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Differential effect of 6-ethylmercaptopurine on c-myc expression in wild-type and HGPRT-deficient HL-60 cells*

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Summary. A variety of compounds inhibit the growth and induce differentiation of human promyelocytic leukemia (HL-60) cells. HL-60 subclones that lack the purine salvage enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) can also be induced to differentiate with purine analogs. Mechanisms by which purine analogs induce differentiation offer unique possibilities for cancer chemotherapy. We have studied the effect of the purine analog 6-ethylmercaptopurine (e^{6}MP) on the growth and induction of differentiation in both wild-type and HGPRT-deficient HL-60 cells. We have previously shown that e^{6}MP inhibits cell growth in both wild-type and HGPRT-deficient HL-60 cells without activation through salvage pathways [8]. In this report we evaluate the effect of e^{6}MP on c-myc mRNA expression. c-Myc mRNA, which is amplified in HL-60 cells, has been shown to play a role in the induction of granulocytic differentiation in HL-60 cells. e^{6}MP transiently down-regulates c-myc mRNA in wild-type cells but has no efect on c-myc mRNA expression in HGPRT-deficient HL-60 cells. Despite the differential effects of e^{6}MP on c-myc mRNA, both wild-type and HGPRT-deficient HL-60 cells appear to engage in terminal differentiation. The morphological changes and nonspecific esterase activity induced by e^{6}MP suggest differentiation down the monocytic pathway. However, early monocytic markers such as the rapid induction of c-fos and the stabilization of c-fms mRNA are not observed. In addition, e^{6}MP inhibits TPA-induced monocytic/macrophage differentiation as characterized by stabilization of c-fms mRNA and cellular adherence.

Introduction

Induction of differentiation by pharmacologic agents in the absence of cytotoxic effects is an attractive mechanism for chemotherapeutic treatment. Activation of purine analogs through salvage pathways results in the disruption of DNA and RNA synthesis. Conversion of purine antimetabolites into their corresponding mononucleotides causes significant cytotoxicity to proliferating cells. Cells that are deficient in the salvage enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) still exhibit growth inhibition by purine antimetabolites such as 6-thioguanine (6-TG), 8-azaguanine (8-AzaG) and 6-mercaptopurine (MP). Growth inhibition in HGPRT-deficient HL-60 cells by the above purine analogs is associated with granulocytic terminal differentiation [5, 17, 19]. Cytotoxic metabolites are not responsible for this induction of differentiation [10], and further studies have shown that the induction of differentiation is directly due to the nucleobase analog [12].

Although these nucleobase analogs are useful as model purine analogs that induce differentiation in HGPRT-deficient HL-60 cells, they suffer from the disadvantage of being highly cytotoxic to wild-type cells. We have previously observed that 6-ethylmercaptopurine (e^{6}MP) inhibits growth and produces a specific terminal end-cell in both types of HL-60 cells [8]. The mechanism appears to be independent of the normal modes of cytotoxic activation through the salvage pathways, since no metabolites were detected by HPLC and the cells maintained good viability after treatment. Thus, the mechanism of action of e^{6}MP differs from that of other nucleobase analogs such as 6-TG, 8-AzaG, and MP.

Induction of cellular differentiation generally involves changes in the expression of cellular oncogenes [2]. The exact role of oncogene expression in the differentiation pathway is not known. Correlations have been made with

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Abbreviations: e^{6}MP, 6-ethylmercaptopurine; 6-TG, 6-thioguanine; MP, 6-mercaptopurine; 8-AzaG, 8-azaguanine; FBS, fetal bovine serum; HGPRT, hypoxanthine-guanine phosphoribosyltransferase

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the down-regulation of certain oncogenes, especially c-myc in granulocytic and monocytic differentiation [1, 3], as well as the activation of others, such as c-fos and c-fms in monocytic differentiation [16, 18].

Involvement of c-myc expression in the precommitment of cell differentiation and the onset of G_0 arrest prior to phenotypic differentiation has been indicated [9, 22]. The role of c-myc in cellular differentiation appears to be complex and system-dependent. Enhanced c-myc expression has been suggested to play a role in the precommitment step of retinoic acid-induced differentiation of HL-60 cells [22]. However, enhanced c-myc expression blocks terminal differentiation in 3T3-L1, mouse erythroleukemia, and U937 cells [7, 13, 14]. Uptake of an antisense oligomer to c-myc mRNA inhibits HL-60 cell growth and is sufficient to induce differentiation [11, 21]. In addition, it has been suggested by Freytag [7] that a transient decrease in c-myc is necessary for terminal differentiation and that high c-myc expression precludes the cells from entering a predifferentiation state. Retinoic acid causes a transient decrease in c-myc expression in HL-60 cells, followed by an enhanced expression of the message [9].

