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Review

DNA methylation – a common denominator in transcriptional repression, genome imprinting, X-inactivation, and carcinogenesis

[1]Andrei C. Grădinaru, [2]Mădălina A. Davidescu, [3,4,5]Ioan V. Petrescu-Mag

[1] Faculty of Veterinary Medicine, "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine of Iași, România; [2] Faculty of Animal Husbandry, "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine of Iași, România; [3]Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, România; [4]SC Bioflux SRL, Cluj-Napoca, România; [5]University of Oradea, Oradea, România.

Abstract. The expression of genetic information in traits depends both on the nucleotide sequence and epigenetic factors, such as DNA methylation. Considered a natural process, the DNA methylation involves a methyl group adding to the 5-position of cytosine through a process of transfer from S-adenyl methionine. Over the time, the obtained effect was linked to gene repression and differentiated expression of the same genes in various cell types, genome imprinting with allele silencing considering their parental origin, X-chromosome inactivation in most mammalian females, and silencing of repetitive or satellite DNA. Unfortunately, the need for methylation has been associated with the risk of cancer development, since many tumor suppressor genes have been inactivated in normal cells through DNA hypermethylation. This paper is a review and its aim is to debate the scientific literature focused on DNA methylation process and the derived consequences.

Key Words: DNA methylation, genes silencing, X-inactivation, cancer

Corresponding Author: A. C. Grădinaru, a.c.gradinaru@uaiasi.ro

Introduction

Nowadays, the DNA structure and its role in the storage and inheritance of genetic information related to protein/enzymes or, more simplified, RNA syntheses, is a well-known fact all over the biological and medical scientific community. Everything we are debated today about this molecule started with simple but very important historical events, such as its first empirical isolation, from human pus contaminating surgical bandages and salmon semen, in 1869 by swiss biologist Johan Friedrich Miescher, followed by the discovery of genetic transformation phenomenon in *Pneumococcus spp*., in 1928 by Frederick Griffith, and subsequent demonstration of DNA as a transforming agent, in 1944 by Oswald Avery and his team (MacLeod C.M. and McCarty M.). In fact, the last mentioned event supported for the first time the DNA role in the process of heredity (Griffiths et al 2005; Lacks 2003).

The process of heredity is undoubtedly related to that of variability, both of them being a component of Genetic inheritance and the basic expression of traits. A common dogma of Genetics established the expression of characters as a result of the interaction between the genotype and environmental factors. Nowadays, there is known that beside the sequence of nucleotides and the variability resulted subsequent to point and/or chromosomal mutations through substitution, insertion or deletion, an important fact is the control exercised in the genetic information expression by epigenetic factors, such as DNA methylation (Moore et al 2013).

The aim of this paper is to briefly review the scientific literature focused on DNA methylation process and the derived consequences.

Material and methods

Some aspects on DNA methylation process and its involvement in transcriptional regulation, X-chromosome inactivation, genomic imprinting, as well as its implications in tumorigenesis, were debated based on the study of 25 scientific papers with relevance in this area. This review is important in terms of its originality and comprehensive presentation of information.

Results and discussions

A modern debate of genome structure includes two types of the encoded information, one related to the sequence of nucleotides which acts as a blueprint for protein syntheses, and a second one, without any modification into their succession as a result of mutations, but by a methyl group adding to the 5-position of cytosine. This newly created structure provides instructions on how, where, and when the genetic information should be used (Robertson 2001). In fact, this justifies why, although normally there is no alteration in the gene nucleotide sequence in cells of the same organism, different cellular organization, with different functions of specialized cells are found in the same body (Jones & Taylor 1980). Therefore, the DNA methylation ensures a differentiated expression of genes in various cell types.

