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Review

Pharmocoepigenetics: a new approach to predicting individual drug responses and targeting new drugs

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Abstract:
Epigenetics is the study of heritable changes in genes and gene expression that do not involve DNA nucleotide sequences. Epigenetic modifications include DNA methylation, several forms of histone modifications, and microRNA expression. Because of its dynamic nature, epigenetics provides a link between the genome and the environment and fills the gap between DNA and proteins. Advances in epigenetics and epigenomics (the study of epigenetics on a genome-wide basis) have influenced pharmacology, leading to the development of a new specialty, pharmacoepigenetics, the study of the epigenetic basis for variations in drug response. Many genes encoding enzymes, drug transporters, nuclear receptors, and drug targets are under epigenetic control. This review describes the known epigenetic regulation of drug-metabolizing enzymes and other proteins that might affect drug response and compounds that modify the epigenetic status.

Key words:
epigenetics, DNA methylation, histone modification, microRNA, pharmacoepigenetics, epigenetic drugs and chemopreventive agents

Abbreviations: ABC – ATP-binding cassette, CYPs – cytochromes P450, DNMTs – DNA methyl transferases, DRE – dioxin responsive element, EGCG – epigallocatechin-3-gallate, ER – estrogen receptor, HDAC – histone deacetylases, MDR – multidrug resistance, miRNA – microRNA, MRP – multidrug resistance protein, NR – nuclear receptor, siRNA – small interfering RNA, SLC – soluble carrier

Introduction

According to the "central dogma of molecular biology" [13], DNA is the only source of genetic information, with information flow running smoothly from DNA to RNA and finally to proteins. Currently, however, many phenomena, including individual responses to drugs, cannot be explained by this dogma. It is remarkable that a single mammalian genome, encoding approximately 30,000 genes, results in different gene patterns in about 200 different cell types at different stages of development [76]. It is obvious that there has to be an additional layer of information encoded in or around the genome that exceeds the information content of the genetic sequence. This additional level of information is achieved by epigenetic modifications. Epigenetics is the study of heritable changes in gene expression that occur without changes in the DNA sequence, while epigenomics re-

fers to the study of epigenetics on a genome-wide basis. Epigenetics involves three interacting molecular mechanisms: DNA methylation, modification of histones in chromatin and RNA-mediated regulation of gene expression [71]. Epigenetic patterns are known to be reversible and to vary with age as well as from tissue to tissue, since an individual has multiple epigenomes [77]. The dynamic aspect of epigenetics provides a link between the genome and the environment and fills the gap between DNA and proteins. Advances in epigenetics and epigenomics have had an impact on pharmacology, leading to the development of several new specialties, pharmacoepigenetics [32], which is the study of the epigenetic basis for variations in drug response, and pharmacoepigenomics [77]. These approaches are particularly useful when variations in gene sequence (pharmacogenetics) cannot explain variability in drug responses. Pharmacoepigenomics involves the study of the roles of epigenomics in intrapersonal and interpersonal variations in response of individuals to drugs, in the effects of drugs on gene-expression profiles, in the mechanism of action of drugs and adverse drug reactions and in the discovery of new drug targets [71]. It is remarkable that most papers on pharmacoepigenetics have been published in the "Future Medicine" sections of various biomedical journals, indicating that this is a very promising area of research. In this review we describe 1) known epigenetic regulation of drug-metabolizing enzymes and other proteins that might affect drug responses and 2) compounds that modify the epigenetic status.

Epigenetic modifications and their effect on drug response

Environmental factors generate a spectrum of phenotypes in the population through their participation in epigenetic mechanisms such as covalent modification of DNA and histones and expression of regulatory non-coding RNA molecules such as microRNAs (miRNAs). The dynamic quality of epigenetic modification, which stands in contrast to static nucleotide sequence information, provides the basis for an individual's response to a constantly changing environment (Fig. 1).

