

HIGHLIGHTED TOPIC | *Epigenetics in Health and Disease*

Epigenetics and environment: a complex relationship

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¹Instituto de Investigaciones Biomédicas Alberto Sols, Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid, Madrid; ²Cancer Epigenetics Laboratory, Instituto Universitario de Oncología del Principado de Asturias, Hospital Universitario Central de Asturias, Universidad de Oviedo, Oviedo; and ³Department of Immunology and Oncology, National Center for Biotechnology, Centro Nacional de Biotecnología-Consejo Superior de Investigaciones Científicas, Cantoblanco, Madrid, Spain

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Aguilera O, Fernández AF, Muñoz A, Fraga MF. Epigenetics and environment: a complex relationship. *J Appl Physiol* 109: 243–251, 2010. First published April 8, 2010; doi:10.1152/jappphysiol.00068.2010.—The epigenomes of higher organisms constantly change over time. Many of these epigenetic changes are necessary to direct normal cellular development and differentiation in the developing organism. However, developmental abnormalities may occur in response to inappropriate epigenetic signaling that occurs secondarily to still poorly understood causes. In addition to genetic and stochastic influences on epigenetic processes, epigenetic variation can arise as a consequence of environmental factors. Here we review the effects of such environmental factors on the epigenomes of higher organisms. We discuss the possible impact of epigenetic changes on physiological and pathophysiological processes, depending in part on whether these changes occur during embryonic development or adulthood.

methylation; chromatin; epigenotype

FIRST PROPOSED BY CONRAD HAL WADDINGTON in 1942 to designate the study of the processes by which the genotype gives rise to phenotypes through programmed changes during development (104), “epigenetics” was subsequently understood to be the heritable changes in gene expression that are not due to any alteration in the DNA sequence (43). Nowadays, epigenetics is more accurately defined as “the study of stable genetic modifications that result in changes in gene expression and function without a corresponding alteration in DNA sequence” (<http://nihroadmap.nih.gov/roadmap15update.asp>).

Epigenetics encompasses all of the mechanisms involved in deploying the genetic program for the many processes operating during the lifespan of a cell. Although epigenetic modifications seem to be stable, they can be modulated by many factors, including physiological and pathological conditions and by the environment (42, 78, 110).

Epigenetic mechanisms include, among other things, DNA methylation, covalent histone modifications, and noncoding RNAs, and are ultimately related to the regulation of gene expression and chromatin structure (11, 30, 97).

DNA methylation is the best known epigenetic modification and has a critical role in the control of gene expression and the architecture of the cell nucleus. This modification mainly occurs in cytosines that precede guanines to yield 5-methylcy-

tosine (5-meC). These dinucleotide sites are usually referred to as CpGs (41). In the human genome, CpGs are asymmetrically distributed into CpG-poor and CpG-dense regions. The latter are called “CpG islands” and span the promoter of approximately one-half of all genes. They are usually unmethylated in normal cells, whereas the sporadic CpG sites in the rest of the genome are generally methylated (47).

DNA methylation is a dynamic process that takes place throughout the course of development in multicellular organisms and ensures the maintenance of the normal expression pattern. It is also involved in genomic imprinting (31), X-chromosome inactivation in females (73), and the silencing of parasitic and foreign elements (24), among other processes. However, hypermethylation of CpG islands in promoter regions is often associated with gene silencing and contributes to the typical hallmarks of a cancer cell that result from tumor suppressor gene inactivation (27, 46).

DNA methylation patterns seem to be established by at least three DNA methyltransferases, DNMT1, DNMT3a, and DNMT3b, which catalyze the transfer of a methyl group from *S*-adenosyl-*L*-methionine to cytosine bases in DNA, and which are restricted to symmetrical CG sequences in most mammals. These methyltransferases can be generalized into ones that maintain or copy methylation marks after DNA replication, and those that initiate new (de novo) methylation marks on DNA. DNMT1 is the most abundant methyltransferase in somatic cells and is responsible for the maintenance of DNA methylation. It is believed to be the enzyme responsible for copying methylation patterns in the new strand after DNA

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replication and is required for correct embryonic development, imprinting, and X-inactivation (7, 59). DNMT3a and DNMT3b are two major DNA methyltransferases that are fundamental to embryonic development in mammals and required for the de novo methylation that occurs in the genome following embryo implantation (10, 65) (Fig. 1). However, several studies show that all three DNMTs not only cooperate, but also may participate in de novo and maintenance methylation functions (29, 52).

