Levels of Phospholipid Metabolites in Breast Cancer Cells Treated with Antimitotic Drugs: A $^{31}$P-Magnetic Resonance Spectroscopy Study

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ABSTRACT

Magnetic resonance spectroscopy (MRS) methods have provided valuable information on cancer cell metabolism. In this study, we characterized the $^{31}$P-MR spectra of breast cancer cell lines exhibiting differences in hormonal response, estrogen receptors (positive/negative), and metastatic potential. A correlation was made between the cytotoxic effect of antimitotic drugs and changes in cell metabolism pattern. Because most anticancer drugs are more effective on proliferating cells, our study attempted to elucidate the metabolic profile and specific metabolic changes associated with the effect of antimitotic drugs on proliferating breast cancer cell lines. Accordingly, for the $^{31}$P-MRS experiments, cells were embedded in Matrigel to preserve their proliferation profile and ability to absorb drugs. The MRS studies of untreated cells indicated that the levels of phosphodiester and uridine diphosphosugar metabolites were significantly higher in estrogen receptor-positive and low metastatic potential cell lines. $^{31}$P-MRS observations revealed a correlation between the mode of action of anticancer drugs and the observed changes in cell metabolic profiles. When cells were treated with antimitotutable drugs (paclitaxel, vincristine, colchicine, noclodoxazole), but not with mithoxetate and doxorubicin, a profound elevation of intracellular glycerophosphorylcholine (GPC) was recorded that was not associated with changes in phospholipid composition of cell membrane. Remarkably, the rate of elevation of intracellular GPC was much faster in cell population synchronized at G2-M compared with the unsynchronized cells. The steady-state level of GPC for paclitaxel-treated cells was reached after ~4 h for synchronized cells and after ~24 h (approximate duration of one cell cycle) for the unsynchronized ones. These observations may indicate a correlation between microtubule status and cellular phospholipid metabolism. This study demonstrates that $^{31}$P-MRS may have diagnostic value for treatment decisions of breast cancer and reveals new aspects of the mechanism of action of antimitotutable drugs.

INTRODUCTION

MRS has the unique ability to measure the biochemical content of living tissue in a dynamic and noninvasive manner. This technique has provided substantial information on cellular metabolism and energy status in a variety of tissues and organs and, in particular, in tumor-igenic tissues and malignant cell cultures (1-6). The ability to follow the cell metabolism pattern in malignant cells before, during, and after drug treatment may assist the prognosis of treatment success.

For optimal experimental purposes, perfused intact cells represent perhaps the best approach to the noninvasive study of metabolism. Intact cells are metabolically stable and homogeneous, and thus are more appropriate for studying cellular mechanisms than cell suspensions or even perfused tissue (7). Several significant metabolic differences between cell lines have been delineated by use of $^{31}$P-MRS (4, 8, 9), and the effects of manipulation with hormones (6, 10-13), drugs (10, 14-16), and other factors on metabolism have been monitored.

Choosing an effective chemotherapeutic treatment for breast cancer is a challenging task. Malignant breast cells may present a spectrum of variant cells, some of which retain their estrogen dependence and sensitivity to tamoxifen treatment, whereas others are estrogen independent and tamoxifen resistant (17). These variants may also present diverse metastatic properties (17). Understanding the molecular processes involved in cellular transformations may assist in the selection of a proper treatment.

The cell lines studied in this work are variants of human breast cancer cells comprising cells at different stages of progression from hormone-sensitive to -insensitive and more metastatic phenotypes (Table 1 and Refs. 12, 18, 19). The state of phosphate-containing metabolites during cell proliferation was recorded for each cell line, and a comparison was made between these cells. Earlier studies presented spectra of phosphate-containing metabolites of MCF7 at several experimental conditions (12, 20). Differences in the metabolic spectra of various breast cancer cell lines may imply differences in cellular properties that are relevant for treatment decisions.

