dose of 100 mg/kg i.v. or i.p. inhibited growth of Burkitt lymphoma xenografts in SCID mice and was well tolerated [134]. *DHC*, a component of *Melilotus officinalis* (sweet clover), is frequently used in cosmetics or as a flavoring agent. DHC was identified as an inhibitor of yeast *Sir2p* and human *SIRT1* activity. Treatment of human TK6 lymphoblastoid cells with DHC led to a dose-dependent induction of *ac-p53*, cytotoxicity, and apoptosis [143]. Kahyo et al. attempted to

identify novel inhibitors of sirtuins (*SIRT*s), also known as class III HDACs. Using acetylated *p53* as a substrate, they identified the synthetic *3,2′,3′,4′-tetrahydroxychalcone* as an inhibitor of *SIRT* activity and *p53* deacetylation in vitro. Treatment of human embryonic kidney cells with the chalcone induced hyperacetylation of endogenous *p53*, increased *p21* expression and suppressed cell growth. Since HDAC inhibitory potential of the compound was not tested, it is difficult to conclude whether *p21* induction is indeed mediated via inhibition of *SIRT1* [135].

An alternative mechanism leading to hyperacetylation of *p53* and apoptosis induction is mediated through the activity of *MTA1*/*HDAC1* in the nucleosome remodeling deacetylation (NuRD) complex. *MTA1* (metastasis-associated protein 1) expressed in various cancers has been associated with aggressiveness and metastasis [165]. Kai et al. identified that treatment of prostate cancer cells with *resveratrol* resulted in down-regulation of *MTA1*. This functionally blocked the *MTA*/NuRD complex and led to hyperacetylation of *p53*, trans-activation of *p21* and *Bax*, and apoptosis induction. This effect was corroborated by knockdown of *MTA1* and further enhanced by cotreatment with the HDACi suberoylanilide hydroxamic acid (SAHA). These combination effects might present an innovative therapeutic strategy for the management of prostate cancer [165].

The tumor suppressor *PTEN* (phosphatase and tensin homolog deleted on chromosome 10) negatively regulates the *phosphatidylinositol 3-kinase* (*PI3K*)-*AKT* pathway that transmits anti-apoptotic survival signals and regulates cell proliferation, growth and motility [245]. Downstream signaling is indirectly mediated via transcription factors such as *NF-κB* and *FOXO* [245, 246]. Somatic *PTEN* deletions and mutations, and epigenetic inactivation of *PTEN* by promoter methylation or miRNA silencing are common in multiple tumor types. Silencing through epigenetic mechanisms frequently occurs in breast, prostate, thyroid, and lung cancer, glioma, and melanoma, whereas mutations and deletions are common in endometrium, bladder, kidney, colorectal cancer, and leukemias. $PTEN^{-/-}$ was shown to lead to early onset of prostate or mammary cancer in mouse models [245, 246].

PTEN is hypermethylated in breast cancer cell lines MCF-7 and MDA-MB-231. Stefanska et al. analyzed whether *PTEN* silencing could be reversed in these cell lines after incubation with the chemopreventive agents *all-trans-retinoic acid* (*ATRA*), *Vitamin* D_3, and *resveratrol* alone and in combination with nucleoside analogs such as 2-chloro-2′-deoxyadenosine (2CdA), 9-β-D-arabinosyl-2-fluoroadenine (F-ara-A), and 5-aza-2′-deoxycytosine (5-Aza) [104]. In MCF-7 cells with a methylation level of about 30% at the *PTEN* promoter, incubation with all three natural products resulted in demethylation and reexpression of *PTEN*. This was associated with down-regulation of *DNMT1* and upregulation of *p21* after incubation with vitamin D_3 and resveratrol. The effects were further enhanced by co-incubation with 2CdA and F-ara-A. In highly invasive MDA-MB-231 cells, the PTEN promoter was >90% methylated. Only Vitamin D_3 treatment was able to reduce methylation and to enhance concomitantly expression of *PTEN*, whereas the combined treatment with nucleoside analogs did not enhance efficacy [104]. Kikuno et al. investigated whether *genistein* might suppress *AKT* signaling via epigenetic mechanisms. In prostate cancer cell lines, genistein treatment led to reexpression of *PTEN* and consequential

inactivation of *AKT*, resulting in induction of *p53* and *FOXO3a*. Genistein treatment also upregulated the endogenous *NF-κB* inhibitor *CYLD* and decreased constitutive *NF-κB* activity. These effects were likely unrelated to inhibition of DNA methylation, as promoter regions of all of these factors were unmethylated in the investigated cell lines. Rather, reexpression was associated with elevated H3K9 acetylation (*PTEN*, *CYLD*, *p53*, and *FOXO3a*) and loss of H3K9 methylation (*PTEN* and *CYLD*). H3K9 hyperacetylation could be associated with reduced expression and nuclear localization of *SIRT1* after genistein treatment [154].

Death-associated protein kinase (*DAPK*) is a pro-apoptotic serine/threonine kinase acting in the extrinsic death receptor-mediated pathway of apoptosis induction [233, 247]. *DAPK* is induced by *p53* activation and in turn elevates *p53* expression, supporting the existence of an autoregulatory feedback loop between *DAPK* and p53 that controls apoptosis. In addition to apoptosis induction, *DAPK* is also involved in the control of autophagy, which can lead to cell survival or cell death depending on the cellular context (review in [247]). *DAPK* expression is reduced in a wide range of cancer types by promoter methylation, including lung, bladder, head and neck, kidney, breast, and B-cell malignancies. Detection of *DAPK* methylation has been suggested as a useful prognostic biomarker for invasive and metastatic potential [247]. *DAPK* is an *NF-κB* regulated gene. Hypermethylation of *DAPK* might be mediated by a targeted recruitment of *DNMT*s to *RelB* (a subunit of NF-κB)-regulated genes via *Daxx*, an apoptosis regulator. *DAPK* function is also lost by deletion and point mutations [247]. In a study by Fang et al. treatment of mouse lung cancer cells with EGCG in combination with trichostatin (TSA) or butyrate synergistically increased mRNA levels of *DAPK* and retinoic acid receptor β (*RARβ*), indicating a reversal of epigenetic silencing. *DAPK* promoter methylation was not investigated in this study.

9 DNA Repair

Cancer genomes are characterized by accumulation of genomic instability and chromosomal aberrations, associated with underlying defects in the DNA repair machinery [248]. Important DNA repair genes, such as the mismatch repair gene *hMLH1* and the DNA-alkyl repair gene *MGMT* (O^6-methylguanine DNA methyltransferase) are commonly inactivated in human cancers by CpG island hypermethylation. Loss of *hMLH1* expression by germ-line mutations and promoter hypermethylation leads to microsatellite instability that is mainly associated with hereditary non-polyposis colorectal cancer (HNPCC), but also observed in endometrial and gastric tumors [249]. *MGMT* repairs promutagenic O^6-methylguanine adducts by transferring the methyl group to a cysteine residue in its active site. Methylated *MGMT* is then degraded by the proteasome. *MGMT* has been shown to be silenced by aberrant methylation in a large spectrum of human tumors, with highest hypermethylation rates in tumors of the testis and colon, in retinoblastoma, glioma, head and neck and cervical cancer, lymphoma, lung, esophageal, gastric

and pancreatic cancer, and several further cancer types. It has been suggested that silencing of *MGMT* is associated with 72% of the mutations observed in the *p53* gene, and with 40% of the colon cancer cases induced through *K-ras* mutations [250]. Noteworthy, although loss of *MGMT* expression contributes to tumorigenesis and is a marker of poor prognosis, glioma patients with reduced *MGMT* activity respond better to treatment with alkylating agents [251].

