Vol. 149, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1987 Pages 276-281

AN ANTIBODY TO THE RECEPTOR FOR INSULIN-LIKE GROWTH FACTOR I INHIBITS THE GROWTH OF MCF-7 CELLS IN TISSUE CULTURE

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Received October 23, 1987

SUMMARY: Alpha IR-3, a monoclonal antibody to the insulin-like growth factor I receptor which blocks insulin-like growth factor I binding and inhibits its activity, inhibits the binding of ^{125}I-insulin-like growth factor I to MCF-7 cells (an estrogen dependent human breast carcinoma cell line) with an IC-50 of approximately 100 ng/ml. It also inhibits the growth of MCF-7 cells cultured in 5% calf serum with approximately the same IC-50. Inhibition of growth occurs both when cells are cultured in the presence and absence of estrogen and is more pronounced when cells are grown at a low density. These findings demonstrate a requirement for insulin-like growth factor I for optimal growth of MCF-7 cells and suggest that it is an autocrine growth factor in these cells.

Several human breast carcinoma cell lines, including estrogen dependent MCF-7 cells, secrete insulin-like growth factor I (1,2). They also have receptors for insulin-like growth factor I and respond to it with increased thymidine incorporation and cell growth (3,4), raising the possibility that insulin-like growth factor I is an autocrine growth factor in these cells. We have used αIR-3, a murine monoclonal antibody that is highly specific for the insulin-like growth factor I receptor and both blocks insulin-like growth factor I binding and biological response but does not affect the closely related insulin and insulin-like growth factor II receptors (5-9), to further evaluate the role of insulin-like growth factor I in the growth of MCF-7 cells.

MATERIALS AND METHODS

MCF-7 cells were obtained from the American Type Culture Collection (designation HTB 22) and grown in phenol red-free Eagle's minimum essential

0006-291X/87 $1.50

medium (Hazelton Laboratories) supplemented with non-essential amino acids, sodium pyravate, 5% serum (HyClone calf bovine-supplemented, lot no. 2151644), 5 ng/ml insulin, and 1 nM estradiol. Where indicated, serum was used that had been incubated with dextran coated charcoal as described (10) to remove endogenous estrogens. For cell growth studies, cells were detached by washing twice with 1 mM EDTA in phosphate buffered saline and once with 0.05% trypsin. For binding studies, cells were detached by washing twice with 1 mM EDTA in phosphate buffered saline and sharply agitating the flask. In some studies, cell number was assessed by neutral red dye uptake (14), which was found to be proportional to cell number.

Alpha IR-3 producing hybridomas were grown as peritoneal tumors in SJL x Balb/C F_1 hybrid mice and ascites fluid harvested as previously described (5). Antibody was purified on a Staphylococcal protein A-agarose column using the Biorad MAP buffer system according to the manufacturer's instructions. Trace impurities were removed by HPLC ion exchange chromatograph as follows: The protein A-agarose eluate was dialysed against 50 mM TrisHCl, pH 8.0 and applied to a Mono Q column equilibrated with the same buffer. The column was eluted with a 120 ml linear gradient of 50 mM TrisHCl, pH 8.0 to 50 mM TrisHCl, pH 8.0, containing 500 mM NaCl run over 30 minutes. The resulting antibody, which eluted as a sharp peak, was more than 95% pure on SDS polyacrylamide gel electrophoresis and was found to be free of mycoplasm and endotoxin.

[T59] insulin-like growth factor I was obtained from Amgen and iodinated with chloramine T. To determine ^{125}I-insulin-like growth factor I binding, MCF-7 cells were washed and suspended in phosphate buffered saline containing 1 mg/ml bovine albumin. The cells were incubated at 15 degrees in 200 µl of this buffer with ^{125}I-insulin-like growth factor I and the indicated additions. After 90 minutes, 150 µl of the cell suspension was removed and layered over 200 µl of ice cold buffer in a 500 µl microfuge tube. The cells were pelleted and washed with another 200 µl of ice cold buffer. After aspiration of the supernatant the tube was cut above the pellet and counted.

