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Report

Hormone dependence of breast cancer cells and the effects of tamoxifen and estrogen: ^{31}P NMR studies

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Abstract

Many breast tumors appear to progress from estrogen-dependent growth to a more malignant phenotype characterized by estrogen-independent growth, antiestrogen resistance, and a high metastatic potential. Utilizing ^{31}P NMR spectroscopy on human breast cancer cells growing *in vitro,* we have investigated the effects of 17β-estradiol and tamoxifen on the metabolic/bioenergetic spectra of a series of human breast cancer cells that vary in their estrogen and antiestrogen responsiveness. A comparison of baseline spectra associates higher levels of phosphodiesters and UDP-glucosides (*e.g.* UDP-glucose, UDP-*N*-acetylglucosamine), and lower phosphocholine/glycerylphosphocholine and phosphocholine/phosphoethanolamine ratios, with the acquisition of estrogen-independent growth in estrogen receptor expressing cells. No metabolic changes are clearly associated with the metastatic phenotype. Whilst estrogen treatment produces no consistently significant spectral changes in any of the cell lines, the estrogen-independent and estrogen-responsive MCF7/MIII cell line responds to tamoxifen treatment by significantly increasing all spectral resonances 30%–40% above baseline values. This may reflect a tamoxifen-induced change to a more differentiated or apoptotic phenotype, or an attempt by the cells to reverse the inhibitory effects of the drug. The ability to detect metabolic changes in response to tamoxifen by NMR spectroscopy may provide a novel means to identify those tumors that are responsive to antiestrogen therapy.

Abbreviations: CCS-IMEM – steroid-deprived Improved Minimal Essential Medium; E2 – 17β-estradiol; ER – estrogen receptor; P_i – inorganic phosphate; GPE – glyceryl-phosphoethanolamine; GPC – glyceryl-phosphocholine; PC – phosphocholine; PE – phosphoethanolamine; PDE – phosphodiesters; PME – phosphomonoesters; TAM – tamoxifen (trans-1-(4-β-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene); UDPG – uridine diphosphoglycoside

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Introduction

Many breast tumors appear to follow a predictable clinical pattern of malignant progression. For example, tumors may be initially responsive to endocrine manipulation, and/or sensitive to cytotoxic chemotherapy. Many of these tumors will progress to a more malignant phenotype, characterized by invasive/metastatic foci which are resistant to both endocrine manipulation and chemotherapeutic intervention [1]. The development of this phenotype is responsible for the high percentage of systemic treatment failures among breast cancer patients, and may reflect a fundamental biological property of many human breast tumors [1, 2]. It remains difficult to predict precisely which patients will initially respond to a specific therapy. While the detectable expression of cellular receptors for estrogen (ER) and progesterone (PGR) can predict response to antiestrogens in 60%–70% of cases, approximately one-third of patients with ER/PGR positive tumors will fail to respond to tamoxifen (TAM) [3].

One major obstacle in studying progression from estrogen-dependent growth to estrogen-independent and antiestrogen resistant growth, has been the lack of suitable models. We have recently isolated and characterized a series of novel MCF-7 variants, which together represent the most critical stages in breast cancer progression [1, 2]. Parental MCF-7 cells are fully dependent upon estrogen (E2) for growth *in vitro* and *in vivo,* and are sensitive to antiestrogens. MCF7/LY2 cells are MCF-7 cells that were selected for growth in the presence of the benzothiophene antiestrogen LY117018 [4]. These cells are resistant to each of the major structural classes of antiestrogens [5]. MCF7/MIII cells are estrogen-independent and estrogen-responsive MCF-7 cells that no longer require E2 for growth in athymic nude mice [6]. However, MCF7/MIII cells are sensitive to the growth inhibitory effects of triphenylethylene, benzothiophene, steroidal and other non-steroidal antiestrogens [5], and also retain sensitivity to inhibition by LH-RH agonists and antagonists [7]. MCF7/LCC2 cells were obtained by a further passage of MCF7/MIII cells *in vivo,* followed by a stepwise selection for growth in the presence of the triphenylethylene antiestrogen 4-hydroxytamoxifen. These cells are resistant to triphenylethylenes, but sensitive to the inhibitory effects of the steroidal antiestrogens ICI 182, 780 and ICI 164, 384 [8, 9]. Both MCF7/MIII [10] and MCF7/LCC2 cells (unpublished observations) are more invasive and metastatic *in vivo* than the parental MCF-7 cells. We have recently demonstrated that the acquisition of an estrogen-independent phenotype is not associated with the presence of detectable amplified DNA sequences in these estrogen-independent MCF-7 variants. Whilst specific E2-regulated genes are differentially expressed [11], the precise molecular, metabolic, and bioenergetic events that enable breast cancer cells to acquire a progressed phenotype remain unclear [12].