In this report we examine the differential effects that e^6MP has on c-myc expression in both wild-type and HGPRT-deficient HL-60 cells.

Materials and methods

Cell culture. HGPRT-deficient HL-60 cells (provided by Dr. Linda F. Thompson, Scripps Clinic and Research Foundation), U937 cells (provided by Dr. Kathleen Clause, Georgetown University), and HL-60 wild-type cells (provided by Dr. Robert Gallo, NCI) were grown at 37° C in RPMI 1640 medium supplemented with 10% fetal bovine serum in a humidified atmosphere containing 10% CO_2. The HGPRT-deficient cells were derived from a clonal isolate of mutagenized HL-60 cells selected with 6-TG. Cells used for induction of differentiation studies were grown to the early stationary phase and then diluted to a concentration of 2×10^5 cells/ml.

Plasmids. The following plasmids were used as probes for Northern blot analysis. pSV2cMyc was used to probe for protooncogene c-myc expression and pSM3 was used to probe for c-fms expression. Both plasmids were obtained from the American Type Culture Collection (ATCC). Linearized plasmids or inserts were radiolabelled by the oligonucleotide primer method according to the manufacturer's instructions (Pharmacia, Piscataway, N.J.).

RNA isolation and Northern blot analysis. Total RNA was isolated by the guanidinium-isothiocyanate CsCl method [4, 15]. Prior to separation by electrophoresis, the RNA was denatured at 60° C for 5 min in a solution containing 50% formamide (v/v), 6% formaldehyde (v/v), and 20 m*M* morpholinepropanesulfonic acid (MOPS, pH 7.0). Identical amounts (20 µg) of total RNA were added to individual lanes of a 1.2% agarose-2.2 *M* formaldehyde-MOPS gel, after which the RNA was fractionated by electrophoresis [15]. Equivalency of RNA loading was then confirmed visually by staining with ethidium bromide. The RNA was transferred to Nytran membranes (Schleicher and Schuell, Keene, N.H.) in 10× SSPE buffer [20× SSPE buffer comprises 3.6 *M* NaCl, 0.2 *M* sodium phosphate (pH 7.7), and 20 m*M* ethylenediaminetetraaceticacid (EDTA)]. The membrane was baked for 2 h at 80° C, wetted with 5× SSPE, and prehybridized for 2 h at 42° C in 50% formamide, 5× Denhardt's, 5× SSPE, 0.1% sodium dodecyl sulfate (SDS), and 200 µg sonicated-denatured herring-testes DNA. The bound RNA was hybridized overnight against DNA labelled with P32 at 42° C in the above hybridization buffer.

Following hybridization, the blot was initially washed for 5 min at room temperature in 1× SSPE, 0.1% buffer; it was then washed twice again for 30 min at 60° C. The filter was wrapped in plastic wrap and exposed overnight to Kodak XAR-5 film (Eastman Kodak Co., Rochester, N.Y.) at –70° C with a Cronex Lightening intensifying screen.

Differential cell staining. Approximately 5×10^5 cells were collected on glass slides by cytocentrifugation for 10 min at 600 rpm in a Shandon Cytospin2. The cells were stained with Wright-Giemsa. For detection of monocytic nonspecific esterase activity, the treated cells were first reacted with α-naphthyl butyrate according to the manufacturer's instructions (Sigma Chemical Co., St. Louis, Mo.) and then cytocentrifuged onto glass slides as described above.

Results

Unlike the guanine analogs 6-TG and 8-AzaG, which induce granulocytic differentiation in HL-60 cells, e^6MP induced a distinctive end-cell morphology (Fig. 1 A). This morphology was characterized by an increase in the cytoplasmic/nuclear ratio as well as increased cytoplasmic vacuole formation. The morphology of e^6MP-treated cells was indicative of a monocytic-like differentiation. The morphologies of wild-type and HGPRT-deficient HL-60 cells were very similar. For purposes of comparison, we also treated the promonocytic U937 cell line with e^6MP and TPA (Fig. 1 A). The morphology of e^6MP-treated U937 cells was similar to that of e^6MP-treated HL-60 cells, further suggesting that e^6MP induces a monocytic-like differentiation of HL-60 cells.