The most important epigenetic modification of DNA is the methylation of cytosine. DNA cytosine methylation occurs in the context of 5'-CpG-3' dinucleotides. Almost all CpG dinucleotides that are randomly localized across the genome are methylated, unlike those that are densely grouped in CpG islands (regions of DNA where the proportion of CpG dinucleotides is much higher than elsewhere in the genome). CpG islands are found in the promoter regions of many genes, and their aberrant methylation in cancer cells leads to the functional silencing of those genes due to chromatin compaction [65]. Methylation of DNA provides an impediment to transcription factors and the transcription machinery by attracting proteins that affect chromatin configuration. Silenced chromatin is rich in deacetylated and methylated histones. Besides acetylation, histones are currently known to be subjected to eight different types of post-translational modifications including methylation,

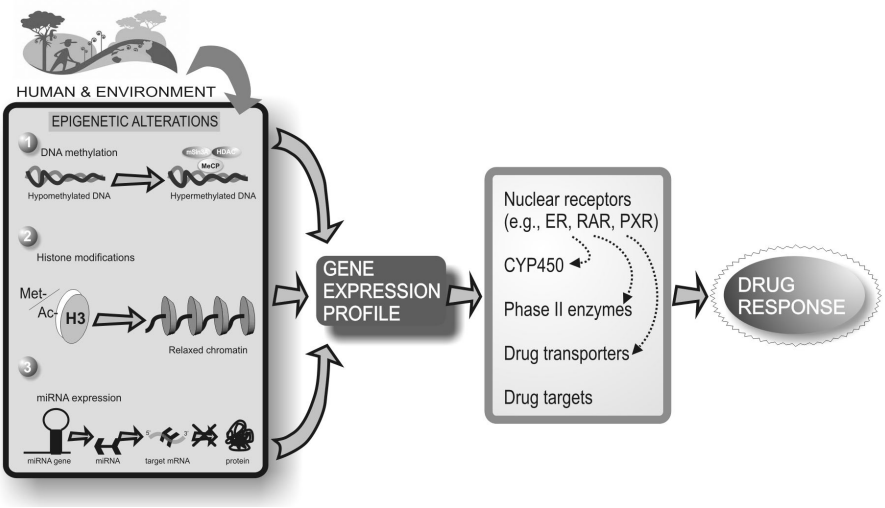

Fig. 1. The effect of epigenetic alterations on drug response

phosphorylation, ubiquitination, sumoylation, ADP-ribosylation, deimination, proline isomerization, and the newly identified propionylation [44, 54]. Covalent modifications of histones occur on histone "tail" residues, and distinct modifications may promote or block modifications on other residues, thus forming a specific histone code. Posttranslational modifications of the N-terminal of the histone proteins affect the compaction of the chromatin; methylation of lysine at position 9 of histone 3 (H3K9me) is a signature of heterochromatin or compact DNA, whereas acetylation of lysine at position 14 of histone 3 (H3K14ac), sometimes in combination with phosphorylation of proline at position 10 of histone 3 (H3P10p), creates a more open chromatin configuration (euchromatin) that allows transcription [36].

miRNAs, another part of the epigenetic machinery, are single-stranded RNA molecules of 21–24 nucleotides in length that arise from miRNA genes, which, when transcribed, can promote posttranscriptional regulation by binding to 3'-untranslated regions (3'UTRs) of target mRNAs and promoting their degradation or cleavage or interfering with their translation [35, 85, 88]. Besides their direct influence on mRNA transcription, some miRNAs, defined here as epi-miRNAs, have an indirect impact on gene transcription by affecting the epigenetic machinery, including DNA methyltransferases, histone deacetylases, and polycomb repressive complex genes [16].

Epigenetic factors affect the expression of drug-metabolizing enzymes, drug transporters, and nuclear receptors that regulate the expression of various genes and ultimately affect the response to drugs [23].

Epigenetic regulation of drug metabolizing enzymes

Cytochromes P450

The cytochromes P450 (CYPs) form a superfamily of hemoproteins that catalyze a huge diversity of enzymatic reactions and use both exogenous and endogenous compounds as substrates [72]. Individual differences in CYP expression have, to a large extent, been attributed to genetic polymorphisms [http://cypalleles.ki.se]. However, for some CYP genes no important functional genetic polymorphisms have been described. Thus, it is evident that epigenetic changes, particularly DNA methylation, can also influence their expression levels. Epigenetic regulation seems to be particularly important for CYP1A1, CYP1A2 and CYP3A, for which interindividual variations have not yet been elucidated, but other CYP isoforms are also affected [33, 72]. Examples of CYPs whose expression might be regulated by epigenetic mechanisms are presented below.

