Retinoid Modulation of Estradiol-stimulated Growth and of Protein Synthesis and Secretion in Human Breast Carcinoma Cells

Joseph A. Fontana, Alicia Burrows Mezu, Brenda N. Cooper, and Dante Miranda

ABSTRACT

All-trans-retinoic acid (RA) has been demonstrated to inhibit the growth of numerous breast carcinoma cell lines. In this study we demonstrated that RA inhibits estradiol stimulation of proliferation of the estrogen-dependent breast carcinoma cell lines MCF-7 and ZR-75. RA inhibition of MCF-7 breast carcinoma cell proliferation is associated with the marked stimulation of the synthesis and secretion of a 75,000 molecular weight protein. Minor stimulation of the synthesis and secretion of other proteins was also noted, including 160,000, 95,000, 80,000, 52,000, and 46,000 molecular weight proteins. Increased synthesis and secretion of the M, 75,000 protein was noted with as little as 10 nM RA. Stimulation of the synthesis and secretion of this protein occurred within 24 h of adding the retinoid and at the time of RA inhibition of cellular proliferation. RA stimulated the production and secretion of this protein both in the absence and presence of estradiol. Stimulation of the secretion and synthesis was not noted in the presence of difluoromethylornithine or tamoxifen, both of which significantly inhibited cellular proliferation. The estrogen-dependent breast carcinoma cell line ZR-75 when exposed to RA also expressed increased synthesis of the M, 75,000 and 46,000 proteins but increased synthesis was not noted in the RA-resistant cell line MDA-MB-231. Stimulation of the synthesis and secretion of the M, 75,000 and/or 46,000 proteins may be intimately involved in RA inhibition of cellular proliferation.

INTRODUCTION

The ability of retinoids to inhibit the proliferation of hormone-dependent breast carcinoma cells has been well documented (1-7). The combination of retinoids and ovariec tomy or of retinoids and antiestrogens has a synergistic effect on the growth inhibition of these tumors (8-11). Most chemical carcinogen-induced rat mammary tumors are hormonally responsive and the ability of either ovariec tomy or antiestrogens to suppress their growth has been demonstrated (11-13). Retinoids have also displayed efficacy in inhibiting the proliferation of these carcinogen-induced mammary tumors (9-12). The mechanism by which retinoids inhibit the growth of these cells remains unclear.

Recently, elegant studies have demonstrated that breast carcinoma cells produce and secrete numerous growth factors, possess receptors for these factors, and respond to their presence by increasing their growth rate (14-17). Estrogens dramatically increase the secretion of numerous growth factors including platelet-derived growth factor, insulin-like growth factor-I, and transforming growth factor α in ER-positive breast carcinoma cells (14-16). Antiestrogens stimulate the secretion and activation of TGF-β, which inhibits the proliferation of these cells; nanomolar concentrations of estrogens prevent antiestrogen stimulation of TGF-β secretion and activation (18). Bronzert et al. (19) and Sheen and Katzenellenbogen (20) have recently demonstrated antiestrogen stimulation of the secretion and production of 37,000 and 39,000 molecular weight proteins, which correlates with growth inhibition by these agents. Other agents which inhibit proliferation do not induce expression of these proteins; thus stimulation of these proteins appears to be restricted to antiestrogens. Estrogens markedly inhibit the expression of these proteins while increasing the proliferation rate (19, 20).

In this report we investigated the ability of retinoids to specifically inhibit estradiol stimulation of ER-positive human breast carcinoma cell growth as well as to modulate the synthesis and secretion of proteins by these cells. A number of these proteins have been identified and it is possible that they may contribute to the antiproliferative action of retinoids on these cells.

MATERIALS AND METHODS

Materials. DMEM, F-12 medium, gentamycin, and fetal bovine serum were obtained from Gibco (Grand Island, NY). RA and TMX were obtained from Sigma Chemical Co. (St. Louis, MO). Phenol red-free DMEM was obtained from Haezelton Research Products (Lenexa, KS) and Gibco. Estradiol was obtained from Steraloids Inc. (Wilton, NH) [3H]Methionine (>800 Ci/mmol) was obtained from Du Pont-New England Nuclear (Boston, MA). The MCF-7, ZR-75, and MDA-MB-231 cells were a gift of Dr. Mark Lippman (National Cancer Institute, Bethesda, MD). Tissue culture 6-well plates (0.75 cm²) were obtained from Corning (Corning, New York).