We studied the effects of antimitotutable drugs on the spectrum of phosphate-containing metabolites. Paclitaxel (Taxol), the drug used for most of our studies, is a novel anticancer drug that has been approved for the treatment of refractory ovarian and breast cancer and is showing promising activity in malignant melanoma and lung cancer (21). Paclitaxel is an antimitotutable drug that at low concentrations inhibits cellular microtubule dynamic properties and at higher concentration causes the formation of highly organized bundles of microtubules. Nocodazole, vincristine, and colchicine are also antimicrotuble drugs that interfere with the assembly process of microtubules (22). Although each of these drugs binds to tubulin, they exert distinct effects on protein organization in the cell, as well as an effect on membrane-related functions. These drugs inhibit cell proliferation and replication; they also induce multidrug resistance and a variety of cellular effects apparently unrelated to their action on tubulin (22). In breast cancer cells and other malignant cell lines, some of the cellular effects of antimitotutable drugs are modulated by the action of steroids, estrogens, and antiestrogens, and vice versa (23-27).

We monitored the metabolic response of the cells to these antimitotutable drugs and found a correlation between drug action and changes in the cellular metabolic pattern.

The correlation between the cellular effects of chemotherapeutic drugs and breast cancer cell metabolism may help to elucidate the potency of anticancer drugs on in vivo malignancies.

MATERIALS AND METHODS

Materials

Matrigel, a basement membrane extracted from a mouse tumor, is composed of 30% type IV collagen, 60% laminin, 5% nidogen, 3% heparan sulfate proteoglycan, and 1% entacin. It was prepared according to a published procedure (28, 29) with modifications. The following materials were purchased: MatrixSpere, a mixture of dispase and collagenase was from Collaborative Biomedical Products (Bedford, MA); DMEM and FCS were from Biological Industries (Beth Haemek, Israel); RNase A, propidium iodide,
alginic acid, vincristine, colchicine, nocodazole, Adriamycin, and methotrexate were from Sigma Chemical Co. (St. Louis, MO), and tissue solubilizer BTS-450 was from Beckman (Fullerton, CA). Paclitaxel was a gift by Bristol Myers Squibb (USA). Unless otherwise specified, the drugs were dissolved in DMSO for cell work. The final DMSO concentration never exceeded 0.1% (v/v) in medium, which has no effect on cell health. One batch of FCS was used for the entire study. Charcoal-stripped FCS was prepared as described previously (30).

**Cell Culture**

Cells were a generous gift from Dr. Robert Clarke (Georgetown University, Washington, DC). The phenotypes of the cells used are shown in Table 1. The cells were treated according to published procedures (18, 19). MB231, MB435, and MCF7 cells were routinely maintained in DMEM supplemented with 5% FCS. LCC2 and MIII cells were maintained in DMEM without phenol red, which has an estrogen-like activity (31), and supplemented with 5% charcoal-stripped calf serum. All cell cultures were maintained at 37°C in a humidified 5% CO2 atmosphere incubator. For each MRS experiment group, cells were plated in 10 Petri dishes (10 cm in diameter). The number of cells planted was calculated to bring the culture to 90% confluence after 5 days (6–7 × 106 cells per experiment). The medium was replaced once after 3 days of culture.

**Preparation of Matrigel Threads**

Cells were harvested and centrifuged, and the preclosed pellet was mixed with liquid Matrigel at 4°C. The concentration of cells in Matrigel was set for synchronization of cells in G2-M. The mixture was pulled into plastic tubing by an attached C. The number of cells was determined by hemocytometer.

**Determination of Cell Growth in Matrigel**

Cells were embedded in Matrigel threads in several concentrations depending on the cell line proliferation profile. Every 24 h, threads were extruded into Petri dishes containing 10 ml of medium and placed in an incubator. At each time point, two threads were dissolved in MatriSperse (for 1 h on ice). The number of cells was determined by hemocytometer.

**Cell Cycle Characterization by FACS**

After trypsinization or release from Matrigel threads, cells were fixed by ice-cold ethanol, resuspended in PBS, treated by RNase A (10 mg/ml) for 30 min at 37°C, and then incubated with propidium iodide (0.5 mg/ml) and 1% Triton for 30 min at room temperature. Measurements were performed on a Becton Dickinson FACScan.