Several studies have investigated the effect of natural products on the methylation status and expression of repair genes. *EGCG* and *genistein* treatment resulted in reduced *MGMT* and *hMLH1* promoter methylation and mRNA/protein re-expression in human esophageal carcinoma cells [37, 55, 78, 252]. Incubation of colon cancer cell lines with *apple polyphenols* also led to reexpression of *hMLH1* by promoter hypomethylation due to reduced *DNMT1* and *DNMT3b* protein expression [241]. This effect on DNA methylation may contribute to the colon cancer preventive efficacy of apple polyphenols (reviewed in [253]). In the transgenic adenocarcinoma of the mouse prostate (TRAMP) model, intervention with *PEITC* given at a dose of 15 μmol daily by gavage for 13 weeks significantly reduced prostate tumor formation and lowered *MGMT* promoter methylation in tumor tissue [101]. In the same model, intervention with *5-aza-2′-deoxycytidine* (*5-Aza*) at a dose of 0.25 mg/kg twice per week completely prevented prostate cancer development at 24 weeks of age, whereas in 54% of the control mice poorly differentiated prostate cancers were detected upon necropsy. Treatment with 5-Aza also prevented lymph node metastases and dramatically extended survival compared with control-treated mice. In tumor tissue, *MGMT* promoter methylation was reduced by 5-Aza treatment, and *MGMT* mRNA expression was induced [254].

10 Inflammation and Regulation of NF-κB

Epidemiological evidence indicates that chronic infections and subsequent inflammation are causally linked to about 15–20% of all cancer deaths [255, 256]. Examples include chronic infections with *Hepatitis B* and *C* virus and risk for hepatocellular carcinoma, infections with *Helicobacter pylori* and gastric cancer, chronic inflammatory bowel diseases and colorectal cancer, and chronic airway irritations and inflammation caused by tobacco smoke and lung cancer [255]. Chronic inflammatory conditions are characterized by the accumulation of inflammatory cells, which are recruited to the tumor tissue and contribute to the stromal tumor microenvironment and the release of tumor-promoting pro-inflammatory mediators [256]. These factors facilitate evasion from host defense mechanisms, promote genomic instability, regulate growth, migration, and differentiation, alter response to hormones and chemotherapeutic agents, and stimulate angiogenesis and metastasis [256, 257].

One of the most important transcription factors controlling inflammatory conditions is *NF-κB* [258]. *NF-κB* is a homodimer or heterodimer of members of the *NF-κB* subunit family, consisting of *RELA* (also known as *p65*), *RELB*, *REL*,

p50, and *p52*. All these members contain a REL homology domain that allows DNA-binding and dimerization (for further detailed information refer to [255, 259]). During carcinogenesis, aberrant *NF-κB* activation regulates transcription of anti-apoptotic genes, cyclins, and oncogenes that promote cell proliferation, pro-angiogenic genes, as well as matrix metalloproteinases and cell adhesion genes [259]. Interestingly, *NF-κB* activity is partly controlled by post-translational modifications, including phosphorylation, acetylation, methylation, and ubiquitinylation [259]. Reversible acetylation at lysine 310 mediated by the HAT *p300* is required for full trans-activating activity [260–262].

NF-κB has been extensively studied as a target for chemopreventive agents [263]. Interestingly, recent research now establishes a link between *NF-κB* and chemopreventive agents via an indirect epigenetic mechanism by inhibition of *NF-κB* acetylation mediated by *p300* HAT. *Anacardic acid* (6-nonadecyl salicylic acid) isolated from cashew nut shell liquid was identified as the first natural product inhibitor of *p300* HAT activity. In a natural product screen it was found to inhibit *p300* and *PCAF* activities with IC_{50} values of 8.5 and 5 μM, respectively [124]. In a study by Sung et al., anacardic acid blocked *NF-κB* activation by *TNF-α* and a series of other stimuli and suppressed acetylation and nuclear translocation of the *NF-κB* subunit *p65*. Anacardic acid-mediated effects could be mimicked by down-regulation of *p300* HAT by siRNA, indicating that *p300* is a key mediator of the effects of anacardic acid on *NF-κB* signaling. In cancer cell lines, anacardic acid potentiated *TNF-α*-, cisplatin-, and doxorubicin-mediated apoptosis induction, and strongly suppressed *TNF-α*-mediated upregulation of *NF-κB* target genes, including the anti-apoptotic proteins *Bcl-2*, *Bcl-xL*, *cFLIP*, *cIAP-1*, and *survivin*, as well as *cyclin D1*, *c-Myc*, *Cox-2*, *VEGF*, *ICAM*-1, and *MMP9* involved in invasion and angiogenesis. Based on these results, anacardic acid might be an interesting lead compound for further development in cancer prevention [126]. *Garcinol* is a polyisoprenylated benzophenone isolated from the Mangosteen tree *Garcinia indica* Choisy (*Clusiaceae*) [264]. Garcinol was identified as a cell-permeable inhibitor of *PCAF* and *p300* HAT activities with IC_{50} values of 5 and 7 μM, respectively. In HeLa cells, garcinol treatment repressed general histone acetylation and induced apoptosis [151]. Similar to the activities of anacardic acid, garcinol reduced the expression of various *NF-κB* target proteins, including anti-apoptotic *survivin*, *Bcl-2*, *XIAP*, and *cFLIP* [265]. Although garcinol has previously been reported to inhibit *NF-κB*, acetylation of *p65* was not analyzed in this study. *Curcumin* was identified as a specific inhibitor of *p300/CBP* in vitro and in cell culture, whereas other histone-modifying enzymes, including *PCAF*, *HDAC*, and *HTM* activities were not inhibited by curcumin. *HAT* inhibition was attributed to a structural modification of *p300*, thereby preventing binding of histones or cofactor acetyl-CoA. Curcumin also inhibited acetylation of *p53* as a non-histone target of *p300/CBP* [137, 138]. In Raji cells, curcumin treatment significantly down-regulated levels of *HDAC1* and *p300* protein and mRNA. Reduction was prevented by co-treatment with MG-132, an inhibitor of the 26S proteasome [136]. Although not specifically addressed in these studies, direct inhibition and down-regulation of *p300* might contribute to the well-known inhibition of *NF-κB* by curcumin [266].

In a natural product screen, Choi et al. identified *gallic acid* from rose flowers, a simple polyphenol found in various fruits, tea, and wine, as a novel inhibitor of *p65* acetylation, leading to suppression of lipopolysaccharide (LPS)-induced *NF-κB* signaling [149]. Gallic acid was found to inhibit uncompetitively *p300* HAT activity with an IC_{50} value of 14 μM. Other HATs, such as *PCAF* and *Tip60*, were inhibited to a lesser extent, whereas *SIRT1*, *HDAC*, and *HMT* activities were not affected. In cell culture, gallic acid prevented *p65* acetylation, binding to the *IL-6* promoter, activation of an *NF-κB* reporter construct by LPS, inhibited inflammatory response to various stimuli, and downregulated the expression of *NF-κB*-dependent inflammatory and anti-apoptotic proteins. Inhibition of *p65* acetylation was also confirmed in vivo in macrophages of LPS-stimulated mice [149]. The same group also identified *EGCG* as a *p300* inhibitor with similar effects on *p65* acetylation and downstream pathways as described for gallic acid. Inhibition of *p65* acetylation reduced EBV-induced B-lymphocyte transformation [147]. Recently, they also reported that *delphinidin*, an anthocyanidin plant pigment isolated from pomegranate (*Punica granatum* L.), potently inhibited *p300* HAT activity and suppressed pro-inflammatory signaling through inhibition of *NF-κB* acetylation in synoviocyte cells and in T lymphocytes [140]. Interestingly, all three compounds structurally share a 1,2,3-trihydroxybenzene moiety. The authors did not discuss whether this structural feature might be important for the observed *p300*-inhibitory activity. Overall these data demonstrate that acetylation of NF-κB seems to play an important role in mediating downstream signaling events, and that regulation of *p65* acetylation by inhibition of *p300* might be an interesting target for chemoprevention.

11 Cell Signaling and Cell Growth

Normal cells do not proliferate without mitogenic stimulatory signals. Consequently, "self-sufficiency in growth signals" was defined as one of the hallmarks of cancer cells [243].