Cell Culture. The breast carcinoma cell lines were Mycoplasma-free and maintained in DMEM/F-12 (1:1 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, and 50 µg/ml gentamycin, in a 5% CO₂ humidified atmosphere at 37°C. Viability was determined by trypsin blue exclusion. In growth inhibition experiments, the cells were seeded at concentrations of 1 x 10⁵ cells/well in 6-well plates. The cells were incubated for 24 h in DMEM/F-12 medium supplemented as described above. The cells were then washed and incubated in phenol red-free DMEM, supplemented with 4% heat-inactivated charcoal and sulfatase-treated fetal bovine serum, for 3 h. Fetal bovine serum was treated with charcoal and dextran, utilizing the procedure of Eckert and Katzenellenbogen (21). The serum was then treated with 2 units of limpet sulfatase/ml for 2 h at 37°C, followed by further charcoal-dextran treatment at 55°C for 30 min to remove sulfated estrogens. The cells were then washed and this procedure was repeated an additional 2 times in order to remove estrogenic compounds. The cells were then incubated in a similar medium in the presence of 10⁻⁸ M estradiol, 1 µM TMX, or a combination of these agents for 6 days. The media and agents were changed every 48 h. A similar amount of vehicle (0.05% ethanol) was added to the control flasks. This concentration of ethanol has no effect on cell growth or viability. Cell counts were performed in triplicate using a hemocytometer. Data were analyzed using the analysis of variance and the least significant difference for comparing adjacent means among treatment groups.

Labeling and Analysis of Released Proteins. MCF-7 and ZR-75 cells were seeded at cell concentrations of 1 x 10⁶ and 2 x 10⁶ cells/well, respectively, in DMEM/F-12 (1:1) medium supplemented with 10% heat-inactivated fetal bovine serum and gentamycin (25 µg/ml). The cells were incubated for 24 h, the estradiol was removed as previously

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The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. This research was supported by a grant from the Veterans Administration. The abbreviations used are: ER, estrogen receptor; RA, retinoic acid; TMX, tamoxifen; cRABP, cytosolic retinoic acid-binding protein; TGF-β, transforming growth factor β; EST, estradiol; DMEM, Dulbecco's modified Eagle medium.
described, and the cells were incubated in phenol red-free DMEM supplemented with 4% heat-inactivated, charcoal sulfatase-treated, fetal bovine serum. Cells were labeled by washing the cultures with 1 ml phenol red-free, methionine-free, minimal essential medium 4 times and then replacing the medium with 300 μl of this medium, containing 100 μCi [35S]methionine and the appropriate agent, for 6 h. The medium was collected and centrifuged to remove cellular debris; for gel electrophoresis, 35,000 cpm were mixed with an equal volume of sample buffer consisting of 2% sodium dodecyl sulfate, 100 mM Tris-HCl, pH 6.8, 20% glycerol, and 2% 2-mercaptoethanol. The samples were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis upon 10% acrylamide gels, using the system of Laemmli (22). The gels were dried and exposed to Kodak AR films. The amount of [35S]methionine incorporated into the various bands was determined by scanning the film with a LKB slab gel densitometer and determining the area under the various peaks.

RESULTS

Estradiol significantly stimulated the growth of MCF-7 cells, approximately 2-fold, in the phenol red-free DMEM supplemented with the charcoal- and sulfatase-treated fetal bovine serum (Table 1), while the addition of RA or TMX resulted in significant inhibition. The addition of RA alone to the cultures resulted in a 50% inhibition of growth noted in the controls, similar to the inhibition noted with TMX. The addition of TMX and RA did not result in a significantly increased inhibition of cellular proliferation over that noted with either agent alone. The addition of TMX or RA to media containing estradiol resulted in a similar but significant reduction in the estradiol-stimulated growth rate to that observed in the control. The combination of TMX and RA did not inhibit MCF-7 proliferation in the presence of estradiol significantly more than either agent alone.

Addition of RA to the ER-positive ZR-75 cells also inhibited estradiol stimulation of proliferation, with reduction of the estradiol-stimulated growth rate to that noted in the control cultures (Table 2). Neither estradiol stimulation of growth nor RA inhibition of growth was noted in the ER-negative MDA MB 231 cells (Table 2).

Since RA inhibited estradiol-stimulated growth of MCF-7 cells, we investigated the effect of RA on estradiol binding by the cells. We observed, as noted previously by Wetherall and Taylor (23), that there was no direct competition between RA and estradiol for the estrogen receptor (data not shown).