**MRS**

31P-MR spectra of perfused cells embedded in Matrigel were recorded on an AMX-400 WB Bruker instrument with a 10-mm probe, operating at 162 MHz (delay time, 2 s; acquisition time, 0.5 s; spectral width and number of points, 8000; flip angle, 60°). Broad-band Watz proton decoupling was used. One thousand scans were accumulated for each spectrum, totaling 1 h, and processed using 10-Hz line broadening. Chemical shifts were referenced to the GPC signal at 0.49 ppm as an internal standard. Peak areas were calculated as the ratio to the δ-ATP signal. We also compared peak areas to total phosphate, excluding Pi, and the

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**Table 1: Characteristics of cell lines used in this study**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Estrogen dependence</th>
<th>ER</th>
<th>Tamoxifen responsive</th>
<th>Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) MDA-MB231</td>
<td>Independent</td>
<td>Unresponsive</td>
<td>Resistant</td>
<td>Yes</td>
</tr>
<tr>
<td>(II) MDA-MB435</td>
<td>Independent</td>
<td>Unresponsive</td>
<td>Resistant</td>
<td>Yes</td>
</tr>
<tr>
<td>(III) MCF7/LCC2</td>
<td>Independent</td>
<td>Responsive</td>
<td>Resistant</td>
<td>ND</td>
</tr>
<tr>
<td>(IV) MCF7/MIII</td>
<td>Independent</td>
<td>Responsive</td>
<td>Sensitive</td>
<td>Yes</td>
</tr>
<tr>
<td>(V) MCF7</td>
<td>Independent</td>
<td>Responsive</td>
<td>Sensitive</td>
<td>No</td>
</tr>
</tbody>
</table>

*Requirement for 17β-estradiol to form tumors in nude mice.

* Respond to 17β-estradiol by inducing specific genes/mitogenesis.

ND, no data.
RESULTS

Cell Growth Characteristics and Cell Cycle Distribution of Different Cell Lines. In this study, we present the effect of drugs on hormone-sensitive and -resistant variants of human breast cancer cells (Table 1). The proliferation rates and cell cycle characteristics of each cell line grown as monolayers or embedded in Matrigel were studied. In general, no significant differences were observed in the cell cycle distribution of cells grown in either of these two conditions (results not shown). Minor differences in the percentage of cells in the S-phase among the different cell lines were found; the percentage was lower for LCC2 and MIII cells (8%) and higher for MB435 cells (17%) compared with MCF7 cells (10%). The proliferation rates of these cell lines in Matrigel were determined. At several time points, Matrigel threads were degraded by MatriSperse, and the released cells were counted. Growth curves of all cell lines (results not shown) exhibited exponential growth patterns. The doubling times obtained for cells in Matrigel threads were: 24, 23, 65, 70, and 30 h for MB231, MB435, LCC2, MIII, and MCF7, respectively. These values are comparable to those obtained for monolayers, although somewhat longer.

The viability of cells embedded in Matrigel and perfused inside the nuclear magnetic resonance spectrometer was assessed. Threads containing lower than usual concentration of MCF7 cells were prepared, and $^{31}$P-MR spectra were recorded at several time intervals between 0 and 72 h after preparation. The signal intensities of ATP increased along with cell proliferation and reached a plateau at confluence, consistent with Gompertzian growth (results not shown). These results confirmed that cells embedded in Matrigel are viable and proliferating as cells grown as monolayers (32).

$^{31}$P-MR Spectra of Breast Cancer Cell Lines. In previous $^{31}$P-MR studies, spectra of these cell lines were obtained in perfused agarose gel threads (12). Unfortunately, because cells cannot multiply in this system, it was not possible to study the effects of cell cycle progression and proliferation on the cell metabolism pattern. As stated above, the use of Matrigel threads enabled the cells to proliferate. In this study, the cells were embedded in Matrigel and incubated in flasks for 48 h before their spectra were recorded. Representative $^{31}$P-MR spectra for five cell lines (Table 1) are presented in Fig. 1. For comparison purposes, cell lines were grown under the same conditions (estrogen-free medium; Fig. 1, I–V). The cells presented in Fig. 1, I–IV, are estrogen independent and therefore are not affected by the lack of estrogen. MCF7 cells, on the other hand, are estrogen dependent and at the above conditions did not proliferate (Fig. 1V). Fig. 1V represents the spectrum for MCF7 under normal culture conditions (estrogen-containing medium). No major differences were observed between the two conditions for MCF7 (Fig. 1, V and VI). The integrals of the resolved and assigned peaks normalized to the $\beta$-ATP peak are presented in Fig. 2. A confirmatory experiment was carried out with cells embedded in Matrigel threads and incubated for 48 h in a perfused MRS tube, after which their MR spectra were recorded (results not shown). There was no significant difference between the two sets of spectra.

