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The Paracrine Stimulation of MCF-7 Cells by MDA-MB-231 Cells: Possible Role in Antiestrogen Failure

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Abstract—*The influence of paracrine factors secreted by the hormone independent breast cancer cell line MDA-MB-231 on the growth of the hormone dependent breast cancer cell line MCF-7 was examined in the absence of estradiol and in the presence of inhibitory concentrations of antiestrogens.*

MDA-MB-231 cells were grown on transwell membranes and co-cultured with MCF-7 cells (50,000) plated in 6-well tissue culture dishes. Growth was maximally increased (80%) when MCF-7 cells were grown in the presence of 150,000 or more MDA-MB-231 cells for 4 days.

The non-steroidal antiestrogens tamoxifen (10^{-10} to 10^{-6} M) and 4-hydroxytamoxifen (10^{-11} to 10^{-7} M), the steroidal antiestrogens ICI 164384 (10^{-10} to 10^{-6} M) and Ru39411 (10^{-11} to 10^{-7} M) all inhibited estradiol (10^{-11} M) stimulated MCF-7 cell growth in a dose related manner when cultured for 4 days. However, the paracrine stimulation of MCF-7 cells produced by co-culture with 250,000 MDA-MB-231 cells was not inhibited by any of these antiestrogens.

These data suggest that in heterogeneous tumors, paracrine factors from hormone independent cells may reverse the growth inhibitory action that antiestrogens have on estrogen-dependent breast cancer cell growth.

INTRODUCTION

APPROXIMATELY 30% of non-selected breast cancers will respond to some form of antihormonal therapy [1]. Antiestrogen therapy in the form of tamoxifen has proven to be as effective in the treatment of hormone-dependent breast cancer as any ablative or additive therapy [2]. However, while tamoxifen has been demonstrated to provide a therapeutic advantage in the treatment of breast tumors [2], eventual tumor progression is consistently seen. The mechanism behind tamoxifen failure is presently unclear although we have reported on a number of possible mechanisms [3–6].

Breast cancer is frequently a heterogeneous mixture of hormone receptor positive and hormone receptor negative cells [7–10]. An antiestrogen would be expected to control the estrogen receptor positive cell population. However, if the drug cannot exert control over the estrogen receptor negative cells, this population would continue to proliferate. Many breast cancer cell lines, both ER positive and negative, secrete a variety of stimulating growth factors [11–14]. We have previously postulated that estrogen receptor negative breast cancer cells in a heterogeneous tumor may stimulate the growth of estrogen receptor positive cells even in the presence of inhibitory concentrations of antiestrogens [3, 6]. In support of this, we [3, 6], along with others [15], have demonstrated that a representative growth factor, epidermal growth factor, can stimulate estrogen-dependent breast cancer cells to proliferate in the presence of inhibitory concentrations of tamoxifen and other antiestrogens.

This study examines this postulate further by developing a co-culture system to detect paracrine influences of an estrogen-independent breast cancer cell line (MDA-MB-231) on the growth of an estrogen-dependent breast cancer cell line (MCF-7). Concentrations of a variety of antiestrogens, shown to be inhibitory to estrogen stimulated MCF-7 cell growth, were then studied in the co-culture system to assess whether such agents could control the stimulating action of these paracrine factors. Tamoxifen and 4-hydroxytamoxifen (see Fig. 1) were used as representatives of the nonsteroidal antiestrogens because of their clinical relevance. ICI 164384 and Ru39411 (see Fig. 1) were chosen as representatives of the new steroidal antiestrogens in case interactions different from those of the triphenylethylene-type antiestrogen were produced.

