Targeting Epigenetic Mechanisms: Potential of Natural Products in Cancer Chemoprevention

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Key words • Epigenetics

histone modifications

DNA methylation

histone acetylation

 received
 April 11, 2008

 revised
 June 6, 2008

 accepted
 June 20, 2008

Bibliography DOI 10.1055/s-2008-1081347 Planta Med 2008; 74: 1593-1601

© Georg Thieme Verlag KG Stuttgart · New York Published online August 14, 2008 ISSN 0032-0943

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Abstract

The term epigenetics is defined as heritable changes in gene expression patterns that occur without changes in DNA sequence. Epigenetic changes according to this definition are achieved by methylation of cytosine bases in the DNA and by histone modifications, such as acetylation, methylation or phosphorylation. These modifications play an important role in regulating gene expression and the existence of an epigenetic code which maintains these modifications even

Introduction

According to Crick and his "Central Dogma of Molecular Biology" [1] from 1970, DNA was supposed to be the only source of genetic information, running fluently from DNA to RNA and finally to proteins. But by now, many phenomena are known which cannot be explained by this "central dogma". It is remarkable that a single mammalian genome encoding about 30,000 genes is able to express different gene patterns in about 200 different cell types at different stages of development [2]. The function and morphology of liver cells, for example, differs completely from those of neural cells, although they have the same set of genes, and most importantly, this phenotype is maintained upon every division of the cells. So it is obvious that there has to be an additional layer of information encoded in or around the genome exceeding the information of the genetic sequence. This additional level of information is achieved by epigenetic modifications which in their entirety are called the epigenome (from the Greek prefix epi-, which means "on" or "over"). In 1999, Wolffe et al. defined the term "epigenetics" as "heritable changes in gene expression that occur without changes in DNA sequence" [3]. These heritable changes are achupon cell division has been underlined by many investigations. Targeting the enzymes which catalyze DNA methylation or histone modifications may be a possibility not only for cancer therapy but also for chemoprevention since disruption of epigenetic balance is known to cause diseases such as cancer. In this review, we want to present the key epigenetic targets. We highlight natural products that modulate these epigenetic mechanisms and show their potential for cancer chemoprevention.

ieved by methylation of cytosine bases in the DNA and by post-translational histone modifications, such as acetylation, methylation or phosphorylation. Epigenetic events occur throughout all stages of tumorigenesis and are accepted as important mechanisms in silencing tumor suppressor genes. Therefore, they are a key driving force in the development of cancer. We present here the major epigenetic targets and mechanisms of biological effects for inhibitors and their relationship to human cancer. We present inhibitors from natural sources and mainly focus on those which are of interest for cancer chemoprevention. Compounds like vitamins that also act rather remotely on epigenetic phenomena as well [4] will not be included. In most cases only links to cancer therapy are documented so far and we discuss selected examples with regard to that as well.

DNA Methylation

The methylation of cytosine bases in the mammalian genome by DNA methyltransferases occurs at cytosines located 5' to a guanosine as part of a CpG dinucleotide and in total about 70-80% of the CpG sites are methylated [4], [5],

[6]. CpG dinucleotides are found in short DNA regions with a length of 0.5 – 4 kb, which are known as CpG islands and which are located in the proximal promoter regions of about half of the genes in our genome. They represent about 1-2% of the genome and contain more than half of the unmethylated CpG sites [4], [5], [7], [8]. Other highly methylated sequences include satellite DNAs, repetitive elements, non-repetitive intergenic DNA and exons of genes [9]. In cancer cells the hypermethylation of certain promoter regions is known as one of the most important epigenetic changes taking place in tumors, leading to transcriptional silencing of tumor suppressor genes [10], [11], [12]. A large variety of genes are aberrantly methylated in cancer cells including genes that are involved in regulating DNA repair (e.g., BRCA1, MLH1), signal transduction (e.g., RASSF1), cell cycle (e.g., p16^{INK4a}, p15^{INK4b}), carcinogen metabolism (e.g., GSTP1), cell adherence (e.g., CDH1, CDH13), apoptosis (e.g., DAPK1, TMS1) or angiogenesis (e.g., THBS1) [11], [12]. But of course, DNA methylation is also an essential function in normal mammalian cells. It is involved in genomic imprinting (restriction of the expression of a gene to only one of the two parental chromosomes) [13] which is important for development. It is also necessary for Xchromosomal gene inactivation [14], the mechanism of dosage compensation in female mammals. The importance of DNA methylation for normal genome function could be shown by the finding that a homozygous mutation of the DNA methyltransferase gene results in embryonic lethality [15]. Thus, unwanted activation of certain genes by DNA methyltransferase inhibitors may pose a risk, e.g., by derepression of proinvasive genes [16]. DNA methyltransferases (DNMTs) use S-adenosyl-Lmethione (SAM) as methyl group donating cofactor. So far, four DNMTs are known in mammals, called DNMT1, DNMT2, DNMT3A and DNMT3B [17], [18], [19].

