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Special Article

Epigenetic mechanisms in health and diseases

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ABSTRACT

Epigenetic represents the programming of the genome to express the appropriate set of genes in specific cells at specific time points in life. The main epigenetic mechanisms are: 1 - methylation status of cytosines within CpG islands located in the promoter region of many genes; 2 - post-translational acetylation or methylation of lysines in the histone N-terminal region, which influence chromatin packaging; and 3 - production of non coding micro-RNAs involved in gene expression modulation. Epigenetic includes both heritable changes in gene activity and expression but also stable, long-term alterations in the transcriptional potential of a cell that are not necessarily heritable. These changes might be produced in particular by the early life environment (pollution, infection, maternal care, etc) and might affect health in adult life influencing the susceptibility to several diseases, such as cancer, psychiatric or neurological disorders.

Key words. Epigenetic mechanisms; disease; environment; behaviour; drugs.

RESUMO

MECANISMOS EPIGENÉTICOS NA SAÚDE E DOENÇA

Epigenética representa a programação do genoma para expressar o conjunto apropriado de genes em células específicas em momentos específicos da vida.

Os principais mecanismos epigenéticos são: 1 - metilação de citosinas nas ilhas CpG localizadas na região promotora de vários genes; 2 - acetilação pós-translacional ou metilação de lisinas na região N-terminal da histona, que influencia a cobertura da cromatina; e, 3 - produção de micro-RNAs não codificantes envolvidos na modulação da expressão gênica. Epigenética inclui mudanças hereditárias na atividade e expressão do gene, mas também alterações estáveis em longo prazo no potencial de transcrição de uma célula que não é necessariamente hereditária. Essas mudanças podem ser produzidas em especial pelo ambiente no início da vida (poluição, infecção, cuidados maternos, etc) e pode afetar a saúde na vida adulta, influenciando a susceptibilidade a diversas doenças, como câncer, psiquiátricas ou neurológicas. Ferramentas farmacológicas e outras formas de intervenção podem modificar potencialmente o padrão epigenético natural, oferecendo um caminho possível para reverter a programação epigenética deletéria

Palavras-chave. *Mecanismos epigenético; doença; meio-ambiente; comportamento; drogas.*

Introduction

Epigenetics refers to heritable (meiotically or mitotically) patterns in phenotype or gene expression produced by mechanisms other than changes in the underlying DNA sequence; it represents the programming of the genome to express the appropriate set of genes in specific cells at specific time points in life.

Epigenetic patterns are generated during cellular differentiation by a highly programmed and organized process, nevertheless, they are dynamic and responsive to the environment especially during the critical periods of gestation and early life, but to a minor extent also later in life. Today the term epigenetics has widened to include both heritable changes in gene

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Received on April 8, 2010. Accepted on May 4, 2010.

activity and expression (in the progeny of cells or of individuals) but also stable, long-term alterations in the transcriptional potential of a cell that are not necessarily heritable. These changes might be produced in particular by the early life environment and might affect the health in adult life influencing the susceptibility to several diseases.

In eukaryotic cells epigenetic modifications are encoded via two primary modes: DNA methylation and histone modifications. Non coding microRNAs production and differences in chromatin-related proteins are additional mechanisms involved in modulating gene expression.

Basic epigenetic mechanisms

DNA methylation

It is the most frequently occurring epigenetic mechanism consisting in a covalent addition of a methyl group to the C5 position of cytosines that precede guanines in CpGs dinucleotides, called CpG islands[1] (figure 1).

CpG islands are short stretches of DNA in which the frequency of the CG sequence is higher than in other regions. "p" simply indicates that cytosine and guanine are connected by a phosphodiester bond. CpG islands are often located around the promoters of housekeeping genes or other genes frequently expressed in a cell (56% of genes).[2] Unmethylated CpG islands are target of transcription factors to start the transcription. By contrast, the CpG sequences in inactive genes are usually methylated to suppress their expression. DNA methylation patterns correlate with chromatin structure: active regions, where genes are expressed, are associated with hypomethylated DNA sequences, whereas hypermethylated DNA is packaged in inactive chromatin.[3]

Two main mechanisms are involved in silencing gene expression by DNA methylation: direct interference of methyl residues in binding of transcription factors[4] and attraction of binding proteins, such as MeCP25 and/or histone modifying enzymes, which leads to a formation of "closed" chromatin configuration associated with silencing of gene expression.[5]

Genomic DNA hypermethylation at one of the two parental alleles is also the basic mechanism of imprinting.[6] Demethylation of imprinted genes represents an important mechanism of aberrant gene expression occurring in some diseases, as cancer. Methylation is also involved in X-chromosome inactivation in females and in the induction of chromosomal stability through the hypermethylation of repetitive genomic sequences.[7,8]

DNA methyltransferases (DNMTs) are the enzymes involved in methylation; at least three functional DNMTs have been identified in eukaryotic systems.

