BG-1 OVARIAN CELL LINE: AN ALTERNATIVE MODEL FOR EXAMINING ESTROGEN-DEPENDENT GROWTH IN VITRO

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SUMMARY

Examination of estrogen-responsive processes in cell culture is used to investigate hormonal influence on cancer cell growth and gene expression. Most experimental studies have used breast cancer cell lines, in particular MCF7 cells, to investigate estrogen responsiveness. In this study we examined an ovarian cancer cell line, BG-1, which is highly estrogen-responsive in vitro. This observation, plus the fact that the cells are of ovarian rather than mammary gland origin, makes it an attractive alternative model. 17β-Estradiol, epidermal growth factor, and insulin-like growth factor induced proliferation of BG-1 and MCF7 cells. Viability was dependent on these growth factors in BG-1 cells, but not in MCF7 cells. Therefore, we examined the differences between these two cell lines with respect to estrogen and growth factor receptors. BG-1 cells have twice as many estrogen receptors as MCF7 cells, and BG-1 cells have higher insulin-like growth factor-1 and epidermal growth factor receptor levels than MCF7 cells. This may also explain why BG-1 cells proliferate 56% more robustly in serum and show more serum dependence in culture. In both BG-1 and MCF7 cells, epidermal growth factor receptor number is low (<20 000/cell), while insulin-like growth factor-1 receptor level was highest in estrogen receptor positive cell lines. For example, insulin-like growth factor-1 receptor was higher in BG-1 and MCF7 cells than in estrogen receptor negative cells (HeLa > MDA-MB-435 > HBL100). In conclusion, BG-1 cells are an excellent model for understanding hormone responsiveness in ovarian tissue and an alternative for examining estrogen receptor-mediated and insulin-like growth factor-1/epidermal growth factor/estrogen cross-talk processes because of their sensitivity to these factors.

Key words: estrogen; growth factors; estrogen receptor; IGF-1; EGF.

INTRODUCTION

Estrogens influence a variety of processes, such as reproduction, immune function, and cell proliferation (Richards, 1980; Luster et al., 1983; Katzenellenbogen et al., 1987; Laidlaw et al., 1995). Furthermore, estrogens have received much attention recently due to their effects on cancer cell proliferation in breast, endometrial, and ovarian carcinomas, and their use to prevent osteoporosis, (Jacobelli et al., 1984; DuPont and Page, 1991; Steinberg et al., 1991).

The most recognized experimental model for studying estrogen-induced proliferation and its molecular effects in cells is the human breast cell line, MCF7, originally described by Soule et al. (1973). Bowman Gray-1 (BG-1) cells are an ovarian adenocarcinoma originating from an ovarian tumor (Geisinger et al., 1989). This cell line is estrogen receptor (ER)-positive (Geisinger et al., 1989) and provides an alternative to using estrogen-responsive breast cell lines. Compared to mammary carcinomas, relatively little work has been done examining estrogen-responsive proliferation in human ovarian cell lines, although many ovarian cancers are estrogen-dependent (Clinton et al., 1996).

17β-Estradiol mediates some of its effects through the production of growth factors, such as transforming growth factor-α (TGFα), epidermal growth factor (EGF), and insulin-like growth factor-1 (IGF-1) (Dickson and Lippman, 1987; van der Burg, 1991; Reddy et al., 1994). These growth factors are called progression growth factors because they abolish competence and progression restriction points in a normal cell cycle (Pastan, 1975; Sporn and Todaro, 1980). Interestingly, IGF-1, EGF, and TGFα have been shown to act synergistically with 17β-estradiol to activate an estrogen responsive element (ERE) in BG-1 cells; however, transcriptional activation of the ERE was not synergistic when using only IGF-1 and TGFα (Ignar-Trowbridge et al., 1995, 1996). Recent work has shown that growth factors, such as EGF and IGF-1 can mediate phosphorylation of the ER at serine 118 and enhance the ligand-binding activity of the ER through a map kinase pathway (Kato et al., 1995). Therefore, we compared the 17β-estradiol proliferative and growth factor responses in MCF7 [American Type Culture Collection (ATCC) HTB-22, Rockville, MD] and BG-1 cells and characterized the BG-1 cells with respect to ER, estrogen-dependent growth and activity (pS2), and relevant growth factor signaling (IGF-1 and EGF) with which estrogens may cross talk.

MATERIALS AND METHODS

Cell culture. All cells were cultured in Dulbecco’s modified Eagle’s medium with Ham’s supplement F12 (DMEM/F12) containing 5% fetal bovine serum.
plates and then with 5% DCC-FBS DMEM/F12 containing 5% dextran-coated charcoal-filtered fetal bovine serum (DCD-FBS) to eliminate all organics such as steroid hormones (estrogen starvation). Cells were then trypsinized and plated for experimentation.

**ER binding assay.** MCF7 and BG-1 cells were grown to confluence and then cultured in DCC-FBS for 24 h before trypsinization, washing with phosphate-buffered saline (PBS), and freezing for the subsequent assays. Cell pellets (~1.5 × 10⁶ cells per sample) were resuspended in ice-cold TEGM buffer (10 mM tris(hydroxymethyl)aminomethane (Tris), 1.5 mM disodium ethylenediaminetetraacetic acid (EDTA), 3 mM MgCl₂, and 10% glycerol, pH 8.0 at 4°C) with protease inhibitors (50 μg/ml leupeptin, antipain, soybean trypsin inhibitor, chymostatin, and 1.5 mM EGTA), and 0.4 M NaCl. The cell lysates were centrifuged in a Dounce homogenizer (20 strokes per sample) until the cell membrane was ruptured, which was visualized with trypan blue stain. The cell lysate was centrifuged at 2500 × g for 10 min to pellet the nuclei. The supernatant was centrifuged at 105 000 × g for 30 min to clear the cytosol.