It should be noted that there were significant differences between the spectra of the different cell lines, which constituted a “fingerprint” of the metabolic state of a given cell line. Specific large differences in metabolite levels may have been related to the differences in their hormonal properties. Significant differences observed between the spectra were as follows. (a) The UDPS signal was much higher (Fig. 1, peak 9) in ER $^+$ lines [LCC2 (III), MIII (IV), and MCF7 (V)] compared with the signal in the ER $^-$ cell lines [MB231 (I) and MB435 (II); $P < 0.01$ except for MB231 versus LCC2 where $P = 0.015$]. (b) PDE levels (GPE and GPC; Fig. 1, peaks 3 and 4) were higher in the ER $^+$ cell lines MIII (IV), and MCF7 (V) compared with the ER $^-$ cell lines (P < 0.01). The level of GPC was not significantly higher in the LCC2 line (III; ER $^-$) compared with the MB231 line (I; ER $^+$); however, LCC2 had a significantly higher GPE level (P < 0.01). A gradual decrease in the PDE signals could be observed in the progression of ER $^-$ cell lines, from those representing an early type of breast cancer (MCF7; V) to the more aggressive cell lines [MIII (IV) and LCC2 (III)]; the results were consistent but not statistically significant. (c) The PME level (Fig. 1, peak 1) was higher in the MB435 line (II; P < 0.01), which may be attributable to its higher metastatic potential compared with the other cell lines.

To assign the UDPS peak, which showed significant differences between cell lines, cells were extracted by PCA as described in “Materials and Methods.” Spectra of extracts had reduced line width, thus enabling resolution of the different compounds in the UDPs peak. Peaks were assigned by spiking a spectrum with known compounds and were found to be N-acetylgalactosamine (−11.32 and
It should be emphasized that in both cell lines compared with threads without cells, indicating drug accumulation inside the cells (results not shown). The absolute amount of radioactive drug per cell was checked for three experimental protocols: (a) cells growing as a monolayer and treated with [3H]paclitaxel; (b) cells were preloaded with the radioactive drug for 1 h before they were embedded in Matrigel and the threads were extruded into a dish containing medium with the radioactive drug; in this case the cells were saturated with the drug at the plateau value even if the Matrigel diminished the penetration; (c) the same protocol as in (b) but without the 1 h drug preloading. In all three experiments the amount of drug in the cells at the plateau stage was similar (2.1 ± 0.2 cpm/1000 cells). These results demonstrate that embedding cells in Matrigel threads does not prevent drug uptake by the cells.

The ED50 of paclitaxel for MB231 cells embedded in Matrigel threads and for cells grown as a monolayer was measured (results not shown). The results indicated that the ED50 for cells embedded in Matrigel was higher by one order of magnitude than that of cells grown in monolayers. As shown above, this cannot be explained as a result of lower drug penetration through Matrigel. Obviously the components of Matrigel or the experimental conditions have some protective action on cells. Consequently, the concentration chosen for work with MB231 cells in Matrigel was 2.5 × 10⁻⁷ M.