RESULTS

When MCF-7 were cultured in 5% calf serum with 1 nM estradiol, αIR-3 significantly decreased cell number at various times up to seven days (Fig. 1). This appeared to be due to a decrease in the rate of cell growth rather than to a cytotoxic effect of αIR-3, since cell viability (assessed by the uptake of neutral red, a vital dye) was greater than 95% in antibody treated cells. The concentration dependence for inhibition by αIR-3 of cell growth and ^{125}I-insulin-like growth factor I binding is shown in Fig. 2. Eighty-five (85%) of the total ^{125}I-insulin like growth factor binding could be displaced by 13 µg/ml αIR-3. This fraction probably represents specific receptor binding. Unlabeled insulin-like growth factor I, 1 µg/ml, caused a similar degree of inhibition, while 100 µg/ml insulin inhibited binding by 74%. The dose response for inhibition of growth was very comparable to the dose response for inhibition of specific ^{125}I-insulin-like growth factor

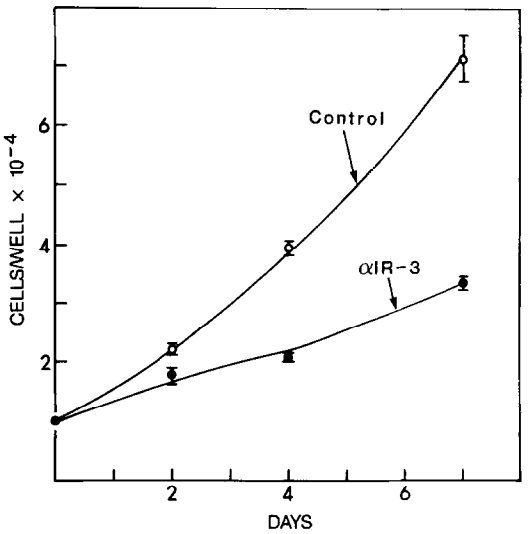

Fig. 1. Effect of αIR-3 on growth of MCF-7 cells. MCF-7 cells were plated in 24 well Costar dishes (10,000 cells per 16 mm well) and grown in phenol red-free modified Eagle medium containing 5% calf serum and 1 nM estradiol. After 2 hours, αIR-3 (13μg/ml) was added. Fresh medium, containing αIR-3 where appropriate, was added after 4 days in culture. At various times, the cells were detached from the wells and counted in a Coulter counter. The results shown are the mean and standard error of quadruplicate determinations.

binding. The IC-50's for both processes were approximately 100 ng/ml. Since both processes were measured under different conditions, and cell number may not be linearly related to growth inhibition, this may be

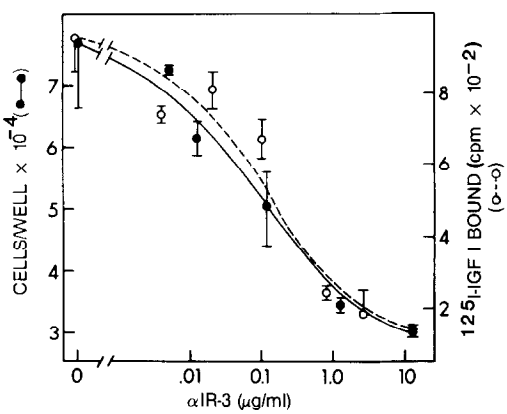

Fig. 2. Concentration dependence of αIR-3 inhibition of cell growth and ^{125}I-insulin-like growth factor I binding. For cell growth (●——●), 10,000 cells were plated in 24 well Costar dishes (16 mm dia.) and grown in phenol red-free modified Eagle medium containing 5% calf serum and 1 nM estradiol. After 2 hours, the indicated concentrations of αIR-3 were added. Fresh medium with appropriate concentrations of αIR-3 was added after 4 days and the cells detached and counted after 6 days. For binding (0----0), 20,000 cpm of ^{125}I-insulin-like growth factor I and indicated concentrations of αIR-3 were incubated with 300,000 MCF-7 cells and bound ^{125}I-insulin-like growth factor I was measured as described in Methods section.