Antiestrogens appear to have the capability of stimulating the production and secretion of a protein which antagonizes the proliferative effect of estrogens (19, 20).

Table 1 Retinoic acid inhibition of estradiol-stimulated growth of MCF-7 cells

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cell no. × 10^4</th>
<th>% of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.3 ± 0.9</td>
<td>200 ± 39</td>
</tr>
<tr>
<td>Estradiol</td>
<td>6.6 ± 1.0</td>
<td>200 ± 39</td>
</tr>
<tr>
<td>RA</td>
<td>1.7 ± 0.5</td>
<td>51 ± 14</td>
</tr>
<tr>
<td>TMX</td>
<td>1.5 ± 0.4</td>
<td>45 ± 11</td>
</tr>
<tr>
<td>RA/TMX</td>
<td>1.1 ± 0.3</td>
<td>33 ± 5</td>
</tr>
<tr>
<td>RA/EST</td>
<td>2.7 ± 0.5</td>
<td>82 ± 15</td>
</tr>
<tr>
<td>TMX/EST</td>
<td>3.9 ± 1.4</td>
<td>118 ± 30</td>
</tr>
<tr>
<td>TMX/RA/EST</td>
<td>2.4 ± 0.4*</td>
<td>73 ± 9</td>
</tr>
</tbody>
</table>

* Significantly different from control (P < 0.01).
* Significantly different from estradiol-treated cells (P < 0.01).

Table 2 Retinoic acid inhibition of estradiol stimulation of ZR-75 and MDA-MB growth

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cell no. × 10^4</th>
<th>% of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZR-75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.0 ± 0.8</td>
<td>147 ± 17</td>
</tr>
<tr>
<td>EST</td>
<td>7.4 ± 1.2*</td>
<td>60 ± 6</td>
</tr>
<tr>
<td>RA</td>
<td>3.0 ± 0.2*</td>
<td>78 ± 18</td>
</tr>
<tr>
<td>RA/EST</td>
<td>3.9 ± 0.7*</td>
<td>103 ± 12</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.4 ± 0.7</td>
<td>96 ± 7</td>
</tr>
<tr>
<td>EST</td>
<td>6.2 ± 0.8</td>
<td>98 ± 8</td>
</tr>
<tr>
<td>RA</td>
<td>6.0 ± 0.6</td>
<td>103 ± 12</td>
</tr>
<tr>
<td>RA/EST</td>
<td>6.3 ± 0.6</td>
<td>98 ± 8</td>
</tr>
</tbody>
</table>

* Significantly different from control (P < 0.01).
* Significantly different from estradiol-treated cells (P < 0.01).

Fig. 1. Effect of RA on protein synthesis and secretion. MCF-7 cells were seeded at a concentration of 1 × 10^4 cells/well in DMEM/F-12 medium and washed and grown in phenol red-free medium as described in "Materials and Methods." RA and estradiol were added to a final concentration of 1 μM and 1 nM, respectively. Control wells (CON) contained an equal concentration of ethanol. Media and contents were changed every 2 days. Total exposure time to RA was 6 days. Labeling of the cells with [35S]methionine and electrophoresis were performed as described in "Materials and Methods."
areas under the bands were determined.

Stimulation of this to retinoic acid for varying periods of time, with total incubation time in the secretion of a M, 37,000 and 39,000 protein, respectively, if the mean of three determinations ± SD. Fluorographs were quantified using a laser densitometer. The area under the corresponding peak and the percentage of the total activity incorporated into the band were determined.

The fluorogram depicted in Fig. 1 was scanned by laser densitometer and the areas under the bands were determined.

The addition of the combination of RA and EST resulted in a 14-fold increase in the density associated with this band. Thus the marked increase in the density of the M, 75,000 region when MCF-7 cells are treated with the RA/EST combination is due predominantly to RA increase of M, 75,000 synthesis and secretion.

and secretion of a protein with a broad band centered at M, 70,000 but extending to some of the area occupied by the M, 75,000 protein. This complicated the laser densitometry analysis of the M, 75,000 region, resulting in an artificially high value (3- to 4-fold of control) in the estradiol lane. However, the addition of RA results in a 10-fold increase in the density associated with the M, 75,000 band, while the combination of RA and EST results in a 14-fold increase in the density associated with this band. Thus the marked increase in the density of the M, 75,000 region when MCF-7 cells are treated with the RA/EST combination is due predominantly to RA increase of M, 75,000 synthesis and secretion.

