IMPORTANT NOTICE

BG1Luc4E2 cells are being renamed VM7Luc4E2 cells

Reason: Recent DNA analysis (STR) revealed that the original cell line used to generate the BG1Luc4E2 cells were not human ovarian carcinoma (BG-1) cells but a variant of human breast cancer (MCF7) cells. Details are provided below.

Background

The recombinant BG1Luc4E2 cell line was originally generated in our laboratory by stable transfection of human ovarian adenocarcinoma Bowman Gray-1 (BG-1) cells we obtained from the National Institute of Environmental Health (NIEHS) with the estrogen receptor (ER) responsive-firefly luciferase reporter gene plasmid pGudLuc7ere (Rogers and Denison, 2000). The recombinant BG1Luc4E2 cells respond to estrogenic chemicals with the induction of firefly luciferase in an ER agonist-, concentration-, ER- and time-dependent manner and they have been extensively for the detection of chemicals and sample extracts with estrogenic/antiestrogenic activity. More recently, the BG1Luc4E2 cell line received official approval by the Organization of Economic Cooperation and Development (OECD) as a cell-based transactivation test method for identifying ER agonists and antagonists (the so-called BG1Luc ER TA bioassay (TG455/457) (OECD) (2012a,b)) and was included as an alternative cell bioassay for estrogenic chemicals in the USEPA Endocrine Disruptor Screening Program.

BG-1 cells were originally described by Geisinger et al. (1989) and were later characterized by researchers at the National Institute of Environmental Health Sciences (NIEHS) (Baldwin et al., 1998). Relatively recently, it was discovered that there exist two different variants of BG-1 cells being used by researchers and in-depth analysis of these two BG-1 variant cell lines was carried out by Li and coworkers (2014). This paper is attached below. The two BG-1 lines were denoted as BG-1 Fr and BG-1 NIEHS, based on their usage and distribution (in France (Fr) and the US (NIEHS) (Li et al., 2014)). Initial comparison revealed significant mophological differences between these two BG-1 variants. While short tandem repeat (STR) analysis of these two BG-1 lines revealed that BG-1 Fr was unique, BG-1 NIEHS was found to have an STR pattern identical to that of human breast cancer (MCF7) cells (Li et al., 2014). Subsequent cytogenetic analysis not only confirmed that BG-1 Fr cells were the same as the originally described BG-1 cells, but that BG-1 NIEHS and MCF7 cells were genetically similar. The studies of Li and coworkers (2014) also demonstrated that BG-1 NIEHS and MCF7 cells were not identical and a significant number of chromosomal translocation differences were found between these two cell lines. Additionally, gene microarray analysis revealed dramatic differences in estrogen-responsive gene expression patterns between BG-1 NIEHS and MCF7 cells. Taken together, these authors concluded that the BG-1 NIEHS cells were not human ovarian adenocarcinoma cells, but are a variant derived from human breast carcinoma MCF7 cells.

Our STR Analysis

We assumed that the original BG-1 cells provided to our lab from investigators at the NIEHS in the late 1990s were actually BG-1 cells. Given the new published information described above that indicated that the BG-1 NIEHS cells were actually a variant of MCF7 cells, we questioned

the actual identity of our BG-1 cells. To determine whether the BG-1 cells that we originally obtained from NIEHS were BG-1 Fr or BG-1 NIEHS cells, we sent BG1Luc4E2 cells to the American Type Culture Collection (ATCC) for STR analysis. The final report of the ATCC STR analysis is attached below. These analyses results revealed that the BG-1 cells we obtained from NIEHS and used to generate the recombinant BG1Luc4E2 cell line were BG-1 NIEHS cells. Thus the recombinant cell line we developed is actually based on a variant of MCF7 cells and are not BG-1 cells as originally assumed. This finding is also relevant to our newly developed BG1LucERβc9 cell line, in which BG1Luc4E2 cells were stably transfected with an ERβ expression vector (Brennan et al., 2016). Accordingly, given this information, we are making the following changes to the name of the original cell line and our recombinant cell lines to the following:

- The original human ovarian carcinoma (**BG-1**) cells from NIEHS will now be designated as human breast carcinoma (**vMCF7**) cells.
- The recombinant human ovarian carcinoma (**BG1Luc4E2**) cells (Rogers and Denison, 2000) will now be designed as human breast carcinoma (**VM7Luc4E2**) cells.
- The recombinant human ovarian carcinoma (**BG1LucERβc9**) cells (Brennan et al., 2016) will now be designated as human breast carcinoma (**VM7LucERβc9**) cells.

Impact

There is already an extensive literature on the validation and application of these recombinant cell lines for the detection and relative quantitation of estrogenic activity of known chemicals and chemical mixtures, as well as extracts containing unknown chemical mixtures. While the information presented above changes the specific cell line upon which our recombinant estrogen cell bioassays are based (Rogers and Denison, 2000; Brennan et al., 2016), the fact that the cell background is different than originally thought does not affect published validation studies nor utility and application of these cells for screening of estrogenic/antiestrogenic chemicals. Analyses can continue, but with a more correct designation for the recombinant cell lines.

Questions

If you have any questions or need any additional information, please don't hesitate to contact Mike Denison (msdenison@ucdavis.edu).

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Research Resource: STR DNA Profile and Gene Expression Comparisons of Human BG-1 Cells and a BG-1/MCF-7 Clonal Variant

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Human ovarian cancer BG-1 cells are a valuable in vitro model that has enabled several laboratories to study the estrogenic responses of ovarian cancers. We recently discovered that there are two different BG-1 cell lines being used for experiments, denoted here as BG-1 FR and BG-1 NIEHS, which exhibit striking morphological differences. The objective of this study was to methodically analyze these two BG-1 variants and compare their characteristics. Short tandem repeat analysis revealed that the DNA profile of BG-1 FR cells was unique, yet the Short tandem repeat pattern of BG-1 NIEHS was identical with that of MCF-7 cells. From a cytogenetic analysis, it became apparent that the BG-1 FR line had the same profile as previously reported, whereas the BG-1 NIEHS and MCF-7 cells share a similar genetic display. A significant number of unique chromosomal translocations were observed between the BG-1 NIEHS and MCF-7 cells, suggesting that acquired genotypic differences resulted in the formation of two lines from a common origin. Although all cell types demonstrated a similar estrogen responsiveness in reporter gene assays, a microarray analysis revealed distinct estrogen-responsive gene expression patterns with surprisingly moderate to low overlap. We conclude that BG-1 FR is the original ovarian cancer cell line, whereas the BG-1 NIEHS is a variant from the MCF-7 cells. These findings provide much needed clarification of the identities and characteristics of key cell line models that are widely used to study estrogen action in female reproductive cancers. **(*Molecular Endocrinology* 28: 2072–2081, 2014)**

Ovarian cancer is the most lethal gynecological cancer in the United States and is the fourth leading cause of cancer deaths in women (1). Surgery and chemotherapy are currently used as first-line treatments (2, 3). Hormonal therapy, a less toxic alternative to chemotherapy, also provides clinical benefits (1). However, there currently exists a need to learn more about the causes and factors involved in the progression of this disease. Thus, ovarian cancer cell lines have been derived from cancer patients, and these are used as in vitro models to characterize the molecular mechanisms underlying ovarian tumorigenesis and to facilitate the development of novel therapeutics targets (4).

Estrogens, including the endogenous ovarian hormone estradiol (E2), play an essential role in the growth, differentiation, and homeostasis of a number of target tissues (5–8). The biological effects of E2 are mediated through estrogen receptors (ERs), including ERα and ERβ, which belong to the nuclear receptor superfamily of ligand-inducible transcription factors (9). The well-known classi-

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Abbreviations: BG-1 FR, BG-1 cells being used for experiments in France; BG-1 NIEHS, BG-1 cells being used for experiments the United States; E2, 17β-estradiol; ER, estrogen receptor; ERE, estrogen-responsive element; FBS, fetal bovine serum; M-FISH, multicolor fluorescence in situ hybridization; NIEHS, National Institute of Environmental Health Sciences; PCA, principal component analysis; sFBS, stripped FBS; STR, short tandem repeats.