Cytosolic ER levels were determined by Scatchard analysis with dilutions of 100 nM radiolabeled 17β-estradiol (H-estradiol, Dupont NEN Research Products, Wilmington, DE) and 20 μM diethylstilbestrol with concentrations of 0.1, 0.25, 0.5, 1.0, and 10.0 nM 17β-estradiol and with or without a 200-fold excess of diethylstilbestrol. Receptor bound fractions were assayed with hydroxyapatite absorption procedures (HAP assay) and were counted in a Beckman 5810C scintillation counter as previously described (Korach, 1979). The data were analyzed with Accufit Saturation Two Site software (Lundon Software, Cagrin Falls, OH). Receptor levels were normalized to DNA levels, determined from the nuclear pellet with a fluorescence assay as previously described (Couze et al., 1995).

**Growth factor binding assays.** Cells were grown to confluence in 12-well plates and then with 5% DCC-FBS DMEM/F12 media for 24 h. Subsets were counted on a hemocytometer or Coulter counter for a representative cell count. [³H]-IGF-1 (138 μCi/μg, Diagnostic Systems Laboratory, Inc., Webster, TX) or [³H]-IGF-1 (302 μCi/μg, Dupont NEN) was added to each well at various concentrations from 18 ng/ml to 0.30 ng/ml of DMEM/F12 with no serum and 0.1% bovine serum albumin at 4°C. Unlabeled EGF or IGF-1 (Calbiochem-Novabiochem Corp., La Jolla, CA) was added to half of the wells at 500 (IGF-1) to 1000 (EGF) times the concentration of labeled growth factor to correct for nonspecific binding. Replicates were done at each concentration. Samples were incubated on ice for 105 min and washed three times in cold media (4°C) to eliminate unbound growth factor before being lysed with 0.2% NaOH/0.1% sodium dodecyl sulfate (SDS). Lysates were decanted and this step was repeated to collect all bound radioactivity. Lysates (bound radioactivity) and washes (free radioactivity) were counted on a gamma counter (Packard Multi-Prias Plus, Downers Grove, IL).

**Cell proliferation.** Following a 4-d estrogen starvation (cells cultured in 5% DCC-FBS, DMEM/F12), cells were trypsinized and plated in 100-mm² dishes at 1 × 10⁶ to 2 × 10⁶ cells per plate. When measuring estrogen (Sigma Chemical Co., St. Louis, MO) induced proliferation, cells were treated with 17β-estradiol 24 h following plating. Fresh medium was added and cells were reexposed to 17β-estradiol at 72 h following the initial exposure. Cells were counted by Coulter counter (Coulter Electronics, Inc., Hialeah, FL) at 72 and 144 h after 17β-estradiol exposure.

When measuring EGF or IGF-1 induced proliferation, cells were plated as described above with the following exceptions. Twenty-four h following plating, cells were washed twice with PBS and then grown in low serum media (0.1% DCC-FBS DMEM/F12) with 0.1 μg kanamycin per ml. EGF or IGF-1 (10 ng/μL) was added at this time and every 24 h for the next 3 d following the initial addition. Fresh medium was added 48 h following the initial addition of growth factor, and cells were counted (Coulter) at 48 and 96 h.

**Northern blotting.** BG-1 and MCF7 cells were estrogen starved for 7 d and then plated at approximately 2 × 10² cells per 100-mm² dish. Cells were exposed to 10⁻¹⁰ M 17β-estradiol for 24 h, washed with PBS, and scraped from the plates with 1.5 ml of Tri-reagent (MRC Inc., Cincinnati, OH). RNA was extracted by the acid guanidinium-phenol-chloroform procedure (Chomczynski and Sacchi, 1987) and quantitated spectrophotometrically on a spectrophotometer (Baldwin et al., 1995). RNA (2 μg) was electroblotted in a 1.2% agarose gel containing formaldehyde and transferred to a Gene Screen Plus Membrane (Dupont). Blots were hybridized with probes that had been radiolabeled with ³²P to a specific activity greater than 10⁶ cpm/μg with the Ready-to-go random priming kit (Pharmacia, Piscataway, NJ), and hybridized overnight in Hybriol 1 solution (Oncor, Gaithersburg, MD). Blots were washed at high stringency (0.1% standard saline citrate and 1% SDS at 60°C) before phosphorimaging and autoradiography. Probes used in this study were a 320-base pair PstI fragment from pS2 (ATCC, Rockville, MD), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Genbank M33197) nucleotides 906–1032.

**RESULTS AND DISCUSSION**

**Growth in culture.** BG-1 cells proliferate in DMEM/F12 media with 5% FBS at a significantly greater rate than MCF7 cells (Fig. 1). Doubling times for BG-1 cells were 56% faster than for MCF7 cells. BG-1 cells doubled in number every 1.01 d, while MCF7 cells doubled every 1.58 d over the 6 d the cells were examined. 17β-estradiol stimulation of BG-1 cells. BG-1 cells had approximately twice the number of ERs as MCF7 cells (Table 1). Both cell lines had similar affinities for 17β-estradiol (Table 1). Correspondingly, BG-1 cells produced estrogen-responsive pS2 mRNA in a more robust fashion (~twofold greater relative induction) than MCF7 cells following exposure to 17β-estradiol at 10⁻⁸ M as measured by North-