Anticancer Research 2012 vol.32 N11, pages 4715-4722

DNA methylation in ATRA-treated leukemia cell lines lacking a PML-RAR chromosome translocation

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Abstract

A deficient retinoic acid signaling has been suggested to be an important cause of the clinical inefficacy of all-trans retinoic acid (ATRA) therapy in nonpromyelocytic (non-PML) forms of acute myeloid leukemia (AML). The general aim of the present work was to explore novel ways to take advantage of the anti-leukemic potential of ATRA, and, specifically, to search for a synergism between ATRA and epigenetic drugs. Because previous reports have found no major influence of ATRA on DNA methylation, we investigated whether ATRA-mediated differentiation of the U937 and HL-60 AML cell lines, both lacking a PML-retinoic acid receptor (RAR) fusion product, is accompanied by early-appearing and weak changes in CpG methylation. We report that in HL-60 cells, by using a highly quantitative analysis of a set of genes found to be abnormally expressed in AML, polymerase chain reaction (PCR)-amplified p16 gene promoter molecules (each with 15 CpG sites), exhibited a CpG methylation level of 0-4% in untreated cells, which increased to 4-21% after treatment with ATRA for seven days. In contrast to HL-60 cells, U937 cells exhibited a very high CpG methylation level in p16, and ATRA did not influence the promoter methylation of this gene. In the total CCGG sites of the genome, analysed using a methylation-sensitive restriction enzyme, CpG methylation was significantly lower in ATRA-treated HL-60 ($p<0.01$) and U937 cells ($p<0.05$) than in controls. Taken together, our findings show that ATRA can influence DNA methylation, and suggest that future research should investigate whether epigenetic modulation may evoke a clinical effect of ATRA in leukemia.

Keywords

ATRA, DNA methylation, Hep-2, HL-60 cells, p16, PML-RAR chromosome translocation, U937