To determine whether e^6MP-treated cells would enter a monocytic-like differentiation pathway, we tested for the induction of nonspecific esterase using α-naphthyl butyrate (Fig. 1 B). Cells treated for 4 days with e^6MP exhibited nonspecific esterase activity. This induction of nonspecific esterase activity was not observed with the granulocytic differentiation inducer 6-TG. However, as compared with TPA, e^6MP was a weak inducer of nonspecific esterase. Both e^6MP- and TPA-induced nonspecific esterase activity was inhibited by sodium fluoride (data not shown).

An additional marker of monocytic differentiation is the stabilization of c-fms mRNA, the message coding for the M-CSF receptor. TPA was evaluated as a positive control, and it clearly stabilized the c-fms message (Fig. 2 A, lane 1). However, when we examined the level of c-fms mRNA following e^6MP treatment, we found that the c-fms mRNA was not stabilized (Fig. 2 A, lane 4). We also tested whether e^6MP might interfere with TPA-mediated stabilization of c-fms mRNA by examining the level of the message after pretreatment with e^6MP followed by treatment with TPA for 24 h. Pretreatment with e^6MP for 6 h blocked TPA-induced stabilization of c-fms (Fig. 2 A, lane 2). The protein synthesis inhibitor cycloheximide also blocked TPA stabilization of c-fms mRNA (Fig. 2 A, lane 3).

Based on the effect of e^6MP on the stabilization of c-fms mRNA by TPA, it was also of interest to determine whether e^6MP could inhibit TPA-induced cellular adherence. As can be seen in Fig. 2 B, HL-60 cells treated with