DNMT1 is involved in maintenance of methylation status during replication (it can methylate only

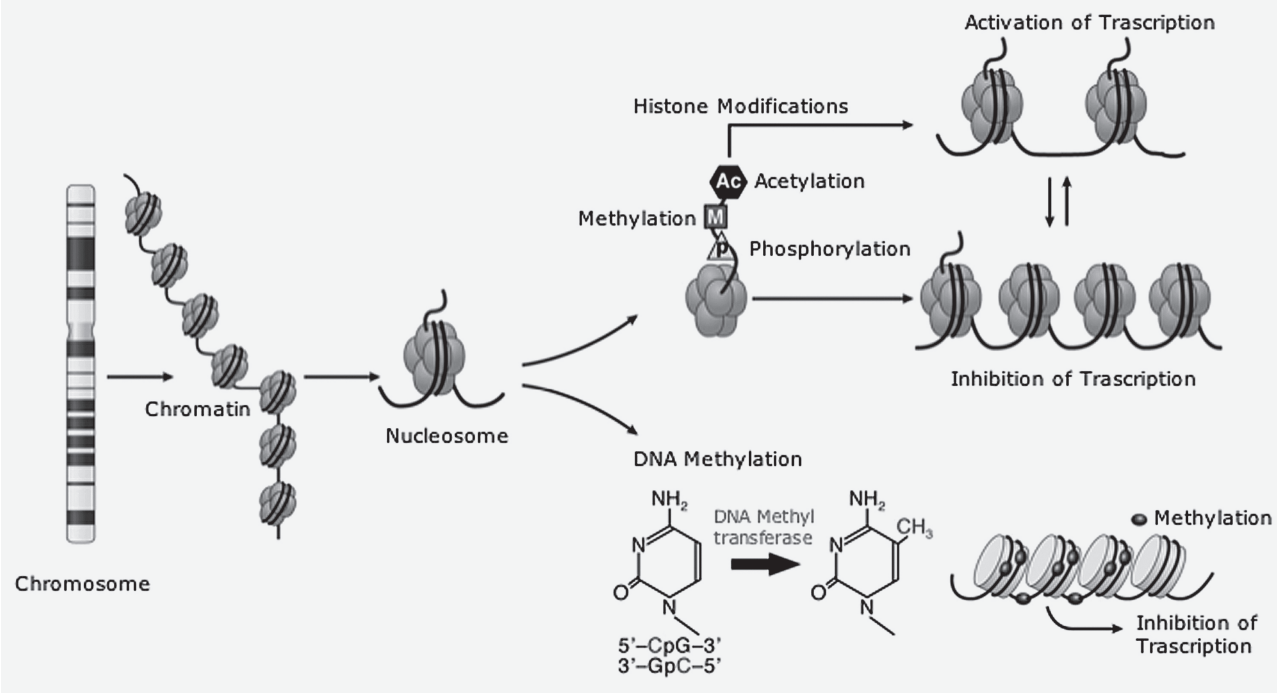

Figure 1. DNA methylation at 5'cytosine and effects on gene transcription

the CG sequence paired with methylated CG), while the other methylases contribute to the 'de novo' methylation pattern first established during embryonic development.[9]

Histone modifications

The basic repeating unit of chromatin, the nucleosome, consists of 146 bp of DNA wrapped around an octameric histone core formed by two copies each of histones H2A, H2B, H3, and H4. Histones beside possessing a definite structural function have a specific role in modulating the physical access of nuclear factors to DNA. It is now clear that post-translational modifications of charged aminoacids of histone tails that protrude from the core structure of the nucleosome can alter chromatin conformation and create binding sites for transcription factors playing a direct regulatory role in gene expression[10] (figure 2). Six types of modification (methylation, acetylation, phosphorylation, ubiquitylation, sumoylation and proline isomerization) are possible on at least 30 sites for each nucleosome; it is therefore apparent that a very strong modulating activity can be produced by the many possible combinations of modifications that can occur on a variety of sites on histones.[11]

