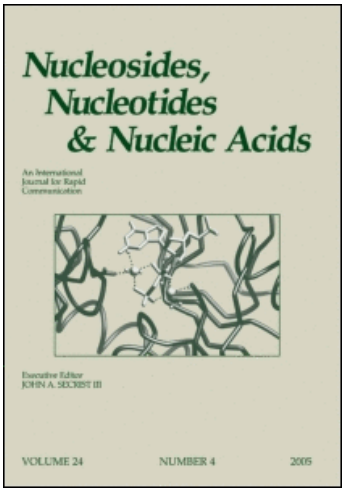


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Alteration of DNA Methylation Status in K562 and MCF-7 Cancer Cell Lines by Nucleoside Analogues

B. Krawczyk[a]; K. Fabianowska-Majewska[a]
[a] Department of Biomedicinal Chemistry, Medical University of Lodz, Lodz, Poland

To cite this Article Krawczyk, B. and Fabianowska-Majewska, K.(2006) 'Alteration of DNA Methylation Status in K562 and MCF-7 Cancer Cell Lines by Nucleoside Analogues', Nucleosides, Nucleotides and Nucleic Acids, 25: 9, 1029 — 1032
To link to this Article: DOI: 10.1080/15257770600890764
URL: http://dx.doi.org/10.1080/15257770600890764

Nucleosides, Nucleotides, and Nucleic Acids, 25:1029–1032, 2006

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770600890764

Taylor & Francis
Taylor & Francis Group

ALTERATION OF DNA METHYLATION STATUS IN K562 AND MCF-7 CANCER CELL LINES BY NUCLEOSIDE ANALOGUES

B. Krawczyk and K. Fabianowska-Majewska □ *Department of Biomedicinal Chemistry, Medical University of Lodz, Lodz, Poland*

□ *The effects of 2-chloro-2′-deoxyadenosine, β-D-arabinofuranosyl-2-fluoroadenine, and 5-aza-2′-deoxycytidine on promoter methylation of the selected tumor suppressor genes (i.e., ERα, BRCA1, E-cadherin, PTEN, and APC) were estimated using methylation-sensitive restriction analysis (MSRA) in K562 cells (human erythroleukemic cell line) and MCF-7 cells (human breast cancer cell line). In both cell lines all tested drugs completely reduced methylation of PTEN and APC promoters. The results indicate that the tested nucleoside analogues, which are known inhibitors of DNA synthesis, also are implicated in indirect (or direct in the case of 5-aza-dCyd) regulation of post-replicative DNA modifications (i.e., DNA methylation).*

Keywords Cladribine; Fludarabine; Decitabine; *PTEN* and *APC* methylation

INTRODUCTION

2-Chloro-2′-deoxyadenosine (2-CdA, cladribine), β-D-arabinofuranosyl-2-fluoroadenine (F-ara-A, fludarabine), and 5-aza-2′-deoxycytidine (5-aza-dCyd, decitabine) have important therapeutic activity in blood cancers. The drugs induce inhibition of DNA synthesis, which results in induction of apoptosis in dividing and resting lymphocytes.[1] Our previous studies indicate that 2-CdA and F-ara-A are involved in alteration of genomic DNA methylation.[2] Their action probably is associated with inhibition of S-adenosyl-L-homocysteine (SAH) hydrolase activity and increase of the level of S-adenosylmethionine (SAM), a donor of methyl group. It can lead to disturbance of SAM -dependent methylation reactions.[2]

The research was supported by the Medical University of Lodz (Grant No. 502-12-302). K562 and MCF-7 cell lines were kind gifts from Professor Jean Claude D'Halluin (INSERM 125, Lille, France) and Doctor Marek Rozalski (Department Biology and Biotechnology, Medical University of Lodz, Poland), respectively.

Address correspondence to Krystyna Fabianowska-Majewska, Department of Medicinal Chemistry, Medical University of Lodz, Mazowiecka Street 6/8, 92-215 Lodz, Poland. E-mail: fabian@csk.umed.lodz.pl

5-Aza-dCyd, however, is a potent inhibitor of DNA methyltransferase activity.

The present studies were aimed at estimation of the effect of the tested adenosine analogues and 5-aza-dCyd on methylation level of promoters of the following tumour suppressor genes: *ERα*, *BRCA1*, *E-cadherin*, *PTEN*, and *APC*. The promoters of *ERα*, *BRCA1*, and *E-cadherin* genes were chosen for our studies due to the high frequency of silencing of their expression (often through alteration of methylation status) in breast cancer.[3] *PTEN* and *APC* promoters were the subject of our interest because these genes are involved in regulation of cell growth, migration, adhesion, and apoptosis as well as, indirectly, in regulation of expression of *DNA methyltransferase* through affecting intracellular signal transduction pathways.[4,5] Moreover, *PTEN* and *APC* promoters are hypermethylated in various types of human cancers.[6,7]

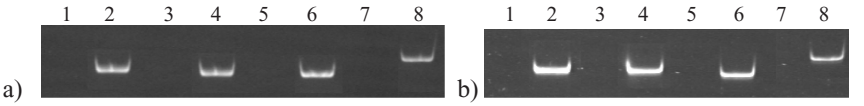
MATERIALS AND METHODS

Chemicals: Basal reagents and 2-CdA, F-ara-A and 5-aza-dCyd were purchased from Sigma Chemical Co. Endonucleases: HpaII, BstU1, and AatII were purchased from New England Bio Labs; Eco72I (Fermentas, Lithuania); Taq polymerase (Polgen, Poland).

