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DIETARY PHYTOCHEMICALS AS EPIGENETIC MODIFIERS IN CANCER: CURRENT CHALLENGES AND PROSPECT

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ABSTRACT

Diet and environment play important role in human health. Most plant-derived natural bioactive compounds (phytochemicals) in our diets are potent antioxidants and cancer chemo-preventive agents. Dietary phytochemicals (Sulforaphane, catechins/epicatechins, Genistein, Resveratrol, Lycopene, and others) have been found to play significant role as epigenetic modifiers in several cancers. Epigenetics was first defined as the complex interplay between the genome and environmental factors that govern cell differentiation and development. At the current time, this term refers to heritable traits that are not a consequence of changes in DNA sequence, rather the result of alterations in gene expression regulated by changes in DNA accessibility or chromatin structure. Epigenetic modifications can be affected by exogenous factors providing a link between genes (or the genome) and environment (or the exposome) in defining phenotype variations. The field of epigenetics is expanding rapidly, with a number of ongoing international research initiatives, including the Human Epigenome Project and the International Human Epigenome Consortium. In past few decades, the role of epigenetic alterations such as DNA methylation, histone modifications and non-coding RNAs in the regulation of mammalian genome had been comprehensively addressed. Defining the determinants of epigenetic regulation offers opportunities for novel strategies for disease prevention and treatment. Several epigenetically active synthetic molecules such as DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) inhibitors, are either approved or, under clinical trials for the treatment of various cancers. However, most of the synthetic inhibitors have shown adverse side effects, narrow therapeutic index and are expensive. Hence, bioactive phytochemicals, which are widely available with lesser toxic effects, have been tested for their role in epigenetic modulatory activities in gene regulation for cancer prevention and therapy. These bioactive phytochemicals showed promising results against various cancers. This review discusses the role of commonly investigated phytochemicals and their epigenetic targets that are of particular interest in cancer prevention and therapy; as well as the progress in cancer chemoprevention with dietary phytochemicals.

KEYWORDS: **Phytochemicals, Epigenetics, DNA methylation, histone modifications, non-coding RNAs.**

INTRODUCTION

Carcinogenesis is considered to be the outcome of deregulated genetic and epigenetic events. Epigenetic alterations are important as they link the behaviour of cells to their environmental interactions and thus determine the susceptibility of a cell to transforming changes. As these changes do not involve alterations in the genome constructs, these epigenetic events occur constantly during the life of the cells. Epigenetics is defined as the study of heritable but reversible changes in gene expression that occur without alterations in the sequences of underlying DNA. Epigenetic modifications often alter gene expression and, in particular, expression of the tumor suppressor, promoter, and oncogenes that are crucial for cellular proliferation, differentiation and

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survival during carcinogenesis. DNA methylation, histone tail modifications, chromatin remodeling and miRNA-mediated multi-gene silencing are considered to be the major epigenetic changes that are involved in maintaining cellular homeostasis and differentiation states. Multiple studies have revealed that global DNA hypomethylation events are a major characteristic of most of types of cancer and contribute to genomic instability by activating retrotransposons and other silent genomic regions [1]. On the other hand, promoter hypermethylation events occur in important tumor suppressor genes, indicating that it is inappropriate DNA methylation that is important in driving the process of carcinogenesis [2]. Histone modifications also occur, including acetylation and methylation of lysine residues, methylation of arginine residues, phosphorylation of serine or threonine residues, ubiquitination and sumoylation of lysine residues, ADP ribosylation of glutamic acid residues and isomerisation of proline residues, *etc.* These modifications define the patterns of chromatin remodelling, thus determining the resultant gene expression and gene silencing patterns. The microRNAs (miRNAs), which are recently identified non-coding RNAs, also have been shown to contain gene-expression regulatory activities and are capable of functioning both to suppress and promote oncogenesis. Deregulated miRNA transcription leads to upregulation of oncogenes and silencing of tumor suppressor genes in the lung, breast and neck as well as bone cancers [3].

Dietary phytochemicals or supplements are not only a rich source of minerals, vitamins and micronutrients but also contain bioactive components such as anti-oxidants, polyphenols and alkaloids, which are not among the basic nutrients. These bioactive compounds have shown great potential against many diseases including cancers through genetic and epigenetic modifications [4-6]. In this review article, we focus on the major types of epigenetic modifications, such as DNA methylation, histone modifications and miRNA-mediated gene silencing in cancer progression, and epigenetic targeting by phytochemicals in cancer prevention and therapy.

Targets/mechanism of Epigenetic Modification

DNA methylation

Among epigenetic modifications, DNA methylation is the most studied [7]. Covalent attachment of a methyl group to the C5 position of cytosine comprises the principal epigenetic modification of DNA. This modification occurs primarily in CpG dinucleotide-containing regions, often in regulatory sequences that suppress gene expression. CpG methylation is important for transcriptional repression of transposons and repeat elements, for imprinting and X-chromosome inactivation, [8] and for tissue-restricted gene expression during development and differentiation.

S-adenosyl methionine (SAM) functions as a universal methyl group donor in the methyl transfer reactions catalyzed by DNA methyltransferases (DNMTs) in the eukaryotic nucleus. Cytosine methylation at CpG dinucleotides is carried out by a family of enzymes, the DNA methyltransferases (DNMTs). There are two types of DNMTs present in eukaryotes including maintenance methyltransferase (DNMT1) and the *de novo* methyltransferases (DNMT3A and DNMT3B). DNMT1 functions in maintaining the pre-established patterns of DNA methylation, while the *de novo* enzymes establish new patterns of methylation in the fully un-methylated DNA.

DNA hypermethylation of tumor suppressor genes is a rather frequent event in most of the cancers both during the initiation or the progression events [9]. Gene hypermethylation might also initiate recruitment of the methylation-dependent DNA binding proteins to the hypermethylated DNA sites. The methylation-dependent DNA binding proteins further help in silencing of methylated genes by recruiting repressor complexes to these regions. These proteins are commonly found occupying the hypermethylated gene promoters in multiple cancers [10]. In addition to the methylation-dependent DNA binding proteins, a transcriptional domain in DNMT1 also recruits histone deacetylase (HDACs) and other chromatin re-modelling proteins to the target sites that can modify acetylation and methylation status of histones, thereby inhibiting transcriptional access to the chromatin [11,12]. Hence, DNMTs inhibitors are important in cancer therapy and some FDA-approved inhibitors of DNMTs, such as 5-azacytidine and 5-aza-2'-deoxycytidine, are already being used as therapeutic drugs against multiple cancer types [13, 14]. Many of the synthetic inhibitors have, however, been shown to cause adverse toxic effects and are narrow in their specificity. Hence, phytochemicals, which are widely available and have lesser toxicities, are being tested for their role in direct or indirect inhibition of DNMTs activity in cancer prevention and therapy.

Although demethylation is known to be an essential process that occurs during certain stages of development, the mechanism of DNA demethylation

is less understood than that of methylation. Demethylation may play an essential role in modulating brain plasticity or transcriptional responses to hormones, and targeted or global loss of methylation has been associated with cancer, cardiovascular disease, and other pathologies [15]. One possible pathway for demethylation involves oxidization of methyl cytosines to hydroxymethyl cytosine by the ten-eleven translocation (Tet) enzymes. Growing evidence suggests that hydroxymethyl cytosine formation is an intermediate stage in DNA demethylation possibly due to its increased susceptibility to deamination [15]. At this time, it is unclear whether 5-hydroxymethyl cytosine may have other essential functions as an epigenetic marker capable of regulating gene expression and chromatin structure; however, the function of the Tet proteins appears to be important for replication-independent DNA demethylation.

Histone modifications and chromatin remodeling

Eukaryotic DNA is organized in a complex structure known as chromatin, which is comprised of DNA, histones and several other DNA-binding proteins. In addition to promoting a compact structure, chromatin organization also helps in the regulation of gene expression by restricting the access of different DNA binding proteins or protein complexes to the genetic material. The processes of "opening up" of chromatin and its compaction are associated with a number of ATP-dependent multi-enzyme complexes known as chromatin remodeling complexes. The chromatin remodeling is triggered by various histone tail modifications, which determine the state of activity of chromatin. The best studied histone modification, lysine acetylation, leads to opening up of the chromatin due to the negative charge conferred by the acetyl moieties, which reduces the histone-DNA interactions. The lysine acetylation reactions are catalyzed by histone acetyltransferases (HATs), which transfer the acetyl groups from acetyl coenzyme A to the lysine moieties in the nucleosomes. HATs also play important roles in regulating cell cycle regulatory protein expression and also can bind directly to the cell cycle regulatory apparatus [16].

Histone deacetylases (HDACs) remove the acetyl groups from lysine residues to reduce the negative charge thus leading to chromatin compaction. Four distinct classes of HDACs in humans have been identified based on their structural similarity to yeast proteins as well as their localization and acetylation activities [17]. Class I, II and IV HDACs are zinc-dependent histone deacetylases, while class III HDACs, also known as sirtuins, is NAD^+-dependent HDACs. Over expression and altered activities of HDACs are associated generally with the silencing of tumor suppressor genes, epithelial to mesenchymal transitions and metastasis [18]. Over-expression of *HDAC1* resulted in downregulation of *p53* and *von Hippel-Lindau* tumor suppressor genes and stimulated angiogenesis of human endothelial cells [18], while HDAC10 suppresses metastasis of cervical cancer through inhibition of matrix metalloproteinases 2 and 9 expression [19, 20].

