Review Article

Theme: Natural Products Drug Discovery in Cancer Prevention

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Epigenetic Cancer Prevention Mechanisms in Skin Cancer

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Abstract. Epigenetics is an important emerging area for study of mechanisms of cancer prevention. In recent years, it has been realized that cancer prevention agents, derived from natural dietary sources, impact cancer cell survival by modulating epigenetic processes. In the present manuscript, we review key epigenetic regulatory mechanisms and examine the impact of sulforaphane and green tea polyphenols on these processes. We also discuss available information on the epigenetics in the context of skin cancer. These studies indicate that diet-derived chemopreventive agents modulate DNA methylation status and histone modification *via* multiple processes and point to additional areas for study of epigenetic mechanisms in skin cancer.

KEY WORDS: chemoprevention; epigenetics; green tea polyphenol; skin cancer; sulforaphane.

EPIGENETICS IN CANCER

The term epigenetics was coined by the biologist Conrad Waddington in 1942 as reversible heritable changes in gene expression that occur without alteration in the DNA sequence (1). The principal epigenetic changes are modifications of DNA and histones. These modifications impact chromatin compaction, DNA folding, and nucleosome position (2) and act in a synergistic and cooperative manner to alter gene expression. Epigenetic changes, including global hypomethylation and promoter specific hypermethylation, are early events in tumor development. Thus, epigenetic change is associated with reduced cellular homeostasis which precedes genetic change. Ultimately, these epigenetic changes lead to genetic instability, oncogene activation, and tumor suppressor silencing (3).

Epigenetics mechanisms represent attractive therapy targets, as epigenetic alterations are potentially reversible (4). Thus, genes silenced by methylation can be activated with DNA methyltransferase (DNMT) inhibitors and genes silenced by deacetylation can be activated by histone deacetylase (HDAC) inhibitors. These characteristics are

ideal for development of chemopreventive and therapeutic strategies. The most extensively studied DNMT inhibitors are 5-azacytidine (Vidaza) and 5-aza-2-deoxycytidine. HDAC inhibitors include trichostatin A, suberoylanilide hydroxamic acid, and phenyl butyrate (5–7). Several of these epigenetic agents are in preclinical and clinical trials for use in solid tumors and hematologic malignancies (8).

Present-generation epigenetic drugs are only partially effective for several reasons. First, DNMT and HDAC inhibitors can activate inappropriate genes, including oncogenes, and in some cases actually accelerate tumor formation (9). Second, drug-stimulated correction of epigenetic status may be lost after termination of the drug treatment (10). These issues and the toxicity observed with some synthetic drugs that target epigenetic processes have stimulated studies to identify safer agents. In this context, dietary agents have received attention, as many diet-derived agents modulate the epigenome, are non-toxic, can be taken continuously, and display anticancer action. We will discuss some of the epigenetic mechanisms that are modulated by diet-derived agents in this review with a focus on skin cancer.

DNA METHYLATION

DNA methylation is an important epigenetic modification that has been extensively studied. DNA methylation generally occurs at the cytosine residue in cytosine–guanine (CpG) dinucleotide pairs to form 5-methylcytosine (11). Regions enriched in CpG repeats, ranging from 0.5–4 kb in length, called CpG islands, are present in the promoter region of many genes (12). Hypermethylation of CpG islands is associated with repression of gene expression, while hypomethylation leads to transcriptional activation (13).

The key enzymes involved in the methylation reaction are the DNMTs. DNMTs catalyze the transfer of methyl groups from

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methyl donors, such as S-adenosylmethionine, to the 5' cytosine in the CpG dinucleotide. The products of the reaction are 5-methylcytosine and S-adenosyl-L-homocysteine (SAH). The human genome encodes three different types of DNMT: DNMT1, DNMT3a, and DNMT3b (14). DNMT1 is a methylation maintenance enzyme that functions during DNA replication to methylate newly synthesized DNA as a mechanism to maintain the preexisting methylation pattern. In contrast, DNMT3a and DNMT3b are *de novo* methyltransferases that methylate CpG islands without a requirement for existing hemimethylation (15). DNA methylation regulates the transcriptional status of genes *via* two major mechanisms. First, DNA methylation in the promoter region inhibits the binding of sequence specific transcription factors to transcription factor-specific DNA binding sites and thereby inhibits transcription (16). Second, methylated cytosine in DNA can act as a binding site for the methyl-CpG-binding domain proteins. These proteins act as a scaffold for chromatin remodeling proteins like HDACs which removes acetyl groups from histones and thereby produce compact and transcriptionally-silent chromatin (17).