Several investigators have used ^{31}P NMR to monitor the bioenergetic and phospholipid metabolism of breast cancer cells both *in vivo* and *in vitro* [13–15]. We have used a method in which cells are embedded in agarose gel threads to investigate the effects of cytotoxic drugs by NMR [16, 17]. This technique enables continuous perfusion that can be made to mimic *in vivo* conditions [18, 19]. ^{31}P NMR spectroscopy can determine the concentrations of several molecules closely involved in cellular metabolism/energy production. For example, we can readily determine changes in the total intracellular concentrations of inorganic phosphate (P_i), phosphoethanolamine (PE), glyceryl-phosphoethanolamine (GPE), phosphocholine (PC), glyceryl-phosphocholine (GPC), phosphocreatine, α-ATP, β-ATP, gamma-ATP, NAD(P), and uridine diphosphoglycoside (UDPG). To facilitate analysis, some related metabolites are grouped together. Thus, GPC and GPE are referred to as the phosphodiesters (PDE), and PC and PE represent the phosphomonoesters (PME). PC and PE are precursors for phosphatidylethanolamine and phosphatidylcholine respectively. PC and PE are also produced by the phospholipase C-mediated degradation of phosphatidylethanolamine and phosphatidylcholine. This reaction releases diacylglycerol, a potent activator of protein kinase C.

In this study, we have investigated potential metabolic and bioenergetic perturbations among estrogen-independent, antiestrogen sensitive, and antiestrogen resistant variants of the estrogen-de-

pendent MCF-7 human breast cancer cell line [5, 6, 11]. We have also examined the ability of E2 and TAM to alter energy metabolism in these cells.

Materials and methods

Cell culture

MCF-7 human breast cancer cells (passage > 500) were originally provided by Dr. Marvin Rich (Michigan Cancer Foundation, MI) and MDA-MB-435 cells were obtained from Dr. Janet Price (M.D. Anderson Cancer Center, Houston, TX). Both cell lines were routinely maintained in Improved Minimal Essential Medium (IMEM) containing phenol red (Biofluids, Rockville, MD) and supplemented with 5% fetal calf serum (FCS-IMEM). MCF7/MIII cells are an estrogen-independent variant of MCF-7 cells derived in this laboratory [6, 11]. MCF7/LCC2 is an antiestrogen resistant variant of MCF7/MIII obtained by a further passage of MCF7/MIII cells *in vivo,* followed by selection for growth in the presence of the triphenylethylene antiestrogen 4-hydroxytamoxifen [8]. These cells were routinely maintained in IMEM without phenol red, and supplemented with 5% steroid-stripped calf serum (CCS-IMEM). MCF7/LY2 cells are antiestrogen resistant MCF-7 cells, and were grown in IMEM with phenol red supplemented with 5% steroid-stripped calf serum [4].