In general, histone acetylation is associated with gene activation and is abundant in the euchromatin whereas deacetylation is linked to gene repression and occurs in the heterochromatin. Nevertheless, gene repression or activation is not completely dependent on histone acetylation or methylation, but rather is dependent on the site and degree of methylation or acetylation on histone tails. Some of the active chromatin markers associated with gene expression are histone methylation on histone H3 at lysine 4 (H3K4), on histone H3 at lysine 36 (H3K36), on histone H3 at lysine 79 (H3K79) and on histone H4 at lysine 20 (H4K20); while the inactivation markers associated with gene repression are methylation on histone H3 at lysine 9 (H3K9) and on histone H3 at lysine 27 (H3K27) [21]. It is recognized widely that HDACs are promising targets for cancer prevention and therapy. Some of the well-studied HDAC inhibitors are trapoxin (TPX), trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA). Among them, Vorinostat or SAHA and Romidepsin (Istotax) are commercially-available FDA-approved HDAC inhibitors for treatment of cutaneous T-cell lymphoma [22]. Some other HDAC inhibitors such as Panobinostat, valproic acid and Belinostat are in different phases of clinical trials. Fig. 1 depicts the relationships between DNA methylation and common histone modifications in epigenetic regulation of gene expression.

Noncoding RNA

Long noncoding RNAs can silence genes, owing, in part, to their recruitment of remodeling complexes, such as the polycomb complex, that promote histone methylation. These RNAs can also recruit RNA-binding proteins that impair histone deacetylation or that inhibit transcription factor binding to promoter regions [23]. Through these and other mechanisms, long noncoding RNAs are essential for imprinting and X-chromosome inactivation and play key roles in cardiac development [24]. Small inhibitory RNAs and dicer-dependent microRNAs, as short noncoding RNAs, have also been shown to play a role in transcriptional suppression through several

mechanisms, including the recruitment of specific argonaute proteins to form epigenetic remodeling complexes that promote histone deacetylation, histone methylation, and DNA methylation [25,26]. The protein interaction world-interacting RNAs (21–30 nt) are a single stranded subclass of these small noncoding RNAs that have been shown to play a role in maintaining the transgenerational inheritance of RNA-induced epigenetic silencing [27].

RNA epigenetics

Posttranscriptional RNA modifications represent another type of epigenetics, RNA epigenetics [28]. In particular, RNA (tRNA, mRNA, and rRNA) can undergo methylation at a variety of positions (Fig. 2) in the nucleotide base, as well as at the 2 position of the ribose, and these methylation events can modulate function [29]. In addition, there is growing evidence for RNA demethylases that may modulate gene expression. RNA methylation has different functional consequences, including stabilization, enhanced function, and quality control. In tRNA, for example, modifications are found in certain regions of the tRNA and can contribute to tertiary structure and the accuracy of tRNA recognition. This field of RNA epigenetics is still developing and holds promises for another level of complex epigenetic regulation of gene expression.

Dietary Phytochemicals and their Epigenetic Modulatory Activities

Enthusiasm for the use of dietary phytochemicals in the prevention and therapy of different diseases has increased in recent years. Possible reasons behind this interest lie in their natural origin, widespread availability, lesser side-effects and the possibility of inclusion in the routine diet. Traditionally, these phytochemicals have been utilized in the treatment of various diseases since ancient times [30,31]. There has been a tremendous increase in the knowledge concerning their mechanisms of actions and molecular targets. Interestingly, these dietary factors have shown a capability to regulate the patterns of expression of multiple genes through epigenetic modulatory mechanisms.

Polyphenols are one of the essential bioactive dietary supplements largely present in fruits, vegetables, seeds and nuts [32]. There are approximately 8000 polyphenols present in the diet and these can be classified into ten different generalized classes according to their chemical structure [4]. The major classifications are catechins/epicatechins, stilbenes, benzoquinones, acetophenones, flavonoids, phenolic acids, proanthocyanidins, ellagitannins and anthocyanins [33]. Among these, the most commonly studied polyphenols are (−)-epigallocatechin-3-gallate (EGCG; found in green tea), resveratrol (present in grape skin) and curcumin (found in turmeric) [33]. These dietary polyphenols have been shown to have chemo-preventive and therapeutic potential in preclinical models against various cancers [34,35]. Significant progress in research has shown that these dietary polyphenols have shown to reverse the epigenetic changes occurring during the process of carcinogenesis [36].

Tea catechins/epicatechins

The tea plant (*Camellia sinensis*) is cultivated in more than 30 countries. Green, black, and oolong teas are the most common varieties derived from the leaves of the tea plant defined but differ according to their manufacturing processes [34]. Studies have shown that green tea possesses significant beneficial effects due to an abundance of monomeric catechins or epicatechins, which include (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin-3-gallate (EGCG) [34]. Of these, EGCG is the major and most active ingredient of green tea polyphenols (GTPs) and is shown to have potent anti-cancer activities both *in vitro* and in *in vivo* models [37, 38]. In particular, EGCG has been shown to inhibit cellular proliferation and to induce apoptosis in many cancer cell types through multiple mechanisms [37]. Recent studies have suggested that the anti-cancer activity of EGCG is mediated, at least in part, through its epigenetic modulatory activities, such as inhibition of DNMTs and HATs [38,39].

EGCG is involved in direct inhibition of DNMTs by forming hydrogen bonds in their active sites that hinder substrate binding [39]. EGCG also has been reported to reduce the available S-adenosyl-L-methionine (SAM), a methyl donor for DNMTs, and induce S-adenosyl-L-homocysteine (SAH), a potent inhibitor of DNMTs, in a mechanism in which EGCG mediates indirect inhibition of DNMTs [22]. The inhibition of DNMTs can lead to reversal of the silencing of tumor suppressor genes in cancer cells. Meeran *et al.,* [40] reported that EGCG and a pro-form of EGCG inhibit *hTERT* expression by inducing gene-specific demethylation and chromatin modifications in human breast cancer cells [40,41]. They demonstrated that EGCG and the pro-form of EGCG induced chromatin alterations that facilitated the binding of many *hTERT* repressors such as MAD1 and E2F-1 to the *hTERT* regulatory region, thereby contributing to their transcriptional repression [40]. In another study using oral carcinoma cells, partial demethylation of *RECK*,

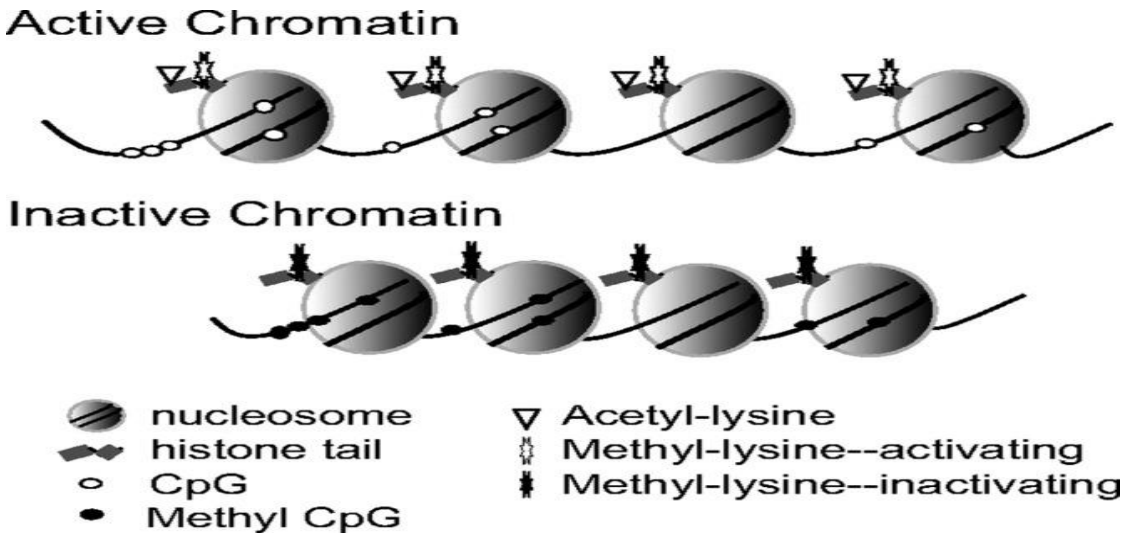

Figure 1: Chromatin structure and epigenetic tags. Nucleosomes are indicated as spheres, with sites of methylation and acetylation on DNA and histone proteins listed in the key.

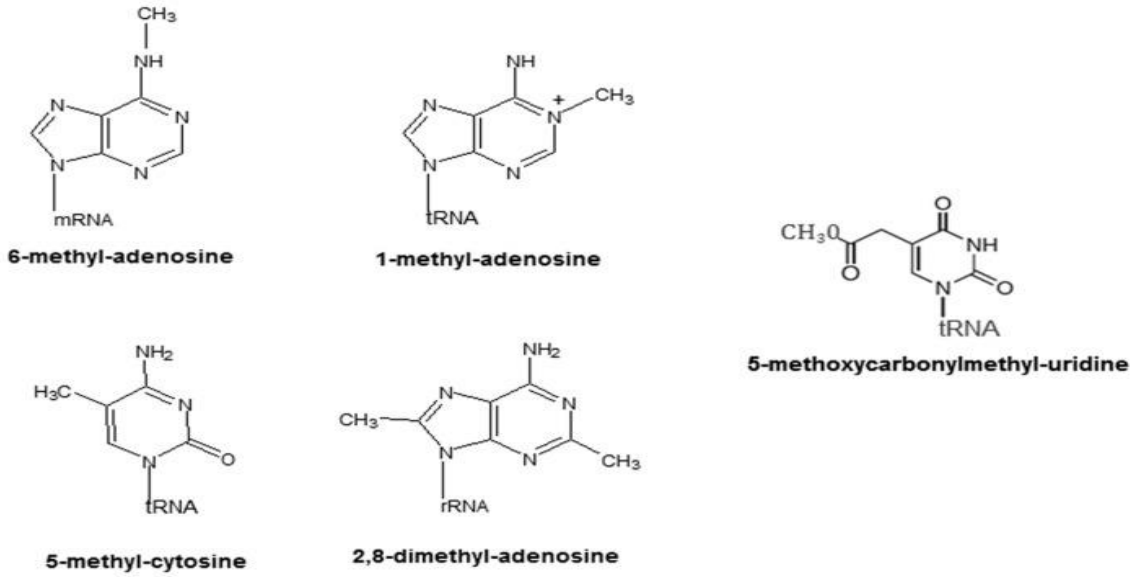

Figure 2: RNA methylation products. Common methylated bases found in mRNA, tRNA, and rRNA.

a tumor suppressor gene, by EGCG was found to be well correlated with inhibition of tumor invasion, angiogenesis and metastasis [42]. Furthermore, EGCG has been demonstrated to inhibit the invasive potential of human pancreatic adenocarcinoma cells by modulation of HDAC activity [43] and EGCG has been shown to induce the degradation of DNMT3A and HDAC3 in methylation-sensitive colon cancer cells, in part, through inhibition of E3 ubiquitin ligase [44].

