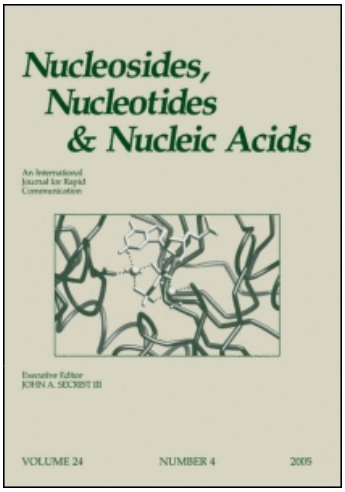

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

The Effects of Nucleoside Analogues on Promoter Methylation of Selected Tumor Suppressor Genes in MCF-7 and MDA-MB-231 Breast Cancer Cell Lines

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

To cite this Article Krawczyk, B. , Rudnicka, K. and Fabianowska-Majewska, K.(2007) 'The Effects of Nucleoside Analogues on Promoter Methylation of Selected Tumor Suppressor Genes in MCF-7 and MDA-MB-231 Breast Cancer Cell Lines', Nucleosides, Nucleotides and Nucleic Acids, 26: 8, 1043 — 1046
To link to this Article: DOI: 10.1080/15257770701509594
URL: http://dx.doi.org/10.1080/15257770701509594

Nucleosides, Nucleotides, and Nucleic Acids, 26:1043–1046, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701509594

Taylor & Francis
Taylor & Francis Group

THE EFFECTS OF NUCLEOSIDE ANALOGUES ON PROMOTER METHYLATION OF SELECTED TUMOR SUPPRESSOR GENES IN MCF-7 AND MDA-MB-231 BREAST CANCER CELL LINES

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

□ *The effects of 2-chloro-2′-deoxyadenosine, 9-β-D-arabinofuranosyl-2-fluoroadenine, and 5-aza-2′-deoxycytidine on promoter methylation of the selected tumor suppressor genes (i.e., ERα, BRCA1, RARβ2, E-cadherin, PTEN, and APC) were estimated using methylation-sensitive restriction analysis. The studies were carried out in hormone-responsive, low-invasive cell line MCF-7 and hormone-insensitive, highly invasive cell line MDA-MB-231. The results demonstrate an implication of the tested adenosine analogues and 5-aza-dCyd in regulation of DNA methylation process. Moreover, the effects of nucleoside analogues on PTEN promoter methylation suggest distinct mechanism of regulation of the epigenetic DNA modification in low-invasive compared to highly invasive breast cancer cells.*

Keywords Nucleoside analogues; Promoter methylation; *PTEN*

INTRODUCTION

2-Chloro-2′-deoxyadenosine (2-CdA, cladribine) and 9-β-D-arabinofuranosyl-2-fluoroadenine (F-ara-A, fludarabine) are antileukemic drugs with therapeutic activity in a variety of blood cancers. Their efficacy mainly results from inhibition of DNA synthesis by their nucleotide derivatives.[1] In previous experiments we proved that the adenosine analogues also inhibit S-adenosyl-L-homocysteine (SAH) hydrolase causing alteration of genomic DNA methylation.[2]

In the present studies we investigated whether the adenosine analogues are able to reduce methylation of regulatory regions of the selected tumor suppressor genes. The effects of the tested adenosine analogues were compared with the effect of 5-aza-2′-deoxycytidine [5-aza-dCyd, decitabine,

The research was supported by the Medical University of Lodz (Grant No. 502-12-302) and Ministry of Science and Higher Education (Grant No. 2 P05A 036 30). MCF-7 cell line was kind gift from Dr. Marek Rozalski (Department of Biology and Biotechnology, Medical University of Lodz, Poland).

Address correspondence to B. Krawczyk, Department of Biomedical Chemistry, Medical University of Lodz, Lodz, Poland. E-mail: bkrawczyk@zdn.am.lodz.pl

a potent competitive inhibitor of DNA methyltransferase (DNMT1) activity] which is known to reduce promoter methylation of tumor suppressor genes in numerous human cancers. For this reason, 5-aza-dCyd was used in our experiments as a reference agent.

Our attention was focused on the promoters of *ERα*, *BRCA1*, *RARβ2*, and *E-cadherin* as well as *PTEN* and *APC* genes frequently silenced in breast cancer (which is often associated with their hypermethylation).[3] The tested genes encode proteins crucial for normal breast tissue development. Moreover, PTEN and APC may participate in regulation of *DNA methyltransferase* (*DNMT1*) expression through controlling main intracellular oncogenic signal transduction pathways, Ras/Raf/MAPK, and Wnt/β-catenin/TCF/LEF, respectively.[4,5]

MATERIALS AND METHODS

Chemicals

Basal reagents and nucleoside analogues (2-CdA, F-ara-A, and 5-aza-dCyd) were purchased from Sigma Chemical Co. Endonucleases were purchased from Fermentas (Lithuania) and Taq polymerase- from Polgen (Poland).