DNA methylation is crucial in the context of cancer development, as evidenced by the fact that the methylation landscape in cancer cells differs from normal cells in two major ways. First, global genome-wide methylation is decreased in cancer cells. A marked reduction in methylation is observed in centromere repeats and other repetitive sequences, and it has been proposed that this contributes to the chromosome instability associated with cancer (18). Second, cancer cells display increased methylation of the CpG islands within tumor suppressor genes (19,20). These islands are normally located within the gene promoter, and the "CpG island methylation phenotype" has been described in multiple cancers. High methylation at these sites leads to reduced tumor suppressor gene expression (21–23). It is not well understood why an overall reduction in global DNA methylation is observed in association with increased DNMT level. However, it is clear that reducing DNMT level/activity and CpG island methylation at tumor suppressor gene loci reduces cancer cell survival. In fact, epigenetic therapy using DNMT inhibitors aims to demethylate hypermethylated CpG islands to restore tumor suppressor gene expression and thereby reduce cancer cell survival/proliferation. This strategy preferentially impacts cancer cells, as DNA surrounding tumor suppressor genes in cancer cells is highly methylated, and the genes are thereby silenced. Reducing the methylation density restores gene expression. DNMT inhibitors have less of an impact on normal cells, as chromatin surrounding these genes is methylated at a normal level, and tumor suppressor gene expression is at normal level. Thus, the high methylation level of CpG islands in cancer cells and low level in normal cells creates a favorable therapeutic window (24).

DNA Methylation in Skin Cancer. Changes in DNA methylation have been examined in human epidermal squamous cell carcinoma. Global hypomethylation is observed in advanced cancer (25). A substantial decrease in 5-methylcytosine content occurs as normal cells transition to benign papilloma, and when benign papilloma transitions to more advanced disease (25). Another important finding is that CpG island hypermethylation-associated silencing of tumor suppressor genes, including MLH-1, BRCA1, and MGMT, is an early event in tumorigenesis in murine skin carcinogenesis

(25). However, hypermethylation can also be observed later in disease progression. For example, hypermethylation-associated silencing of the E-cadherin gene expression occurs during the later stages (25).

Changes in DNA methylation are also observed in human melanoma. Hypermethylation of tumor-related genes, including WIF1, TFPI2, RASSF1A, and SOCS1, is associated with advanced melanoma and poor prognosis (26). Methylation status of the long interspersed nuclear element-1 (LINE1) is an important index of global methylation status (27), and is an important prognostic marker in patients with Stage IIIC cutaneous melanoma. Patients with LINE1-hypomethylation survive better than patients with LINE1-hypermethylation (28). Stage III melanoma patients display elevated levels of DNMT3a and DNMT3b, and this correlates with reduced overall survival (29). These changes impact expression of a host of genes (30). However, the biological impact of altered expression of individual genes is not known and is an important area for future study. Taken together, these studies indicate that altered DNA methylation is an important epigenetic event in melanoma and nonmelanoma skin cancer that contributes to disease progression, but also point to the fact that more studies are necessary to understand the role of individual changes in gene expression on disease progression.

HISTONE MODIFICATION

Histone modification is a key epigenetic mechanism that regulates gene expression (3). Nucleosomes serve as building blocks to pack eukaryotic DNA into highly organized chromatin fibers. Each nucleosome subunit is composed of approximately 146 base pairs of DNA wound around a histone octamer. Histones are basic proteins that are rich in lysine and arginine. The core histone octamer consists of double subunits each of H2A, H2B, H3, and H4 (31). The degree of packaging distinguishes tightly packed heterochromatin, which is transcriptionally inactive and inaccessible to the transcription factors, and loosely packed euchromatin, which is accessible to transcription factors and is transcriptionally active (32). The N-terminal histone tails protruding from the nucleosome are sites of posttranslational modification that regulate access to the DNA (33). Posttranslational histone modifications include methylation, acetylation, phosphorylation, ubiquitination, SUMOylation, and biotinylation (33). The principal residues targeted for modification are lysine, arginine, and serine in the histone tail (33). The most intensively studied events are acetylation and methylation.

