HIGHLIGHTED TOPIC | *Epigenetics in Health and Disease*

Epigenetics and environment: a complex relationship

Oscar Aguilera,[1]* **Agustín F. Fernández,**[2]* **Alberto Muñoz,**[1] **and Mario F. Fraga**[3]

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

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/japplphysiol.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-methylcytosine (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

* O. Aguilera and A. F. Fernández contributed equally to this work.
Address for reprint requests and other correspondence: Mario F. Fraga, Dept. of Immunology and Oncology, National Center for Biotechnology, CNB-CSIC, Cantoblanco, Madrid E-28049, Spain (e-mail: mffraga@cnb.csic.es).

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 NH_2-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
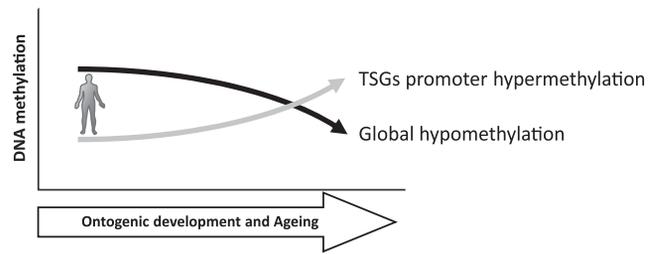

Fig. 2. DNA methylation changes during ontogenic development and aging. Global DNA methylation decreases with age in many tissue types, while several specific regions of the genomic DNA become hypermethylated. TSGs, tumor-suppressor genes.

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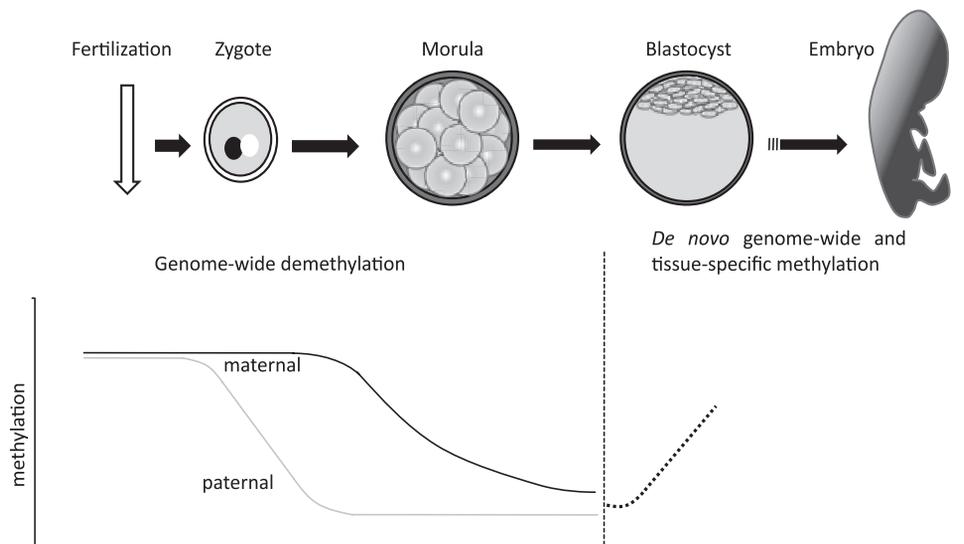
ously been found in cultured fibroblasts (19). Curiously, global DNA hypomethylation and promoter hypermethylation of specific genes have also been found in cancer (27), which suggests a genuine connection between aging and malignant transformation.