Androgen receptor (*AR*) *signaling* provides the most important growth stimulus in hormone-dependent prostate cancer. Androgen action is mediated via circulating testosterone levels. Free testosterone enters prostate cells and is converted by 5α-reductase to dihydrotestosterone (DHT) with higher affinity to the *AR* than testosterone. *AR* is sequestered in the cytosol by complexation with heat shock proteins (HSP) such as *HSP90*. After DHT binding, receptor dimerization, phosphorylation, and nuclear translocation, the receptor-ligand complex binds to the androgen-response element in promoter regions of androgen-responsive genes. This leads to recruitment of co-activators, which then facilitate transcription of androgen-sensitive target genes, resulting in increased proliferation and survival [267]. In early stages of prostate cancer, androgen signaling primarily controls cellular growth and proliferation [268], and therefore androgen ablation therapy is carried out as a first line of treatment [269]. An initial response is often followed by an androgen-resistant, lethal disease state. This transition has been attributed to

aberrant reactivation of *AR*-signaling that is hypothesized to occur through multiple mechanisms, including *AR* amplification, *AR* mutations, ligand-independent *AR* activation, excessive production of co-activators, and enhanced local production of androgens [270, 271].

Anti-androgen therapy is achieved by compounds binding to the androgen receptor. Alternatively, compounds inhibiting 5α-reductase and the formation of DHT (such as finasteride) are used, but their application in the prevention of prostate cancer is controversial [272].

Chemopreventive agents might indirectly target *AR* signaling via epigenetic mechanisms. *HDAC6* was shown to deacetylate and activate non-histone proteins, including the AR-chaperone *heat shock protein 90* (*HSP90*). Basak et al. reported that *genistein* treatment of LNCaP cells led to enhanced proteosomal degradation of *AR*. Genistein downregulated the expression of *HDAC6*, which resulted in hyperacetylation of *HSP90* and consequent dissociation of the *AR*. Genistein-mediated effects of *HDAC6* downregulation on *AR* were mimicked by *HDAC6* siRNA. These data indicate that prostate cancer preventive potential of genistein may be mediated through modulating the complex of *HDAC6* with *HSP90* and *AR* [152]. Similarly, *SFN* treatment of LNCaP cells induced rapid hyperacetylation of *HSP90* and dissociation of the *AR* by inhibition of *HDAC6* activity. *AR* degradation led to decreased expression of AR target genes such as prostate specific antigen (PSA) and the androgen-regulated fusion of *TMPRSS2* with the oncogene *ERG*. SFN-mediated effects on *AR* were mimicked by *HDAC6* siRNA or treatment with TSA, whereas overexpression of *HDAC6* restored the effects of *HDAC6* inhibition. Therefore, similar to genistein [152], SFN may act as a prostate cancer preventive agent by affecting the complex of *HSP90-AR* through *HDAC6* inhibition [171]. Recently, *EGCG* was shown to affect acetylation of *AR* via inhibition of HAT activity. This was associated with reduced acetylation and nuclear translocation of AR, leading to inhibition of cell proliferation, especially in hormone-dependent prostate cancer cells [148]. In summary, these indirect epigenetic mechanisms might be interesting tools to counteract androgen signaling as a means for prostate cancer prevention.

Wnt signaling plays an important role during embryonic tissue development and tissue homeostasis in adults. Aberrant *Wnt* signaling has been implicated in cancer development in various organs, including colon, skin, liver ovary, breast, and lung [273]. The main function of canonical *Wnt* signaling is controlling the levels of the transcriptional co-activator *β-catenin*. In the absence of Wnt, *β-catenin* levels in the cytosol are regulated through interaction and complex formation with the scaffolding protein *Axin*, *APC* (the gene product of the *adenomatous polyposis coli* gene), *casein kinase* (*CK1*), and *glycogen-synthase kinase 3β* (*GSK3β*). Phosphorylation by *CK1* and *GSK3β* marks *β-catenin* for ubiquitinylation and degradation through the proteasome. Under these conditions, *β-catenin* levels in the nucleus are low, and *Wnt*-target genes are repressed by binding of the *Tcf*/*Lef* (T cell factor/lymphoid enhancer factor) family of proteins in conjunction with *Groucho* corepressors [274]. Binding of a *Wnt* ligand to the transmembrane receptor *Frizzled* activates the Wnt signaling pathway and ultimately results in the recruitment of *Axin* to the membrane. Consequently, the *CK1*/*APC*/*GSK3β* destruction complex gets

disrupted, and *β-catenin* is stabilized, accumulates in the cytosol, and finally translocates to the nucleus, where it interacts with *Tcf/Lef* and activates the transcription of *Wnt* target genes, including *c-Myc*, *cyclin D1* and many others [274].

Components of the *Wnt* signaling pathway are mutated or altered in over 90% of human colorectal cancers and in high fractions of other cancer types. In addition to these genetic alterations, endogenous *Wnt* antagonists that inhibit *Wnt* signaling through direct binding to *Wnt* are frequently disrupted by DNA methylation in various cancers. These include *secreted frizzled-related proteins* (*sFRP*s) and *Wnt-inhibitory factor 1* (*WIF-1*) [274].

Several recent studies indicate that the chemopreventive agents *EGCG*, *genistein*, and *black raspberries* reactivate silenced *Wnt* pathway antagonists by promoter demethylation [41, 60, 80]. In lung cancer cell lines treated with *EGCG*, promoter methylation of *WIF-1* was potently reduced, resulting in reexpression of *WIF-1*. This was associated with decreased *β-catenin* levels and reduced *Tcf/Lef* reporter activity, indicating that EGCG can inhibit aberrant *Wnt* signaling in vitro [60]. Wang and Chen reported variable methylation and expression levels of the *Wnt* receptor ligand *Wnt5a* in colon cancer cell lines [80]. In the SW1116 cell line derived from an early stage colorectal cancer, *Wnt5a* promoter methylation correlated with lowest expression compared to cell lines derived from later stage tumors that were not methylated. Treatment with *genistein* reduced SW1116 cell viability by about 80%. Under these conditions, *Wnt5a* mRNA levels increased upon treatment, accompanied by about a 10% decrease in *Wnt5a* promoter methylation [80]. Dose-dependent effects were not analyzed in this study.

Wang et al. performed a small human Phase 1 pilot study with 20 colorectal cancer patients to investigate the effects of intervention with 60 g/day freeze-dried *black raspberries* (*BRB*) for 1–9 weeks on biomarkers of colorectal cancer [41]. Promoter sequences of *Wnt*-inhibitory genes *WIF1*, *sFRP2*, and *sFRP4*, as well as *p16* and the developmental gene *PAX1* were analyzed for methylation changes. Also, expression of downstream *Wnt* target genes, including *β-catenin*, *E-cadherin*, and *c-Myc*, as well as of markers of proliferation, apoptosis, and angiogenesis, was measured in colorectal cancer and adjacent normal tissue. At least a 4 weeks intervention was necessary to detect a significant reduction in promoter methylation of *sFRP2* and *Pax6* in both normal and tumor tissue, comparing samples from before and after intervention. In tumor tissue, promoter methylation of *WIF1* was also significantly lower in the group with higher BRB uptake than in the group with uptake for only about 2 weeks. Reduced methylation levels correlated with lowered expression of *DNMT1* in both normal and tumor tissue in the high BRB dose group. Overall, demethylation of *Wnt* inhibitors led to reduced expression of *β-catenin*, *E-cadherin*, and *Ki67* as a proliferation marker in tumor tissue, and induced apoptosis [41]. This is one of the first studies demonstrating modulation of epigenetic markers and downstream effects in human target tissue after chemopreventive intervention.