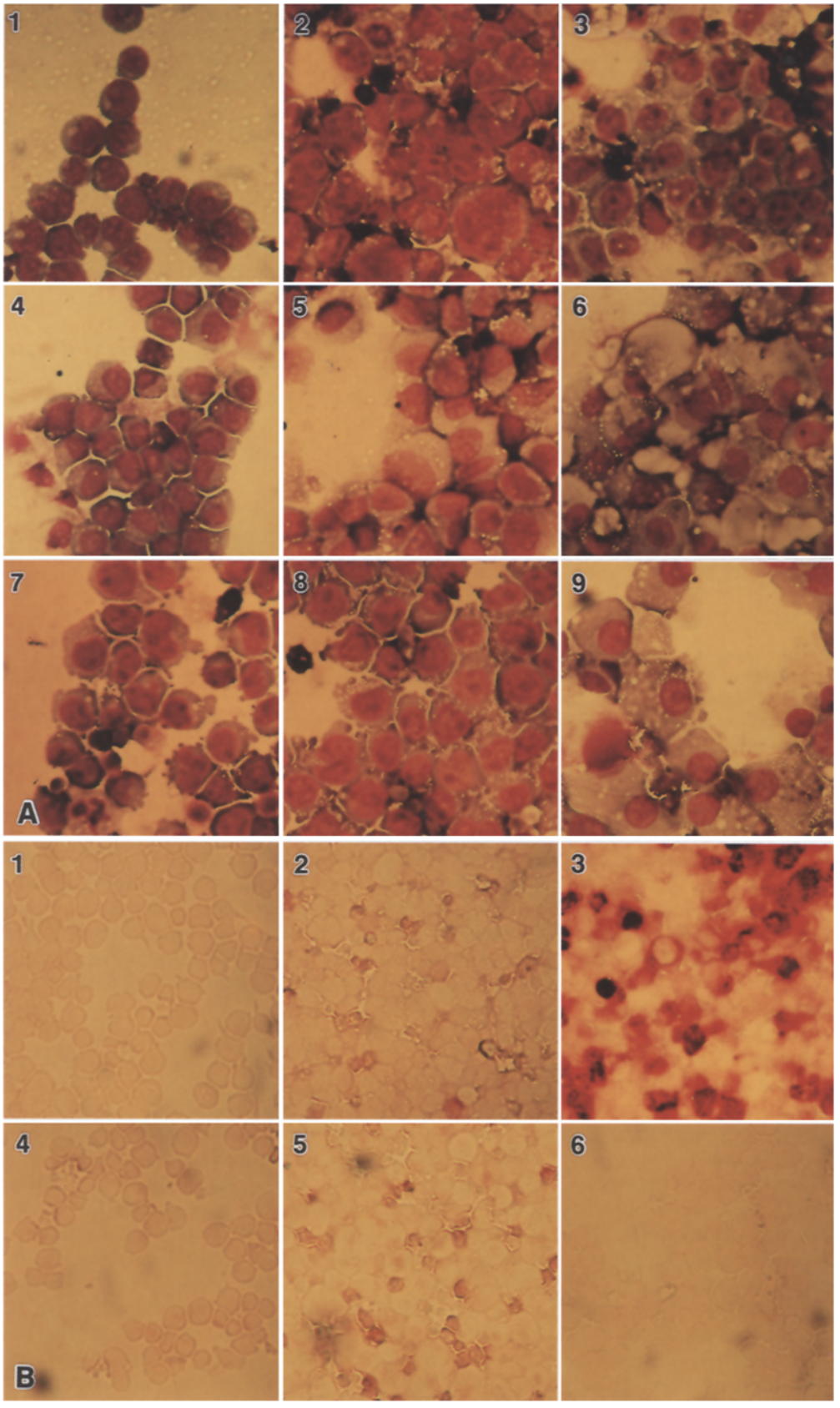

Fig. 1 A, B. A Photomicrographs of cells treated with e^6MP and TPA ($\times$ 350). *1*, Untreated wild-type HL-60 cells; *2*, e^6MP-treated wild-type HL-60 cells; *3*, TPA-treated wild-type HL-60 cells; *4*, untreated HGPRT-deficient HL-60 cells; *5*, e^6MP-treated HGPRT-deficient HL-60 cells; *6*, TPA-treated HGPRT-deficient HL-60 cells; *6*, untreated U937 cells; *8*, e^6MP-treated U937 cells; *9*, TPA-treated U937 cells. **B** Photomicrographs of e^6MP- and TPA-treated cells stained for non-specific esterase activity ($\times$ 220). *1*, Untreated HGPRT-deficient HL-60 cells; *2*, e^6MP-treated HGPRT-deficient HL-60 cells; *3*, TPA-treated HGPRT-deficient HL-60 cells; *4*, untreated wild-type HL-60 cells; *5*, e^6MP-treated wild-type HL-60 cells; *6*, 6-TG-treated HGPRT-deficient HL-60 cells. Cells treated with either 0.6 m*M* e^6MP, 1×10^{-7} *M* TPA, or 0.4 m*M* 6-TG were initially seeded at a cell density of 2×10^5 cells/ml and then incubated for a period of 4 days before being stained with Wright-Giemsa and evaluated for non-specific esterase activity