Histone Modifications

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The structure of chromatin plays an important role in gene expression. Chromatin is a macromolecular complex existing of DNA, histone and non-histone proteins, and is responsible for the controlled storage of the genetic information within the nucleus [20]. The basic repeating units of chromatin are the nucleosomes that consist of 147 bp of DNA wrapped around a histone octamer, which is composed of an H3/H4 tetramer and two H2A/ H2B dimers [21]. This alignment of nucleosomes is compacted into a fiber of 30 nm, which is then further condensed to form the chromosomes. This higher order folding is stabilized by the linker histone H1 [22]. There are different levels of chromatin organization and subsequently transcriptional activity. Usually condensed, inaccessible chromatin ("heterochromatin") mediates transcriptional repression, while transcriptionally active genes are in regions of the more open and accessible "euchromatin" [23]. Importantly, histones have amino-terminal tails protruding out of the nucleosomes. These tails are open for posttranslational modifications, such as acetylation, methylation, phosphorylation, sumoylation and ubiquitination. [24] To this date at least eight different histone modifications, taking place mainly at the N-terminal histone tails of histone H3 and histone H4, have been documented [25]. These modifications result in changes in the accessibility of the DNA to transcription factors and in protein interactions determining the chromatin structure [26]. So, chromatin is highly dynamic and can change its structure in response to cellular signals, which in turn affects gene expression [27]. Because of the diverse and complex nature of histone modifications, which interact with each other, the term "histone code" has been coined. The modifications of histone proteins are also linked with DNA methylation, as DNA methyltransferases recruit histone deacetylases (HDACs), leading to histone deacetylation and subsequently to transcriptional repression.

Several in vitro studies have shown the cooperation of DNA methylation and histone deacetylation in order to repress transcription [28], [29]. Therefore, it is not surprising that the combination of DNMT and HDAC inhibition has been shown to induce differentiation, apoptosis and cell growth arrest in several cancer cell lines [30]. This synergistic effect is seen in the increase in expression of specific cancer-related genes. For example, the re-expression of the hypermethylated genes MLH1, TIMP3, CDKN2B and CDKN2A was greatly enhanced when histone deacetylase inhibitors were combined with low doses of an inhibitor of DNA methyltransferases, whereas HDAC inhibition alone was not able to reactivate transcription [31]. The expression of the cyclooxygenase 2 gene, whose promoter is hypermethylated in gastric cancer cells, can be restored with a combined treatment of DNMT and HDAC inhibitors, while DNMT inhibition itself only induces partial re-expression [32]. More links between the "histone code" and the "cytosine methylation code" are becoming evident [33].

Certain post-translational modifications on distinct amino acids in the N-terminal tails of histones have been linked to either active or repressed transcription. The acetylation of certain lysine residues by histone acetyltransferases (HATs), for example, is associated with transcriptionally active regions, whereas transcriptionally repressed chromatin usually is hypoacetylated [34]. But there are also examples where histone hyperacetylation has been shown to lead to gene repression [35]. For other modifications on the histone tails examples have been provided for both activators and inhibitors of transcription. The methylation of lysine residues leads either to transcriptional activation or repression, depending on the site of lysine methylation [36]. As there are different states of methylation (mono-, di- or trimethylation) possible for one lysine residue, the biological consequences of methylation may differ [37]. Although the whole process of selection of histone marks along our genome is not fully understood, it is obvious that the enzymes, which catalyze post-translational histone modifications, are actively involved in carcinogenesis. It has been shown that global loss of monoacetylation and trimethylation of histone H4 is a common hallmark of human tumor cells [38]. Another example is the correlation of phosphorylated H3T11 with Gleason scores of prostate carcinomas [39].