The addition of the combination of RA and EST resulted in an increase in the synthesis and secretion of the M, 75,000 protein over that noted when RA alone was added. This was an interesting observation in light of the findings of Bronzert et al. (19) and Sheen and Katzenellenbogen (20) that there was a marked increase in the antiestrogen-induced synthesis and secretion of a M, 37,000 and 39,000 protein, respectively, if estrogens were also present.

Exposure of MCF-7 cells to 1 μM RA resulted in significant inhibition of proliferation by 24 h of exposure (Table 4). We therefore investigated whether RA stimulated the synthesis and secretion of these proteins prior to or at the time of the inhibition of cellular proliferation (Fig. 2). There is an approximately 4-fold increase in the amount of secreted M, 75,000 and 82,000 proteins within 24 h of RA addition (Table 4) and prior to maximum RA-induced inhibition of cellular proliferation, strengthening the possibility that modulation of these proteins may be involved in the RA-mediated inhibition of cellular proliferation (Fig. 2; Table 4). Stimulation of this protein is not noted when MCF-7 proliferation is inhibited by approximately 50 to 80% by TMX or difluoromethylornithine. This would suggest that these proteins are not simply induced as a result of cessation of cellular growth but are specifically modulated by RA. One again observes the intermittent increased labeling of M, 43,000, 46,000, 100,000 and 160,000 proteins; however, the apparent increased synthesis and secretion of these proteins is sporadic and does not appear to correlate with the extent of inhibition of cellular proliferation. The ability of varying concentrations of RA to modulate the production and secretion of these proteins was also investigated (Fig. 3; Table 5). Modulation of the M, 75,000 protein was...
Table 5  Inhibition of MCF-7 proliferation and stimulation of the synthesis and secretion of M, 75,000 and M, 82,000 proteins by retinoic acid

Cells were grown in 6-well plates and exposed to RA as described in Fig. 3. Inhibition results represents the mean of three determinations ± SD. Fluorographs were quantified using a laser densitometer. The area under the corresponding peak and the % of the total activity incorporated into the peaks were determined.

<table>
<thead>
<tr>
<th>Concentration (nM)</th>
<th>Cell counts × 10^4</th>
<th>% Inhibition</th>
<th>Area^a</th>
<th>Relative area^b</th>
<th>Area^a</th>
<th>Relative area^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.5 ± 0.8</td>
<td>0.47</td>
<td>4.5</td>
<td>0.03</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5.9 ± 0.4</td>
<td>10 ± 4</td>
<td>2.15</td>
<td>8.1</td>
<td>0.05</td>
<td>1.9</td>
</tr>
<tr>
<td>30</td>
<td>4.6 ± 0.5^a</td>
<td>29 ± 6</td>
<td>4.50</td>
<td>15.1</td>
<td>0.10</td>
<td>2.8</td>
</tr>
<tr>
<td>100</td>
<td>4.3 ± 0.4^a</td>
<td>34 ± 4</td>
<td>5.10</td>
<td>17.4</td>
<td>0.15</td>
<td>2.7</td>
</tr>
<tr>
<td>300</td>
<td>4.2 ± 0.3^a</td>
<td>36 ± 7</td>
<td>4.65</td>
<td>17.2</td>
<td>0.46</td>
<td>7.8</td>
</tr>
<tr>
<td>1000</td>
<td>2.8 ± 0.5^a</td>
<td>43 ± 7</td>
<td>4.25</td>
<td>18.3</td>
<td>0.41</td>
<td>7.9</td>
</tr>
</tbody>
</table>

^a Area under peak (see Table 3).
^b Percentage relative area (% of total secreted protein).
^c Significantly different from control (P < 0.05).

DISCUSSION

Retinoids markedly inhibit the proliferation of numerous breast carcinoma cell lines (1–7). Retinoids appear to be cytostatic, since cultures resume growth after retinoid removal (4). In the early stage of treatment, there appears to be an enhancement of DNA synthesis, as indicated by an increased number of cells in S phase (7). This is followed within 48 to 96 h by a decrease in cell proliferation and accumulation of cells in the G0-G1 phase (7). It is of interest that the antiestrogen TMX is also cytostatic and arrests cells in the G1 phase (25). In addition, TMX and retinoids have an additive effect in inhibiting the proliferation of breast carcinoma cells, perhaps suggesting a common mechanism of action, although much more definitive studies need to be performed (23, 26). Several studies have suggested that retinoids may act as antiestrogens (27–29). Growth of human squamous cervical carcinoma C41 cells is stimulated by the addition of estradiol or diethylstilbestrol, while the addition of RA in the presence of steroid inhibits growth and converts the cells to a fibroblast-like morphology (27). Vitamin A has been demonstrated to prevent estrogen-induced proliferation and cornification in the vaginal epithe-

