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HUMAN BREAST CANCER CELLS SYNTHESIZE AND SECRETE AN EGF-LIKE IMMUNOREACTIVE FACTOR IN CULTURE

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A human breast cancer cell line, strain MCF-7, in culture synthesized and secreted a large amount of a polypeptide (designated as MCF-7 EGF) immunologically related to human epidermal growth factor (hEGF). The molecular weight of MCF-7 EGF estimated by gel filtration on Sephadex G-75 was similar to that of hEGF from human urine. On isoelectric focusing analysis, MCF-7 EGF gave a major peak at pH 4.6 and a minor peak at pH 5.0. In our enzyme immunoassay system, however, the dose-response curve of MCF-7 EGF did not show good parallelism with that of standard hEGF. From these results, it is suggested that MCF-7 cells synthesize and secrete a polypeptide immunologically related to hEGF into the culture medium.

Epidermal growth factor (EGF), a small polypeptide originally isolated from the submaxillary glands of the male mouse (1), is a potent mitogen for a great variety of cell types (2). Subsequently, human EGF (hEGF), isolated from human urine, was found to be identical or closely related to urogastrone. hEGF differs from mouse EGF by 16 of 53 amino acid residues (3,4). hEGF has many seemingly independent effects, particularly on the endocrine glands (5), and is an inhibitor of gastric acid secretion (4). Despite this wide range of effects, the physiological role of EGF and its site of synthesis have not been elucidated yet, but much evidence that the factor plays important roles in regulating cell proliferation and differentiation is accumulating (6,7).

In recent years, several lines of evidence have implicated EGF in the process of neoplastic transformation. It has been shown that EGF stimulates cell proliferation in cultured tumor cells (8,9), enhances the carcinogenicity of methylcholanthrene in mouse skin tumors (10), and potentiates the tumorigenicity of Kirsten sarcoma virus in rat ovarian granulosa cells (11). More recently, Kurachi et al. (12) have shown that sialoadenectomy of tumor-bearing mice caused rapid and sustained cessation of

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the growth of mammary tumors, and this effect of sialoadenectomy was reversed by EGF treatment, indicating that the growth of the mammary tumor was dependent on EGF. Further, Downward et al. (13) have recently reported that the EGF receptor has a high sequence homology with a product of an oncogene found in the avian erythroblastosis virus (v-erbB).

The information presented here reveals that cells derived from human breast cancer, strain MCF-7, synthesize and secrete a large amount of hEGF-like immunoreactive material into the culture medium.

MATERIALS AND METHODS

Materials.

Human EGF and anti-hEGF antiserum were prepared as described previously (14). RPMI 1640 was obtained from Nissui Pharmaceutical Co., Ltd. (Tokyo); fetal calf serum (FCS), from GIBCO; insulin, from NOVO; β-estradiol, from Tokyo Kasei Co., Ltd. (Tokyo).

Cell Culture.

MCF-7 cells were cultured in RPMI 1640 medium supplemented with 10% FCS, insulin (2×10^{-6}M), and β-estradiol (10^{-8}M) in an humidified atmosphere of 5% CO_2/95% air at 37°C. Cells were fed every other day.

Two-Site Enzyme Immunoassay.

The enzyme immunoassay for hEGF sample was performed using anti-hEGF antibody IgG-coated polystyrene beads and anti-hEGF antibody Fab'-linked peroxidase as described (15). The amounts of immunoreactive material are represented as equivalents of the amounts of hEGF isolated from urine.

Protein Determination.

Protein was determined by the method of Lowry et al. (16) with some modifications Bovine serum albumin was used as a standard.

Isoelectric Focusing.

Isoelectric focusing was carried out according to the methods of Vesterverg and Svensson (17). The column (3.4 x 24 cm) contained 1.0% Ampholine (LKB, Sweden) having a pH range of 3.5 to 5.0 in a sucrose gradient (0 to 50%). Electrofocusing was carried out at 4°C for 25 h at a constant voltage of 800 V.

RESULTS AND DISCUSSION

During our study of the site of EGF biosynthesis, we found that normal human fibroblast (WS-1) cells synthesize and secrete hEGF in culture (18). In addition, it was demonstrated that a high level of hEGF exists in urine of patients with gastric cancer (19). The above findings, coupled with the hypothesis proposed by Sporn and Roberts (20) that cancer cells possess the ability to produce and respond to their own growth factors, promoted us to test whether human cancer cells might be capable of secreting such factors.

Previously, we developed a highly sensitive and reliable two-site enzyme immunoassay system (EIA) for hEGF which is based on the sandwiching of an antigen between anti-hEGF antibody IgG-coated polystyrene beads and anti-hEGF antibody Fab'-linked peroxidase (EC. 1.11.1.7., horseradish, Type IV) conjugate. The limit of detection was as low as 0.1 pg/assay tube (15).