Interestingly, a study by Huang et al. indicates that *Wnt* inhibitory genes are repressed not only by DNA methylation but also by histone lysine methylation. As outlined above, histone lysine methylation is regulated by the balance between HMT and HDMs (compare also Fig. 3). *LSD1* is a FAD-dependent amine oxidase

which demethylates mono-methylated and di-methylated H3K4 as part of a multiprotein co-repressor complex and thereby broadly represses gene expression ([187] and references cited therein). Since *LSD1* has high homology with monoamine and polyamine oxidases and histone lysine residues resemble polyamines, Huang et al. tested the hypothesis that polyamine analogs might inhibit *LSD1* activity and lead to reexpression of epigenetically silenced genes. Treatment of colon cancer cells with polyamine analogs indeed resulted in re-expression *sFRP1*, s*FRP4*, *sFRP5*s, and transcription factor *GATA5* [186]. This was accompanied by a dose-dependent global increase in H3K4me1 and H3K4me2 levels and enhanced occupancy of these activating histone marks and H3K9ac at the promoters of all re-expressed genes, whereas binding of the repressive marks H3K9me1 and H3K9me2 was reduced. Knockdown of *LSD1* by siRNA recapitulated the effects of the *LSD1* inhibitors on *sFRP* and *GATA5* gene expression [186]. These results were further strengthened by a follow up study that identified two decamine analogs, *PG11144* and *PG11150*, as *LSD1* inhibitors with similar effects on histone methylation and *sFRP* reexpression leading to reduced proliferation and apoptosis induction in colon cancer cell lines. Combined treatment with PG11144 and 5-Aza strongly repressed tumor growth of HCT116 colon cancer xenografts [187]. These data indicate the potential value of *LSD1* inhibitors for the reactivation of silenced genes in cancer prevention or therapy.

hTERT is a catalytic subunit of the enzyme telomerase, which is often upregulated in cancer cells. Telomerase activity is responsible for the maintenance of telomeres which protect chromosome ends from degradation and repair activities to ensure chromosomal stability. Loss of telomeres is associated with ageing, whereas gain of telomerase activity during carcinogenesis enables unlimited cell division [275]. Sequence variations at the *hTERT* locus on chromosome 5 have been associated with many types of cancer, including acute myelogenous leukemia and tumors of the lung, bladder, prostate, cervix, and pancreas (review in [275]). *hTERT* transcription is repressed through binding of the repressor *E2F* to its promoter region. In tumor cells, methylation at the *E2F* binding site prevents *E2F* binding, contributing to elevated expression [54].

ATRA treatment is used in differentiation therapy of leukemia. In human promyelocytic leukemia (HL60) and human teratocarcinoma (HT) cells, ATRA treatment induced cell differentiation and led to progressive histone hypoacetylation. This was coupled with gradual accumulation of *hTERT* promoter methylation, reduced *hTERT* expression, and lower telomerase activity [107]. *hTERT* methylation was not influenced by ATRA treatment in SKBr3 breast cancer cells [276]. In two studies with estrogen receptor (ER)-positive and negative breast cancer cell lines in comparison with an immortalized breast epithelial cell line, treatment with *EGCG* or a prodrug of EGCG with enhanced bioavailability and stability differentially reduced promoter methylation of *hTERT* at selected CpG sites in the cancer cell lines. This allowed enhanced binding of the *E2F* repressor measured by chromatin immunoprecipitation (ChIP), and reduced expression of *hTERT* mRNA. Concomitantly, cell proliferation was reduced in the cancer cell lines by apoptosis induction [54, 62]. Similarly, *genistein* treatment inhibited

hTERT transcription by increasing the binding of the repressor *E2F-1* to the *hTERT* core promoter. This was facilitated by site-specific hypomethylation of the *E2F-1* binding site. Reduced methylation was concomitant with genistein-mediated downregulation of *DNMT* expression [81]. Only recently Meeran et al. identified SFN as a DNA demethylating agent. SFN treatment of breast cancer cell lines inhibited telomerase activity and repressed *hTERT* mRNA expression. SFN intervention reduced *DNMT1* and *DNMT3a* protein expression and significantly lowered *hTERT* methylation at CpG sites in exon 1. These sites were identified as binding region for the transcription factor *CTCF* that is also known to act as an *hTERT* repressor. Activating histone marks, including ac-H3, H3K9ac, and ac-H4, were enhanced at the *hTERT* promoter, whereas the inactivating marks H3K9me3 and H3K27me3 were decreased. SFN-induced histone hyperacetylation facilitated binding of *hTERT* repressors *MAD1* and *CTCF* and decreased binding of *c-Myc*. The importance to *CTCF* for SFN-mediated effects was demonstrated by knockdown of *CTCF* that restored *hTERT* expression and decreased the apoptosis-inducing potential of SFN. In addition, SFN treatment inhibited *HDAC* activity and may modulated histone methylation by increased expression of the histone demethylase *RBP2* [173, 178].

12 Cell Differentiation

Retinoid acid receptors (*RAR*) belong to the steroid hormone receptor superfamily of nuclear receptors that play important roles in embryonic development, maintenance of differentiated cellular phenotypes, metabolism, and cell death. Dysfunction of nuclear receptor signaling is implicated in the development of proliferative, reproductive or metabolic diseases such as obesity, diabetes, and cancer [277]. Genetic studies have identified three isoforms of *RAR*, namely *RARα*, *RARβ*, and *RARγ*, that are activated by binding of ATRA and function as heterodimers with a member of the *9-cis retinoic acid receptor* (*RXR*) family represented by *RXRα*, *RXRβ*, and *RXRγ*. *RXR* heterodimerization with *RAR*s or other steroid hormone receptors allows fine-tuning of nuclear hormone receptor signaling [277].

Alterations in *RAR* function may contribute to cancer development in two ways.

A fusion of *RARα* with the *promyelocytic leukemia* (*PML*) gene caused by translocation of *RARα* leads to formation of a *PML-RARα fusion protein* that acts as a co-repressor of ATRA-responsive genes and is involved in the development of acute promyelocytic leukemia (APL). This defect is efficiently treated by differentiation therapy with ATRA. Some ATRA-resistant leukemia cells fail to respond to ATRA treatment [278]. Treatment of these ATRA-refractory APL blasts with ATRA plus *HDAC* inhibitors or with demethylating agents restored ATRA sensitivity and cell differentiation [226].

RARβ has been identified as silenced by promoter methylation in various tumor types, including colorectal, breast, prostate, head and neck, stomach, and liver cancer, and lymphoma (overview in [279]). Combination of ATRA with natural

or synthetic *DNMT* or *HDAC* inhibitors has been suggested to facilitate reexpression of *RARβ* and may provide beneficial effects for chemoprevention [280]. This was recently demonstrated by the combined intervention with ATRA and *butyrate* as an HDACi in colon cancer cell lines that led to demethylation and reexpression of *RARβ*. Butyrate treatment alone resulted in demethylation of single CpG sites in the *RARβ* promoter. Its effect on *RARβ* reexpression was further enhanced by cotreatment with the soy isoflavone genistein alone or in combination with ATRA [42]. Loss of expression of the *RARβ2* gene is commonly observed during breast carcinogenesis. ATRA therapy failed to induce *RARβ2* in primary breast tumors if the *RARβP2* promoter was methylated. When breast cancer cell lines were treated with ATRA alone or in combination with trichostatin A (TSA) to induce histone acetylation, reactivation of *RARβ2* transcription was facilitated, accompanied by inhibition of cell growth and apoptosis induction [105, 110]. Treatment of APL cells with ATRA reduced *RARβ2* promoter methylation linked with *RARβ2* mRNA reexpression [106]. In the same cell line, Nouzawa et al. were unable to detect ATRA-mediated alterations in *RARβ* CpG island methylation. However, following ATRA-induced differentiation, more than 100 CpG islands within 1 kB of transcription start sites of a known human gene became hyperacetylated [108]. Tang et al. investigated the effect of ATRA at two concentrations alone and in combination with 5-Aza on carcinogen-induced oral cavity carcinogenesis in mice. Both compounds alone and in combination reduced the average number of oral lesions per mouse; combined treatment additionally reduced severity of tongue lesion. Reduction of *RARβ2* mRNA expression in tongue tissue as a consequence of the carcinogen treatment was partly prevented by the combined intervention, whereas carcinogen-induced *Cox-2* and *c-Myc* mRNA expression was inhibited [281].

In studies with natural products, treatment of esophageal cancer cell lines with *EGCG* led to demethylation and reexpression *RARβ2* in a time-dependent and dose-dependent manner [37, 55]. Similar effects were observed with *genistein* in the same cell line [78]. In breast cancer cell lines, Lee et al. reported a slight reduction of *RARβ2* promoter methylation by EGCG intervention [44]. Also, treatment with two coffee polyphenols, *caffeic acid* and *chlorogenic acid*, led to a partial demethylation of the *RARβ2* promoter. Both compounds were potent inhibitors of DNMT activity in vitro [43]. King-Batoon et al. investigated the effects of *lycopene* and *genistein* on *RARβ2* methylation in breast (cancer) cells. A single low dose of *lycopene*, a caroteinoid isolated from tomatoes, reduced *RARβ2* and *HIN1* promoter methylation in immortalized MCF10A human breast cells, but not in MCF-7 breast cancer cells [79]. The mechanism of DNA demethylating activity was not further investigated. In the same study, *genistein* treatment did not result in demethylation of the *RARβ2* promoter in MCF-7 and MDA-MB468 breast cancer cell lines [79]. In a 4-week human intervention trial in 34 healthy premenopausal women, soy isoflavones at two doses led to dose-dependent changes in *RARβ2* and *CCND2* promoter methylation in mammary tissue. Before treatment, methylation levels of both genes were very low. The low dose of isoflavones further reduced methylation, whereas the high dose weakly increased methylation levels of both genes [92].