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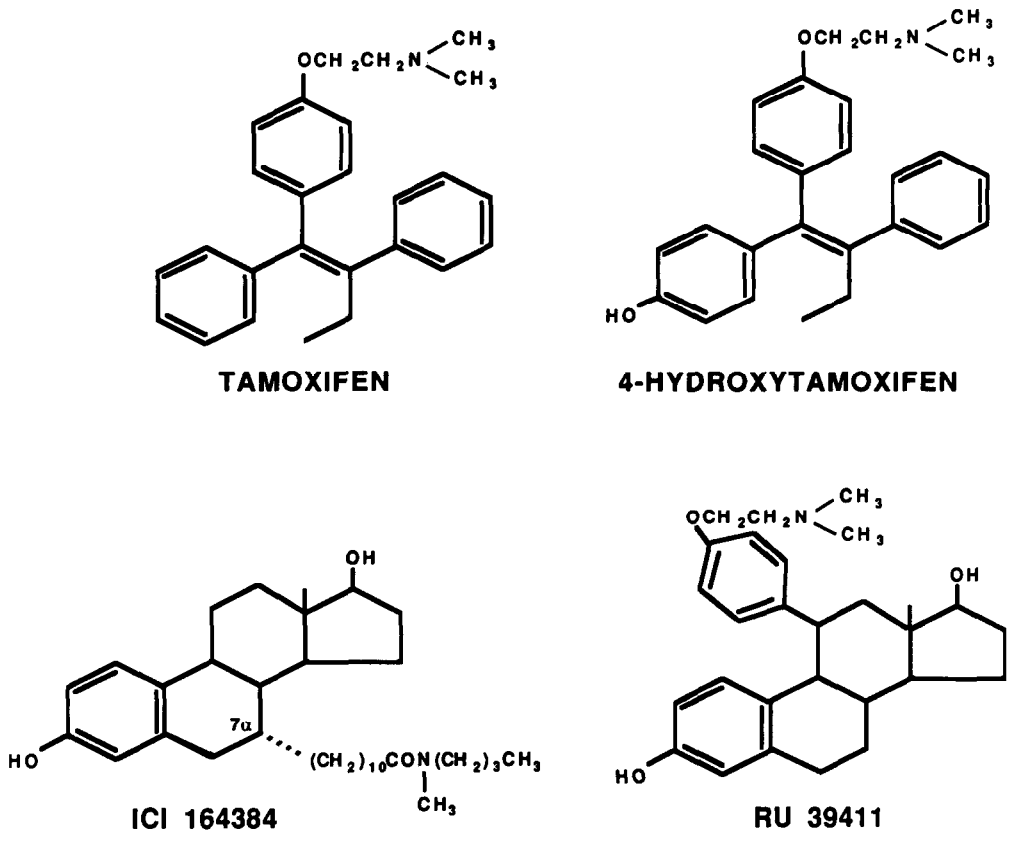

Fig. 1. Chemical structures of the nonsteroidal antiestrogens tamoxifen and 4-hydroxytamoxifen and the steroidal antiestrogens ICI 164384 and Ru39411.

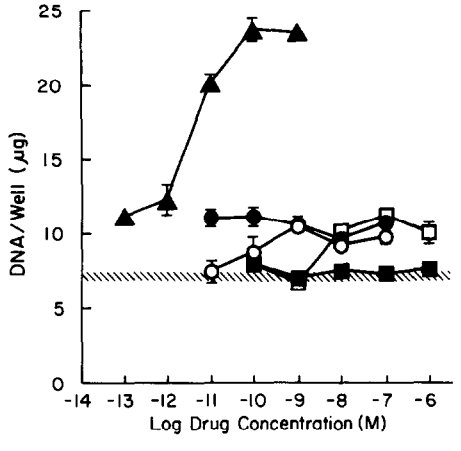

Fig. 2. Effect of estradiol (▲—▲), Ru39411 (●—●), 4-hydroxytamoxifen (○—○), tamoxifen (□—□), and ICI 164384 (■—■) on the growth of MCF-7 cells. MCF-7 cells were grown for 4 days in the presence of various concentrations of these drugs. DNA determinations ± S.E.M. were made for each concentration including control (hatched bar).

MATERIALS AND METHODS

Tamoxifen, 4-hydroxytamoxifen and ICI 164384 were a gift from Imperial Chemical Industries plc (Pharmaceutical Division, Macclesfield, Cheshire, U.K.). Ru39411 was a gift from Roussel UCLAF (Romainville, France). 17β-Estradiol was purchased from Sigma Chemical Company (St. Louis, MO).