Histone Acetylation and Methylation. Regions of chromatin enriched in acetylated histones are transcriptionally active regions of chromatin. Histone acetylation neutralizes the DNA-histone interaction, facilitating an open chromatin structure that allows transcription factor access to DNA recognition elements (32). The principal controllers of histone acetylation are HDAC and histone acetyltransferases (HAT). HATs catalyze addition of acetyl groups to the lysine residues of histones, while HDACs remove these groups (34,35). HATs are classified into the GNAT family (e.g., GCN5,

PCAF), the MYST family (e.g., TIP60), the p300/CBP family, the SRC family, and the TAFII250 family (34). Histone deacetylation leads to transcriptional repression by increasing the total positive charge density on the N-termini of the target core histones. This increases the strength of the DNA-histone tail interaction, thereby restricting access of the transcription machinery to the DNA template. The HDACs are classified into four classes depending on localization. Class I are present in the nucleus and include HDAC1, HDAC2, HDAC3, and HDAC8 (36). Class II HDACs shuttle between the nucleus and the cytoplasm and include HDAC4, HDAC5, HDAC6, HDAC7, HDAC9, and HDAC10 (36).

Histone methylation occurs on lysine and arginine residues. The reaction is catalyzed by different enzymes that produce distinct histone modification and distinct biological outcomes. The effect of lysine methylation on transcription depends upon which lysine in the histone is modified (37). Trimethylation of histone H3 on lysine 4 (H3K4me3) activates transcription, while trimethylation of lysine 27 or lysine 9 on histone H3 represses transcription (33,38). Histone lysine methylation is catalyzed by proteins containing the suppressor of variegation, enhancer of zeste, and trithorax (SET) domain, and by the non-SET domain proteins DOT1/DOT1L (39). S-adenosyl methionine (SAM) is the cofactor and methyl group donor in these reactions.

Of particular interest are the polycomb group proteins (PcG) where enhancer of zeste homolog 2 (Ezh2) is the major histone methyltransferase (40). The PcG proteins are an important family of histone modification proteins that have been extensively studied in skin cancer. These proteins were first identified in *Drosophila melanogaster* as repressors of homeotic gene expression. In mammals, PcG proteins function in multiprotein polycomb repressive complexes, PRC2 and PRC1, to repress transcription by chromatin modification (41). The PRC2 multiprotein complex includes Ezh2, EED, Suz12, and RbAp46. Ezh2, the catalytic component of the PRC2 complex, is a histone methyltransferase that specifically catalyzes trimethylation of lysine 27 of histone H3 (H3K27me3) (42). Interaction of Suz12 and EED with Ezh2 leads to a 1,000-fold increase in Ezh2 methyltransferase activity (43,44). The PRC1 complex includes Bmi-1, PH1, CBX, and Ring1B (45,46). Ring1B, the catalytic subunit of the PRC1 complex, is a histone ubiquitin ligase that requires interaction with a second PRC1 subunit, Bmi-1, for optimal activity (47). The CBX protein of the PRC1 complex specifically binds to H3K27me3 to anchor the PRC1 complex to chromatin (48), which provides an environment for Ring1B to ubiquitinate histone H2A at lysine 119 (H2AK119-ubi) (41,49–51). PRC2 and PRC1 act sequentially. First, Ezh2 catalyzes H3K27me3 formation, the PRC1 complex then binds to trimethylated lysine 27, and this is followed by Ring1B-dependent H2AK119-ubi formation (52). These sequential trimethylation and ubiquitination events lead to chromatin compaction and silencing of tumor suppressor gene expression (46).

