Vol. 164, No. 1, 1989
October 16, 1989

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 277-283

STIMULATION OF GROWTH OF HUMAN BREAST CANCER CELL LINES IN CULTURE BY LINOLEIC ACID

David P. Rose and Jeanne M. Connolly

Division of Nutrition and Endocrinology, American Health Foundation, Valhalla, New York 10595

Received September 4, 1989

Linoleic acid, an omega-6 unsaturated fatty acid, stimulated growth of the MDA-MB-231 and MCF-7 human breast cancer cell lines in culture. Responses of the estrogen-independent MDA-MB-231 cells both in serum-free medium and with 1% fetal bovine serum added were positively correlated with linoleic acid concentration over the entire range examined (5-750 ng/ml). Growth stimulation of the estrogen-responsive MCF-7 cell line was maximal at a LA concentration of 500 ng/ml when cultured in 1% fetal bovine serum-containing medium with added estradiol. Linoleic acid had no mitogenic effect on three human cancer cell lines derived from sites other than breast, or on untransformed 3T3 cells.

Evidence from epidemiological studies suggests a role for dietary fat in the etiology of breast cancer (1-3). While an effect on prognosis is less certain, there is some indication that high fat consumption is associated with a reduced survival time in patients with breast cancer which has spread to distant sites (4). In animal models, the level of dietary fat intake has been shown to influence both the development of chemically-induced mammary tumors (5,6), and the growth and metastatic behavior of transplantable mammary carcinomas (7,8). However, not only does the quantity of fat consumed need to be considered, but so also does its FA composition; those fats high in LA, an omega-6 unsaturated FA, enhance the growth (5,6) and metastasis (7) of rat mammary tumors, whereas fish oil, rich in omega-3 FA, has an inhibitory effect (8,9). In contrast to these results from ani-

Abbreviations: LA, linoleic acid; FA, fatty acid; IMDM, Iscove's modified Dulbecco's medium; FBS, fetal bovine serum; DMEM, Dulbecco's modified Eagles medium; DMBA, dimethylbenz-(a)anthracene.

0006-291X/89 $1.50

mal experiments, a recent epidemiological study of human breast cancer associated risk of the disease with high consumption of fats rich in the saturated FAs (10).

The present study was performed to determine the effect of LA on the growth of two human breast cancer cell lines in vitro. The MDA-MB-231 breast cancer cell line represents the autonomous type of tumors which does not respond to estrogens (11) whereas the MCF-7 breast cancer cell line has retained hormone responsiveness and is stimulated to grow in culture by estradiol (12). For comparison, human cell lines derived from cancers of the prostate (DU 145) and lung (A421), an epidermoid carcinoma (A431), and the untransformed NIH 3T3 cell line were included in the study.

MATERIALS AND METHODS

Cell Culture. All six cell lines were obtained from the American Type Culture Collection. Cells were grown at 37°C in a humidified 95% air-5% CO_2 atmosphere. The medium for routine culture maintenance of the breast cancer cells was IMDM (Gibco, Grand Island, NY) with the addition of 5% FBS (Gibco), 100,000 units/l penicillin and 100 mg/l streptomycin. The cells were trypsinized and replated into fresh medium every 7 days. For the growth experiments, breast cancer cells were cultured for 24 hr in 24-well plates (Costar, Cambridge, MA) containing 5% FBS-supplemented medium, and then washed with IMDM. The initial plating density of the MCF-7 cells was 1.0×10^5 and of the MDA-MB-231 cells 5.0×10^4 cells/ml/well. After the FBS-supplemented medium was replaced by the basal medium plus the desired additions, each being set up in triplicate, incubation was continued for 5 or 6 days, with feeding after 3 days. The cell number was then determined with a Coulter Counter after harvesting by trypsinization.