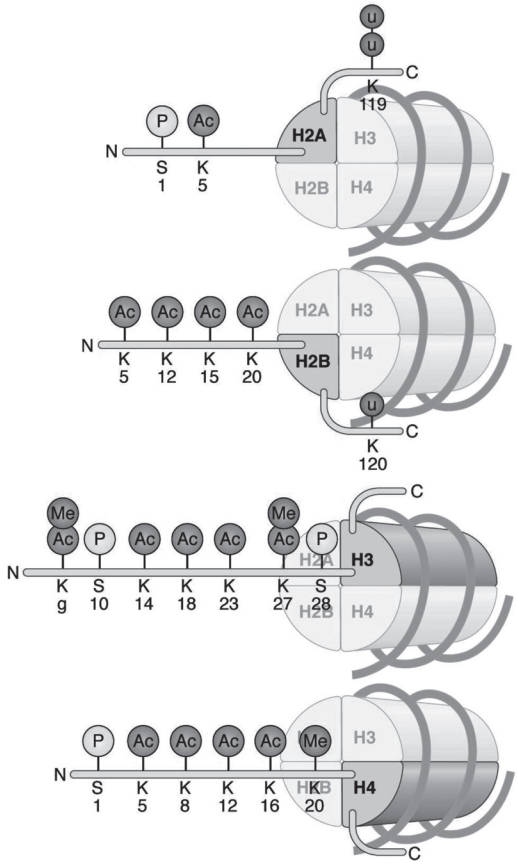

Figure 2. Post-translational histone modifications

Some modifications, such as acetylation and phosphorylation, are reversible and dynamic and are thought to be involved in inducible expression of individual genes, while other modifications, such as methylation, are more stable and are possibly involved in the long-term maintenance of the expression status.[11]

Acetylation of histones decreases the affinity between the protein tail and DNA, thus relaxing the chromatin structure and allowing the recruitment of transcriptional machinery. Moreover it facilitates the binding of additional coactivators with domains that recognize acetylated lysines.

Generally, histone acetylation is associated with transcriptional activation,[12] while the effect of histone methylation depends on the histone type and on the lysine position in the tail, even if methylation of histones is primarily found in transcriptionally silenced heterochromatin regions.[13]

Among all the post-translational modifications of histones, methylation and acetylation of lysine residues in the amino-terminal tails of histones H3 and H4[14] are the best studied: methylation of histone H3 at lysine 9 (H3K9) usually leads to transcriptional repression and can inhibit acetylation of the H3 tail at several lysines.

Histone methylation is catalyzed by histone lysine methyltransferases (HLMTase), while histone acetyltransferase (HAT) and histone deacetylases (HDACs) regulate, respectively, the acetylation and deacetylation of lysine residues.[15] Recently described histone demethylases are involved in removing the methylation marks.[16] It is noteworthy that CREB binding protein (CBP) is one known HAT and therefore chromatin structure might be regulated as part of CREB dependent activation of gene transcription in intracellular signalling cascades involving cAMP, Ca2+ and extracellular signal regulated kinase (ERK).[17] The DNA remodelling produced by intracellular signalling is apparently very important in the neurobiological adaptations associated with long-lasting behaviours in animal models and in the brains of humans with psychiatric conditions.[17]

Histone modifying enzymes are generally not gene-specific. In response to putative environmental and other signals, specific transcription factors and transcription repressors recruit histone-modifying enzymes to specific genes and define the gene-specific profile of histone modification.[15]

The processes of histone modification and DNA methylation are interdependent and both contribute to the overall state of chromatin and its epigenetic control of gene expression.[18] For example the transcriptional repressor, methyl-CpG binding protein 2 (MeCP2), which binds to methylated CpG islands recruits histone deacetylases (HDAC) and corepressors, such as Sin3a.[19] On the other hand histone H3-K9 methylation creates a binding site for the Heterochromatin Protein (HP1) which recruits a DNA methyl transferase, capable to methylate DNA. In both cases, DNA methylation and histone modifications act synergistically to repress transcription.