The structure of DNA is well-known since 1953, when James Watson and Francis Crick, based on investigations carried out by Rosalind Franklin and Maurice Wilkins among others, published in *Nature* the B conformational model, double-stranded, helical, and antiparallel, with nitrogenous bases pairing off by complementarity, with double hydrogen bounds among adenine (A) and thymine (T), and three, among guanine (G) and cytosine (C) (Watson & Crick 1953).

As previously mentioned, the process of DNA methylation involves a chemical modification into its structure determined by the addition of a methyl group (CH_3) at the carbon 5 position of the cytosine ring. The methylation of cytosine mostly occurs within 5`CG3` sequence, also known as the CpG dinucleotide, although there were reported other methylation sites, such as CpA and CpT dinucleotides (Das & Singal 2004; Robertson 2001). However, mammalian genomes are considered to be depleted in CpG, with about 28 million palindromes in human genome (~1% of total DNA bases), for example, among which 60-80% is generally methylated (Bird 2002; Kass et al 1997; Law & Jacobsen 2010; Ng & Bird 1999; Robertson & Jones 2000; Smith & Meissner 2013).

In normal cells, just certain regions of the genome contain methylated DNA, here including satellite DNA and parasitic elements, for example Long Interspersed Transposable Elements (LINES), Short Interspersed Transposable Elements (SINES), and endogenous retroviruses (Kulis & Esteller 2010; Robertson 2005). Short CpG-rich regions, known as CpG islands (CGis), found in more than half of the genes in vertebrate genomes, about 1 kb in size but greater than 200 bp (Jones 1999; 2012; Jones & Takai 2001), and occurring on average every 100 kb, are protected from methylation in normal cells (Roberson & Jones 2000), although some exceptions were identified (Robertson 2005). CpG islands are GC rich (60-70%), with a CpG to GpC ratio of at least 0.6, being prevalent in promotors or transcription start sites of housekeeping and/or the first exon region (Jones & Takai 2001; Kulis & Esteller 2010), and developmental regulator genes (Das & Singal 2004; Smith & Meissner 2013), representing about 15% of all CpGs, at least in human DNA (Ng & Bird 1999). Also for human DNA, there were predicted 29,000 CpG islands (Bird 2002). Jones & Takai (2001) reviewed their presence also in genes regions more toward the 3` end.

The process of DNA methylation is catalyzed by a group of enzymes known as DNA methyltransferases (DNMTs). Broadly speaking, up to date, five members of this group are known, DNMT1, DNMT2, DNMT3a, DNMT3b, and DNMT3L (Kulis & Esteller 2010), although other forms as DNMT1b, DNMT1o, DNMT1p were also reported (Das & Singal 2004). However, it seems that only DNMT1, DNMT3a, and DNMT3b are involved in the transfer of a methyl group from S-adenyl methionine (SAM) to the fifth carbon of a cytosine residue, and forming 5-Methylcytosine (Moore et al 2013; Razin & Riggs 1980). Among them, DNMT3 family is considered to be as a *de novo* DNA methyltransferase, establishing a new methylation pattern to unmodified DNA, when CpG dinucleotides on both DNA strands are unmethylated, while DNMT1 is of maintenance, functioning during the DNA replication in order to copy the DNA methylation pattern from the parental DNA strand onto the newly synthesized daughter strand; therefore, in this second case, the CpG dinucleotides on one DNA strand are methylated (Das & Singal 2004; Law & Jacobsen 2010; Moore et a 2013; Robertson 2001). However, some data suggest that either DNMT1 has *de novo* as well as of maintenance methyltransferase activity (Das & Singal 2004) or all three of them (DNMT1, DNMT3a, DNMT3b) possess both *de novo* and of maintenance functions (Robertson 2001).

At the level of DNA, three categories of CpG are found: unmethylated, half-methylated, and fully methylated, during the process of replication, half-methylated sites becoming fully methylated due to DNA methyltransferases (DNMTs) activity of maintenance. It seems that partially methylated DNA is a better substrate than unmethylated DNA for DNMT1, at least in vitro experiments, justifying the fully methylation process as a result of a self-perpetuating methylated state through more methylation attracted by already methylated sites (Jones & Takai 2001).

