Effects of Difluoromethylornithine and Dicyclohexlammonium Sulfate on the Transformed State of AKR-MCA Cells

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ABSTRACT

The effect of two inhibitors of polyamine biosynthesis, difluoromethylornithine and dicyclohexlammonium sulfate, on the transformed fibroblastic cell line AKR-MCA and its parental counterpart AKR-2B was investigated. Treatment of monolayer AKR-MCA cells with either agent results in morphological changes akin to AKR-2B; the cells appear to be flattened with a polygonal shape. The ability of the inhibitors to alter the phenotype is lost when the cells are cocultured with polyamines. More specifically, putrescine and spermidine abrogate the effects of difluoromethylornithine while only spermidine is effective in reverting the dicyclohexlammonium sulfate induced phenomenon. Further evidence that these enzyme inhibitors are reversing the transformed state of AKR-MCA is obtained from soft agarose experiments. AKR-MCA cells will generate colonies only in the absence of either difluoromethylornithine or dicyclohexlammonium sulfate.

Polyamine levels were determined in parental AKR-2B and AKR-MCA cells. The levels of putrescine and spermine were similar in both cell types. In contrast, significantly more ($P \leq 0.01$) spermidine was expressed by the malignant line [7.3 ± 0.8 (SD) nmol/10^6 cells] when compared with the untransformed AKR-2B (5.4 ± 0.8 nmol/10^6 cells). Intracellular putrescine and spermidine were sensitive to difluoromethylornithine, dicyclohexlammonium sulfate, and dimethylformamide, a planar, polar solvent which has been reported to "normalize" the transformed phenotype. AKR-MCA treated with difluoromethylornithine or dimethylformamide manifested time dependent reductions in both polyamines which preceded morphological changes. Dicyclohexlammonium sulfate similarly caused a 70% reduction in spermidine, but in contrast to the other agents there was a marked accumulation of putrescine. These data concur with the established molecular actions of the two enzyme inhibitors as blockers of ornithine decarboxylase (difieuromethylornithine) and spermidine synthase (dicyclohexlammonium sulfate). The normalizing capacity of dimethylformamide was not compromised by cotreatment with putrescine or spermidine.

Both difluoromethylornithine and dicyclohexlammonium sulfate inhibited the growth of monolayer AKR-2B and AKR-MCA. In view of the well documented cytostatic effects of polyamine inhibitors, it is suggested that a decrease in growth by these agents triggers a more normal phenotype in AKR-MCA cells.

INTRODUCTION

Some cancers may be composed of primitive stem cells characterized by their inability to mature into a terminally differentiated population (1). The deficiency in the maturation (differentiation) process may not, however, be irreversible inasmuch as exposure of transformed stem cell lines including human leukemia (2), neuroblastoma (3), and teratocarcinoma (4) to a differentiation signal. For example, the induction of Friend cell erythroiddifferentiation by dimethyl sulfoxide and hexamethylen bisacetamide is thought to be triggered by an early stimulation of ODC, the rate limiting enzyme in polyamine biosynthesis (5). In a similar vein, Bethel and Pegg (6) have found that conversion of 3T3 L1 fibroblasts into adipocytes can be blocked with DFMO, an irreversible inhibitor of ODC. Kufe et al. (7) have established the requirement of polyamines in HL60 promyelocyte maturation. In this case, depletion of intracellular spermidine with DFMO abrogated the normalizing effects of leukocyte conditioned media.

In some cases, however, reduced levels of polyamines will result in cell maturation. For example, treatment of melanocytes with DFMO is associated with a 10-fold increase in melanin production and reduced growth rate (8).

To further investigate the role of polyamines in cell differentiation (normalization) we have investigated the effects of 2 polyamine synthesis inhibitors, namely DFMO (9) and DCHS (10, 11), on the growth characteristics and intracellular polyamine profiles of the transformed fibroblastic cell line AKR-MCA and compared it with its parental counterpart AKR-2B.