Histone modifications operate throughout differentiation and development to specify gene expression but it is not yet clear if the modifications that have been generated, called histone marks, are heritable through generations in multicellular organisms.[20]

Small non coding RNAs

Another mechanism of gene regulation is mediated by small, non coding RNAs. Among these small RNAs are the microRNAs (miRNAs) and short interfering RNAs (siRNAs); they are both 20-30 nucleotide-long double-stranded RNA molecules, encoded by their own set of genes (miRNAs) or introduced into the cell from outside sources (siRNAs).

The miRNAs are typically expressed in the nucleus in the form of longer precursors which are subsequently exported into the cytoplasm and processed by the enzyme Dicer into ~22 nucleotide long miRNA. One single strand of these short RNAs, the mature guide or antisense strand, will next be loaded into the RNA-induced silencing complex RISC together with one or more Ago (Argonaute) protein, which will allow it to bind to its mRNA target and induce silencing (figure 3) (see 21, 22 for reviews).

The siRNAs are more often produced by long, linear, perfectly base paired double strand RNA (dsRNAs) precursors introduced into the cell by virus, taken up from the environment or experimentally or clinically induced. More rarely precursors are endogenous products expressed by centromers, transposons or other repetitive sequences. These dsRNAs are then processed by Dicer enzymes and incorporated into RISC complexes in the same way as miRNAs.

Dicer proteins cleave dsRNA precursors into 20-30 nucleotide-long fragments through the action of two RNase III domains. The double-stranded products of Dicer enter into a RISC assembly pathway that involves separation of the two strands, followed by

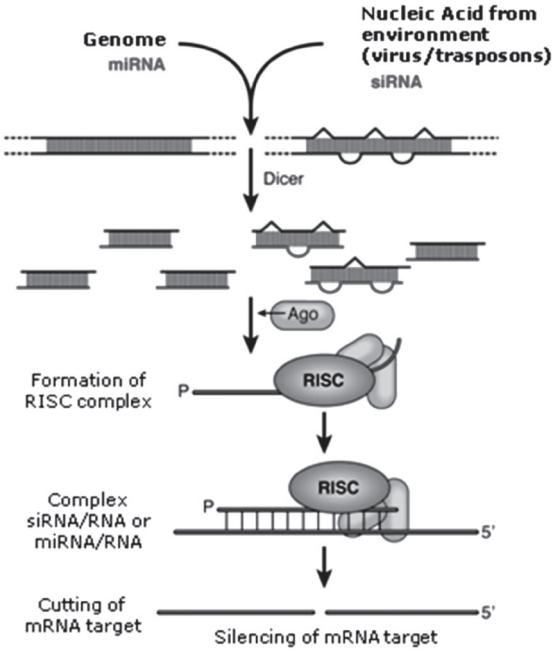

Figure 3. Mechanism of miRNA/siRNA maturation

the stable association of only one of the strands with an Ago effector protein. This guide strand recognizes each target messenger RNA by base pairing, whereas the other strand of the original small RNA duplex (the passenger strand) is discarded. Argonaute proteins are RNA silencing effectors endowed with an endonucleasic effect on the RNA target. The degradation effect of the Ago protein cleaves precisely the phosphodiester linkage between the target nucleotides that are base paired to siRNA residues 10 and 11 (counting from the 5' end). The generated fragments are then attacked by exonucleases to complete the degradative process. MicroRNAs play important roles in various biologic processes: they might regulate apoptosis, proliferation, differentiation, development, and metabolism affecting the expression of signalling molecules, such as cytokines, growth factors, transcription factors, proapoptotic and antiapoptotic genes etc.

It is noteworthy that the availability of exogenously delivered siRNAs or endogenously expressed microRNAs (by viral vectors) has generated a great optimism regarding potential RNA interference-based therapies for human disease (see below).