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cal mechanism of receptor action involves hormone binding and association of the activated ERs with estrogen responsive elements (EREs) located in the regulatory regions of target genes (9, 10). Most ovarian cancers are epithelial in origin, and there is decreased expression of ERβ mRNA levels in epithelial ovarian cancers compared with normal ovarian tissues (11). Likewise, low or absent ERβ expression is associated with more aggressive tumors, suggesting a protective role of the receptor (11–14). There is additional evidence that the ratio of ERα to ERβ is higher in ovarian tumors than in normal tissues due to lower expression of ERβ (15).

Estrogens regulate a number of target genes through the ERs, and some of these genes have been used as biomarkers in clinical cancer research. The human *FBLN1C*, an isoform of the *FBLN1* (fibulin-1) gene, is highly expressed in ovarian carcinomas and is estrogen-inducible in ovarian tumor cells (16, 17). The human *GREB1* (gene regulated by estrogen in breast cancer 1) gene was reported as an ER-responsive gene (18, 19), and this factor appears to be a critical regulator of hormone-dependent breast cancer growth (20). The human *pS2/TFF1* and *PGR* (progesterone receptor) genes are well-characterized ER-target genes (21, 22). Both genes are up-regulated by E2 in a subclass of ER-positive human breast cancer cells and are prognostic indicators of hormonal tumor responsiveness (23).

The human ovarian epithelial cancer cell line BG-1 was established in 1989 from a solid primary tumor of a patient with poorly differentiated stage III ovarian adenocarcinoma (24). Since that time, BG-1 cells have been used as an in vitro model to study estrogen-responsive ovarian cancers. Recently we discovered that there are two different variants of BG-1 cells being used for experiments: BG-1 FR and BG-1 NIEHS. These are names we assigned to the individual cell lines based on their uses and distribution in France (BG-1 FR) (3, 17, 25–29) and the United States (BG-1 NIEHS) (30–32), respectively.

In this study, we performed an extensive characterization of the BG-1 FR and BG-1 NIEHS cell lines and compared their features with human breast cancer MCF-7 cells, another model of estrogen responsiveness. This included cellular morphology studies, short tandem repeats (STR) analysis, also known as DNA fingerprinting (33), and molecular cytogenetic analysis. We also evaluated the basal expression levels of the ERs and ER-target genes and the cellular responses to E2 in ER/ERE-mediated activation using luciferase reporter assay in the three cell lines. Finally, we profiled whole-genome gene expression by microarray analysis.

Materials and Methods

Reagents

E2 was purchased from Sigma-Aldrich, and ICI 182780 (ICI) was purchased from Tocris Bioscience.

Plasmids

The expression vector pcDNA3 was purchased from Invitrogen. An internal control plasmid for transfection efficiency, pRL-TK, was purchased from Promega. The luciferase reporters, 3xERE Luc (synthetic vitellogenin ERE-TATA fused to a luciferase reporter gene) and pS2 Luc (endogenous human pS2 gene promoter region containing an ERE fused to a luciferase reporter gene) have been described previously (34–36).

Cell lines and tissue culture

The BG-1 FR cell line was acquired directly from Dr Charles Welander at the Wake Forest University (Winston-Salem, NC) and maintained by the laboratory of Dr Vincent Cavailles at the University of Montpellier (Montpellier, France) (3, 17, 25–29). The BG-1 NIEHS cell line was acquired from Dr Carl Barrett [National Institute of Environmental Health Sciences (NIEHS)/National Institutes of Health (NIH)] (30–32). The MCF-7 cell line was purchased from American Type Culture Collection. All three cell lines were maintained in the same culture conditions: phenol red free DMEM-F12 medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS; Gemini Bio Products) and 4 mM L-glutamine (Invitrogen). When assessing the estrogen responsiveness, 10% charcoal/dextran stripped FBS (sFBS; HyClone, Gemini Bio Products) was substituted for the FBS in the medium for E2 treatment.

DNA isolation and STR analysis

For the BG-1 FR cell line, preparation of the DNA samples was carried out using a FTA sample collection kit (100-FTA; American Type Culture Collection), and STR analysis was performed using the Cell Authentication Testing Service at American Type Culture Collection. For the BG-1 NIEHS and MCF-7 cell lines, genomic DNA (5–10 μg) was extracted using a DNeasy blood and tissue kit (QIAGEN) according to the manufacturer's protocol. The concentration and quality of DNA were determined with an ND-1000 Nanodrop spectrophotometer. STR analysis was carried out at the Cancer Institute Cell Culture and Cytogenetics Facility Core at the University of Pittsburgh.

Cytogenetic analysis

The multicolor fluorescence in situ hybridization (M-FISH) karyotype was performed in the Comparative Molecular Cytogenetics Core Facility at the National Cancer Institute/NIH. The aim of the karyotype studies was the identification of any complex chromosomal rearrangements. Briefly, chromosome preparations were obtained from established cell cultures with the addition of colcemid (KaryoMax colcemid solution; 10 μg/mL; Invitrogen) 3 hours prior to harvest. Cells were collected and treated with a hypotonic solution (KCL 0.075 M) for 15 minutes at 37°C and fixed with methanol-acetic acid 3:1. Slides were prepared and incubated overnight for use in a Hyperspectral Karyotyping (SKY) analysis. Cell metaphases were hybridized with the 24-color human SKY paint kit (Applied Spectral

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Imaging Inc) according to the manufacturer's protocol (37). Hybridization was carried out in a humidity chamber at 37°C for 16 hours. The posthybridization rapid wash procedure was used with 0.4× saline sodium citrate at 72°C for 4 minutes. Detection was carried out after the manufacturer's protocol. Spectral images of the hybridized metaphases were acquired using a SD301 SpectraCube system (Applied Spectral Imaging Inc) mounted on top of an epifluorescence microscope Axioplan 2 (Zeiss). Images were analyzed using Spectral Imaging 6.0 acquisition software (Applied Spectral Imaging Inc). A minimum of 10 mitoses of comparable staining intensity and quality was examined per cell line, and each was further compared for chromosomal differences. G-banding was simulated by electronic inversion of 4′,6′-diamino-2-phenylindole counterstaining.

Cell morphological imaging

The cells were seeded in six-well plates and cultured overnight. Differential interference contrast images were taken on a Zeiss AxioObserver Z1 inverted microscope using a linkage disequilibrium Achroplan ×20/0.40 objective with a Zeiss AxioCam MRm camera.

E2 treatment and RNA extraction

Cells were cultured in phenol red-free DMEM-F12 + 10% sFBS medium for 2 days and then treated with vehicle control (EtOH) or 10 nM E2 for 18 hours. Total RNA was extracted using an RNeasy minikit (QIAGEN). First-strand cDNA synthesis was performed using Superscript reverse transcriptase according to the manufacturer's protocol (Invitrogen).

Real-time PCR analysis

The mRNA levels of ER target genes were measured using SYBR green assays (Applied Biosystems). The sequences of real-time PCR primers used in this study are shown in Supplemental Table 1. Cycle threshold values were obtained using the ABI PRISM 7900 sequence detection system and analysis software (Applied Biosystems). Each sample was normalized to β-actin expression. Experiments were repeated three times and results are presented as mean ± SEM. Fold change of basal gene expression in the three cell lines was calculated relative to the BG-1 FR cell line. Changes of endogenous gene expression by E2 were calculated as fold change relative to the vehicle control group in each cell line.

Protein extraction and Western blot analysis

Whole-cell lysates were prepared by using a BD TransFactor extraction kit (BD Biosciences). For Western blot, the samples (40 μg) were loaded on a SDS-PAGE gel and separated by electrophoresis. The proteins were electrotransferred onto nitrocellulose membranes, and membranes were subsequently blocked in PBS with 5% nonfat milk for 2 hours. The blots were incubated with primary antibody (human ERα, clone HC-20, catalog number sc-543 or human ERβ, clone H-150, catalog number sc-8974; Santa Cruz Biotechnology Inc) at 4°C overnight, washed with PBS-T and then incubated with antirabbit IRDye 800CW secondary antibody (catalog number 926-32211; LI-COR Biosciences) at room temperature for 1 hour. The immunoreactive products were detected by the Fc ODYSSEY image system (LI-COR Biosciences). Anti-α-tubulin (clone B-5-1-2, catalog number T5168; Sigma) was used as a loading control.