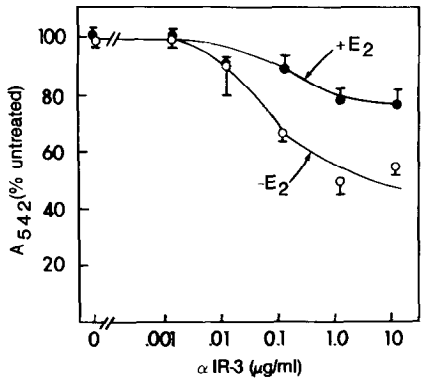

Fig. 3. Effect of estradiol on growth inhibition by αIR-3. MCF-7 cells were grown as in Fig. 1 except that 2% charcoal stripped serum was used with (●) or without (○) 1 nM estradiol. After four days, cell number was assessed by neutral red dye uptake. Results are expressed as percent of control which was A_{452}=.548±.015 for cells grown with 1 nM estradiol and A_{542}=.359±.004 for cells grown without estradiol.

somewhat coincidental; however, it does suggest that the inhibitory action of the antibody was mediated through the insulin-like growth factor I receptor.

When MCF-7 cells were grown for four days in 2% serum that had been stripped with dextran coated charcoal to remove endogenous estrogens, the addition of 1 nM estradiol increased cell number 1.54 fold (Fig. 3). Alpha IR-3 decreased the number of cells grown both in the absence and presence of estradiol but was considerably more inhibitory when cells were grown in the absence of estradiol.

The effect of cell density on the inhibitory activity of αIR-3 is shown in Table I. Although αIR-3 inhibited cell growth when cells were plated at both low and high density, it was considerably more effective at low cell density.

Table 1. Effect of cell density on growth inhibition by αIR-3

Plating density	Cells per well after 6 days	
Cells per well	Control	αIR-3 (13 ug/ml)
10,000	76,600 ± 10,400	30,313 ± 800
100,000	679,000 ± 3,500	579,800 ± 8000

Cells were plated in Costar 24 well dishes (16 mm diameter wells) in phenol red free modified Eagles medium containing 5% calf serum and 1nM estradiol. After 2 hours αIR-3 was added to the indicated wells. Six days later, the cells were detached with EDTA and counted in a Coulter counter. Results are the mean and standard error of quadruplicate determinations.

DISCUSSION

Previous studies have shown that MCF-7 cells have insulin-like growth factor I receptors and in culture both secrete insulin-like growth factor I and respond to it with increased cell growth (1-4). Tumor formation by MCF-7 cells in nude mice is estrogen dependent (10); however, the requirement for estrogen can be partially replaced by administering insulin-like growth factor I (4), indicating that it is an important growth factor for this cell line in vivo as well as in tissue culture.

The main finding of the present study is that an insulin-like growth factor I receptor antagonist, αIR-3, inhibits the growth of cultured MCF-7 cells. Since the cells were grown in 5% calf serum, which may contain small amounts of insulin-like growth factor I, the present study does not definitively establish an autocrine role for insulin-like growth factor I; however, in conjunction with information from previous studies (1-4), this possibility seems quite likely. Since serum might contain other growth factors that could conceivably diminish the requirements for insulin-like growth factor I, the fact that αIR-3 inhibited the growth of MCF-7 cells in serum is in some respects more significant in establishing that insulin-like growth factor I is necessary for optimal growth than if the studies were carried out in serum free defined media.

Although insulin-like growth factor I secretion is enhanced somewhat by estrogen, MCF-7 cells secrete substantial amounts of insulin-like growth factor I in its absence(1). Consistent with the limited estrogen dependence of insulin-like growth factor I secretion, αIR-3 inhibited their growth both in the presence and absence of estrogen. In fact, αIR-3 was more effective in the absence of estrogen. A possible explanation for this is as follows: MCF-7 cells secrete a number of growth factors in a fashion that is more highly estrogen dependent than insulin-like growth factor I (12). One of these, TGF α, is a progression factor like insulin-like growth factor I and in some cells can substitute for insulin-like growth factor I. In the presence of estrogen, high levels of secreted TGF α may partially overcome

the requirement for insulin-like growth factor I. Alpha IR-3 was found to be more effective in inhibiting the growth of cells plated at a low density. This can similarly be explained by a higher concentration of other secreted growth factors in the medium of cells grown at high density, although other explanations are also possible. These results suggest that a combination of growth factor receptor antagonists might be more effective than αIR-3 alone in inhibiting the growth of MCF-7 cells.

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