Elevated PcG protein expression in cancer cells and tumors correlates with increased cancer cell proliferation and survival (53–55). The Bmi-1, Ezh2, and Suz12 PcG proteins are increased in many cancer types (56–60). PcG target genes include tumor suppressor genes like Ink4A, Ink4B, Arf (61,62), and a host of additional genes including Kruppel-like

factor 4, Wilms's tumor 1, retinoic acid receptor- β , and others (63). In addition, PcG proteins have been reported to function in conjunction with the DNA methylation machinery, as Ezh2 physically associates with DNMTs (64), and numerous PcG target genes are susceptible to methylation-induced silencing (65). It is likely that PcG proteins and DNMTs, act together, to aberrantly silence tumor suppressor genes and pro-differentiation genes rendering the resulting cancer cells nonresponsive to growth control agents.

Histone Acetylation and Methylation in Skin Cancer. Studies of histone acetylation in skin cancer have focused on melanoma. However, information is limited. Altered histone covalent modification is associated with the conversion of normal tissue and benign nevi to malignant melanoma. Reduced histone acetylation is associated with reduced expression of p21^{Cip1} and the proapoptotic proteins APAF-1, Bax, Bak, Bid, and Bim (66). It is potentially relevant that human melanoma cells and tumors express high levels of wild-type p53, which would normally act to increase expression of these genes and thereby reduce proliferation and drive apoptosis. However, the fact that histones surrounding the p53 target genes (p21^{Cip1}, etc.) are hypoacetylated may explain why melanoma cells do not undergo p53-dependent cell death.

Methylation status may also have a role. Elevated expression of the Ezh2 methyltransferase, which trimethylates lysine 27 of histone H3, is associated with reduced p21^{Cip1} tumor suppressor expression in human melanoma (67–69). In human tumor cells, p21^{Cip1} expression is restored upon Ezh2 knockdown *via* a mechanism that requires p53. The increase in p21^{Cip1} contributes to cell cycle arrest and induction of senescence. These events are also associated with reduced HDAC1 interaction at the p21^{Cip1} gene transcriptional start site, leading to increase histone acetylation and increased p21^{Cip1} transcription (68).

EPIGENETIC IMPACT OF DIETARY AGENTS (SULFORAPHANE AND GREEN TEA)

Dietary agents are being tested as potential chemopreventive agents. These agents have the advantage in that they are biologically active against cancer, and are nontoxic and so can be consumed on a continuing basis. Two of the most extensively studied agents are sulforaphane from broccoli and (–)-epigallocatechin-3-gallate (EGCG) from green tea. These are discussed in the following sections.

Sulforaphane. Sulforaphane (SFN) [1-isothiocyanato-4-(methylsulfinyl) butane] is an isothiocyanate principally found in broccoli and broccoli sprouts (70), and is a promising diet-derived cancer prevention agent. SFN is highly bioavailable in human subjects and produces biochemical changes following broccoli consumption. For example, a single ingestion of 8 g of broccoli inhibits HDAC activity and alters histone acetylation in circulating peripheral blood mononuclear cells at 3 h post-consumption in human subjects (71). SFN suppresses proliferation and increases apoptosis of cancer cell lines (72–74). SFN also suppresses tumor formation in epidermis. For example, SFN treatment increases the level of phase I and phase II drug metabolizing enzymes in human epidermis (75,76). SFN treatment reduces UVB-dependent inflammation in HR-1 hairless mice and

suppresses UVB-induced skin carcinogenesis in SKH-1 mice (77,78).

Work on epigenetic regulation of skin cancer cell function by SFN has focused on polycomb group protein function. Several studies report a role for PcG proteins in the pathogenesis of skin cancer. PcG protein levels are elevated in human squamous cell carcinoma, basal cell carcinoma, and melanoma tumors (57,79,80). Bmi-1 is elevated in human squamous cell carcinoma (79,81), and Bmi-1, Ezh2, and Suz12 are elevated in cultured human squamous carcinoma cells (81). Moreover, overexpression of Bmi-1 in HaCaT cells, a relatively normal non-tumorigenic epidermal keratinocyte cell line, leads to malignant transformation characterized by colony formation in soft agar and tumor formation in immune-compromised mice (82).