Calf serum was stripped of endogenous steroids by treatment with dextran-coated charcoal and sulfatase as previously described [5]. For studies in CCS-IMEM, endogenous steroids were removed by washing cells three times in CCS-IMEM and refed CCS-IMEM for five days. All cell cultures were maintained at 37° C in a humidified 5% CO_2/95% air atmosphere. All cell lines used in this study are free from contamination with *Mycoplasma spp.*

Cell preparation for ^{31}P NMR studies

Cells were grown in CCS-IMEM to 90% confluence, harvested with 0.05% (w/v) trypsin/0.53 mM EDTA (Gibco, Grand Island, NY), centrifuged at 4° C at 1000 × g for 5 min, and washed twice in CCS-IMEM. This treatment is sufficient to remove any endogenous E2 from the cells [20]. A 1 ml aliquot of packed cell volume ($2–3 \times 10^8$ cells) was mixed with an equal volume of agarose solution (1.8% w/v low temperature gelling agarose; 10 mM 0.02 M N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid in saline; pH 7.4), and embedded in agarose threads by extrusion through 0.5 mm internal diameter tubing into a Wilmad NMR 10 mm tube [19]. The gel threads were concentrated without compression at the bottom of the NMR tube by using an insert with inlet and outlet tubing. The cell thread mixture was continuously perfused with the appropriate media, at a constant perfusion rate of 1 ml/min. The medium was gently bubbled with 5% CO_2:95% air to ensure sufficient oxygenation and to maintain a pH of 7.4. The NMR tube containing the cells and perfusion apparatus was placed in a standard 10 mm NMR probe, and maintained at a constant temperature of 37° C [19].

Magnetic resonance spectroscopy and data analysis

Cells were grown and studied in the same culture medium, unless otherwise stated. This minimizes the effects of media changes on cell proliferation. Cells were continually perfused with growth medium throughout each experiment. ^{31}P NMR spectra were recorded on a Varian XL-400 operating at 162 MHz, and analyzed on a Varian 4000 ADS data station. We used a three second repetition time and a 60° flip angle [17]. Readers unfamiliar with NMR methods are referred to references [18, 19] for detailed descriptions of the methodology. Since we are comparing the same phosphorus metabolites from different cell lines, it was not necessary to allow complete relaxation. One thousand two hundred scans were accumulated for each spectrum totaling one hour, and processed using 10–15 Hz line broadening. Spectra were analyzed either by peak height, since the line width of the resonances did not change by more than 3% throughout the time course, or by integration and estimation of the area on a Varian ADS 4000 data station. Ratios of peak

heights (or areas), and not peak heights alone, are comparable among experiments. Thus, we determined each peak as a ratio of the β-ATP signal standardized at – 18.7 ppm. It should be noted that the α and gamma ATP peaks are larger than the β-ATP peak, due to the presence of underlying resonances of other origin.

NMR data acquisition and processing were performed with identical parameters throughout all experiments. Since some cell loss can occur during thread casting and placing of the perfusion insert, protein content was used to standardize the actual amount of cellular material in each experiment, and to enable quantitative comparisons [21]. Protein was measured by the bicinchoninic acid assay (Pierce, Rockfort, IL). Agarose in the threads does not interfere with the determination of protein content [22]. Therefore, all comparisons of peak intensities are normalized to an independent standard, *i.e.* cellular protein content [21].

For TAM and E2 studies, two baseline spectra were recorded before drugs were added to the media. A 1 mM solution of drug was prepared in ethanol and added to the perfusion reservoir, producing a final concentration of 0.5 μM for TAM and 0.5 nM for E2. These concentrations are ≥ the approximate ED_{50}'s required for the regulation of proliferation of sensitive MCF-7 cells through ER-mediated pathways, but are below concentrations known to produce significant toxicities independent of the ER system [23]. The cells were continually perfused with the appropriate solution for 15–20 hours while spectra were obtained. NMR data acquisition and processing were performed with identical parameters throughout all experiments.