CYP1 family

CYP1A1 is mainly involved in the metabolic activation of the polycyclic aromatic hydrocarbons, which are common environmental pollutants. No important functional polymorphisms in this gene have been described. In contrast, several epigenetic mechanisms for the regulation of *CYP1A1* expression have been documented [72]. DNA methylation contributes to the regulation of *CYP1A1* in prostate cancer cells. The lack of *CYP1A1* expression in the prostate cancer cell line LNCaP has been associated with methylation of the promoter region of the *CYP1A1* gene, which prevents the binding of the AhR complex to the dioxin response element (DRE). On the other hand, hypomethylation of this region in non-cancer cell lines such as PWR-1E and RWPE-1 provides easier access for nuclear receptors to the DRE, allowing *CYP1A1* expression in cells exposed to dioxin [62]. Another article arguing for the significance of DNA methylation in *CYP1A1* expression provided analysis of the methylation status of *CYP1A1* in heavy smokers, light smokers and non-smokers and showed the amounts of methylation to be 33%, 71% and 98%, respectively; methylation was also increased in smokers up to 7 days after quitting smoking [4]. Histone modifications and chromatin configuration have also been suggested to be important for the regulation of *CYP1A1* [72]. An analysis of a possible association of miRNA levels with mRNA expression in lymphoblastoid cell lines by Wang et al. [22, 83] showed that the expression of *CYP1A1*, along with that of other phase I enzymes, such as members of the aldehyde dehydrogenase 2 family and flavin-containing monooxygenase 4, was correlated with the levels of miRNA-18b and miRNA-20b.

CYP1A2, which, like CYP1A1, is induced by smoking and affected by diet, is involved in the metabolism of several drugs, including estrogens, glucocorticoids and interferon. The known genetic polymorphisms do not explain its constitutive variation among individuals. However, methylation of a CCGG site (bp 2.759) located adjacent to an activator protein-1 site in the 5'-flanking region of *CYP1A2*

gene is apparently correlated with CYP1A2 expression [28]. Ingelman-Sundberg's group noticed a CpG island close to the translation start site in the second exon and found an inverse correlation of the methylation status of this CpG island with hepatic CYP1A2 mRNA levels, thereby suggesting that individual CpG sites may have important roles in CYP1A2 regulation by, for example, affecting binding to transcriptional regulation elements [21].

CYP1B1 catalyzes the metabolic activation of numerous procarcinogens. Overexpression of CYP1B1 in various tumor tissues leads to increased conversion of estradiol to 4-hydroxyestradiol, which may be responsible for the initiation of breast and endometrial carcinogenesis. *CYP1B1* is polymorphic, with many rare null alleles that cause glaucoma and other common alleles leading to amino acid substitutions that slightly affect enzyme function [72]. *CYP1B1* is also regulated epigenetically. Aberrant methylation of the promoter and enhancer regions of the *CYP1B1* gene affects the binding of transcription factors and enhancers such as AhR/ARNT and Sp1, which contain CpG dinucleotides in their binding sites. Hypomethylation of these sites is suggested to be associated with prostate cancer [79]. Methylation of the *CYP1B1* gene is also associated with the development of colorectal cancer [27]. CYP1B1 expression in breast cancer is influenced by miRNA-mediated translational repression or by mRNA cleavage. miRNA-27b, which is expressed at lower levels in breast cancer, allows the translation of CYP1B1 mRNA [80].

CYP2 family

Among the members of the *CYP2* family, the *CYP2A6, CYP2C19, CYP2D6, CYP2E1, CYP2J2, CYP2R1, CP2S1* and *CYP2W1* genes contain putative important CpG islands, suggesting a potential role for DNA methylation in their regulation [33]. Besides the role of CpG island methylation, the expression of some *CYP2* genes is affected by histone modifications. However, only a few studies have previously considered this. In the case of *CYP2A6*, histone acetylation analysis of human hepatocytes showed that histone H4 acetylation of the proximal promoter was increased by dexamethasone, leading to a more relaxed chromatin state. This might allow increased binding of hepatic nuclear factor 4α to the nuclear factor α response element and upregulate *CYP2A6* expression [64]. It was shown that methylation of specific 5' residues in the *CYP2E1* gene may be responsible for the lack of transcription of this gene in fetal liver [39]. Other epigenetic mechanisms may also be involved, as suggested by the variable levels of CYP2E1 mRNA in full-term placenta [39]. CYP2E1, in addition to acting on many drugs and carcinogens, metabolizes ethanol, which also induces expression of the enzyme at the transcriptional and post-translational level. This induction could be caused by the ability of ethanol to change the DNA methylation pattern. The increased expression of *CYP2E1* and the stabilization of the protein observed even at low ethanol levels have been implicated in various cancers [63]. *CYP2W1* is expressed in fetal stages of colon and in colon cancers but not in normal adult tissues [24]. It was also found to be expressed in adrenal tumors [40] and the tumor cell line HepG2 [41]. The first exon/intron junction in this gene is CpG dinucleotide-rich, and DNA methylation has been shown to be involved in the regulation of *CYP2W1* expression [24, 40]. The expression of *CYP2W1* during development suggests that it could be involved in the metabolism of endogenous substrates necessary for cell growth or development. Because CYP2W1 is re-expressed during carcinogenesis, it would be interesting to explore its role in this process [72].

