Bridging the Gap

The meaning of the methylation of genomic DNA in the regulation of gene expression levels

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Summary

Introduction: Methylation of genomic DNA is one of the major mechanisms that deactivates genes and regulates their tissue-specific transcription levels. Its patterns are based on clonal inheritance that occurs in the early stages of embryogenesis. All changes in the DNA methylation levels occurring especially in the promoter region of the genes, which involve hypo- as well as hyper-methylation, lead to cell differentiation and growth disorders. Therefore it can become an impulse that initiates different pathological processes including carcinogenesis.

Material and Methods: The purpose of this review was to present the recent knowledge concerning methylation of genomic DNA based on recent references and authors' experience.

Results and Conclusion: Genome stability disorders could be caused either by mutations, which damage the structure of the genes and have not been formerly removed, or as the consequence of an epigenetic mechanism. Methylation plays a decisive role in the activity of many genes and could be a natural weapon of an organism against the expression of foreign genetic material that degrades the original genome structure.

Key words: Methylation, Methyltransferases, Gene expression, Transcription silencing.

Introduction

Methylation of the genomic DNA is one of the major mechanisms that deactivates genes and regulates their levels of tissue-specific transcription. Its patterns are based on clonal inheritance that occurs in the early stages of the embryogenesis. It plays a decisive role in the activity of many genes [1]. It might be modified during the individual development and therefore influence its final patterns [2, 3]. DNA methylation is catalyzed by several 5-cytosine methyltransferases that transfer the methyl group from S-adenosyl-L-methionine to the fifth carbon of the pyrimidine ring of deoxycytidine (m5dC) [4]. They usually show higher affinity for half-methylated DNA than for non-methylated DNA which helps maintain the continuity of the inheritably transmitted methylation patterns [3]. Between 3-5% of the whole pool of cytosine present in human DNA undergoes this process. Methylated cytosine, like cytosine, pairs with guanosine in the sister DNA strand of the DNA helix.

Methylated cytosine is mostly present in human cells in CpG dinucleotide sequences of which about 70% are methylated [5] and mostly clustered in so-called CpG islands (1-2 kb long), and are about 50-100 kb from each other (the interspaced regions are very poor in methylated deoxycitosines) [6]. They are called methylation zones of low density. The number of CpG islands in the human genome is about 45,000. They account for about 1% of the whole genome and are mostly located in the promoter regions of the different genes. There is however an exception to these rules in as far as such methylated CpG islands are also located in the inactive chromosome X. The CpG islands are said to be hot spots for mutations and up to 30% of all detectable point mutations take place there [7].

Most of the CpG dinucleotides that occur in the regions of so-called methylation zones of low density are concentrated in the retrotransposon and parasitic DNA elements where origins are endogenic retroviruses [8, 9]. The retrotransposons are large zones in the DNA of many thousands of pairs of nucleotides in length that are capable of moving (transposition) within the genome. Therefore it is clear that these motions could cause large structural and functional changes in the genes where this takes place. Most of the parasitic viral

elements and the retrotransposons include promoter sequences, which bind the RNA polymerase. They occupy up to 40% of the human genome structure and therefore their integration into the transcriptional region in "sense" orientation could initiate the transcription process whereas their integration in an "antisense" orientation could in counterpart possibly block the expression of the genes [10].

Methylation of the promoter sequences could be a natural weapon of an organism against the expression of foreign genetic material that degrades the original genome structure.

The silence of transcriptions, which is dependent on cytosine methylation in the DNA chain, could be the effect of several mechanisms. It could be due to:

i. a higher polarization of m5dC which could cause an increase in the energetic demand necessary to obtain the transcription space for the RNA polymerase [11],

ii. the methyl groups present in the major groove of the DNA helix which could change the position of the histones and therefore the result could be a stronger clench of the chromosomal structure that could stop the access of the RNA polymerase [12],

iii. the methylation of cytosines in a site where transcription factors are bound with DNA (RE - responsive elements) could change their affinity for these factors.