Transient transfection and luciferase assay

Cells were seeded in DMEM-F12 + 10% sFBS. After 24 hours, the ER-responsive reporter plasmids (3xERE Luc or pS2 Luc; 0.2 μg/well) and pRL-TK renilla luciferase plasmid (0.1 μg/well) were transiently transfected using the Effectene transfection reagent (QIAGEN) according to the manufacturer's protocol. After 8 hours, the cells were changed to fresh DMEM-F12 + 10% sFBS medium overnight and then were treated with vehicle control (EtOH) or E2 (0, 1, or 10 nM) in the absence or presence of 1 μM ICI. Luciferase assays were performed using the dual luciferase reporter activity system (Promega). Transfection efficiency was normalized to renilla luciferase. Fold changes were calculated relative to vehicle controls. All experiments were repeated at least three times. Data shown are the average of triplicate determinations in a representative experiment. Values were calculated relative to vehicle control and presented as ± SEM.

Microarray analysis

Gene expression analysis was performed at the NIEHS Microarray Core Facility using Agilent whole human genome 4 × 44 multiplex format oligo arrays (014850) (Agilent Technologies) following the Agilent 1-color microarray-based gene expression analysis protocol. Three biological replicates were examined in each treatment group. The Agilent Feature Extraction Software performed error modeling, adjusting for additive and multiplicative noise. Feature extraction data files were then imported into Partek software (Partek Genomics Suite version 6.6). A principal component analysis (PCA) was performed on all samples and all probes to identify any variability present in the data. The resulting data were then analyzed for differentially expressed genes by ANOVA. Contrasts were set at BG-1 FR vehicle control vs E2, BG-1 NIEHS vehicle control vs E2, and MCF-7 vehicle control vs E2. Gene numbers displayed in the Venn diagram were derived from the data set using a ±1.5-fold cutoff and $P < .05$.

Data access

Microarray expression data have been submitted to the National Center for Biotechnology Information Gene Expression Omunibus (http://www.ncbi.nlm.nih.gov/geo) under accession number GSE58324.

Statistical analysis

One-way ANOVA with a multiple comparison test (**, $P < .01$; ***, $P < .001$ or ****, $P < .0001$, Figure 3) and a two-way ANOVA with a multiple comparison test (***, $P < .001$ or ****, $P < .0001$, Figures 4 and 6) were performed using Graph Pad Prism version 6.0.

Results

Differential morphology of BG-1 FR, BG-1 NIEHS, and MCF-7 cell lines

Our initial assumption that the BG-1 NIEHS cell line was an ovarian cancer cell line was due to their unique morphology compared with MCF-7 cells. The compara-

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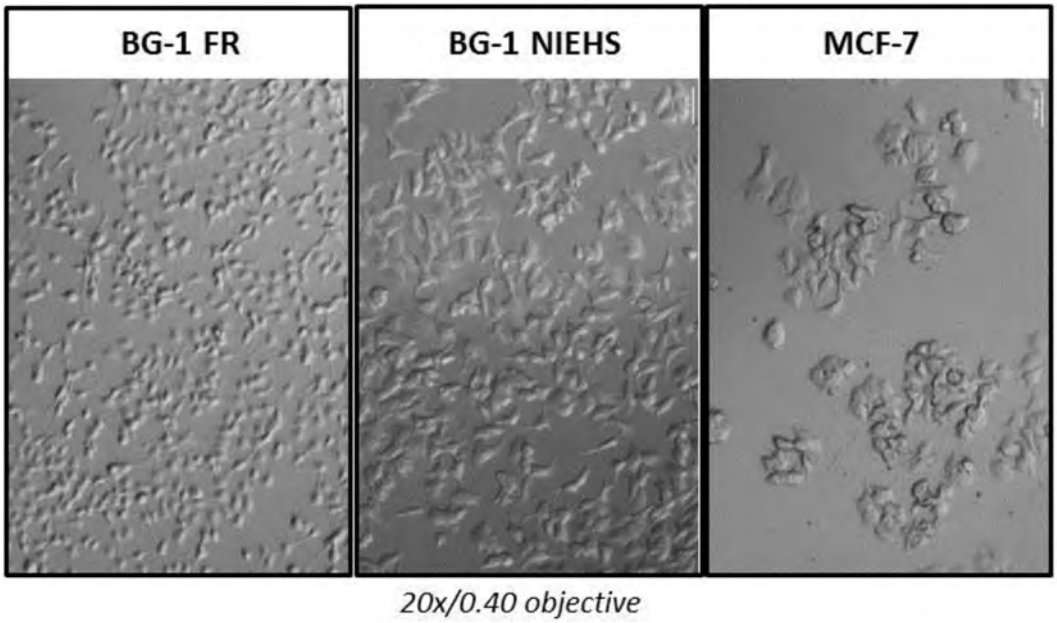

Figure 1. Cell morphology imaging of BG-1 FR, BG-1 NIEHS, and MCF-7 cell lines. Cells were seeded in six-well plates and cultured overnight. Differential interference contrast images were taken on an inverted microscope using a ×20/0.40 objective lens with a Zeiss AxioCam MRm camera.

tive morphology of the BG-1 FR, BG-1 NIEHS, and MCF-7 cells are shown in Figure 1. We found that the morphology of the BG-1 FR cells differed from the BG-1 NIEHS cells. Specifically, BG-1 FR cells were smaller and more adherent than the BG-1 NIEHS cells. The MCF-7 cells grew in clumps, were more adherent, and possessed rounded cytoplasms, whereas BG-1 NIEHS cells possessed cytoplasmic spindles and were more dispersive.

STR DNA and cytogenetic profiles of the BG-1 FR, BG-1 NIEHS, and MCF-7 cell lines

Noting the morphological differences between the BG-1 FR, BG-1 NIEHS, and MCF-7 cells, we next performed an STR analysis of the genetic background of those cell lines. The STR DNA profiles differed significantly between the BG-1 FR and BG-1 NIEHS cells (Table 1). Based on the STR database of the American Type Culture Collection, there was no cell line corresponding with the DNA profile of BG-1 FR cells. However, the DNA profile of BG-1 NIEHS cells matched with the profile of MCF-7 cells (Table 1).

Next, we performed a molecular cytogenetic analysis in the three cell lines. The M-FISH karyotypes of the representative metaphases are shown in Figure 2. The BG-1 FR cells revealed a uniform karyotype of 46,XXX,-4,+derdic,t(1:4)(p11;q33),-14,t(14;15),-15 (Figure 2A). This karyotype was matched with the original BG-1 cells, which was reported by Geisinger et al (24). In contrast, the karyotype of the BG-1 NIEHS cells was similar to that of the MCF-7 cells (Figure 2, B and C). However, we observed a significant number of differential chromosomal translocations between the BG-1 NIEHS and MCF-7 cells. The comparison of chromosomal aberrations in the BG-1 NIEHS and the MCF-7 cells are shown in Supplemental Table 2 (red for BG-1 NIEHS and blue for MCF-7 cells).

Differential gene expression in the BG-1 FR, BG-1 NIEHS, and MCF-7 cell lines

The basal expression levels of *ESR1* (ERα) and *ESR2* (ERβ) genes in the BG-1 FR, BG-1 NIEHS, and MCF-7 cell lines were examined (Figure 3A). The values are represented as fold change relative to the level of BG-1 FR

Table 1. Summary of STR DNA Profile of Human Cancer Cell Lines

Cell Lines	BG-1 FR	BG-1 NIEHS	MCF-7
Amelogenin	X	X	X
CF1PO	10, 11	10	10
D13S317	10	11	11
D16S539	9, 11	11, 12	11, 12
D18S51	12, 15	14	14
D19S433	13, 15	13, 14	13, 14
D21S11	29, 33.2	30	30
D2S1338	22, 23	21, 23	21, 23
D3S1358	16, 17	16	16
D5S818	12	11, 12	11, 12
D7S820	12	8, 9	8, 9
D8S1179	12, 13	10, 14	10, 14
TH01	8, 9.3	6	6
TPOX	8, 11	9, 12	9, 12
vWA	14, 17	14, 15	14, 15

American Type Culture Collection suggests that cell lines with 80% or greater match are considered to be related. BG-1 FR shows no match for any profile in the American Type Culture Collection STR database. BG-1 NIEHS and MCF-7 show greater than 80% value of the STR loci.