Epigenetics and human diseases

The importance of epigenetics in human disease[23-25] has been initially proposed in some rare inherited diseases such as the Prader-Willi syndrome, the Angelmann syndrome and the Beckwith-Wideman

syndrome which are characterized by an epigenetic aberration of imprinted genes. More recently several genetic alterations of the enzymes involved in the epigenetic mechanisms have been discovered. For example, the Rett syndrome is a severe neurodevelopmental disease produced by mutations in the MeCP2 gene which encodes the methyl CpG-binding protein 2, a protein important in transcriptional repression (see above). The hypometilation of CpG sites is, on the other hand, the hallmark of the Immunodeficiency Centromeric Instability-Facial Anomalies Syndrome (ICF) characterized by immunodeficiency and facial anomalies. The syndrome is due to mutations in the coding region of the DNMT3b gene, which encodes a DNA methylase enzyme. Epidemiologically more relevant, even if still largely unexplored, is the association of epigenetic aberrations produced by environmental factors and the risk of developing frequently occurring diseases as cancer or immunological, cardiovascular and psychiatric disorders.

Cancer

A vast and rapidly growing number of publications indicate that the epigenetic mechanisms have an important role in the development and progression of cancer (see 25, 26 for recent reviews). One of the first epigenetic alterations shown in human cancer is the hypomethylation of DNA occurring, in particular, in the repetitive DNA sequences and in the intronic sequences. During the development of a neoplasm, the degree of hypomethylation of genomic DNA increases as the lesion progresses from a benign proliferation of cells to a metastatic cell. Widespread hypomethylation has been associated with many cancers including: stomach, kidney, colon, pancreas, liver, uterus, lung and cervix.

Four mechanisms might explain the contribution of DNA hypomethylation to the development of a cancer cell:

1. Generation of chromosomal instability: favours mitotic recombination leading to deletions, translocations, chromosomal rearrangements.

2. Reactivation of intragenomic transposable elements e.g. L1 (long interspersed nuclear elements) and Alu (recombinogenic sequence) repeats: these transposons can be transcribed or translocated to other genomic regions, thereby further disrupting the genome.

3. Loss of imprinting: for example loss of imprinting of IGF2 gene produces an increased risk of colorectal cancer.

4. Frequently global hypomethylation correlates with hypomethylation of CpG islands in promoter regions of activating proto-oncogens increasing their expression and activating cell proliferation.

Paradoxically, along with global hypomethylation, cancer cells also show localized regions of de novo hypermethylation, predominantly in CpG islands of tumor suppressor genes and microRNA (miRNA) genes. Inactivation of tumour suppressor genes through hypermethylation of CpG islands within promoter regions is a major event in the origin of cancer.

Initially hypermethylation of the CpG islands was discovered in the promoter region of the retinoblastoma tumor suppressor gene (Rb) but now the hypermethylation of the CpG island is considered a frequent mechanism of inactivation of genes involved in cell cycle control, DNA repair, metabolism of carcinogens, cell-to-cell interaction, apoptosis and angiogenesis, all of which are involved in the development of cancer.

Due to the above mentioned interaction between DNA methylation and histone modifications, it is not surprising that in many human cancers wide variations of the histone modification patterns occurs as a consequence of the wide changes of DNA methylation status.

For example hypermethylation of the CpG islands in the promoter regions of tumor-suppressor genes in cancer cells is associated with a particular combination of histone markers characterized by the hypoacetylated and hypermethylated histones H3 and H4. These changes are associated with silencing of certain tumor-suppressor genes; they appear early and accumulate during the development of the tumor. Changes in histone modification patterns independent of the CpG methylation have also been directly linked to the development of cancer; they are due to the altered expression patterns of the histone-modifying enzymes which have been shown to occur in different cancer cells according to tumor type.

Recent studies have shown that profiles of miRNA expression differ between normal tissues and tumor tissues and among tumor types.

Down-regulation of subgroups of miRNAs, a common finding, might produce a loss of a tumor-suppressor function for miRNAs, as shown for example for RAS and BCL2 oncogenes.

DNA hypermethylation in the miRNA regulatory region is a mechanism that can account for the down-regulation of miRNA in tumors.

Aging

Although aging should not considered itself a human disease, the well-documented age-related phenotypes, such as functional decline and the development of age-related diseases, might have a partial explanation in epigenetic changes accumulating during life (see 20, 23, 27, 28 for reviews).

Two specific alterations of DNA methylation occur during aging:

1. Global hypomethylation

2. Hypermethylation of specific loci (primarily CpG island promoters).

The same modifications are known epigenetic alterations in cancer. This suggests that the accumulation of epigenetic alterations during aging may directly contribute to malignant transformation.

Other epigenetic mechanisms, such as histone modifications, are also known to change during aging but have been less investigated.