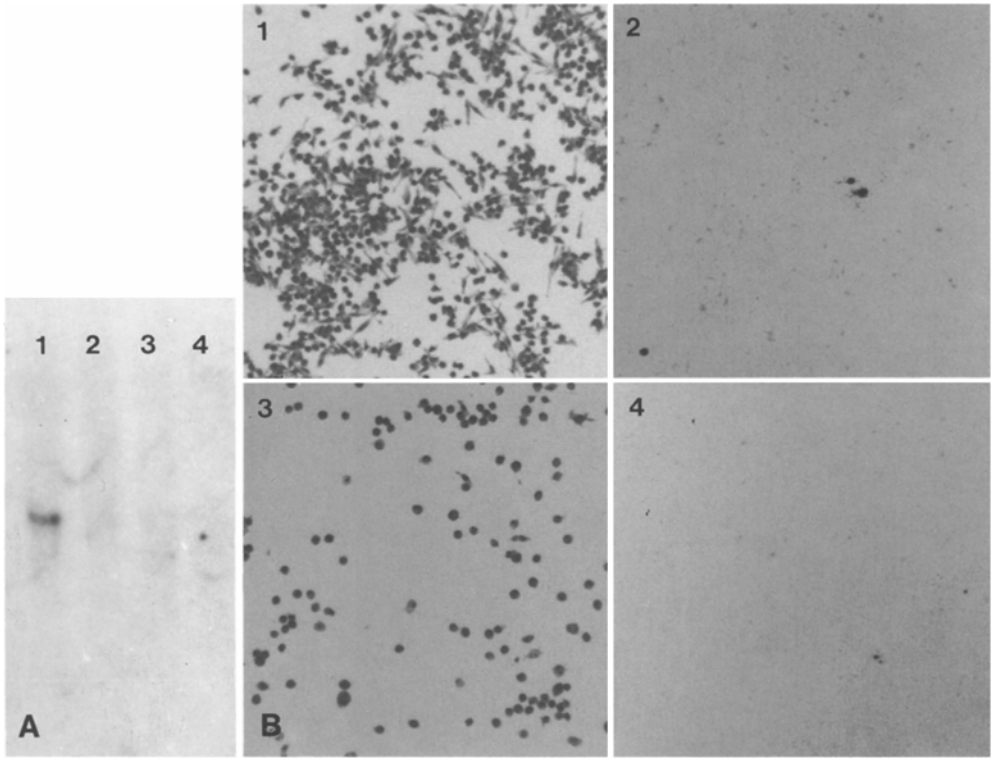

Fig. 2 A, B. A Northern analysis of c-fms mRNA levels in HGPRT-deficient HL-60 cells. Cells were treated for 24 h with 1×10^{-7} *M* TPA either alone (*lane 1*) or after a 6-h pretreatment with 0.6 m*M* e^6MP (*lane 2*) or 0.5 μg cycloheximide/ml (*lane 3*). Alternatively, the cells were treated for 24 h with 0.6 m*M* e^6MP alone (*lane 4*). **B** Photomicrographs of adherent cells following treatment with TPA (× 35). All cells were treated for 24 h with 1×10^{-7} *M* TPA either alone (*1*) or after a 6-h pretreatment with 0.6 m*M* e^6MP (*2*), 0.15 m*M* e^6MP (*3*), or 0.5 μg cycloheximide/ml (*4*). Cells were rinsed with PBS, fixed with formaldehyde, and stained with Wright-Giemsa

TPA for 24 h exhibited a significant degree of adherence (panel 1), whereas cells pretreated with 0.6 m*M* e^6MP showed no such response (panel 2). Partial inhibition of TPA-induced adherence was observed using 0.15 m*M* e^6MP (Fig. 2B, Panel 3), whereas cycloheximide (0.5 μg/ml) elicited a response more comparable with that obtained at the higher concentration of e^6MP (Fig. 2B, Panel 4).

Differentiation of HL-60 cells is generally characterized by c-myc mRNA down-regulation. Therefore, we examined the effect of e^6MP on c-myc mRNA regulation as compared with that of other nucleobase analogs known to be inducers of granulocytic differentiation. Both wild-type and HGPRT-deficient HL-60 cells were treated for 2 h with various nucleobase analogs. The level of c-myc mRNA was then examined by Northern blot analysis (Fig. 3). The nucleobase analogs 6-TG, MP, and e^6MP all decreased the level of c-myc mRNA in wild-type HL-60 cells. However, only 6-TG and MP had an effect on c-myc mRNA levels in HGPRT-deficient HL-60 cells.

The level of c-myc mRNA was then examined during the course of e^6MP-induced differentiation to determine whether further changes in c-myc mRNA expression would occur (Fig. 4). A large reduction in the level of c-myc mRNA was observed 2 h after e^6MP treatment in wild-type HL-60 cells. On day 4 of e^6MP treatment, by which time morphological differentiation of the cell had occurred, the level of c-myc mRNA had returned to that of the untreated control. HGPRT-deficient HL-60 cells showed no reduction in c-myc mRNA at 2 h or 2 days following e^6MP treatment.

Because the level of c-myc expression in HGPRT-deficient HL-60 cells appeared to be resistant to e^6MP treatment, the level of c-myc mRNA was evaluated at various times over a 6-day period (Fig. 5). No reduction in c-myc mRNA was observed in HGPRT-deficient HL-60 cells following e^6MP treatment at any time during the observation period.

Discussion

We have been studying the induction of differentiation in wild-type HL-60 cells and in an HGPRT-deficient subclone. Agents such as 6-TG and 8-AzaG are known granulocytic inducers in HGPRT-deficient HL-60 cells. These compounds, which are highly cytotoxic to wild-type cells, induce differentiation in HGPRT-deficient HL-60 cells without metabolic activation [10, 12]. The mechanism by which this occurs is of interest in terms of its chemotherapeutic potential. In the course of studying the compound e^6MP, we previously noted that this purine analog inhibits the growth of wild-type and HGPRT-deficient HL-60 cells as well as that of the promonocytic U937 cell line [8]. e^6MP inhibits cell growth without the cytotoxic side effects that are usually noted for nucleobase analog inhibition of cellular growth. As with 6-TG inhibition of cell growth in HGPRT-deficient HL-60 cells, e^6MP ap-