The other well-studied and more complex epigenetic mechanism is histone modification. The core histones (H2A, H2B, H3, and H4), in conjunction with the 147 base pairs of genomic DNA wrapped around them, comprise the nucleosomes, which are the basic units of chromatin. One of the main functions of histone modifications is to establish different chromatin “environments”; chromatin with a low-condensation state (euchromatin) that is “accessible” to transcription factors, and chromatin with a high degree of compaction (heterochromatin) that is “inaccessible” to transcription (56). Additionally, interactions between histone modifications and DNA regulate many biological processes, such as gene expression, DNA repair, chromatin compaction, and genome stability, as well as important genetic processes, such as X-inactivation (30).

Modifications have been detected in over 60 different residues on histones, and the NH₂-terminal “tail” domains are the most heavily modified portions (56). There are at least eight types of modifications found on histones, of which acetylation, methylation, phosphorylation and ubiquitination are the most thoroughly studied, and have been associated with activation or repression of transcription. Acetylation, performed by histone acetyltransferase enzymes, is the most extensively researched histone modification and is generally associated with active gene transcription (3), while methylation, performed by histone methyltransferases (HMTs), may be linked to activation (i.e., H3K4, H3K36, and H3K79) or repression (i.e., H3K9, H3K27, and H4K20), depending on the conditions or residue modified (56).

Micro-RNAs are included as another regulation mechanism in the field of epigenetics (22). They are small, noncoding RNAs (~22 nucleotides) that regulate gene expression by

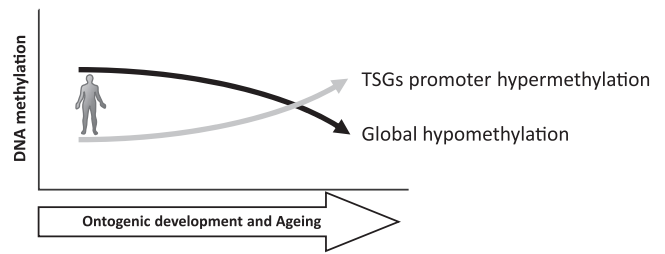


Fig. 2. DNA methylation changes during ontogenic development and aging. Global DNA methylation decreases with age in many tissue types, while several specific regions of the genomic DNA become hypermethylated. TSGs, tumor-suppressor genes.

binding to the 3'-UTR of their target messenger RNA transcripts, usually resulting in their silencing. Most of the studies of the epigenetic effects of environmental factors have concerned DNA methylation and histone modifications, so new projects based on micro-RNAs need to be explored.