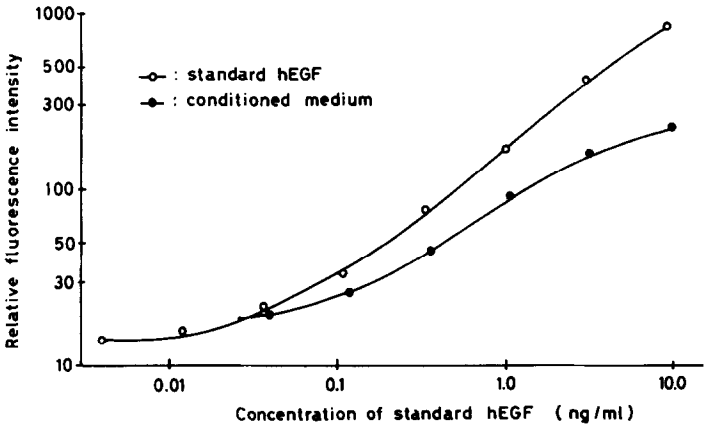

Fig. 1. EIA of Standard hEGF and Immunoreactive Material in the Medium Conditioned by MCF-7 Cells.

Open circles show points for the serial dilution curve of standard hEGF, the amount of which is represented on the abscissa. The ordinate indicates fluorescence intensity. Closed circles show points for the serial dilution curve of the immunoreactive materials in the medium conditioned by MCF-7 cells. Each sample was serially diluted 3-fold with 0.1 M phosphate buffer, pH 7.0, containing 0.1% BSA, 0.3 M NaCl, 0.1% NaN_3, and 1 mM $MgCl_2$. A 0.1 ml aliquot was used for EIA.

Human breast cancer cells, strain MCF-7, were initially derived from malignant effusions of a woman with metastatic breast cancer (21) and characterized with respect to human origin (22). Recently, it was reported that many human breast cancer cells have EGF receptors on their surface and that some of them including MCF-7 cells respond to exogenous EGF with proliferation (23).

Thus, we determined whether the MCF-7 tumor cells synthesize and secrete EGF in culture. When the medium conditioned by MCF-7 cells was assayed by our EIA system, a considerable amount of hEGF-like immunoreactive material was detected. However, quantification of the material was difficult, because the dose-response curve was not parallel with the standard curve of hEGF (Fig. 1).

So, in the following experiments the concentration of the hEGF-like immunoreactive material was estimated from its fluorescence intensity after appropriate dilution as related to that given by hEGF at 200 pg/ml. Therefore a tentative estimation (pg/ml) of hEGF-related material was attempted by assaying diluted samples and by multiplying the dilution factor by 200 pg/ml. The reason why the dose-response curve of the material was not parallel with that of the standard hEGF remains to be clarified. At present, we consider that the reason may depend on interfering species in our EIA system or on the similar but not identical antigenic structure of the material

MCF-7 cells in culture synthesized a molecule which immunologically cross-reacted with hEGF from human urine during the course of incubation (Fig. 2). The amount of the immunoreactive material (ng per day, represented as equivalents of hEGF as estimated by the above method) increased with the increase in cell number. The amount per

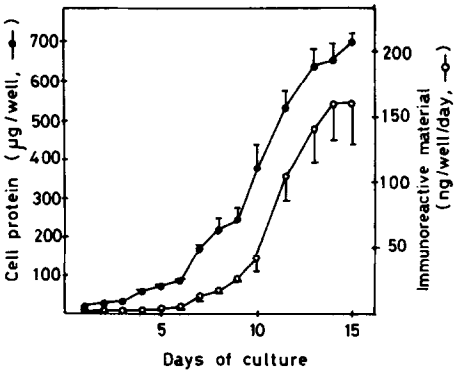

Fig. 2. Changes in Amount of Immunoreactive Material in the Conditioned Medium and of Cell Protein during Culture.

MCF-7 cells were subcultured in 24-well plates at a density of 2 x 10^4 cells/well (well surface 2.1 cm^2) from day 0. On the indicated days, the medium was changed and the cultures incubated further. After 24 h, the medium was collected. Cells were washed with PBS and then lyzed with 0.5 ml of 0.1 N NaOH. The cell protein content (●) was determined as described in MATERIALS AND METHODS. The amount of immunoreactive material (○) was tentatively estimated by the method described in the text. Each point is the mean ± S.E. of three determinations.

cell protein also gradually increased during the culture period. hEGF in the medium used in culture for MCF-7 cells was not detected by our EIA system.

Recently, we reported that normal human fibroblast (WS-1) cells synthesize and secrete hEGF into culture medium. WS-1 cells secreted 20 to 100 pg of hEGF per well per day during culture in 24-well plates (18). The amount of hEGF-like immunoreactive material secreted by MCF-7 cells was remarkably higher than that secreted by WS-1 cells.