Methylation Assay

MCF-7 and MDA-MB-231 breast cancer cell lines were cultured for 72 hours in DMEM and L-15 media, respectively, in the presence of the tested drugs at IC_{50} concentration (μM): 0.2, 15.0, and 0.6 for 2-CdA, F-ara-A, and 5-aza-dCyd, respectively, in MCF-7 cells; and 0.2, 4.0, and 4.0, respectively, in MDA-MB-231 cells. In addition, MDA-MB-231 cells were treated with the nucleoside analogues at concentration higher than IC_{50}. The methylation status of the tested gene promoters was examined by methylation-sensitive restriction analysis (MSRA)[6] including: (1) digestion of cellular DNA with methylation-sensitive restriction endonucleases: HpaII [C$^{\downarrow}$CGG], BstU1 [CG$^{\downarrow}$CG], AatII [(G/T)ACGT$^{\downarrow}$C], and Eco72I [CAC$^{\downarrow}$GTG]; (2) amplification (PCR) of digested DNA; (3) electrophoretic analysis of amplified DNA fragments in 6% polyacrylamide gel; (4) densytometric analysis of gels to estimate methylation status of promoter fragments.

RESULTS AND DISCUSSION

The results of the present studies indicated that in MCF-7 cells *ERα* and *E-cadherin* promoters were non-methylated. Whereas *BRCA1*, *RARβ 2*, *PTEN*, and *APC* promoters were methylated in the following percentages: 100, 25, 30, and 25, respectively. In MDA-MB-231 cells only *PTEN*, and *BRCA1* pro-

TABLE 1 Effects of nucleoside analogues on methylated promoters in MCF-7 cells

	Inhibition of promoter methylation [%][a]			
	Methylated genes			
Drugs at IC_{50}	*BRCA1*	*RARβ2*	*PTEN*	*APC*
2-CdA (0.2 μM)	0	20	34	12
F-ara-A (15.0 μM)	0	65	70	70
5-aza-dCyd (0.6 μM)	0	65	85	60

[a]The inhibition of methylation was expressed as a percentage of methylation of digested control DNA from cells cultured without drugs.

moters were methylated in 85% and 100%, respectively. (Methylation level was expressed as a percentage of undigested control DNA from cells cultured without drugs.)

The effects of 2-CdA, F-ara-A, and 5-aza-dCyd (used at IC_{50} concentration) on methylation of methylated gene promoters (i.e., *BRCA1*, *RARβ2*, *PTEN*, and *APC*) in MCF-7 cells are shown in Table 1. The results indicated the highest inhibitory effect of all tested drugs on methylation of *PTEN* promoter. The effects of F-ara-A and 5-aza-dCyd were over 2-fold higher than the effect of 2-CdA. All tested nucleosides had no effects on methylation of *BRCA1* promoter.

The effects of the tested nucleoside analogues on methylated gene promoters (i.e., *PTEN* and *BRCA1*) in MDA-MB-231 cells are shown in Table 2. The results indicated that adenosine analogues (i.e., 2-CdA and F-ara-A) used at IC_{50} concentration had no effects on *PTEN* promoter methylation. However, these drugs used at 5-fold higher concentration reduced the methylation of *PTEN* promoter by 20% (2-CdA) and 25% (F-ara-A). The higher inhibitory effect on *PTEN* methylation was noted in the case of 5-aza-dCyd (with similar dependence on concentration as in the case of 2-CdA

TABLE 2 Effects of nucleoside analogues on methylated *PTEN* and *BRCA1* promoters in MDA-MB-231 cells

	Inhibition of promoter methylation [%][a]										
	Drug concentration										
	2-CdA [μM]				F-ara-A [μM]				5-aza-dCyd [μM]		
Methylated genes	0.2 IC_{50}	0.3	0.6	1.0	4.0 IC_{50}	8.0	15.0	30.0	4.0 IC_{50}	15.0	30.0
PTEN	0	12	15	20	0	10	20	25	25	45	70
BRCA1	0	0	0	0	0	0	0	0	0	0	0

[a]The inhibition of methylation was expressed as a percentage of methylation of digested control DNA from cells cultured without drugs.

and F-ara-A). In MDA-MB-231 cells methylation of *BRCA1* promoter was not affected.

The findings indicate that the tested breast cancer cell lines differ with both the profile of methylated genes and the state of *PTEN* promoter methylation. Moreover, the results confirm other authors' data that the effects of 2-CdA, F-ara-A and 5-aza-dCyd on DNA methylation are distinct in the tested breast cancer cell lines and depend on the stage of cancer progression.[7] For this reason, the epigenetic therapy focused on inhibition of promoter methylation of tumor suppressor genes seems to be more effective in early stage breast cancer cells. In addition, the results reveal enhanced effect of 5-aza-dCyd on inhibition of DNA methylation (especially in MDA-MB-231 cells) in comparison with adenosine analogues. It can be due to direct inhibition of DNMT1 activity by 5-aza-dCyd which traps the enzyme. Owing to distinct action mechanisms and different efficacies of 5-aza-dCyd and adenosine analogues, an investigation of the effects of cytidine analogue and adenosine analogues used in combination will be undertaken in our future studies.

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. Campbell, P.M.; Szyf, M. Human DNA methyltransferase gene *DNMT1* is regulated by the APC pathway. *Carcinogenesis* **2003**, 24, 17–24.
6. 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. *Br. J. Cancer* **1999**, 80, 1982–1986.
7. Guo, Y.; Pakneshan, P.; Gladu, J.; Slack, A.; Szyf, M.; Rabbani, S.A. Regulation of DNA methylation in human breast cancer. *J. Biol. Chem.* **2002**, 277, 41571–41579.