Results

Baseline comparisons

We studied six different breast cancer cell lines, including E2-dependent MCF-7 cells, E2-independent and E2-responsive MCF7/MIII cells (MCF-7 variant), MDA-MB-435 and MDA-MB-231 cells that are E2-independent and ER-negative, and MCF7/LY2 and MCF7/LCC2 cells that are ER-positive and antiestrogen resistant MCF-7 variants (Table 1). Figure 1 represents the baseline spectra of the cell lines studied, with the exception of MDA-MB-231. The baseline for the ER-negative MDA-MB-435 (Fig. 1c) and MDA-MB-231 cells (not shown) are similar, differing significantly from each other only in the PDE region. When compared with the ER-positive MCF-7 cells and variants, MDA-MB-231 cells and MDA-MB-435 cells have significantly less GPC (peak 5). Both ER-negative cell lines exhibit low GPE levels (peak 4). PC and PE have similar ratios to the MCF-7 cells.

Relative to the parental MCF-7 cells, the baseline spectra for both the estrogen-independent MCF7/MIII (Fig. 1d) and MCF7/LCC2 cells exhibit higher UDPG levels (Fig. 1f). The MCF7/LCC2 cells that are TAM but not ICI 182, 780 resistant [8, 9] exhibit increased PE signals (peak 1) relative to MCF-7 cells (Fig. 1f). In contrast, the baseline PC levels (peak 2) in MCF7/LY2 cells (both TAM and ICI

Table 1. Phenotypes of cells used in this study

Cell line	ER	Estrogen dependence[1]	Estrogen responsivity[2]	TAM responsivity	ICI 182, 780 responsivity	Metastases
MCF-7	+ ve	dependent	responsive	sensitive	sensitive	no [6]
MCF7/MIII	+ ve	independent	responsive	sensitive	sensitive	yes [6]
MCF7/LCC2	+ ve	independent	responsive	resistant	sensitive	ND[4]
MCF7/LY2	+ ve	NT[3] [5]	responsive	resistant	resistant	NT[3] [5]
MDA-MB-231	– ve	independent	unresponsive	resistant	resistant	yes [35]
MDA-MB-435	– ve	independent	unresponsive	resistant	resistant	yes [35]

[1] Requirement for E2 to form tumors in nude mice; [2] respond to E2 by inducing specific genes/mitogenesis; [3] non-tumorigenic; [4] no data. Numbers in parentheses represent the appropriate literature citations