Several histone-modifying enzymes are also known to have an important role in aging. Of these, the Sirtuin family of enzymes, which have histone deacetylase (HDAC) activity, deserves special attention. They are involved in the regulation of expression of genes controlling stress responses, DNA repair, apoptosis, cell cycle and insulin regulation. SIRT1 is particularly relevant with respect to aging since it is expressed in most tissues and down-regulated in senescent cells and during aging. Recent evidence that small molecule activators of Sirtuins increase lifespan reinforces the importance of these HDACs in aging.

The demonstration of age-accumulating epigenetic modifications supports the idea of age-related loss of normal epigenetic patterns as a possible mechanism for the late onset of common human diseases.

One of the key issues in understanding the role of epigenetic modifications in the aging process is how epigenetic changes are generated. It is very likely that they may depend at least in part on environmental factors. If true, epigenetic represents a link between the environment, disease and aging. This assumption opens the possibility of targeted interventions aimed at improving life span or healthy aging.

Environmental influences

Accumulating evidences indicate that the epigenome is an important target of environmental modifications, which might produce effects on disease susceptibility and aging. Although epigenetic programs are established early in life, and therefore the developmental period represents the phase of maximal sensitivity to environmental influences, epigenetic alterations might occur during the whole life.

The early life and the postnatal environment have an important impact on health trajectories later in life and play a crucial role in generating vulnerabilities to chronic disease.[16] It is established that adverse socioeconomic status during childhood anticipates vulnerability to developing chronic disease in adult life, related, for example, to blood pressure, cholesterol, fibrinogen and depressive symptoms.[29] The memory of the intrauterine or early life exposure is maintained as stable change in gene expression programming: for example, maternal care in the rat can affect the long term programming of expression in the glucocorticoid receptor gene in the hippocampus.[30]

Several environmental factors might be involved, alone or in combination, in producing changes in the epigenetic profiles of individuals. For example it has recently been shown that elderly monozigotic twin pairs, living separately in different locations, exhibited more epigenetic differences than young monozigotic twin pairs reared together. The demonstration of age-accumulating epigenetic modifications supports the idea of age- and environment-related loss of normal epigenetic patterns as a possible mechanism for the late onset of common human diseases.

Pharmacological tools and other forms of intervention may potentially modify the natural epigenetic pattern or reverse deleterious epigenetic programming. Indeed, several epigenetic drugs are now at different stages of clinical trials for cancer and psychiatric disease.

Among the environmental agents affecting the epigenome profile, diet, infection, environmental toxins, endocrine disruptor compounds, wasting and smoking are the most studied.

S-adenosyl-methionine (SAM), introduced by diet, is the universal methyl donor for methyl transferases and is exclusively provided by folate-mediated one-carbon metabolism. Therefore a diet enriched or poor in folate can affect the activity of DNA methyl transferase and the methylation profile of DNA, consequently influencing gene expression.[31] In rats, maternal protein restriction during pregnancy leads, in the offspring, to a loss of promoter methylation at gene associated with glucose metabolism, in tissues such as liver, lung and kidney.[32] In humans, dietary restriction in methyl donors (no folate and choline, plus low methionine) as well as genetic polymorphisms in folate metabolism, have been associated with abnormal DNA methyl transferase expression,

global DNA hypomethylation, and increased cancer risk.[31]

Alcohol-induced alterations of the epigenome relates to its ability to increase methylation at gene promoters: this has been associated with methylation-induced silencing of tumor suppressor genes in colorectal cancer;[33] also smoke has been demonstrated to increase methylation in tumor suppressor genes in human case-control studies and in mice and to reduce the methylation status of oncogenes in human cancer cell lines.[34]

The epigenetic profile of a host animal may be directly modified by bacterial infection, increasing or decreasing methylation, depending on the infectious agent. Aberrant methylation of gastric mucosa genes is a common finding in humans infected with Helicobacter Pylori and is an early event in gastric carcinogenesis.[35] Change in the epigenetic profile in response to infection may play a role in the development of immune related disorders and cancers previously associated with infectious agents.[31]

Finally, the exposure to heavy metals, such as nickel, cadmium, and arsenic as well as to ionizing and ultraviolet radiations have all been associated with altered epigenetic profiles.[36]

The best studied epigenome modifiers are endocrine disruptors, a wide group of environmental compounds with a large spectrum of disrupting actions on different endocrine systems; not only these compounds are able to affect the epigenome in animals exposed in utero, but also their alterations can be transmitted from generation to generation when they occur in germ cells.