Methylation assay: K562 and MCF-7 cells were cultured (48 and 72 hours, respectively) in the presence of the tested drugs at IC_{50} concentrations. The used values of concentration of 2-CdA, F-ara-A, and 5-aza-dCyd were equal to 0.1 μM, 3.0 μM, and 0.9 μM, respectively, in K562 cells, and 0.4 μM, 35.0 μM, and 4.0 μM, respectively, in MCF-7 cells. The methylation status of the tested gene promoters was examined according to Iwase's method.[8] The assay included 4 steps: (1) isolation and purification of cellular DNA from K562 or MCF-7 cells; (2) digestion of cellular DNA with methylation-sensitive restriction endonucleases: HpaII [C↓CGG], BstU1 [CG↓CG], AatII [(G/T)ACGT↓C], and Eco72I [CAC↓GTG]; (3) amplification (PCR) of digested DNA; (4) electrophoresis of amplified DNA fragments in 6% polyacrylamide gel followed by computer analysis.

RESULTS AND DISCUSSION

The analysis of methylation status of promoters of *ERα*, *BRCA1*, *E-cadherin*, *PTEN*, and *APC* genes showed that in control K562 cells (i.e., cells growing without nucleoside analogues) promoters of all tested genes were hypermethylated. Whereas in control MCF-7 cells only *PTEN*, *BRCA1*,



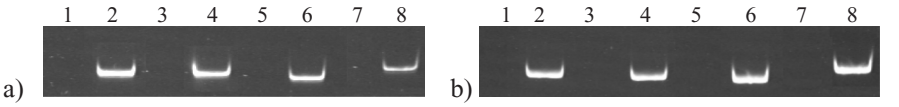
Channels:
1 – control of digestion (HpaII) of non-methylated DNA from human placenta
8 – control of amplification of DNA from cells growing without drugs
2, 4, and 6 – undigested DNA from cells treated with 2-CdA, F-ara-A and 5-aza-dCyd respectively
3, 5, and 7 – digested (HpaII) DNA from cells treated with 2-CdA, F-ara-A and 5-aza-dCyd respectively

FIGURE 1 *PTEN* promoter; (a) K562 cells, (b) MCF-7 cells (lack of a band means that specific sequences were non-methylated).

and *APC* promoters were hypermethylated. 2-CdA, F-ara-A and 5-aza-dCyd completely reduced methylation of CpG sequences of analyzed fragments of *PTEN* promoter (Figures 1a and 1b, channels 3, 5, 7) and *APC* promoter (Figures 2a and 2b, channels 3, 5, 7) in both cell lines.

Additionally, in K562 cells 5-aza-dCyd reduced methylation of CpG sequences of promoters of other tested genes (i.e., *ERα* and *E-cadherin*, except *BRCA1* promoter; results are not shown).

Our findings demonstrate that the action of 2-CdA, F-ara-A, and 5-aza-dCyd leads to reduction of methylation of promoters of tumour suppressor genes, which are important for normal development of breast cells. The change of promoter methylation may bring about re-expression of tumour suppressor genes and restoration of normal cell growth, which requires confirmation by further studies.



Channels:
1– control of digestion (HpaII) of non-methylated DNA from human placenta
8 – control of amplification of DNA from cells growing without drugs
2, 4, and 6 – undigested DNA from cells treated with 2-CdA, F-ara-A and 5-aza-dCyd respectively
3, 5, and 7 – digested (Eco72I) DNA from cells treated with 2-CdA, F-ara-A and 5-aza-dCyd respectively.

FIGURE 2 *APC* promoter; (a) K562 cells, (b) MCF-7 cells (lack of a band means that specific sequences were non-methylated).

REFERENCES

1. Pettitt, A.R. Mechanism of action of purine analogues in chronic lymphocytic leukaemia. *Br. J. Haematol.* **2003**, 121, 692–702.
2. Wyczechowska, D.; Fabianowska-Majewska, K. The effects of cladribine and fludarabine on DNA methylation in K562 cells. *Biochem. Pharmacol.* **2003**, 65, 219–225.
3. Widschwendter, M.; Jones, P.A. DNA methylation and breast carcinogenesis. *Oncogene* **2002**, 21, 5462–5482.
4. Yamada, K.M.; Araki, M. Tumor suppressor PTEN, modulator of cell signaling, growth migration and apoptosis. *J. Cell Science* **2001**, 114, 2375–2382.
5. Goss, K.H.; Groden, J. Biology of the adenomatous polyposis coli tumor suppressor. *J. Cli. Oncol.* **2000**, 18, 1967–1979.
6. Garcia, J.M.; Silva, J.; Pena, C.; Garcia, V.; Rodriguez, R.; Cruz, M.A.; Cantos, B.; Provencio, M.; Espana, P.; Bonilla, F. Promoter methylation of the *PTEN* gene is a common molecular change in breast cancer. *Genes Chromosomes Cancer* **2004**, 41, 117–124.
7. Virmani, A.K.; Rathi, A.; Sathyanarayana, U.G.; Padar, A.; Huang, C.X.; Cunningham, H.T.; Farinas, A.J.; Milchgrub, S.; Euhus, D.M.; Gilcrease, M.; Herman, J.; Minna, J.D.; Gazdar, A.F. Aberrant methylation of the *adenomatous polyposis coli* (*APC*) gene promoter 1A in breast and lung carcinomas. *Int. J. Cancer Res.* **2001**, 7, 1998–2004.
8. Iwase, H.; Omoto, Y.; Iwata, H.; Toyama, T.; Hara, Y.; Ando, Y.; Ito, Y.; Fuji, Y.; Kobayashi, S. DNA methylation analysis at distal and proximal promoter regions of the oestrogen receptor gene in breast cancers. *Brit. J. Cancer* **1999**, 80, 1982–1986.