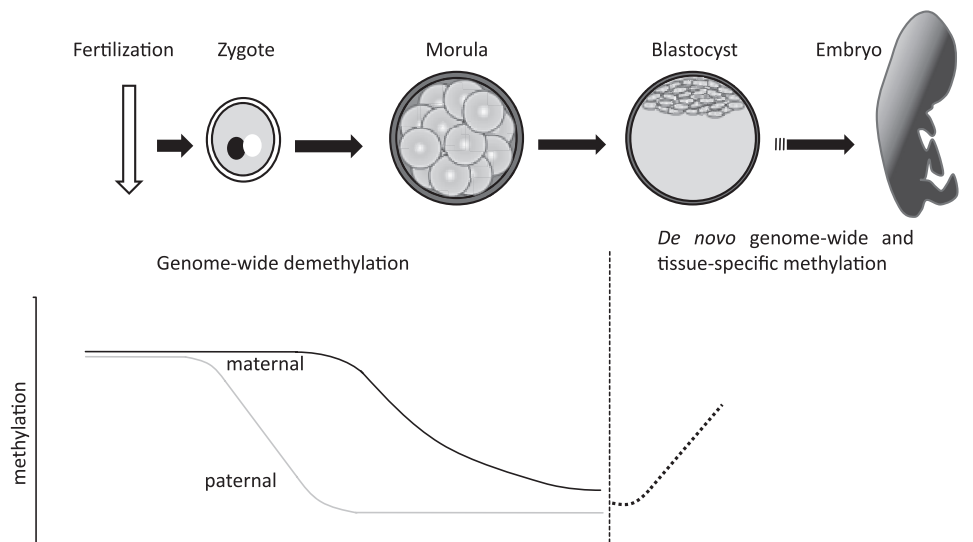
LIFETIME EPIGENETIC CHANGES

Although it is well established that epigenetic marks are quite stable, there is increasing evidence that they change over time (reviewed in Ref. 18).

The association between epigenetics and aging first came to light in a study showing that genomic global DNA methylation (measured as 5-methyldeoxycytidine) decreases with age in spawning Pacific salmon (*Oncorhynchus gorbuscha*) (9). Several studies have subsequently shown similar patterns in rats, mice, and humans (12, 98, 112). This loss of global DNA methylation could be the result of the passive demethylation of heterochromatic DNA as a consequence of failures in DNMT1 functions (19).

Global DNA hypomethylation during aging is usually accompanied by hypermethylation of several specific regions, including CpG island promoters of specific genes or ribosomal DNA clusters (44, 70) (Fig. 2), and may be correlated with functional senescence (111). This CpG island promoter hypermethylation can be due to the overexpression of DNMT3b, a de novo (not maintenance) methyltransferase, as has previ-

Fig. 1. DNA methylation changes during embryonic development. Epigenetic DNA methylation marks are largely erased during early embryonic development. De novo, genomewide DNA methylation marks are made soon after, during early embryogenesis. At later developmental stages, tissue-specific epigenetic marks are laid down. After fertilization, active demethylation of the paternal genome (gray) and passive demethylation of the maternal genome (black) occur. De novo methylation occurs at the blastocyst stage (dotted line).



ously been found in cultured fibroblasts (19). Curiously, global DNA hypomethylation and promoter hypermethylation of specific genes have also been found in cancer (27), which suggests a genuine connection between aging and malignant transformation.

A comprehensive review by Calvanese et al. (18) yielded a list of genes [e.g., estrogen receptor (ER) or myogenic differentiation antigen 1] that present promoter-specific hypermethylation associated with aging (18). The case of the INK4/ARF locus (Cdkn2a-Cdkn2b genes that encode for p16^{INK4a}, p19^{Arf}, and p15^{INK4b}) and its dual functionality in aging and cancer is particularly interesting. In human non-neoplastic gastric epithelia, it has been shown that there is an increase in p16^{INK4a} promoter hypermethylation with age (91). However, p16^{INK4a} is also upregulated in an epigenetically independent manner in elderly individuals (54, 96). It has been proposed that age-dependent p16^{INK4a} upregulation is involved in the decrease of self-renewal of mature progenitor cells (45). Thus, although the promoter DNA methylation status of p16^{INK4a} seems to increase during aging, the functional role of this epigenetic alteration is still unclear. Histone modification profiles are another epigenetic feature that changes during cell transformation and aging. Several papers have reported age-associated changes in global and specific histone profiles, as well as in several histone-modifying enzymes (reviewed in Ref. 18). For example, an increase in trimethyl histone H4 lysine 20 (H4k20me3) is associated with aging processes (81) in rat kidney and liver tissues, and senescence cells are associated with a lower level of trimethyl histone H3 lysine 27 (H3K27me3) in assays carried out using human diploid lung embryonic fibroblast cell line TIG3 (14).