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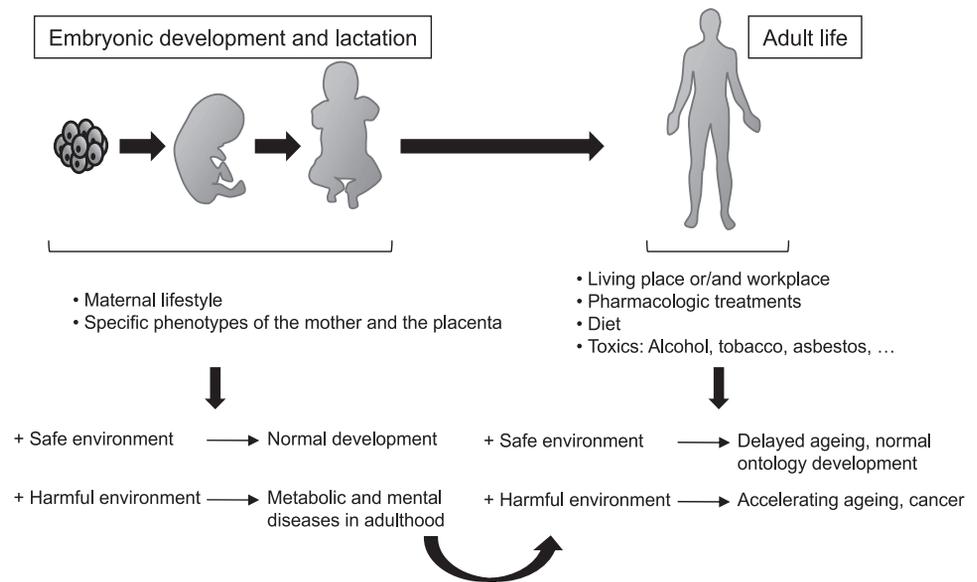
Fig. 3. Effects of environmental factors on epigenetics. Two different scenarios are illustrated (embryonic/lactation stages and adult life), in which different factors associated with safe or harmful environment can determine alterations during life development. The effects in adulthood of alterations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

REFERENCES

1. **Agrelo R, Cheng WH, Setien F, Ropero S, Espada J, Fraga MF, Herranz M, Paz MF, Sanchez-Cespedes M, Artiga MJ, Guerrero D, Castells A, von Kobbe C, Bohr VA, Esteller M.** Epigenetic inactivation of the premature aging Werner syndrome gene in human cancer. *Proc Natl Acad Sci U S A* 103: 8822–8827, 2006.
2. **Agrelo R, Setien F, Espada J, Artiga MJ, Rodriguez M, Perez-Rosado A, Sanchez-Aguilera A, Fraga MF, Piris MA, Esteller M.** Inactivation of the lamin A/C gene by CpG island promoter hypermethylation in hematologic malignancies, and its association with poor survival in nodal diffuse large B-cell lymphoma. *J Clin Oncol* 23: 3940–3947, 2005.
3. **Allfrey VG, Faulkner R, Mirsky AE.** Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc Natl Acad Sci U S A* 51: 786–794, 1964.
4. **Anway MD, Leathers C, Skinner MK.** Endocrine disruptor vinclozolin induced epigenetic transgenerational adult-onset disease. *Endocrinology* 147: 5515–5523, 2006.
5. **Avila MA, Garcia-Trevijano ER, Martinez-Chantar ML, Latasa MU, Perez-Mato I, Martinez-Cruz LA, del Pino MM, Corrales FJ, Mato JM.** *S*-adenosylmethionine revisited: its essential role in the regulation of liver function. *Alcohol* 27: 163–167, 2002.
6. **Bardag-Gorce F, French BA, Joyce M, Baires M, Montgomery RO, Li J, French S.** Histone acetyltransferase p300 modulates gene expression in an epigenetic manner at high blood alcohol levels. *Exp Mol Pathol* 82: 197–202, 2007.
7. **Beard C, Li E, Jaenisch R.** Loss of methylation activates Xist in somatic but not in embryonic cells. *Genes Dev* 9: 2325–2334, 1995.
8. **Belinsky SA, Palmisano WA, Gilliland FD, Crooks LA, Divine KK, Winters SA, Grimes MJ, Harms HJ, Tellez CS, Smith TM, Moots PP, Lechner JF, Stidley CA, Crowell RE.** Aberrant promoter methylation in bronchial epithelium and sputum from current and former smokers. *Cancer Res* 62: 2370–2377, 2002.