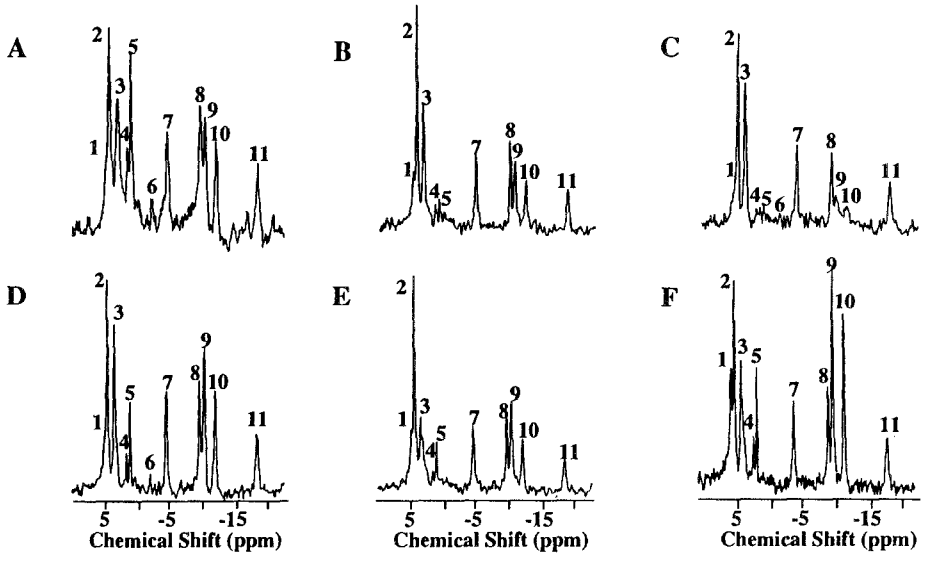

Fig. 1. Comparison of different ^{31}P spectra at 165 MHz of (a) MCF-7 cells perfused with FCS-IMEM, (b) MCF-7 cells perfused with CCS-IMEM media, (c) MDA-MB-435 cells, (d) MCF7/MIII cells, (e) MCF7/LY2 cells, (f) MCF7/LCC2 cells. All spectra were obtained at 37° C with 1200 scans during 1 hour and processed with 15 Hz line broadening. The peak assignments are: (1) PE; (2) PC; (3) P_i; (4) GPE; (5) GPC; (6) phosphocreatine; (7) gamma-ATP; (8) α-ATP; (9) NAD(P) and UDPG; (10) UDPG; (11) β-ATP.

182,780 resistant) are significantly higher than in MCF-7 cells (Fig. 1e). Other peaks in the MCF7/LY2 spectra were not different.

The peak at 10.7 ppm has been attributed to the two NMR-identical phosphorus groups in both NAD and NADP. However, NAD(P) levels can be estimated by subtracting UDPG. Comparisons of the subtracted baseline spectra showed no significant differences in the steady-state total ATP and NAD(P) levels (peak 9) among the cell lines (Fig. 2).

Effects of E2 and TAM on baseline spectra

Following acquisition of the baseline spectra, all cells were perfused either with 0.5 μM TAM or with 0.5 nM E2 for 16 to 20 hours. The baseline spectra of the ER-positive MCF-7 cells in FCS-IMEM and CCS-IMEM (Figs. 1a and 1b respectively) exhibit differences in the PDE region (GPC; GPE). Perfusion of MCF-7 cells in CCS-IMEM with E2 produces inconsistent increases in PDE. This may reflect the small levels of PDEs in these cells, which limits the resolution associated with small changes in PDE concentrations. Perfusion with TAM induces no significant changes in the spectra in any cell lines, with the exception of the MCF7/MIII cells. MCF7/MIII cells respond to TAM within three hours of its administration. A 30% to 40% final increase in all resonances is observed, and these changes are maintained throughout the course of the analyses (Fig. 3). The spectra of MCF7/MIII cells were not altered following perfusion with E2 (Fig. 4).

Discussion

The development of estrogen-independence, antiestrogen resistance, and increased metastatic potential, are among the most important phenotypic changes associated with malignant progression in breast cancer [1, 12]. We have isolated and characterized a series of MCF-7 variants in which to identify the cellular, molecular, and bioenergetic changes associated with malignant progression in breast cancer. The advantages of these variants include their common MCF-7 parentage, and the biological relevance of their respective phenotypes [12, 24]. Using this system we have previously demonstrated that estrogen-independence, antiestrogen-resist-