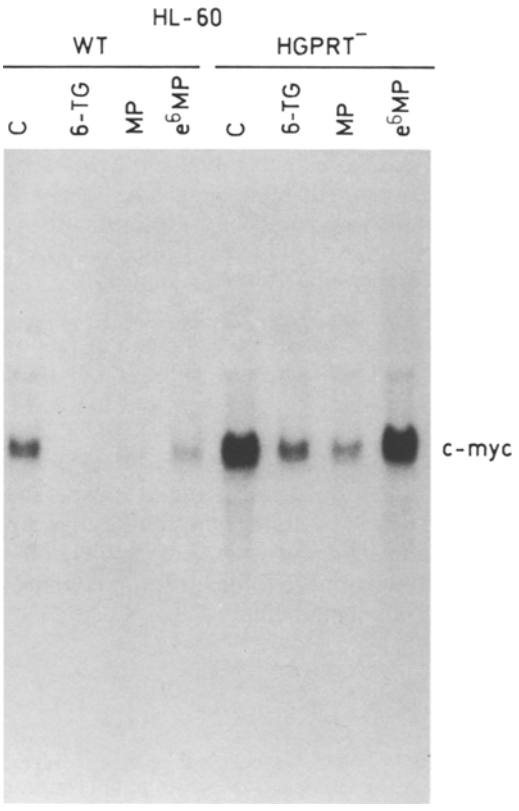

Fig. 3. Northern analysis of c-myc mRNA levels in wild-type and HGPRT-deficient HL-60 cells following treatment with purine analogs. Cells were left untreated (*lane C*) or were treated for 2 h with 0.4 m*M* 6-TG, 0.4 m*M* MP, or 0.6 m*M* e⁶MP

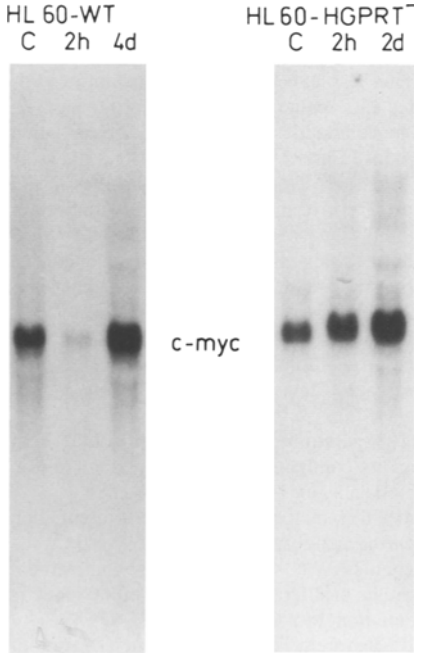

Fig. 4. Northern analysis of c-myc mRNA levels in wild-type and HGPRT-deficient HL-60 cells at various times following treatment with 0.6 m*M* e⁶MP. Cells were left untreated (*lane C*) or were treated for 2 h (*2 h*), 2 days (*2 d*), or 4 days (*4 d*)

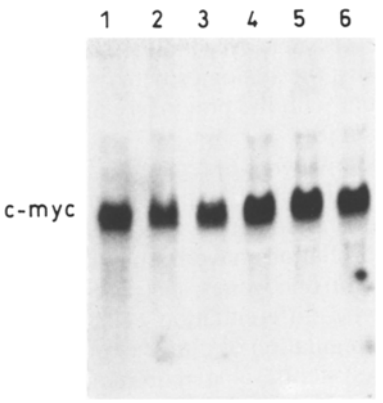

Fig. 5. Northern analysis of c-myc mRNA levels in HGPRT-deficient HL-60 cells at various times following treatment with 0.6 m*M* e⁶MP. *Lane 1*, Zero time control; *lane 2*, 20 min; *lane 3*, 1 day; *lane 4*, 2 days; *lane 5*, 3 days; *lane 6*, 6 days

peared to inhibit cell growth without metabolic activation. The morphological changes that occurred in the presence of e⁶MP suggested that the growth inhibition was a result of cellular differentiation.

The morphological changes observed after e⁶MP treatment were similar in both wild-type and HGPRT-deficient HL-60 cell lines, which showed an increase in cytoplasmic/nuclear ratio as well as in cytoplasmic vacuole formation (Fig. 1A). Because of the similarity of morphological changes seen in HL-60 cells treated with e⁶MP and those treated with TPA, we tested whether e⁶MP would induce HL-60 cells to differentiate down the monocytic pathway. e⁶MP-treated HL-60 cells (both wild-type and deficient) were positive for non-specific esterase activity (Fig. 1B). Induction of nonspecific esterase appeared to be unique to e⁶MP. The granulocytic inducer 6-TG did not induce this activity in HGPRT-deficient HL-60 cells. However, e⁶MP was a weak inducer of nonspecific esterase as compared with the potent monocytic/macrophage inducer TPA.

Induction of monocytic/macrophage differentiation by phorbol esters results in the rapid induction of c-fos and the stabilization of the c-fms oncogene. We detected no induction of c-fos expression by e⁶MP in either of the HL-60 cell lines (data not shown). Another early marker of monocytic differentiation is an increased level of c-fms mRNA, which codes for the M-CSF receptor. This increase in c-fms mRNA has been shown to be due to the induction of a labile protein that is involved in the stabilization of the former [20]. Protein synthesis inhibitors such as cycloheximide block TPA-induced stabilization of c-fms mRNA [20].