The AKR-MCA and AKR-2B cell lines provide an excellent system for defining events associated with cell transformation and consequently have been extensively characterized. In monolayer culture, AKR-2B cells are polygonal whereas AKR-MCA cells assume a fibrillar morphology (12). Accordingly, quantitation of in vitro cell saturation densities reveals higher values for the transformed cell line (12). Further differences between the 2 lines are illustrated by the ability of AKR-MCA but not AKR-2B to form colonies in semisolid media, an indication of tumorigenic potential (13). Indeed, inoculation of nude mice with a suspension of AKR-MCA cells will give rise to solid tumors. On the other hand, similar studies with the parental AKR-2B cells are negative. Previous results have suggested, however, that the transformed phenotype of AKR-MCA is reversible inasmuch as exposure of these cells to the polar solvent DMF or retinoic acid leads (14) to (a) in vitro morphological changes resembling AKR-2B, (b) a loss of colony forming potential in semisolid media, (c) restoration of growth control, and (d) alterations of membrane antigen profiles to AKR-2B patterns (12, 15).

In addition to investigating the biological and molecular effects of DFMO and DCHS on the transformed and parental fibroblasts we have attempted to define a role for polyamines in the normalizing actions of the polar solvent DMF.

MATERIALS AND METHODS

Putrescine dihydrochloride, spermidine phosphate, spermine diphosphate, and dicyclohexylammonium sulfate were purchased from Sigma Chemical Co., St. Louis, MO. [14C]Putrescine, spermidine, and spermine were obtained from Amersham, Arlington Heights, IL. Ethyl acetate, cyclohexane, benzene, triethanolamine, isopropyl alcohol (high...
with dansyl chloride. The reaction was allowed to proceed overnight and centrifuged at 3000 x g to remove particulate matter and derivatized compounds were harvested with trypsin 48 h later unless stated otherwise and analyzed for polyamines. Further details are given in “Results.”

Cell Lines. The murine embryonic fibroblastic cell line AKR-2B and its transformed counterpart AKR-MCA used in these studies were derived from passages 20 and 30. The propagation of these lines in vitro has been described elsewhere (12). Briefly, cells were maintained at 37°C under a 5% CO₂ atmosphere in McCoy’s 5A medium supplemented with 10% fetal bovine serum (Gibco, NY). The subculturing of cells was carried out with 0.05% trypsin (Gibco) in the presence of 3 mM EDTA.

Morphology Studies and Cell Saturation Densities. Cells were seeded at 5.0–7.5 x 10⁴/25-cm² flask (Corning). After a 24-h period to allow cell attachment, DCHS, DFMO, or DMF were added to achieve final concentrations of 1 mM or 1% (v/v) in the case of the polar solvent. At 95–100% confluency (approximately 5 and 9 days of culture with DCHS and DFMO, respectively; 5 days of growth for untreated AKR-MCA or polyamine rescued cells) phase contrast micrographs were taken. The time required to reach confluency was similar for DCHS treated AKR-MCA and untreated cells since this agent caused a reduction in saturation density for the transformed fibroblasts. To assess the effects on saturation density, cultures were harvested at confluency, and viable cells were enumerated with the aid of a hemocytometer. Cell viability was defined as those cells which excluded trypan blue dye and was routinely in excess of 90%.

Growth of Transformed Cells in Semisolid Medium. These studies were carried out as described previously (16) but with minor modifications. AKR-MCA cells (5 x 10⁴ cells/well) were suspended in the 0.4% agar overlay in the presence or absence (control) of the various agents to be tested. Cultures were incubated at 37°C in a humidified atmosphere for 3 weeks. After this time colonies were stained overnight with 1 mg/ml tetrazolium violet (Sigma) and scored the following day on a semiquantitative scale.

Effects of Pharmacological Agents and Exogenous Polyamines on Intracellular Polyamine Profiles. For these experiments, cells were propagated in 75-cm² flasks (Corning). At an approximate density of 1.5 x 10⁶ cells/flask exogenous agents were added to the cultures. Cells were harvested with trypsin 48 h later unless stated otherwise and analyzed for polyamines. Further details are given in “Results.”

Quantitation of Polyamines. Polyamine analysis was essentially that of Dion and Herbst (17). Briefly, 5–15 x 10⁶ cells were resuspended in 2% perchloric acid and sonicated for 5 s. The acid extract was then extracted with benzene and then subjected to thin layer chromatography (Kodak 13179 plates) using an ethyl acetate/cyclohexane (2:3) mobile phase. The plates were sprayed with triethanolamine/isopropanol (1:4) and dried under a N₂ atmosphere. Fluorescent spots located with a long wave UV lamp and coincident with authentic standards were extracted with ethyl acetate and quantified using an Aminco SPF 500 spectrophotometer set at an excitation wavelength of 365 nm and a transmission wavelength of 512 nm. Recoveries were determined with ¹⁴C tracers. Preliminary studies established the linearity of the assay up to 15 x 10⁶ cells.

