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The Theragnostic Relevance of DNA Methylation Analysis in Cancer

Tibor Hortobágyi, Renáta Szabó, Balázs Murnyák*

Division of Neuropathology, Institute of Pathology, University of Debrecen, Debrecen, Hungary

Abstract

Epigenetics has become a focus of interest in tumour research, also because it has theragnostic implications. DNA methylation is frequently observed epigenetic alteration in cancer. Impaired transcription of genes caused by DNA methylation is associated with tumorigenesis and malignant transformation. To date numerous methylated genes were identified as candidate biomarkers by means of high-throughput molecular techniques in glioblastoma, lung, breast, and colon cancer. We review and assess the current DNA methylation analysis methods with emphasis on their relevance to biomarker research and clinical utilization. Although not yet in the routine diagnostic protocols, further research and technical advance may render DNA methylation analysis an integral diagnostic tool to further the cause of personalized medicine.

Introduction

DNA methylation is a well-studied epigenetic modification and generally associated with transcriptional silencing. This epigenetic mechanism could alter gene expression without changing the DNA sequence itself [1]. DNA methylation is the reversible and heritable attachment of an addition of a methyl (CH_3) group to the fifth carbon atom of the cytosine residues resulting in the formation of 5-methylcytosine [2].

This phenomenon is essential in normal development and differentiation of the organisms. Impaired DNA methylation can result in serious outcomes, such as Angelman- and Prader-Willi syndrome [3]. Moreover, impaired methylation pattern is a hallmark of cancer playing critical role in initiation, progression, and maintenance of the tumour. Altered DNA methylation was the first epigenetic mark shown to be associated with cancer [4]. Besides numerous somatic mutations [5], the global hypomethylation of the genome or DNA promoter hypermethylation of certain genes is related to tumorigenesis [2]. Despite recent advances, only some dozens of genes have been specified as possible biomarkers in cancer.

Methods for DNA Methylation Analysis

Most methylation detection techniques are based on the bisulfite treatment of samples. During the bisulfite conversion, unmethylated cytosines convert to uracils, and methylated cytosines remain untouched [6]. To date, high-throughput techniques are available for the analysis of DNA methylation [7,8].

Methylation Specific PCR (MSP) was one of the first methods for the detection of methylated nucleotide. It has high analytical sensitivity due to CpG dinucleotides containing PCR primers. The initial step is the conversion of DNA by bisulfite treatment. Next, the selective amplification is dependent on the designed primer [9]. The only disadvantage of MSP is that its utilization in clinical practice has some limitations, because the degree of methylation could be overrated in normal tissues [10].

Genome-scale mapping of methylation provides new insights into the pathological processes. Next generation molecular analytical methods have been established to examine the methylation levels of the whole genome (e.g. methylome). Epigenome-Wide Association Studies (EWAS) represent a specified version of Genome-Wide Analysis Studies (GWAS). EWAS can detect methylated DNA at cytosine-guanosine dinucleotides (CpG islands), which appear as Methylation Variable Positions (MVP). CpG islands are short DNA sequences in the promoter of the genes with greater than 50% GC content [1]. Methylation of CpGs in promoter regions plays an important role in both chromatin structure control and gene expression. Each MVP represents a single locus. Methylated DNA detection on a higher level is also possible. Using this technique the methylation

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Correspondence:
Tibor Hortobágyi, Division of Neuropathology, Institute of Pathology, Faculty of Medicine, University of Debrecen, 98. Nagyerdei krt., Debrecen, H-4032, Hungary, E-mail: hortobagyi@med.unideb.hu

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pattern can be studied also on longer, so called Differentially Methylated Regions (DMR). EWAS can reveal methylation profile of loci associated with complex diseases [11].

Similarly to the abovementioned methods, mass spectrometry-based platforms also require bisulfite-treated DNA. This high-throughput method utilizes Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). The data acquired are (semi-) quantitative regarding the PCR product following a base-specific cleavage. This information uncover the methylation status of specific region-of-interests [12].

Bisulfited pyrosequencing is a next generation sequencing-by-synthesis method. It uses bisulfite-treated DNA as input material. Due to its high throughput approach, it generates high amount of data which may result in data storage capacity problems [6].

An unquestionable advantage of high throughput methods is the 'parallelization'. It means that millions of tests can be run simultaneously in a time- and cost-effective way. The Reduced Representation Bisulfite Sequencing (RBSS) procedure includes restrictive enzymatic digestion of input DNA, library preparation, selection by size, adapter ligation, bisulfite conversion, PCR amplification and bisulfite sequencing steps. Finally, alignment and mapping of reference genome is required [13].

Contrary to the above-mentioned methods, EpiTect Methyl II PCR Assays do not require bisulfite conversion. This real-time PCR based method quickly detects DNA methylation status of CpG islands within individual genes. Currently this is used only for research purposes [14].

Clinical Relevance

From the clinical point of view, DNA methylation patterns can serve as diagnostic and prognostic biomarkers. In gliomas silenced *MGMT* by promoter methylation serves as useful biomarker in gliomas, because this renders the tumour susceptible to anti-alkylating therapy by temozolomide and therefore extends survival [15,16]. The O^6-methylguanine DNA Methyltransferase (MGMT) gene encodes a DNA repair protein, which is essential for maintaining genome stability. In lung cancer the *short stature of someobox 2* (*SHOX2*) gene is a useful blood-based biomarker, detectable by the HeavyMethyl approach [1]. In colon cancer methylation status of *vimentin* (*VIM*), *septin 9* (*SEPT9*), *adenomatosis polyposis coli* (*APC*), *Ras association domain family 1A* (*RASSF1A*) and *syndecan 2* (*SDC2*) are diagnostic biomarkers [1,14,18]. In breast cancer, *BRCA1* [17], *paired-like homeodomain 2* transcription factor encoded by *PITX2* [1], *chloride channel accessory 2* (*CCA2*) [19], *Ras and Rab interactor 1 (RIN1*) [20] genes have been displayed.

Conclusion

The exact role of gene methylation in cancer and its biomarker potential is far from being fully elucidated. Carefully designed randomised controlled studies are needed, which could also contribute to development of more effective and personalized therapeutic protocols. The integration of DNA methylation analysis into the routine diagnostic workflow remains a challenge due to financial, bioinformatics and time constraints.

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