9. **Berdyshev GD, Korotaev GK, Boiarskikh GV, Vaniushin BF.** [Nucleotide composition of DNA and RNA from somatic tissues of humpback and its changes during spawning]. *Biokhimiia* 32: 988–993, 1967.

10. **Bestor TH.** The DNA methyltransferases of mammals. *Hum Mol Genet* 9: 2395–2402, 2000.

11. **Bird A.** DNA methylation patterns and epigenetic memory. *Genes Dev* 16: 6–21, 2002.

12. **Bjornsson HT, Sigurdsson MI, Fallin MD, Irizarry RA, Aspelund T, Cui H, Yu W, Rongione MA, Ekstrom TJ, Harris TB, Launer LJ, Eiriksdottir G, Leppert MF, Sapienza C, Gudnason V, Feinberg AP.** Intra-individual change over time in DNA methylation with familial clustering. *JAMA* 299: 2877–2883, 2008.

13. **Bleich S, Lenz B, Ziegenbein M, Beutler S, Frieling H, Kornhuber J, Bonsch D.** Epigenetic DNA hypermethylation of the HERP gene promoter induces down-regulation of its mRNA expression in patients with alcohol dependence. *Alcohol Clin Exp Res* 30: 587–591, 2006.

14. **Bracken AP, Kleine-Kohlbrecher D, Dietrich N, Pasini D, Gargiulo G, Beekman C, Theilgaard-Monch K, Minucci S, Porse BT, Marine JC, Hansen KH, Helin K.** The Polycomb group proteins bind throughout the INK4A-ARF locus and are disassociated in senescent cells. *Genes Dev* 21: 525–530, 2007.

15. **Brait M, Ford JG, Papaiahgari S, Garza MA, Lee JI, Loyo M, Maldonado L, Begum S, McCaffrey L, Howerton M, Sidransky D, Emerson MR, Ahmed S, Williams CD, Hoque MO.** Association between lifestyle factors and CpG island methylation in a cancer-free population. *Cancer Epidemiol Biomarkers Prev* 18: 2984–2991, 2009.

16. **Brosh RM Jr, Bohr VA.** Roles of the Werner syndrome protein in pathways required for maintenance of genome stability. *Exp Gerontol* 37: 491–506, 2002.

17. **Burdge GC, Slater-Jefferies J, Torrens C, Phillips ES, Hanson MA, Lillycrop KA.** Dietary protein restriction of pregnant rats in the F0 generation induces altered methylation of hepatic gene promoters in the adult male offspring in the F1 and F2 generations. *Br J Nutr* 97: 435–439, 2007.

18. **Calvanese V, Lara E, Kahn A, Fraga MF.** The role of epigenetics in aging and age-related diseases. *Ageing Res Rev* 8: 268–276, 2009.

19. **Casillas MA Jr, Lopatina N, Andrews LG, Tollefsbol TO.** Transcriptional control of the DNA methyltransferases is altered in aging and neoplastically-transformed human fibroblasts. *Mol Cell Biochem* 252: 33–43, 2003.

20. **Chen H, Ke Q, Kluz T, Yan Y, Costa M.** Nickel ions increase histone H3 lysine 9 dimethylation and induce transgene silencing. *Mol Cell Biol* 26: 3728–3737, 2006.

21. **Christensen BC, Houseman EA, Marsit CJ, Zheng S, Wrensch MR, Wiemels JL, Nelson HH, Karagas MR, Padbury JF, Bueno R, Sugarbaker DJ, Yeh RF, Wiencke JK, Kelsey KT.** Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. *PLoS Genet* 5: e1000602, 2009.

22. **Chuang JC, Jones PA.** Epigenetics and microRNAs. *Pediatr Res* 61: 24R–29R, 2007.

23. **Dashwood RH, Ho E.** Dietary histone deacetylase inhibitors: from cells to mice to man. *Semin Cancer Biol* 17: 363–369, 2007.