We recently reported that SFN suppresses Bmi-1 and Ezh2 level leading to reduced H3K27me3 formation in SCC-13, A431, and HaCaT cells (83). This correlates with cessation of cell proliferation associated with a decrease in cdk1, cdk2, cyclin B, and cdc25 levels and an increase in p21^{Cip1} level. Additionally, SFN treatment causes apoptosis as evidenced by increased cleavage of procaspase 3, 8, and 9 and poly ADP ribose polymerase (PARP) (83). An interesting finding is that forced maintenance of Bmi-1 expression reverses the SFN-induced effects in SCC-13 cells and restores cell survival and proliferation (83). This indicates that polycomb protein expression drives skin cancer cell survival and that a reduction in PcG protein level is required for SFN suppression of cell proliferation. One key finding is that SFN treatment results in the loss of PcG protein expression *via* a posttranscriptional/translational mechanism, in that Bmi-1 and Ezh2 are ubiquitinated and degraded by the proteasome (83). Moreover, treatment with lactacystin, a proteasome inhibitor, reverses the SFN-mediated loss of Bmi-1 and Ezh2, confirming the role of the proteasome. These findings are novel, as they are the first to identify polycomb proteins as a target of SFN in the skin cancer model and indicate the role of proteasome in SFN-mediated suppression of polycomb gene levels. They also indicate that the impact of SFN on PcG protein level is indirect, such that the reduction is mediated *via* a proteasome activation mechanism.

The mechanism of SFN action differs in the normal keratinocytes as compared to skin cancer cells. In keratinocytes, SFN increases p53 protein level by reducing p53 turnover (84). The increase in p53 is accompanied by increased p53 binding to the response elements on the p21^{Cip1} promoter (84). An important inference is that SFN slows proliferation of normal human keratinocytes *versus* driving apoptosis in transformed keratinocytes. This is encouraging from the clinical standpoint, as topical or oral treatment with SFN may cause cancer cells to undergo apoptosis while only slowing the growth of the normal epidermal keratinocytes. Moreover, the range of SFN concentrations utilized in these studies, ranging from 5–10 μ M, can be achieved *in vivo* in humans (71).

SFN and DNA Methylation. Less has been done in skin cancer regarding the impact of SFN on DNA methylation. However, SFN has been shown to inhibit DNMTs and modulate gene expression in other cell types. For example, the human telomerase reverse transcriptase (hTERT) gene encodes the catalytic subunit of telomerase. hTERT level is elevated in 90% of the human cancers and is a potential

target of chemopreventive agent action (85,86). SFN treatment causes a concentration-dependent demethylation at the CpG island in exon 1 of the hTERT gene promoter in MCF-7 and MDA-MB-231 breast cancer cells. This results in increased binding of the CTCF transcriptional repressor (CCCTC binding factor) to the hTERT gene regulatory region (87). This is associated with reduced hTERT transcription, reduced telomerase activity, and reduced cell colony forming ability. Additionally, SFN treatment reduces the level and activity of DNMT1 and DNMT3a in these cells (87).

The cell cycle is a closely orchestrated process regulated by cyclins, cyclin-dependent kinases (cdks), and cyclin-dependent kinase inhibitors. Cdks are serine threonine kinases which require association with a cyclin subunit for activation. Each phase of the cell cycle is controlled by a specific cyclin-cdk pair, which governs the progression from one cell cycle phase to the next. Deregulation of the cell cycle is an important hallmark of cancer, and hence identifying cancer prevention agents that target regulators of the cell cycle constitutes an important area of research. Cyclin D2 is involved in G1/S transition. It acts as a tumor suppressor gene or as a proto-oncogene depending on the type of cancer. Reduced cyclin D2 correlates with increased pathological features in prostate cancer (88,89). SFN treatment reduces DNMT1 and DNMT3b mRNA and protein level in LNCaP prostate cancer cells. This is associated with demethylation at the cyclin D2 promoter (90). An interesting observation is that reduced methylation at the c-Myc and Sp1 transcription factor DNA binding site is associated with increased accumulation of cyclin D2 transcripts (90). Collectively, these results suggest that SFN-mediated restoration of cyclin D2 may be mediated by c-Myc and Sp1 transcription factors, and that access of these factors to DNA in the cyclin D2 gene is controlled by methylation. These pathways should be examined as epigenetic targets in skin cancer, as hTERT and DNMTs have been implicated in the pathogenesis of epidermal squamous cell carcinoma (91,92).