Combination of HDAC inhibitors with EGCG (a known DNMT inhibitor) is another effective approach for cancer prevention and therapy. Studies conducted by Li *et al.* [45] showed that a combination of EGCG with an HDAC inhibitor reactivates estrogen receptor-α (ERα) expression in ERα-negative human breast cancer cells through a process associated with chromatin modifications. Not only synthetic inhibitors, but also the dietary combination of green tea polyphenol (GTPs), a DNMT inhibitor, with sulforaphane (SFN), a HDAC inhibitor, reactivate ERα expression in ERα-negative human breast cancer cells and this was found to correlate consistently with ERα promoter hypomethylation and hyperacetylation [46]. These reactivations are a promising strategy for the effective treatment of hormonal refractory breast cancer with available anti-estrogen drugs like tamoxifen. In addition to ERα reactivation, GTPs and SFN combinations also tend to enhance cisplatin-induced apoptosis and G2/M phase cell cycle arrest through upregulation of $p21^{CIP1/WAF1}$, thereby enhancing the efficacy of cisplatin on both cisplatin-sensitive and cisplatin-resistant ovarian cancer cells [47]. Taken together, these data suggest that GTPs alone or in combination with dietary HDAC inhibitors can modulate epigenetic regulation of gene expression and can play a vital role in cancer chemoprevention and therapy.

Sulforaphane

Sulforaphane (SFN) is one of the major isothiocyanates present in many cruciferous vegetables and has been shown to have anti-cancer activities in several cancer models [48]. Although SFN has been shown to mediate anti-cancer activity through several mechanisms, including cell cycle arrest, induction of cellular apoptosis and phase-2 detoxification enzymes, there is a growing interest in their HDAC inhibitory activity [49, 48]. As aforementioned, HDACs are often upregulated in cancers and HDACs inhibitors play a major role in cancer prevention and therapy. SFN-mediated inhibition of DNMTs and histone methylations also have been found to play a major role in the alterations of gene expressions found in cancer prevention studies. In accordance, SFN-mediated downregulation of DNMTs also is associated positively with *hTERT* promoter demethylation, which is followed by binding of CTCF, a repressor protein, to the *hTERT* gene regulatory region in human breast cancer cells [50]. Further, SFN-mediated transcriptional repression of *hTERT* correlates positively with its inhibition of cellular proliferation and induction of apoptosis in human breast cancer cells [50]. SFN also has been shown to inhibit DNMT1 and DNMT3b in human prostate cancer cells. Interestingly, SFN-induced demethylation at cyclin D2 promoter regions corresponded to an increase in cyclin D2 transcript levels, thereby exerting anti-proliferative effects on prostate cancer cells [51]. In addition, SFN treatment led to demethylation of the first five CpGs in the promoter region of the *Nrf2* gene in TRAMP C1 cells, suggesting a modulatory role on anti-oxidative stress pathways [52]. Furthermore, SFN reduced the expression of DNMTs and downregulated the expression of HDAC1, HDAC2, HDAC3 and HDAC4, thereby reactivating Nrf2, a transcription factor for anti-oxidant enzymes [53]. A recent study by Wong *et al*. [54] evaluated genome-wide effects of SFN on promoter methylation in normal prostate epithelial cells and two prostate cancer cell lines and found that the cancer cell lines showed widespread changes in promoter methylation patterns, including both increased and decreased methylation.

Curcumin

The anti-inflammatory, anti-septic, wound-healing, anti-oxidant, anti-angiogenic and anti-cancer activities of turmeric (*Curcuma longa*) have been attributed to the yellow pigment curcumin, which is a diferuloylmethane polyphenolic compound [55]. Multiple epigenetic activities of curcumin have been reported [55]. Curcumin treatment has been shown to induce global hypomethylation in leukemia cells, further strengthening the notion of curcumin-mediated inhibition of DNMT activity [56]. Curcumin also has been shown to mediate inhibition of HDACs and HATs activity in multiple *in vitro* cancer models [56]. Significant inhibition in the expression of HDACs 1, 3 and 8, as well as of HAT p300, were found after curcumin treatment leading to repression of NF-κB and Notch 1 in Raji cells, an *in vitro* model of Burkitt's lymphoma [57]. Curcumin also has been reported to alter HDAC2 expression by chemically preventing its degradation in human monocytes [57]. The α, β-unsaturated carbonyl groups in the side-chain of curcumin are considered to be structurally important for its HAT inhibitory activity [58].

Curcumin also was found to promote proteasomal degradation of p300 and related HATs in prostate cancer cells and peripheral blood lymphocytes [57]. Recently, curcumin was found to reduce the acetylation of histone H3 in the IL-6 promoter leading to its decreased expression in rheumatoid arthritis synovial fibroblasts [59]. A number of investigators have reported curcumin-mediated alterations in miRNA expression profiles. Treatment of human pancreatic cancer cells with curcumin led to upregulated expression of 11 miRNAs, with miR-22 as the most highly over expressed miRNA, as well as the down-regulation of 18 miRNAs, with miR-199a being the most significantly down-regulated miRNA [60]. Curcumin altered the expression of the tumor suppressor miRNA, miR-203, in a panel of bladder cancer cell lines [61]. These results suggest that curcumin is a very important epigenetically bioactive compound with multiple epigenetic modifying capabilities.

Genistein

Genistein (4',5,7- trihydroxyisoflavone), an isoflavonoid, is found in different varieties of beans and is especially abundant in soy beans. The role of genistein as a chemopreventive phytoestrogen against carcinogenesis is well established [62]. This compound is a strong anti-oxidant and a potent tyrosine kinase inhibitor. Other important mechanisms through which genistein exert its anti-cancer effects includes prevention of mutations in DNA strands; inhibition of cancer cell proliferation and angiogenesis; and proapoptotic effects [63]. Genistein has been found to be capable of modulating important epigenetic events, such as DNA methylation and histone tail modifications [64]. The inhibitory effect on DNMTs and histone modifying activities of genistein has been established in many similar reports [65] Genistein, in combination with other DNMT or HDACs inhibitors, has shown synergistic epigenetic reactivation of hypermethylated tumor suppressor genes [66]. It also inhibits the expression of DNMT1, DNMT3a and DNMT3b and inhibition of tumor promoter *hTERT*, the catalytic subunit of telomerase in human breast cancer cells [64].

Another study demonstrated that genistein reactivates ERα expression in ERα-negative breast tumors [45]. This is of importance in utilization of the available anti-estrogen treatments *in vivo* in breast xenograft and spontaneous breast tumor mouse models [45]. Based on these findings, the authors further demonstrated that the ERα reactivation effect was enhanced synergistically when combined with a HDAC inhibitor in ERα-negative MDA-MB-231 breast cancer cells. In contrast, some *in vivo* studies have shown that genistein induced hypermethylation of cancer-related genes [67]. These results correlate with a recent human trial, in which 34 healthy premenopausal women fed isoflavones, including genistein, daily through one menstrual cycle, showed hypermethylation in some of the key cancer-related genes [68].

Resveratrol

Resveratrol is abundant in grape skin, and also found in berries and peanuts, *etc*. Resveratrol has been shown to possess potent anti-cancer properties. It acts on cancer cells by regulating the pathways of cell division and cell growth, apoptosis, inflammation, angiogenesis, and metastasis [69,70]. Resveratrol has been shown to have a moderate inhibitory effect on DNMTs [71]. Resveratrol treatment led to decreased DNMT1 and 3b expression *in vitro*. In a rodent model of estrogen-dependent mammary carcinoma, resveratrol treatment decreased DNMT3b expression in tumor samples but not in the normal tissues. miRNA expression was also found to be altered after resveratrol treatment in tumor *vs.* normal tissues *in vivo* [68]. Resveratrol treatment of human bladder cancer (EJ) cells was found to lead to remarkable S-phase arrest and apoptotic cell death accompanied by loss of phosphorylation of STAT-3, leading to downregulation of the STAT-3 pathway as well as decreased nuclear translocation of SIRT1 and p53 [72]. Collectively, these studies indicate that resveratrol possesses anti-cancerous activities and that these are mediated through multiple genetic and epigenetic modes of actions.

Quercetrin from *Toona sinensis* leaves

Leaves of *Toona sinensis* (*T. sinensis*) M.Roem, a popular vegetable in China, were reported to have various biologically activated effects including antioxidative [73], anticancer [72], anti-inflammatory [53] and anti-hyperglycemic [74]. A previous study demonstrated that *T. sinensis* leaf extracts are rich in active ingredients such as flavonoids, volatile oils and alkaloids [75]. The major identified flavonoids in *Toona sinensis* leaves (QTL) are quercetrin, kaempferol-3-*O*-α-L-rhamopyranoside, astragalin, kaempferol, methyl and ethyl gallate, and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose [75,73].

Crude water extracts of *T. sinensis* leaves have been shown to inhibit cell proliferation and induce apoptosis of a number of human cancers, including lung adenocarcinoma (A549) cells, human ovarian cancer cell lines [76], HL-60 leukemia cells [75],

H661 lung and cervical carcinoma HeLa cells [77], HepG liver cancer and MCF-7 breast cancer cell [78], and prostate cancer cells [79]. Antiproliferative effects on Caco-2 human colon cancer cells have also been reported [78]. This phytochemicals (flavanoids) can inhibit CRC by disrupting multiple mechanisms that are central to cancer progression [80,81]. Although *T. sinensis* leaves have been used medicinally for a long time, its effects are still not fully understood.