24. **Doerfler W.** Patterns of DNA methylation–evolutionary vestiges of foreign DNA inactivation as a host defense mechanism. A proposal. *Biol Chem Hoppe Seyler* 372: 557–564, 1991.

25. **Epstein J, Williams JR, Little JB.** Deficient DNA repair in human progeroid cells. *Proc Natl Acad Sci U S A* 70: 977–981, 1973.

26. **Eriksson M, Brown WT, Gordon LB, Glynn MW, Singer J, Scott L, Erdos MR, Robbins CM, Moses TY, Berglund P, Dutra A, Pak E, Durkin S, Csoka AB, Boehnke M, Glover TW, Collins FS.** Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature* 423: 293–298, 2003.

27. **Esteller M.** Epigenetics in cancer. *N Engl J Med* 358: 1148–1159, 2008.

28. **Fang M, Chen D, Yang CS.** Dietary polyphenols may affect DNA methylation. *J Nutr* 137: 223S–228S, 2007.

29. **Fatemi M, Hermann A, Gowher H, Jeltsch A.** Dnmt3a and Dnmt1 functionally cooperate during de novo methylation of DNA. *Eur J Biochem* 269: 4981–4984, 2002.

30. **Feinberg AP.** Phenotypic plasticity and the epigenetics of human disease. *Nature* 447: 433–440, 2007.

31. **Feinberg AP, Cui H, Ohlsson R.** DNA methylation and genomic imprinting: insights from cancer into epigenetic mechanisms. *Semin Cancer Biol* 12: 389–398, 2002.

32. **Feinberg AP, Ohlsson R, Henikoff S.** The epigenetic progenitor origin of human cancer. *Nat Rev Genet* 7: 21–33, 2006.

33. **Fernandez-Ruiz J, Gomez M, Hernandez M, de Miguel R, Ramos JA.** Cannabinoids and gene expression during brain development. *Neurotox Res* 6: 389–401, 2004.

34. **Fleming JL, Huang TH, Toland AE.** The role of parental and grandparental epigenetic alterations in familial cancer risk. *Cancer Res* 68: 9116–9121, 2008.

35. **Fraga MF.** Genetic and epigenetic regulation of aging. *Curr Opin Immunol* 21: 446–453, 2009.

36. **Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suner D, Cigudosa JC, Urioste M, Benitez J, Boix-Chornet M, Sanchez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector TD, Wu YZ, Plass C, Esteller M.** Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci U S A* 102: 10604–10609, 2005.

37. **Fraga MF, Esteller M.** Epigenetics and aging: the targets and the marks. *Trends Genet* 23: 413–418, 2007.

38. **Gabory A, Attig L, Junien C.** Sexual dimorphism in environmental epigenetic programming. *Mol Cell Endocrinol* 304: 8–18, 2009.

39. **Gartner K.** A third component causing random variability beside environment and genotype. A reason for the limited success of a 30 year long effort to standardize laboratory animals? *Lab Anim* 24: 71–77, 1990.

40. **Guarente L, Picard F.** Calorie restriction–the SIR2 connection. *Cell* 120: 473–482, 2005.

41. **Herman JG, Baylin SB.** Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med* 349: 2042–2054, 2003.

42. **Holliday R.** Epigenetics: a historical overview. *Epigenetics* 1: 76–80, 2006.

43. **Holliday R.** The inheritance of epigenetic defects. *Science* 238: 163–170, 1987.

44. **Issa JP.** Age-related epigenetic changes and the immune system. *Clin Immunol* 109: 103–108, 2003.

45. **Janzen V, Forkert R, Fleming HE, Saito Y, Waring MT, Dombkowski DM, Cheng T, DePinho RA, Sharpless NE, Scadden DT.** Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16INK4a. *Nature* 443: 421–426, 2006.

46. **Jones PA, Baylin SB.** The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 3: 415–428, 2002.

