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Review

DNA methylation-based biomarkers in serum of patients with breast cancer

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ABSTRACT

Alterations of genetic and epigenetic features can provide important insights into the natural history of breast cancer. Although DNA methylation analysis is a rapidly developing field, a reproducible epigenetic blood-based assay for diagnosis and follow-up of breast cancer has yet to be successfully developed into a routine clinical test. The aim of this study was to review multiple serum DNA methylation assays and to highlight the value of those novel biomarkers in diagnosis, prognosis and prediction of therapeutic outcome. Serum is readily accessible for molecular diagnosis in all individuals from a peripheral blood sample. The list of hypermethylated genes in breast cancer is heterogeneous and no single gene is methylated in all breast cancer types. There is increasing evidence that a panel of epigenetic markers is essential to achieve a higher sensitivity and specificity in breast cancer detection. However, the reported percentages of methylation are highly variable, which can be partly explained by the different sensitivities and the different intra-/inter-assay coefficients of variability of the analysis methods. Moreover, there is a striking lack of receiver operating characteristic (ROC) curves of the proposed biomarkers. Another point of criticism is the fact that 'normal' patterns of DNA methylation of some tumor suppressor and other cancer-related genes are influenced by several factors and are often poorly characterized. A relatively frequent methylation of those genes has been observed in high-risk asymptomatic women. Finally, there is a call for larger prospective cohort studies to determine methylation patterns during treatment and follow-up. Identification of patterns specific for a differential response to therapeutic interventions should be useful. Only in this way, it will be possible to evaluate the predictive and prognostic characteristics of those novel promising biomarkers.

Abbreviations: 5-Aza-CdR, 5-aza-2′-deoxycytidine; 5-Aza-CR, 5-azacytidine; 5mC, 5-methylcytidine; AIMS, amplification of inter-methylated sites; APC, adenomatous polyposis coli; BiMP, bisulfite methylation profiling; BRCA1, breast cancer gene 1; BsPP, bisulfite padlock probes; CA15.3, carcinoma antigen 15-3; CEA, carcinoembryonic antigen; cfDNA, cell-free DNA; CGH, comparative genomic hybridization; CHARM, comprehensive high-throughput arrays for relative methylation; CIMP, CpG island methylator phenotype; COBRA, combined bisulfite restriction analysis; COX-2, Cyclooxygenase-2; CpG island, regions of DNA where a cytosine nucleotide occurs next to a guanine nucleotide in the linear sequence of bases along its length; DCIS, ductal carcinoma in situ; DMH, differential methylation hybridization; DNMT, DNA methyltransferase; dNTP, deoxynucleotide triphosphate; ECCG, epigallocatechin-3-gallate; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; ER, estrogen receptor; FNA, fine needle aspiration; GSTP1, glutathione S-transferase 1; HDAC, histone deacetylase; HELP, HpaII tiny fragment enrichment by ligation mediated PCR; HME, human mammary epithelial-specific marker; ICF, immunodeficiency, centromeric instability, facial anomalies; IDC, invasive ductal carcinoma; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MCA, methylated CpG island amplification; MCAM, methylated CpG island amplification and microarray; MDGI, mammary-derived growth inhibitor; MeDIP, methylated DNA immunoprecipitation; MGB, minor groove binder; MGMT, O6-methylguanine-DNA methyltransferase; MIP, methylation-independent PCR; MIRA, methylated CpG island recovery assay; MLPA, multiplex ligation-dependent probe amplification; MMAss, microarray-based methylation assessment of single samples; MMP, matrix metalloproteinase; MS, methionine synthase; Ms-AC-PCR, methylation-sensitive arbitrarily primed PCR; MSCC, methyl-sensitive cut counting; MS-HRM, methylation-sensitive high-resolution melting; MS-MLPA, methylation-specific multiplex ligation-dependent probe amplification; MSP, methylation-specific polymerase chain reaction; MSRE, 5mC-methylation-sensitive restriction enzymes; Ms-SNuPE, methylation-sensitive single-nucleotide primer extension; MTRR, methionine synthase reductase; NaBi, sodium bisulfite; OS-MSP, one-step methylation-specific polymerase chain reaction; PAX5, paired box protein 5; PCR, polymerase chain reaction; PPi, pyrophosphate; PR, progesterone receptor; PR PROX, PR proximal promoter; QAMA, quantitative analysis of methylated alleles; Q(M)MSP, quantitative (multiplex) methylation-specific polymerase chain reaction; RAR-β, retinoic acid receptor-β; RASSF1A, Ras-association domain family 1; RDA, representational difference analysis; RLGS, restriction landmark genome scanning; ROC, receiver operating characteristic; RRBS, reduced representation bisulfite sequencing; RT-PCR, reverse transcription polymerase chain reaction; SAM, S-adenosyl methionine; SFN, sulforaphane; TIMP-3, tissue inhibitor of metalloproteinase-3; TMS-1, target of methylation induced silencing-1; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