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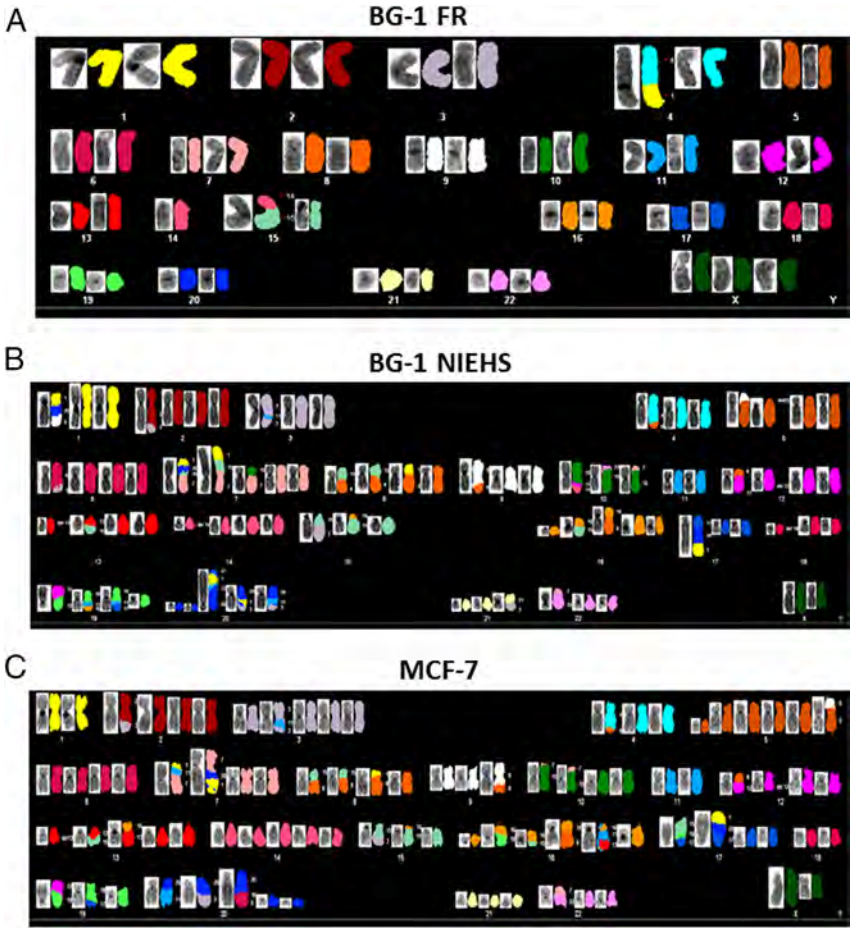

Figure 2. The molecular cytogenetic analysis. G-banded and M-FISH karyotype of a representative metaphase of BG-1 FR cells (A), BG-1 NIEHS cells (B), and MCF-7 cells (C).

cells (set as 1). We found that the expression level of the *ESR1* gene in the BG-1 NIEHS and the MCF-7 cells was 2-fold higher than that in the BG-1 FR cells. On the other hand, the expression level of *ESR2* in the BG-1 FR cells was 3- to 4-fold higher than that of the BG-1 NIEHS and MCF-7 cells. In addition, using a Western blot analysis, the ERα protein (66 kDa) was detected in all three cell lines, but the ERβ protein (55 kDa) was seen only in BG-1 FR cells (Supplemental Figure 1).

Because all three cell lines had ER expression, we examined the basal expression levels for several ER-target genes. The real-time PCR results are shown in Figure 3B. We found that the *FBLN1C* gene was highly expressed in BG-1 FR cells, but this transcript was not detectable in the BG-1 NIEHS and MCF-7 cells. In contrast, the basal expression levels of the *pS2* and *GREB1* genes were significantly higher in the BG-1 NIEHS and MCF-7 cells than in the BG-1 FR cells. A similar expression level of the *PGR* gene was detected in the BG-1 FR and MCF-7 lines, and this expression was 1.5-fold higher in the BG-1 NIEHS cells. These data demonstrated that there are different basal gene expression profiles among the three cell lines.

Transcriptional activity of endogenous ER

To investigate ER-mediated transcriptional activities in the three cell lines, we examined promoter activation using two E2-responsive luciferase reporters: 3xERE Luc (synthetic vitellogenin ERE fused with luciferase reporter) and pS2 Luc (endogenous human pS2 gene promoter region containing an ERE fused to a luciferase reporter gene). The E2-mediated activities of 3xERE Luc are shown in Figure 4A. At 1 nM E2 treatment, the BG-1 FR and the BG-1 NIEHS cells displayed a 4-fold induction compared with the control, whereas a 6-fold induction was seen in the MCF-7 cells. With 10 nM of E2, the maximal response efficacies were observed in the BG-1 NIEHS cells with 6-fold or in the MCF-7 cells with 7-fold induction.

The E2-mediated activities on pS2 Luc are shown in Figure 4B. With 1 or 10 nM of E2, maximal response efficacies were observed in all cell lines with a 4-fold induction in the BG-1 FR cells or a 3-fold induction in the BG-1 NIEHS and MCF-7 cells.

In addition, E2-dependent activity was attenuated in all instances by coadministration of the pure ER antagonist ICI, thus confirming that the ERE-mediated promoter activities were ER dependent (Figure 4).

Gene expression profiles in the BG-1 FR, BG-1 NIEHS, and MCF-7 cell lines

To compare the cellular responses to E2 in each cell line, we profiled whole-genome gene expression by using microarray analysis after 10 nM E2 treatment for 18 hours. PCA mapping is shown in Figure 5A. The graph shows a correlation among the triplicate samples in each treatment group (control group: triangle shape; E2 group: square shape). Notably, the PCA mapping indicates that the gene expression profile of the BG-1 FR cells (green) is clearly different from the profiles of the BG-1 NIEHS (pink) or MCF-7 (blue) cells.

Microarray data were analyzed by examining fold change between the control and E2 in each cell line. Venn