47. **Jones PA, Takai D.** The role of DNA methylation in mammalian epigenetics. *Science* 293: 1068–1070, 2001.

48. **Jung-Hynes B, Reiter RJ, Ahmad N.** Sirtuins, melatonin and circadian rhythms: building a bridge between aging and cancer. *J Pineal Res* 48: 9–19, 2010.

49. **Kaminsky ZA, Tang T, Wang SC, Ptak C, Oh GH, Wong AH, Feldcamp LA, Virtanen C, Halfvarson J, Tysk C, McRae AF, Visscher PM, Montgomery GW, Gottesman II, Martin NG, Petronis A.** DNA methylation profiles in monozygotic and dizygotic twins. *Nat Genet* 41: 240–245, 2009.

50. **Ke Q, Davidson T, Chen H, Kluz T, Costa M.** Alterations of histone modifications and transgene silencing by nickel chloride. *Carcinogenesis* 27: 1481–1488, 2006.

51. **Keyes MK, Jang H, Mason JB, Liu Z, Crott JW, Smith DE, Friso S, Choi SW.** Older age and dietary folate are determinants of genomic and p16-specific DNA methylation in mouse colon. *J Nutr* 137: 1713–1717, 2007.

52. **Kim GD, Ni J, Kelesoglu N, Roberts RJ, Pradhan S.** Co-operation and communication between the human maintenance and de novo DNA (cytosine-5) methyltransferases. *EMBO J* 21: 4183–4195, 2002.

53. **Kim KC, Friso S, Choi SW.** DNA methylation, an epigenetic mechanism connecting folate to healthy embryonic development and aging. *J Nutr Biochem* 20: 917–926, 2009.

54. **Kim WY, Sharpless NE.** The regulation of INK4/ARF in cancer and aging. *Cell* 127: 265–275, 2006.

55. **Kotsopoulos J, Sohn KJ, Kim YI.** Postweaning dietary folate deficiency provided through childhood to puberty permanently increases genomic DNA methylation in adult rat liver. *J Nutr* 138: 703–709, 2008.

56. **Kouzarides T.** Chromatin modifications and their function. *Cell* 128: 693–705, 2007.

57. **Kovalenko VM, Bagnyukova TV, Sergienko OV, Bondarenko LB, Shayakhmetova GM, Matvienko AV, Pogribny IP.** Epigenetic changes in the rat livers induced by pyrazinamide treatment. *Toxicol Appl Pharmacol* 225: 293–299, 2007.

58. **Launay JM, Del Pino M, Chironi G, Callebert J, Peoc'h K, Megnien JL, Mallet J, Simon A, Rendu F.** Smoking induces long-lasting effects through a monoamine-oxidase epigenetic regulation. *PLoS One* 4: e7959, 2009.

59. **Li E, Beard C, Jaenisch R.** Role for DNA methylation in genomic imprinting. *Nature* 366: 362–365, 1993.

60. **Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC.** Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J Nutr* 135: 1382–1386, 2005.

61. **Lillycrop KA, Phillips ES, Torrens C, Hanson MA, Jackson AA, Burdge GC.** Feeding pregnant rats a protein-restricted diet persistently alters the methylation of specific cytosines in the hepatic PPAR alpha promoter of the offspring. *Br J Nutr* 100: 278–282, 2008.

62. **Maffini MV, Rubin BS, Sonnenschein C, Soto AM.** Endocrine disruptors and reproductive health: the case of bisphenol-A. *Mol Cell Endocrinol* 254–255: 179–186, 2006.

63. **Maze I, Covington HE, 3rd Dietz DM, LaPlant Q, Renthal W, Russo SJ, Mechanic M, Mouzon E, Neve RL, Haggarty SJ, Ren Y, Sampath SC, Hurd YL, Greengard P, Tarakhovsky A, Schaefer A, Nestler EJ.** Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science* 327: 213–216, 2010.