Epigenetic Targets and their Inhibitors ▼

DNA methyltransferases

In contrast to those tumor suppressor genes which are inactivated by genetic alterations, the genes that are silenced due to epigenetic modifications are still intact and can be reactivated by intervention of small molecules acting as modifiers of epigenetic mechanisms. For example, many genes that are hypermethylated in cancer can be reactivated with inhibitors of DNA methyltransferases [40]. Several DNA methyltransferase inhibitors have been developed so far. Analogues of cytidine, such as 5-azacytidine or 5-aza-2'-deoxycytidine have long been known for their ability of inhibiting DNA methyltransferases [40], [41]. Several studies have shown the ability of DNA methyltransferase inhibitors to prevent cancer using different pathways [42], [43], [44], [45]. There are also some natural products that were identified as inhibitors of DNA methyltransferases, thus having potential in preventing cancer, too. For example, some disulfide bromotyrosine derivatives, such as psammaplin A, isolated from the sponge Pseudoceratina purpurea, were found to be potent inhibitors of DNMT1 but also histone deacetylases [46]. (-)-Epigallocatechin 3-gallate (EGCG), a main polyphenol from green tea, inhibits DNA methyltransferases and can thereby reactivate silenced genes like P16^{INK4a} and hMLH1 in tumor cells [47]. EGCG is a pleiotropic drug that has received a lot of attention for cancer chemoprevention but it remains to be determined which of its biological activities are the most important for its preventive abilities [48]. Two other polyphenols from coffee, caffeic acid and chlorogenic acid, have also been reported to be inhibitors of DNMT1 in the low micromolar range [49] and also apple polyphenols have similar activity [50]. The major isoflavone from soy bean, genistein, has been reported to prevent cancer in animal models [51]. The effects of genistein have been studied in different cancer cell lines, where it was able to inhibit cancer cell growth [52], to induce apoptosis and cell cycle arrest and to inhibit angiogenesis [53], [54], but the exact mechanisms behind these effects are not fully understood so far. Several mechanisms, such as estrogenic and antiestrogenic effects, are discussed to explain this activity, but one possible mechanism of particular interest is the regulation of transcriptional activity by modulating DNA methylation. Genistein inhibits methylation of DNA and thus can lead to re-expression of methylation-silenced genes, such as $RAR\beta$, p16INK4 and MGMT in the esophageal squamous cell carcinoma cell line KYSE 510 [55]. As the histone acetylation status is also known to have an impact on the re-expression of these genes, the weak inhibition of histone deacetylase activity observed after treatment of the cells with genistein is possibly contributing to the gene reactivation, too. Biochanin A and daidzein, two other isoflavones, are weaker inhibitors of DNMTs and as they also are less effective in reactivating the *RAR*β gene, a direct correlation between the inhibition of DNA methyltransferases and the reactivation of the silenced genes can be assumed [55].

Reversible histone acetylation

The steady state of reversible protein acetylation is maintained by a dynamic equilibrium between histone acetyltransferases (HATs) and histone deacetylases (HDACs). The effect of these chromatin modifying enzymes on gene expression has been studied widely. In 1964, Allfrey was the first to suggest that acetylation of histones was involved in regulation of transcription [56]. HAT enzymes can be classified into several groups, including the GNAT family (e.g., GCN5, PCAF), the MYST group (e.g., Tip60), the p300/CBP family, the SRC group and the TAFII250 family [57]. The histone deacetylases can be divided into four classes [58]. The HDACs of class I, which have homology to the yeast HDAC Rpd3 [59], are located in the nucleus, where they interact with transcriptional repressors and cofactors. This class I includes HDAC1, HDAC2, HDAC3 and HDAC8 [60], HDAC11 was first classified as a class I enyzme but lately is attributed a class of its own (class IV HDAC [61]). The HDACs of class II are able to shuttle between the cytoplasm and the nucleus and show homology to the yeast deacetylase HdaI [62]. Class II histone deacetylases include HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10. Interestingly, one of these deacetylases, HDAC6 has

two catalytic domains, one for histone deacetylation, one for deacetylation of tubulin [63], showing that HDACs, as well as HATs, also target non-histone protein substrates. The histone deacetylases of classes I, II and IV have a zinc ion at the base of their catalytic pocket and a hydrophobic pocket that allows the acetylated lysine residue to insert.