Jha et al. investigated *RARβ2* promoter methylation in cervical cancer cell lines [51]. Both genistein and curcumin resulted in demethylation of the *RARβ2* promoter and led to the reactivation of the gene, especially after incubation for 6 days. Concomitantly with reduction of *RARβ2* promoter methylation, both compounds induced apoptosis in the cervical cancer cell lines at higher concentrations [51]. Since DNMT bears a cysteine in its active center, Lin et al. speculated that *disulfiram* as a thiol-reactive dithiocarbamate might inhibit DNMT activity. Disulfiram is an inhibitor of aldehyde dehydrogenase currently used clinically for the treatment of alcoholism [282], and has been shown to prevent chemically-induced carcinogenesis in various animal models. Lin et al. demonstrated that disulfiram dose-dependently inhibited *DNMT1* enzyme activity in vitro. In prostate cancer cell lines, global levels of 5me-C decreased upon disulfiram treatment. At the same time, disulfiram intervention decreased *APC* and *RARβ2* promoter methylation and led to reexpression of the genes. Cell growth and clonogenic survival of prostate cancer cell cultures were inhibited in vitro. In vivo, there was a trend for reduced growth of prostate cancer xenografts. So far, a direct causal relationship between tumor growth inhibition and demethylating effects has not been established. Volate et al. analyzed the effect of *green tea* intervention on azoxymethane-induced colon carcinogenesis in the $APC^{Min/+}$ mouse model that is characterized by a defect in *Wnt* signaling due to a mutation in the *APC* gene [64]. Intervention with green tea as a 0.6% solution for 8 weeks significantly reduced the number of colonic tumors by 28%. Expression of *β-catenin* and *cyclin D1* as a *Wnt* target gene was reduced in tumors of the green tea group. Interestingly, *RXRα* expression was selectively downregulated early during colon carcinogenesis due to an increase in promoter methylation, whereas other retinoic acid receptors (*RARα*, *RARβ*, *RXRβ*, and *RXRγ*) were all expressed. *RXRα* silencing was independent of *β-catenin*, and could be reversed by green tea intervention [64]. This study showed that dietary levels of GTP were sufficient to reexpress silenced *RXRα* at the mRNA and protein level and to inhibit colon carcinogenesis.

13 Summary and Conclusions

As outlined above, major cellular pathways and cell functions, including drug metabolism, cell cycle regulation, potential to repair DNA damage or to induce apoptosis, response to inflammatory stimuli, cell signalling, cell growth control and differentiation, become deregulated during carcinogenesis by defects in epigenetic gene regulation. These include, among others, silencing by promoter methylation of detoxifying enzymes, tumor suppressor genes, cell cycle regulators, apoptosis-inducing and DNA repair genes, nuclear receptors, signal transducers and transcription factors, as well as modifications of histones and non-histone proteins such as *p53, NF-κB*, and *HSP90* by acetylation or methylation. Accumulating evidence indicates that dietary chemopreventive agents can prevent or reverse these alterations by affecting global DNA methylation, reexpressing tumor suppressor

genes silenced by promoter methylation, and upregulating genes by altering histone and non-histone acetylation and methylation, at least in cell culture systems.

There are several challenges for future nutri-epigenetic research in cancer chemoprevention:

1. A definite link between cancer chemopreventive efficacy in animal models or human pilot studies and targeting of epigenetic mechanisms is often missing. Future investigations will have to demonstrate that chemopreventive efficacy is mediated by epigenetic gene regulation.
2. Some of the described nutri-epigenetic effects appear to be cell type or organ-specific. Underlying mechanisms for these differences have not yet been addressed.
3. Given the fact that epigenetics plays an important role in gene regulation during development, timing of dietary chemopreventive interventions might be critical to target epigenetic deregulation during tumorigenesis. Epigenetic alterations are considered as early events during cancer development. Consequently, interventions with chemopreventive agents might have to start early after birth to be most effective, and cancer preventive effects through epigenetic mechanisms might have been underestimated in studies performed so far. The question of "critical time windows" for application should be addressed in more detail in the future, both in direction of cancer prevention and with respect to potential harmful effects.
4. Frequency of application might also be a critical determinant of chemopreventive efficacy. Several studies have reported that inhibition of HDACs and consequent histone hyperacetylation is a transient effect. Although these activities have been demonstrated in rodent models and in humans, it is not yet clear whether occasional consumption of dietary HDAC inhibitors, for example from cruciferous vegetables would result in long-term epigenetic regulation of gene expression and downstream chemopreventive effects. This also applies to other epigenetic mechanisms.
5. Some interventions are apparently more effective when applied in combination, as exemplified by the combined application of ATRA with DNMT or HDAC inhibitors. This aspect has not been systematically investigated in nutri-epigenetics, but might be relevant when comparing activities of isolated compounds with complex extracts or food items.
6. Most investigations on epigenetic effects have so far only been performed in a targeted candidate gene approach. It becomes more and more clear that epigenetic gene regulation is coordinated in an intricate network and involves a crosstalk between effects on DNA methylation, histone modifications, and miRNA expression. To understand fully the potential impact of epigenetic gene regulation and to target it for chemoprevention, we need to consider the epigenome as an interactive three-dimensional system. Future investigations on DNA methylation changes and the modulation of activating and repressive histone marks at a genome-wide level will improve our understanding of mechanistic links. These analyses will also provide important clues as to whether

Appendix

Table 1 Effect of natural compounds on DNA methylation in cancer models in vitro and in vivo (for a review see [20–36])

Agent	Source	Mechanism	Organ	Target, effect	Reference
Apigenin	Celery, chamomile	DNMTi			[37]
Apple polyphenols	Apples	↓ Promoter meth ↓ DNMT expr	Colon	*hMLH1*, *p14ARF*, *p16*	[38]
Apple polyphenols (in vivo)	Apples	↑ Global DNA meth ↑ Promoter meth	$Apc^{Min/+}$ mice	↓ Adenoma numbers *Line-1*, *Igf2*, *P2rx7*	[284]
B vitamins (B_2, B_6, B_{12})	Meat, nuts	Synthesis of SAM from methionine			Review in [39]
Baicalein	*Scutellaria baicalensis*	DNMTi			[40]
Betaine	Spinach, beets, wheat	Synthesis of SAM from methionine			Review in [39]
Betanin	Beetroot	DNMTi			[40]
Black raspberry extract	Black raspberries	↓ DNMT expr ↓ Promoter meth	Colon (phase 1 clinical trial)	*SFRP2*, *SFRP5*, *WIF1*, *PAX6*, *Line-1*	[41]
Butyrate		↓ Promoter meth	Colon	*RARβ2*	[42]
Caffeic acid	Coffee	DNMTi	Breast	*RARβ*	[43]
Catechins	Green tea	DNMTi, SAM: SAH			[44]
		DNMTi	Prostate	*GSTP1*, *MBD2*	[45]
Chlorogenic acid	Coffee, apples	DNMTi	Breast	*RARβ*	[43]
Chlorogenic acid derivatives	Synthetic	DNMTi	Rec. DNMT3a		[46]