Effect of SFN on Histone Acetylation. SFN regulates HDAC activity in LNCaP, PC3, and BPH1 prostate cancer cells leading to subsequent increase in global acetylation and increased p21^{Cip1} and Bax mRNA and protein expression which leads to G2/M cell cycle arrest and apoptosis (93). SFN treatment also inhibits HDAC activity in PC3 xenografts and in human subjects (72), and in breast and colon cancer (94,95). Here again, p21^{Cip1} and other cell cycle proteins are important targets in skin cancer, and the impact of SFN on methylation of these targets should be studied, as SFN is known to increase p21^{Cip1} level and alter the regulation of other cell cycle regulatory proteins in skin cancer cells (83).

Impact of (–)-Epigallocatechin-3-Gallate on PcG Proteins in Skin Cancer. Green tea is a highly studied chemopreventive mixture (96). It is a rich source of cancer preventive agents including EGCG, (–)-epicatechin-3-gallate, and epicatechin. EGCG is the major polyphenol present in green tea and is an effective agent against cutaneous inflammatory disorders and cancer (97–100). EGCG ingestion and topical treatment has been shown to suppress skin cancer development in mice (101,102). Over the past decade, substantial progress has been made in unraveling the mechanisms of EGCG-mediated prevention of cancer. Downstream targets of EGCG include genes involved in apoptosis and cell cycle, including key signal

transduction pathways such as NF κ B and MAPK (103). In addition, the anticarcinogenic activity of EGCG in skin cancer has been attributed to its antiinflammatory, antioxidant, and DNA repair properties (104,105).

Our laboratory was the first to consider the impact of green tea polyphenols on PcG protein function. Polycomb proteins, including Bmi-1, Ezh2, and Suz12, are elevated in immortal and transformed epidermis-derived cell lines (SCC-13, HaCaT, and A431 cells) (81). Additionally, skin cancer cells have elevated methylation of H3K27me3, a pro-survival chromatin modification that results from action of the Ezh2 histone methyltransferase (81). PcG protein levels are reduced by treatment with EGCG. EGCG treatment of skin cancer cells causes a dose- and time-dependent reduction in Ezh2, Bmi-1, and Suz12. This is accompanied by a reduction in H3K27me3 formation (81). The functional endpoint of EGCG treatment is a reduction in SCC-13 cell number, and this is attributed to reduced levels of cell cycle regulatory proteins including cdk1, cdk2, cdk4, and cdk6 and cyclins (cyclin D1 and cyclin E). EGCG treatment also increases the level of the cdk inhibitors, p21^{Cip1} and p27. In addition to suppressing cell proliferation, EGCG treatment induces apoptosis characterized by cleavage of procaspase 3, 8, and 9 and PARP, leading to accumulation of subG1 cells (81). An interesting finding is that forced expression of Bmi-1 in EGCG-treated cells leads to restoration of Ezh2 level, implying that feedback regulation operates to maintain an optimal balance of PcG proteins in the cell (81). In addition, exogenous expression of Bmi-1 in SCC-13 skin cancer cells reverses the growth inhibitory effects of EGCG, suggesting that a reduction in PcG protein expression is required for the anticancer effects of EGCG (81,106–108). In a subsequent study, we reported that EGCG treatment reduces PcG protein levels *via* a proteasome-dependent mechanism (108).

Recent experience suggests that treatment with a single cancer preventive agent might not be sufficient to prevent cancer. This has led to the consideration that the simultaneous use of multiple agents may be effective. With the aim of finding new prevention combinations, we studied the effect of co-treatment with 3-deazaadenosine (DZNep) and EGCG on the skin cancer cells. DZNep, a deazaadenosine analog, is an inhibitor of the enzyme S-adenosyl homocysteine (AdoHcy) hydrolase (109–111). Inhibition of this enzyme results in accumulation of AdoHcy which limits the methyl groups available for use by S-adenosyl-L-methionine-dependent methyl transferases (111). Recent studies suggest that DZNep reduces Ezh2 methyltransferase activity (112). Combined treatment with DZNep and EGCG leads to more substantial reduction in cell number and an increase in the subG1 cell population, as compared to each individual treatment (108). The combined treatment is also more effective than individual treatments in reducing the level of PcG proteins including Ezh2, EED, Suz12, Mel18 and Bmi-1 (108).

