

Editorial

Understanding Epigenetic Status: DNA Methylation and Cancer

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EDITORIAL

In the past, cancer development was generally considered to be a disease that is caused by genetic alterations (e.g., mutations, chromosomal abnormalities) in tumor-suppressor genes and/or oncogenes. However, it has become increasingly apparent that cancer is also caused by epigenetic changes. DNA methylation is recognized as one of the most important epigenetic mechanisms, and is widely studied in humans. In many different cancer types, aberrant DNA methylation has been shown to play a critical role in tumorigenesis and cancer progression. Understanding DNA methylation status is important for early diagnosis of cancer, monitoring of tumor behavior, and evaluating the response of tumors to targeted therapy. Epigenetic evaluation in pathological states has become the focus of many studies, and cancer epigenetics is rapidly moving into a translational phase. In this editorial, we discuss the most widely used and latest DNA methylation analytical methods with the potentiality of clinical applications in the study of cancer.

DNA methylation, which most commonly occurs at the 5th carbon within CpG dinucleotides, plays an important role in gene silencing, and generally, near the transcriptional start site of a silenced gene is hypermethylated [1]. In cancer, it is generally believed that a high level of DNA methylation in the vicinity of the transcriptional start site is associated with the silencing of tumor-suppressor genes. To distinguish between methylated and unmethylated cytosine, sodium bisulfite is most commonly used. Bisulfite treatment converts unmethylated cytosines to uracils, and methylated cytosines remain cytosines [2]. After PCR amplification, uracils that represent unmethylated cytosines are read as thymidines.

Methylation-specific PCR (MSP) analysis

Methylation-specific PCR (MSP) and real-time quantitative MSP (QMSP) analysis are bisulfite conversion-based PCR techniques and are continually used to distinguish between methylated and unmethylated allele in the genome. In a MSP analysis, two primers are designed to detect methylated and

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unmethylated CpG, the methylated CpG (M primer) and the unmethylated CpG (U primer), respectively. MSP analysis, including QMSP, is the most commonly used technique for analysis of clinical samples [3,4]. Most recently, We showed that oral squamous cell carcinoma could be detected with >90% sensitivity and specificity using QMSP analysis based on the promoter DNA methylation status of some tumor-related genes obtained from oral rinse samples [5]. In contrast, to increase the sensitivity of MSP analysis in clinical samples, nested-MSP was developed [6]. Nested-MSP enabled us to detect DNA methylation more sensitive in low quality/quantity of starting DNA. Although MSP analysis identifies only a limited number of CpGs in a primer sequence, it is regarded as a high sensitivity, rapid and inexpensive method.

Pyrosequencing analysis

Pyrosequencing is a sensitive real-time sequencing methodology for the investigation of DNA methylation level at specific CpG sites [7]. To carry out the pyrosequencing reaction, biotin-labelled amplicon generated from bisulfite-converted DNA is used as a template. This technique relies on the luminometric detection of released pyrophosphate (PPi) during DNA synthesis. Pyrosequencing is a highly reliable method and has become the gold standard for DNA methylation analysis. Recently, Pyrosequencing has also become one of the most commonly used techniques for analysis of clinical specimens [8,9]. An important limitation of pyrosequencing is that it can be performed only for relatively short DNA sequence reads (<100bp).

MassARRAY analysis

MassARRAY is one of the main methods for genome-wide DNA methylation analysis. MassARRAY combines bisulfite treatment, PCR and base-specific cleavage with matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry analysis [10]. MassARRAY allows for the quantitative DNA methylation analysis of multiple CpG sites in a large number of clinical samples [11]. Yin et al. suggested the possibility that the aberrant DNA methylation within the *PTEN*

promoter may potentially serve as a candidate biomarker for soft tissue sarcomas using 110 different clinical specimens [12]. Although MassARRAY analysis has a disadvantage in the cost of the instrument, there are many significant advantages such as highly accurate, sensitive, and high-throughput capability.

Methylation specific electrophoresis (MSE) analysis

A novel DNA methylation analysis approach named methylation specific electrophoresis (MSE) was recently developed [13]. MSE is the modification of the Bisulfite-DGGE (denaturing gradient gel electrophoresis) [14] using a nested PCR technique. Following the conversion of bisulfite, the MSE technique requires the amplification of the target DNA using nested PCR. After DNA amplification, samples are applied to denaturing gradient gel. The MSE method decreases the amount of input DNA (minimum detectable amount of DNA is 20 pg) and lowers the detection limit for detecting the difference in methylation status is <0.1%.

The MUC1 transmembrane glycoprotein is a poor prognostic factor of the potential for malignancy [15], and is ranked as one of the most important cancer antigens [16]. The methylation status of MUC1 promoter indicated a good correlation with the level of MUC1 expression [17]. Most recently, we showed that the level of MUC1 promoter methylation in pancreatic ductal adenocarcinoma was significantly lower than in intraductal papillary mucinous neoplasm using pancreatic juice samples [13]. MSE analysis can efficiently detect DNA methylation patterns or continuity in the target region. Therefore this method can be quite useful for evaluating CpG methylation status and patterns in the region of interest. In the future, it is expected that more quantification approaches will be developed.

Clinical perspectives

In cancer, DNA methylation is a promising biomarker for early detection, diagnosis, prognosis and prediction of response to therapies. However, there are many problems that need to be overcome in studying DNA methylation using clinical specimens. The most critical issue is heterogeneous population of cells. CpG methylation status can change by cell/tissue type. Thus, interpretation of altered CpG methylation in these mixed populations requires caution and prudence. Meanwhile, we have to face problems of limited availability of starting DNA. Also, the amount of DNA recovered will be strongly influenced by cell/tissue type and the difference in clinical specimen (e. g. , body fluids, formalin-fixed paraffin-embedded tissue). In addition, there must also be consideration of analysis cost per sample depending on the number of samples and regions of target genes. A large number of methods can be used for the DNA methylation analysis. There is more than one appropriate approach of DNA methylation analysis in clinical application. Responding to a variety of sample conditions and purpose of the examination, investigators can select the most optimal method or various combinations of the approaches for their specific research needs.

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