The DU 145 cells were cultured routinely in RPMI-1640 medium plus 5% FBS, and the A431, A427, and 3T3 cells in DMEM (Gibco) plus 5% FBS. For the growth experiments the initial plating densities were: DU 145 cells, 5×10^3; 3T3 cells, 15×10^3; A431 cells, 15 and 30×10^3; A427 cells, 30×10^3 cells/ml/well. All cultures were performed in 24-well plates, in the presence of 1% FBS plus the desired additions of LA, and for a 6 day incubation period.

Delipidized BSA was obtained from Collaborative Research (Lexington, MA), and insulin, 17β-estradiol, transferrin, and LA-BSA conjugate from Sigma. The LA-BSA comprised 1 μg of LA/200 μg of complex.

RESULTS

MDA-MB-231 Cell Line. The growth experiments were performed in IMDM with or without 1% FBS, plus delipidized BSA, and LA-BSA

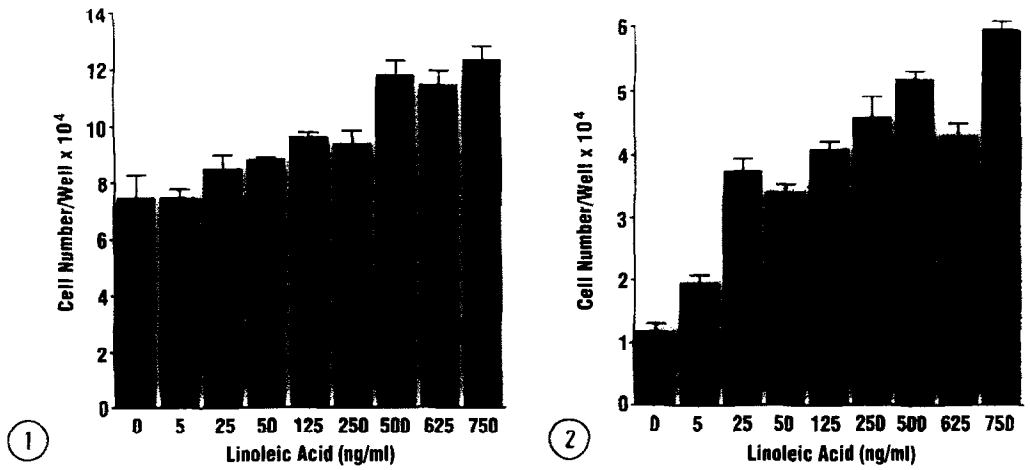

Fig. 1 The growth response of MDA-MB-231 breast cancer cells, cultured in 1% FBS-containing medium, to increasing concentrations of LA. Cell proliferation was evaluated after 5 days incubation. Error bars represent the SEM.

Fig. 2 The growth response of MDA-MB-231 breast cancer cells to increasing concentrations of LA when grown under serum-free conditions for 6 days. Error bars represent the SEM.

complex additions over the range 1 - 150 μg/ml (5-750 ng/ml of LA), but with the final BSA concentration always being held constant at 1.25 mg/ml. Cell growth was stimulated by LA when cultured in IMDM with 1% FBS for 5 days, the increase in cell number being directly related to the LA concentration and reaching 165% of control with 750 ng/ml of LA (Fig. 1) When FBS was omitted from the test culture medium, cell number was reduced compared with that observed in the presence of 1% FBS, but there was again an unequivocable stimulation of cell proliferation which was positively correlated with concentration over the entire range of LA levels, and was increased approximately five-fold over the control with 750 ng/ml of LA after 6 days incubation (Fig. 2). We also performed an experiment with 1% FBS-containing medium and a 6 day incubation, but found that the observed stimulation of MDA-MB-231 growth by LA gave a "bell-shaped" dose-response curve with a peak at 250 ng/ml (data not shown).