Even if most genomic DNA methylation is erased between fertilization and pre-implantation,[37] a lot of epigenetic modifications, acquired in pregnancy or in the foetal period in germ cells, can be transmitted down the generations.

An example of a transgenerational effect on epigenome by an endocrine disruptor compound is the effect of vinclozolin. Indeed, the antifungide vinclozolin, which shows an antiandrogenic activity, affects DNA methylation patterns in the epydidimal sperm of treated animals (hypermethylation and hypomethylation events) and the same modifications are evident also in sperm samples of vinclozolin-treated F2 and F3 generations. These modifications in the vinclozolin generations go in parallel with a significant reduction of the testicular spermatid number, as well as the epididymal sperm number and motility, are significantly reduced in the vinclozolin generations compared to the control animals.[38]

In humans, prenatal exposure with diethystilbestrol, a synthetic estrogen, increases the risk of cervical and vaginal cancer in the female offspring (first generation, F1),[39] whereas second generation (F2, daughters of the exposed offspring) shows menstrual irregularity and possible infertility; these data are compatible with speculation regarding transgenerational transmission of DES-related epigenetic alterations in humans.[40] Indeed, in the reproductive tissues of DES exposed animals, an increase in promoter methylation of DNMTs inhibits their enzimatic activity, affecting the correct methylation pattern of reproductive tissues.[41]

Finally Bisphenol A is associated with higher body mass, impaired reproductive function and specific changes in DNA methylation in agouti mouse: these DNA alterations are present also in germ cells and are transmitted transgenerationally.[42] Furthermore male rat offspring, perinatally exposed to Bisphenol A shows an impaired spermatogenesis starting from the first up to the third generation. This impairment seems to be related to a perturbation in protein expression profile of steroid receptor coregulators (such as steroid receptor coactivator-1, SRC-1, or 300/CBP/cointegrator-associated protein, p/CIP) involved in regulation of the spermatogenesis.[43]

Maternal behaviour

A new and very interesting field of epigenetic alterations related to environmental stimuli is the effect on epigenome of different degrees of maternal care.

For example, in rats, the adult offspring of mothers that exhibit high levels of pup licking/grooming (LG) (i.e., High LG mothers) over the first week of life show increased hippocampal glucocorticoid receptor (GR) expression, related to an enhanced glucocorticoid feedback sensitivity; indeed these animals show low DNA methylation in the regulatory regions of the glucocorticoid receptor (GR exon 17 promoter) gene in the hippocampus. Furthermore pups of high LG mothers show decreased hypothalamic corticotrophin releasing factor (CRF) expression, more modest HPA stress responses and increased behavioral exploration related to decreased levels of stress-induced corticosterone.[44]

These differences in epigenetic programming emerge early in life in response to differences in maternal LG and remain stable into adulthood.

The mechanism by which maternal behavior triggers the epigenetic modification seems to include a

signalling pathway that involves the serotonin receptor, increase in cAMP, recruitment of the transcription factor NGFI-A (a transcription factor induced by nerve growth factor), which in turn recruits a DNA demethylase MBD2 to the GR promoter.[45]

Differences in methylation degree of promoter region of estrogen receptor alpha are also related to low/high maternal care: the offspring of High LG mothers show a low degree of ER-alpha methylation, whereas low LG induces high methylation, inhibiting ER-alpha expression (figure 4).

The infusion to adult offspring of trichostatin-A (TSA), a demethylating agent, reverses the effects of low maternal LG; conversely, the central administration of methionine, a methyl donor that promotes methylation, reverses the effect of high maternal LG.[46] Moreover, epigenetic studies suggest that the accumulation of epigenetic variation in gene promoters, through early-life environmental or stochastic means, may account for changes in brain development, behaviour, stress responsivity, and risk of mental illness. Recent studies in humans suggest that epigenetic processes, similar to those experimentally observed in rats, may play a significant role in shaping human behavioral plasticity.