Indeed many proteins have been discovered, e.g., AP-2, c-Myc, E2F, CRE and NF-kB, that mediate the silencing of the promoter when they bind with methylated DNA regions [13]. Another protein, MeCP2, also plays a major role when binding to methyl groups. This protein recognizes methylated CpG dinucleotides in the nucleosome which selectively restrains the gene's transcription [14]. It is possible to mark off two different domains in its structure that have different biological functions: firstly the domain that recognizes and binds methylated CpG (MBD) through contact with the major groove of a double DNA helix, and secondly the transcriptional repression domain (TRD) which reacts with several other regulatory proteins. In the aftermath of tethering the TRD domain with specific DNA binding elements (GAL4) the MeCP2 represses transcription processes. The consequence of this process is the blockage of the activity of transcriptional factor D (TFIID) for RNA polymerase II [15, 16]. The protein MeCP2 also causes the repression of transcription by binding with the co-repressor complex that includes histone deacetylases. The transcription repression which is mediated by the activity of the MeCP2, Sin3A and histone deacetylases complex, in normal conditions, may be inhibited by the activity of the deacetylases-trichostatin inhibitor (TSA) [17-19].

The pattern of DNA methylation is the result of three methyltransferases: DNMT1, DNMT3A, DNMT3B. The DNMT1 methyltransferase is referred to as a conservative. It modifies cytosine in the sister strand of DNA according to the pattern of the parental strand and is therefore responsible for copying parental methylation patterns of the DNA. The DNMT1 is situated in the proliferative focal points where it interacts with the PCNA (proliferating cell nuclear antigen). The methyltransferase DNMT1 is included into the replication fork that enables the copy of the methylation patterns in newly synthesized DNA chains [20]. Such localization of the enzyme allows a transfer of genetic information of the methylation pattern to the sister DNA strand in the replication process. DNMT1 has 10-40 times higher affinity for hemi-methylated DNA than for unmethylated DNA. Protein p21, which can stop the cell cycle to facilitate possible repair of the DNA, competes with the DNMT1 for the same binding site with a PCNA, which regulates the activity of DNA polymerases and that can also modify the methylation patterns. It has been discovered recently, that the DNMT1 can also react with the histone deacetylase and in this way it could repress the gene information for transcription. This process can be blocked by a histone deacetylase inhibitor – trichostatin [21].

The second type of methylases, DNMT3, includes two enzymes: DNMT3A coded by a gene located on the short arm of chromosome 2, and DNMT3B coded by a gene located on chromosome 20. Their biological functions include especially the catalysis of *'de novo'* methylation processes [22]. This phenomenon is observed within the CpG dinucleotides present in the DNA regions that are derived from retrotransposons and retroviruses. The aim of the *de novo* methylation is to repress the expression of foreign genetic material incorporated into the cell [23]. Usually this process does not disclose in the regions of high dinucleotide density, i.e., the CpG islands in gene promoters [3, 24]. However, in some cases, the methyltransferases can also cause the activation of *de novo* methylation processes in correct gene promoter regions which can influence the changes in their expression levels.

Changes in methyltransferase expression levels can lead to changes in the parental methylation pattern. If this occurs in the gene promoter regions that play an important role in maintaining proper cell functioning (e.g. protooncogenes or suppressor genes of cancer transformation), it will initiate tumor development. In

some cases the methyltransferases can also activate *de novo* methylation in regions of the correct gene promoters, which leads to changes in their expression level. If this phenomenon takes place in the promoter regions of suppressor genes, its effects may be catastrophic for the organism because of the promotion of oncogenic transformation [25-28].

Methylation processes themselves could also contribute to the formation of mutations. Such situations arise when, in the aftermath of deacetylation, 5-methyldeoxycytosine is turned into thymine, another DNA base. This phenomenon is however very difficult to recognize and is often eliminated during the miss-match repair process. However if this repair fails, after a cell's division, i.e., DNA replication, transition could occur in the gene (which is the exchange of a guanosine-cytosine pair to an adenine-thymine pair). This exchange will then strengthen spontaneous point mutations. The aftermath of such mutations could be either changes in gene expression or gene translation products. Probably, in most cases, such transitions in the CpG islands of a gene promoter region could belong to the first mutation appearing in human oncogenic cells.