The sirtuin family is an especially interesting subset of the various histone-modifying enzymes that are known to be associated with aging. Sirtuins are nicotinamide adenine dinucleotide-dependent histone deacetylase enzymes (HDACs) and are named after their homology with the *Saccharomyces cerevisiae* gene silent information regulator 2. Seven sirtuins (SIRT1–7) have been identified in mammals, and, although all of them play important roles in cell cycle control, metabolism, DNA repair, and rDNA transcription (reviewed in Ref. 48), SIRT1 deserves special attention because it modulates important processes of glucose and lipid homeostasis in key metabolic tissues and is especially relevant to aging and cancer (37, 48, 82, 92). SIRT1 may act not only by deacetylating histone polypeptides with a preference for histone H4 lysine 16 (H4K16Ac) and H3 lysine 9 (H3K9Ac) (76, 99), but also on key transcription factors, including tumor proteins such as p53 (reviewed in Ref. 37).

Progeria syndromes (human premature aging-like syndromes) offer great opportunities for studying changes associated with aging and epigenetic factors. Werner's syndrome (WS) and Hutchinson-Gilford progeria syndrome (HGPS) are two classic and well-characterized examples of such syndromes. WS is a rare autosomal recessive disorder associated with a defect in the WRN gene (which codes for a DNA helicase) and is characterized by premature aging and several features indicative of accelerated aging, including type 2 diabetes and osteoporosis, at a relatively young age (16, 25, 72). HGPS is a childhood disorder caused by a point mutation of the lamin A/C gene (LMNA) and, unlike WS, is not caused by defective DNA repair (26). Furthermore, LMNA is one of the

elements that participate in the structure and organization of the nuclear compartment and that is involved in aging (84, 85). The promoter hypermethylation of both genes (WRN and LMNA) has been found to be associated with malignant transformation in human colon, breast, and leukemia cancer cell lines (1, 2), and altered histone modification patterns, including a decrease in the level of trimethyl histone H3 lysine 9 (H3k9me3) and of H3K27me3 or an increase in that of H4k20me3, have been found in HGPS (86, 88). These data, therefore, suggest that epigenetics affects aging processes, as well as those of cancer.

As the epigenome of an organism can accumulate alterations overtime, the question arises as to what factors are involved in the process. Recent data indicate that epigenetic variation over time can depend on the genotype (intrinsic factors), the environment (extrinsic factors), and stochastic (undetermined) factors (35), although the relative contribution of each is still unknown.

The role of hereditary factors in epigenetic variation over time is supported by studies of monozygotic twins [fewer intrapair than interindividual epigenetic differences (36), more epigenetic differences between dizygotic than monozygotic twins (49)] and by the familial clustering of methylation changes observed in longitudinal studies (12). Epigenetic variation over time can also be influenced by undetermined or stochastic factors, as indicated by the presence of phenotypic and epigenetic differences in isogenic laboratory animals living under the same environmental conditions (39, 103). The last possibility is that environmental factors affect the epigenotype during the lifetime of organisms (Fig. 3). This is an attractive idea because, if true, epigenetics could provide a link between the environment and gene function. Although the effect of the environment on epigenetic factors has been widely reported (reviewed in Ref. 30), the mechanistic pathways by which environmental factors affect the epigenotype remain largely unclear. Possible mechanisms include the substrate-dependent activation or inhibition of epigenetic enzymes (i.e., dietary intake of methyl donors for DNA methyltransferases and/or HMTs and histone acetyltransferase inhibitors/activators such as resveratrol valproate) and the activation/inhibition of signaling pathways that results in specific chromatin changes (38).