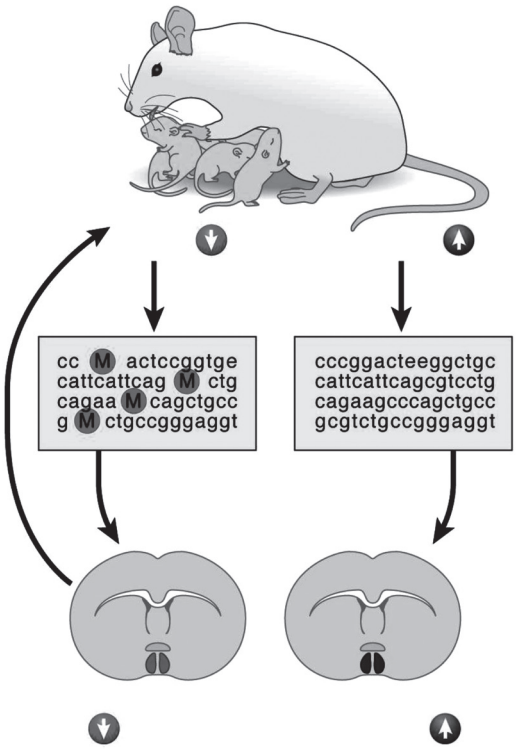

Figure 4. Differences in methylation degree of promoter region of ER-alpha related to maternal care

Brain-derived neurotrophic factor (BDNF) is a gene known to play an important role in cognition and aberrant regulation of this gene has been implicated in the etiology and pathogenesis of several cognitive and mental disorders, including schizophrenia: postmortem reports have indicated that in the prefrontal cortex and hippocampus of schizophrenic patients there is both decreased BDNF protein and BDNF mRNA levels.[47]

In rats, early negative social experiences trigger lasting changes in DNA methylation of BDNF CpG islands that are associated with decreases in BDNF expression in the adult prefrontal cortex.[48]

Even if there has been little investigation on BDNF DNA methylation in schizophrenia, the hypothesis of the hypermetylation triggered by an early adverse social environment and the consequent reduced gene expression is an attractive hypothesis.

To conclude, we can consider epigenetic as a mechanism that interfaces between nurture and nature, as Szyf suggests in his paper about "The early life environment and the epigenome".

Epigenetic drugs

As said before, pharmacological tools and other forms of intervention may potentially modify the natural epigenetic pattern offering a possible route to reverse deleterious epigenetic programming.

Indeed, several epigenetic drugs are now at different stages of clinical trials in cancer and psychiatric disease.

The first epi-drugs to be synthesized were the DNA Methyltransferase (DNMT) Inhibitors nucleoside analogues 5-azacytidine (azacitidine; Vidaza) and 5-aza-2′-deoxycytidine (decitabine; Dacogen), initially developed as cytotoxic agents to treat leukemia.

Azacitidine and decitabine have cytotoxic effects, but at lower doses these agents are predominantly epigenetic modulating. The nucleoside analogues are S-phase specific and mainly exert their effects after incorporation into the DNA: the DNA/nucleoside-analog complex stoichiometrically binds to DNMTs. This results in a cellular depletion of DNMTs and subsequent hypomethylation of newly synthesized DNA strands.[49, 50] FDA approved Azacitidine and decitabine for the treatment of myelodysplastic syndrome in 2004 and 2006, respectively. Other inhibitors of DNMTs are non nucleoside analogs, as procaine, procainamide and hydralazine; they act as low inhibitors of DNMTs, without requiring DNA incorporation. These compounds are still experimental drugs.[51]

Histone deacetylase inhibitors (HDAC inhibitors) are to the second class of epigenetic drugs. They are structurally heterogeneous but share a common ability recognize and bind to the catalytic zinc-pocket on class I and II HDAC (valproic acid or hydroxamic acid). HDAC inhibitors can induce in vitro cell cycle arrest and differentiation. These drugs are currently under clinical phase II or I trials.[51]

SiRNA can be engineered to specifically silence any gene with a known sequence; their delivery, either directly or by means of viral vectors, represents a potential new therapeutic tool.

A series of successful pre-clinical studies in small animals and initial clinical studies in humans have been done (Phase I, Phase II and III trials);[52] although some disadvantages, as specificity of siRNA, delivery of siRNA to target tissue, immunogenicity and liver damage, still remain, it is possible that future developments might provide valuable drugs to suppress unwanted gene expression.

Conflicts of interests

Nothing to declare by authors.

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