The third class of histone deacetylases is called Sirtuins after their homology to the yeast silent information regulator 2 (Sir2) [64], [65], [66]. They differ completely from the zinc-dependent HDACs of classes I, II and IV in their mode of action in removing the acetyl group from lysine residues, as they are dependent on nicotinamine adenine dinucleotide (NAD⁺). For class III HDACs also a large number of non-histone substrates, such as p53 or tubulin have been reported [65], [66], [67].

Modulators of histone deacetylases

The first inhibitors of histone deacetylases classes I and II were isolated from natural sources and based upon those, a variety of synthetic inhibitors have been developed. The general structure contains a binding region responsible for enzyme specifity, which is separated by a spacer from a group that effects inactivation of the enzyme [68], [69]. This inactivating group needs to be able to bind the zinc ion in the active site, often referred to as the zinc binding group (ZBG). The HDAC inhibitors are classified into different groups depending on their structure, including (a) small-chain fatty acids, (b) hydroxamic acids, (c) cyclic tetrapeptides, (d) benzamides and others that do not fit in these classifications [70], [71].

The largest of these groups of HDAC inhibitors is that which carries a hydroxamic acid as the zinc binding group with the natural product trichostatin A (TSA) as lead structure (see • Fig. 1 for selected inhibitor structures). TSA, isolated from *Streptomyces hygroscopicus*, inhibits HDACs of group I and group II in the nanomolar range and induces differentiation, cell cycle arrest and apoptosis [72]. It has been reported that HDAC inhibition can affect changes in gene expression of genes that have an impact on apoptosis and the cell cycle, such as p21^{Cip1/Waf1}, cyclins (A, E, B1, D1 and D3), apoptosis mediators (e.g., CD95, Bax, Bcl-2), transcription factors (e.g., GATA-2, c-Myc) and retinoic acid receptors (RAR) [73], [74].

Acute promyeolocytic leukemia (APL) is one disease of particular interest concerning RAR as targets of retinoids, as APL patients respond with disease remission, when treated with pharmacological doses of retinoic acid. In APL, the RAR/PML oncogenic transcription factor binds to the retinoic acid response element (RARE), recruiting the CoR/SIN3/HDAC complex and repressing transcription. Agonists, such as retinoic acid, displace CoR/SIN3/ HDAC by interacting with the RAR and thereby activate gene expression in association with HATs [71]. Therefore, it is apparent that the therapeutic efficacy in cases of APL can be improved, when synthetic or naturally occurring retinoids are combined with inhibitors of histone deacetylases [75].

Inhibitors of HDACs are able to disrupt the cell cycle in the G_2 phase, so that the cells enter the M phase prematurely and furthermore they have been reported to interfere directly with the mitotic spindle checkpoint [76]. Cell cycle arrest and/or apoptosis are induced by the activation of repressed genes, such as *P21* and *BAX*. Strikingly, tumor cells seem to be more sensitive to the actions of HDAC inhibitors than normal cells. The mechanisms behind this "cancer selectivity" are not fully understood, but it has been reported that thioredoxin, the intracellular thiol status and the accumulation of reactive oxygen species as well as the



Fig. 1 Structures of important epigenetic modifiers.

induction of TRAIL, DR4 and DR5 could be responsible for it [77], [78]. Some HDAC inhibitors have been reported to be able to lead to an increasing level of CD95/CD95 L [79] and FAS/FASL [80], consequently leading to receptor-mediated apoptosis. HDACs interact directly with the tumor suppressor protein p53 by removing acetyl groups from its C-terminal tail, resulting in decreased transactivation and on the other hand, HDAC inhibitors can lead to an increase in acetylation levels of the *p53* gene and therefore to an increase of transcriptional activity [71].

So far, there are several studies that have tried to point out the chemopreventive potential of HDAC inhibitors [81], [82], [83]. For example, suberoylanilide hydroxamic acid (SAHA), a synthetic inhibitor of zinc-dependent HDACs, decreases the incidence of *N*-methyl-*N*-nitrosourea-induced rat mammary tumors and the multiplicity of carcinogen-induced lung tumors in mice [83]. SAHA (INN: Vorinostat, Zolinza[™]) has been approved for the treatment of cutaneous T-cell lypmphoma by the FDA [84]. Although inhibitors of histone deacetylases have a direct impact on tumor cells, it is discussed that interaction with the cancer cell environment also plays a role in chemoprevention. In case of metastasis, for example, the primary tumor needs vasculari-

zation to supply nutrients and oxygen, before it is able to finally metastasize. Hypoxia, one of the factors inducing angiogenesis, is known to increase expression levels and activity of HDAC1 [85] and therefore it has been hypothesized and already been demonstrated that inhibition of HDAC1 can lead to inhibition of angiogenesis *in vitro* and *in vivo* [86]. Another pathway leading to cancer prevention is the up-regulation of stimulary factors of the immune system. HDAC inhibition induces expression of CD40, CD80 and CD86, as well as major histocompatibility (MHC) proteins of class I and class II and interferons [71], thus making it more difficult for tumor cells to survive.