THE EFFECTS OF ENVIRONMENTAL FACTORS ON THE EPIGENOME

To understand the impact of the environment on the epigenotype, it is necessary to consider two scenarios: embryonic development and adult life (Fig. 3). The need to differentiate between prenatal and postnatal life arises from the differential impact that an epigenetic change can have on the organism. In principle, the epigenetic changes occurring during embryonic development will have a much greater impact on the overall epigenetic status of the organism because, as they can be transmitted over consecutive mitotic divisions, alterations occurring in single embryonic stem cells will affect many more cells than those occurring in adult stem and/or somatic cells during postnatal development. Although this possibility is plausible, it remains to be firmly established. In the final analysis, the type of change and the genomic location of the change(s) may dictate the clinical significance of the epigenetic

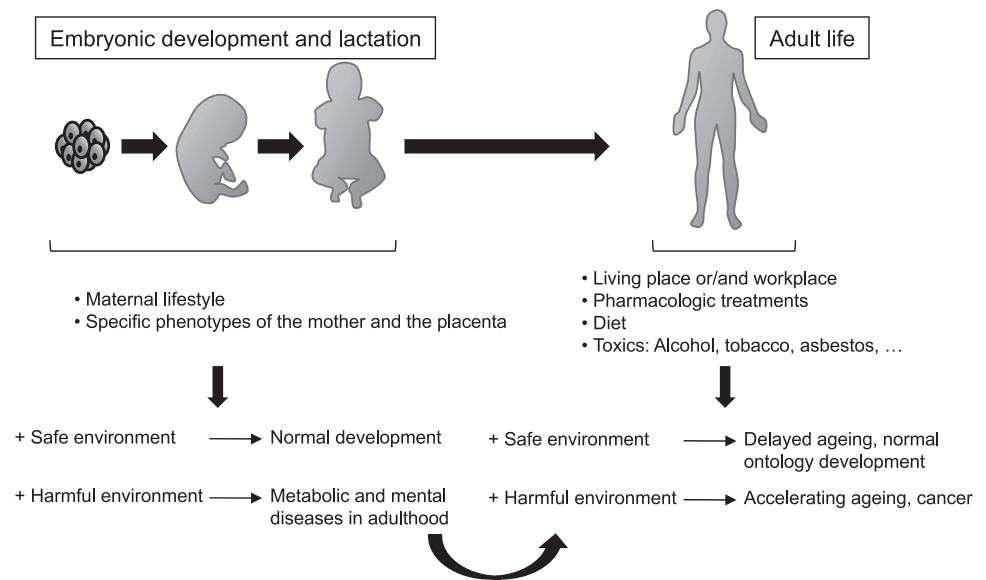


Fig. 3. Effects of environmental factors on epigenetics. Two different scenarios are illustrated (embryonic/lactation stages and adult life), in which different factors associated with safe or harmful environment can determine alterations during life development. The effects in adulthood of alterations occurring in early stages are also shown.

changes, along with genetic background and random other variables.

The Impact of the Environment on the Epigenome During Embryonic Development

The environmental conditions in the uterus can determine phenotypic alterations in the offspring that, since they persist throughout life, can give rise to stable changes in gene expression. Recent studies suggest that such stable gene regulation can be mediated, at least in part, by epigenetic mechanisms. In principle, the environmental conditions during embryonic development must be primarily determined by two factors: 1) the specific phenotypes of the mother and the placenta, which determine characteristics, such as the size of the uterus and the availability of nutrients; and 2) the mother's lifestyle, which determines the environmental factors to which the embryo is exposed (Fig. 3, Table 1). Although little is currently known about how the phenotypes of the mother and the placenta can affect the epigenotypes of the offspring [it has been suggested that maternal obesity can lead to specific epigenetic alterations of the offspring (109)], there is a great deal of information

about how the environmental conditions of the mother can affect specific epigenetic factors of their offspring.