Choline	Egg, milk, meat	Synthesis of SAM from methionine			Review in [39]
Cruciferous vegetables (in vivo)		↓ DNA meth ↔ Promoter meth	Human PBMC of heavy smokers 4 weeks intervention	*Line1* *RASSF1A*, *ARF*, *CDKN2*, *MLH1*, *MTHFR*	[47]
Curcumin	Turmeric	DNMTi, ↓ 5mC	Leukemia		[48]
		↓ Promoter meth	Prostate (mouse)	*Nrf-2*	[49]
		↓CGI meth ↑ MeCP2 binding ↓ H3K27me3 binding	Prostate	*Neurog-1*	[50]
		↓ Promoter meth	Cervix	*RARβ2*	[51]
		↓ Promoter meth	Leukemia	*p53* pathway	[52]
Cyanidin	Blueberries	DNMTi			[40]
Disulfiram	Synthetic	DNMTi ↓ 5mC levels ↓ Promoter meth	Prostate	*APC*, *RARB*	[53]
Ellagic acid	Berries	DNMTi			[40]
Epicatechin	Green tea	DNMTi, SAM: SAH			[44]
(−)-Epigallocatechin gallate (EGCG)	Green tea	DNMTi, SAM: SAH	Breast	*RARβ*	[44]
		↓ Promoter meth	Breast	*hTERT*	[54]
		DNMTi	Prostate	*GSTP1*	[45]
		DNMTi	Esophagus, Colon, prostate	*p16*, *RARβ*, *MGMT*, *hMLH1*	[55]
		↔ Methylation	Bladder, colon, prostate	*p16*, *MAGE-A1*, *Alu*, *LINE*	[56]
		↔ 5mC level	Colon, leukemia		[57]
		↓ Promoter meth	Colon	*p16* via folate metabolism	[58]

(continued)

Table 1 (continued)

Agent	Source	Mechanism	Organ	Target, effect	Reference
		↑ mRNA expr	Esophagus	*p16*, *MGMT*	[37]
		↑ mRNA expr	Lung, esophagus	*RARβ*, *p16*, *DAPK*	[37]
		↓ Promoter meth	Oral cavity	*RECK*	[59]
		↓ Promoter meth	Lung	*WIF-1*	[60]
		DNMTi act/expr ↓ 5mC	Skin	*p16*, *p21*	[61]
proEGCG	Prodrug	DNMTi ↓ Promoter meth	Breast	*hTERT*	[62]
EGCG (in vivo)	Green tea	↓ SAM levels ↔SAH, methionine, homocysteine	Plasma, small intestine, liver in healthy mice		[37]
Green tea polyphenols (in vivo)	Green tea	↔ DNA meth, 5mC ↔ Promoter meth	Prostate, gut, liver in TRAMP mice	*B1 repetitive elements*, *MAGE-a8 IRX3*, *CACNA1A*, *CDKN2A*, *NRX2*	[63]
		↓ Promoter meth	Colon, small intestine in AOM-treated mice	*RXRα* ↓ Colon tumors	[64]
		↓ Promoter meth ↔ Promoter meth	Gastric cancer patients	↓ *CDX2, BMP2* ↔ *p16*, *CACNA2D3*, *GATA5*, *ER*	[65]
Fisetin	Strawberries	DNMTi, SAM: SAH			[44]
Flavonoids (in vivo)	Green tea and soy products	↓ DNA meth ↔ Promoter meth	Human PBMC of heavy smokers 4 weeks intervention	*Line1* ↔ *RASSF1A*, *ARF*, *CDKN2*, *MLH1*, *MTHFR*	[47]
Folate	Green vegetables	Synthesis of SAM from methionine	Various	Maintenance of genomic stability, regulation of purine and pyrimidine biosynthesis ⇨ DNA biosynthesis, DNA repair, proliferation	Reviewed in [23, 39, 66–74]

Folic acid (in vivo)	Supplement	↑ CGI meth	Colorectal mucosa	*ERα*, *SFRP1*	[75]
Folate (in vivo)	Green vegetables		Liver, colon in mouse, rat; healthy individuals; patients with colonic adenoma and colon cancer		reviewed in [23, 66–68, 76]
Folate, green vegetables, multivitamins		Protection against methylation	Cohort-based study with 1,100 participants	*p16*, *MGMT*, *RASSF1A*, *DAPK*, *GATA4*, *GATA5*, *PAX5α*, *PAX5β*	[77]
Galangin	Propolis, galangal root	DNMTi			[40]
Garcinol	Mangosteen tree	DNMTi			[37]
Genistein, daidzein	Soy beans	DNMTi, ↓ promoter meth	Esophagus, prostate	*p16*, *RARβ*, *MGMT*	[78]
		↓ Promoter meth	Breast	*GSTP1*	[79]
		↓ Promoter meth	Colon	*RARβ2*	[42]
		↓ Promoter meth	Colon	*Wnt5a*	[80]
		↓ DNMT expr ↓ Promoter meth	Breast	*hTERT*	[81]
		DNMTi ↓ Promoter meth	Kidney	*BTG*	[82]
		↓ Promoter meth	Prostate	↓ *GSTP1*, *EPHB2* ↔ *RASSF1A*, *BRCA1*	[83]
		↓ Promoter meth ↑ Protein expr	Prostate	↓ *BRCA1*, *GSTP1*, *EPHB2*	[84]
		↓ Promoter meth	Cervix	*RARβ2*	[51]
		↓ Promoter meth Genome wide analysis	Embryonic stem cells	*Ucp1*, *Sytl1*	[85]
Genistein (in vitro and in vivo)	Soy beans	↓ Promoter meth	Endometrium	*SF-1*	[86]

(continued)

Table 1 (continued)

Agent	Source	Mechanism	Organ	Target, effect	Reference
Genistein (in vivo)		↑ DNA meth	Bone marrow	*Repetitive elements*	[83]
		↑ DNA meth in prostate	Brain, kidney, liver, spleen, prostate, testes of healthy mice		[87]
		↑ Methylation	tail, brain, kidney, liver in A^{vy} mice	*A^{vy} intracisternal A particle (IAP) murine retrotransposon*	[88]
		↑↓ Promoter meth	Cynomolgus monkeys	Fat tissue: *ABCG5*, *TBX5*, *HoxB1* Muscle*: HoxA5*, *HoxA11*, *NTRK3*	[89]
		↓ Promoter meth	Uterus in healthy mice, intact and ovarextomized (OVX)	*Nsbp*1	[90]
Soy isoflavones	Soy beans	↓ Promoter meth ↔ Promoter meth	prostate	*GSTP1* and *EPHB2* *BRCA1* and *RASSF1A*	[83]
Soy isoflavones (in vivo)	Soy beans	↓ Promoter meth	Pancreas, liver in healthy mice	*ActaI*	[91]
		Promoter meth	Human intervention trial	↓ *RARβ2*, *CCDN2* ↔ *ER*, *p16*, *RASSF1A*	[92]
Hesperetin	Citrus fruit	DNMTi			[37]
Hydroxycinnamic acid	Fruit	DNMTi			[37]
Luteolin	Parsley, celery	DNMTi			[37]
Lycopene	Tomatoes	↓ Promoter meth	Breast	*GSTP1*, *RARβ*, *HIN1*	[79]
Mahanine	Asian vegetables	DNMTi	Prostate, lung, breast, pancreas, vulva, ovaries	*RASSF1A*	[93]
Mahanine derivative	Synthetic	DNMTi	Prostate	*RASSF1A*	[94]
Mahanine derivative (in vivo)	Synthetic		Prostate xenograft	↓ Tumor volume	[94]
Methionine	Dairy products, nuts, fish	Synthesis of SAM			Review in [39]
Mithramycin A (MMA)		↓ Promoter meth ↓ DNMT1 expr	Lung	*SLIT2*, *TIMP3*	[95]

Myricetin	Fruit, herbs, vegetables	DNMTi, SAM: SAH			[37, 40, 44]
Naringenin	Citrus fruit	DNMTi			[37]
Nordihydroguaiaretic acid (NDGA)	Creosote bush	↓ Promoter meth	Breast	*E-cadherin*, *p16*	[96, 97]
		↔ DNA meth	Liver	*LINE-1*	[98]
Parthenolide	Feverfew	DNMTi ↓ DNMT expr ↓ 5mC	Breast	*HIN-1*	[99]
Parthenolide (in vivo)		↓ DNA meth ↓ DNMT expr	Human leukemia Xenograft	↓ Tumor volume	[99]
Phenylethyl isothiocyanate (PEITC)	Watercress	↓ Promoter meth	Prostate	*GSTP1*	[100]
PEITC (in vivo)		↓ Promoter meth	Prostate of TRAMP and wt mice	*MGMT* ↓ Tumor incidence	[101]
Phenylhexyl isothiocyanate (PHI)	Synthetic	↓ Promoter meth	Myeloma	*p16*	[102]
Phloretin	Apples	DNMTi			[40]
Piceatannol	Grapes	DNMTi			[40]
Protocatechuic acid	Açaí oil, olives	DNMTi			[40]
Quercetin	Ubiquitous	DNMTi, SAM: SAH			[37, 44]
		↓ Promoter meth	Colon	*p16*	[283]
Resveratrol	Grapes	DNMTi		*BRCA1*	[40]
		↓ *MBD2* recruitment	Breast	*BRCA1*	[40, 103]
		↓ Promoter meth ↓ DNMT expr	Breast	*PTEN*	[104]
		↓ Promoter meth	Breast	*RARβ2*	[105]