Cell lines

MCF-7 cells, as used previously, were maintained and passaged as described [6]. MDA-MB-231 (ATCC Passage 25) were maintained in the same media as the MCF-7 [minimum essential medium (MEM)] containing phenol red with 0.29 mg L-glutamine/ml, 100 units penicillin/streptomycin/ml, 6 ng insulin/ml, 0.35 g $NaHCO_3$/l, 25 mM hepes, and 5% charcoal-stripped calf serum; media components were obtained from Gibco (Grand Island, NY). Experiments were performed in phenol red-free MEM (Sigma) made as described for phenol red-containing media.

DNA growth assay

MCF-7 cells were washed and plated (6-well plates, Costar Corporation, Cambridge, MA) at 50,000 cells/well in 5 ml of phenol red-free media. Media on the cells was changed after 24 and 72 h before incubation with media containing compounds or co-culture.

MDA-MB-231 cells were plated in phenol red-free media on transwell-COL® membranes (24 mm diameter, 3.0 µm pore size, Costar Corporation) 24 h before growth assay or co-culture. In co-cultures these microporous membranes containing plated MDA-MB-231 cells were placed into wells containing 2.6 ml of media covering the plated

MCF-7 cells. An additional 1.5 ml of media was subsequently added into each transwell.

DNA determination

Following aspiration of media (and removal of transwells containing MDA-MB-231 cells), MCF-7 cells were sonicated in 2 ml of Hank's balanced salt solution diluted 1:9 with water. MDA-MB-231 cells were similarly disrupted in 10 × 75 mm glass tubes following dissection of the microporous membrane from the transwell. DNA was measured on samples fluorimetrically using Hoechst 33258 (Calbiochem-Behring, La Jolla, CA) according to the method of Labarca and Paigen [16].

RESULTS

The incubation of MCF-7 cells with estradiol (10^{-13} to 10^{-9} M) for a 4-day period produced a dose-related increase in DNA/well. Maximal stimulation with estradiol (10^{-10} to 10^{-9} M) produced more than a 3-fold increase over control (see Fig. 2). Similar incubation of these cells with the non-steroidal antiestrogens, tamoxifen and 4-hydroxytamoxifen (see Fig. 1), produced only a slight increase in DNA/well over control, consistent with these agents being weak partial agonists (see Fig. 2). The weak stimulatory effect of 4-hydroxytamoxifen occurred at a lower concentration than that of tamoxifen. The steroidal antiestrogen, Ru39411 (see Fig. 1), was also a weak partial agonist in the assay whereas a different steroidal antiestrogen, ICI 164384 (see Fig. 1), produced no increase in DNA content over control (see Fig. 2).

All these antiestrogens were demonstrated to be capable of preventing estradiol (10^{-11} M) stimulated MCF-7 cell growth in a dose related manner when incubated for the 4-day period (see Fig. 3). The hormone-independent breast cancer cell line MDA-MB-231 was found to plate and grow on the collagen coated transwell membrane (data not shown). No whole cells were observed to pass through the membrane and no detectable DNA was measurable in the well beneath.

Following plating of MCF-7 cells in tissue culture dishes and MDA-MB-231 cells on transwell membranes the two cell types could be co-cultured as shown in Fig. 4. Co-culture of MDA-MB-231 cells plated at 150,000 cells/transwell with MCF-7 cells produced an increase (80%) in the DNA/well from MCF-7 cells after 4 days of incubation (see Fig. 5). A greater stimulation of the MCF-7 cells could not be produced by increasing the number of MDA-MB-231 cell (see Fig. 5). Co-culture of MCF-7 cells with 250,000 MDA-MB-231 in the presence of concentrations of either the steroidal or non-steroidal antiestrogens previously shown to be inhibitory to estrogen stimulated growth, failed to prevent the MDA-MB-231 cells from stimulating the MCF-7 cells (see Fig. 6). Furthermore, the weak partial