The best studied environmental condition of the mother affecting the epigenotype of the offspring is maternal diet. A good example of how this can affect specific epigenetic pathways of the offspring comes from agouti mice. The agouti alleles regulate the production of pigment in individual hair follicles. Yellow and mottled mice are obese and prone to diabetes and cancer, in contrast to fully agouti mice, known as pseudoagoutis, which are lean and nondiabetic. The phenotype of the agouti mice depends on the expression of the agouti protein, which is regulated by the DNA methylation status of a repeated DNA region at the agouti promoter (66), and, interestingly, methyl donor supplementation of female mice before and during pregnancy permanently increases tissue-specific DNA methylation of the agouti gene in offspring (106–108). In keeping with this observation, in utero exposure to a high-fat diet modifies the methylation pattern of leptin and ER promoters in rats (64, 114). Interestingly, maternal diet-dependent methylation changes at the ER promoter boost expression of this gene with aging and, consequently, prompt a higher

Table 1. List of some environmental factors/conditions that can affect the epigenetic status

Environmental Factor	Effect	Reference No.
Methionine, folic acid, vitamin B6, vitamin B12, SAM, choline, betaine	SAM synthesis, methionone synthesis	53, 68, 95
Genistein	Increased methylation, cancer prevention	105
Sulphoraphane, butyrate	Modification of histone acetylation	67, 77
Nicotine	MAO-B promoter epigenetic demethylase activity	58
Resveratrol	inhibition of SIRT1, a member of the sirtuin family of NAD-dependent deacetylases	40
High-fat diet	Reduction of natural ER promoter hypermethylation with aging in rats and causes stronger expression of ER and a higher incidence of tumors in offspring	114
Metal ions: chromium, cadmium, nickel	Reduction of methylation levels at genetic loci by inhibiting the activity of DNA methyltransferases	80, 87, 93
Nickel	Repression of gene expression by decreasing global histone H4 acetylation and increase histone H3 lysine 9 dimethylation at the promoter level	20, 50
Diethylstilbestrol, bisphenol A, vinclozolin	Alteration of DNA methylation at a global and/or local level	4, 62, 100

SAM, S-adenosylmethionine; MAO-B, monoamine oxidase-B; SIRT1, sirtuin 1; ER, estrogen receptor.

incidence of tumors in offspring (114). A high-fat diet during pregnancy has also been linked to genomewide and locus-specific methylation changes in the placenta (C. Junien, personal communication).

Other examples of epigenetic modulation in response to environmental factors include the epigenetic downregulation of genes involved in pancreatic B-cell function in abnormal intrauterine environments (89) and the specific DNA methylation profiles of offspring associated with maternal diet (60, 61). Lillycrop and colleagues have shown that, either feeding a protein-restricted diet to pregnant rats and mice, or exposing them to undernutrition, causes stable changes to the epigenetic regulation of glucocorticoid receptor and peroxisomal proliferator-activated receptor- α genes in the livers of juvenile (60, 61) and adult (17) offspring. Interestingly, diet-dependent epigenetic changes are associated with altered messenger RNA expression of these genes and their target genes.

The role of maternal diet in establishing epigenetic marks has recently been confirmed at the genomewide level by Sinclair and colleagues (90). Using restriction landmark genomic scanning, they analyzed the methylation status of 1,400 CpG sites in the offspring of mature female sheep in response to the restriction of the supply of specific B vitamins and methionine from the periconceptual diet (74). They found that the offspring from vitamin-restricted maternal diets had numerous phenotypic alterations, such as increased body mass, altered immune responses to antigenic challenge, insulin resistance, and elevated blood pressure. In addition, 4% of the CpG sites analyzed featured altered methylation, which suggests that specific maternal diets can lead to widespread epigenetic alterations to DNA methylation in offspring and modify adult health-related phenotypes (90).

In humans, it has also recently been found that maternal diet may have an important role in establishing the long-term epigenotype of offspring (94). Tobi and colleagues (94) showed that individuals who were prenatally exposed to famine during the Dutch Hunger Winter exhibited less DNA methylation of the imprinted *IGF2* gene 6 decades later compared with their unexposed, same-sex siblings. The epigenetic alterations were established during periconceptual exposure, thereby supporting the concept that very early mammalian development is a crucial period for establishing and maintaining epigenetic marks. This work provides evidence that environmental conditions early on in human life can cause epigenetic changes that persist throughout life. Future studies will decipher the functional role of these epigenetic marks acquired during embryonic development in the establishment of specific phenotypes during adult life.