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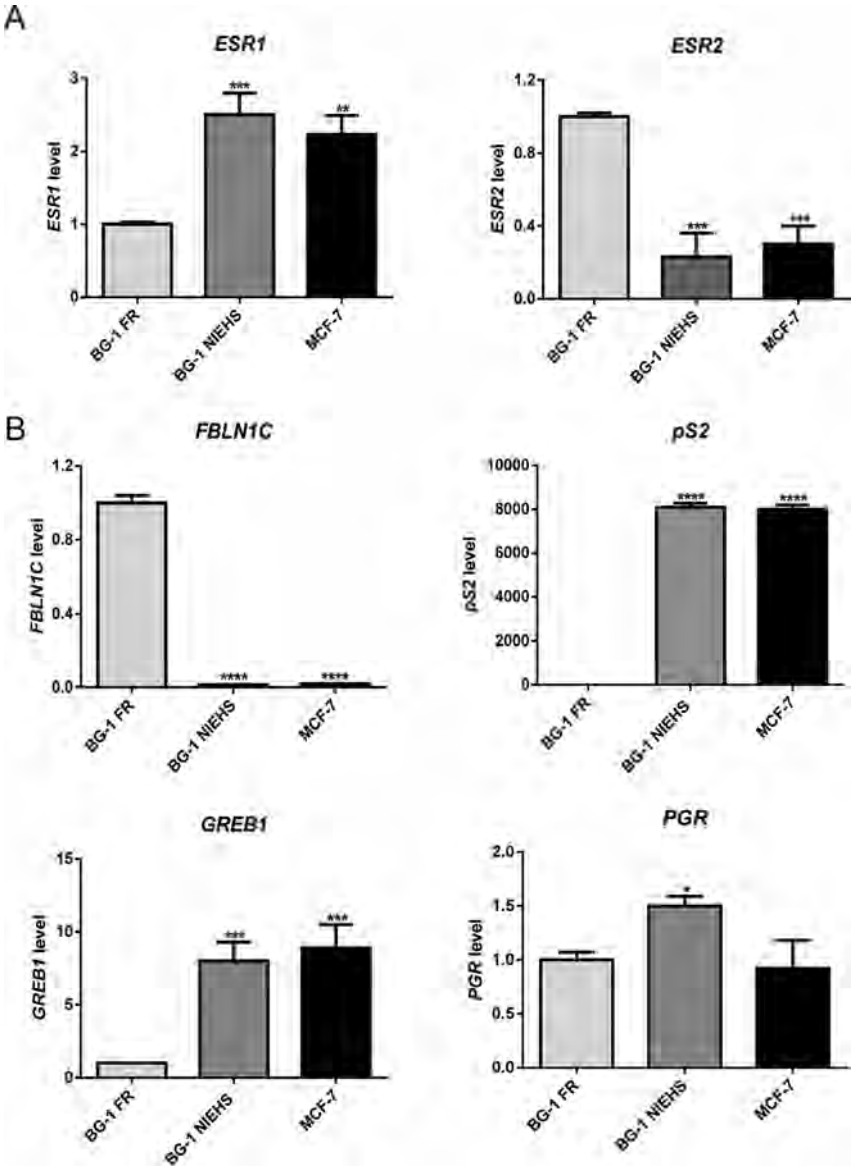

Figure 3. Expression of *ESR1* (ERα), *ESR2* (ERβ), and ER-target genes in BG-1 FR, BG-1 NIEHS, and MCF-7 cell lines. A, *ESR1* and *ESR2* gene expression. Total RNA was extracted from the cells, and the mRNA levels were quantified by real time-PCR. Values were normalized by the expression level of β-actin. Data shown are representative of triplicates and fold change is calculated relative to the BG-1 FR cell line (set as 1) ± SEM. **, $P < .01$; ***, $P < .001$; ****, $P < .0001$. B, *FBLN1C*, *pS2*, *GREB1*, and *PGR* gene expression. The assay and data analysis were described as above in panel A.

diagram analysis for fold change (± 1.5-fold cutoff) and overlap between cell lines is shown in Figure 5B. We found that the BG-1 FR cells had only 506 E2-responsive genes, whereas 3058 genes were identified in the BG-1 NIEHS cells and 2979 in the MCF-7 cells. When comparing genes between the cell lines, a total of 119 genes overlapped between all three cell lines. A list of the 119 genes and their hormonal regulation attributes (up or down) is shown in Supplemental Table 3. There were 187 (68 + 119) genes overlapping between the BG-1 FR and BG-1 NIEHS cells, 164 (45 + 119) genes between the BG-1 FR and MCF-7, and 1481 (1362 + 119) genes between the BG-1 NIEHS and MCF-7 cell lines.

To confirm the microarray results, we examined the effect of E2 on several ER target genes from the microarray using real time-PCR analysis. Fold changes in gene expression, relative to the control, are shown in Figure 6. E2 induced a 4-fold increase of *FBLN1C* gene expression in the BG-1 FR cells, but this induction was not seen in the BG-1 NIEHS and MCF-7 cells (Figure 6A). In contrast, E2 induced the expression of the *pS2* gene in the BG-1 NIEHS (5-fold) and MCF-7 cells (4-fold) but not in the BG-1 FR cell line (Figure 6B). In addition, the *GREB1* and *PGR* genes were induced by E2 in all three cell lines (Figures 6, C and D). These results are consistent with the data from the microarray analysis.

Discussion

We and others have been using the human ovarian cancer cell line BG-1 to study ER signaling in ovarian cancer since the early 1990s when this cell line was established (24). Recently we discovered that there are two different variants of the BG-1 cells being used for experiments. We named those cell lines BG-1 FR and BG-1 NIEHS, depending on whether they were distributed from the University of Montpellier in France (3, 15, 17, 25–29) or the NIEHS/NIH in the United States (30–32, 38–40).

STR DNA profiling, also known as DNA fingerprinting, identifies variants in tetranucleotide microsatellite loci on multiple human chromosomes (33). The American Type Culture Collection developed a standard for authentication of human cell lines by comparison with established STR DNA profiling databases (4, 41, 42). In this study, the STR analysis revealed that the BG-1 FR cell line had a completely novel profile because it did not match any STR DNA profile in the American Type Culture Collection databases. When comparing our cytogenetic anal-

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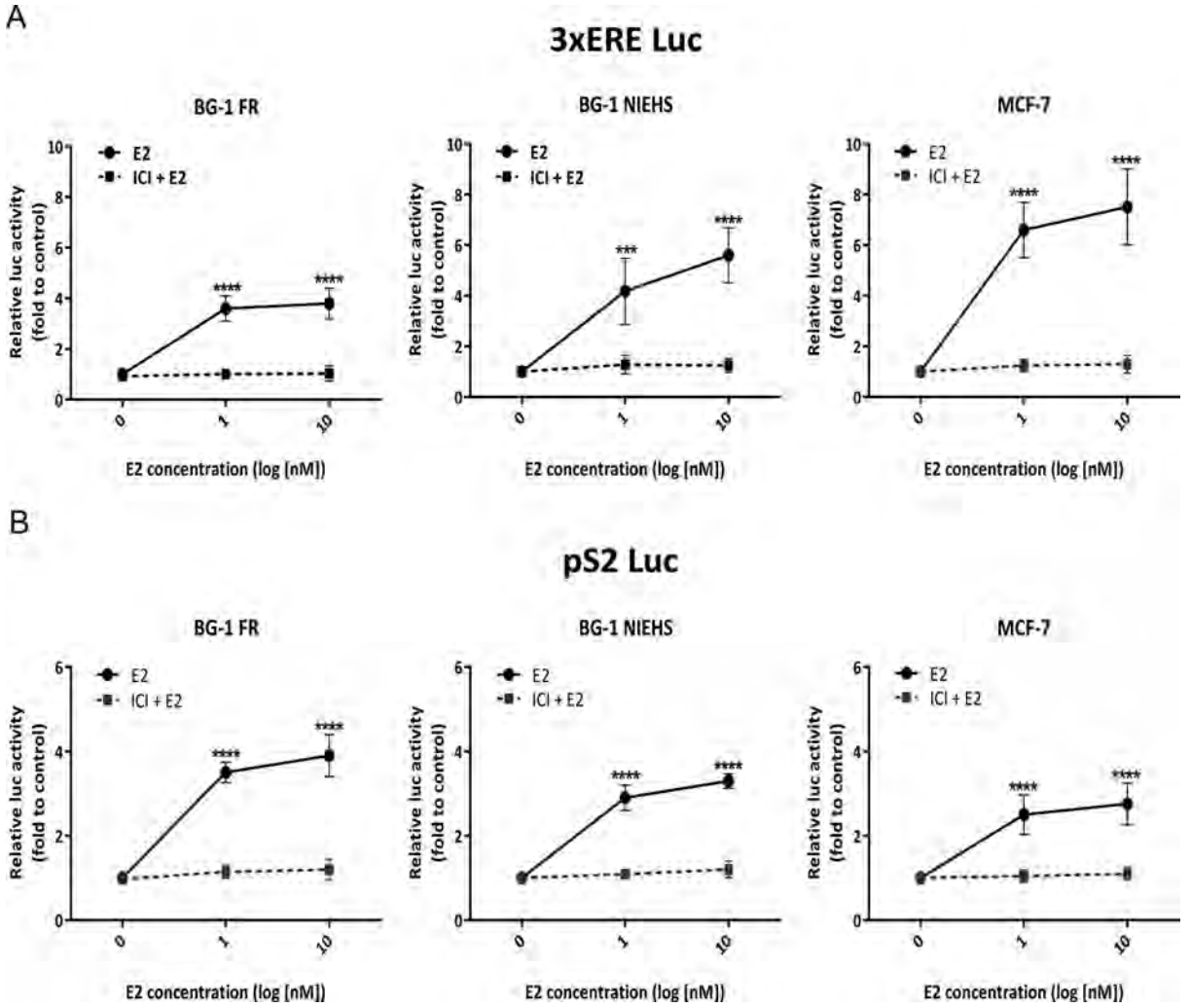