It was reported that aqueous extracts from the leaves of *T. sinensis* arrested SKOV3 ovarian cancer cells at the G2/M phase and induced the apoptotic pathway [76]. Cell cycle arrest or apoptosis in response to DNA damage was mediated primarily by transcription factor p53 [82]. Moreover, p21 is a very important checkpoint gene in the cell cycle, and it is also regulated by the transcription of p53. The findings are consistent with previous studies demonstrating that QTL increased the expression level of p53 and p21 proteins [83]. Those results revealed that the QTL-induced inhibition of SW620 cell growth was partially due to the induction of G2/M arrest.

Mitochondria are key organelles crucial for cell survival, and are conversely a source of ROS during apoptosis. The loss of mitochondrial membrane potential ($\Delta\Psi m$) is often, but not always observed to be associated with the early stages of apoptosis [84]. The collapse of this potential is believed to coincide with the opening of the mitochondrial permeability transition pores, leading to the release of cytochrome *c* into the cytosol. In the cytoplasm, cytochrome *c* combines with caspase-9, Apaf-1 and dATP to form the apoptosome complex which in turn activates caspase-9, −3 and −7. Moreover, enhancement of ROS production has long been associated with the apoptotic response induced by anticancer agents [85,86]. Several natural compounds, such as phenolic phytochemicals used for cancer treatment have been shown to decrease the $\Delta\Psi m$ leading to the increased generation of intracellular ROS and apoptosis [85]. Studies therefore revealed that QTL acts as an antiproliferative agent via the overproduction of ROS and the loss of $\Delta\Psi m$.

Furthermore, previous *in vitro* anticancer studies of anticancer agents revealed that the increased ROS levels in primary cancer cells were associated with a decrease of antioxidants, such as SOD, GPx and catalase (CAT) [87]. It is also known that many types of human cancer cells can exist in a highly oxidative state due to the decreased levels of protective antioxidant enzymes as compared to their normal tissue counterparts [88]. Natural phytochemicals are known to deplete intracellular GSH and increase intracellular ROS to a level that can cause cell death [89,90].

Lycopene for the Prevention and Therapy of Prostate Cancer

Lycopene is a phytochemical that belongs to carotenoids. It is red, lipophilic and naturally occurring in many fruits and vegetables, with tomatoes and tomato-based products containing the highest concentrations of bioavailable lycopene. Several epidemiological studies have linked increased lycopene consumption with decreased prostate cancer risk [91,92]. These findings are supported by *in vitro* and *in vivo* experiments showing that lycopene not only enhances the antioxidant response of prostate cells, but that it is even able to inhibit proliferation, induce apoptosis and decrease the metastatic capacity of prostate cancer cells [91,92]. However, there is still no clearly proven clinical evidence supporting the use of lycopene in the prevention or treatment of prostate cancer, due to limited number of published randomized clinical trials and the varying quality of existing studies.

The interest on lycopene rich diets and supplements for the prevention or therapy of prostate cancer has extremely increased during the last years. Lycopene products are well tolerated and meet the requirements of the US Food and Drug Administration for the designation of Generally Recognized as Safe (GRAS).

Carotenoids are divided into two main groups; highly unsaturated hydrocarbons (consisting of lycopene, α-, β-, and γ-carotene), and xanthophylls, such as β-cryptoxanthin, lutein, and zeaxanthin. Xanthophylls are considered as the second big carotenoid-group. Lycopene with the molecular formula of $C_{40}H_{56}$, has an acyclic open-chain structure consisting of 13 double-bonds, two of them are non-conjugated, whereas eleven are conjugated double bonds, thereby building a chromatophore [93].

Anti-Oxidant Properties

Due to its polyene-structure, providing an electron-rich system, lycopene is an eligible target for electrophilic reagents. Thus, it performs an uttermost reactivity towards oxygen and free radicals [94]. Carotenoids like lycopene act as antioxidants through several mechanisms. Lycopene and other carotenoids are also known for their antioxidant activities towards impeding free radical reactions [94]. Peroxyl radicals are built during the process of lipid peroxidation, and inactivation of these reactive species results in the development of radical adducts

that build a resonance-stabilized carbon-centred radical [95]. Inhibition of these radical reactions by lycopene may shelter membranes from lipid peroxidation [95]. Furthermore, lycopene is able to repair vitamin E and C radicals *in vivo* [96].

Lycopene-Content in Different Sources

Natural sources of lycopene include watermelon, rosehips, pink grapefruit, guava, apricots and, above all, tomatoes [97]. As all tomato products contain high concentrations of lycopene, they are the most important source of this carotenoid for humans, accounting for over 85% of all dietary sources [97]. However, the lycopene concentration in fresh fruits shows a great variability, depending on environmental conditions, geographic location, climatic situation, species and maturity, but with an average of about 5 to 10 mg lycopene per 100 g tomato [98]. Up to 15 mg lycopene in 100g fruit has been found for deep-red tomato varieties, whereas yellow species are less rich in lycopene, with a content of only about 0.5 mg per 100 g tomato [98]. Processed and, therefore, less hydrated tomato products have been found to be richer in lycopene than whole, raw tomatoes [99].

Molecular Mechanisms of Lycopene on Prostate Cancer Cells

Prevention of DNA Damage

Androgen has been reported to induce an increase of ROS in prostate cancer cells [100]. Goo *et al*., [101] investigated changes in protein-expression of androgen-depleted and androgen-sufficient LNCaP (*hormone sensitive prostate cancer cell line*) by conducting quantitative proteomic analysis [101]. After treatment with 0.2 µM lycopene, they found increased numbers of detoxification proteins, such as epoxide hydrolase-1, which is involved in the hydrolysis of epoxides to transfer these proteins to less reactive species. Furthermore, the proteins, superoxide dismutase-1(which degrades radicals in cells) and catalase (which protects cells from hydrogen peroxide) have been found to be increased. Lycopene has been found to induce a moderate increase of these detoxification proteins in androgen-sufficient cell and a significant increase in androgen-depleted LNCaP cells. Matos *et al*., [102] induced oxidative damage to DNA in green monkey kidney fibroblasts, measured via the formation of 8-hydroxydeoxyguanosine (8-OHdG), which is a specific marker for oxidative DNA damage. Addition of lycopene in a concentration of 20 Pmol/10^6 cells has been shown to decrease 8-OHdG levels in DNA by 77%, thereby indicating a protective effect of lycopene against oxidation of DNA in mammalian cells [102,103].

Effects on Tumour Cell Proliferation and Growth

Kotake-Nara *et al*., [104] examined 15 different carotenoids with regard to the potential of growth inhibition to the prostate cancer cell lines, PC3, DU145, and LNCaP. The authors found significantly reduced cell viability after treatment with acyclic carotenoids at concentrations of 20 µmol/L. Another study, using a hexane extract of tomato paste resulted in a time- and dose-dependent decrease of cell proliferation of LNCaP cells, with maximal effects detected at a concentration of 5 µM lycopene [105]. After 48 hours of incubation, growth inhibition reached 67%. Ivanov *et al*., [106] compared two lycopene preparations, a 3% lycopene formulation from tomato powder (Lycopen™) and a tomato extract (LycoTrue™) to assess their potential to affect the proliferation of androgen-responsive LNCaP prostate cancer cells, as well as androgen-independent PC3 prostate cancer cells. They found a significant reduction in cell proliferation of LNCaP cells upon treatment with 1 µM or more Lycopen™.

Effects on the Cell-Cycle

Hwang *et al.,* [107] demonstrated that a tomato-paste extract induced arrest in both the G_0/G_1 phase and the G_2/M phase of the cell-cycle of LNCaP cells via flow cytometry. Effects have been detected after 24 h with treatment of 0.5 µM of the extract and increased with higher concentrations and expanded time periods. These results showed the efficiency of the tomato paste extract to inhibit tumour cell proliferation at physiological concentrations. Palozza *et al.,* [108] reported lycopene-induced cell-cycle arrest by describing molecular mechanisms [108]. After lycopene treatment, they revealed a reduced binding ability of NF-κB in LNCaP cells, which controls cell growth by influencing cell-cycle related proteins, and different apoptosis mediating proteins, like cyclin D1, p21, p27, p53, Bax or Bcl-2. A 24 hours exposure of lycopene resulted in a dose-dependent decrease of the G_0/G_1 phase-related protein, cyclin D1, and an increase in the cyclin kinase inhibitors, p53, p21 and p27 [108].

Potential to Induce Apoptosis

Hwang *et al.,* [107] detected apoptosis in the hormone sensitive prostate cancer cell line, LNCaP, after treatment with tomato paste extract for 24 and 48 h using an annexin V-FITC detection kit. Apoptosis has been detected predominantly in late stages, and most of the treated cells responded after 24 h of exposure to the tomato paste extract. Importantly, a significant increase in apoptosis has

been reported after treatment with concentration of 1 µM [108]. Exposure of LNCaP cells to physiologically relevant concentrations of lycopene induced a dose-dependent pro-apoptotic effect.

Other Effects of Lycopene on Prostate Cancer Cells

It has been shown that lycopene inhibits the androgen receptor element, resulting in decreased PSA velocity, and may, therefore, provide an anti-hormonal potential [48]. Lycopene might also have an impact on invasion and migration of prostate cancer cells by reducing the expression of integrins, which are known to be involved in signaling processes regarding adhesion and invasion. Bureyko *et al.,* [109] reported a decrease of $\alpha_2\beta_1$-integrin-expression in 22Rv1-, LNCaP- and PC3-cells upon lycopene exposure.

Additionally, lycopene has been shown to inhibit signaling of insulin-like growth factor-I (IGF-I) and, therefore, disrupts one pathway in the development of prostate cancer [110]. Signalling of IGF-I and IGF-II via their receptor, IGF-IR, facilitates survival and proliferation of cancer cells using PI3K/Akt and MAPK pathways [110]. After exposure of LNCaP cells to lycopene, a reduction of IGF-IR expression, as well as an Akt activation and an increase in the expression of insulin-like growth factor binding protein 2 (IGFBP2) has been shown. Combining docetaxel therapy with lycopene may, therefore, especially be effective in cancer types expressing high levels of IGF-IR activity [111]. The above-mentioned effects of lycopene are summarized in Fig. 4 [52].