We examined e⁶MP-treated wild-type and HGPRT-deficient HL-60 cells for c-fms stabilization as an indicator for monocytic differentiation. We could not discern c-fms mRNA in e⁶MP-treated cells (Fig. 2A). This result suggests the possibility that e⁶MP-induced monocytic differentiation of HL-60 cells is abortive. Likewise, the ability of e⁶MP pretreatment to block TPA-induced adherence and c-fms mRNA stabilization in a manner comparable with that exhibited by cycloheximide further supports the possi-

bility of an abortive response (Fig. 2). Although we have yet to establish the mode of acton by which e^6MP elicits these effects on HL-60 cells, the analogies with cycloheximide are at least consistent with the previously proposed tRNA-mediated mechanism [8].

Many oncogenes are known growth regulators, and the induction of differentiation alters their expression [2]. The exact role of oncogene expression in the differentiation pathway is not known. Correlations have been made with the down-regulation of certain oncogenes, such as c-myc in granulocytic and monocytic differentiation [1, 3]. Evidence suggests that down-regulation of c-myc expression, albeit transient in some systems, is a requirement for granulocytic differentiation [7]. In addition, down-regulation of c-myc by antisense oligomers is sufficient to enable differentiation to proceed in some cell systems [11, 21]. Compounds such as dimethylsulfoxide (DMSO), which also induce granulocytic differentiation and cause c-myc down-regulation, do not act synergistically with antisense c-myc oligomers [21]. These results suggest that multiple pathways of induction exist and that these pathways differ from simple down-regulation of c-myc mRNA.

The human promyelocytic leukemia HL-60 cell line is pluripotent in vitro. When this cell line is treated with compounds such as DMSO, 6-TG, and 8-AzaG, the cells are induced to differentiate down the granulocytic pathway, whereas after treatment with compounds such as TPA and vitamin D3, they are induced toward monocytic differentiation [2]. The different behavior of various cell lines in response to inducers of differentiation may lead to insight into the mechanisms by which these compounds act.

Although both wild-type and HGPRT-deficient HL-60 cells treated with e^6MP had similar morphological characteristics and showed moderate nonspecific esterase activity, they exhibited distinct characteristics with regard to c-myc regulation during e^6MP-induced differentiation. c-Myc mRNA was down-regulated transiently in wild-type HL-60 cells (Fig. 4), whereas e^6MP had no effect on c-myc mRNA levels in the HGPRT-deficient cell line (Figs. 4, 5). It seems unlikely that this differential effect on c-myc mRNA expression would be due to differences in salvage enzymes between the two cell lines. HPLC analysis revealed no indication of e^6MP-derived nucleotides in wild-type HL-60 cells, and neither hypoxanthine nor adenine blocked e^6MP effects on the cells [8]. Also, one would not expect a transient effect on c-myc mRNA expression in wild-type HL-60 cells to be due to a salvage-mediated mechanism. Most importantly, however, induction of differentiation by e^6MP occurred in HGPRT-deficient HL-60 cells despite any observable down-regulation of c-myc mRNA.

Subclones of HL-60 cells that have been shown to be resistant to phorbol-induced differentiation differed in c-myc expression [6]. The resistant cell line exhibited a transient decrease in c-myc expression, whereas the parental cell line maintained a reduced level of c-myc mRNA after phorbol treatment. Neither wild-type nor HGPRT-deficient HL-60 cells were resistant to growth inhibition and differentiation by e^6MP, although c-myc mRNA was only transiently down-regulated in wild-type HL-60 cells and was not affected in HGPRT-deficient HL-60 cells. Thus, it would appear that e^6MP-mediated effects on HL-60 cells do not require c-myc down-regulation to be maintained during the course of differentiation.

Induction of differentiation as a mode of chemotherapeutic intervention is appealing. The ability of agents such as 6-TG and 8-AzaG to induce granulocytic differentiation in HGPRT-deficient HL-60 cells enables their use as model purines for studying the molecular mechanisms involved. However, the use of 6-TG in chemotherapy results in a high degree of cytotoxicity to wild-type cells. Unlike these guanine analogs, the compound e^6MP inhibits cell growth and initiates an abortive mechanism of monocytic differentiation in wild-type and HGPRT-deficient HL-60 cells without producing the associated cytotoxic effects. These data suggest that some nucleobase analogs have the potential to induce cellular differentiation and growth inhibition in transformed cells without causing the high degree of cytotoxicity associated with analogs that are activated through salvage pathways. As a result, compounds such as e^6MP may prove to be useful in conjunction with other forms of chemotherapy.

