Aberrant DNA methylation of regulatory regions of tumor-suppressor genes is shown for many types of cancer. DNA methyltransferase DNMT3 is responsible for hypermethylation and subsequent silencing of tumor suppressor genes (TSG) by converting RCGY sites into R(5mC)GY in the gene regulation regions (Handa V. et al, 2005).

Phenol-chloroform extraction was used for DNA isolation. Recently we have discovered and characterized a new DNA-endonuclease GlaI, which belongs to a novel type of site-specific methyl-directed DNA-endonucleases. These enzymes hydrolyze only methylated DNA. GlaI recognizes DNA sequence Pu(5mC)GPy which is identical to the modification site of DNMT3 (Tarasova G.V. et al, 2008).

GlaI hydrolysis and Ligation Adapter Dependent PCR (GLAD PCR) is the novel method to determine R(5mC)GY sites produced by methylation with DNMT3A and DNMT3B. GLAD PCR analysis may be performed in one tube and includes 3 steps: DNA hydrolysis with site-specific methyl-directed DNA-endonuclease GlaI, universal adapter ligation to the cleaved fragments, and Real-time PCR with Taqman probe. One genome primer is designed for DNA region of interest, structure of another hybrid primer is based on an adapter sequence. Hybrid primer is a DNA sequence 5’-CCTGCTCTTTCATCGGYNN-3’ wherein 5’-end of 15-dinucleotide primer corresponds with universal adapter and tetranucleotide part at the 3’-end (underlined) is complementary to the genomic sequence at DNA hydrolysis point by MD DNA-endonuclease GlaI. This structure implies the existence of 32 variants of hybrid primers corresponding to different possible terminal sequences after hydrolysis by GlaI of all possible variants of NNR(5mC)GY sequence.

GLAD-PCR ASSAY

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GLAD PCR analysis allows to detect even several copies of methylated DNA and thereby may be used in routine laboratory and clinical practice in earlier cancer detection.
List of STUDIED GENES

ESR1 1518796

ELMO1 3744850

RESULTS

GLAD-PCR assay of two selected sites was performed three times for each DNA sample containing 10^3 copies of the ELMO1 or ESR1 gene. Tables demonstrate the results of GLAD-PCR assay where each number means the average number of amplification cycle (Cq) with standard deviation (p<0.01).

APPLICATIONS

We are planning to continue GLAD PCR analysis of RCGY sites in regulation regions of other tumor suppression genes and to obtain a panel of TSG regulation regions which are methylated in the most of tumor samples. At the next step the obtained panel of TSG regulation regions will be tested on the samples of the blood cell-free DNA in order to create the simple, reliable, and sensitive diagnostic test for CRC.

PERSPECTIVES

GLAD-PCR assay may be used as a universal tool for methylation analysis of R(5mC)GY site of interest in the human genome instead of DNA bisulfite conversion. Therefore it can be used as epigenetic instrument for diagnostics of aging diseases, such as: the most kinds of cancer, coronary artery disease, type 2 diabetes (T2D) and others connected with epigenetic genome alterations. For such tests development a panel of reference genes should be comprised by examination of candidate genes based on their methylation status .

REFERENCES


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