Lycopene for the Prevention and Therapy of Prostate Cancer

Lycopene, with its abundant availability, low costs and lack of side effects, would be a suitable antitumorigenic drug, but there is still no clear clinical evidence whether to support or refute its use for the prevention or therapy of prostate cancer [112]. There are epidemiological studies [113,114] analysing the effects of tomato-based products on prostate cancer, but research that specifically investigates lycopene supplementation is limited. Most of the published studies have a low level of evidence, and there are only a limited number of published randomized clinical trials [115].

Attempts to recapitulate the antitumorigenic activity of lycopene in animal models have been in some parts highly significant and showed interesting data, suggesting the potential of lycopene to reduce tumour growth rate. However, the results have mainly been inconsistent [116]. Stringent animal models and a clear definition of the lycopene-preparations used are strongly recommended before results can be transferred to the clinical setting. Nevertheless, the use of lycopene in the clinic will still rest on findings derived from randomized controlled clinical trials. Recent systematic review could show that lycopene is able to decrease the serum PSA-levels in patients with prostate hyperplasia or cancer, demonstrating its effect on proliferating prostate cells [115].

Other dietary phytochemicals

In addition to the various dietary phytochemicals described above, some other dietary phytochemicals have shown epigenetic modulatory activities in various cancers. For example, allyl derivatives from garlic such as allyl mercaptan, diallyl disulfide, S-allylcysteine, S-allylmercaptocysteine and allicin inhibit HDAC activities in human cancer cells [117]. Grape seed proanthocyanidins (GSPs) are also bioactive phytochemicals that have been shown to have anti-cancer activities both *in vitro* and in *in vivo* models [118,119]. Treatment of A431 and SCC13 skin cancer cells with GSPs revealed inhibition of DNA methylation and histone modification leading to the activation of tumor suppressor genes [120].

In addition to the above described epigenetic modulatory phytochemicals, there are several other compounds for which some evidence of epigenetic modulation in cancer prevention and therapy is available but require more elaborative studies to pin-point their exact epigenetic modes of action [4,121]. Examples of these phytochemicals are apigenin, caffeic acid, anacardic acid and lycopene. However, all these dietary bioactive compounds induce epigenetic modulations to variable extents and with different patterns, indicating their potential in cancer chemoprevention.

ADVANTAGES, PRESENT CHALLENGES AND PROSPECTS OF EPIGENETIC CANCER CHEMOPREVENTION

Epigenetic therapy has emerged as a gleaming beam of hope in the field of cancer therapeutics. Epigenetic alterations are the early events during carcinogenesis, therefore understanding the course and nature of these alterations may help to set biomarker profiles for different cancers. Dietary phytochemicals have shown potential of modulating all the major epigenetic pathways such as DNA methylation, histone modifications and miRNAs [5]. These epigenetic alterations culminate into the alterations in the activity of cellular regulatory and metabolic pathways leading to loss of carcinogenicity of transformed cells. Natural dietary

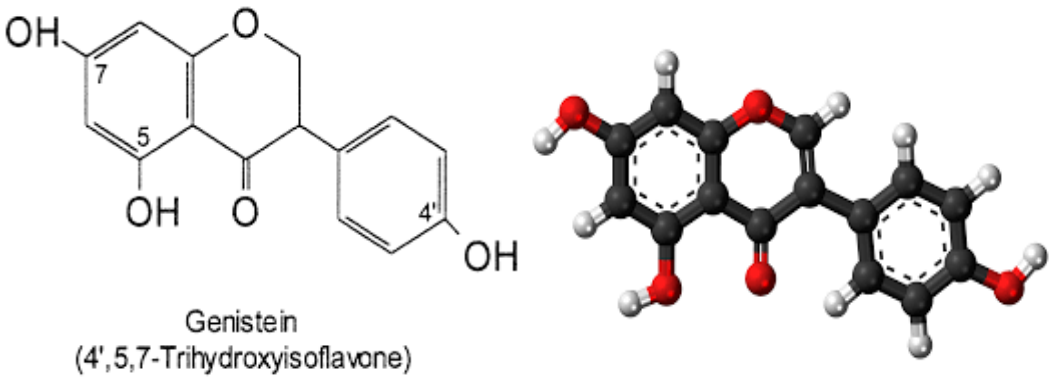

Figure 3: Chemical structure of genistein.

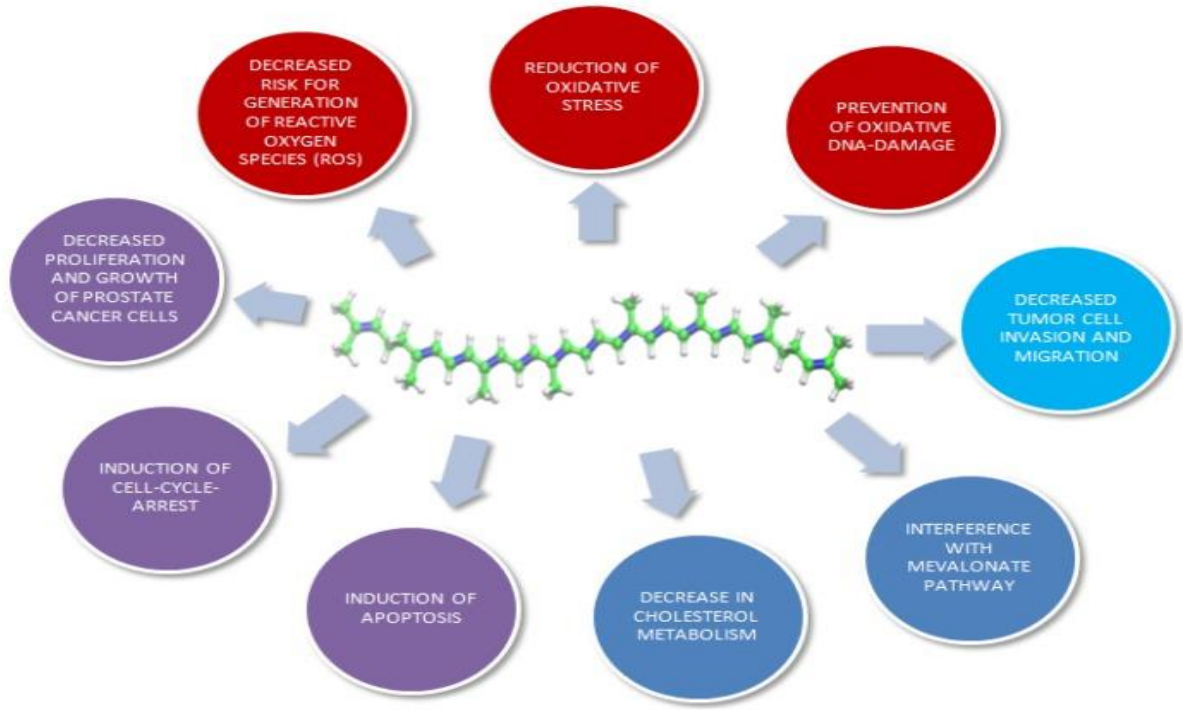

Figure 4: Potential effects of lycopene on prostate cancer cells.

phytochemicals cause lesser cytotoxicity to the normal cells and they are widely available. The dietary epigenetically active compounds have added advantage of cost-effectiveness, oral bioavailability and wide range of gene targets. In addition, they are also capable of functioning as sensitizers for chemotherapeutic drugs in the drug-resistant cancer types. The combination of chemotherapy and epigenetic therapy has shown promising results against multiple cancers [122,123].

In spite of several preclinical and clinical evidences supporting the potentiality of epigenetic therapy, there are multiple challenges which remain to be solved. Firstly, the dietary compounds function *via* multiple mechanisms. This lack of specificity places a major hindrance in the druggability of these compounds. Secondly, since the epigenetic processes are reversible, unnecessary reversal of dietary compound-mediated epigenetic modifications might prevent effective cancer therapy. Thirdly, although the epigenetically active compounds have shown better promises in chemoprevention, chemo-sensitization and maintenance therapy, their efficiency in monotherapy is compromised and variable among different cancers. Therefore, these compounds may not be good choices for the first-line cancer therapy. Fourthly, the early nature of epigenetic alterations requires early diagnosis of the disease to be treated by epigenetic therapy. Use of epigenetic therapy at later stages is not as effective due to accumulation of multiple genomic alterations in the tumor cells [123]. A better understanding of the global patterns of epigenetic modifications induced by dietary compounds can establish improved insights into the chemopreventive strategies and the potential of dietary phytochemicals to inhibit carcinogenesis. Development of early diagnostic techniques might also help in the prevention and therapy of cancer by epigenetically active dietary compounds/phytochemicals.

CONCLUSION

Dietary phytochemicals are of particular interest in the field of cancer prevention and therapy. Many of these phytochemicals establish their anti-cancer activities through multiple pathways and mechanisms. Currently, there is a greater focus on their epigenetic modulatory and gene regulatory activities due to the transgenerational nature and reversibility of these mechanisms of action. These characteristics, as well as their generally low toxicity, position these bioactive natural compounds as crucial cancer chemo-preventives. This review paper has provided a brief insight into the mechanisms of action of some selected dietary phytochemicals and their epigenetic targets, including the inhibition of DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) during the course of cancer prevention and therapy. Since cancer is a multi-stage process, it requires multi-targeted approaches for the development of an effective treatment regimen. Many of the bioactive phytochemicals possess more than one epigenetic target, and are capable of additive or synergistic effect in up-regulating tumor suppressor genes, down-regulating tumor promoters and/or down-regulating oncogenes in cancer. Furthermore, combinations of bioactive phytochemicals having two different epigenetic modulatory capacities or targets are needed as such strategies could represent a major advance in the development of effective therapeutic and preventive approaches against cancer.