To further characterize the hEGF-like immunoreactive material, we gel-filtered the conditioned medium on a Sephadex G-75 column in 1% AcOH to estimate its molecular size. An aliquot of each fraction was then assayed for hEGF-like immunoreactive material by our EIA system. One symmetrical peak with hEGF-like immunoreactivity appeared at a position identical with that of standard hEGF. From calibration of the column using marker proteins, the molecular weight of the hEGF-like immunoreactive material in the conditioned medium was found to be 7,500 - 8,000 (Fig. 3). Even after gel filtration, the dilution curve of the peak fraction with hEGF-like immunoreactivity was also not parallel to that of the standard hEGF.

For determination of the isoelectric point of the hEGF-like immunoreactive material, the conditioned medium was subjected to isoelectric focusing. As shown in Fig. 4, the immunoreactive material gave a major peak at pH 4.6 and a minor one at pH 5.0.

Type alpha transforming growth factor (TGF-α), one of the potent mitogens synthesized by various cancer cells, has been shown to share amino acid sequence homology with hEGF (24). Previous attempts to measure TGF-α by radioimmunoassay system for hEGF have failed (25), suggesting that both factors have immunologically different antigenic sites in their molecules.

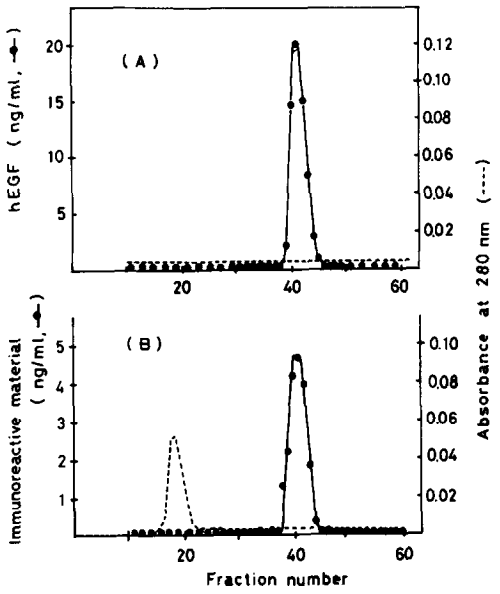

Fig. 3. Chromatographic Profiles of hEGF and MCF-7 EGF on a Column of Sephadex G-75 .

One hundred ng of purified hEGF (A) or 0.1 ml of medium conditioned by MCF-7 cells (B) was fractionated on a column of Sephadex G-75 (superfine, 1.0 x 45 cm) equilibrated with 1% acetic acid. Fractions of 0.82 ml were collected at a flow rate of 3.9 ml/hr, and a 0.01 ml aliquot of each was used for EIA. Calibration of the column for estimation of the molecular weight of MCF-7 EGF was carried out using standard proteins: BSA [1], ovalbumin [2], chymotrypsinogen A [3], cytochrome c [4], and aprotinin [5].

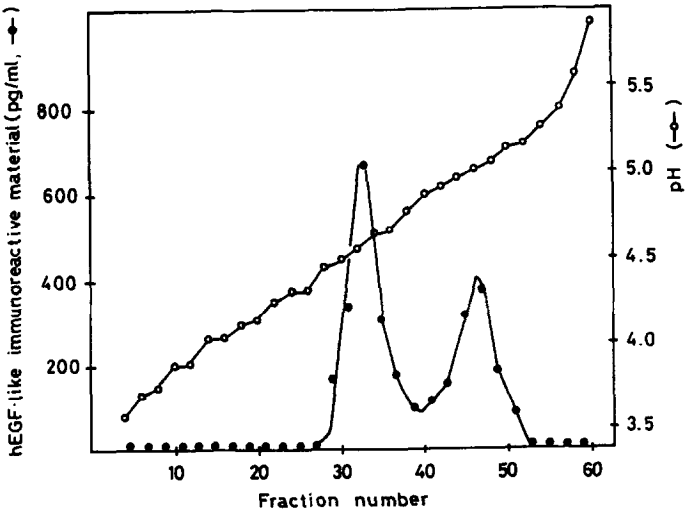

Fig. 4. Isoelectric Focusing Analysis of MCF-7 EGF.

Two hundred µl of medium conditioned by MCF-7 cells were mixed with 22.5 - 30% sucrose and then applied at half the distance from the top of the column (3.4 x 24 cm). After electrophoresis at 800 V, 1 mA, for 25 hr at 4°C, fractions of 1.5 ml were collected and a 0.01 ml aliquot of each fraction was used for EIA.

The presently described experiments give an unequivocal demonstration that MCF-7 human breast cancer cells synthesize and secrete into the medium a novel polypeptide which immunologically crossreacts with hEGF isolated from human urine. Extensive studies on the biochemical and physiological properties of this hEGF-like material are now in progress.

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