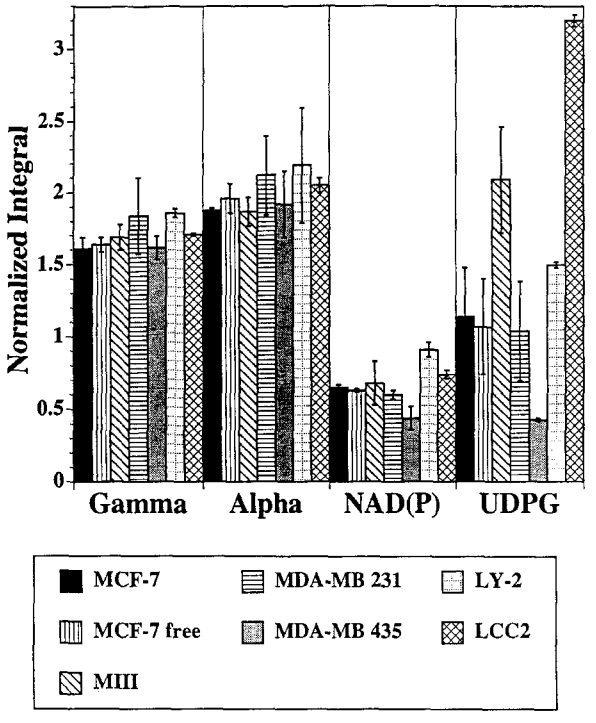

Fig. 2. Comparison of the high energy phosphates in different cell lines. The normalized integral was standardized by obtaining the ration of each peak area to that of β-ATP (– 18.7 ppm). The NAD/NADP peak was measured by subtracting the resonances at 12.5 and 10.7 ppm. Values represent the mean of 5 determinations ± SD.

ance [5, 11, 12], cytotoxic drug resistance [2], and increased metastatic potential [10] are phenotypes that can be independently acquired [5, 12]. We now report the use of these cells to investigate possible bioenergetic/metabolic changes associated with the acquisition of these characteristics (Table 1).

To identify bioenergetic/metabolic factors that might contribute to specific phenotypic changes, comparisons of the baseline ^{31}P spectra among the cell lines and variants were performed. The levels of high energy phosphates (ATP, phosphocreatine) were similar in all the cell lines (Fig. 2). Spectral subtraction of peak 10 (UDPG) from peak 9 (UDPG + NAD(P)) indicates low NAD(P) levels. For equal concentrations of both molecules, the ratio between resonance 9 and resonance 10 should be 3:1. In most of the cell lines, this ratio is only 1:1.5. These differences are most frequently due to perturbations in UDPG concentrations, primarily UDP-glucose and UDP-*N*-acetylglucosamine [25]. UDP-glucose is the glycosyl donor for glycogen and glucosyl disaccharides that can also participate in glycoprotein/proteoglycan biosynthesis.

Estrogen and estrogen-independent growth

MCF7/MIII and MCF7/LCC2 cells have significantly elevated levels of UDPG. These levels are approximately 50% higher than MCF-7 cells (± E2), and thereby associate perturbations in glucose/glycoprotein/proteoglycan metabolism in the acquisition of an intermediate estrogen-independent and estrogen-responsive/ER-positive phenotype. The activity of the hexose monophosphate shunt enzyme glucose-6-phosphate dehydrogenase, which is critical for the production of fatty acids in normal lactating breast, is clearly regulated by estrogens in MCF-7 cells and overexpressed in malignant breast tissues [26, 27]. Since we have previously demonstrated the altered regulation of E2-regulated genes in the estrogen-independent and estrogen-responsive MCF-7 variants [11], the increased levels of UDPG in the MCF7/MIII and MCF7/LCC2 cells could primarily reflect UDP-glucose from increased glucose-6-phosphate dehydrogenase activity. This may reflect a fundamental difference in how the intermediate phenotype of the

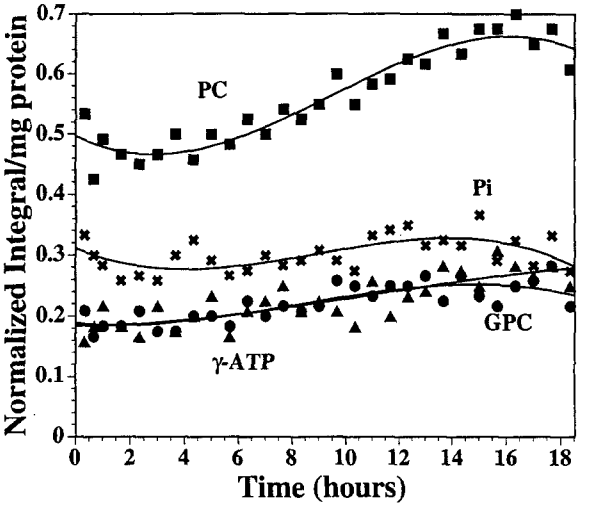

Fig. 3. Effect of TAM on MCF7/MIII cells. TAM (final concentration 0.5 μM) was added to perfusion media after 120 min of baseline data acquisition. Symbols are (■) PC; (★) P_i; (●) GPC; (▲) γ-ATP.