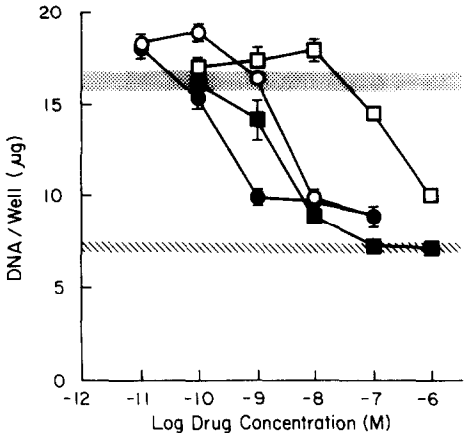

Fig. 3. Inhibition of estradiol (10^{-11} M) stimulated MCF-7 cell growth by various concentrations of Ru39411 (●—●), 4-hydroxytamoxifen (○—○), tamoxifen (□—□), and ICI 164384 (■—■). MCF-7 cells were grown with drugs for 4 days before DNA determination ± S.E.M. were made. Estradiol (10^{-11} M; stippled bar) and control containing no estradiol or antiestrogen (hatched bar) show the mean ± S.E.M.

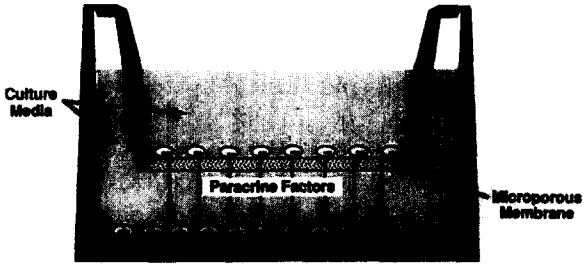

Fig. 4. Diagrammatic representation of MDA-MB-231 cells (⊖) plated on a transwell-col microporous membrane being co-cultured with MCF-7 cells (⊕) plated on the bottom of a 6-well tissue culture dish. The microporous membrane (3 µm) will allow paracrine factors secreted from the MDA-MB-231 cells to interact with the MCF-7 cells, however, the membrane will prevent cell mixing.

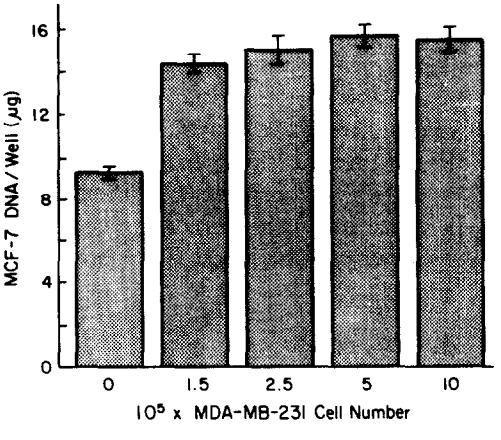

Fig. 5. Stimulation of MCF-7 cell growth by co-culture with MDA-MB-231 cells (see Fig. 4). MCF-7 cells were co-cultured for 4 days with transwells containing plated MDA-MB-231 cells (numbers as shown) before DNA determinations (± S.E.M.) were made on the MCF-7 cells in the 6-well culture dishes following removal of the transwells containing the MDA-MB-231 cells.

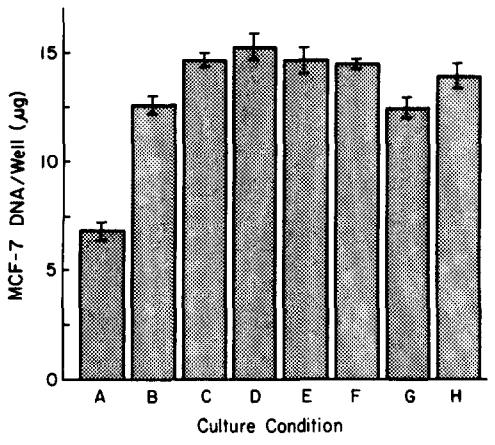

Fig. 6. Effect of antiestrogens on the stimulation of MCF-7 cell growth by co-culture with MDA-MB-231 cells (see Fig. 4). MCF-7 cells were co-cultured for 4 days with transwells containing either: (A) no MDA-MB-231 cells in the presence of no antiestrogen, or 250,000 MDA-MB-231 cells in the presence of (B) no antiestrogen, (C) 10^{-8} M tamoxifen, (D) 10^{-6} M tamoxifen, (E) 10^{-9} M 4-hydroxytamoxifen, (F) 10^{-7} M 4-hydroxytamoxifen, (G) 10^{-6} M ICI 164384, (H) 10^{-7} M Ru39411. DNA determinations ± S.E.M. were made on the MCF-7 cells in the 6-well culture dishes following removal of the transwells containing the MDA-MB-231 cells.

agonist action of tamoxifen, 4-hydroxytamoxifen and Ru39411 was still present in addition to the paracrine stimulation of MCF-7 cells produced by the MDA-MB-231. Consistent with ICI 164384 being a pure antiestrogen, this agent did not produce this additional stimulation of MCF-7 cells. However, in keeping with the other antiestrogens tested, ICI 164384 did not reduce MDA-MB-231 stimulation of MCF-7 cells.

