Changes in Receptor Occupancy and Growth Factor Responsiveness Induced by Treatment of a Transformed Mouse Embryo Cell Line with N,N-Dimethylformamide

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ABSTRACT

Treatment of the transformed mouse embryo fibroblast cell line (AKR-MCA) with N,N-dimethylformamide (DMF) results in a reversion to the nontransformed AKR-2B cell line phenotype. AKR-MCA cells grown in the presence of 1% DMF showed a 2-fold increase in the sites for epidermal growth factor (EGF) binding. However, most of these sites were occupied by an endogenous ligand. The EGF receptor was unoccupied in untreated AKR-MCA cells. The increased receptor occupation was paralleled by an increase in the mitogenic response to EGF. Treatment of these cells with 1% DMF resulted in a 6-fold stimulation of mitogenesis by EGF. The ability to respond to nutrient replenishment (a property of growth-arrested AKR-MCA cells) was lost within 24 h of DMF treatment. Upon removal of DMF from the cells, both the mitogenic response to EGF and the occupation of the EGF receptor by endogenous ligands were lost. Treatment of the AKR-2B cell line with DMF had little effect on its growth properties. Therefore, DMF altered the growth control response and growth factor binding of AKR-MCA cells in a reversible, noncytotoxic manner.

INTRODUCTION

A variety of chemicals have been used to induce differentiation in transformed cells. These include planar polar compounds such as sodium butyrate (1-4), dimethyl sulfoxide (2, 4-6), and DMF (7-10) and analogues of retinoic acid (4, 11, 12). These compounds were shown to cause molecular alterations in several different types of tumor cells which may be related to a more differentiated phenotype.

We have found that DMF treatment of AKR-MCA cells (a chemically transformed mouse embryo fibroblast cell line) resulted in a number of significant changes in these cells. The DMF-treated AKR-MCA cells assumed a more differentiated phenotype as judged by changes in growth properties, cell morphology, membrane antigens (13), phosphoprotein pattern (14), and cell surface labeling patterns (15). In terms of these properties, the DMF-treated AKR-MCA cells resembled the nontransformed AKR-2B cells. None of these changes was observed when AKR-2B cells were treated with DMF. Treatment of the AKR-MCA cells restored the ability of these cells to respond to EGF.

DMF treatment of AKR-MCA cells resulted in increased extra-
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various exposures to DMF and removal of DMF from the cells.

Dissociation constants and receptor numbers were obtained by the method of Scatchard (22) using a least-squares analysis. Cells were grown in 24-well plates (Falcon) as described above, except that the assay was performed using confluent cells (Day 5 or 6) with no changes in serum concentration. Binding buffer contained 1–50 ng of 125I-EGF per ml, and nonspecific binding was determined in the presence of a 500-fold excess of unlabeled EGF.

Mitogenesis Assays. AKR-2B or AKR-MCA cells were plated at 50,000 cells/well in 35-mm, 6-well plates (Costar) in McCoy’s Medium 5A containing 10% FBS on Day 1. After 24 h, the medium was replaced with fresh medium (containing 0 or 1% DMF). On Day 5, medium was removed and replaced with McCoy’s Medium 5A containing 0.5% FBS with or without 1% DMF. The medium was replaced on Day 6 by medium with or without 1% DMF for 1 day or 4 days resulted in an apparent 40% decrease in the amount of EGF bound to the cells compared to controls (Table 1). This decrease could reflect changes in the number of EGF receptors, in the rate of internalization and metabolism of the EGF-receptor complex, or occupation of the receptors by endogenous ligands.

To determine the reason for the apparent decrease in EGF binding to DMF-treated cells, the amount of EGF bound following pH 4.5 treatment was determined. This brief exposure to a lower pH has been shown to unmask receptor sites by removing endogenous ligands from the cell surface (17, 18). The total number of EGF binding sites was increased approximately 2-fold by DMF treatment. Control AKR-MCA cells (Table 1) showed no significant change in the amount of EGF bound after pH 4.5 treatment. The cells exposed to DMF for 1 day (Table 1) showed a 2-fold increase in the amount of EGF bound after acid treatment. DMF treatment for 4 days resulted in a 4-fold increase in the amount of EGF bound (Table 1). Therefore, growth of AKR-MCA cells in the presence of DMF increased the total number of EGF receptors, but approximately 75% of these receptors were occupied by an endogenous ligand (Chart 1).