This area of research requires further clinical studies, especially controlled randomized clinical trials, which will help to standardize the doses, routes of administration, organ specificity and bioavailability in humans. This present review indicates that the use of these bioactive natural compounds regularly in the diet can serve as a preventive approach, as well as therapeutic strategy for cancers of different organs and origins.

Therefore, there is need to facilitate interest in this area of research because it may provide a leeway to abate the rising incidence of cancers in our environment.

REFERENCES

1. Jones PA, Gonzalgo ML. Altered DNA methylation and genome instability: a new pathway to cancer? *Proceedings of the National Academy of Sciences*. 94, 1997: 2103-2105.
2. Esteller M. CpG island hypermethylation and tumor suppressor genes: a booming present, a brighter future. *Journal of Oncogenesis.* 21, 2012: 5427–5440.
3. Nugent M. MicroRNA function and dysregulation in bone tumors: the evidence to date. *Cancer Management Research.* 6, 2014: 15–25.
4. Link A, Balaguer F, Goel A. Cancer chemoprevention by dietary polyphenols: promising role for epigenetics. *Journal Biochemical Pharmacology.* 80, 2010: 1771–1792.

5. Meeran SM, Ahmed A, Tollefsbol TO. Epigenetic targets of bioactive dietary components for cancer prevention and therapy. *Journal of Clinical Epigenetics.* 1, 2010: 101–116.
6. Tollefsbol TO. Dietary epigenetics in cancer and aging. *Journal Cancer Treatment Research*. 159, 2014: 257–267.
7. Ratel D, Ravanat JL, Berger F, Wion D. N6-methyladenine: the other methylated base of DNA. Bioessays. *Journal of Biomedical Science*. 28, 2006: 309–315.
8. Reik W. Stability and flexibility of epigenetic gene regulation in mammalian development. *Journals of Biosciences.* 447, 2007: 425–432.
9. Leonhardt H, Page AW, Weier HU. A targeting sequence directs DNA methyltransferase to sites of DNA replication in mammalian nuclei. *Cell Biology*. 71, 1992: 865–873.
10. Lopez-Serra L, Ballestar E, Fraga MF, Alaminos M, Setien F, Esteller M. A profile of methyl-CpG binding domain protein occupancy of hypermethylated promoter CpG islands of tumor suppressor genes in human cancer. *Journal of Cancer Research*. 66, 2016: 8342–8346.
11. Fuks F, Burgers WA, Brehm A, Hughes-Davies L, Kouzarides T. DNA methyltransferase Dnmt1 associates with histone deacetylase activity. *Journal of Natural Genetics*. 24, 2014: 88–91.
12. Ambrosi C, Manzo M, Baube T. Dynamics and context-dependent roles of DNA methylation. *Journal of Molecular Biology*. 429, 2017: 1459–1475.
13. Muller A and Florek M. 5-Azacytidine/Azacitidine. *Recent Results Cancer Research*. 184, 2010: 159–170.
14. Momparler RL, Cote S, Momparler LF. Epigenetic action of decitabine (5-aza-2'-deoxycytidine) is more effective against acute myeloid leukemia than cytotoxic action of cytarabine (ARA-C). *Journal of Leukemia Research*. 37, 2013: 980–984.
15. Guo JU, Su Y, Zhong C, Ming GL, Song H. Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. *Cell Biology*. 145, 2011: 423–434.
16. Wang C, Fu M, Mani S, Wadler S, Senderowicz AM, Pestell RG. Histone acetylation and the cell-cycle in cancer. *Frontiers of Bioscience*. 6, 2001: D610–629.
17. Dalvai M and Bystricky K. The role of histone modifications and variants in regulating gene expression in breast cancer. *Journal of Mammary Gland Biology/Neoplasia*.15, 2010: 19–33.
18. Kim MS, Kwon HJ, Lee YM, Baek JH, Jang JE, Lee SW, Moon EJ, Kim HS, Lee SK, Chung HY, Kim CW, Kim KW. Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. *Journal of Natural Medicine.*7, 2001: 437–443.
19. Song C, Zhu S, Wu C, Kang J. Histone deacetylase (HDAC) 10 suppresses cervical cancer metastasis through inhibition of matrix metalloproteinase (MMP) 2 and 9 expression. *Journal of Biological Chemistry.* 288, 2017: 28021–28033.
20. Dong C, Yang G. Inhibition of matrix metalloproteins in attenuated Candida albicans induced inflammation in mouse cornea. *Journal of Cellular and Molecular Biology*. 62(12), 2018: 45.
21. Shukla S, Khan S, Tollefsbol TO, Meeran SM. Genetics and epigenetics of lung cancer: Mechanisms and Future Perspectives. *Current Cancer Therapy Reviews*. 9, 2016: 97–110.
22. Shukla S, Meeran SM. Epigenetic Factors in Breast Cancer Progression. In: AA, editor. Breast Cancer Metastasis and Drug Resistance. Springer Publications. 2013: 341–365.
23. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Natural Review of Genetics*. 10, 2009: 155–159.
24. Schonrock N, Harvey RP, Mattick JS. Long noncoding RNAs in cardiac development and pathophysiology. *Journal of Circulation Research*. 111, 2012: 1349–1362.
25. Kim DH, Villeneuve LM, Morris KV, Rossi JJ. Argonaute-1 directs siRNA-mediated transcriptional gene silencing in human cells. *Journal of Natural and Molecular Biology*. 13, 2006: 793–797.
26. Kim DH, Saetrom P, Snove O J, Rossi JJ. MicroRNA-directed transcriptional gene silencing in mammalian cells. *Proceedings of the National Academy of Sciences*.105, 2008: 16230–16235.
27. Luteijn MJ, Ketting RF. PIWI-interacting RNAs: from generation to transgenerational epigenetics. *Review of Genetics.* 14, 2013: 523–534.
28. He C. Grand challenge commentary: RNA epigenetics? *Journal of Natural Chemistry Biology*. 6, 2010: 863–865.
29. Motorin Y, Helm M. RNA nucleotide methylation. Wiley Interdisciplinary Review of RNA. *Journal of carcinogenesis.* 2, 2017: 611–631.
30. Boeing H, Bechthold A, Bub A, Ellinger S, Haller D. Critical review: vegetables and fruit in the

prevention of chronic diseases. *Europian Journal of Nutrition*. 51, 2012: 637-663.
31. Kotzekiduo P, Gainnakidis P, Boulamatsis A. Antimicrobial activity of some plant extracts and essential oils against food borne pathogens *in vitro* and on the fate of inoculated pathogens in chocolate. *Food Science and Technology Journal*. 41, 2008: 119-127.
32. Pérez-Jiménez J, Neveu V, Vos F, Scalber A. Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. *European Journal of Clinical Nutrition.* 64, 2010: 112–120.
33. Zhou Y, Zheng J, Li Y, Xu DP, Li S, Chen YM. Natural polyphenols for prevention and treatment of cancer. *Nutrients*. 8, 2016: 515.
34. Katiyar S, Elmets CA, Katiyar SK. Green Tea and Skin Cancer: Photo-immunology. Angiogenesis and DNA Repair. *Journal of Nutritional Biochemistry*. 18, 2007: 287–296.
35. Khan N, Mukhtar H. Modulation of signaling pathways in prostate cancer by green tea polyphenols. *Journal of Biochemical Pharmacology*. 85, 2013: 667–672.
36. Weng CJ, Yen GC. Chemo-preventive effects of dietary phytochemicals against cancer invasion and metastasis: phenolic acids, monophenol, polyphenol, and their derivatives. *Cancer Treatment Review*. 38, 2017: 76–87.
37. Meeran SM, Katiyar SK. Cell cycle control as a basis for cancer chemoprevention through dietary agents. *Frontiers of Bioscience*. 13, 2008: 2191–2202.
38. Nandakumar V, Vaid M, Katiyar SK. (−)-Epigallocatechin-3-gallate reactivates silenced tumor suppressor genes, Cip1/p21 and p16INK4a, by reducing DNA methylation and increasing histones acetylation in human skin cancer cells. *Journal of Carcinogenesis*. 32, 2011: 537–544.
39. Fang M, Wang Y, Ai N, Hou Z, Sun Y, Lu H, Welsh W, Yang C. Tea polyphenol (−)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. *Journal of Cancer Research*. 63, 2007: 7563–7570.
40. Meeran SM, Patel SN, Chan TH, Tollefsbol TO. A novel prodrug of epigallocatechin-3-gallate: differential epigenetic hTERT repression in human breast cancer cells. *Cancer Preview Research*. 4, 2011: 1243–1254.
41. Deb G, Thakur VS, Limaye AM, Gupta S. Epigenetic induction of tissue inhibitor of matrix metalloproteinase-3 by green tea polyphenols in breast cancer cells. *Journal of Molecular Carcinogenesis*. 34, 2016: 204-214.
42. Kato K, Long NK, Makita H, Toida M, Hara A, Mori H, Shibata T. Effects of green tea polyphenol on methylation status of RECK gene and cancer cell invasion in oral squamous cell cancinoma cells. *British Journal of Cancer*. 99, 2008: 647-654.
43. Kim SO, Kim MR. (−)-Epigallocatechin 3-gallate inhibits invasion by inducing the expression of Raf kinase inhibitor protein in AsPC1 human pancreatic adenocarcinoma cells through the modulation of histone deacetylase activity. *International Journal of Oncology*. 42, 2013: 349–358.
44. Moseley VR, Morris J, Knackstedt RW, Wargovich MJ. Green tea polyphenol epigallocatechin 3-gallate, contributes to the degradation of DNMT3A and HDAC3 in HCT 116 human colon cancer cells. *Journal of Anticancer Research.* 33, 2013: 5325–5333.
45. Li Y, Meeran SM, Patel SN, Chen H, Hardy TM, Tollefsbol TO. Epigenetic reactivation of estrogen receptor-alpha (ERalpha) by genistein enhances hormonal therapy sensitivity in ERalpha-negative breast cancer. *Journal of Molecular Cancer*. 12, 2015: 521-522.
46. Meeran SM, Patel SN, Li Y, Shukla S, Tollefsbol TO. Bioactive dietary supplements reactivate ER expression in ER-negative breast cancer cells by active chromatin modifications. *Public Library of science One*. 7, 2012: e37748.
47. Chen H, Landen CN, Li Y, Alvarez RD, Tollefsbol TO. Enhancement of cisplatin-mediated apoptosis in ovarian cancer cells through potentiating G2/M arrest and p21 upregulation by combinatorial epigallocatechin gallate and sulforaphane. *Journal of Ethnopharmacology*. 87(1), 2017: 29-57.
48. Myzak MC, Tong P, Dashwood WM, Dashwood RH, Ho E. Sulforaphane retards the growth of human PC-3 xenografts and inhibits HDAC activity in human subjects. *Journal of Experimental Biology and Medicine.* 232, 2015: 227–234.
49. Ho E, Clarke JD, Dashwood RH. Dietary sulforaphane, a histone deacetylase inhibitor for cancer prevention. *Journal of Nutrition*. 139, 2011: 2393–2396.
50. Meeran SM, Patel SN, Tollefsbol TO. Sulforaphane causes epigenetic repression of hTERT expression in human breast cancer cell lines. *Public Library of science One*. 5, 2010: e11457.