CYP2A13, which acts to activate tobacco-specific nitrosamines, is selectively expressed in the respiratory tract, in which it is believed to play an important role in the initiation of carcinogenesis. Human lung cancer cells treated with 5-aza-'2-deoxycytidine (a DNA demethylating agent) and trichostatin A (an inhibitor of HDAC), showed a ~10-fold increase in the level of *CYP2A13* expression, suggesting a role for epigenetic modulation in the tissue-specific expression of this CYP [53].

CYP3 family

CYP3A plays a role in the metabolism of most therapeutic drugs. Treatment of HepG2 cells with 5-aza-2'-deoxycytidine and/or trichostatin A (inhibitors of DNA methyltransferase and histone deacetylase, respectively) and the analysis of changes in gene expression at genome level showed an effect on the expression of *CYP3A4*, *CYP3A5* and *CYP3A7* [14]. Histone methylation has also been shown to play a role in the control of murine *Cyp3a* expression; alterations in histone H3 methylation and acetylation are involved in the switch from *Cyp3a16* expression in the fetus to *Cyp3a11* expression in the adult mouse [51].

CYP24 family

The *CYP24A1* gene encodes 25-hydroxyvitamin D 24-hydroxylase, which mediates 24-hydroxylation of $1\alpha25(OH)_2D_2$ to much less active vitamin D metabolites. *CYP24A1* is deregulated in a wide range of tumors, and increases in expression are associated with a poor diagnosis in some human cancers. Upregulation of *CYP24A1* expression may counteract the antiproliferative activity of calcitriol, presumably by decreasing the calcitriol level. Recently, it was shown that the CYP24A1 promoter is methylated in a tissue-specific manner in normal human tissues. The *CYP24A1* gene is methylated in human placenta, while no methylation was detected in somatic tissues [61]. Moreover, in human prostate cancer cells, the *CYP24A1* gene was found to be hypermethylated in malignant lesions compared with matched benign lesions, indicating that *CYP24A1* repression is mediated in part by promoter DNA methylation and repressive histone modifications [55].

As is true for the cytochrome P450 genes, phase II enzyme expression is also subject to epigenetic regulation. It was found that the extent of methylation of glutathione-S-transferase genes depends on the haplotype of the glutathione S-transferase P1 (*GSTP1*) in cancer patients [73]. The mRNA expression of *GSTP1* was also associated with miRNA-192 and miRNA-194 levels [22, 83].

Drug transporters and nuclear receptors: epigenetic modulation

Drug transporters

Human genome sequence analysis suggests the presence of ~1,000 genes that encode transporters, comprising approximately 4% of all genes [12]. Two major superfamilies of membrane transporter proteins that influence drug pharmacokinetics are the ATP-binding cassette (ABC) and soluble carrier (SLC) transporter groups. ABC transporters are frequently associated with decreased cellular accumulation of anticancer drugs and multidrug resistance of tumors [1, 30]. SLC transporters such as the folate, nucleoside, and amino acid transporters commonly increase chemosensitivity by mediating the cellular uptake of hydrophilic drugs such as gemcitabine and other nucleoside analogues [9].