(continued)

Table 1 (continued)

Agent	Source	Mechanism	Organ	Target, effect	Reference
Retinoic acid		↓ Promoter meth	Leukemia	*RARβ2*	[105, 106]
		↓ Promoter meth	Leukemia	*hTERT*	[107]
		↔ DNA meth	Leukemia	*RARβ*	[108]
		↓ Promoter meth	Breast	*PTEN*	[104]
		↓ Promoter meth	Breast	*RARβ2*	[105]
		↓ Promoter meth (genome wide) ↓ *DNMT1*, *3B* expr	Neuroblastoma	*iNOS*	[109]
Retinoic acid (in vivo)		↔ Promoter meth	Breast cancer patients	*RARβ2*	[110]
Rosmarinic acid	Rosemary	DNMTi			[40]
Selenomethionine		↔ Promoter meth	Prostate	*GSTP1*, *RASSF1A*	[111]
Sinapic acid	Rapeseed	DNMTi			[40]
Sodium selenite	Inorganic	DNMTi			[112]
		↑ Global DNA meth	Colon	*p53*	[113]
		↑ Global DNA meth	Colon		[114]
		↓ DNMT1 expr	Colon		[114]
		↓ DNMT1 expr	Prostate	*GSTP1*, *APC*, *CSR1*	[115]
		↓ Global DNA meth			[115]
		↓ Liver SAM: SAH	Intestine in DMH-treated rats	↓ Aberrant crypt foci formation	[114]
		↓ Global DNA meth	Rat intestine	↓ Aberrant crypt foci formation	[116]

Sodium selenite (in vivo)	Anorganic	↓ Global DNA meth	Liver, colon in rats		[113]
Sulforaphane	Broccoli	↓ DNMT expr ↓ Promoter meth	Breast	*hTERT*	[31]
Syringic acid	Açaí oil	DNMTi			[40]
Thearubigins	Black tea	DNMTi	Recombinant DNMT3a		[46]
Vitamin D		↓ Weak promoter meth	Breast	*RARβ2*	[105]
		↓ Promoter meth ↓ DNMT expr	Breast	*PTEN*	[104]
Vitamin E (in vivo)	Seed oils	↔ DNA meth ↔ Promoter meth	Rat liver	*SDR5A1*, *GCLM*	[117]

CGI CpG island, *MeCP2* methylated CpG binding protein 2, *DNMTi* inhibition of DNMT activity, *expr* expression, *meth* methylation, *SAM:SAH* modulation of the SAM to SAH ratio through alternative mechanisms, ↓ reduction, inhibition, ↔ no effect, ↑ induction, stimulation

Table 2 Effect of natural compounds on acetylation of histones and non-histone substrates in cancer models in vitro and in vivo (for a review see [20, 22, 25–27, 29–36, 121, 122])

Agent	Source	Mechanism	Organ/cell type	Target, effect	Reference
Allylmercaptan	Garlic	HDACi ↑ ac-H3 and ac-H4	Colon	*p21*	[123]
Anacardic acid	Cashew nuts	HATi: *p300*, *PCAF*			[124]
		HATi: *Tip60*	Cervix, embryonic kidney	*ATM*, *DNA PKs*	[125]
		HATi	Leukemia, tongue, lung, prostate	*IκBα*, ↓ *p65ac*, *NF-κB*-dependent *IAP1*, *XIAP*, *Bcl-2*, *Bcl-xL*, *c-FLIP*, *cyclin D1*, *c-Myc*, *Cox-2*, *VEGF*, *ICAM-1*, *MMP-9*	[126]
Apicidin	Fungal metabolite	HDACi ↑ ac-H4	Cervix and others	*Gelsolin*, *p21*,*DNMT1*	[127, 128]
Butyrate	Fermentation	HDACi ↑ ac-H3 and ac-H4	Colon		[129]
		↑ ac-H3 and ac-H4	Colon	↑ *p21* ↓ Cell proliferation	[130]
		HDACi	T lymphocytes	↓ *Bcl-2* *DR5*, *caspases 8* and *10*	[131]
		HDACi ↑ ac-histones	Leukemia	*Cyclin D1*, *B1*, *c-Myc* ↑ *p21*	[132]
Butyrate (in vivo)	Supplement	↑ ac-H3	DMH-treated mice		[133]
Cambinol	Synthetic	SIRTi	Lung, lymphoma	↑*p53ac*	[134]
Cambinol (in vivo)			Burkitt lymphoma xenograft	↓ Tumor growth	[134]
Chalcone derivative	Synthetic	SIRTi	Embryonic kidney	↑*p53ac*, *p21*	[135]
Curcumin	Turmeric	↓ *HDAC1*, *HDAC3* expr ↓ *p300* (HAT) expr	B-cell lymphoma	↓ Proliferation *Notch 1*	[136]
	Turmeric	HATi: *p300/CBP* ↓ ac-H3, H4		↓ *p53ac*	[137, 138]

Curcumin (in vitro and in vivo)	Turmeric	↓ *HDAC*4 expr	Medulloblastoma	*Tubulin* ↓ Xenograft growth	[139]
Delphinidin	Pomegranate	HATi: *p300* ↔ *PCAF*, SIRT1, HDACs, HMTs	Synoviocytes	↓ *p65ac*, ↑ *cytosolic IκBα*, ↓ *NF-κB-dependent Cox-2*, *IL-6*, *IL-1β*, *TNF-α*	[140]
Diallyldisulfide	Garlic	↑ ac-H3, ac-H4	Leukemia, colon, liver, breast, prostate	*p21*	[141]
Diallyldisulfide (in vivo)		↑ Transient ac-H4	Rat colon		[142]
Dihydrocoumarin	Sweet clover	SIRTi	Leukemia	↑ *p53*ac	[143]
Diindolylmethane (DIM)	Broccoli metabolite	↑ *HDAC1*, *2*, *3*, *8* degradation ↓ *HDAC1*, *2*, *3* expr	Colon	*p21*, *p27*, apoptosis	[144]
		↓ acH4	Breast	↓ *Cox2* expression	[145]
Diindolylmethane (in vivo)		↓ *HDAC1*, *2* expr	Colon cancer xenografts	*p21*	[144]
(−)-Epigallocatechin gallate (EGCG)	Green tea	↑ HAT *p300* expr ↓ HDAC1 exp ↑ ac-H3, H3K9ac, ac-H4	Breast	*ERα*	[146]
		↓ HDAC activity ↑ ac-H3, ac-H4	Skin	*p16*, *p21*	[61]
		HATi: *p300* *PCAF*, *Tip60* ↔ SIRT1, HDACs, HMTs	Leukemia, B-cells	↓ *p65ac*, ↑ cytosolic *IκBα*, ↓ *p65*-binding to IL6 promoter, ↓ *NF-κB*-dependent *Cox-2*, *IL-6*, *NOS-2*, *XIAP*, *Bcl-2*, *Bcl-xL*,, *cyclin D1*, *c-Myc* ↓ EBV-mediated *IL-6*, *IL-12*, Cell transformation	[147]
		HATi: *p300* ↓ ac-H3 ↔ SIRT1, HDACs, HMTs	Prostate	↓ *AR*-mediated *PSA*, *NKX3.1* ↓ ac-*AR*, ↓ *AR* nuclear translocation, ↓ Proliferation	[148]

(continued)

Table 2 (continued)