The Relationship Between Environment and Epigenetics During Adult Life

The effect of specific environmental factors on the epigenetic status of adult organisms has been widely reported (Table 1) (30, 32). However, the precise molecular mechanisms by which the environment can alter the epigenetic marks are still poorly understood and represent a fascinating field of study.

To understand how environmental factors can affect the epigenotype of an organism, it is important to take into account that higher organisms are composed of multiple tissues and that, as epigenetic status is tissue or cell specific, the effects of

environmental factors on the epigenotype of an organism can depend on the tissue type (i.e., because the specific epigenetic status of a tissue makes this tissue more resistant to environmentally induced epigenetic alteration). The degree of exposure of a tissue to a specific environmental factor can also determine its ability to induce specific epigenetic alterations within that tissue. For instance, it is easy to imagine that, if UV radiation has a specific effect on a particular epigenetic factor, then this would be much more evident in skin, since this is exposed to solar radiation, than in muscle, which is not.

In humans, the environmental factors that may affect epigenetic status during adult life can be divided into four groups: diet, living place and/or workplace, pharmacological treatments, and unhealthy habits (Fig. 3).

One of the most obvious examples of how the type of diet can affect epigenetic factors is folate intake. Folic acid (vitamin B9) is important for epigenetics, because it is necessary for the remethylation of homocysteine, a key chemical reaction in the metabolic pathway that systematizes *S*-adenosyl methionine, the methyl donor group of the histone and DNA methylation reactions. The amount of dietary folate intake may be associated with the epigenetic status of the organism (51, 55), and, interestingly, deficiencies in dietary folate result in numerous health alterations that are most evident when they occur during embryonic development (75). Methionine, another dietary compound involved in the metabolic pathway that synthesizes *S*-adenosyl methionine, is also thought to be involved in epigenetic-dependent hepatic diseases (5). Selenium is a dietary substance that modifies the epigenetic status of an organism at the DNA methylation and histone modification levels (113). Moreover, it has been proposed that cancer prevention by selenium may be mediated by its epigenetic effects (113). Certain dietary polyphenols, such as (-)-epigallocatechin 3-gallate from green tea and genistein from soybean are also thought to prevent cancer by means of epigenetic mechanisms (28). Recently, other substances occurring naturally in some foods, like butyrate in cheeses, diallyl disulphide in garlic, and sulphoraphane in broccoli, have been identified as HDAC inhibitors, and a putative role for some of these has been proposed in cancer chemoprevention through the disruption of the uncontrolled progression of cell cycle or by the induction of apoptosis via increased acetylation and derepression of genes, such as P21 and BAX (18, 23). The effect of diet on the epigenetic status of an organism can be so important that it has even been described that a high-fat diet can be associated with promoter DNA hypermethylation of specific tumor-suppressor genes (15).

Although it seems obvious that, if epigenetic factors can be altered by dietary compounds, then they should also be susceptible to alteration by pharmacological substances; the evidence for the latter is limited. One example is diethylstilbestrol, a drug used by millions of pregnant women to prevent miscarriages and many other disorders in pregnancy, which is currently known to be associated with an increased risk of breast cancer, clear cell adenocarcinoma of the vagina and cervix, and reproductive anomalies (100). Interestingly, it has been proposed that the dramatic health effects of diethylstilbestrol can be mediated by epigenetic mechanisms, since this drug has been shown to alter expression of DNA methyltransferases and methylation of genomic DNA (34, 83). Another example is sodium valproate, an anticonvulsant classically used to treat

some mental disorders that is now a well-known and potent inhibitor of HDACs (101). Procaine, a drug that was used in the past as a local anesthetic, is now known to induce DNA demethylation (102). Another example is the antituberculosis drug pyrazinamide, which has recently been shown to induce a decrease in cytosine DNA methylation of long-interspersed nucleotide element-1 and aberrant promoter hypermethylation of p16(INK4A) in rats (57). Several antibiotics have also been shown to induce significant epigenetic alterations. For instance, the anthracycline antibiotic doxorubicin is known to induce conditional apoptosis in cancer cells by the inhibition of the DNA methyltransferase DNMT1 (115).