MCF-7 Cell Line: When these cells were cultured in IMDM plus 1% FBS, BSA, 3 μg/ml of insulin and 1 x 10^{-10}M estradiol, LA stimulated mitogenesis, with an increase in cell number which was maximal at a LA concentration of 500 ng/ml (180% of control) after 6 days (Fig. 3). Under serum-free conditions, but with 5

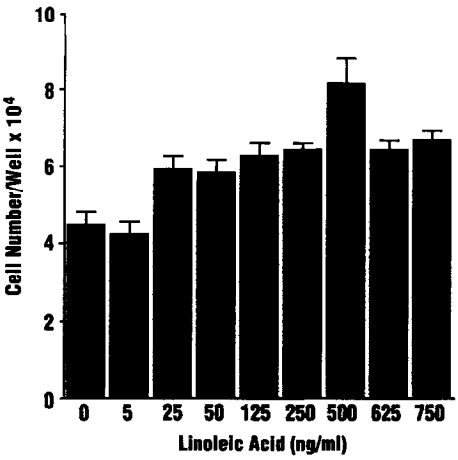

Fig. 3 The growth response of MCF-7 breast cancer cells, cultured in 1% FBS-containing medium supplemented with 3 μg/ml of insulin, 1 x 10^{-10}M estradiol and increasing concentrations of LA.

μg/ml of insulin, 5 μg/ml of transferrin, and 1.25 mg/ml of BSA added to the basal IMDM, LA had no effect on MCF-7 cell growth (data not shown).

Other Cell Lines: None of the human cancer cell lines derived from sites other than breast, or the untransformed mouse 3T3 cells showed a mitogenic response to LA over the 5-750 ng/ml range of concentrations (Table 1).

DISCUSSION

Wicha et al. (14) studied the influence of free FAs, dissolved in ethanol, on DMBA-induced rat mammary carcinoma cells,

Table 1. Changes in the cell number (x 10^4) of four non-breast cancer cell lines grown for 6 days without or with increasing concentrations of linoleic acid

Cell line	Linoleic acid (ng/ml)								
	0	5	25	50	125	250	500	625	750
DU 145	8.6±0.4	7.2±0.5	6.8±0.9	7.2±0.9	6.9±0.1	7.8±0.1	5.8±0.6	5.5±0.5	5.5±0.9
A 427	6.4±0.3	5.0±0.4	6.1±1.2	6.3±0.3	5.8±0.7	6.5±0.6	5.1±0.6	6.4±0.9	5.4±0.9
A 431 (a)	3.4±0.7	2.2±0.2	3.3±0.4	2.7±0.3	3.1±0.7	1.6±0.3	1.6±0.3	1.9±0.2	1.8±0.1
(b)	24.5±1.3	24.2±2.3	22.8±2.8	24.9±2.6	22.7±2.0	21.5±0.3	23.0±2.9	21.6±1.8	21.3±1.8
3T3	8.2±0.7	7.8±0.9	7.1±0.9	7.6±0.4	6.9±0.1	6.6±0.1	6.1±0.1	5.7±0.5	5.2±0.4

grown as primary cultures in medium containing 5% delipidized FBS. Maximal stimulation was observed with oleic acid, although LA was also effective. When the growth-promoting effects of LA were compared in the presence or absence of insulin, hydrocortisone, prolactin, estradiol and progesterone, it was found that this combination of hormones enhanced the stimulatory effect of LA on both ^{3}H-thymidine incorporation and cell growth.

Our use of an LA-BSA complex approximates more closely to physiological conditions than does the addition to culture medium of FA dissolved in ethanol (15). In serum-free IMDM, there was an unequivocable stimulation of MDA-MB-231 cell growth which was positively correlated with concentration over the entire range of LA. Cell growth was also stimulated by LA when cultured in IMDM with 1% FBS, and again the response was directly related to the LA level after 5 days incubation. The peak effect which we observed with 250 ng/ml of LA after 6 days incubation in 1% FBS-containing medium, most likely resulted from inhibition of further growth due to the high cell densities attained with the higher LA concentrations.

The MDA-MB-231 cell line was originally derived from an anaplastic autonomous tumor metastasis, and its growth is not steroid hormone-dependent (11). However, growth of MCF-7 breast cancer cells is stimulated by estrogens (12), and in the present study LA increased cell proliferation when added to estradiol-containing medium with 1% FBS. Culture of MCF-7 cells in serum-free medium with supplemental albumin, insulin, transferrin, and estradiol showed no response to LA, suggesting that, in contrast to MDA-MB-231 cells, other nutrients and/or growth factors present in FBS were necessary in order to obtain a mitogenic response.

