REVIEW OF CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS, INTERNATIONAL
EDITION 18: 40-42 (2004)

DNA/RNA Methylation Inhibitors Block Murine Erythroleukemia Cell Differentiation and Promote Accumulation of Discrete Small RNA Species: Implications in Activation of Silent DNA Sequences by Pharmacological Agents

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S u m m a r y. Abnormalities in DNA methylation are considered to be frequent epigenetic modifications detected in cancer cells. Since methylation status and transcriptional activity are inversely related, aberrant DNA methylation in promoter region of tumor-related genes might be a key mechanism for silencing of tumor suppressor genes. By trying to analyze the effect of methylation inhibitors N^6-methyladenosine, neplanocin A, 3-deazaneplanocin A, and cycloleucine in the blockade of MEL cell differentiation in vitro, we detected by Northern blot analysis discrete small molecular weight RNA transcripts hybridized only with DNA probes derived from 3'-flanking sequences of β^{major} globin gene locus. Interestingly, such new RNA transcripts lack polyA-tail and their appearance in the cytoplasm occurred only upon exposure of MEL cells to methylation inhibitors. Although, it is not known by now whether these discrete polyA$^-$ RNA molecules may contribute to blockade of commitment of MEL cells to terminal maturation by methylation inhibitors, it would be of great importance, however, to see how these previously silent DNA regions are activated as well as to assess their possible involvement in MEL cell differentiation program.

INTRODUCTION

Murine eryhtroleukemia (MEL) cells have been used as a suitable model system to uncover the molecular and cellular events of differentiation along the erythrocytic cell lineage (1). Previous studies have shown that agents that inhibit DNA methylation, like 5-azacytidine, induce erythroid differentiation in a substantial proportion of MEL cells (2). Moreover, studies from our laboratory have shown that changes in RNA methylation are also occurred during MEL cell differentiation (3,4). In fact, we observed that DNA/RNA methylation inhibitors, [N^6-methyladenosine (N^6mAdo), neplanocin A, 3-deazaneplanocin A, and cycloleucine], block initiation of MEL cell differentiation suggesting that methylation of DNA and RNA is involved in the commitment process.

In the present study we extended previous observations and further analyzed the effect of methylation inhibitors neplanocin A, 3-deazaneplanocin A, and cycloleucine in the blockade of MEL cell differentiation by assessing specific gene expression patterns by Northern blot hybridization analysis.

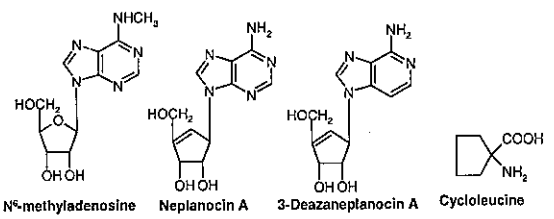

Figure 1. Chemical structures of DNA and RNA methylation inhibitors.

RESULTS AND DISCUSSION

Previous work has suggested that induction of hemoglobin synthesis and terminal erythroid maturation in MEL cells is associated with changes in

methylation of both polyA$^+$ and polyA$^-$ RNA as well as with alterations in S-adenosyl-homocysteine/S-adenosylmethionine (SAH/SAM) ratio (3,4). This conclusion was further supported by the observation that N^6mAdo (Figure 1) inhibits commitment of MEL cells to terminal maturation through its intracellular conversion into S-N^6-methyladenosylhomocysteine (N^6-SAH), an active intermediate that affects methylation of RNA. This notion has also been supported with the use of neplanocin A, 3-deazaneplanocin A, and cycloleucine (Figure 1), agents that inhibit RNA and DNA methylation and block MEL cell differentiation *in vitro* by causing selective degradation of RNA transcripts (4). The observations that discrete patterns of gene expression operate in differentiating MEL cells suggest that the steady-state cytoplasmic accumulation of RNA transcripts result either from transcriptional activation, or repression, or posttranscriptional processes (1,5). In fact, previous studies have considered DNA hypomethylation in differentiating MEL cells responsible for activation of transcription of globin genes (2). It is also known that sodium butyrate, an inhibitor of histone deacetylases, can induce differentiation of MEL cells *in vitro* (6). Since histones are considered crucial components of the DNA condensation process, it is reasonable to correlate these changes with chromatin structure alterations that could result in gene expression modulation (5). Furthermore, the recent findings that the methyl-CpG binding protein, MeCP2, interact with the histone deacetylase-involved complex have provided evidence to the coupling of DNA methylation and histone acetylation processes in the mechanisms involved in chromatin structure modification and gene transcription silencing (7).

In an effort to better understand how do the methylation inhibitors block MEL cell differentiation, we assessed the kinetics of several RNA transcripts encoded by developmentally regulated genes (β^{major} globin, α_1-globin, TfR), as well as house-keeping genes (rpS5, β-actin, GAPDH). During the course of these experiments, assessment of cytoplasmic RNA isolated from MEL cells exposed to either neplanocin A, or 3-deazaneplanocin A, and/or cycloleucine by Northern blot hybridization analysis, with multi-primed ^{32}P-labeled EcoRI/EcoRI ~7.3 kb mouse genomic DNA fragment bearing β^{major} globin gene as well as flanking sequences, revealed the existence of full length β^{major} globin mRNA as well as discrete RNA transcripts of relatively small molecular weight. It is noteworthy, within this ~7.3 kb DNA fragment, the only known coding sequence is that for β^{major} globin gene (4)., This fact forced us to proceed towards the characterization of the new RNA transcripts accumulated in the cytoplasm specifically only upon exposure of MEL cells to methylation inhibitors. Notably, the detection of short-end RNA transcripts was specific to genomic probes derived from the β^{major} and α_1-globin gene loci. Furthermore, genomic mapping analysis spanning the β^{major} globin gene locus region indicated that these nascent RNA transcripts are hybridized only with DNA probes derived from transcriptionally silent 3'-downstream non-coding sequences of β^{major} globin gene. Such new RNA transcripts lack polyA-tail and their appearance occurred only upon exposure of MEL cells to methylation inhibitors and seemed to represent RNA species that accumulate in the cytoplasm, although with unknown function as yet.

These data tend to propose that the detected new RNA transcripts lack polyA-tail giving thus a new direction towards the elucidation of their structure and function. As a matter of fact, it would be of great importance in trying to clone and characterize them, since these discrete polyA$^-$ RNA molecules may contribute to the blockade of commitment of MEL cells to terminal maturation caused by methylation inhibitors through the activation of previously silent non-coding DNA sequences. Such an attempt could provide new evidence by trying to delineate specific mechanisms correlating with chromatin structure, DNA methylation and gene expression patterns seen during commitment of MEL cells and uncovering key components involved in these processes. This need is further strengthen by the accumulated data suggesting that chromatin structure and DNA methylation are closely related in pathological and physiological processes during development of higher organisms (8,9). Furthermore, such information would also be of great importance in the process of developing methylation inhibitors as potential chemotherapeutic agents, as it happened in the case of 5-aza-2'-deoxycytidine (Decitabine), whose phase II clinical trials have recently been completed and are related with its potential use as drug in myelodysplastic syndrome and chronic myelogenous leukemia therapy (10).

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