Figure 4. ER/ERE-mediated estrogenic responses in the BG-1 FR, BG-1 NIEHS, and MCF-7 cell lines. A, Activation on the 3xERE Luc reporter. Cells were transfected with 3xERE Luc and pRL-TK (control) plasmid overnight. After changing to fresh 10% sFBS DMEM-F12 medium, cells were treated with 0, 1, or 10 nM E2 for 18 hours in the absence or presence of 1 μM ICI. E2/ERE-mediated activation was detected by luciferase reporter assays as described in *Material and Methods*. Data shown are the average of triplicate determinations in a representative experiment. Value were calculated relative to vehicle control and presented as ±SEM. ***, $P < .001$, or ****, $P < .0001$. B, Activation on the pS2 Luc reporter. Cells were transfected with pS2 Luc and pRL-TK (control) plasmid overnight. The treatment and luciferase reporter assay were performed as described above in panel A.

ysis results with the original karyotype report of the BG-1 cells (24), we discovered an identical profile in the BG-1 FR cells. Furthermore, the basal expression level of the *ESR2* (ERβ) gene and its protein level were significantly higher in the BG-1 FR cell line than in the BG-1 NIEHS and MCF-7 cells. *ESR2*-expressing tissues in the whole body are quite restricted in comparison with *ESR1*-expressing tissues (43). Ovarian tissue and granulosa cells specifically are one of the highest expressing cell types for *ESR2* in the body (44). The elevated expression of *ESR2* in the BG-1 FR cells may be due to the cell origin, a solid primary tumor tissue in a patient with stage III ovarian adenocarcinoma (24). Regardless, our studies suggest that the BG-1 FR cell line is the original human ovarian cancer cell from the research group of Geisinger and colleagues (24). In addition, this study is the first report characterizing the STR DNA and gene expression profiles of this original BG-1 cell line.

A previous study reported that the BG-1 NIEHS cell line has a similar STR DNA profile to the MCF-7 cell line (4). We also observed identical STR DNA profiles between the BG-1 NIEHS and MCF-7 cells. Interestingly, we confirmed our earlier observations (26–28) that the BG-1 NIEHS cells were morphologically different from the MCF-7 cells, suggesting that similarities in STR profiles do not completely correlate with phenotypic features. In addition, we found that the gene expression level of *ESR1* (ERα) in both the BG-1 NIEHS and the MCF-7 cells was much higher than the level in the BG-1 FR cells.

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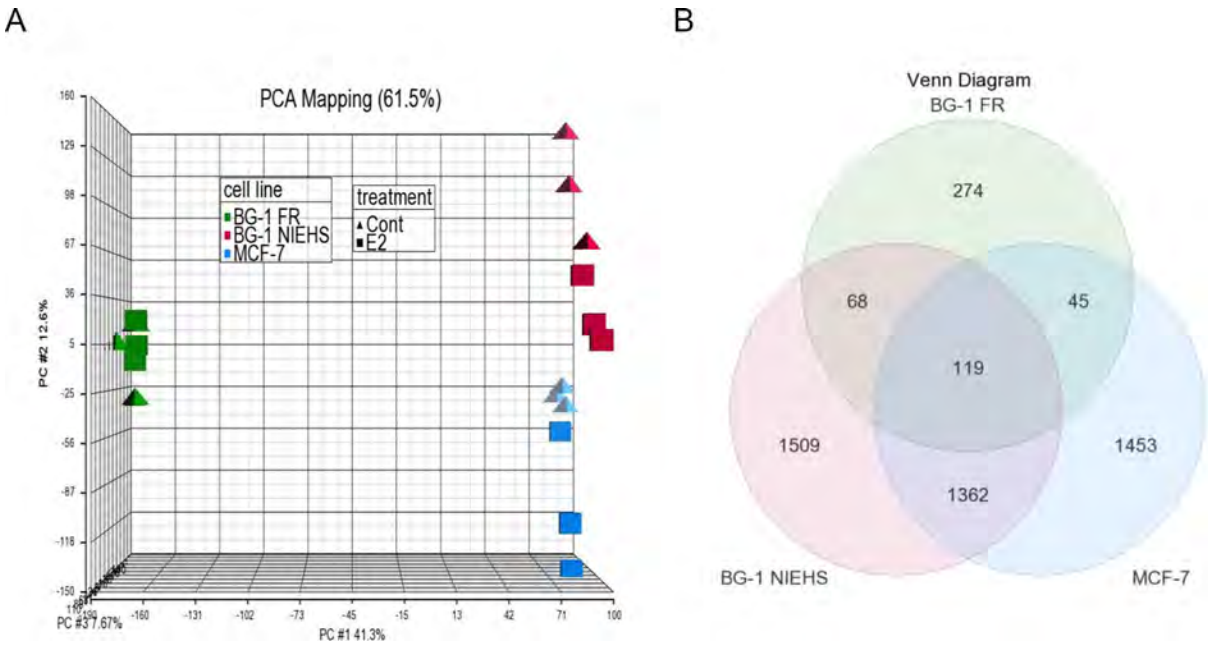

Figure 5. Microarray analysis of E2-responsive gene expression in BG-1 FR, BG-1 NIEHS, and MCF-7 cells. A, PCA mapping. The PCA was performed on all samples and all probes to characterize the variability among triplicates for each treatment group and between different treatment groups. B, Venn diagram. Contrasts were set at BG-1 FR vehicle control vs E2 (10 nM, 18 h treatment), BG-1 NIEHS vehicle control vs E2 (10 nM, 18 h treatment), and MCF-7 vehicle control vs E2 (10 nM, 18 h treatment). The gene numbers in the graph were determined with ±1.5-fold cutoff, $P < .05$.

This observation suggests that BG-1 NIEHS cells are a derivative of MCF-7 cells.

Using microarray analysis, we found that although BG-1 NIEHS is a variant of MCF-7 cells the E2-responsive gene expression profiles between the three cell lines are quite different. Specifically, there was an approximate 40%–50% overlap in E2-responsive genes identified between any two cell lines. One specific marker of E2 responsiveness in the breast and ovary is fibulin-1, a secreted glycoprotein that binds with extracellular matrix

