

QUANTIFICATION OF GLOBAL DNA METHYLATION LEVELS IN ACUTE LEUKEMIA CELL LINES BEFORE AND AFTER DECITABINE TREATMENT USING A RAPID, SPECIFIC AND SENSITIVE LC-MS/MS METHOD

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Global DNA methylation (GDM) relates to genomic stability and is an important epigenetic event in malignant transformation of human cancers. As DNA hypermethylation is being explored as a therapeutic target in cancer, its changes might serve as a relevant endpoint for the pharmacodynamic effects of hypomethylating agents. Herein, we report quantification of GDM in 5 leukemia cell lines before and after exposure to the DNA hypomethylating agent decitabine or the histone deacetylase inhibitor FK-228, using a rapid, specific and sensitive electrospray (ESI) LC-MS/MS method. Kasumi 1, K562, Jurkat, Eol-1, THP-1 K562 cells were cultured in RPMI medium supplemented with 10% fetal bovine serum in a humidified 5% CO₂ atmosphere at 37°C. Each cell line was treated with 2.5 μM decitabine or 5 nM FK-228 for 48 hr. DNA was extracted from 10⁶ cells using DIamp DNA blood midi Kit. A 1 μg aliquot of the extracted DNA from each cell line was sequentially hydrolyzed using nuclease P1 for 1 hr, venom phosphodiesterase I for 2 hr, and alkaline phosphatase for 1 hr. The genomic DNA methylation levels of these cell lines before and following drug treatment were measured using a modified ESI LC-MS/MS method. The method utilized the precursor/product ion pair at m/z 242/127 for 5-methyl-2-deoxycytidine (5mdC) and at m/z 268/152 for 2-deoxyguanosine (2dG) as the internal standard. Separation of 5mdC from 2dG and other nucleosides was accomplished on a C18 Aquasil column eluting with 30% methanol in 10 mM HCOONH₄, with post-column addition of methanol. The LC-MS/MS method was validated in mobile phase with a linear range from 1 to 500 ng/mL. The within-day precision values ranged from 2.8 % to 9.9 % and the between-day ranged from 1.1 % to 17.6 % (n=6). The accuracy values of the assay varied from 96.7 % to 109.5 %. The baseline DNA methylation mean values of Kasumi 1, K562, Jurkat, Eol-1, THP-1 were 4.98, 2.43, 4.25, 4.45, and 4.79%, respectively. K562 cell was also used as the low quality control with a 7.0 %CV (n=6) and Kasumi 1 as the high quality control with a 6.8 %CV (n=6). Following treatment with decitabine, the GDM levels of Kasumi 1, K562, Jurkat, Eol-1, THP-1 were reduced to 57.1, 62.9, 63.7, 59.8, and 55.1% of those pre-treatment, respectively. Consistently, no alteration of the DNA methylation level was found in these cell lines following treatment with FK-228. A significant hypomethylating effect of decitabine for 5 tumor cell lines was found. As a specificity control, we demonstrated that no hypomethylation occurred following treatment with FK-228. The modified ESI LC-MS/MS method described here provides an unambiguous measurement of GDM with high reproducibility and high sensitivity and can be adapted for high-throughput rapid screening of DNA methylation effects by DNA hypomethylating agents.

Supported by NIH-NCI-RO1-CA102031.