An additional interesting observation is that DZNep and EGCG increase formation of acetylated histone H3 (108). This increase in H3Ac formation is due to reduced HDAC1 level as a result of combined EGCG and DZNep treatment (108). This is consistent with previous reports showing that the PcG protein complex interacts with HDACs and transports them to the site of PcG protein complex interaction with chromatin, where PcG proteins and HDACs act together to

produce closed chromatin (113,114). It is important to note that normal human keratinocytes are less responsive to the combined action of EGCG and DZNep, as compared to skin cancer cells, making this combination therapy a promising option for prevention of skin cancer (108).

EGCG Impacts Methylation in Skin Cancer. EGCG has been heavily studied as an agent that stimulates demethylation. This occurs *via* several mechanisms. First, it generates S-adenosyl-L-homocysteine (SAH). EGCG is methylated by catechol-O-methyltransferase which transfers a methyl group to the catecholamine group of EGCG. The donor molecule in this case is S-adenosylmethionine (115). Demethylation of SAM leads to SAH formation which is a DNMT inhibitor. Second, EGCG binds to the active site residue of the DNMT1 to directly inhibit activity. This prevents methylation of newly synthesized DNA and ultimately leads to expression of previously silenced genes (116,117). *Via* these mechanisms, EGCG reduces tumor suppressor gene methylation in a host of cancer types (115,118).

EGCG is an epigenetic regulator of skin cancer cell function which suppresses DNA methylation in A431 and SCC-13 skin cancer cells in a time- and dose-dependent manner (92). This is accompanied by decreased DNMT1, DNMT3a, DNMT3b mRNA, and protein level and DNMT activity. Additionally, EGCG decreases methylation of lysine 9 of histone H3. These changes are associated with increased levels of p16^{Ink4a} and p21^{Cip1} mRNA and protein (92). Moreover, inhibition of DNA methylation is one of the mechanisms responsible for the antimetastatic action of EGCG in oral squamous cell carcinoma cells, which are related to skin cancer cells (118). RECK is a tumor suppressor protein that acts to reduce metalloproteinase expression to inhibit metastasis. Reduced RECK protein level correlates with increased metastasis. Hypermethylation of the RECK gene promoter leads to low RECK mRNA levels in oral SCC cell lines, and EGCG treatment reduces methylation of the promoter resulting in restoration of RECK mRNA and protein level (118). Functionally, this is associated with decreased cell migration. In addition, *in vivo* topical application of EGCG has been reported to inhibit UVB-induced global hypomethylation in SKH-1 hairless mice (119). These studies suggest that inhibition of DNA methylation is an important mechanism responsible for chemotherapeutic action of EGCG in skin cancer. However, the exact mechanism whereby EGCG selectively reduces methylation at tumor suppressor genes has not yet been elucidated.

EGCG and Histone Acetylation. The role of EGCG in the context of histone acetylation in cancer has not been extensively studied. EGCG inhibits HDAC activity and suppresses mRNA level of class 1 HDACs, including HDAC1, HDAC2, and HDAC3 in other cancer types, and this is associated with increased acetylation of histones H3 and H4 (120). EGCG produces similar effects in human skin cancer cells (92). These studies suggest that EGCG can act as a potential HDAC inhibitor in addition to being a DNA methylation inhibitor.

CONCLUSION

Although the chemopreventive effects of the dietary polyphenols are promising, many questions remain. For example, many of the mechanistic details remain to be discovered

including the precise mechanism, whereby cancer prevention agents regulate expression of HDAC, PcG proteins, etc. In addition, most of the abovementioned studies involve the use of a single dietary factor. It will certainly be beneficial to study the effect of combined nutrient mixtures as epigenetic modifiers in skin cancer. This may pave the way for the development of an epigenetic diet, wherein dietary anticancer nutrients are incorporated into the daily routine to impede the development of skin cancer.

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