DNA Methylation, Mitotic Arrest Deficient 2 (MAD2) And Cancer A Brief Review

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Abstract

DNA methylation is an epigenetic mechanism in controlling gene expression which involves the control of gene promoter region. Aneuploidy is also commonly found in cancers which showed abnormal cell cycle control and cell division, partly as a consequence of abnormal spindle formation. This review aims to summarise the mechanism of DNA methlyation in the promote control of gene expression of a spindle formation-controlling gene, MAD2, and its roles in beast cancer.

Key words

Epigenetics, CpG island, DNA methylation, tumor marker, cancer therapy

What is DNA methylation?

DNA methylation is an epigenetic event leading to a heritable change in the pattern of gene expression that is mediated by mechanisms other than changes in the primary sequence of a gene. It involves covalent modification of post-replicative DNA. In humans, this modification is imposed only on cytosines that precede a guanosine in the DNA sequence (the CpG dinucleotide). The enzyme DNA methyltransferases (DNMTs) add a methyl ($-CH_3$) group to the cytosine ring to form methyl cytosine. S-adenosyl-methionine serves as a methyl donor[1].

The molecular role of DNA methylation

In concert with histone deacetylation and chromatin-binding proteins, methylation in a gene promoter region generally correlates with a silenced gene. Methyl groups project into the major groove of DNA and inhibit transcription[2]. It prevents the transcription of large parts of the genome that consist of repeat elements, inserted viral sequences, and transposons. Almost half the genes in our genome have CpG-rich promoter regions. In the bulk of the genome, about 80% of the CpG dinucleotides are heavily methylated. In contrast, the dinucleotides in CpG islands (small regions of DNA, approximately 0.5 to 4.0 kb in size, harbor the expected number of CpG sites), especially those associated with gene promoters, are usually unmethylated, whether or not the gene is being transcribed[1,3].

Exceptions to this rule are the fully methylated CpG islands associated with many transcriptionally silent genes on the inactive X chromosome of females and the silenced alleles of some "imprinted genes," which through parental determination are programmed such that only one allele of the gene is expressed in normal tissues[1,3].

DNA methylation in cancer

In cancer cells, the DNA-methylation and chromatin patterns are shifted. Many CpG sites in the bulk of the genome and in coding regions of genes, which should be methylated, become unmethylated (hypomethylated)[1]. Global hypomethylation has been implicated in chromosomal instability, loss of imprinting, and reactivation of transposons, retroviruses and oncogenes, which may all contribute to tumourigenesis[3]. Concurrently, a growing list of genes has been identified as having abnormal methylation of promoters containing CpG islands, with associated transcriptional silencing[4]. Aberrant silencing of tumour suppressor genes (TSG) through DNA methylation occurs as frequently as mutations and deletions[2]. This has led to a revision of the Knudson's two-hit hypothesis for TSG inactivation by adding a new pathway to gene inactivation by DNA

methylation. The two alleles of a TSG may be inactivated by any combination of genetic (mutations and deletions) and epigenetic events[1,3]. Abnormal patterns of DNA methylation in cancer cells have been recognized for over 20 years. The number of CpG islands aberrantly methylated in a single tumour is about 3000 and the average number is about 400[5].

Clinical Implications

The most attractive nature of epigenetic gene silencing over mutations is that the former are potentially reversible and the latter are irreversible. Strategies to reverse gene silencing to prevent and treat neoplastic diseases have been developed. DNA methylation profile of a tumour also serves as markers for risk assessment, early diagnosis, and prognosis[1].

Reversal of Gene Silencing as Cancer Therapeutics

The demethylating agents 5-azacytidine and its deoxy derivative, decitabine (deoxy-5-azacytidine) are powerful inhibitors of DNA methylation. Moreover, inhibitors of histone deacetylase exerts additive or synergistic effects if some demethylation is first achieved by low doses of deoxy-5-azacytidine[1,2]. Clinical studies clearly demonstrate that these drugs have benefit in many patients with haematologic malignancies such as myelodysplastic syndromes (MDS) and leukaemias[2,6].

DNA Methylation as a Molecular Marker for Cancer

Molecular signatures of cancers of all types can be used to improve cancer detection and the assessment of cancer risk. Hypermethylation of gene promoters is common in all cancers, and profiles or marker panels can be designed in which the examination of a few gene-hypermethylation markers will be positive in over 70% of major types of cancer[1,4].

Detecting DNA Methylation

Detection of methylated cytosine at specific sites in DNA from clinical specimens is very difficult because the methylation signature is erased during conventional procedures used to amplify DNA, including cloning and polymerase chain reaction (PCR). Southern blot analysis of DNA digested with methylation sensitive restriction endonucleases has previously been an indispensable tool in the study of DNA methylation, but has now been replaced by PCR methods that are based on initial modification of DNA with bisulfite. Bisulfite selectively deaminates cytosine residues to uracil, leaving methylated cytosines intact, and the modified DNA can be used as a template in a standard PCR using primers specific for the gene of interest. Sequence analysis of the resulting PCR product provides an accurate display of methylated cytosines, but may be technically difficult and labor intensive. A variety of PCR-based methods have been developed, including methylation-specific PCR (MSP), methylation-sensitive single nucleotide primer extension (Ms-SNuPE), and methods based on the use of restriction endonucleases, which are simple to use but all suffer from the drawback that only a limited number of CpG sites can be analyzed in each assay[1,3,7,8].