64. **Milagro FI, Campion J, Garcia-Diaz DF, Goyenechea E, Paternain L, Martinez JA.** High fat diet-induced obesity modifies the methylation pattern of leptin promoter in rats. *J Physiol Biochem* 65: 1–9, 2009.

65. **Morgan HD, Santos F, Green K, Dean W, Reik W.** Epigenetic reprogramming in mammals. *Hum Mol Genet* 14, *Spec No* 1: R47–R58, 2005.

66. **Morgan HD, Sutherland HG, Martin DI, Whitelaw E.** Epigenetic inheritance at the agouti locus in the mouse. *Nat Genet* 23: 314–318, 1999.

67. **Myzak MC, Dashwood WM, Orner GA, Ho E, Dashwood RH.** Sulforaphane inhibits histone deacetylase in vivo and suppresses tumorigenesis in Apc-minus mice. *FASEB J* 20: 506–508, 2006.

68. **Nafee TM, Farrell WE, Carroll WD, Fryer AA, Ismail KM.** Epigenetic control of fetal gene expression. *BJOG* 115: 158–168, 2008.

69. **Novikova SI, He F, Bai J, Cutrufello NJ, Lidow MS, Undieh AS.** Maternal cocaine administration in mice alters DNA methylation and gene expression in hippocampal neurons of neonatal and prepubertal offspring. *PLoS One* 3: e1919, 2008.

70. **Oakes CC, Smiraglia DJ, Plass C, Trasler JM, Robaire B.** Aging results in hypermethylation of ribosomal DNA in sperm and liver of male rats. *Proc Natl Acad Sci U S A* 100: 1775–1780, 2003.

71. **Oliva J, Dedes J, Li J, French SW, Bardag-Gorce F.** Epigenetics of proteasome inhibition in the liver of rats fed ethanol chronically. *World J Gastroenterol* 15: 705–712, 2009.

72. **Opresko PL, Cheng WH, von Kobbe C, Harrigan JA, Bohr VA.** Werner syndrome and the function of the Werner protein; what they can teach us about the molecular aging process. *Carcinogenesis* 24: 791–802, 2003.

73. **Payer B, Lee JT.** X chromosome dosage compensation: how mammals keep the balance. *Annu Rev Genet* 42: 733–772, 2008.

74. **Plass C, Shibata H, Kalcheva I, Mullins L, Kotelevtseva N, Mullins J, Kato R, Sasaki H, Hirotsune S, Okazaki Y, Held WA, Hayashizaki Y, Chapman VM.** Identification of Grf1 on mouse chromosome 9 as an imprinted gene by RLGS-M. *Nat Genet* 14: 106–109, 1996.

75. **Poulsen P, Esteller M, Vaag A, Fraga MF.** The epigenetic basis of twin discordance in age-related diseases. *Pediatr Res* 61: 38R–42R, 2007.

76. **Pruitt K, Zinn RL, Ohm JE, McGarvey KM, Kang SH, Watkins DN, Herman JG, Baylin SB.** Inhibition of SIRT1 reactivates silenced cancer genes without loss of promoter DNA hypermethylation. *PLoS Genet* 2: e40, 2006.

77. **Rada-Iglesias A, Enroth S, Ameur A, Koch CM, Clelland GK, Respuela-Alonso P, Wilcox S, Dovey OM, Ellis PD, Langford CF, Dunham I, Komorowski J, Wadelius C.** Butyrate mediates decrease of histone acetylation centered on transcription start sites and down-regulation of associated genes. *Genome Res* 17: 708–719, 2007.

78. **Rakyan VK, Beck S.** Epigenetic variation and inheritance in mammals. *Curr Opin Genet Dev* 16: 573–577, 2006.

79. **Romieu P, Host L, Gobaille S, Sandner G, Aunis D, Zwiller J.** Histone deacetylase inhibitors decrease cocaine but not sucrose self-administration in rats. *J Neurosci* 28: 9342–9348, 2008.