Effects of Paclitaxel on Cell Metabolism. A time course experiment in which MB231 cells were treated with 2.5 × 10⁻⁷ M paclitaxel was performed. Cells were embedded in Matrigel and perfused in a MRS tube for the entire duration of the experiment. Spectra were taken at several time points during 48 h. The same procedure (adding 0.1% DMSO, the amount of solvent introduced to each sample with the drug) was repeated for control. As can be seen in Fig. 3, A and B, in both cases GPC (peak 4) and PME (peak 1) increased during the progression of the experiment. However, compared with the controls (Fig. 3A), the increase in GPC was much more profound in the paclitaxel-treated cells (Fig. 3B). The moderate increase in PME and GPC in control cells is probably characteristic for cells proliferating in Matrigel. An increase in PME was reported previously in proliferating cancer cells (5), and an increase in GPC has been reported for cells embedded in Matrigel (35). A confirmatory experiment was carried out with cells embedded in Matrigel threads that were initially incubated in the presence or absence of paclitaxel in flasks for 48 h before being placed in MRS tubes and the spectra recorded. There was no significant difference between the spectra taken for cells grown in the MRS tube versus those grown in flasks. In both cases, GPC was significantly higher in paclitaxel-treated cells than in control cells (0.49 ± 0.03 and 0.27 ± 0.02, respectively; P < 0.01).

Because paclitaxel treatment blocks the cell cycle at the G2–M phase, we hypothesized that its effect would be even more profound if cells were treated after they were synchronized in the G2–M phase. Cells were synchronized with nocodazole (3.3 × 10⁻⁷ M, for 12 h) while they were being grown as monolayer in dishes, washed with fresh medium for 3 h, checked for the degree of synchronization by FACS, and preloaded with paclitaxel; Matrigel threads were then prepared. ³¹P-MR time course experiments were performed (Fig. 3C). Remarkably, the rate of GPC signal elevation was significantly faster for the synchronized preparation (Fig. 4). Whereas the GPC level of the synchronized cells treated with paclitaxel reached its maximum value in ∼4 h, the GPC level in the unsynchronized cells treated with paclitaxel reached its maximum in 24–26 h (a time duration of one cell cycle for MB231 in Matrigel). The final GPC level was similar for both the synchronized and unsynchronized experiments (Fig. 4). The faster increase in GPC in the synchronized preparation was not attributable to the pretreatment with nocodazole. Replacement with fresh medium after the nocardazole treatment resulted in a fully recovered microtubule network (confirmed by immunofluorescence studies using antitubulin antibody; results not shown). The increase in GPC in cells that were pretreated with nocardazole, washed several times with medium over 3 h, and incubated for up to 48 h in drug-free medium was similar to that of the control presented in Fig. 4, and FACS analysis after 48 h revealed normal cell cycle phase distribution (results not shown).

The MRS studies at each time point were accompanied by an analysis of cell cycle distribution (Fig. 3). Fig. 3A shows that the untreated cells were viable (as confirmed by the ATP level) and had normal cell cycle distribution in Matrigel. Conversely, paclitaxel treatment led to a G2–M arrest and induced apoptosis (Fig. 3B). Unexpectedly, 2.5 × 10⁻⁸ M paclitaxel caused more apoptosis than 2.5 × 10⁻⁷ M in MB231 cells (data not shown). Nevertheless, am increase in GPC was observed after treatment with 2.5 × 10⁻⁷ M but not with 2.5 × 10⁻⁸ M (data not shown). Thus, apoptosis was probably not the cause of the increase in GPC.

To confirm the origin of the changes in GPC, we carried out the
following control experiment. Cells embedded in Matrigel were extracted with PCA at the end of the MRS experiments (Fig. 5). It was clearly apparent that the increase in GPC in paclitaxel-treated cells was attributable to an increase in GPC content and not to a change in GPC relaxation times, e.g., possibly from transport of GPC to another cell compartment. Nor was it attributable to phospholipid head groups because phospholipids are not extracted by PCA. To verify that the type of embedding material was not determining the intensity of the GPC signal, monolayers of MB231 cells were treated with paclitaxel and at the relevant time points were embedded in alginate capsules, according to a published procedure (36), and $^{31}$P-MR spectra were acquired. A profound increase in the GPC signal in paclitaxel-treated cells relative to control was also recorded for this system (results not shown).

The elevated GPC signal was attributable to its intracellular concentration in viable cells and not to extracellular GPC released from disrupted cells. The supernatant medium from the MRS experiment was collected and checked by MRS. Only the $P_i$ signal was observed in the effluent spectrum (results not shown). In a supplementary
were dissolved at the relevant time, and the phase distribution of the cells was determined by FACS. The results are presented in Table 2.