Remarkably, HDAC inhibitors have a large variety of targets in different stages of tumorigenesis and therefore are promising candidates both in therapy and in prevention of cancer. Therefore, it is not surprising that there are several HDAC inhibitors in clinical trials, tested against different kinds of tumors [87]. Among them is the natural product FK-228 (also known as FR-90122 or depsipeptide) which is in clinical trials for the treatment of cancer (INN: romidepsin) [88]. Some natural products that have been reported to have potential in cancer prevention acting as inhibitors of histone deacetylases shall be discussed in the following.

Butyrate, a short-chain fatty acid, which inhibits HDAC activity at high micromolar concentrations, is formed in the digestive tract by fermentation of dietary fiber. Butyrate affects different signalling pathways [89], [90], suggesting multiple mechanisms of action, of which not all involve alteration in histone acetylation. But it is apparent that butyrate modulates transcriptional regulation in a similar manner as other HDAC inhibitors do, with similar consequences concerning cellular differentiation, cell cycle arrest, apoptosis, invasion and metastasis. Like other HDAC inhibitors, butyrate is able to induce apoptosis by a mechanism involving the activation of caspases and decreased levels of Bcl-2 [91]. Apoptosis mediated by activation of death receptors has also been proposed for butyrate, with increasing death receptor 5 (DR5) expression, a receptor for TRAIL, consequently leading to activation of caspase 8 and caspase 10 [92]. Additionally, butyrate inhibits tumor invasion and metastasis, as it induces expression of proteins that are known to inhibit invasion in a cell culture model [93] and reduced liver metastasis of rat colon cancer cells in vivo and resistance to oxidative stress in vitro [94]. Although therapeutic intervention with butyrate is not promising, an ongoing exposure stemming from the microbial degradation of dietary fiber in the colon could have chemopreventive effects, at least in part by histone deacetylase inhibition [95]. Ester prodrugs of butyrates might have potential for an improvement of fatty acid based HDAC inhibitors [96].

Diallyl disulfide (DADS) is an organosulfur compound, which is found in garlic and other *Allium* species. Upon administration of DADS, differentiation, cell cycle arrest and apoptosis has been demonstrated in several cell lines [97], for example, by inducing a G2/M arrest in different cancer cell types, such as colon and neuroblastoma cells and by increasing the expression levels of BAX in human lung and breast cancer cells [98], [99]. *S*-Allylmercaptocysteine, a metabolite of DADS, has also been shown to have an effect on the levels of histone acetylation. It induced growth arrest in human colon and breast cancer cells [100] and also a G2/M arrest, as well as apoptosis in human colon cells has been described [101]. Inhibition of histone deacetylases seems to be a general mechanism for cancer prevention by organosulfur agents from garlic or other *Allium* vegetables. Sulforaphane (SFN), an isothiocyanate present in cruciferous vegetables, such as broccoli, has been made responsible for several chemopreventive mechanisms [102]. For example, SFN increases histone H4 acetylation in P21 promoter regions, subsequently leading to an increasing expression of p21 in HCT116 cells. In addition, the higher acetylation levels achieved by SFN led to G2/M arrest [103] and apoptosis. Remarkably, the metabolite SFN-cysteine and not the parent compound itself, is the active, HDAC-inhibiting principle. [104]. Interestingly, it was demonstrated that a combination of sulforaphane and the DMNT-inhibiting isoflavone genistein enhances the reactivation of the DNA methylation-silenced genes p16INK4a and MGMT [55], implicating synergistic effects. Recently, ACS 2, a sulfurated derivative of valproic acid (VPA), has been demonstrated to show HDAC inhibition [105]. It displayed much stronger inhibition of deacetylase activity than VPA, which is also known as an HDAC inhibitor. Also its sulfur containing moiety, 5-(4-hydroxyphenyl)-3H-1,2-dithiole-3-thione, a metabolite of anethole trithione is contributing to this inhibition of HDACs. Anethole trithione has shown potential in clinical trials for lung cancer prevention [106]. Inhibition of sirtuins has been shown for hyperforin and derivatives [107] but it is unclear whether the antiproliferative activity of these compounds can be tied to sirtuin inhibition. Resveratrol, a polyphenol from grapes, has received a lot of attention in cancer chemoprevention [108]. It has been postulated to be an activator of sirtuins [109] but there is discussion about whether these effects are artefacts from the assay procedure [110]. Sirtuin activators have demonstrated anti-inflammatory effects, e.g., decreased release of TNF- α , that make them interesting for the prevention of damages by metabolic diseases [111], [112] which could also play a role for cancer chemoprevention. But again, it will be difficult to dissect the decisive effects for such an activity in a pleiotropic drug like resveratrol.

