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PROGESTIN REGULATION OF EGF-RECEPTOR mRNA ACCUMULATION IN T-47D HUMAN BREAST CANCER CELLS

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Incubation of T-47D human breast cancer cells with the synthetic progestin, medroxyprogesterone acetate (MPA), resulted in a time and dose-dependent increase in epidermal growth factor-receptor (EGF-R) mRNA. Concentrations of MPA as low as 1 nM resulted in a greater than five fold increase in EGF-R mRNA. A significant increase (2-3 fold) in EGF-R mRNA was apparent 12 hr after exposure to MPA and a further increase was seen 12-48 hr after addition of MPA to the cultures. From these studies it is concluded that the increased EGF binding in progestin-treated T-47D cells results at least in part from increased EGF-R gene expression. We believe this is the first report of a steroid hormone modulating expression of this growth factor receptor gene.

Previously we have shown by classical ligand binding studies that the level of the epidermal growth factor receptor (EGF-R) is increased by progestins in T-47D human breast cancer cells (1). The mechanisms whereby progestins increased EGF binding was not clear. By analogy with other receptor systems a number of mechanisms may mediated the increase in EGF binding apparent in progestin-treated T47-D cells. These include an increase translocation of latent receptors from intracellular compartments to the cell membrane, increased membrane fluidity allowing for exposure of cryptic receptors, enhanced receptor recycling, decreased receptor degradation and increased receptor synthesis (2). Although inhibition of protein synthesis prevented the progestin-induced increase in EGF-R in T47-D cells (1), it was not clear at what levels progestins exerted an effect on EGF-R. We now present evidence which demonstrates that progestin regulation of EGF-R in

Abbreviations used; MPA, medroxyprogesterone acetate; EGF, epidermal growth factor; EGF-R, epidermal growth factor receptor.

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T-47D cells occurs, at least in part, at the level of EGF-R mRNA accumulation.

Materials and Methods

Cell Culture. T-47D human breast cancer cells and other human breast cancer cells were obtained from sources listed previously (3) and were grown in Dulbecco's minimal essential medium supplemented with 10% fetal bovine serum or 10% charcoal-stripped fetal calf serum, glutamine, glucose and penicillin-streptomycin. For experiments the cells were grown to confluence in the above medium, the medium was replaced with fresh medium and MPA was added directly from 1000X stock solutions in ethanol to achieve the concentrations indicated. Cells were harvested by scraping the cells off the monolayer with a rubber policeman. After centrifugation the cell pellet was snap frozen and stored at -70^{o}C until RNA preparation.

RNA extraction and Northern blot analysis. RNA was isolated by the guanidinium thiocyanate/cesium chloride method (4). Poly (A^+) RNA was isolated by one cycle of oligo(dT) cellulose chromatography (5). Ten to 15 ug of poly (A^+) RNA was analysed by electrophoresis and transfered to nitrocellulose paper which was hydridized with the ^{32}cDNA insert of pE7 (kindly provided by Dr. I. Pastan) labelled with ^{32}P by nick-translation (Amersham, Oakville, Canada). Hybridizations were performed at 42C in the presence of 50% (v/v) deionized formamide. At the end of the hybridization period the blots were washed as previously described with the final wash in 0.1% SDS, 0.1 x SSC, (1x SSC = 0.15M NaCl, 0.015M sodium citrate) for 45-60 min at 65^{o}C. Filters were also hybridized with chicken beta-actin cDNA (6) as a control for differences in the amount of RNA loaded on the gel. Hybridization signals were quantitated by densitometric scanning of multiple autoradiograms of various exposures, and was expressed relative to the control, arbitrarily assigned a value of 1.

RESULTS

The effect of various concentrations of MPA on the level of EGF-R mRNA was examined in the first instance. As previously described, EGF-R cDNA hybridized to two major transcripts of approximately 11.5 and 6.7 kilobases in T-47D cell mRNA (Fig. 1). In addition a minor transcript of 7.7 kilobases was apparent in some experiments. In cultures incubated for 24 hrs in the presence of the MPA a 6 - 8 fold increase in abundance of both major EGF-R transcripts. This effect was demonstrable with as little as 1 nM MPA while 0.1 nM MPA had only a small effect (less than 2 fold increase) on EGF-R mRNA abundance. These initial experiments were performed in medium containing 10% charcoal-stripped fetal calf serum using cells which had been passaged twice in medium containing charcoal-stripped fetal calf serum. Subsequently we found that a similar effect was seen in cells cultured in medium containing 10% non-charcoal stripped fetal calf