In mammalian cells, the DNA methylation is the major form of epigenetic information, whose effects are seen at the level of transcriptional repression inducing and influencing the protein-DNA interaction, chromatin structure and function. However, all of these are mechanisms of epigenetic regulation, including here the compensatory effect of DNA demethylation. The reverse process of DNA methylation, the demethylation, occurs either as an active or passive phenomenon. The active form takes place both in dividing and non-dividing cells, being involved enzymatic reactions in order to remove the methyl group added to cytosine which are preferred to the thermodynamically cleavage of the covalent bond carbon-to-carbon between cytosine and the methyl group, although the last variant was also reviewed (Bird 2002; Jones 2012). It is worth mentioning that chemical reactions of deamination and or oxidation at the methylated base lead to a modified product which is subsequently recognized and replaced with an unmethylated cytosine through a mechanism of Base Excision Repair (BER). The active demethylation by deamination involves an enzymatic complex named AID/APOBEC (Activation-Induced cytidine Deaminase/Apolipo-Protein B mRNA-editing Enzyme Complex) that induces the deamination of the amino group to a carbonyl group, converting 5mC into thymine. In this way, a G/T mismatch was created and the prompt intervention of BER mechanism will correct the improper base. If the oxidation reaction is discussed, then a group of enzymes named Ten-Eleven Translocation (Tet) enzymes or Tet-methyl-cytosine dioxygenases is used to add a hydroxyl group onto the methyl group of 5mC. In this way, a 5hmC is formed, which is further converted into cytosine either through its oxidation catalyzed by Tet enzymes, or by deamination, more exactly by AID/APOBEC. The intermediate products of these reactions are 5-formyl-cytosine and then 5-carboxy-cytosine, in the case of oxidation, and 5-hydroxymethyl-uracil, in the case of deamination (Moore et al 2013). It seems that the protection of CpG islands for their methylation is related, in fact, with the mechanism of active DNA demethylation (Cedar & Bergman 2009; Law & Jacobsen 2010; Schübeler 2015).

The passive DNA demethylation occurs only in dividing cells and its mechanism involves the DNMT1 (as DNA methylation maintaining enzyme) inhibition or dysfunction (Moore et al 2013). Incorporating cytidine analogs containing an altered 5 position have also been reported to inhibit the process of DNA methylation. Such analogs are 5-azacytidine (5-aza-CR), 5-aza-2`-deoxycytinine or decitabine (5-aza-CdR), pseudo-iso-cytidine

(ΨICR), 5-fluoro-2`-deoxycytidine (FcdR) (Jones 2002; Jones & Taylor 1980; Kass et al 1997; Ng & Bird 1999; Roberson & Jones 2000).

In mammalian cells, the genes function is affected by the reaction of methylation of cytosine residues in CpG dinucleotides and by reactions of acetylation, but also of methylation, phosphorylation and ubiquitylation of the N-terminal tails of histone proteins (chromatin-associated proteins). Some of these processes may be in a co-dependence relationship, in the sense of which, for example, the histone methylation can help to direct the DNA methylation patterns [the methylation on Lys 9 of H3 (H3K9) tails might recruit DNA methyltransferases, but not always; the same it was reported for the 27th position (H3K27)] and the DNA methylation might serve as a template for some histone modifications after DNA replication; on the other hand, the acetylation of H3 or H4, or the methylation of Lys 4 of H3 (directed by sequence-directed binding of RNA polymerase II to add specific methyltransferases) exclude the action of DNA methyltransferases. It seems that DNMT3L included in complexes with DNMT3a and DNMT3b, although it lacks of methyltransferase activity, is able to recruit methyltransferases to DNA by binding to H3 in nucleosome, but any kind of methylation of Lys 4 of H3 (H3K4) inhibits the contact between DNMT3L and the nucleosome, blocking in this way the DNA methylation (Bird 2002; Cedar & Bergman 2009). However, both types of events have the same results in controlling the genes expression. This control is variable, considering the fact that the methylation of CpGs of promoters represents a potent suppressor of gene activity, while the methylation process in the transcribed region does not block the formation of a transcript (Das & Singal 2004; Jones 1999; 2002).