Data Analysis. Differences in mean values were tested for statistical significance by Student’s t test.

RESULTS

Effect of DFMO and DCHS on Morphology and Saturation Densities. Exposure of AKR-MCA cells to DFMO, DCHS (1 mM), or DMF 1% (12) resulted in a flattened polygonal cell shape resembling AKR-2B (Fig. 1). These changes manifested themselves between 24 and 48 h, and in the presence of inhibitor were maintained throughout the life span of the culture. The morphological appearance was unchanged by the level of confluency and was independent of saturation density. In the presence of DFMO, AKR-MCA cells required a total of 10 days to reach confluency whereas similar cultures treated with DCHS needed 6 days. In contrast, untreated AKR-MCA cells achieved 100% confluency after 5 days in culture. It is worthy of comment that the marginal time difference (24 h) between untreated AKR-MCA and DCHS treated AKR-MCA cells in reaching confluency does not reflect an unaltered growth rate. The saturation density data in Fig. 2 clearly show a 60% reduction in this parameter brought about by this agent. Thus AKR-MCA cells treated with DCHS have to undergo fewer rounds of replication than their untreated counterparts to achieve confluency.

To accommodate growth inhibitory effects of DFMO,
2B fibroblasts did not manifest a reduction in saturation densities when treated with DFMO or DCHS in logarithmic or stationary phases of growth. Similarly, data not shown indicate that the effects of DMF on AKR-2B fibroblasts are marginal.

The effects of both DFMO and DCHS on morphology can be reversed by concomitant treatment with exogenous polyamine (Fig. 1). AKR-MCA cells were rescued from the effects of DFMO by simultaneous exposure to either 0.1 mM putrescine (Fig. 1e) or 10 μM spermidine (Fig. 1g). On the other hand spermidine (Fig. 1h) but not putrescine (Fig. 1f) will abrogate the morphological effect of DCHS. These findings are in contrast to those with DMF treated AKR-MCA where putrescine and spermidine are unable to reverse the effect of the polar solvent (data not shown).

Growth of AKR-MCA in Semisolid Media: Effects of DFMO, DCHS, and DMF. The DFMO and DCHS induced morphological changes in monolayer AKR-MCA suggested that these agents might in fact be reversing the transformed state. In an attempt to give further substance to this hypothesis AKR-MCA cells were tested for colony formation in semisolid media. Anchorage independent growth is one of the best in vitro indicators of tumorigenic potential (18, 19). In the absence of any exogenous agent, AKR-MCA cells formed numerous colonies (Table 1). However, if the cultures were coincubated with either DFMO, DCHS (1 mM), or DMF (1%) colony formation was totally abolished. The inability of AKR-MCA cells to generate colonies in the presence of DFMO could be restored by simultaneous exposure to spermidine (0.1 mM). The observation that spermidine (1 or 10 μM) did not rescue AKR-MCA cells from the inhibiting actions of DCHS is not surprising. High levels of catabolizing diamine oxidase present in fetal bovine serum (20) and the long period of incubation (3 weeks) probably generate acutely cytotoxic metabolites of spermidine (21).

Polyamine Profiles in AKR-MCA. DFMO and DCHS have been characterized as inhibitors of the rate limiting enzyme ODC and spermidine synthase, respectively. Since experiments with AKR-MCA growing in monolayer and semisolid media indicated that DFMO and DCHS might be producing a more normal phenotype similar to that of AKR-2B, it was of interest to determine whether decreases in polyamine levels might be involved. As a first step in this direction the levels of polyamine were compared in 70% confluent AKR-2B and AKR-MCA. It is apparent from Fig. 4 that the levels of putrescine and spermidine expressed in AKR-2B and transformed fibroblasts proliferating at identical rates are very similar. However, this effect is not the case for spermidine which is consistently elevated in AKR-MCA [7.3 ± 0.8 (SD) nmol/10⁶ cells] compared with (5.4