Fig. 4. Effect of RA on protein synthesis and secretion in ZR-75. ZR-75 cells were seeded at a concentration of 6 × 10^4 cells/well, as described in the legend to Fig. 1. Growth of cells, addition of RA and estradiol, labeling with [35S]methionine, and electrophoresis were performed as described in Fig. 1. C, control; E, estradiol.

noted with as little as 10 nM RA but maximal stimulation required 30 to 100 nM RA. The addition of 30 nM RA to the culture resulted in the stimulation of the synthesis and secretion of the M, 82,000 protein (Fig. 3). RA-induced increases in the synthesis and secretion of a number of other proteins were also observed. The increased presence of these proteins was only intermittently observed and did not always correlate with the dose of RA. Increased synthesis and secretion of the M, 46,000 protein occurred at 10 nM RA, when inhibition of cellular proliferation was minimal and not significantly different from the control, while it was not noted at higher concentrations of RA, when inhibition of cellular proliferation was significantly greater (Table 5). Significant inhibition of cellular proliferation occurred at 30 nM RA and appeared to correlate with the increased synthesis and secretion of both the M, 75,000 and 82,000 proteins.

RA produced an approximately 50% inhibition of proliferation of the ER-positive cell line ZR-75. Exposure of this cell line to RA resulted in the increased synthesis and secretion of the M, 46,000, 75,000, and 18,000 proteins and a decrease in a M, 100,000 protein (Fig. 4). Thus, RA stimulated the synthesis and secretion of the M, 75,000 and 46,000 proteins in both the MCF-7 and ZR-75 cells. Stimulation of the synthesis and secretion of the M, 82,000 protein was not noted. Estradiol treatment of ZR-75 cells resulted in increased synthesis and secretion of the M, 160,000 and 52,000 proteins. The growth of the ER-negative cell line MDA-MB-231 is not inhibited by RA (Table 2). Modulation of the production or the secretion of either the M, 75,000, 82,000, or 46,000 proteins was not noted in this cell line following exposure to RA (Fig. 5). The addition of RA to the MDA-MB-231 cells did result in the inhibition of the synthesis and secretion of M, 66,000 and approximately M, 220,000 proteins but had no effect on cellular proliferation.

Fig. 5. Effect of RA on protein synthesis and secretion in MDA-MB-231. MDA-MB-231 cells were seeded at a concentration of 3 × 10^4 cells/well in DMEM/F-12 medium and washed and grown in phenol red-free medium, as described in "Materials and Methods." RA and estradiol were added to concentrations of 1 μM and 1 nM, respectively. The media and contents were changed every 2 days. Cells were incubated for a total of 6 days. Labeling of the cells with [35S]methionine and electrophoresis were performed as described in "Materials and Methods." C, control; E, estradiol.
lium as well as keratinizing metaplasia in the uterus (28). However, studies in breast carcinoma cells have not demonstrated any direct competition between retinoids and estrogens or progestérones for their respective receptors (23). Therefore, it is not clear if and how retinoids might directly antagonize the action of estrogens.

The mechanism by which retinoids inhibit cellular proliferation remains unclear. A positive correlation exists between binding of some retinoids to cRABP and their biological actions in various tissues (30, 31). High levels of cRABP have been demonstrated in a majority of human breast carcinoma tissues (32, 33). However, there appears to be no correlation between the level of cellular binding proteins and the susceptibility to retinoid-induced growth inhibition (34, 35). In addition, several cell lines which are sensitive to the antiproliferative effects of these agents do not possess cRABP (36, 37). Recently two investigators have cloned complementary DNA which encodes a protein that binds retinoic acid with high affinity (38, 39). This protein appears to be a ligand-dependent DNA-binding protein which may be a necessary intermediary for retinoid action.

The ability of retinoids to modulate the synthesis of numerous proteins in a variety of systems has been demonstrated (40). Many of these proteins are constituents of the cytoskeleton of the extracellular matrix (40). In addition, the expression of various other proteins including alkaline phosphatase, plasminogen activator, and the receptor for epidermal growth factor is modulated by retinoids (40–44). The stimulation of synthesis of these proteins often occurs during inhibition of proliferation (44).