The most widely used of the above methods is MSP, which uses primers that are specific for bisulfite modified DNA and discriminate between methylated and unmethylated alleles of a given gene. A potential pitfall inherent in the use of this method is the very high sensitivity, implying that a methylcytosine-positive signal can be detected even when only 0.1% of the DNA molecules present such base modification[3,8,9].

Early Detection of Cancer

Methylation alteration usually occurs before apparent malignant changes and thus may be useful for early detection of cancer. Abnormally methylated gene sequences have been detected in DNA from serum, plasma, sputum, bronchial-lavage fluid, urine, ductal fluids, and lymph nodes from patients with many different types of cancer[4,6]. Studies of sputum from patients with lung cancer have yielded promising evidence that cancer can be detected, up to three years before clinical detection of tumors in persons who smoke[3].

Hypermethylation and Prognosis in Cancer

The O6-methylguanine-DNA methyltransferase (O6-MGMT) gene encodes DNA-repair proteins. When O6-MGMT function is lost, the cells have a diminished capacity to repair alkylation damage to the base guanosine and they become susceptible to guanosine-to-adenine mutations. Such lost function sensitizes cells to the effects of chemotherapy that depend on an alkylating mechanism. Thus, early studies indicate that brain tumors that carry hypermethylated O6-MGMT respond better to alkylation therapy than those that do not, and the same may be true for lymphomas[1,2,5].

The death-associated protein (DAP) kinase gene encodes

an anti-apoptotic factor. Patients with lung tumors containing a hypermethylated DAP kinase gene have decreased survival after diagnosis than those with tumors that do not[4,5]. Hypermethylated *p16* was also found to be associated with more advanced disease and reduced disease-free survival in non-small-cell lung cancer[4].

Mitotic Arrest Deficient 2 (MAD2)

Most human cancer display some degree of aneuploidy, especially those at later stages of the disease[10]. Aneuploidy and associated chromosome instability may be important in the progression of mammary tumorigenesis. It is prevented during normal cell division in part through regulation of a mitotic spindle checkpoint where mitotic arrest prevents segregation of misaligned chromosomes into daughter cells at anaphase[11].

Defects in spindle checkpoint contribute to chromosome instability leading to aneuploidy[11], e.g. frequently in many solid tumors and occasionally few uterine carcinoma and prostatic carcinoma[12]. Mitotic arrest genes, including the mitotic arrest deficient (MAD) and budding uninhibited by benzimidazole (Bub) family[13], which was originally characterized in yeast[11], help regulate normal function of the mitotic spindle checkpoint. MAD2 is a central player in the regulation of spindle checkpoint during anaphase onset[14,15]. Using fluorescence *in situ* hybridization (FISH), MAD2 human homolog like-1 (MAD2L1) is located at 4q27[16] and has 5 coding exons[17]. The MAD2L1 protein, which is localized to the nucleus, is widely expressed in all fetal and adult tissues[17]. Normally, this protein localizes as part of a protein complex at unattached kinetochores after chromosome condensation but not after metaphase[17]. If chromosomes are not correctly attached at the kinetochore, MAD2L1 mediates cell cycle arrest by associating with CDC20 and the anaphase promoting complex (APC)[16,19,20]. Some reports have correlated a reduce level of MAD2 protein expression with defective mitotic checkpoint control in breast cancer and nasopharyngeal cancer cell lines[19,21-27].

At the same time, studies on the underlying mechanistic connections between genetic alteration of the MAD2 gene and impaired mitotic checkpoints in human cancers have not identified MAD2 mutations in human cancer cell lines[18]. Therefore, epigenetic changes of MAD2 may contribute to impairment of the mitotic checkpoint. For example, transcriptional failure or promoter methylation may be responsible for changes in checkpoint protein levels and so induce mitotic checkpoint dysfunction and chromosomal instability[18]. It has been described that many genes are inactivated by promoter hypermethylation in breast[28], lung[27,29,30] and hepatocellular carcinomas[3,4]. By means of MSP and immunohistochemistry in formalin-fixed paraffin-embedded tissues, we are studying the relationship between MAD2 gene and breast cancers. We hope this would further elucidate the role of epigenetic events in the formation of breast carcinomas.

Conclusion

DNA methylation is gaining more and more interest from the research field as indicated by the upsurge in the number of related reports in recent years. It is now clear that such epigenetic event plays an important role in normal gene regulation as well as tumour development. Clinically, the application of DNA methylation in cancer risk assessment, early diagnosis, prognosis and ultimately treatment is likely to increase greatly in the near future.

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