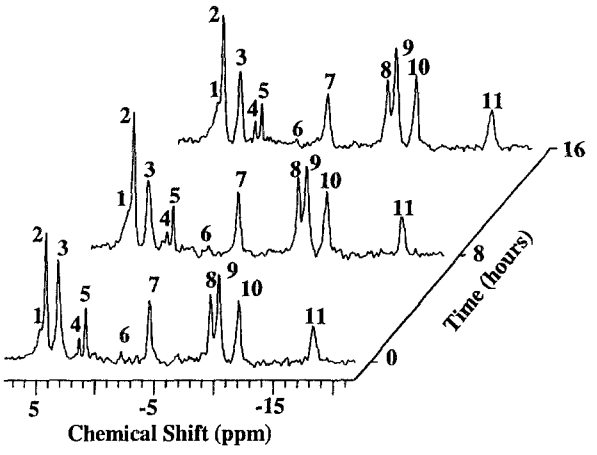

Fig. 4. ^{31}P spectra of MCF7/MIII cells at 37° C after E2-perfusion for 8 and 16 hours. Baseline spectra are also shown. Each trace consists of 1200 transients. The spectra were plotted with 15 Hz line broadening. The peak assignments are: (1) PE; (2) PC; (3) P_i; (4) GPE; (5) GPC; (6) phosphocreatine; (7) gamma-ATP; (8) α-ATP; (9) NAD(P) and UDPG; (10) UDPG; (11) β-ATP.

MCF7/MIII and MCF7/LCC2 cells (E2-independent and E2-responsive *in vivo*) utilize glucose metabolites relative to ER-positive, E2-responsive cells (*e.g.* MCF-7). There are no metabolic functions consistently associated with an increased metastatic potential (*i.e.* MCF-7 vs MCF7/MIII; MCF7/LCC2; MDA-MB-231; MDA-MB-435). This may reflect the multistep nature of the metastatic cascade, aspects of which can apparently be randomly acquired [1].

A few previous studies have attempted to address the effects of estrogen stimulation on breast cancer cell metabolism. For example, Neeman & Degani observed a 10%–30% increase in the levels of PC following E2 greatment of T47D cells [15]. TAM treatment produced a further 33%–140% increase in T47D PC levels when compared to E2-treated cells [28]. In contrast, ovariectomy increases phosphocreatine levels in N-nitrosomethylurea-induced rat mammary adenocarcinomas [29]. E2-treatment of the responsive OESHR1 rat mammary tumor is associated with elevated PC and reduced GPC levels [30]. We did not observe any significant response to E2 in MCF-7 cells, perhaps either indicating that T47D and MCF-7 cells respond differently to stimulation with E2, or reflecting differences in analytic procedures. We would not have anticipated any significant effects of E2 in the ER negative MDA-MB-231 or MDA-MB-435 cells. MCF7/MIII [5, 6] and MCF7/LCC2 cells [8] are mitogenically unresponsive/less responsive to E2 stimulation *in vitro*, but some genes remain E2-regulated [11]. MCF7/LY2 cells exhibit a significantly reduced mitogenic response to E2 [4]. Thus, it is perhaps not surprising that MCF7/MIII, MCF7/LCC2, and MCF7/LY2 cells do not produce detectable metabolic changes following E2-treatment.