The living place and/or workplace can determine the level of exposure to many environmental factors that are potentially able to alter epigenetic status. For example, it is obvious that the chemical and xenobiotic composition of the atmosphere and water in a city is different from that in a rural environment. Although it is not entirely certain that these environmental factors are responsible for epigenetic alterations, strong associations among them have been found. One of the best examples of these is the recent observation that, in pleural tissues, at least 24 CpG loci have asbestos-related alterations in methylation, all of which featured increases in methylation (21). Other types of chemical entities that are known to alter DNA methylation levels are metal ions. For instance, the environmental pollutants chromium (87), cadmium (93), and nickel (80) have been shown to reduce methylation levels by inhibiting the activity of DNA methyltransferases. In the case of nickel, carcinogenic nickel compounds can reduce global histone H4 acetylation and increase histone H3 lysine 9 dimethylation at the promoter level, repressing expression of specific genes (20). Other chemicals and xenobiotics to which organisms may be exposed can also change DNA methylation at a global and/or local level. These include bisphenol A (62), used in the plastics industry, and vinclozolin (4), a fungicide used in vineyards. These molecules are considered to be endocrine disruptors and have been convincingly related to DNA methylation alteration of specific promoters, developmental disorders, and tumorigenesis.

Unhealthy habits, such as alcohol and tobacco consumption and drug abuse, can also alter specific epigenetic factors. Indeed, one of the best current examples of how environmental factors can alter the epigenetic status of an organism is the promoter hypermethylation of tumor-suppressor genes that occurs in nontumorigenic lung tissues of smokers, but not in the corresponding tissues of nonsmokers (8). The relationship between tobacco use and alterations of DNA methylation was recently confirmed by Christensen and colleagues (21), who reported that, in lung tissues, smoking status (smoker vs. never smoker) is associated with altered methylation at 138 CpG loci. Interestingly, these authors also showed that, in adult blood cells, increasing pack-years of smoking was significantly associated with *MLH1* and *RIPK3* methylation, which suggests that tissues less exposed to cigarette smoke are also prone to smoking-dependent epigenetic alterations. The effect of alcohol consumption on epigenetic factors in humans has been much less extensively studied. One of the few published studies describes how the *HERP* gene is significantly down-regulated by promoter DNA hypermethylation in patients with alcohol dependence compared with healthy controls (13) and that over 30 CpG loci have significantly altered methylation in

never-drinkers compared with drinkers (21). However, it has been widely reported that chronic alcohol feeding in rats results in many epigenetic alterations. For instance, chronic ethanol feeding causes the inhibition of the ubiquitin proteasome pathway in the nucleus, which leads to changes in the turnover of transcriptional factors, histone-modifying enzymes, and, therefore, altered epigenetic mechanisms (6, 71).

The abuse of some drugs has also been shown to alter specific epigenetic factors. Indeed, cannabinoids, heroin, and cocaine have been shown to produce deep epigenetic alterations (33).

The best studied epigenetic alterations caused by drug abuse are those caused by cocaine. Maternal cocaine administration in mice is known to alter DNA methylation and gene expression in hippocampus neurons of neonatal and prepubertal offspring (69). In addition, gene regulation by the HMT G9a has an essential role in cocaine-induced plasticity (63), and HDAC inhibitors decrease cocaine self-administration in rats (79). These observations suggest that a number of the physiological effects of cocaine in vivo are mediated by epigenetic mechanisms. Further research is needed to determine the real impact of the abuse of other drugs, such as cannabinoids and heroin, on the epigenome of the organism.

Although it is evident that most of the substances described above can alter specific epigenetic factors, it should be stressed that it is not known whether all of them can be considered authentic epigenetic modifiers, because it has not yet been demonstrated whether the epigenetic modifications that they induce are stable over time.

DISCLOSURES

No conflicts of interest (financial or otherwise) are declared by the author(s).

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