Agent	Source	Mechanism	Organ/cell type	Target, effect	Reference
EGCG (in vivo)			TNFα-stimulated mice	↓ Serum *IL-6*, ↓ *p65ac* in macrophages, ↓ cytokine expression	[149]
ProEGCG	Prodrug	HDACi ↓ ac-H3, H3K9ac	Breast	*hTERT* ↑ Promoter binding *MAD1*, *E2F1*; ↓ binding *c-Myc*	[62]
Gallic acid	Rose flowers	HATi: *p300* *PCAF*, *Tip60* ↔ SIRT1, HDACs, HMTs	Lung	↓ *p65ac*, ↑ cytosolic *IκBα*, ↓ *p65*-binding to IL6 promoter, ↓ *NF-κB*-dependent *Cox-2*, *IL-6*, *IL-1β*, *NOS-2*, *XIAP*, *Bcl-2*, *Bcl-xL*,, *cyclin D1*, *c-Myc*	[149]
Gallic acid (in vivo)			LPS-stimulated mice	↓ Serum *IL-6*, ↓ *p65ac* in macrophages, ↓ cytokine expression	[149]
Garcinol	Mangosteen tree	HATi: *p300*			[150]
		HATi: *p300*, *PCAF* ↑ ac-H4, acH2B	Cervix	↓ Global gene expression	[151]
Genistein	Soy beans	↓ HDAC expr	Prostate	↑ *HSP90*ac promotes dissociation and degradation of AR	[152]
		↑ HAT expr *p300*, *PCAF*, *CREBBP*, *HAT1* ↑ ac-H3, ac-H4	Prostate	*p21*, $p16^{INK4a}$	[153]
		↑ ac-H3K9 ↓ SIRT1 expr	Prostate	↓ *Akt* signaling through *PTEN*, *CYLD*, *p53*, *FOXO3a*	[154]
Genistein, equol, AglyMax	Soy beans	↑ ac-histones	In vitro	*ERα*-mediated	[155]

β-Methylselenopyruvate α-Keto-γ-methylseleno-butyrate	Metabolites of selenium compounds	HDACi	Colon	↑ ac-H3, $p21^{WAF1}$	[156]
Parthenolide	Feverfew	↑ HDAC1 degrad ↑ ac-H3	Breast	*HDAC1* degradation through *ATM*, ↑ cell death	[99, 157]
Phenylethyl isothiocyanate (PEITC)	Watercress	↑ ac-H3	Prostate	*p27*, *p21*, *c-Myc*	[158]
Phenylhexyl isothiocyanate (PHI)	Synthetic	HDACi HATa ↑ ac-histones	Prostate, leukemia, myeloma, hepatoma	*p21*, *p27* *Bcl-2*	[102, 159–162]
In vivo		↑ ac-H3 and ac-H4	Bone marrow of AML patients		[163]
Resveratrol	Grapes	*SIRT1*a	Bone	↓ *p53ac*	[164]
		↓ MTA1/NuRD corepressor complex	Prostate	↑ *p53ac*, recruitment to *p21* and *Bax* promoters ↑ Apoptosis	[164, 165]
Retinoic acid (ATRA)		↑ ac-histones at CpG islands	Leukemia	↑ ac-H4 binding at *HOXA1* and *satellite DNA* ↑*RARβ*, *CD11b*, *HCK*, *OS-9*, *HOXA1*, *c-myc*, *c-myb*, *hTERT* mRNA expr	[108]
			Leukemia, breast	↓ H3K9ac at *hTERT* promoter	[107, 166, 167]
Retinoic acid (in vitro and in vivo)			Breast, prostate, larynx	↑ ac at *RARβ* P2 promoter ↓ T47D xenograft growth	[110]
Silimarin	Milk thistle	SIRTa	Melanoma	*Bax*	[168]
Sulforaphane (SFN)	Broccoli	HDACi ↑ ac-H3, ac-H4	Embryonic kidney, colon, prostate	*p21*, *Bax*	[169, 170]
		HDACi	Prostate	↑ *HSP90*ac promotes dissociation and degradation of AR	[171]

(continued)

Table 2 (continued)

Agent	Source	Mechanism	Organ/cell type	Target, effect	Reference
		HDACi ↓ HDAC cl. I/II expr ↑ ac-H3	Prostate cancer vs. normal prostate	*p21*, ↑*ac-tubulin*	[172]
		HDACi ↑ ac-H3, H3K9ac ↔ HAT activity	Breast	*hTERT* *MAD1, c-Myc, CTCF*	[173]
		HDACi ↔ ac-H3, H4	Breast	↑ G_2/M phase arrest ↑ Apoptosis	[174]
		↓ HDAC3 expr	Colon	*SMRT* corepressor complex *Pin1*, *14-3-3*	[175]
Sulforaphane (in vivo)	Broccoli	HDACi ↑ ac-H3, ac-H4	Colon mucosa, ileum, colon, prostate, PBMC of wt mice; ileum, colon of $APC^{Min/+}$ mice	*p21*, *Bax*	[176]
		HDACi ↑ ac-histones	Prostate cancer xenografts	*Bax* ↓ growth of PC3 xenograft	[177]
Broccoli sprouts (in vivo)		HDACi ↑ ac-H3, ac-H4 (both transient)	Human PBMC		[172, 178]
Ursodeoxycholic acid	Endogenous secondary bile acid	↓ ac-histones ↔ HDACi ↑ *HDAC6* expr	Colon	*E-cadherin*, *CK8*, *18*, *19*	[179]

ac acetylation, *AglyMax* fermented soybean germs, *AR* androgen receptor, *degrade* degradation, *HDACi* HDAC inhibitor, *HATa* activator of HAT proteins, *HATi* inhibitor of HAT activity, *SIRTi* inhibitor of SIRT deacetylases, *SITRa* activator of SIRT, ↓ reduction, inhibition, ↔ no effect, ↑ induction, stimulation

Table 3 Effect of natural compounds on histone methylation in cancer models in vitro and in vivo (for a review see [29, 30, 34])

Agent	Source	Mechanism	Organ	Target, effect	Reference
Chaetocin		HMTi: *SUV39* ↓ H3K9me2, ↓ H3K9me3		*p21*	[180, 181]
		↓ H3K9me2 and H3K9me3 at *p15* and *E-cadherin* promoter	Leukemia	$p15^{INK4B}$ and *E-cadherin*	[182]
Curcumin	Turmeric	↓ *EZH2* expr ↓ H3K27me3	Breast	Via *MAPK* pathway	[183]
(−)-Epigallocatechin gallate (EGCG)	Green tea	↓ HMT expr: *BMI-1*, *SUZ12*, *EZH2*, *Eed* ↓ H3K27me3	Skin	*p21, p27*, *Bax*, *Bcl-xL* ↑ Effect in combination with SAH hydrolase inhibitor	[184, 185]
		↓ H3K9me	Skin	*p16*, *p21*	[61]
		↓ HMT *SUV39H1* expr ↑ H3K4me2, ↓ H3K9me3	Breast	*ERα*	[146]
Genistein	Soy beans	↓ H3K9me2	Prostate	*Akt* signaling through *PTEN*, *CYLD*, *p53*, *FOXO3a*	[154]
Polyamine analogs	Synthetic	HDMi*: LSD1* ↑H3K4me2, ac-H3K9	Colon	*sFRP1*, s*FRP4*, *sFRP5*, *GATA5*	[186]
PG11144 (*cis*), PG11150 (*trans*)	Synthetic	HDMi*: LSD1* ↑ H3K4me, H3K4me2	Colon	*SFRP1*, *SFRP2*	[187]
n-3 Polyunsaturated fatty acid (*n*-3 PUFA) DHA, EPA	Fish oil	↓ *EZH2* expr ↓ H3K27me3 and H3K9me3	Breast	*E-cadherin, IGFBP3*	[188]

HTMi inhibitor of histone methyltransferases, *HDMi* inhibitor of histone demethylases, *expr* expression, ↓ reduction, inhibition, ↔ no effect, ↑ induction, stimulation

nutri-epigenetic effects are specific for certain pathways or selective for subsets of genes. The emergence of novel technologies such as next-generation sequencing for genome-wide assessment of DNA methylation and localization of histone marks, the expected drop in sequencing costs, and the development of bioinformatic tools to integrate systematically available information will facilitate this type of analyses in future chemoprevention studies.

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