51. Hsu A, Wong CP, Yu Z, Dashwood RH, Ho E. Promoter de-methylation of cyclin D2 by sulforaphane in prostate cancer cells. *Journal of Clinical Epigenetics*. 3, 2011: 56-58.
52. Zhang X, Wang Q, Neil B, Chen X. Effect of lycopene on androgen receptor and prostate-specific antigen velocity. *China Medical Journal.* 123, 2010: 2231–2236.
53. Su YF, Yang YC, Hsu HK, Hwang SL, Lee KS, Lieu AS, Chan TF, Lin CL. *Toona sinensis* leaf extract has antinociceptive effect comparable with non-steroidal anti-inflammatory agents in mouse writhing test. *Biomedical Journal of Complementary and Alternative Medicine*. 15, 2015: 70.
54. Wong CP, Hsu A, Buchanan A, Houseman EA, Williams DE, Dashwood RH, Ho E. Effects of sulforaphane and 3,3'-diindolylmethane on genome-wide promoter methylation in normal prostate epithelial cells and prostate cancer cells. *Public Library of Science One.* 9, 2014: e867-87.
55. Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF, Walters MA. The Essential Medicinal Chemistry of Curcumin. *Journal of Medicinal Chemistry*. 60 (5), 2017: 1620–1637.
56. Fu S, Kurzrock R. Development of curcumin as an epigenetic agent. *Journal of Cancer Research*. 116, 2010: 4670–4676.
57. Chen Y, Shu W, Chen W, Wu Q, Liu H, Cui G. Curcumin, both histone deacetylase and p300/CBP-specific inhibitor, represses the activity of nuclear factor kappa B and Notch 1 in Raji cells. *Journal of Basic Clinical Pharmacology and Toxicology*. 101, 2007: 427–433.
58. Marcu MG, Jung YJ, Lee S, Chung EJ, Lee MJ, Trepel J, Neckers L. Curcumin is an inhibitor of p300 histone acetylatransferase. *Medical Chemistry*. 2, 2006: 169–174.
59. Wada TT, Araki Y, Sato K, Aizaki Y, Yokota K, Kim YT, Mimura T. Aberrant histone acetylation contributes to elevated interleukin-6 production in rheumatoid arthritis synovial fibroblasts. *Journal Biochemistry and Biophysics Research*. 444, 2017: 682–686.
60. Sun M, Estrov Z, Ji Y, Coombes KR, Harris DH, Kurzrock R. Curcumin (diferuloylmethane) alters the expression profiles of microRNAs in human pancreatic cancer cells. *Journal of Molecular Cancer and Therapy*. 7, 2008: 464–473.
61. Saini S, Arora S, Majid S, Shahryari V, Chen Y, Deng G, Yamamura S, Ueno K, Dahiya R. Curcumin modulates microRNA-203-mediated regulation of the Src-Akt axis in bladder cancer. *Cancer Preview Research*. 4, 2011:1698–1709.
62. Banerjee S, Li Y, Wang Z, Sarkar FH. Multi-targeted therapy of cancer by genistein. *Journal of Cancer research*. 269(4), 2008: 226–242.
63. Singh AV, Franke AA, Blackburn GL, Zhou JR. Soy phytochemicals prevent orthotopic growth and metastasis of bladder cancer in mice by alterations of cancer cell proliferation and apoptosis and tumor angiogenesis. *Journal of Cancer Prevention Research.* 66, 2006: 1851–1858.
64. Li Y, Chen H, Hardy TM, Tollefsbol TO. Epigenetic regulation of multiple tumor-related genes leads to suppression of breast tumorigenesis by dietary genistein. *Public Library of science One*. 8, 2013: e54369.
65. Zhang Y, Chen H. Genistein, an epigenome modifier during cancer prevention. *Journal of Epigenetics*. 67, 2011: 888–891.
66. Raynal NJ, Charbonneau M, Momparler LF, Momparler RL. Synergistic effect of 5-Aza-2'-deoxycytidine and genistein in combination against leukemia. *Journal of Oncology Research*. 17, 2008: 223–230.
67. Guerrero-Bosagna CM, Sabat P, Valdovinos FS, Valladares LE, Clark SJ. Epigenetic and phenotypic changes result from a continuous pre- and post-natal dietary exposure to phytoestrogens in an experimental population of mice. *Basic Medical Physiology*. 8, 2008: 17.
68. Qin W, Zhang K, Clarke K, Weiland T, Sauter ER. Methylation and miRNA effects of resveratrol on mammary tumors vs. normal tissue. *Journal of Nutrition and Cancer*. 66, 2017: 270–277.
69. Mao QQ, Bai Y, Lin YW, Zheng XY, Qin J, Yang K, Xie LP. Resveratrol confers resistance against taxol via induction of cell cycle arrest in human cancer cell lines. *Journal of Molecular and Nutritional Food Research.* 54, 2010: 1574–1584.
70. Vanamala J, Reddivari L, Radhakrishnan S, Tarver C. Resveratrol suppresses IGF-1 induced human colon cancer cell proliferation and elevates apoptosis via suppression of IGF-1R/Wnt and activation of p53 signaling pathways. *Journal of Cancer Research*. 10, 2010: 238.
71. Mirza S, Sharma G, Parshad R, Gupta SD, Pandya P, Ralhan R. Expression of DNA methyltransferases in breast cancer patients and to analyze the effect of natural compounds on DNA methyltransferases and associated

proteins. *Journal of Breast Cancer*. 16, 2013: 23–31.
72. Wu ML, Li H, Yu LJ, Chen XY, Kong QY, Song X, Shu XH, Liu J. Short-term resveratrol exposure causes *in vitro* and *in vivo* growth inhibition and apoptosis of bladder cancer cells. *Public Library of Science One.* 9, 2014: e898-906.
73. Yang H, Gu Q, Gao T, Wang X, Chue P, Wu Q, Jia X. Flavonols and derivatives of gallic acid from young leaves of *Toonasinensis* and evaluation of their anti-oxidant capacity by chemical methods. *Pharmacognosy Magazine*. 10, 2014: 185–190.
74. Liu HW, Huang WC, Yu WJ, Chang SJ. *Toonasinensis* ameliorates insulin resistance via AMPK and PPARγ pathways. *Journal of Food Function*. 6, 2015: 1855–1864.
75. Kakumu A, Ninomiya M, Efdi M, Adfa M, Hayashi M, Tanaka K, Koketsu M. Phytochemical analysis and antileukemic activity of polyphenolic constituents of *Toonasinensis*. *Journal of Bioorganic Medical Chemistry.* 24, 2014: 4286–4290.
76. Chang HL, Hsu HK, Su JH, Wang PH, Chung YF, Chia YC, Tsai LY, Wu YC, Yuan SS. The fractionated *Toonasinensis* leaf extract induces apoptosis of human ovarian cancer cells and inhibits tumor growth in a murine xenograft model. *Journal of Gynecological Oncology.* 102(3), 2006: 309–314.
77. Wang CY, Lin KH, Yang CJ, Tsai JR, Hung JY, Wang PH, Hsu HK, Huang MS. *Toona sinensis* extracts induced cell cycle arrest and apoptosis in the human lung large cell carcinoma. *Journal of Medical Science*. 26, 2010: 68–75.
78. Liu J, You L, Wang C, Liu R. Antioxidization and antiproliferation of extract from leaves of *Toonasinensis*. *Journal of Biomedical Science*. 37, 2012: 42–47.
79. Chen HM, Wu YC, Chia YC, Chang FR, Hsu HK, Hsieh YC, Chen CC, Yuan SS. Gallic acid, a major component of *Toona sinensis* leaf extracts, contains a ROS-mediated anti-cancer activity in human prostate cancer cells. *Journal of Oncology*. 286, 2009: 161–171.
80. Aghajanpour M, Nazer MR, Obeidavi Z, Akbari M, Ezati P, Kor NM. Functional foods and their role in cancer prevention and health promotion: A comprehensive review. *American Journal of Cancer Research.* 327(3), 2017: 740–769.
81. Redondo-Blanco S, Fernández J, Gutiérrez-Del-Río I, Villar CJ, Lombó F. New insights toward colorectal cancer chemotherapy using natural bioactive compounds. *Frontiers of Pharmacology*. 8, 2017: 109.
82. Mirzayans R, Andrais B, Kumar P, Murray D. Significance of wild-type p53 signaling in suppressing apoptosis in response to chemical genotoxic agents: Impact on chemotherapy outcome. *International Journal of Molecular Science*. 18, 2017: 18.
83. Fischer M, Quaas M, Steiner L, Engeland K. The p53-p21-DREAM-CDE/CHR pathway regulates G2/M cell cycle genes. *Journal of Nucleic Acids Research*. 44, 2016: 164–174.
84. Suzuki Y, Hasegawa H, Tsuji T, Tsuruda K, Sasaki D, Ishihara K, Nagai K, Yanagihara K, Yamada Y, Kamihira S. Relationships of diverse apoptotic death process patterns to mitochondrial membrane potential (Δψm) evaluated by three-parameter flow cytometric analysis. *Journal of Cytotechnology*. 65, 2013: 59–70.
85. Bishayee K, Ghosh S, Mukherjee A, Sadhukhan R, Mondal J, Khuda-Bukhsh AR. Quercetin induces cytochrome-*c* release and ROS accumulation to promote apoptosis and arrest the cell cycle in G2/M, in cervical carcinoma: Signal cascade and drug-DNA interaction. *Journal on Cell Proliferation*. 46, 2013: 153–163.
86. Kim J, Kim J, Bae JS. ROS homeostasis and metabolism: A critical liaison for cancer therapy. *Journal of Experimental and Molecular Medicine*. 48, 2016:e269.
87. AbdalDayem A, Hossain MK, Lee SB, Kim K, Saha SK, Yang GM, Choi HY, Cho SG. The role of reactive oxygen species (ROS) in the biological activities of metallic nanoparticles. *International Journal of Molecular Science*. 18(6), 2017:18.
88. Banerjee K, Mandal M. Oxidative stress triggered by naturally occurring flavone apigenin results in senescence and chemotherapeutic effect in human colorectal cancer cells. *Journal Redox Biology*. 5, 2015: 153–162.
89. Ferraresi R, Troiano L, Roat E, Lugli E, Nemes E, Nasi M, Pinti M, Fernandez MI, Cooper EL, Cossarizza A. Essential requirement of reduced glutathione (GSH) for the anti-oxidant effect of the flavonoid quercetin. *Journal of Free Radical Research*. 39, 2005:1249–1258.
90. Ramos AM, Aller P. Quercetin decreases intracellular GSH content and potentiates the apoptotic action of the antileukemic drug arsenic trioxide in human leukemia cell lines. *Journal Biochemistry and Pharmacology*. 75, 2008: 1912–1923.