Expression of drug transporter proteins is influenced by DNA methylation. The human multidrug resistance gene 1 (*MDR1*), a member of the ABC transporter family, was shown to be overexpressed upon treatment of the breast cancer cell line MCF-7 with the demethylating drug 5-azacytidine, with accompanying changes in chromatin structure [15, 47]. Hypomethylation of the *MDR1* gene accounts for glycoprotein P overexpression and results in aggressive behavior in invasive ductal carcinomas of the breast [75]; it may also explain the P-glycoprotein-mediated multidrug-resistance in some cell lines [47]. It was also shown that the epigenetic status of the *MDR1* locus dictates its expression following treatment with chemotherapeutic drugs like daunorubicin and etoposide, such that the chemotherapeutic drugs activate *MDR1* transcription only when the promoter is significantly hypomethylated. Upregulation of *MDR1* was also associated with histone modification. Increases in histone 3 (H3) acetylation and H3 methylation at lysine 4 (K4) correlate with *MDR1* upregulation [5]. Multidrug resistance mediated by MRP-1 protein is, as reported by Liang et al. [52], influenced by miRNA-326, which has an impact on the chemotherapeutic response of breast cancer cells. It was found that miRNA-326 is downregulated in a panel of advanced breast cancer tissues, and its expression is inversely correlated with that of MRP-1, suggesting that this miRNA may be an efficient agent for the prevention and treatment of MDR in tumor cells. Moreover, in the studies of Wang et al. [22, 83], miRNA-363 levels were associated with mRNA expression of *ABCB4*, which encodes *MDR-3*.

The gene for solute carrier family 5 (iodide transporter) member 8 (*SPC5A5*), which has been characterized as a tumor suppressor, was also reported to be downregulated by promoter methylation in pancreatic and prostatic carcinomas; its expression was rescued by treatment with DNA methylation inhibitors [69]. Other transporters have also been demonstrated to be epigenetically downregulated. Aberrant hypermethylation of the reduced folate carrier (*RFC*) gene has been associated with resistance to methotrexate in cancer cell lines, primary osteosarcomas, lymphoproliferative disorders [42] and breast cancer [84]. Preliminary data provided by Canadelaria et al. [9] showed that cervical cancer cell lines with acquired resistance to gemcitabine downregulate expression of the nucleoside transporter hENT1 methylating its promoter; this effect is reversed by treatment with a demethylating agent, leading to re-sensitization to gemcitabine. It was also shown that miRNA-221 influences

the expression of another nucleoside transporter, *SLC29A1*, whereas miRNA-181a, miRNA-181b, and miRNA-213 have an impact on *SLC47A1* [22, 83].

These examples indicate that intervention in drug transporter expression at the epigenetic level may represent one way to overcome drug resistance.

Nuclear receptors

Nuclear receptors (NRs) form one of the largest superfamilies of transcription factors and play essential roles in the regulation of a wide array of developmental and physiological pathways, including the transcription control of genes encoding drug transporters and enzymes. Nuclear receptors themselves are dynamically modulated by several types of posttranslational modification including phosphorylation, methylation, acetylation, ubiquitination, and sumoylation. DNA methylation regulates the expression of members of the retinoic acid receptor family. Loss of retinoic acid receptor-β2 expression in head and neck squamous cell carcinomas was correlated with its hypermethylation, which occurs early in head and neck carcinogenesis [87]. The genes for this receptor and the steroid hormone receptor estrogen receptor α (ERα) were found to be regulated by DNA methylation and histone modification in breast cancer cells [7]. In mammals, DNA methylation is considered to be a very stable marker for ERα and androgen receptor silencing in cancer cells and in normal brain development [31]. Treatment of ERα-negative human breast cancer cells with the DNMT1 inhibitor 5-aza-2'-deoxycytidine rescues ERα mRNA and protein expression [19]. ER transcripts may also be induced by the HDAC inhibitor trichostatin A [86].

miRNAs contribute to the regulation of the final output of several NR signaling pathways at three different levels [68]. They can directly target the 3'UTR of the NR mRNA itself and/or the 3'UTRs of the mRNAs of the NR co-regulators or even NR target genes, thereby regulating NR signaling in an indirect manner. ER α was one of the first NRs whose 3'UTR was shown to be targeted by miRNAs. Studies focusing on miRNAs that are differentially expressed in ER α-positive and ER α-negative breast tumors found that the levels of one of the miRNAs, miRNA-206, are negatively correlated with ER α expression [34]. Furthermore, it was shown that miRNA-206 inhibits ER α expression by directly targeting one of the miRNA sites present in the 3'UTR of the ER α mRNA [2].