The stimulatory effect of LA appeared to be restricted to the two breast cancer cell lines. The hormone unresponsive DU 145 prostate cancer cell line appeared to show some inhibition of cell growth at the higher LA concentrations, as did the 3T3 cells.

To our knowledge, the present data are the first to demonstrate that LA, an unsaturated FA, stimulates directly the growth of human breast cancer cells. While the mechanism remains to be determined, LA is the precursor for arachidonic acid synthesis, which itself is the principal substrate for eicosanoid production. Indomethacin, a cyclo-oxygenase inhibitor, suppresses the development of chemically-inducible rat mammary tumors, an effect which is believed to result from a block in the synthesis of prostaglandins by the mammary cells (16), and in preliminary experiments we have found that indomethacin inhibits the growth of both MCF-7 and MDA-MB-231 human breast cancer cells in vitro.

Further studies are planned to examine the influence of dietary LA on the metastatic potential of human breast cancer cell lines when implanted into athymic nude mice. The outcome of such experiments has obvious relevance to clinical trials of a low-fat dietary intervention which have been proposed as an approach to preventing recurrence in surgically-treated breast cancer patients (17).

REFERENCES

1. Rose, D.P., Boyar, A.P., and Wynder, E.L. (1986) Cancer, 58:2363-2371.
2. DeCarli, A., and La Vecchia, C. (1986) Oncology, 43:116-126.
3. Toniolo, P., Riboli, E., Protta, F., Charrel, M., and Cappa, A.P.M. (1989) J. Natl. Cancer Inst. 81:278-286.
4. Gregorio, D.I., Emrich, L.J., Graham, S., Marshall, J.R., and Nemoto, T. (1985) J. Natl. Cancer Inst. 75:37-41.
5. Carroll, K.K., and Braden, L.M. (1984) Nutr. Cancer, 6:254-259.
6. Cohen, L.A., Thompson, D.O., Maeura, Y., Choi, K., Blank, M.E., and Rose, D.P. (1986) J. Natl. Cancer Inst. 77:33-42.
7. Katz, E.B., and Boylan, E.S. (1987) J. Natl. Cancer Inst. 77:351-358.
8. Kort, W.J., Weijma, I.M., Bijma, A.M., van Schalkwijk, W.P., Vergroesen, A.J., and Westbroek, D.L. (1987) J. Natl. Cancer Inst. 79:593-599.
9. Jurkowski, J.J., and Cave, W.T. Jr. (1985) J. Natl Cancer Inst. 74:1145-1150.
10. Toniolo, P., Riboli, E., Protta, F., Charrel, M., and Cappa, A.P.M. (1989) J. Natl. Cancer Inst. 81:278-286.
11. Dickson, R.B., Bates, S.E., McManaway, M.E., and Lippman, M.E. (1986) Cancer Res. 46:1707-1713.
12. Karey, K.P., and Sirbasku, D.A. (1988) Cancer Res. 48:4083-4092.
13. Berthois, Y., Katzenellenbogen, J.A., and Katzenellenbogen, B.S. (1986) Proc. Natl. Acad. Sci. USA, 83:2496-2500.

14. Wicha, M.S., Liotta, L.A., and Kidwell, W.R. (1979) Cancer Res. 39:426-435.
15. Spector, A.A. (1986) Meth. Enzymol. 128:320-339.
16. Carter, C.A., Milholland, R.J., Shea, W., and Ip, M.M. (1983) Cancer Res. 43:3559-3562.
17. Rose, D.P., and Boyar, A.P. (1986) In: B.S. Reddy and L.A. Cohen (eds.), Diet, Nutrition and Cancer: A Critical Evaluation, Vol. 1, CRC Press, New York, pp. 151-166.