The reversibility of this effect was tested by growing the cells in 1% DMF for 3 days and then removing the DMF (for 1 day) at the time of growth arrest. Under these conditions (Table 1), the total amount of EGF bound was similar to that measured for control cells that were not treated with DMF. The increase in endogenous ligand binding was also reversible, since removal of DMF did not produce an increase in endogenous binding following pH 4.5 pretreatment (Chart 1).

Mitogenesis Response in AKR-MCA and AKR-2B Cells. The response to nutrient replenishment and EGF by AKR-MCA cells

Table 1

<table>
<thead>
<tr>
<th>Exposure to DMF</th>
<th>pmol of EGF bound/µg of DNA</th>
<th>Relative binding of acid-treated/ non-acid-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-acid-treated cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 4.5-treated cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>748</td>
<td>823</td>
</tr>
<tr>
<td>1 Day</td>
<td>485</td>
<td>1015</td>
</tr>
<tr>
<td>4 Days</td>
<td>476</td>
<td>1908</td>
</tr>
<tr>
<td>3 Days, remove DMF for 1 day</td>
<td>880</td>
<td>1112</td>
</tr>
</tbody>
</table>

Chart 1. Effect of DMF on EGF receptor occupancy in AKR-MCA cells. The percentage of sites occupied by endogenous ligands was calculated from the data in Table 1. The dashed line indicates the reversal experiment in which the cells were removed from 1% DMF on Day 3, and the EGF binding was measured on Day 4.
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was examined by a mitogenesis assay where cells were serum starved. As reported by other laboratories (27, 28), \(^{3}H\)thymidine incorporation by transformed AKR-MCA cells was stimulated 2-fold by nutrient replenishment (data not shown). There was no additional increase in thymidine incorporation if EGF was included (Chart 2A).

AKR-MCA cells grown in the presence of 1% DMF no longer responded to nutrient replenishment (data not shown). If EGF was added to these cells with fresh medium, a 6-fold stimulation of \(^{3}H\)thymidine incorporation was observed (Chart 2A). In order to determine if DMF shifted the lag time between EGF addition and the onset of mitogenesis, a second mitogenesis experiment was performed as described above except that the cells were exposed to \(^{3}H\)thymidine for 24 h instead of 1 h. Similar results were obtained in that the AKR-MCA cells grown in the presence of 1% DMF no longer responded to nutrient replenishment and EGF stimulated mitogenesis (data not shown). This indicated that a change in lag time was probably not occurring upon DMF treatment.

Nontransformed AKR-2B cells showed a 7-fold stimulation of \(^{3}H\)thymidine incorporation when exposed to EGF (Chart 2B) as previously described (13, 27). These cells did not show a response to nutrient replenishment. A similar stimulation (12-fold) occurred when AKR-2B cells were exposed to 1% DMF during their growth (Chart 2B).

Uptake of \(^{3}H\)Thymidine. Cellular uptake of \(^{3}H\)thymidine was measured for both cell lines in experiments similar to those described for thymidine incorporation. The pattern of uptake of \(^{3}H\)thymidine paralleled the results obtained for thymidine incorporation. AKR-MCA cells did not show an EGF-stimulated uptake of thymidine (Chart 3A), while DMF treatment of these cells resulted in a 5-fold increase in \(^{3}H\)thymidine uptake (Chart 2A). EGF stimulated uptake of \(^{3}H\)thymidine approximately 15-fold in AKR-2B cells (Chart 3B) and was not affected by addition of 1% DMF (Chart 3B).

Time Course of Response. To determine the time course of the changes in responsiveness to nutrient replenishment and EGF addition, the cells were exposed to 1% DMF for 0, 1, or 4 days. In addition, the reversibility of these changes was examined by removal of DMF from the culture medium after 3 days of treatment. The results of these experiments (using AKR-MCA cells) are shown in Chart 4A. The AKR-MCA cells grown in the presence of 1% DMF showed a time-dependent increase in their responsiveness to EGF. The response was 2-fold at 1 day and 6-fold after 4 days of exposure to DMF. Removal of DMF from the AKR-MCA cells at Day 3 resulted in the loss of response to EGF (Chart 4A) within 24 h of DMF removal. The loss of response to medium replenishment in AKR-MCA cells grown in the presence of 1% DMF was complete within 24 h (data not shown). The response to EGF by AKR-2B cells was not affected by exposure to DMF for 1 day (Chart 4B). However, exposure to DMF for 4 days increases the responsiveness of the AKR-2B cells to DMF.