References

1. Bentley DL, Groudine M (1986) A block to elongation is largely responsible for decreased transcription of c-myc in differentiated HL-60 cells. Nature 321: 702–706
2. Birnie GD (1988) The HL-60 cell line: a model system for studying human myeloid cell differentiation. Br J Cancer 58: 41–45
3. Cayre Y, Marie-Cecile R, Darzynkiewjcz Z, Dorner MH (1987) Model for intermediate steps in monocytic differentiation: c-myc, c-fms and ferritin as markers. Proc Natl Acad Sci USA 84: 7619–7623
4. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18: 5294–5299
5. Collins SJ, Bodner A, Ting R, Gallo RC (1980) Induction of morphological and functional differentiation of human promyelocytic leukemia cells (HL-60) by compounds which induce differentiation of murine leukemia cells. Int J Cancer 25: 213–218
6. Ely CM, Leftwich JA, Chenevix-Trench G, Hall RE, Westin EH (1987) Altered regulation of c-myc in an HL-60 differentiation resistant subclone, HL-60-1E3. Cancer Res 47: 4595–4600
7. Freytag SO (1988) Enforced expression of the c-myc oncogene inhibits cell differentiation by precluding entry into a distinct predifferentiation state in G_0/G_1. Mol Cell Biol 8: 1614–1624
8. Gibboney DS, French BT, Patrick DE, Trewyn RW (1989) 6-Ethylmercaptopurine-mediated growth inhibition of HL-60 cells in vitro irrespective of purine salvage. Cancer Chemother Pharmacol 25: 189–194
9. Guernsey DL, Yen A (1988) Retinoic-acid-induced modulation of c-myc not dependent on its continued presence: possible role in pre-commitment for HL-60 cells. Int J Cancer 42: 576–581
10. Gusella J, Housman D (1976) Induction of erythroid differentiation in vitro by purines and purine analogs. Cell 8: 263–269
11. Holt JT, Redner RL, Nienhuis AW (1988) An oligomer complementary to c-myc mRNA inhibits proliferation of HL-60 promyelocytic cells and induces differentiation. Mol Cell Biol 8: 963–973
12. Ishiguro K, Schwartz EL, Sartorelli AC (1984) Characterization of the metabolic forms of 6-thioguanine responsible for cytotoxicity and induction of differentiation of HL-60 acute promyelocytic leukemia cells. J Cell Physiol 121: 383–390
13. Kaneko-Ishino T, Kume TU, Sasaki H, Obinata M, Oishi M (1988) Effect of c-myc gene expression on early inducible reactions required for erythroid differentiation in vitro. Mol Cell Biol 8: 5545–5548

14. Larsson L, Ivhed I, Gidlund M, Pettersson U, Vennstrom B, Nilsson K (1988) Phorbol ester-induced terminal differentiation is inhibited in human U-937 monoblastic cells expressing a v-myc oncogene. Proc Natl Acad Sci USA 85: 2638–2642
15. Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor
16. Mitchell RL, Zokas L, Schreiber RD, Verma IM (1985) Rapid induction of the expression of proto-oncogene fos during human monocytic differentiation. Cell 40: 209–217
17. Papac RJ, Brown AE, Schwartz EL, Sartorelli AC (1980) Differentiation of human promyelocytic leukemia cells in vitro by 6-thioguanine. Cancer Lett 10: 33–38
18. Sariban E, Mitchell T, Kufe D (1985) Expression of the c-fms proto-oncogene during human monocytic differentiation. Nature 316: 64–66
19. Schwartz EL, Blair OW, Sartorelli AC (1984) Cell cycle events associated with the termination of proliferation by cytotoxic and differentiation-inducing actions of 6-thioguanine on HL-60 cells. Cancer Res 44: 3907–3910
20. Weber B, Horiguchi J, Luebbers R, Sherman M, Kufe D (1989) Posttranscriptional stabilization of c-fms mRNA by a labile protein during human monocytic differentiation. Mol Cell Biol 9: 769–775
21. Wickstrom EL, Bacon TA, Gonzalez A, Freeman DL, Lyman GH, Wickstrom E (1988) Human promyelocytic HL-60 proliferation and c-myc protein expression are inhibited by an antisense pentadecadeoxynucleotide targeted against c-myc. Proc Natl Acad Sci USA 85: 1028–1032
22. Yen A, Guernsey DL (1986) Increased c-myc RNA levels associated with the precommitment state during HL-60 myeloid differentiation. Cancer Res 46: 4156–4161