All changes in the DNA methylation levels occurring especially in the promoter region of the genes, which involve hypo- as well as hyper-methylation, lead to cell differentiation and growth disorders. This can therefore become an impulse that initiates different pathological processes including carcinogenesis. The decrease of the methylcytosine content found especially in the parasitic regions of DNA and retrotransposons regions is frequently observed in tumor cells. The hypomethylation of a genome can lead to the activation of many non-active gene promoters, including protooncogenes. In comparison with normal cells oncogenic cells usually have a lower mean methylation value, and the first place its low level occurs is in the parasitic regions of DNA and their repeats, which in physiological conditions consist of a great amount of methylcytosines [29-32]. The decrease of the methylation level in the mentioned regions is often connected with its increase in the normally non-methylated CpG islands of gene promoters. The result of this phenomenon leads to either decreased or inhibited activity of the cell growth regulatory genes.

It has also been discovered that in various tumors an increase of the methylcytosine content in promoter regions of different genes causes a decrease or complete loss of their activity. Such changes in methylation patterns have been detected in many suppressor genes like: p16, p15, pRB, PTEN, BRCA1, VHL, WT1, p53, E – cadherins. Incorrect levels of m5dC have also been discovered in the genes responsible for the DNA miss-match repair systems – hMLH1 [33-35].

The reasons for the changes in the methylation patterns are not fully known yet. Nevertheless, the final effect is always a change in the expression level of many different genes. The total value of DNA methylation depends on the relation between its level in the promoter regions and the regions outside the promoter where low concentrations of CpG dinucleotide are found [36]. The promoters of many genes are rich in CpG and therefore may be methylated at many sites. The after-effect of promoter-region methylation is the inhibition of a transcription process described as the silencing of a gene. The changes of the methylation patterns are considered to be epigenetic transformations that modify the expression of a gene without causing changes to its structure. Therefore, when a low level of the random methylation is kept in a cell, it could gradually lead to the silencing of the gene expression. Such situation could occur in the promoter regions of suppressor genes, where silencing, which is the effect of hypermethylation, could initiate tumoral development. However, for such situation to take place, further processes of genetic changes have to occur i.e., the promotors of both copies of the suppressor genes have to be hypermethylated and passed on to the descendant cells in the next cell cycle [37-39]. The appearance of a mutated gene in the genome could activate the sudden methylation processes that quickly would cause silencing of a gene, preventing the disclosure of biological-end phenotypic effects of mutations. Such situation is visible e.g., in the cases of virus insertions made into the promoter region of a gene. Moreover, the changes in gene methylation patterns are also considered to be the mechanisms responsible for chromosome segregation during mitosis [40, 41].

In every cell there are many different mechanisms, acting at various levels, that protect the genome from destabilization. Disorders in the normal functioning of gene repair systems have to occur practically at the same time in all elements included in the genome control and stability to facilitate phenotypical disclosure of the genome changes. Partial functioning of the repair system is usually sufficient to protect the cell from initiating tumoral transformation [2]. Genome instability caused by the accumulation of mutations in the same genes over many years is said to be the basic cause of the process of changing a single cell clone into an invasive cancer. Although many defense mechanisms maintain the correct genome structure, sometimes their function is not adequate and leads to genome structure disorders. The stable genome is finally establi-

shed when a cell owns the complete set of genes identical to the ones in the parental cells and shows an adequate expression level to its phenotype function. Genome stability disorders could therefore be caused either by mutations, which damage the structure of the genes and have not been formerly removed, or as the consequence of an epigenetic mechanism [2]. During the life of each organism a large number of mutations, that are potentially carcinogenic, occur. However, the efficient functioning of the genome protection system causing that frequency of new cancer occurrences is very low compared to the mutation numbers [42, 43].

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