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKR-MCA control</td>
<td>+ + *</td>
</tr>
<tr>
<td>AKR-MCA DFMO (1 mM)</td>
<td>-</td>
</tr>
<tr>
<td>AKR-MCA DMF (1%)</td>
<td>-</td>
</tr>
<tr>
<td>AKR-MCA DCHS (1 mM)</td>
<td>-</td>
</tr>
<tr>
<td>AKR-MCA DFMO + putrescine (0.1 mM)</td>
<td>+ + *</td>
</tr>
<tr>
<td>AKR-MCA DFMO + spermidine (1 and 10 μM)</td>
<td>-</td>
</tr>
<tr>
<td>AKR-MCA DMF + putrescine (0.1 mM)</td>
<td>-</td>
</tr>
<tr>
<td>AKR-MCA DMF + spermidine (10 μM)</td>
<td>-</td>
</tr>
<tr>
<td>AKR-MCA DCHS + putrescine (0.1 mM)</td>
<td>-</td>
</tr>
<tr>
<td>AKR-MCA DCHS + spermidine (1 and 10 μM)</td>
<td>-</td>
</tr>
</tbody>
</table>

++, 400–600 colonies/well; –, colonies absent.

Figure 2. Effect of DFMO, DCHS, and DMF on saturation densities of AKR-MCA cells. Cells were plated (5 x 10⁴/25-cm² flask) in McCoy’s 5A media in the absence of any agent. The following day, DFMO, DCHS, or DMF was added to give final concentrations of 1 mM or 1% (v/v) for the polar solvent. At confluence, i.e., after 9 days of culture with DFMO or 5 days with DCHS, the cells were harvested with trypsin and enumerated. Control flasks with untreated AKR-MCA reached confluency 5 days after plating. The experiment was repeated twice. Data, mean ± SD (bars).

Figure 3. Effect of DFMO and DCHS on proliferation rates of AKR-MCA and AKR-2B cells. AKR-MCA (A) or AKR-2B (B) cells were seeded in duplicate at 3x the normal seeding density of untreated controls and then exposed to inhibitors. Again, the morphological effects accrued just after 24 h and were maintained throughout the culture life span.

These observations are in accordance with the cell saturation density data (Fig. 2). It is evident that there is a 60–75% reduction in saturation densities of transformed cells treated with 1 mM DFMO, DCHS, or 1% DMF. AKR-MCA cells exposed to DFMO reached saturation density after a treatment period of 9 days. On the other hand, transformed fibroblasts treated with DCHS attained saturation density after a 5-day exposure while untreated cells required a similar period. The reason for this apparent paradox as explained previously reflects the fact that AKR-MCA cells exposed to DFMO grow at a slower rate when compared to those treated with DCHS (Fig. 3). The marginal growth retarding effect of DCHS is, however, offset by its effect on saturation density.

Noteworthy is the finding that transformed fibroblasts grown to confluence and then treated with DFMO also underwent similar morphological changes after 24 h.

In contrast to that of their transformed counterparts, AKR-
transformed fibroblasts were exposed to no treatment (control), DMF 1%, or DMF in combination with 0.1 mM putrescine or 10 μM spermidine. After 48 h, the cells were harvested and intracellular polyamines were analyzed as described in the legend to Fig. 4. The experiments were performed twice. Data, mean percentage of values; bars, range of the 2 separate experiments.

Table 2 Effect of Various Agents on Polyamine Depletion: a Time Course Study

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Agent</th>
<th>Putrescine (%)</th>
<th>Spermidine (%)</th>
<th>Spermine (%)</th>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>DFMOM</td>
<td>&lt;10^-6</td>
<td>85 ± 8</td>
<td>83 ± 13</td>
</tr>
<tr>
<td></td>
<td>DMF</td>
<td>50 ± 4</td>
<td>67 ± 11</td>
<td>85 ± 12</td>
</tr>
<tr>
<td></td>
<td>DCHS</td>
<td>120 ± 20</td>
<td>63 ± 15</td>
<td>100 ± 4</td>
</tr>
<tr>
<td>12</td>
<td>DFMOM</td>
<td>&lt;10^-6</td>
<td>60 ± 7</td>
<td>80 ± 4</td>
</tr>
<tr>
<td></td>
<td>DMF</td>
<td>12 ± 13</td>
<td>40 ± 6</td>
<td>82 ± 11</td>
</tr>
<tr>
<td></td>
<td>DCHS</td>
<td>280 ± 120</td>
<td>38 ± 10</td>
<td>130 ± 14</td>
</tr>
<tr>
<td>24</td>
<td>DFMOM</td>
<td>&lt;10^-6</td>
<td>30 ± 12</td>
<td>82 ± 10</td>
</tr>
<tr>
<td></td>
<td>DMF</td>
<td>&lt;10^-6</td>
<td>35 ± 18</td>
<td>80 ± 15</td>
</tr>
<tr>
<td></td>
<td>DCHS</td>
<td>370 ± 130</td>
<td>15 ± 3</td>
<td>136 ± 17</td>
</tr>
</tbody>
</table>

* P < 0.01.
* P < 0.05.