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Contents

1. Introduction

Detection of breast cancer at an early stage is the key to successful treatment and patient's outcome [1]. The late onset of symptoms, the poor accessibility of the malignancy and the unpredictable course of the disease often contribute to the high mortality in cancer [2]. The traditional triple test for breast cancer diagnosis includes physical examination, mammography and aspiration cytology [3]. The development of strategies capable of detecting metastatic or recurrent disease in preclinical or pre-symptomatic phases of the disease is desirable. A search for more sensitive and specific prognostic indicators, reflecting the presence or absence of tumor-specific alterations in the bloodstream, is still going on.

Several studies have demonstrated that cancer patients have abnormally high serum levels of tumor-specific DNA alterations, with more than 90% of the total circulating cell-free DNA (cfDNA) derived from tumor tissue [4–7]. The nucleic acid markers described in blood include oncogene mutations, microsatellite alterations, gene rearrangements and epigenetic alterations [8]. Epigenetic silencing of tumor-related genes due to methylation of gene promoter regions plays an important role in breast carcinogenesis [9]. DNA methylation is a common early event in carcinogenesis and can be a potential predictor of cancer risk. It is suggested that this epigenetic alteration remains involved during the whole process of oncogenic transformation [10].

Many methylation markers are characterized by a tumor prevalence that is higher than that of tumor-gene and proto-oncogene mutations and deletions. Similarities of methylation patterns found in primary tumor specimens and in plasma indicate the potential utility of a blood-based molecular detection of breast cancer [3,11–15]. However, studies comparing methylation patterns in tumor and serum DNA in early or late stages of breast cancerogenesis are limited. Besides its use in diagnostics, DNA methylation biomarkers could have the potential to predict disease outcome and therapeutic response [3].

In this review, we will focus on the analytical methods of serum epigenetic biomarkers and highlight their importance in risk assessment, earlier diagnosis and monitoring of breast cancer.

2. General characteristics of DNA methylation

Epigenetic alterations are changes in gene expression that arise independently of DNA sequence changes [16–19]. In mammals, so far five members of the DNA methyltransferase (*DNMT*) family have been discovered (DNMT1, DNMT2, DNMT3A, DNMT3B and DNMT3L) of which only three have catalytic activity (DNMT1, DNMT3A and DNMT3B) [20,21]. The remaining members, DNMT2 and DNMT3L, lack cytosine methyltransferase activity. DNMT3L binds only very weakly to DNA, but can stimulate DNA methylation mediated by DNMT3A, DNMT3B and their splice variants [22,23]. DNMT3L has dual functions of binding the unmethylated histone tail and activating DNMT3A/B *in vivo*. More and more evidence accumulates that the function of all these proteins overlaps with a synergistic activity of DNMT3A and DNMT1 in *de novo* methylation even without physical interaction. Furthermore, DNMT3A/3B can compensate for inefficient maintenance methylation by DNMT1 [12].

Studies have been carried out to analyze the expression of *DNMTs 1*, *3A* and *3B* in normal tissues and tumors [24,25]. Using Nothern blotting with poly(A)-selected RNA and semi-quantitative reverse transcription polymerase chain reaction (RT-PCR), a high expression of all *DNMTs* was detected in the fetal liver (probably due to residual hematopoiesis). In contrast to *DNMT3B*, transcripts of *DNMT1* and *DNMT3A* were also found in a large amount in all