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Letter to the Editor

DNA Hypermethylation and Changes in Gene Expression may be Related to the Chemotherapeutic Action of Cytarabin*

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CYTARABIN (1-β-D-arabinofuranosylcytosine), a pyrimidine analog used in the therapy of various types of malignancies, has been shown to induce differentation of malignant myeloid cells in a low-dose regimen [1]. Cytarabin also induces hypermethylation of cellular DNA [2]. Since an inverse correlation between DNA methylation and expression of certain genes exists [3], we were interested in whether cytarabin in low concentrations causes changes in gene expression in treated cells. Here we have studied the expression of the β-major globin gene in P815 mastocytoma cells treated with this compound. 5-Aza analogs of cytidine were also studied since these compounds can activate transcriptionally silent genes, most likely through the inhibition of enzymatic methylation in these sequences [4]. Cells grown in the presence of cytarabin and 5-azacytosines at concentrations causing 50% growth inhibition for one cell cycle (Table 1) were further cultured in the absence of the drugs for two additional cell cycles. The extent of enzymatic DNA methylation in 5′-CCGG sequences was studied by use of 5-methylcytosine-sensitive restriction endonucleases [2]. 5-Azacytidine and 5-azadeoxycytidine inhibit DNA methylation in their sequences, whereas cytarabin treatment causes a significant hyper-

Table 1. *Effect of cytidine analogs on enzymatic DNA methylation and expression of the β-major globin gene in mouse P815 cells*

Drug	Concentration (μM)*	Extent of enzymatic methylation of DNA (% of control)†	β-Major globin mRNA level (No. of copies per cell)‡
None	-	100	0.50 ± 0.05
5-Azacytidine	0.40	72 ± 10	0.45 ± 0.05
5-Azadeoxycytidine§	0.30	70 ± 15	1.00 ± 0.10
Cytarabin	0.02	137 ± 12	0.02 ± 0.03

*At indicated concentrations, the drugs used caused 50% growth inhibition after 72 hr incubation.

†Assayed by digestion with HpaII and MspI [2]; the means and standard deviations of three experiments are given.

‡Quantitative analysis based on the amount of radioactivity which hybridized to the individual dots as measured by densitometric analysis of the autoradiographs and liquid scintillation counting, respectively, and by standard curves obtained by hybridization to plasmid DNA (the recombinant plasmid McβG1 was kindly provided by Dr N. Mantei); the means and standard deviations of four measurements are given.

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methylation (Table 1). The presence of RNA coding for the β-major globin gene was quantified by dot-blot hybridization of nitrocellulose-immobilized poly(A) $^{+}$-mRNA with a ^{32}P-nick-translated β-major globin cDNA probe (Fig. 1, Table 1). The data indicate a dramatic decrease in the expression of this gene in cells treated with cytarabin. Although both 5-aza analogs caused a hypomethylation of DNA, only 5-azadeoxycytidine was capable of altering the expression of these gene in P815 cells. The reason for this difference is unknown as yet, but it may point to the fact that hypomethylation of DNA *per se*, although necessary, is not sufficient for the activation of transcriptionally silent genes [4]. Nevertheless, the results obtained with cytarabin provide the first evidence for a decrease in the expression of a single gene in cells treated with this drug. Since it is likely that the expression of the β-major globin gene was aberrantly induced during the malignant transformation of this mastocytoma line, the present results suggest that the expression of aberrantly expressed genes may be reduced by cytarabin treatment. Since cytarabin also induces hypermethylation of total cellular DNA [2] and of '-CCGG sequences in the β-major globin gene region itself [manuscript in preparation], these two effects may be functionally related. Thus the cytarabin-induced hypermethylation of DNA may be responsible for the differentiation of malignant cells by low-dose treatment with cytarabin [1].

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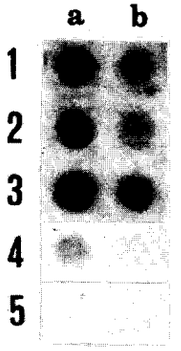

Fig. 1. Dot-blot hybridization assay of nitrocellulose-immobilized poly(A)$^+$-mRNA with ^{32}P-nick-translated β-major globin cDNA. The isolation of RNA and the assays for its integrity [5] *and the hybridization conditions* [6] *have been described. Poly(A)$^+$-mRNA from control cultures (row 1), 5-azacytidine-treated (row 2), 5-azadeoxycytidine-treated (row 3) and cytarabin-treated (row 4) cells were immobilized to nitrocellulose in two different amounts: lane a, 2 μg and lane b 1 μg. Yeast tRNA (row 5) was included as a background control. RNase-treatment diminished all hybridization signals (not shown).*