± 0.8 nmol/10^6 cells; P = 0.01) of that of AKR-2B.

Further studies were undertaken to determine the effects of DMF, DFMO, and DCHS on fibroblast polyamines. Exposure of AKR-MCA cells in the log phase of growth to either DMF or DFMO gave similar trends. There is a time dependent reduction of both putrescine and spermidine after exposure to the enzyme inhibitor or polar solvent (Table 2). The kinetics of the spermidine decrease was similar requiring a minimum period of 6 h and reaching a low of 30% by 24 h. However, the DFMO induced disappearance of putrescine was rapid, occurring within 6 h. In contrast while DMF was able to reduce AKR-MCA putrescine below assay limits (0.112 nmol/10^6 cells or 10% of control) the rate of decline was slower, requiring a 24-h exposure. Longer exposure to these inhibitors (48 h) did not result in further decreases in intracellular polyamines.

The effect of DCHS on fibroblast polyamines was as predicted based on its published antiaspermidine synthase activity. Accordingly, AKR-MCA cells treated with DCHS for 24 h manifested an 85% reduction in intracellular spermidine with a marked accumulation of the putative substrate, putrescine (Table 2).

Since exogenous spermidine was capable of abrogating the effects of DFMO and DCHS on AKR-MCA morphology, it was unclear why DMF treated cells were insensitive to the addition of this polyamine. One possible explanation could be that exogenous spermidine added to DMF cultures does not restore intracellular levels of the depleted polyamines. The data in Fig. 5 indicate that this may very well be the case. Addition of spermidine to DMF treated AKR-MCA cells was without effect on the depleted level of the intracellular polyamines. Likewise, exogenous putrescine was unable to return spermidine to control values. On the other hand, spermine completely restored its own intracellular profile in parallel cultures exposed to DFMO.

Effect of Polyamine Inhibitors on Growth of AKR-2B and AKR-MCA Cells. Both DFMO and DCHS slowed the growth rates of AKR-2B and AKR-MCA (Fig. 3). The effect of DFMO on both cell lines was more marked than DCHS. Doubling times of AKR-MCA and AKR-2B were increased from 17-24 and 25 h, respectively, in the presence of DCHS. In contrast the generation time of DFMO treated AKR-MCA was 40 h while that of AKR-2B fibroblasts with this inhibitor was 32.4 h.

DISCUSSION

Morphological observations of monolayer AKR-MCA fibroblasts together with studies in soft agarose suggest that both DFMO and DCHS are capable of inducing a more benign phenotype similar to that of their parental AKR-2B cells. It seems unlikely that this effect is a result of nonspecific toxic insult insofar as removal of either agent and continued cultivation in standard medium restored both the transformed phenotype and growth kinetics after 48 h (data not shown). More importantly the effects of these inhibitors appeared selective on transformed fibroblast morphology with little apparent change of the parental cells. A more viable proposition is that the specific depletion of cellular polyamines in response to both agents either directly or indirectly reverses the transformed state of AKR-MCA cells.

In agreement with this proposition are the findings of time dependent decreases in putrescine and spermidine after exposure to DFMO and DCHS. The reduction in the polyamines can be regarded as an early event since they preceed morphological change in the AKR-MCA cells which manifests itself between 24 and 48 h. In addition, the observation that coinbination of DFMO and DCHS treated AKR-MCA cells in monolayer with exogenous polyamines abrogate the morphological effects of the inhibitor lends support to the hypothesis that reduced polyamine levels are required for the expression of a

4 Unpublished observations.
more normal phenotype. It would appear that spermidine is the key polyamine in governing cellular phenotype and behavior since (a) there is significantly more spermidine in AKR-MCA cells than AKR-2B, (b) spermidine but not putrescine will reverse the effects of DCHS on AKR-MCA morphology, (c) the intracellular level of the endogenous compound is reduced by 70% in both DFMO and DCHS treated cultures, and (d) the inability of exogenous putrescine and spermidine to rescue AKR-MCA cells from the "redifferentiating" effects of DMF correlates with their ineffectiveness in restoring intracellular spermidine but not putrescine to control values.