Picard and colleagues [68] performed a study on the role of 14 miRNAs which might be involved in ER α expression and found that miRNA-22 exerted the strongest inhibition estrogen signaling, by directly targeting ER α mRNA. Several other studies have shown that 3'UTR of ER α is targeted by yet other miRNAs, including miR-221. The latter was found to be overexpressed in a tamoxifen-resistant variant of the human breast cancer cell line MCF7 compared to its tamoxifen-sensitive parent line, suggesting a role for miRNAs in the acquisition of drug resistance during adjuvant therapy of breast cancer. The protein level of the cell cycle inhibitor p27(Kip1), a known target of miR-221/222, was reduced by 50% in tamoxifen-resistant and by 28–50% in miR-221/222-overexpressing MCF-7 cells [58].

The transcription factor pregnane X receptor, which regulates the expression of a number of CYP members, has been shown to be regulated by miR-148a [78].

Thus, different mechanisms may be involved in the epigenetic regulation of individual drug response.

Epigenetic targets and their inhibitors: perspectives for the development of new drugs and chemopreventive agents

In contrast to loci that undergo genetic alterations, the genes that are silenced due to epigenetic modification are still intact and can be reactivated by small molecules that act as modifiers of epigenetic mechanisms. Therefore, targeting of epigenetic modifications is a very promising strategy, particularly in cancer therapy or chemoprevention [29].

In cancer cells, a general decrease in the methylated cytosine level (genome hypomethylation) is accompanied by local CpG island hypermethylation [48, 65]. Both hypo- and hypermethylation may promote cancer development. Genomic hypomethylation may lead to genome instability and hypomethylation of proto-oncogenes, which results in upregulation of their expression. On the other hand, local promoter CpG island hypermethylation induces the functional silencing of tumor suppressor genes, mimicking genetic mutation. Epigenetics is thought to play a major role not only in cancer but also in the pathogenesis of some other multifactorial diseases such as schizophrenia and bipolar disorder, both of which are due to epigenetic defects rather than genetic effects [70]. Recently it was shown in animal models that epigenetic

changes including histone modification and aberrant DNA methylation affects diverse pathways leading to depression-like behaviors. For instance, early life stress can change the gene expression profiles of the glucocorticoid receptor and brain-derived neurotrophic factor, and these altered expression profiles can be reversed by treatment with epigenetic drugs. Postmortem studies of depressed suicide victims also revealed epigenetic changes in frontal cortex [74]. There are, however, reports indicating that in major depressive disorders, the differential expression of the glucocorticoid receptor is not epigenetically programmed [3]. Recent data indicate also that epigenetic changes play an important role in the development of cardiac hypertrophy and heart failure, which may affect response to therapy [57]. Movassagh and colleagues [59] reported that differential DNA methylation occurs in human end-stage cardiomyopathy. Differential methylation of three angiogenesis-related loci (PECAM1, ARHGAP24, and AMOTL2) correlated with altered gene expression in the various cardiac samples investigated. Hypermethylation within the PECAM1 and AMOTL2 genes correlated with their reduced expression, whereas hypermethylation within the body of the ARHGAP24 gene correlated with an increase in its expression. Unraveling additional epigenetic changes in gene expression leading to cardiovascular diseases could help improve therapeutic options and alter patient management. There are several neurological diseases that are associated with deficiencies in enzymes or proteins required for epigenetic modification of histones. Neurological disorders in which an epigenetic gene is mutated include Rett syndrome, α thalassemia/mental retardation X-linked syndrome (ATRX), Rubinstein-Taybi, and Coffin-Lowry syndromes. Rubinstein-Taybi syndrome is associated with the dysfunction of a histone acetyltransferase, while Coffin-Lowry syndrome is a neurological disease caused by deficiencies in a histone phosphorylase. ATRX and Rett syndromes are both X-linked disorders caused by mutations in a chromatin remodeling protein and in methyl-CpG binding domain protein 2 (MeCP2), respectively [81].

All these pathological conditions require the development of epigenetic therapy.