Endogenous Growth Factor Effects on Mitogenesis. A
crude growth factor preparation from AKR-MCA conditioned medium was added to AKR-MCA cells to determine the effects of endogenous factors on mitogenesis. This growth factor preparation stimulated the incorporation of \( ^{3}H \)thymidine 2-fold more than nutrient replenishment (Chart 5). When this crude growth factor preparation was added to AKR-MCA cells grown in the presence of 1% DMF, a 5-fold stimulation of \( ^{3}H \)thymidine incorporation was observed (Chart 5). This was similar to the stimulation observed when the growth factor preparation was added to AKR-2B cells (data not shown), indicating that AKR-2B and DMF-treated AKR-MCA cells respond to endogenous growth factors in a similar manner.

**DISCUSSION**

Treatment of transformed AKR-MCA cells with 1% DMF resulted in a reversible change in the expression of some molecular features of the malignant phenotype and the acquisition of a molecular phenotype more like that of the nontransformed AKR-2B cell line (13-15). In this paper, we show that DMF treatment of AKR-MCA cells caused a reversible increase in the number of EGF receptors on these cells. However, 75% of the EGF receptors on DMF-treated cells were occupied by an endogenous ligand. The EGF receptors were unoccupied on AKR-MCA cells not treated with DMF. One possibility for the endogenous ligand is an EGF-receptor competing activity which we have previously described (16). This factor was not mitogenic towards growth-arrested mouse fibroblasts and therefore was not EGF or TGF-\( \alpha \) (29). In addition, this factor eluted from reverse-phase high-pressure liquid chromatography at a different acetonitrile concentration (20% compared to 31% for TGF-\( \alpha \)) (16, 30). This EGF-receptor competing activity may be a member of the TGF-\( \alpha \) family, but our current data indicate that it is different than the TGF-\( \alpha \) previously described (29, 30).

Growth of the transformed AKR-MCA cells in the presence of 1% DMF resulted in an increased mitogenic response to EGF and the loss of the ability to respond to nutrient replenishment. These effects of DMF were reversible. Within 24 h of DMF removal, the AKR-MCA cells responded to nutrient replenishment and did not respond to EGF in a manner similar to untreated AKR-MCA cells.

The restoration of growth factor dependence was not immediate. AKR-MCA cells exposed to 1% DMF for 1 day showed a 2-fold stimulation of mitogenesis compared to the 6-fold increase observed in cells treated with DMF for 4 days. This pattern of stimulation was paralleled by a time-dependent increase in binding of endogenous ligands to the EGF receptor. Marks et al. (15) have also shown a similar time-dependent increase in changes in cell surface fibronectin in response to DMF treatment of AKR-MCA cells. Maximum cell surface fibronectin was measured after 4 days of DMF treatment, with only small increases observed in 1–2 days.

Recently, it has been shown that TGF-\( \beta \) can inhibit cellular proliferation (31, 32). TGF-\( \beta \), the EGF receptor competing activity, or other factors produced in the presence of DMF could act as inhibitors of cell growth. Therefore, DMF could inhibit cell growth by altering the factors produced by the AKR-MCA cells or by altering the cell’s response to these factors. Marks et al. (15) have shown that the simultaneous addition of DMF and TGF to AKR-2B cells prevents the phenotypic transformation normally associated with TGF treatment. Another type of differentiation agent, retinoic acid, has also been shown to alter embryonal carcinoma cell response to growth factors (33).

The changes in growth factor synthesis (16) and response are only some of the phenotypic changes brought about by differentiation agents such as DMF. While DMF may induce a nontransformed phenotype with respect to the hormonal control of growth, the mechanism of this response may not be the same as in parental nontransformed cells. Further study of the mechanism by which DMF restores this normal cell function may provide insight into the mechanisms which control neoplastic cell growth.

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**REFERENCES**


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