CORRESPONDENCE

Cell Line Designation Change: Multidrug-Resistant Cell Line in the NCI Anticancer Screen

Since 1990, the Developmental Therapeutics Program (DTP) of the National Cancer Institute (NCI) has screened over 60,000 compounds and a larger number of natural product extracts for their capacity to inhibit the growth of 60 different human tumor cell lines (1). These cell lines have been maintained in cryopreservation and in culture, and they have been subjected to strict quality controls, including adventitious agent testing, human isoenzyme analysis, karyology, morphological and immunocytochemical characterization (2), and DNA fingerprinting. One of these cell lines, previously designated as MCF-7/ADR-RES, has been included in the in vitro cell line screening panel because of its stable multidrug-resistant (MDR) phenotype (3) characterized by high levels of MDR-1 and P-glycoprotein expression (4,5). Recently, we submitted cell lines from the screening panel for DNA fingerprinting analysis by three different laboratories. Included in the tested cell lines were MCF-7 and MCF-7/ADR-RES. Utilizing restriction fragment length polymorphism (RFLP) testing, CellMark Diagnostics (Germantown, MD) concluded that their DNA fingerprinting data were consistent with each of the cell lines (MCF-7 and MCF-7/ADR-RES) having different donors. The other laboratories—American Type Culture Collection (Rockville, MD) used both RFLP and amplification fragment length polymorphism (AmpFLP) methods, and Children’s Hospital of Michigan Cell Culture Laboratory (Detroit, MI) used the AmpFLP method—reached the same conclusions. Based on the reports from these DNA fingerprinting analyses, we have concluded that the preponderance of the information available suggests that the MCF-7/ADR-RES multidrug-resistant cell line that is included in the DTP screening program is not related to the MCF-7 cell line that is a part of the screening panel. Thus, we have changed the nomenclature of the MCF-7/ADR-RES multidrug-resistant cell line. The new designation of this cell line is NCI/ADR-RES. This nomenclature change will soon appear in all DTP databases, including the worldwide web. The DTP web site address is: http://epws1.ncifcrf.gov:2345/dis3d/dtp.html

Irrespective of its origin, this cell line has served as a valuable sentinel for compounds interacting with the multidrug-resistant mechanism (5).

DOMINIC A. SCUDIERO
ANNE MONKS
EDWARD A. SAUSVILLE

References


Notes

Affiliations of authors: D. A. Scudiero, A. Monks, Science Applications International Corp., National Cancer Institute–Frederick Cancer Research and Development Center, Frederick, MD; E. A. Sausville, Developmental Therapeutics Program, Division of Cancer Treatment, Diagnosis, and Centers, National Cancer Institute, Bethesda, MD.

Correspondence to: Dominic A. Scudiero, Ph.D., Science Applications International Corp., National Cancer Institute–Frederick Cancer Research and Development Center, Box B, Bldg. 432, Frederick, MD 21702–1201.

Influence of Genistein and Daidzein on Brca1 Protein Levels in Human Breast Cell Lines

Phyto-estrogens potentially have anticarcinogenic biologic activities and could play a role in the dietary etiology of breast cancer. They have estrogenic as well as antiestrogenic properties and can also inhibit specific enzyme activities. In vitro and in vivo studies have shown that phyto-estrogens are tumor inhibitory. Moreover, Asian populations who consume large amounts of phyto-estrogens derived from a soy-rich diet have a lower frequency of breast tumors than western populations who consume much lower quantities of these compounds (1).

The BRCA1 gene is involved in breast cancer families with a pattern of autosomal-dominant inheritance of the disease (2). Comparison of BRCA1 messenger RNA levels in a series of normal breast tissues and sporadic breast cancer tissues demonstrated an apparent decrease in BRCA1 gene expression by tumor cells (3). Moreover, there is some evidence that BRCA1 gene transcription is directly and indirectly regulated by estrogen (4).