To date the drugs that have been studied in the greatest detail are inhibitors of DNA methyltransferase and histone deacetylase, which have potential for use in the management of cancer [20]. Drugs from both of these classes have started receiving approval from the US FDA for treatment of patients [71]. Many genes that are hypermethylated in cancer can be reactivated upon treatment with inhibitors of DNA methyltransferase. Multiple DNMTs with varying degrees of specificity towards unmethylated and hemi-methylated DNA substrates appear to be present in humans. DNMT1 shows higher specificity towards hemimethylated DNA substrates and is responsible for the maintenance of DNA methylation profiles during cell division. Interestingly, DNMT1 seems to be more frequently required for aberrant DNA methylation in cancer cells than other DNMTs, making it the major target for anticancer drugs [67]. Analogues of cytidine, such as 5-azacytidine or 5-aza-2'-deoxycytidine (decitabine) and zebularine (a cytidine analogue containing a 2-(1H)-pyrimidinone ring), have long been known for their ability to inhibit DNA methyltransferases [10, 38]. Apart from these nucleoside analogues, other DNMT inhibitors, such as procainamide and procaine, are undergoing preclinical trials [70]. However, the side effects and toxicity of these compounds are serious concerns. A particularly important problem with all epigenetic drugs, not only DNMT inhibitors, is lack of specificity, which can result in effects on non-target genes. Thus, there is a great need for the development of effective and non-toxic inhibitors of DNMTs for not only therapy but also chemoprevention. It has been shown that several potential chemopreventive/chemotherapeutic phytochemicals are able to inhibit DNMTs.

(–)-Epigallocatechin-3-gallate (EGCG) inhibits DNMT and reactivates the suppressor genes *RAR*β, *p16* (*CDKN2A*), O6-methylguanine methyltransferase (*MGMT*) and human mutL homologue 1 (*hMLH1*) in tumor cells [17]. The other polyphenols in coffee, caffeic acid and chlorogenic acid, have also been reported to be strong DNMT1 inhibitors, especially in the presence of COMT [50]. Further studies provided evidence that other dietary components, e.g., genistein, nordihydoguaiaretic acid, lycopene, parthenolite and Annurca apple polyphenols, may also affect DNA methylation [67]. This activity, however, was often gene-specific and cell line-dependent. For example, EGCG was not effective in reducing DNA methylation in T24 (urinary bladder transitional cell carcinoma), PC3 (prostate adenocarcinoma) and HT29 (colorectal adenocarcinoma) cancer cells and did not allow the reactivation of *p16* in T24 cells [11].

Our study showed that a wide range of dietary phytochemicals were able to inhibit DNA methyltransfe-

rase activity in a cell-free system, with betanin being the weakest and rosmarinic and ellagic acids the strongest modulators of the enzyme's activity. However, while decitabine led to partial demethylation and reactivation of genes, none of the phytochemicals tested affected the methylation pattern or the expression of *RASSF1A, GSTP1* or *HIN1* in human breast cancer MCF7 cells [67]. Thus, the results of our study suggest that non-nucleoside agents are not likely to be effective epigenetic modulators. However, long-term exposure to these chemicals in the diet might be sufficient for chemoprevention. In this regard, it was shown that even a nucleoside analogue like decitabine is able to restore the expression of hypermethylated genes after prolonged exposure (for many generations) of the cells to this compound [49]. Evidence also exists of the role for inflammation in the induction of aberrant DNA methylation, e.g., through chlorination of cytosine residues [82]. Thus, dietary phytochemicals, which often have anti-inflammatory properties, could indirectly affect the epigenome by the modulation of inflammatory reactions.

The steady state levels of protein acetylation are maintained by a dynamic equilibrium between histone acetyltransferases (HATs) and histone deacetylases (HDACs). While acetylation is associated with activation of gene transcription, deacetylation of histones is associated with silencing of gene transcription [70]. It is now well established that methylation of DNA and histone modifications are intimately interconnected. DNMTs can bind to HDACs, thereby repressing gene transcription through histone deacetylation [8]. There are three classes of HDACs (I, II and IV) with at least 11 isozymes identified, which were shown to be zinc-dependent amidohydrolases [26]. The members of the fourth class of HDACs (class III) are dependent on NAD^+ for their activity, which results in the formation of nicotinamide and O-acetyl-ADP ribose. Based on the homology to the yeast histone deacetylase Sir2p, the NAD^+-dependent deacetylases have been termed sirtuins; seven members (SIRT1–7), which are localized either in the nucleus, cytoplasm, or mitochondria, have been described in humans. While class I and II HDACs have been identified as valid anticancer targets and clinical studies of their inhibitors as new anticancer agents are under way, much less is known about the consequences of class III histone deacetylase inhibition, which seems to be more complex and not univocal. While their activation may be beneficial for metabolic diseases, sirtuin overexpression is also related to Parkinson's disease and cancer [60]. Recently, SIRT1 (and the sirtuins in general) have been intensely investigated, since it was shown that sirtuin inhibitors induce apoptosis and could be useful for the treatment of cancers [46].