Two mechanisms, usually found into a combination of them (Bird 2002; Roberson & Jones 2000), are used to justify the transcriptional repression by DNA methylation: the first one involves the presence of CpG dinucleotides within a specific binding site at which normally several specific transcription factors, including here AP-2, c-Myc/Myn, E2F, NFkB, the cyclic AMP-dependent activator CREB, EBP-80, MIB-1, MLTF/USF, VBP1, recognize these sequences to bind them in order to take part in the mechanism of transcription (Kass et al 1997; Tate & Bird 1993). If these sites are marked by methylation, the binding of transcriptional factors is inhibited and the transcription repressed. In a second chance, the repression of transcription may occur as a result of coupling of some methyl-CpG-binding proteins [MeCP1 and MeCP2 are well-known, being a part of MBD (Methyl-CpG Binding Domain) family] at the site of methylated DNA, leading to a competition with transcriptional factors for these sites or to a reorganization of DNA packing in much more condensed chromatin structures which are incompatible with the process of transcription (Das & Singal 2004; Kass et al 1997; Roberson & Jones 2000). MeCP2 appears to be more abundant than MeCP1, neither of them being able to bind hemi-methylated DNA or DNA in which methyl-C is not a part of a CpG dinucleotide (Tate & Bird 1993). MeCP2 is essential for normal embryonic development (Kass et al 1997) and its gene mutation was reported to be associated in humans and mice with neurological dysfunctions (Bird 2002).

One of the DNMTs family group, DNMT3L (DNMT3-Like), was shown to stimulate *de novo* methylation by DNMT3a, and to mediate transcriptional repression as a result of interaction with histone deacetylase 1 (Kulis & Esteller 2010). In fact, the acetylation of histones is believed to maintain an open state of chromatin for transcriptional activities; when histone proteins, as a part of nucleosome` structure, are deacetylated, a tighter packaging of DNA will result, with a reduction of the access of transcriptional factors to their local binding sites (Baylin 2005; Cedar & Bergman 2009; Robertson 2001). Therefore, both DNA methylation and histone deacetylation are involved in gene transcription and, subsequently, their expression, with physical changes into the chromatin structure: methyl groups are projected into the major groove of DNA, heavy methylated DNA and deacetylated histones are found at the level of chromosomal heterochromatin, becoming highly condensed and transcriptionally inactive, in contrast with unmethylated and acetylated histone of euchromatin regions (Baylin 2005; Jones & Takai 2001).

There is a well-known tissue-specific expression patterns of the same genes, and this is related to the methylation of CpGs located close to their promoters, with a subsequent inverse correlation between their methylation and transcriptional activity, including here the criteria of the number and density of methylated CpGs at the promoter rather their individual presence at specific sites. Surprisingly, but some genes activation can depend on single CpG sites hypo methylation (Tate & Bird 1993).

