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Mini-review

Epigenetic regulation by selected dietary phytochemicals in cancer chemoprevention

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ABSTRACT

The growing interest in cancer epigenetics is largely due to the reversible nature of epigenetic changes which tend to alter during the course of carcinogenesis. Major epigenetic changes including DNA methylation, chromatin modifications and miRNA regulation play important roles in tumorigenic process. There are several epigenetically active synthetic molecules such as DNA methyltransferase (DNMTs) and histone deacetylases (HDACs) inhibitors, which are either approved or, are under clinical trials for the treatment of various cancers. However, most of the synthetic inhibitors have shown adverse side effects, narrow in their specificity and also 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. Encouragingly, many bioactive phytochemicals potentially altered the expression of key tumor suppressor genes, tumor promoter genes and oncogenes through modulation of DNA methylation and chromatin modification in cancer. These bioactive phytochemicals either alone or in combination with other phytochemicals showed promising results against various cancers. Here, we summarize and discuss the role of some commonly investigated phytochemicals and their epigenetic targets that are of particular interest in cancer prevention and cancer therapy.

Introduction

Carcinogenesis is considered to be the outcome of deregulated genetic and epigenetic events. Epigenetic alterations are important as they link the behavior 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. 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 remodeling, 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 lung, breast, head and neck as well as bone cancers [3–6].

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 more than the basic nutrients. These bioactive compounds have shown great potential against many diseases including cancers through genetic and epigenetic modifications [7–10]. In this review article, we focus on the major types of epigenetic modifications, such as DNA methylation, histone modifications and miRNA-mediated

Abbreviations: DNMT, DNA methyltransferase; MBD, methyl binding proteins; HDAC, histone deacetylase; HAT, histone acetyltransferase; EGCG, (−)-epigallocatechin-3-gallate.

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gene silencing in cancer progression, and epigenetic targeting by phytochemicals in cancer prevention and therapy.

DNA methylation

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 survival during carcinogenesis. Among epigenetic modifications, DNA methylation is the best studied modification of DNA [11]. 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. 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 [2]. Gene hypermethylation also might initiate recruitment of the methylation-dependent DNA binding proteins (MBDs) to the hypermethylated DNA sites. The MBDs 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 [12]. In addition to the MBDs, a transcriptional domain in DNMT1 also recruits histone deacetylase (HDACs) and other chromatin re-modeling proteins to the target sites that can modify acetylation and methylation status of histones, thereby inhibiting transcriptional access to the chromatin [13]. An example of such aberrant methylation-mediated gene silencing was demonstrated in ultraviolet-B (UVB) radiation-induced skin tumors in the SKH-1 mouse model. This study clearly demonstrated the positive correlation between transcriptional repression of tumor suppressor $p16^{INK4a}$ and *RASSF1A* genes and the UVB-mediated hypermethylation and subsequent recruitment of MeCP2 and MBDs at gene regulatory regions in skin cancer model *in vivo* [14]. MeCP2 is the founding member of the MBD family of transcriptional repressors, which creates a repressive environment at the target DNA site through recruitment of HDAC-containing transcriptional repressor complexes to the methylated DNA [15]. In addition, MeCP2 is also linked with higher H3K9 methylation, which is an important heterochromatin mark [16]. 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 [17,18]. 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 side effects or toxicities, are being tested for their role in direct or indirect inhibition of DNMTs activity in cancer prevention and therapy. DNMTs-mediated differential effects on promoter methylation and histone acetylation on gene regulation by bioactive phytochemicals are depicted in Fig. 1.

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 are classified into three families, the GCN5 N-acetyltransferase (GNAT), the MOZ/YBF2/SAS2/TIP60 (MYST) and the p300/CBP families [19,20]. HATs also play important roles in regulating cell cycle regulatory protein expression and also can bind directly to the cell cycle regulatory apparatus [21].

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 [20]. Class I, II and IV HDACs are zinc-dependent histone deacetylases, while class III HDACs, also known as sirtuins, are NAD^+-dependent HDACs. Different HDACs function in distinct ways and on different downstream targets leading to their diverse tumor suppressor and oncogenic activities. Overexpression and altered activities of HDACs are associated generally with the silencing of tumor suppressor genes, epithelial to mesenchymal transitions and metastasis [22–24]. Over-expression of *HDAC1* resulted in downregulation of *p53* and *von Hippel–Lindau* tumor suppressor genes and stimulated angiogenesis of human endothelial cells [22], while HDAC10 suppresses metastasis of cervical cancer through inhibition of matrix metalloproteinases 2 and 9 expression [25].

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) [26]. 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 [27]. Some other HDAC inhibitors such as Panobinostat, valproic acid and Belinostat are in different phases of clinical trials.

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. There now has been a tremendous increase in the knowledge concerning their mechanisms of actions and molecular targets.