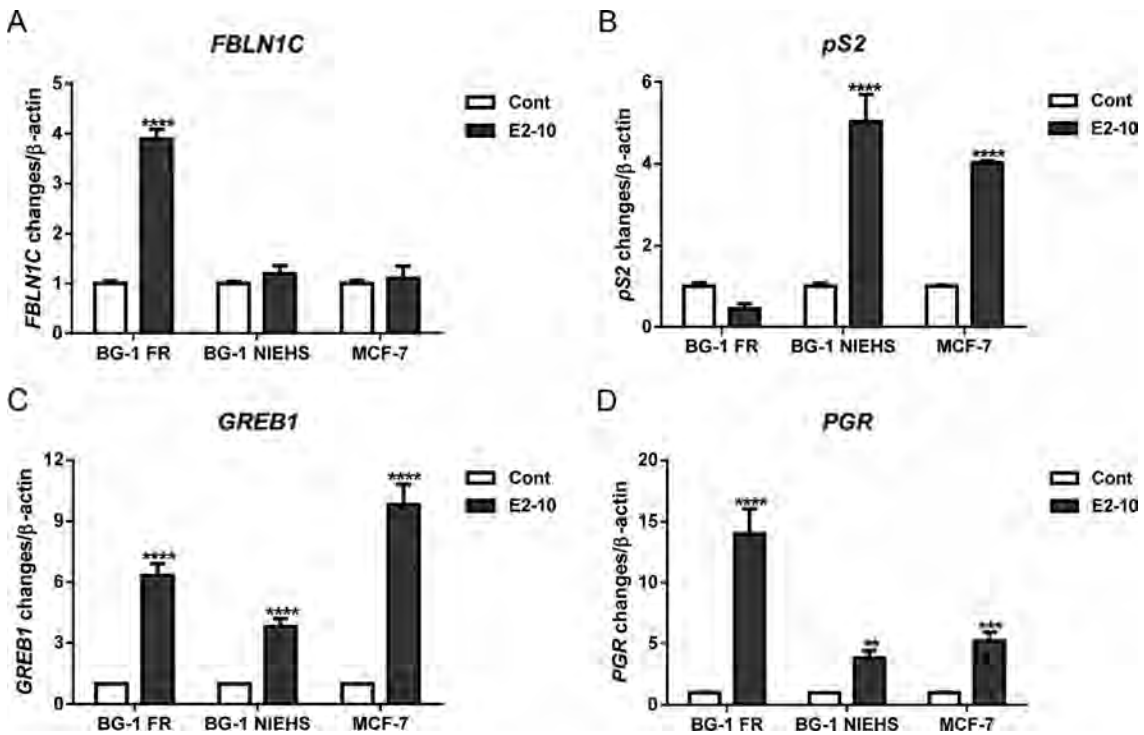

Figure 6. The effects of E2 on expression of *FBLN1C*, *pS2*, *GREB1*, and *PGR* in BG-1 FR, BG-1 NIEHS, and MCF-7 cells. Cells were treated with vehicle control (Cont) or 10 nM E2 for 18 hours. Total RNA was extracted and used as a template for cDNA synthesis. Gene expression was quantitated by real time-PCR. Experiments were repeated three times and results are presented as mean ±SEM. **, $P < .01$; ***, $P < .001$; ****, $P < .0001$.

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proteins (16, 17). Several reports suggested that the elevated expression of fibulin-1 protein is associated with mammary (45) and ovarian tumors (46). However, the E2 responsiveness of the *FBLN1* gene is differentially stimulated by E2 in mammary and ovarian tumors. Namely, the expression level of a specific isoform of the *FBLN1* gene, *FBLN1C*, is selectively induced by E2 only in human ovarian cancer but not in breast cancer cells (17, 25, 46). In this study, we found that the *FBLN1C* mRNA was highly expressed in BG-1 FR cells and up-regulated by E2. However, the expression of *FBLN1C* mRNA and hormonal responsiveness were not observed in BG-1 NIEHS and MCF-7 cell lines, thereby suggesting that the BG-1 NIEHS cell line shares more characteristics with MCF-7 cells than with ovarian cancer cells.

Several human breast cancer cell lines, including MCF-7, have a range of 76–88 chromosomes (47). Numerous common chromosomal translocations have been identified in the MCF-7 clonal variants (48). These chromosomal abnormalities may influence the biologic and pharmacological response of the cells (47). In this study, we also found different chromosomal aberrations from the cytogenetic analysis in BG-1 NIEHS and MCF-7 cells. These differences could explain the distinct gene expression profiles and morphologies observed. To evaluate the effect of distinct gene expression in BG-1 NIEHS and MCF-7 cells, we performed Ingenuity pathway analysis for the cell specific E2-regulated genes (Supplemental Table 4). Interestingly, cell morphology- and cell function-related genes were ranked in BG-1 NIEHS, whereas the cell cycle-related genes were ranked in MCF7 cells. These profiles may reflect the differential cell morphology and cell growth between BG-1 NIEHS and MCF7 cells. Although a clear correlation between chromosomal locations and gene expression profiles was not apparent, further analyses could be pursued in future studies and may need to be considered in comparing experimental results from different laboratories using MCF-7 cells.

In summary, we recently discovered that there are two different BG-1 cell lines being used for experimental studies, denoted here as BG-1 FR and BG-1 NIEHS. Based on STR DNA and gene expression profiling, we conclude that the BG-1 FR cell line is the original human ovarian cancer cells from the research group of Geisinger and colleagues (24). This study is the first report characterizing the STR DNA and gene expression profiles of the original BG-1 cell line. More importantly, we concluded that the BG-1 NIEHS variant cells were derived from MCF-7 cells and listed in the publications that used BG-1 NIEHS cells in Supplemental Table 5. This information may possibly require a reevaluation of the reported results. Altogether these findings provide much-needed clarification of the identities and characteristics of these in vitro cell models that are widely used to study estrogen responses in female ovarian and breast cancers.

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Disclosure Summary: The authors have nothing to disclose.

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Cell Line Authentication Service

STR Profile Report

Sample Submitted By: Michael Denison
UC Davis

Email Address: msdenison@ucdavis.edu

ATCC Sales Order: SOJ44477

FTA Barcode: STRA3399

Cell Line Designation: BGI ERE

Date Sample Received: June 2, 2016

Report Date: June 3, 2016

Methodology: Seventeen short tandem repeat (STR) loci plus the gender determining locus, Amelogenin, were amplified using the commercially available PowerPlex® 18D Kit from Promega. The cell line sample was processed using the ABI Prism® 3500xl Genetic Analyzer. Data were analyzed using GeneMapper® ID-X v1.2 software (Applied Biosystems). Appropriate positive and negative controls were run and confirmed for each sample submitted.

Data Interpretation Cell lines were authenticated using Short Tandem Repeat (STR) analysis as described in 2012 in ANSI Standard (ASN-0002) *Authentication of Human Cell Lines: Standardization of STR Profiling* by the ATCC Standards Development Organization (SDO) and in Capes-Davis et al., Match criteria for human cell line authentication: Where do we draw the line? Int. J. Cancer. 2012 Nov 8. doi: 10.1002/ijc.27931

ATCC performs STR Profiling following ISO 9001:2008 and ISO/IEC 17025:2005 quality standards.

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Test Results for Submitted Sample					ATCC Reference Database Profile			
Loci	**Query Profile: BGI ERE**				**Database Profile: MCF7**			
D3S1358	16							
TH01	6				6			
D21S11	30							
D18S51	14							
Penta_E	7	12						
D5S818	11	12			11	12		
D13S317	11				11			
D7S820	8	9			8	9		
D16S539	11	12			11	12		
CSF1PO	10				10			
Penta_D	12							
Amelogenin	X				X			
vWA	14	15			14	15		
D8S1179	10	14						
TPOX	9	12			9	12		
FGA	23	24	25					
D19S433	13	14						
D2S1338	21	23						

Number of shared alleles between query sample and database profile:	14
Total number of alleles in the database profile:	14
Percent match between the submitted sample and the database profile:	100

The allele match algorithm compares the 8 core loci plus amelogenin only, even though alleles from all loci will be reported when available.

NOTE: *Loci highlighted in grey (8 core STR loci plus Amelogenin) can be made public to verify cell identity. In order to protect the identity of the donor,* ***please do not publish*** *the allele calls from all the STR loci tested. Electropherograms showing raw data are attached.*

Explanation of Test Results

Cell lines with ≥ 80% match are considered to be related; i.e., derived from a common ancestry. Cell lines with between a 55% to 80% match require further profiling for authentication of relatedness.