91. Chen P, Zhang W, Wang X, Zhao K, Singh DN, Zhuo L, Qi M, Wang X, Zhang X. Lycopene and Risk of Prostate Cancer: A Systematic Review and Meta-Analysis. *Medicine*. 94(33), 2015: e1260.
92. Zu K, Mucci L, Rosner BA. Dietary lycopene, angiogenesis, and prostate cancer: a prospective study in the prostate-specific antigen era. *J ournal of National Cancer Institute.* 106, 2014: 430.
93. Shi J, Le Maguer M. Lycopene in tomatoes: Chemical and physical properties affected by food processing. *Critical Review of Biotechnology*. 20, 2000: 293–334.
94. Krinsky NI. The antioxidant and biological properties of the carotenoids. *Annual Review of Science*. 854, 1998: 443–447.
95. Stahl W, Sies H. Antioxidant activity of carotenoids. Molecular Aspects. Medicine. 24, 2003: 345–351.
96. Bast A, Haenen GR, van den Berg R, van den Berg H. Antioxidant effects of carotenoids. *International Journal of Vitamines and Nutural Resources.* 68, 1998: 399–403.
97. Mangels AR, Holden JM, Beecher GR, Forman MR, Lanza E. Carotenoid content of fruits and vegetables: An evaluation of analytic data. *Journal of American Dietry. Association*. 93, 1993: 284–296.
98. Hart DJ, Scott KJ. Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. *Journal of Food Chemistry.* 54, 1995: 101–111.
99. Tonucci LH, Holden JM, Davis CS, Mulokozi G. Carotenoid content of thermally processed tomato-based food-products. *Journal of Agriculture/Food Chemistry*. 43, 1995: 579–86.
100. Pathak SK, Sharma RA, Steward WP, Mellon JK, Griffiths TR, Gescher AJ. Oxidative stress and cyclooxygenase activity in prostate carcinogenesis: Targets for chemopreventive strategies. *European Journal of Cancer.* 41, 2005: 61–70.
101. Goo YA, Li Z, Pajkovic N, Shaffer S, Taylor G, Chen J, van Breemen RB. Systematic investigation of lycopene effects in LNCaP cells by use of novel large-scale proteomic analysis software. *Journal of Proteomics and Clinical Applications.* 1, 2007: 513–523.
102. Matos HR, di Mascio P, Medeiros MH. Protective effect of lycopene on lipid peroxidation and oxidative DNA damage in cell culture. *Archieves of Biochemistry and Biophysics*. 383, 2000: 56–59.
103. Karaham F, Dede S, Ceylan E. The effect of lycopene treatment on oxidative DNA damage of experimental diabe tic rats. *The Open Clinical Biochemistry Journal*. 9, 2018: 2588-778.
104. Kotake-Nara E, Kushiro M, Zhang H, Sugawara T, Miyashita K, Nagao A. Carotenoids affect proliferation of human prostate cancer cells. *Journal of Nutrition.* 131, 2001: 3303–3306.
105. Hwang ES, Bowen PE. Effects of tomato paste extracts on cell proliferation, cell-cycle arrest and apoptosis in LNCaP human prostate cancer cells. *Journal of Biochemistry and Molecular Biology*. 23(2), 2005: 97-107.
106. Ivanov NI, Cowell SP, Brown P, Rennie PS, Guns ES, Cox ME. Lycopene differentially induces quiescence and apoptosis in androgen-responsive and -independent prostate cancer cell lines. *Journal of Nutrition*. 26, 2007: 252–263.
107. Hwang ES, Bowen PE. Effects of tomato paste extracts on cell proliferation, cell-cycle arrest and apoptosis in LNCaP human prostate cancer cells. *Journal of Cancer Research*. 23, 2005: 75–84.
108. Palozza P, Colangelo M, Simone R, Catalano A, Boninsegna A, Lanza P, Monego G, Ranelletti FO. Lycopene induces cell growth inhibition by altering mevalonate pathway and Ras signaling in cancer cell lines. *Journal of Carcinogenesis*. 31, 2010: 1813–1821.
109. Bureyko T, Hurdle H, Metcalfe JB, Clandinin MT, Mazurak VC. Reduced growth and integrin expression of prostate cells cultured with lycopene, Vitamin E and fish oil *in vitro*. *British Journal Nutrition.* 101(2), 2009: 990–997.
110. Wertz K. Lycopene effects contributing to prostate health. *Journal of Nutrition and Cancer*. 61, 2009: 775–783.
111. Tang Y, Parmakhtiar B, Simoneau AR, Xie J, Fruehauf J, Lilly M, Zi X. Lycopene enhances docetaxel's effect in castration-resistant prostate cancer associated with insulin-like growth factor 1 receptor levels. *Journal Neoplasia*. 13, 2017: 108–119.
112. Rackley JD, Clark PE, Hall MC. Complementary and alternative medicine for advanced prostate cancer. Urology. *North America Clinical Journal.* 33, 2006: 237–246.
113. Peters U, Leitzmann MF, Chatterjee N, Wang Y, Albanes D, Gelmann EP, Friesen MD, Riboli E, Hayes RB. Serum lycopene, other carotenoids, and prostate cancer risk: A nested case-control study in the prostate, lung, colorectal, and

ovarian cancer screening trial. *Cancer Epidemiology Biomarker Preview.* 16, 2007: 962–968.
114. Kristal AR, Till C, Platz EA, Song X, King IB, Neuhouser ML, Ambrosone CB, Thompson IM. Serum lycopene concentration and prostate cancer risk: Results from the prostate cancer prevention trial. *Cancer Epidemiology/Biomark Preview*. 20, 2011: 638–646.
115. Ilic D, Misso M. Lycopene for the prevention and treatment of benign prostatic hyperplasia and prostate cancer: A systematic review. *Journal of Cancer Research*. 72, 2017: 269–276.
116. Sporn MB, Liby KT. Is lycopene an effective agent for preventing prostate cancer? *Journal of Cancer Prevention Research.* 6, 2016: 384–386.
117. Lea MA, Rasheed M, Randolph VM, Khan F, Shareef A, DesBordes C. Induction of histone acetylation and inhibition of growth of mouse erythroleukemia cells by S-allylmercaptocysteine. *Journal of Nutrition and cancer.* 43, 2002: 90–102.
118. Nian H, Delage B, Pinto JT, Dashwood RH. Allyl mercaptan, a garlic-derived organosulfur compound, inhibits histone deacetylase and enhances Sp3 binding on the P21WAF1 promoter. *Journal of Carcinogenesis*. 29, 2008: 1816–1824.
119. Mittal A, Elmets CA, Katiyar SK. Dietary feeding of proanthocyanidins from grape seeds prevents photocarcinogenesis in SKH-1 hairless mice: relationship to decreased fat and lipid peroxidation. *Journal of Carcinogenesis*. 24, 2016: 1379–1388.
120. Vaid M, Prasad R, Singh T, Jones V, Katiyar SK. Grape seed proanthocyanidins reactivate silenced tumor suppressor genes in human skin cancer cells by targeting epigenetic regulators. *Journal of Toxicol and Applied Pharmacology*. 263, 2012: 122–130.
121. Paluszczak J, Krajka-Kuzniak V, Baer-Dubowska W. The effect of dietary polyphenols on the epigenetic regulation of gene expression in MCF7 breast cancer cells. *Journal of Ethnopharmacology*. 192, 2010: 119–125.
122. Rong S, Hong-Xia W, Song S, Yang L, Yan-Hun Z, Wang J. Combination therapy with epigenetic targeted and chemotherapeutic drugs enhance the chemotherapy response overcome resistance by breast cancer stem cells. *Journal of Controlled Release.* 205, 2016: 7-14.
123. Dapeng H, Wang L, Wang H, Yuan W, Zhao Z, Peipei L, Deng C. Epigenetic targeting drug potentiate chemotherapeutic effects in solid tumor therapy. *Journal of Natural Research.* 7, 2017: 4035.