DISCUSSION

The hormone-independent breast cancer cell line MDA-MB-231 has been reported to secrete IGF-1 [14] and TGFα [12], both of which have been shown to be stimulatory to MCF-7 cells [17, 18]. Our findings that at certain ratios in co-culture MDA-MB-231 cells can stimulate the growth of MCF-7 cells in a paracrine manner is consistent with these data. As described in the Introduction, on the basis of a representative growth factor (EGF) antiestrogens cannot prevent the growth stimulation of MCF-7 cells by this class of agent, although at least one report has indicated otherwise [19]. The co-culture system may be more representative of a heterogeneous tumor because of the multiple growth factors secreted by MDA-MB-231 cells and the possible inhibitory influence that MFC-7 cells could have on the MDA-MB-231 cells [20]. Using concentrations of antiestrogens demonstrated to be inhibitory to estrogen mediated MCF-7 cell growth, no decrease was produced in the maximal paracrine stimulation produced by MDA-MB-231 cells. This was observed for both the non-steroidal (4-hydroxytamoxifen and tamoxifen) and the steroidal antiestrogens (ICI 164384 and Ru39411). The only differences seen between the antiestrogens was the relative potencies in inhibiting estradiol stimulated MCF-7 cell growth and the degree of partial agonist activity. Tamoxifen and 4-hydroxytamoxifen have previously been reported to have partial agonist activity [21, 22]. The new steroidal antiestrogen Ru39411 has a similar degree of partial agonist activity as tamoxifen and 4-hydroxytamoxifen, however it is a more potent antagonist than either of these agents. Consistent with previous reports, ICI 164384 was a pure antiestrogen [23] and produced no stimulation of MCF-7 cells. Interestingly, not only were the antiestrogens incapable of preventing the paracrine stimulation of MCF-7 cells but the weak partial agonist action of these agents was produced above and beyond the maximal paracrine stimulation. Since the paracrine stimulation was maximal the additional stimulation must be mediated via systems other than those already activated.

These data suggest that the paracrine influences of hormone independent breast cancer cells may affect the tumoristatic action of antiestrogens, and ultimately be involved in tumor progression. Furthermore, if estrogen receptor positive cells can be stimulated to replicate in heterogeneous tumors in the absence of estrogen then the presence of the estrogen receptor need not be an indication of a tumor that requires estrogens for the maintenance of growth. This may partially account for why only 60% of estrogen receptor containing tumors are seen to respond to endocrine therapy [24] and why the presence of both estrogen and progesterone receptors is a better predictive marker than estrogen receptors alone [1].

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REFERENCES

1. McGuire WL. Steroid receptors in breast cancer treatment strategy. *Recent Prog Hormone Res* 1980, **36**, 135–146.
2. Furr BJA, Jordan VC. The pharmacology and clinical use of tamoxifen. *Pharmacol Ther* 1984, **25**, 127–205.
3. Jordan VC, Robinson SP, Welshons WV. Resistance to antiestrogen therapy. In: Kessel D, ed. *Resistance to Antineoplastic Drugs*. Boca Raton, CRC Press, 1988. 403–427.