TAM and antiestrogen resistance

As expected, TAM treatment did not induce any detectable metabolic changes in either the antiestrogen resistant and ER-positive (MCF7/LY2; MCF7/LCC2), or ER-negative (MDA-MB-231; MDA-MB-435) cells. The lack of any inhibitory effect in the sensitive parental MCF-7 cells is consistent with the absence of E2/phenol red in the culture media [31]. Using these conditions, MCF-7 cells generally perceive the weak partial agonist (estrogenic) activities of TAM [5], and E2 alone had no effect on these cells.

Of the cell lines we have utilized, only MCF7/MIII cells are significantly inhibited by TAM when growing without E2 [5, 11]. However, MCF7/MIII cells respond to TAM treatment by significantly increasing all spectral resonances approximately 40% above baseline values. This could reflect a fundamental change from a proliferating to a more differentiated or apoptotic phenotype. Alternatively, these metabolic changes may be an attempt by the cells to reverse the inhibitory effects of TAM, which generally induces a blockade in G_0/G_1 of the cell cycle [32]. The metabolic changes induced by TAM could provide intermediate endpoints for following response to TAM treatment in breast cancer patients. Significantly, the lack of a detectable TAM-induced metabolic response in tumors that express ER but are resistant to the inhibitory effects of TAM (*i.e.* MCF-7 cells growing in CCS-IMEM; MCF7/LCC2; MCF7/LY2 versus MCF7/MIII), may provide a means for identifying the 30%–40% of these tumors that fail to respond to TAM treatment [3]. Clearly, this possibility requires further study.

There were no spectral characteristics common

to MCF7/LCC2 and MCF7/LY2 cells that could be clearly associated with antiestrogen resistance in these ER-positive cells. However, PC levels were increased in MCF7/LY2 cells when compared to the other MCF-7 variants and the parental MCF-7 cells. This could reflect alterations in the rate of phosphoinositol turnover, which we do not observe in the estrogen-independent/antiestrogen-sensitive MCF-7 variant cells [11]. MCF7/LY2 cells are the only variant resistant to all the major structural classes of antiestrogens [5]. The high baseline PC levels in these cells may reflect the metabolic changes more closely associated with cross-resistance to other antiestrogens, rather than specific resistance to TAM. We are currently investigating this possibility by using additional MCF-7 variants that are resistant to ICI 182,780.

Conclusions

Using ^{31}P NMR, we have investigated the approximate intracellular steady-state concentrations of P_i, PC, GPC, PE, GPE, phosphocreatine, α-ATP, β-ATP, gamma-ATP, NAD, and UDPG in a series of human breast cancer cell lines and variants. The data associate higher levels of phosphodiesters and UDPG, and lower phosphocholine/glycerylphosphocholine and phosphocholine/phosphoethanolamine ratios, with the acquisition of estrogen-independent growth by ER positive cells (MCF7/MIII; MCF7/LCC2). Increased PC levels (MCF7/LY2) could be associated with resistance to steroidal antiestrogens, or cross-resistance to all antiestrogens. Further studies are in progress to determine the possible functional relevance of these observations.

The ability to use NMR spectroscopy to identify specific phenotypes could have considerable clinical potential in the management of breast cancer. The location of the primary tumors in the breast can greatly facilitate their imaging in what is essentially a physically peripheral tissue [33]. The breast tissue surrounding the tumors is generally composed of soft fatty tissue that has a low level of phosphate metabolism relative to the tumor [34]. Merchant *et al.* [33] have recently demonstrated the ability to discriminate among signals obtained from malignant, benign, and normal breast tissue in women using NMR spectroscopic analyses. Clearly, this area of research, and its potential for clinical applications, requires further study. We are further evaluating this potential using human breast tumors growing in nude mice. NMR spectroscopy could be used to identify the rapid bioenergetic responses or patterns of response to antiestrogen treatment in ER positive breast tumors, and thereby provide an important new tool for the identification of antiestrogen sensitive versus antiestrogen resistant ER positive breast tumors.

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