Inhibition of histone acetyltransferases

Although direct cancer prevention of specific modulators of histone acetyltransferases has not been demonstrated so far, they have shown their capability in cancer therapy, as they are involved in transcriptional regulation. Several studies support the assumption that HATs, such as p300/CBP, act as transcriptional integrators for physiological cues to coordinate regulation of cell cycle, differentiation, DNA repair and apoptosis [113], [114]. Taking the inhibitors of HDACs as an example, it is possible that HAT inhibitors have potential in cancer prevention as well.

Different types of inhibitors of histone acetyltransferases have been reported and also several natural products have shown their ability to inhibit HATs. For example, anacardic acid, found in the apple of the cashew (*Anacardium occidentale*), shows antitumor activity due to an inhibition of p300 and PCAF in the low micromolar range [115], [116], with the problem, however, that cells are poorly permeable for this compound. Garcinol, a polyisoprenylated benzophenone derivative from *Garcinia indica* fruit rind, has been described as a cell-permeable HAT inhibitor, showing inhibition towards p300 and PCAF [117]. Another natural compound, which has been shown to specifically inhibit p300/CPB, is curcumin, a major curcuminoid in turmeric [118]. But again, curcumin is a pleiotropic agent with numerous possible mechanisms that could contribute to a chemopreventive effect [48].

Ursodeoxycholic acid (UDCA, ursodiol), a tertiary bile acid used in treatment of biliary cirrhosis and ulcerative colitis has been demonstrated to have potential in colon cancer prevention in preclinical and in animal models [119], [120], but the mechanisms behind it were unknown. Recently, it has been shown that UDCA induces differentiation and senescence by modulating histone acetylation [121]. But in contrast to HDAC inhibitors, UDCA induces hypoacetylation of histones like HAT inhibitors would do, although histone acetylase activity is not affected directly by UDCA *in vitro*. Further investigation revealed that HDAC6 is up-regulated in cells treated with ursodeoxycholic acid [121]. Not only because of this finding has it already been suggested that modulation of HAT or HDAC activity in any direction might induce differentiation and senescence [121]. At present, a randomized phase II clinical trial is ongoing, comparing UDCA to acetylsalicylic acid or sulindac for its activity in preventing colorectal cancer [122].

Modulation of histone methylation

Histone methylation is another widely described modification, taking place at lysine and arginine residues. Histone methylation marks play a role in regulation of transcriptional activity as well. Methylation of histones, for example, has been shown to be important for the establishment of a checkpoint control following DNA damage [123]. The methylation of arginine residues contributes to both transcriptionally active and repressed genes and is catalyzed by protein arginine methyltransferases (PRMTs) [124], [125], [126]. Arginine methylation generally is correlated with transcriptional activation and can occur in three different states, monomethylated, symmetrically dimethylated or asymmetrically dimethylated. PADI4, a peptidyldeiminase is able to remove methyl groups from methylated arginines, leading to citrulline [127]. But because of the fact that unmethylated arginines are deiminated to citrullines as well, the role of PADI4 in antagonizing histone arginine methylation needs further clarification. Nevertheless, it is clear that demethylimination of arginines contributes to regulation of transcription. Importantly, PADI4 is not able to demethylate dimethylated arginines. Recently, a new enzyme with arginine demethylase activity has been discovered [128]. This Jumanji domain-containing protein 6 (JMJD6) is an iron- and α -ketoglutarate-dependent dioxygenase that demethylates histone H3 at arginine 2 (H3R2) and histone 4 at arginine 3 (H4R3). In contrast to the deiminase PADI4, JMJD6 is able to remove methyl groups from dimethylated arginines [128].