80. **Salnikow K, Costa M.** Epigenetic mechanisms of nickel carcinogenesis. *J Environ Pathol Toxicol Oncol* 19: 307–318, 2000.

81. **Sarg B, Koutzamani E, Helliger W, Rundquist I, Lindner HH.** Postsynthetic trimethylation of histone H4 at lysine 20 in mammalian tissues is associated with aging. *J Biol Chem* 277: 39195–39201, 2002.

82. **Sasaki T, Maier B, Bartke A, Scrable H.** Progressive loss of SIRT1 with cell cycle withdrawal. *Aging Cell* 5: 413–422, 2006.

83. **Sato K, Fukata H, Kogo Y, Ohgane J, Shiota K, Mori C.** Neonatal exposure to diethylstilbestrol alters expression of DNA methyltransferases and methylation of genomic DNA in the mouse uterus. *Endocr J* 56: 131–139, 2009.

84. **Scaffidi P, Misteli T.** Lamin A-dependent misregulation of adult stem cells associated with accelerated ageing. *Nat Cell Biol* 10: 452–459, 2008.

85. **Scaffidi P, Misteli T.** Lamin A-dependent nuclear defects in human aging. *Science* 312: 1059–1063, 2006.

86. **Scaffidi P, Misteli T.** Reversal of the cellular phenotype in the premature aging disease Hutchinson-Gilford progeria syndrome. *Nat Med* 11: 440–445, 2005.

87. **Shiao YH, Crawford EB, Anderson LM, Patel P, Ko K.** Allele-specific germ cell epimutation in the spacer promoter of the 45S ribosomal RNA gene after Cr(III) exposure. *Toxicol Appl Pharmacol* 205: 290–296, 2005.

88. **Shumaker DK, Dechat T, Kohlmaier A, Adam SA, Bozovsky MR, Erdos MR, Eriksson M, Goldman AE, Khuon S, Collins FS, Jenuwein T, Goldman RD.** Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging. *Proc Natl Acad Sci U S A* 103: 8703–8708, 2006.

89. **Simmons R.** Developmental origins of adult metabolic disease: concepts and controversies. *Trends Endocrinol Metab* 16: 390–394, 2005.

90. **Sinclair KD, Allegrucci C, Singh R, Gardner DS, Sebastian S, Bispham J, Thurston A, Huntley JF, Rees WD, Maloney CA, Lea RG, Craigon J, McEvoy TG, Young LE.** DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. *Proc Natl Acad Sci U S A* 104: 19351–19356, 2007.

91. **So K, Tamura G, Honda T, Homma N, Waki T, Togawa N, Nishizuka S, Motoyama T.** Multiple tumor suppressor genes are increasingly methylated with age in non-neoplastic gastric epithelia. *Cancer Sci* 97: 1155–1158, 2006.

92. **Sommer M, Poliak N, Upadhyay S, Ratovitski E, Nelkin BD, Donehower LA, Sidransky D.** DeltaNp63alpha overexpression induces downregulation of Sirt1 and an accelerated aging phenotype in the mouse. *Cell Cycle* 5: 2005–2011, 2006.

93. **Takiguchi M, Achanzar WE, Qu W, Li G, Waalkes MP.** Effects of cadmium on DNA-(cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation. *Exp Cell Res* 286: 355–365, 2003.

94. **Tobi EW, Lumey LH, Talens RP, Kremer D, Putter H, Stein AD, Slagboom PE, Heijmans BT.** DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum Mol Genet* 18: 4046–4053, 2009.

95. **Tremolizzo L, Carboni G, Ruzicka WB, Mitchell CP, Sugaya I, Tueting P, Sharma R, Grayson DR, Costa E, Guidotti A.** An epigenetic mouse model for molecular and behavioral neuropathologies related to schizophrenia vulnerability. *Proc Natl Acad Sci U S A* 99: 17095–17100, 2002.