One can speculate that retinoids may either increase the production and secretion of a growth-inhibitory protein or decrease the synthesis and secretion of a growth-stimulating protein, as has been shown with other growth-regulatory agents. Levine et al. (45) demonstrated that treatment of the transformed mouse embryoid fibroblast cell line AKR-MCA with N,N-dimethylformamide resulted in the increased synthesis and secretion of TGF-β, which is a potent inhibitor of AKR-MCA proliferation. Knabbe et al. (18) have demonstrated that the addition of antiestrogens resulted in increased secretion in ER-positive breast carcinoma cells of the growth inhibitor TGF-β. Levine and Crandall (46) have recently demonstrated that RA prevents the mitogenic response of AKR-2B cells to TGF-β by blocking the synthesis of c-sis mRNA normally induced by TGF-β and inhibiting the subsequent synthesis and secretion of platelet-derived growth factor.

We have shown that RA can inhibit estradiol-stimulated growth of several breast carcinoma cell lines. The addition of RA to media in the presence of exogenous estradiol reduces growth to that noted in the control cultures. The inhibition of proliferation by TMX in the control cultures may in part be attributed to inhibition of the minimal growth-stimulatory effect of residual estrogenic compounds; that the effect of any residual endogenous estrogenic compounds is minimal is supported by the marked stimulatory effect of exogenous estradiol on the growth rate and on the estrogen-dependent M, 52,000 and 160,000 protein secretion by the ER-positive cells. Vignon et al. (47) have recently demonstrated that antiestrogens can inhibit ER-positive breast carcinoma cell proliferation in the total absence of estrogen when cell proliferation is stimulated by insulin or epidermal growth factor. This effect of antiestrogens appears to still be mediated through the ER site.

In addition, we have demonstrated that, during RA inhibition of breast carcinoma growth, there is marked modulation of numerous proteins. Bronzert et al. (19) and Sheen and Katzenellenbogen (20) have demonstrated antiestrogen stimulation of the synthesis and secretion of a M, 39,000 and 37,000 protein, respectively. The stimulation of the synthesis and secretion of these proteins by antiestrogens only occurs in the presence of estrogens. We have detected RA modulation of the synthesis and secretion of a number of proteins. Major modulation of the synthesis and secretion of the M, 82,000, 75,000 and 46,000 proteins was noted. RA modulation of the synthesis and secretion of the M, 75,000 and 46,000 proteins was noted in both the MCF-7 and ZR-75 cells during RA inhibition of cellular proliferation, while the M, 82,000 protein was only noted in the MCF-7 cells. Ervin et al. (48) recently described the isolation of M, 47,000 and 67,000 polypeptides from the conditioned media of normal mammary cells. These polypeptides, termed mammostatin, markedly inhibit the proliferation of numerous breast carcinoma cell lines. RA modulation of other proteins was also noted in the MCF-7 cells, including M, 160,000, 100,000, 95,000, 89,000, and 52,000 proteins. Modulation of the M, 100,000, 95,000 and 82,000 proteins by RA occurs only in the MCF-7 cells and RA stimulation of the secretion and synthesis of the M, 160,000, 100,000, 95,000, and 52,000 proteins was inconsistent, was not related to the dose of RA utilized, and/or did not correlate with the extent of inhibition of cellular proliferation. Estradiol markedly stimulated MCF-7 secretion of the M, 160,000 and 52,000 proteins, while TMX had a minimal effect on M, 52,000 secretion and synthesis. Westley et al. (49) have reported that TMX has no effect on the synthesis and secretion of either the M, 52,000 or 160,000 proteins.

RA addition to the ER-negative MDA-MB-231 cells did not result in the inhibition of cellular proliferation or stimulation of the synthesis and secretion of the M, 46,000, 75,000, and 82,000 proteins; an inhibition of the synthesis and secretion of a M, 66,000 and approximately 220,000 proteins was noted. Synthesis and secretion of the M, 75,000 protein was consistently increased by RA during RA inhibition of the MCF-7 and ZR-75 cells. Whether the levels of the M, 75,000 protein are intimately associated with retinoid inhibition of growth remains to be determined.

REFERENCES

10. Welsh, C. W., and DeHoog, J. V. Retinoid feeding, hormone inhibition and/


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