Global DNA methylation level sensing system for early cancer diagnosis

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Introduction

In human genomic DNA, fifth position of the cytosine in cytosine-guanine dinucleotide (CpG) is methylated by DNA methyltransferase. DNA methylation plays an important role in regulation of gene expression. When gene promoter is methylated, the gene expression is suppressed. In normal cells, normal DNA methylation pattern has been established during the development. In contrast, abnormal DNA methylation pattern is detected in abnormal cells such as cancer. The cancer genomic DNA has two characteristics: (1) hypermethylation at tumor suppressor genes, and (2) globally hypomethylation on genomic DNA, indicating that the abnormal DNA methylation pattern can be utilized as a biomarker for cancer diagnosis (Fig. 1).

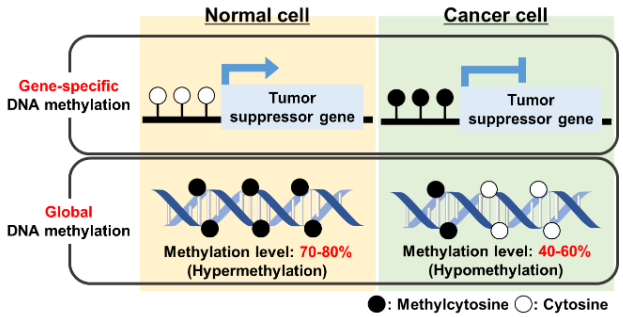

Fig. 1 Abnormal DNA methylation pattern in cancer cells.

In this study, we aimed to develop a fluorescence-based global DNA methylation level sensing system using methyl-CpG binding domain (MBD)-fused green fluorescent protein (MBD-GFP) for early cancer diagnosis. The principle is shown in Fig. 2. First, genomic DNA is immobilized on the well. Next, MBD-GFP is added on the well. After washing the well, fluorescence intensity of the GFP is measured to detect the global DNA methylation level. MBD specifically bind to methylated CpG on genomic DNA, indicating that fluorescence intensity of the GFP depends on the global DNA methylation level. Therefore, the global DNA methylation level is quantified by measuring the fluorescence intensity.

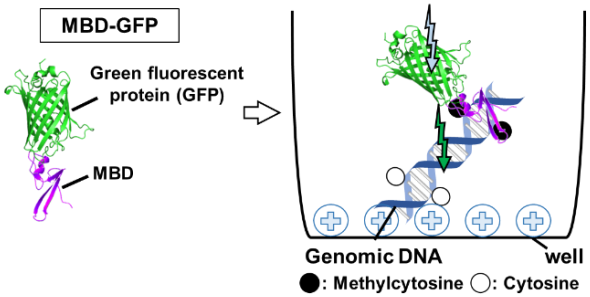

Fig. 2 Schematic representation of quantification of global DNA methylation level by means of MBD-GFP. The global DNA methylation level of genomic DNA immobilized on well depends on the binding amount of MBD-GFP, which can be determined based on the fluorescence intensity.

Methods

1. Expression and purification of the MBD-GFP

pET30c-*Streptag II-MBD-GFP* was constructed. *Escherichia coli* (*E. coli*) BL21 (DE3) competent cells were transformed by pET30c-*Streptag II-MBD-GFP*. The *E. coli* was cultured in 150 mL LB medium at 37°C. To induce gene expression, 1.2 mM isopropyl β-D-

thiogalactopyranoside (IPTG) was added when the OD_{600} was 0.5. The harvested cells were lysed by BugBuster Protein Extraction Reagent. The water-soluble fraction obtained from the lysate was applied to a Strep-Tactin column to purify MBD-GFP. In each fraction, fluorescence intensity of MBD-GFP was measured using a SpectraMax iD5 microplate reader. The eluted fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

2. Characterization of the MBD-GFP

Biotinylated methylated or unmethylated DNA were synthesized and mixed to prepare DNA of which methylation levels were 0, 25, 50, 75 or 100%. These DNAs were immobilized on streptavidin coated plate. The purified MBD-GFP was added on the plate. After washing the well, the fluorescence intensity of GFP was measured to analyze binding activity of MBD-GFP to methylated DNA.

3. Detection of global DNA methylation level

Human genomic DNA was prepared from HeLa cells. To prepare hypomethylated human genomic DNA, genomic DNA was purified from HeLa cells that were treated with DNA methyltransferase inhibitors (5-Aza-2'-deoxycitidine). These genomic DNAs were individually immobilized on amino plate well. MBD-GFP was added on the well. After washing the well, the fluorescence intensity of GFP was measured.

Results

1. Expression and purification of the MBD-GFP

In the purification process, the fluorescence intensity of MBD-GFP in each fraction were monitored. The highest intensity was detected in the second elution fraction. In the SDS-PAGE analysis, a single band corresponding to MBD-GFP was detected in the elution fractions (Fig. 3). These results indicated that MBD-GFP was successfully expressed in *E. coli* BL21 (DE3) and purified by the Streptag/Strep-Tactin system.

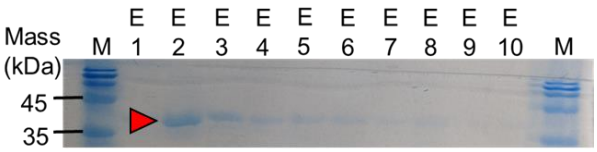

Fig. 3 SDS-PAGE analysis of the elution fraction 1-10. Molecular weight of MBD-GFP: 3.8×10^4

2. Characterization of the MBD-GFP

To investigate whether purified MBD-GFP binds to methylated DNA, amount of MBD-GFP that bound to immobilized methylated DNA was determined. The fluorescence intensity of MBD-GFP increased with increasing the methylation level of the immobilized DNA. These results indicated that MBD-GFP specifically bound to methylated DNA.

3. Detection of global DNA methylation level

The HeLa genomic DNA or hypomethylated genomic DNA were immobilized on the amino plate well and then the amount of MBD-GFP that bound to immobilized genomic DNA was measured. The higher fluorescence intensity of MBD-GFP was detected in the HeLa genomic DNA immobilized well compared with hypomethylated genomic DNA immobilized well. These results indicated that global DNA methylation level was detected by measuring fluorescence intensity of MBD-GFP.

Conclusion

In this study, MBD-GFP fusion protein was expressed and purified from in *E. coli*. The MBD-GFP had methylated DNA binding and fluorescence activities. The MBD-GFP bound to methylated CpG sites on the genomic DNA; therefore, the global DNA methylation level was detected by measuring the fluorescence intensity of the MBD-GFP. The global DNA methylation level sensing system would be applied for early cancer diagnosis, which contribute to provide appropriate treatment approaches.