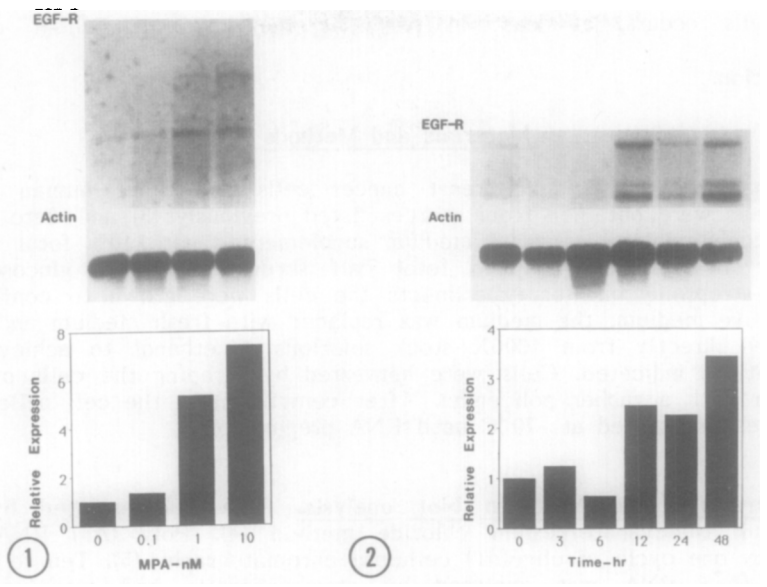

Fig. 1. Dose-dependence of progestin-induced epidermal growth factor-receptor mRNA accumulation. Confluent T-47D cell monolayers were incubated with varying concentrations of medroxyprogesterone acetate for 24 hr in full growth medium. Upper panel shows the pattern of hybridization obtained with pE7 cDNA while the middle panel shows hybridization of the same nitrocellulose filter with actin cDNA as a control for gel loading. In the lower panel the EGF-R mRNA abundance has been quantitated by densitometry, corrected for difference in actin abundance and expressed relative to control cultures.

Fig. 2. Time course of progestin-induced epidermal growth factor-receptor expression. Confluent T-47D cell monolayers were incubated with 10 nM medroxyprogesterone acetate for varying times in full growth medium. Upper panel shows the pattern of hybridization obtained with pE7 and actin cDNAs are shown.

serum. For convenience the subsequent experiments have been carried out with cells grown only in medium containing 10% fetal calf serum.

The time-course of progestin-induced EGF-R expression is shown in Fig. 2. A significant increase in EGF-R mRNA was apparent at 12 hrs after addition of MPA. A co-ordinate increase in both major EGF-R mRNAs and the minor transcript was seen. Since previous reports of EGF-R transcripts in breast cancer and other cell lines have not reported the 7.7 kilobase transcript (7,8) we examined EGF-R transcript size in other human breast cancer cell lines including the MDA 468 cell line which has been reported to contain high levels of EGF-R mRNA (7). Fig. 3 shows the hybridization obtained with the EGF-R cDNA in a number of human breast cancer cell lines. The 7.7 kilobase transcript was detected in most of the cell lines

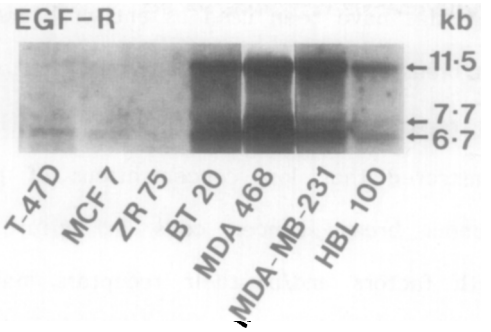

Fig. 3. Epidermal growth factor-receptor expression in human breast cancer cell lines. Ten micrograms of poly (A^+) RNA was extracted from confluent cell monolayers was analysed by electrophoresis and Northern transfer. The pattern of hybridization obtained with pE7 cDNA is shown.

examined. EGF-R mRNA abundance varied among cell lines with the highest levels being found in MDA-MB-231, BT 20 and MDA 468. The latter two cell lines have been shown previously to have amplified EGF-R gene (7, 9).

DISCUSSION

We have previously reported that progestins induce ^{125}I-EGF binding in T-47D human breast cancer cells (1). We have also demonstrated that in this cell line, the effect is specific for progestins and that the magnitude of the progestin effect in various other human breast cancer cell lines correlates with progesterone receptor concentration (1). In this report we have demonstrated that the progestin-increased EGF-R can be at least in part explained by increased expression of the EGF-R gene. Although we can not exclude other mechanisms including EGF-receptor recycling, turn-over or exposure of cryptic receptors, the time-course of the increased EGF-R mRNA accumulation show striking similarity to that previously demonstrated by ligand binding studies. The magnitude of the progestin effect on EGF-R mRNA level in T-47D cells is considerably more marked than that seen when EGF binding activity was measured (2-3 fold) and suggests that the increased EGF-R gene expression would be more than sufficient to explain the increased binding observed. Furthermore, Lippman et al., have shown that there is a good correlation between EGF receptors as measured by direct binding assays and EGF-R mRNA in a series of human breast cancer cells (10).

Although progestins have been used as anti-proliferative agents in the treatment of human breast cancer (11) and early in vitro studies with human breast cancer cells reported growth inhibitory effects (12,13) more recent studies have demonstrated that low concentrations of progestin stimulate proliferation of human breast cancer cells in culture (14). Enhanced expression of growth factors and/or their receptors may be part of the mechanism whereby progestins modulate cellular proliferation.

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