The methylation of lysine residues can lead either to activation or repression of gene expression, depending on the particular histone lysine residue [36]. Methylation of H3K4, H3K36 and H3K79 are associated with transcriptional activation [129], whereas methylation of H3K9, H3K27 and H4K20 are linked to repressed genes [130]. As mentioned above, also the methylation state (mono-, di- or trimethylation) is variable, having different effects on transcription [37]. Histone lysine methylation is carried out by a family of proteins containing a SET (suppressor of variegation, enhancer of zeste and trithorax) domain and by the non-SET-domain proteins DOT1/DOT1 L [131], [132]. All lysine methyltransferases use S-adenosyl-L-methionine (SAM) as the cofactor. Until recently, it was assumed that histone lysine methylation was an irreversible modification, although a group led by Paik had described a demethylating activity in rat kidney already in 1964 [133]. But this activity was never linked to a particular enzyme. In 2004, the assumption of "permanent methylation" really was shaken, when the lysine specific demethylase 1 (LSD1), known as a component of several histone deacetylase complexes, was identified as an amine oxidase which selectively removes methyl groups from H3K4 (mono- or dimethylated)

[134]. Interestingly, LSD1 can change its selectivity to H3K9 (mono- or dimethylated) when it interacts with the androgen receptor [135]. Caused by its monoamine oxidase (MAO) type mechanism of the demethylation reaction, LSD1 is not able to demethylate trimethylated lysine residues. Hence it was assumed that there have to be demethylases that operate by a different mechanism. An oxidation reaction similar to that of AlkB from E. coli, a demethylating DNA repair enzyme was predicted [136]. AlkB is a α -ketoglutarate- and iron-dependent dioxygenase which hydroxylates methyl groups from damaged DNA leading to the release of formaldehyde. In search for histone lysine demethylating enzymes with a similar mechanism, the group of the already mentioned Jumanji domain-containing (JmjC) enzymes was identified [137]. In fact, the members of this family are able to demethylate trimethylated lysine residues in an oxidation reaction also dependent on Fe(II) and α -ketoglutarate.

So far, only few small molecule modulators of the histone lysine demethylating enzymes have been described. But evidence suggests that LSD1 is a promising target both in cancer prevention and in therapy. It is up-regulated in mammary epithelial cells that have been exposed to dietary and environmental carcinogens, resulting in the assumption that induction of LSD1 represents an early response to carcinogen exposure [138]. It has also been reported that inhibition of LSD1 in colon carcinoma cells results in re-expression of aberrantly silenced genes, which are important in the development of colon cancer [139]. The colocalization of LSD1 and the androgen receptor in androgen-dependent tissue led to investigations showing that LSD1 stimulates androgen-receptor-dependent transcription [135]. JMJD2C, a member of the Jumanji domain-containing enzymes has been reported to regulate the androgen receptor as well [140]. It was found that JMJD2C colocalizes with LSD1 and the androgen receptor and that both demethylases cooperatively stimulate androgen receptor-dependent gene expression [140]. Thus, modulation of the activity of LSD1 and JMJD2C represents novel strategies to regulate androgen receptor functions and accordingly control specific gene expression. Therefore, the inhibition of LSD1 and the JmjC proteins will be interesting options in cancer prevention and therapy.

Perspectives

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Epigenetics is an emerging field in cancer research, as the impact of DNA methylation and histone modifications on transcriptional activity is becoming more and more clear. Thus, the search for agents that target these mechanisms in order to elucidate the role of DNA methyltransferases and histone modifying enzymes in cancer development is being pursued with increasing efforts. Natural products can be important tools to further characterize the chromatin-modifying enzymes. In many cases they are the first agents identified as inhibitors or modulators of the particular enzyme and thereby serve as lead structures for new druglike compounds or may be drug candidates themselves.

In this review we have presented natural products that target epigenetic mechanisms, more precisely inhibitors of DNA methylation and modulators of histone acetylation and histone methylation. But as mentioned above, there are other modifications to histones, such as ubiquitinylation [141], sumoylation [142] and poly-ADP-ribosylation [143] which have already been associated with altered transcription as well. Natural and chemical products acting as novel small molecule inhibitors of the enzymes involved in these modifications will help us to further improve our understanding of the epigenetic mechanisms on the whole and may result in new candidates for cancer prevention and therapy.

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