The first inhibitors of class I and II HDACs were isolated from natural sources; based upon these compounds, a variety of synthetic inhibitors have been developed. The general structure contains a binding region responsible for enzyme specificity, which is separated by a spacer from a group that inactivates the enzyme [29]. This inactivating group should be able to bind a zinc ion in the active site. The HDACs inhibitors include small-chain fatty acids, hydroxamic acids, cyclic tetrapeptides, benzamides and other compounds that do not fit in these classifications, such as electrophilic ketones [37, 56]. The largest group of HDAC inhibitors are represented by those that carry a hydroxamic acid as the zinc binding group, with the natural product trichostatin A as a leading structure [29]. There is some evidence that histone deacetylase inhibitors could act as novel, effective antidepressants by counteracting previously acquired adverse epigenetic modifications [74]. In general, HDACs deacetylate both histone and non-histone targets. This lack of specificity underlies the pleiotropic effects of HDAC inhibitors, which are not limited to alteration of gene expression but extend into a wide array of cellular (nuclear and/or cytoplasmic) processes [6].

Although most research on epigenetic drugs and chemopreventive agents to date has been concentrated on developing inhibitors of DNMT or HDAC, other potential therapeutic targets, such as histone acetyltransferase and histone methyltransferase, deserve attention [29, 45].

Some authors hold that instead of using epigenetic drugs for the treatment of disease, one should target biochemical pathways that have been disturbed epigenetically using more conventional drugs [18].

In addition to the extensive ongoing work on DNA methyltransferase and histone deacetylase inhibitors, there are also extensive efforts aimed at developing drugs associated with the other major aspect of epigenetic regulation, RNA-mediated regulation of gene expression. This description refers to the regulation of gene expression by small non-coding RNAs, which include the previously mentioned miRNAs as well as small interfering RNAs (siRNA). The precursors of these non-coding RNAs are longer double-stranded

RNAs that are processed to small RNAs by specific sets of enzymes and other proteins. Although in some conditions small RNAs can activate gene expression, they are mainly involved in silencing of gene expression [25]. Small RNAs are known to regulate the expression of more than 30% of protein-coding genes by blocking mRNA translation [43, 89]. Aberrant miRNA expression is known to contribute to the pathogenesis of diseases such as cancer and cardiovascular disease, and therefore, miRNAs may serve as novel targets for therapy [89].

A better understanding of the interplay between DNA methylation, histone modification and RNA-mediated regulation of gene expression, which is expected to result from the ongoing Human Epigenome Project [http://www.epigenome.org/index.php], will lead to a better understanding of human diseases and a new range of molecular targets for epigenetic drugs.

Conclusions and perspectives

Pharmacogenetics has been instrumental in describing interindividual variation in drug metabolism. Epigenetic factors offer another layer of information that could help develop more personalized therapy. The dynamic aspects of epigenetics may not only provide more precise clues to the roles of changing environmental factors in the drug response, thus linking the environment to the genome, but also offer a way to reactivate silenced genes.

Histone modification, miRNA regulation of gene expression, and methylation of genes involved in DNA repair, cell cycle and the maintenance of genomic integrity, are all reported to influence sensitivity to chemotherapeutic drugs, suggesting that epigenetic factors could serve as molecular markers predicting the responsiveness of tumors and other diseases to therapy. However, a comprehensive study of pharmacoepigenomics awaits the advent of genome-wide analysis of DNA methylation using microarrays and next-generation sequencers. Natural and chemical substances acting as novel small molecule inhibitors of enzymes involved in epigenetic modification will further improve our understanding of epigenetic mechanisms and possibly provide new candidates for the prevention and treatment of many diseases, particularly cancer.

Ultimately, to optimize chemotherapy and/or chemoprevention, both pharmacogenetics and pharmacoepigenetics must be taken into account.

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