We investigated whether two selected phyto-estrogens—genistein and daidzein—modulate the expression of Brca1 protein in human breast cells. The effects of these phyto-estrogens were compared in three human mammary cell lines, MCF7 cells (estrogen receptor-positive cells), MDA-MB231 cells (estrogen receptor-negative adenocarcinoma-derived cells), and MCF10a cells (estrogen receptor-positive epithelial cells). Each cell line was treated for 96 hours at the IC50 of each phyto-estrogen—genistein (5 μg/mL) or daidzein (20 μg/mL).

For Brca1 protein determinations following either genistein or daidzein treatment, total cell extracts were prepared from cells metabolically labeled with [35S]methionine. Levels of Brca1 protein were determined by use of a magnetic protein purification protocol (Dynabeads M-450 coated with Brca1 antibodies D–20; Santa Cruz Biotechnologies, Santa Cruz, CA). The beads...
were incubated with the radiolabeled cell extracts, collected by use of a magnet, and washed three times with lysing solution. The Brca1 proteins were eluted with 0.5 M acetate buffer (pH 2.4). Brca1-specific immunoselected protein was quantified by liquid scintillation counting. The amount of Brca1 protein was expressed as a percentage, calculated as follows: 100 × (disintegrations per minute [dpm] Brca1 immuno-selected protein/dpm total cell extract). The results of the Brca1 protein determination are shown in Fig. 1. Genistein had no effect on Brca1 protein expression in MCF7 cells (\(P = .35\); values are two-sided), MDA-MB231 cells (\(P = .20\)) or MCF10a cells (\(P = .35\)) when compared with untreated cells. Daidzein had no effect on Brca1 protein expression in MCF7 cells (\(P = .35\); \(P = .05\)). Brca1 protein levels were decreased in MCF10a cells (\(P = .05\)). Because daidzein increased Brca1 protein levels in estrogen receptor-negative MDA-MB231 cells, the response was not mediated via the estrogen receptor.

Isoflavones have biologic properties that are quite separate from classic estrogen action and may influence carcinogenesis (5). Our results suggest that the lack of an effect by genistein on Brca1 protein expression may be explained by nonestrogenic biologic properties of isoflavones. Genistein apparently signals via a separate mechanism. For example, genistein augments signaling mechanisms mediated by transforming growth factor \(\beta\), which inhibits progression of cells through the \(G_1/S\) phase of the cell cycle (6). It is of interest that the BRCA1 gene is expressed in late \(G_1/S\) phase (7). Further research on dietary phyt-oestrogens should be undertaken by use of such approaches as a quantitative reverse transcriptase-coupled polymerase chain reaction assay to determine whether regulation of the BRCA1 gene at the level of messenger RNA is influenced by phyt-oestrogens.

**Fig. 1.** Relative amount of Brca1 proteins expressed by human mammary cell lines (MCF7, MDA-MB231, and MCF10a) following genistein (5 \(\mu g/mL\)) and daidzein (20 \(\mu g/mL\)) treatments. Each value corresponds to mean ± standard deviation of three assays for each group (two-sided \(P\) values by Wilcoxon rank-sum tests). *\(P = .05\).

**References**


**Diagnostic Drift in the Reporting of Cancer Incidence**

Giving a patient a label that says ‘‘cancer’’ is a fateful event, and there are strong reasons for minimizing the number of errors, both false-negative and false-positive. The first step toward reducing both kinds of errors is, surely, to improve agreement among pathologists about how they read specific specimens.

A second, and even more important, area needs further development. Over a period of many decades, we have become accustomed to thinking of the interpretation of the pathologist as the ‘‘gold standard’’ against which other diagnostic measures are evaluated. However, we must question this practice in light of developments in cancer screening and improved understanding of the biology of proliferative lesions. The work of Wells et al. (1) and the commentary of Page et al. (2) do not deal with changes over time in the boundaries that define what we call ‘‘cancer.’’ At any one fixed time, agreement will be good if most pathologists have changed their standards in much the same way, but apparent trends in cancer incidence rates may still be created or masked.

For example, the reported incidence rate for cancer of the prostate approximately doubled over a 10-year period, without notable impact then or later on the mortality rate. This was a reflection