It should be noted that Adriamycin (1.2 × 10^{-6} \text{M} for 48 h) caused G2-M arrest but no increase in GPC, implying that the increase in GPC is probably not attributable to G2-M arrest. At an increased Adriamycin concentration (3.4 × 10^{-6} \text{M} for 48 h), the GPC signal was elevated (data not shown). To clarify this point and to further study the cause of GPC elevation by antimicrotubule drugs, we used MRS to evaluate membranal phospholipid extracts for cells treated with paclitaxel (2.5 × 10^{-7} \text{M} for 48 h) and Adriamycin (3.4 × 10^{-6} \text{M} for 48 h; Fig. 7). It can be observed that paclitaxel did not cause any significant change in the phospholipid profile (Fig. 7, A and B); consequently, the increase in intracellular GPC produced by this drug is not from the membrane phospholipid pools. Possible explanations might be an elevation of PtdC content and its faster degradation to GPC, or decreased GPC degradation. A high concentration of Adriamycin caused a significant effect on the phospholipid profile (Fig. 7C, mainly a decrease in PtdC in relation to other components). Thus, the increase in GPC observed for high concentrations of Adriamycin was attributable to cell membrane degradation, which is a well-known effect of this drug.

The effects of the above-mentioned drugs on other cell lines were studied, and the level of GPC was recorded (results not shown). As depicted in Fig. 1 for untreated cells, the base level of GPC was low for MB435 and MB231 and significantly higher for LCC2, MIII, and MCF7. The results revealed that antimicrotubule drugs induced increases in intracellular GPC in MB435 as well as in MB231 cells, whereas insignificant changes were recorded for LCC2, MIII, and MCF7 cells. The unchanged GPC levels in the latter set of cell lines may be explained by their higher natural level of GPC, which may be at or near the maximal viable level of free cytosolic GPC, or by the presence of ERs (ER+) in these cells.

**DISCUSSION**

For the MRS studies, perfused intact cells were embedded in Matrigel, as described by Daly et al. (32, 37). The embedded cells were morphologically and functionally similar to their in vivo counterparts and established a "model tumor," which is most suitable for metabolic and pharmacological studies (38). Studies were performed on the five cell lines listed in Table 1. The spectra of these cell lines and the calculated concentrations of relevant phosphate-containing compounds are shown in Fig. 6 for untreated cells and three sets of spectra are presented. Signal areas are normalized to the β-ATP peak in each spectrum (bars, SD). For untreated cells (bars, SD), the following drugs were dissolved at the relevant time and used in the MRS system: I, paclitaxel (2.5 × 10^{-7} \text{M} for 48 h); II, nocodazole (3.3 × 10^{-7} \text{M} for 24 h); III, vincristine (3 × 10^{-7} \text{M} for 48 h); IV, colchicine (2.5 × 10^{-7} \text{M} for 48 h); V, Adriamycin (1.2 × 10^{-6} \text{M} for 48 h); VI, methotrexate (1.1 × 10^{-5} \text{M} for 48 h).

**Fig. 5** 31P-MRS spectra of PCA extracts of cells after *ex vivo* experiments. MB231 cells embedded in Matrigel threads were perfused in the MRS system for 48 h with medium containing 0.1% DMSO (A) or in the presence of 2.5 × 10^{-7} \text{M} paclitaxel (B). After completion of the experiments, the cell contents were extracted by PCA, and analyzed by 31P-MRS. Assignments of signals are as follows: peak 1, PME; peak 2, phosphorylcholine; peak 3, P; peak 4, GPE; peak 5, GPC; peak 6, γ-ATP; peak 7, α-ADP; peak 8, β-ADP; peak 9, α-ATP; peak 10, UDP5 and NADP+; peak 11, UDP5; peak 12, β-ATP.