Although we were somewhat surprised to find two levels of spermidine (1 or 10 μM) incapable of restoring colony formation in AKR-MCA soft agarose cultures exposed to either DFMO or DCHS, these data are not necessarily incompatible with those obtained from monolayer studies. The presence of the spermidine catabolizing enzyme diamine oxidase in fetal bovine serum has been well documented and it is likely that over the 3 weeks of incubation (compared with 3 days in monolayer culture) there was substantial degradation of this polyamine to a toxic metabolite thus preventing proliferation (21). Giving further substance to this theory was the finding that a 5-day exposure of monolayer AKR-MCA to 10 μM spermidine had a deleterious effect on these cells.5

Although DFMO and DCHS abolish the growth of AKR-MCA cells in soft agarose and this is taken to imply reversal of the transformed phenotype, the cytostatic effects of these agents must be taken into consideration. As shown in Fig. 3, these inhibitors slow monolayer growth of the transformed fibroblasts. This effect could quite plausibly translate into a severe reduction in plating efficiency of AKR-MCA in soft agarose, resulting ultimately in the absence of colony formation.

The finding that 2 polyamine synthesis inhibitors were capable of invoking a more normal phenotype in AKR-MCA prompted investigation of the mechanism of action of DMF. Previous work has demonstrated that exposure of transformed cells to the planar solvent will, in a number of cases (22, 23) reverse this state. This reversal is evident for AKR-MCA fibroblasts whereby treatment with DMF leads to flattened, polygonal cells akin to AKR-2B, with an associated reduction in saturation densities (12). The loss of transforming potential is further indicated by the failure of DMF exposed AKR-MCA to propagate in soft agarose.

In investigating polyamine levels in transformed fibroblasts, we found similar alterations by both DFMO and DMF; putrescine was reduced to below assay limits, spermidine declined to 30% of untreated controls, and spermine remained unchanged. The kinetics of putrescine disappearance did differ in response to both agents. The reduction of the diamine by DFMO was rapid, requiring 6 h or less for its disappearance. This contrasted with a slower decline by DMF with putrescine levels approaching assay limits after 12 h. These data suggest that DMF and DFMO may be acting in different ways. Further studies will be necessary to clarify these observations.

If the depletion of intracellular spermidine is a requirement for the reversal of the transformed state then addition of this polyamine to AKR-MCA exposed to DMF might be expected to abrogate the effects of the polar solvent as it does in DFMO/ DCHS treated cells. This is evidently not the case. In fact, these observations were correlated with the inability of exogenous spermidine to restore intracellular levels back to control (DMF untreated) values. On the other hand, DFMO induced depletion of spermidine could be entirely overcome by coculturing with exogenous polyamines (data not shown). It is not clear why the spermidine profile produced by DMF treatment is insensitive to addition of either putrescine or spermidine. It is unlikely that the polar solvent imposes a nonspecific block of amine uptake since the diamine is capable of restoring its own intracellular profile. A block of spermidine synthase could, however, account for this observation. On the other hand, this blocking fails to provide for the finding that coincubation of DMF treated cultures with spermidine has little effect on depleted intracellular levels. The existence of an additional blockade of spermidine uptake could be argued or alternatively an enhanced degradation of the polyamines to N-acetylated derivatives might be considered. Quite clearly, further studies are required to define the molecular effects of DMF on polyamine uptake and metabolism. Alternatively, and a possibility that cannot be excluded at this stage, is that the normalizing effects of DMF may not involve polyamine depletion.

It is worthy of comment that the reduction in polyamines by DFMO and DCHS is not specific to AKR-MCA. Studies with the parental AKR-2B indicate similar reductions with identical kinetics.6 In fact, these reductions were paralleled by decreases in apparent growth rate of both transformed and parental fibroblasts. Thus, it is possible that the primary effect of polyamine depletion is a slowed proliferative rate; this in turn may trigger a more normal program in the AKR-MCA cells.

REFERENCES

15. Levine, A., McRae, L., and Brattain, M. Changes in receptor occupancy and growth factor responsiveness induced by treatment of a transformed mouse

5 Unpublished observation.
6 Unpublished studies.


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