96. **Tsygankov D, Liu Y, Sanoff HK, Sharpless NE, Elston TC.** A quantitative model for age-dependent expression of the p16INK4a tumor suppressor. *Proc Natl Acad Sci U S A* 106: 16562–16567, 2009.

97. **Umlauf D, Fraser P, Nagano T.** The role of long non-coding RNAs in chromatin structure and gene regulation: variations on a theme. *Biol Chem* 389: 323–331, 2008.

98. **Vanyushin BF, Nemirovsky LE, Klimenko VV, Vasiliev VK, Belozersky AN.** The 5-methylcytosine in DNA of rats. Tissue and age specificity and the changes induced by hydrocortisone and other agents. *Gerontologia* 19: 138–152, 1973.

99. **Vaquero A, Scher M, Lee D, Erdjument-Bromage H, Tempst P, Reinberg D.** Human SirT1 interacts with histone H1 and promotes formation of facultative heterochromatin. *Mol Cell* 16: 93–105, 2004.

100. **Veurink M, Koster M, Berg LT.** The history of DES, lessons to be learned. *Pharm World Sci* 27: 139–143, 2005.

101. **Villar-Garea A, Esteller M.** Histone deacetylase inhibitors: understanding a new wave of anticancer agents. *Int J Cancer* 112: 171–178, 2004.

102. **Villar-Garea A, Fraga MF, Espada J, Esteller M.** Procaine is a DNA-demethylating agent with growth-inhibitory effects in human cancer cells. *Cancer Res* 63: 4984–4989, 2003.

103. **Vogt G, Huber M, Thiemann M, van den Boogaart G, Schmitz OJ, Schubart CD.** Production of different phenotypes from the same genotype in the same environment by developmental variation. *J Exp Biol* 211: 510–523, 2008.

104. **Waddington CH.** The epigenotype. *Endeavour* 1: 18–20, 1942.

105. **Warri A, Saarinen NM, Makela S, Hilakivi-Clarke L.** *The role of early life genistein exposures in modifying breast cancer risk. Br J Cancer* 98: 1485–1493, 2008.

106. **Waterland RA.** Assessing the effects of high methionine intake on DNA methylation. *J Nutr* 136: 1706S–1710S, 2006.

107. **Waterland RA.** Do maternal methyl supplements in mice affect DNA methylation of offspring? *J Nutr* 133: 238; author reply 239, 2003.

108. **Waterland RA, Dolinoy DC, Lin JR, Smith CA, Shi X, Tahiliani KG.** Maternal methyl supplements increase offspring DNA methylation at axin fused. *Genesis* 44: 401–406, 2006.

109. **Waterland RA, Travisano M, Tahiliani KG, Rached MT, Mirza S.** Methyl donor supplementation prevents transgenerational amplification of obesity. *Int J Obes (Lond)* 32: 1373–1379, 2008.

110. **Whitelaw NC, Whitelaw E.** How lifetimes shape epigenotype within and across generations. *Hum Mol Genet* 15, *Spec No* 2: R131–137, 2006.

111. **Wilson VL, Jones PA.** DNA methylation decreases in aging but not in immortal cells. *Science* 220: 1055–1057, 1983.

112. **Wilson VL, Smith RA, Ma S, Cutler RG.** Genomic 5-methyldeoxycytidine decreases with age. *J Biol Chem* 262: 9948–9951, 1987.

113. **Xiang N, Zhao R, Song G, Zhong W.** Selenite reactivates silenced genes by modifying DNA methylation and histones in prostate cancer cells. *Carcinogenesis* 29: 2175–2181, 2008.

114. **Yenbutr P, Hilakivi-Clarke L, Passaniti A.** Hypomethylation of an exon I estrogen receptor CpG island in spontaneous and carcinogen-induced mammary tumorigenesis in the rat. *Mech Ageing Dev* 106: 93–102, 1998.

115. **Yokochi T, Robertson KD.** Doxorubicin inhibits DNMT1, resulting in conditional apoptosis. *Mol Pharmacol* 66: 1415–1420, 2004.