A different genic expression is related to the phenomenon of genomic imprinting, in which the genetic material with paternal and maternal origins is expressed differently, contributing to mono allelic expression of imprinted genes. This lead to a non-equivalent contribution of paternal and maternal genomes for mammalian development (Li et al., 1993). In fact, as reviewed for mouse, pig, and bovine, the paternal genome is the subject of an active demethylation process after fertilization occurs. The same demethylation takes place also for maternal genome, but as a passive failure of methylation of progeny strands of DNA and not by direct disarming of methylation imprints, as the oocyte do to the sperm cell (Bird 2002). However, although the DNA methylation pattern is erased is the early embryo, is then re-established in each individual at the time of implantation, through histone modification, becoming a subject of various alterations by both *de novo* methylation and demethylation events (Cedar & Bergman 2009). At the same time of implantation, *de novo* methylation of X-linked genes in mammalian females at the level of their promoters occurs on the inactive X-chromosome and not in the active counterpart (Jones & Takai 2001). This is a long-term repression by DNA methylation what follows to a random inactivation of one of the X-chromosome in somatic cells of mammalian females, initially by changing of chromatin structure in order restrict the access of DNA to protein factors in the process of silencing. Its reactivation in this place is extremely rare, fact that is in contrast with that found in marsupials, where X-inactivation takes place without DNA methylation and the included genes slowly became reactivated as a function of age (Cedar & Bergman 2009). This suggests that although both DNA methylation and histone deacetylation are reversible, the first one is dominant to de second one, and the genes reactivation and their subsequent transcription cannot occur without inhibiting methylation (Baylin 2005).

Even CpG islands are generally non-methylated, their methylation at specific sites is used by mammals for transcriptional repression, to silence the genes located on X-chromosome in females, to maintain a chromosomal stability and integrity at the level of satellite sequences and constitutive heterochromatin. In an undesirable way, the DNA methylation patterns is substantially altered during the process of aging and carcinogenesis (Jones 2002). In cancer, both hypomethylation and hypermethylation are likely to occur, changing the methylome of tumor cells comparing to the normal ones. In fact, the hypomethylation of already silenced oncogenes lead to their activation, while the hypermethylation of tumor suppressor genes of normal cells contributes to the process of tumorigenesis (Kulis & Esteller 2010). Jones (2002) reviewed that undermethylation of highly and moderately repeated DNA sequences may affect the stability of karyotype and might initiate altered heterochromatic-euchromatic interactions that favor the process of oncogenesis. On the other side, there is known the process of hypermethylation of CpG islands located in the promoter regions of more than 40 genes in lung cancer, and also in breast cancer in humans. The process of hypermethylation was also incriminated in hematopoietic malignancies or leukemic transformations. In these last cases, in which hypermethylation is the causal process, it is taken into account that such epigenetic changes are reversible and inhibitors of DNA methylation, such as 5-azacitidine and decitabine, or histone deacetylases (HDACs) inhibitors, such as trichostatin, hydroxamic acid derivatives, the cyclic tetrapeptide depsipeptide, benzamide derivatives like MS-275 and CI-994, aliphatic acids such as valproic acid and phenylbutyrate, may be used as a promising therapy in cancer. Considering the dominance of methylation over histone deacetylation, to derepress the silenced tumor suppressor genes and to restore their normal function is necessary that the initial treatment with azacitidine or decitabine to be followed by HDAC inhibitors, in order to obtain an additive or synergic effect (Baylin 2005; Jones 2002). As a probe for DNA methylation, several restriction enzymes may be used in molecular techniques, since they not cut the DNA if the CpG sequence is methylated. In this group of restriction endonucleases are included *Hpa*II (CCGG), *Msp*I (CCGG), *Hha*I (GCGC), *Xho*I (CTCGAG), *Ava*I (CPyCGPuG), *Sal*I (GTCGAC), *Sma*I (CCCGGG). Although MspI recognizes the same sequence as HpaII, it cuts the DNA regardless of the methylation state of the internal cytosine (Razin & Riggs 1980).

Conclusions

Long time ago it was believed that the mutational process is the only one related to the occurrence and development of cancer. In recent decades, the process of DNA methylation has been discovered to be increasingly involved in this pathological state. However, this is a natural process occurring as a result of methyl-group transfer from S-adenyl methionine to the fifth carbon of a cytosine residue, being in a relationship with chromatin structure and other molecular processes involving transcription repression, gene silencing, X-chromosome inactivation, imprinting genome and, as mentioned, the undesirable inactivation of tumor suppressor genes. The DNA methylation pattern is established at the time of implantation, it is a part of cancer predisposition, of tissue-specific expression patterns of the same genes, and a differential expression of paternal and maternal alleles of imprinted genes.