- ☐ The submitted sample profile is human, but not a match for any profile in the ATCC STR database
- ☒ The submitted profile is an exact match for the following ATCC human cell line(s) in the ATCC STR database (8 core loci plus Amelogenin): HTB-22 (MCF7)
- ☐ The submitted profile is similar to the following ATCC human cell line(s):

Additional Comments:
Submitted sample, STRA3399 (BGI-ERE) is an exact match to ATCC HTB-22 (MCF7).

e-Signature, Technician:	snicholson 06/03/16
e-Signature, Reviewer:	gsykes 06/03/16

CELL LINE AUTHENTICATION SERVICE ATCC VERIFIED EST. 1925

Cell Line Authentication Service
STR Profile Report

FTA Barcode: STRA3399
ATCC Sales Order: SOJ44477

Addendum: Comparative Output from the ATCC STR Profile Database

% Match	ATCC® Cat. No.	Designation	D5S818	D13S317	D7S820	D16S539	vWA	THO1	AMEL	TPOX	CSF1PO
100	HTB-22	MCF7	11,12	11	8,9	11,12	14,15	6	X	9,12	10
100	STRA3399	BGI ERE	11,12	11	8,9	11,12	14,15	6	X	9,12	10

Definitions of terms used in this report:

Peak Area Difference (PAD):
Refers to a heterozygous peak imbalance.
Two alleles at a single locus should amplify in a similar manner; and therefore produce peaks of similar height and area. Peaks which are above threshold (50 rfu) but are not of similar area, within 50% of each other, are referred to as a PAD. Due to their nature cell lines do not amplify in the same manner as a sample taken from a fresh buccal swab. PAD is far more common in cell line samples.

Stutter:
A stutter peak is a small peak which occurs immediately before the true peak. It is defined as being a single repeat unit smaller than the true peak. The stutter peak should be less than 15% of the true peak. The stutter is caused by the polymerase.

+4 Peak:
A +4 is similar to a stutter but occurs immediately after the true peak. A stutter peak should be less than 5% for a homozygous and 10% for a heterozygous.

Below Threshold Peak(s):
Cell lines can produce unusual profiles and occasionally a peak will amplify poorly and be below threshold. Where we find a below threshold peak which we believe is valid we indicate it as a below threshold peak. Our cell line analysis criteria, Homozygous and Heterozygous peaks must be equal to or above the set height threshold for it to be considered a true peak.

Ladder/ Off Ladder Peak(s):
The allelic ladder consists of most or all known alleles in the population and allows for precise assignment of alleles. Those which do not align are termed ‘off ladder.

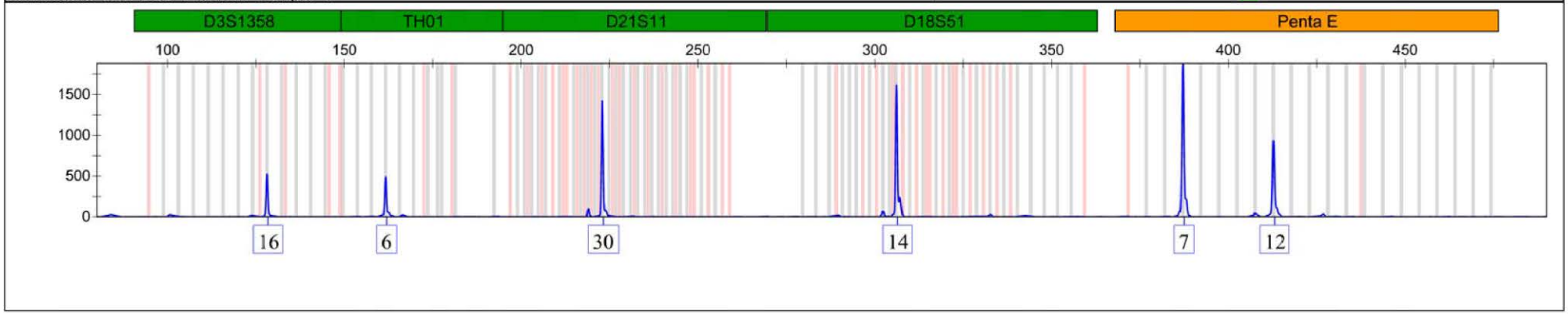
Artifact:
A non-allelic product of the amplification process, an anomaly of the detection process, or a by-product of primer synthesis

Pull-up:
A term used to describe when signal from one dye color channel produces artificial peaks in another, usually adjacent, color.

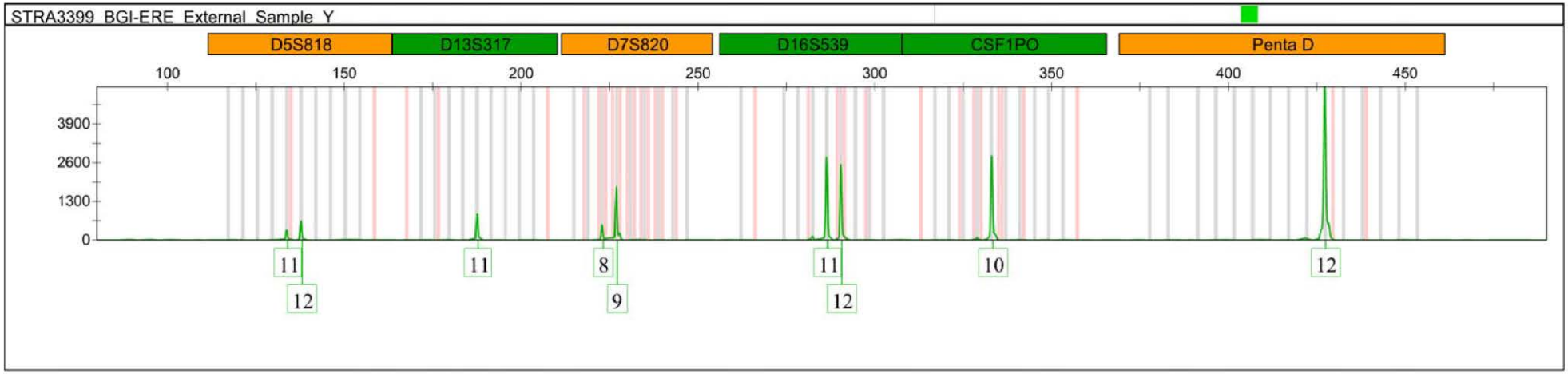
Spike:
An extraneous peak resulting from dust, dried polymer, an air bubble, or an electrical surge.

Dye blob:
Free dye not coupled to primer that can be injected into the capillary (A known and documented dye blob is often found at the D3S1358 locus.)

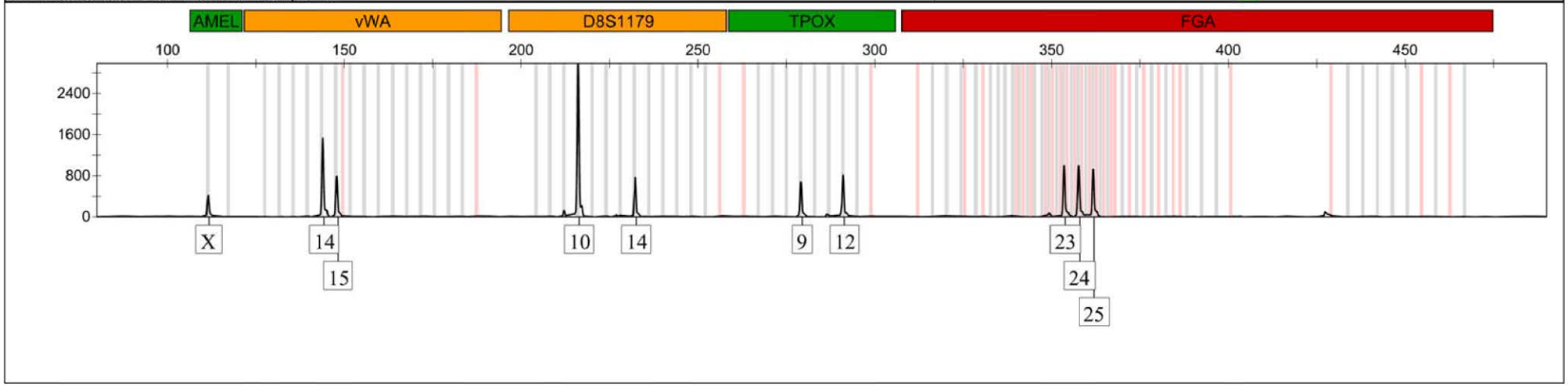
Sample Name	SQ
STRA3399_BGI-ERE_External_Sample_Y	



STRA3399_BGI-ERE_External_Sample_Y



Sample Name	SQ
STRA3399_BGI-ERE_External_Sample_Y	



STRA3399_BGI-ERE_External_Sample_Y