4. Gottardis MM, Robinson SP, Satyaswaroop PG, Jordan VC. Contrasting actions of tamoxifen on endometrial and breast tumor growth in the athymic mouse. *Cancer Res* 1988, **48**, 812–815.
5. Robinson SP, Jordan VC. Reversal of the antitumor effects of tamoxifen by progesterone in the 7,12-dimethylbenzanthracene-induced rat mammary carcinoma model. *Cancer Res* 1987, **47**, 5386–5390.
6. Cormier EM, Jordan VC. Contrasting ability of antiestrogens to inhibit MCF-7 growth stimulated by estradiol or epidermal growth factor. *Eur J Cancer Clin Oncol* 1989, **25**, 57–63.
7. King WJ, DeSombre ER, Jensen EV, Greene GL. Comparison of immunocytochemical and steroid-binding assays for estrogen receptor in human breast tumors. *Cancer Res* 1985, **45**, 293–304.
8. Shimada A, Kimura S, Abe K *et al.* Immunocytochemical staining of estrogen receptor in paraffin sections of human breast cancer by use of monoclonal antibody: comparison with that in frozen sections. *Proc Natl Acad Sci USA* 1985, **82**, 4803–4807.
9. Perrot-Applanat M, Groyer-Picard M-T, Lorenzo F *et al.* Immunocytochemical study with monoclonal antibodies to progesterone receptor in human breast tumors. *Cancer Res* 1987, **47**, 2652–2661.
10. Van Netten JP, Algard FT, Coy P *et al.* Heterogeneous estrogen receptor levels detected via multiple microsamples from individual breast cancers. *Cancer* 1985, **56**, 2019–2024.
11. Lippman ME, Dickson RB, Bates S *et al.* Autocrine and paracrine growth regulation of human breast cancer. *Breast Cancer Res Treat* 1986, **7**, 59–70.
12. Dickson RB, Bates SE, McManaway ME, Lippman ME. Characterization of estrogen responsive transforming activity in human breast cancer cell lines. *Cancer Res* 1986, **46**, 1707–1713.
13. Salomon DS, Zwiebel JA, Bano M, Losonczy I, Fehnel P, Kidwell WR. Presence of transforming growth factors in human breast cancer cells. *Cancer Res* 1984, **44**, 4069–4077.
14. Huff KK, Kaufman D, Gabbay KH, Spencer EM, Lippman ME, Dickson RB. Secretion of an insulin-like growth factor-I-related protein by human breast cancer cells. *Cancer Res* 1986, **46**, 4613–4619.
15. Koga M, Sutherland RL. Epidermal growth factor partially reverses the inhibitory effects of antiestrogens on T47D human breast cancer cell growth. *Biochem Biophys Res Comm* 1987, **146**, 739–745.
16. LaBarca C, Paigen K. A simple, rapid, and sensitive DNA assay procedure. *Anal Biochem* 1980, **102**, 344–352.
17. Karey KP, Sirbasku DA. Differential responsiveness of human breast cancer cell lines MCF-7 and T47D to growth factors and 17β-estradiol. *Cancer Res* 1988, **48**, 4083–4092.
18. Dickson RB, Kasid A, Huff KK *et al.* Activation of growth factor secretion in tumorigenic states of breast cancer induced by 17β-estradiol or v-Ha-*ras* oncogene. *Proc Natl Acad Sci USA* 1987, **84**, 837–841.
19. Vignon F, Bouton MM, Rochefort H. Antiestrogens inhibit the mitogenic effect of growth factors on breast cancer cells in the total absence of estrogens. *Biochem Biophys Res Comm* 1987, **146**, 1502–1508.
20. Knabbe C, Lippman ME, Wakefield LM *et al.* Evidence that transforming growth factor-β is a hormonally regulated negative growth factor in human breast cancer cells. *Cell* 1987, **48**, 417–428.
21. Reddel RR, Sutherland RC. Tamoxifen stimulation of human breast cancer cell proliferation *in vitro*: a possible model for tamoxifen tumor flare. *Eur J Cancer Clin Oncol* 1984, **20**, 1419–1424.
22. Darbre PD, Curtis S, King RJB. Effects of estradiol and tamoxifen on human breast cancer cells in serum free culture. *Cancer Res* 1984, **44**, 2790–2793.
23. Wakeling AE, Bowler J. Novel antiestrogens without partial agonist activity. *J Endocrinol* 1987, **112**, 7–10.
24. McGuire WC, Chamness G, Horwitz KB, Zava DT. Hormones and their receptors in breast cancer. In: O'Malley BW, Birnbaumer L, eds. *Receptors and Hormone Action*. New York, Academic Press 1978, Vol. II, 402–431.