References

Baylin SB. DNA methylation and gene silencing in cancer. Nature clinical Practice Oncology. 2005; 2(1):S4-S11;

Bird A. DNA methylation patterns and epigenetic memory. Genes & Development. 2002; 16:6-21;

Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. Nature Reviews Genetics. 2009; 10:295-304;

Das P, Singal R. DNA methylation and cancer. Journal of Clinical Oncology. 2004; 22(22):4632-4642;

Griffiths AJ, Wessler SR, Lewontin RC, Gelbart WM, Suzuki DT, Miller JH. Introduction to Genetic Analysis. 2005; 8th Edition, Freeman W.H. & Company, New York;

Jones PA. The DNA methylation paradox. Trends in Genetics:TIG. 1999; 15(1):34-37;

Jones PA. DNA methylation and cancer. Oncogene. 2002; 21:5358-5360;

Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nature Reviews. 2012; 13:484-492;

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

Jones PA, Taylor SM. Cellular differentiation, Cytidine analogs and DNA methylation. Cell. 1980; 20:85-93;

Kass SU, Pruss D, Wolffe AP. How does DNA methylation repress transcription? Trends in Genetics:TIG. 1997; 13(11):444-449;

Kulis M, Esteller M. DNA methylation and cancer. Advances in Genetics. 2010; 70:27-56;

Lacks SA. Rambling and scrambling in bacterial transformation – a historical and personal memoir. Journal of Bacteriology. 2003; 185(1):1-6;

Law JA, Jacobsen SE. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nature Reviews Genetics. 2010; 11:204-220;

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

Moore LD, Le T, Fan G. DNA methylation and its basic function. Neuropsychopharmacology Reviews. 2013; 38:23-38;

Ng HH, Bird A. DNA methylation and chromatin modification. Current Opinion in Genetics & Development. 1999; 9:158-163;

Razin A, Riggs AD. DNA methylation and gene function. Science. 1980; 210(4470):604-610;

Robertson KD. DNA methylation, methyltransferases, and cancer. Oncogene. 2001; 20:3139-3155;

Robertson KD. DNA methylation and human disease. Nature Reviews Genetics. 2005; 6:597-610;

Roberson KD, Jones PA. DNA methylation: past, present and future directions. Carcinogenesis. 2000; 21(3):461-467;

Schübeler D. Function and information content of DNA methylation. Nature. 2015; 517:321-326;

Smith ZD, Meissner A. DNA methylation: roles in mammalian development. Nature Reviews Genetics. 2013; 14:204-220;

Tate PH, Bird AP. Effects of DNA methylation on DNA-binding proteins and gene expression. Current Opinion in Genetics and Development. 1993; 3:226-231;

Watson J, Crick F. Molecular structure of nucleic acids: a structure of Deoxyribose Nucleic Acid. Nature. 1953; 171:737-738.

Authors

•Andrei Cristian Grădinaru, Department of Preclinics, Faculty of Veterinary Medicine of Iași, 8 Mihail Sadoveanu Alee, 700489, Iași, Romania; e-mail: a.c.gradinaru@uaiasi.ro;

•Mădălina Alexandra Davidescu, Department of Fundamental Sciences in Animal Husbandry, Faculty of Animal Sciences of Iași, 8 Mihail Sadoveanu Alee, 700489, Iași, Romania; e-mail: mada.davidescu@uaiasi.ro;

•Ioan Valentin Petrescu-Mag, Department of Environmental Engineering and Protection, Faculty of Agriculture, University of Agricultural Science and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăștur Street, Cluj-Napoca 400488, Romania; e-mail: zoobiomag2004@yahoo.com

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