![Fig. 5](image)

**Fig. 6** GPC content in control *versus* treated MB231 cells: The average intensities of GPC signals from three sets of spectra are presented. Signal areas are normalized to the β-ATP peak in each spectrum (bars, SD): I, paclitaxel (2.5 × 10^{-7} \text{M} for 48 h); II, nocodazole (3.3 × 10^{-7} \text{M} for 24 h); III, vincristine (3 × 10^{-7} \text{M} for 48 h); IV, colchicine (2.5 × 10^{-7} \text{M} for 48 h); V, Adriamycin (1.2 × 10^{-6} \text{M} for 48 h); VI, methotrexate (1.1 × 10^{-5} \text{M} for 48 h).

![Fig. 6](image)
metabolites are presented in Figs. 2 and 3. The spectra demonstrated differences composing a fingerprint of each cell line, serving as an identification tool for the various stages of tumor progression or hormonal dependence. Higher UDPs and PDE levels were seen in ER+ lines. A gradual decrease in PDE level was observed as we moved from the early type of breast cancer (MCF7) to the more aggressive cell lines, MB231 and MB435. These findings correlate with the “GPC to PC switch” proposed for changes observed in cells acquiring higher malignant properties (39). An elevation of GPC has also been reported for transfected MB435 mutants that exhibit lower metastatic potential, suggesting that GPC may inhibit phospholipases involved in signal transduction pathways that lead to the higher metastatic potential of malignant cells (40). Our findings correlate with the published results mentioned above, with higher GPC levels observed for cells with lower metastatic potential. The levels of some metabolites (mostly UDPs and PDE) were found to depend on the glucose concentration in the culture medium. The decrease in PDE levels with the acquisition of estrogen-independent growth and tamoxifen resistance in ER+ lines, as well as the difference in UDPS levels between ER+ and ER− lines, were observed only at normal glucose conditions (25 mm). However, differences in PDE levels (GPE and GPC) between ER+ and ER− lines were observed in both normal and low-glucose culture conditions.

The cell line MB231, which proliferates faster than the other cell lines, served as a representative for phenomena observed in all cell lines. 31P-MRS results of cells incubated up to 48 h in cytotoxic concentrations of paclitaxel showed progressive elevation of the intracellular concentration of GPC with time (Fig. 3B). Interestingly, when we performed the same experiment on presynchronized cells in G2-M, the GPC signal reached the same final level as in the experiment performed on unsynchronized cells, but at much shorter time (~4 h compared with ~24 h; Fig. 4). The duration of one cell cycle of MB231 cells is ~24 h, suggesting that the cytotoxic effect of paclitaxel is manifested mainly during the G2-M stage. Cell synchronization by itself did not contribute to the rapid elevation of GPC because untreated synchronized and unsynchronized cells showed similar GPC levels throughout the 48 h of the experiment. Moreover, the activation of cytosolic phospholipase A2, a major contributor to GPC formation, is not associated with a specific phase of the cell cycle (41). A profound elevation of the intracellular GPC level following drug treatment was observed only for the cell lines MB231 and MB435, which contain a low natural level of GPC. The final intracellular level of GPC was approximately the same (a “saturation” level) for cells treated with all antimicrotubule drugs, including synchronized cells. Taken together, these results suggest the existence of a dynamic equilibrium that limits the maximum concentration of compounds involved in phospholipid pathways and maintains a steady total mass of phospholipids in the cell (42). Excess GPC may be further degraded or secreted into the medium (42); thus, when treated with drugs, cells with natural high levels of GPC may not display an elevation in intracellular GPC content, although the total amount of GPC production was higher than in the untreated sample.

Our results indicated that whereas antimicrotubule drugs increased the concentration of intracellular GPC, no such effect was observed for non antimicrotubule drugs such as methotrexate and Adriamycin (Fig. 6). Microtubule dynamics may affect the phospholipid balance in certain cell types. Microtubule-disturbing agents affect the affinity of agonists to their membranal receptors (43), the lateral diffusion of phospholipids in cultured Chinese hamster lung fibroblasts (44), and the PtdC synthesis in phagocytic cells (45). Cytosolic microtubules, tubulin, and microtubule-associated proteins may interact with membranal phospholipids (46–48). GPC is one of numerous phospholipid metabolites in the cytosol that represent the status of the cellular phospholipid system and thus may reflect cytoskeletal dynamics and stability. GPC and GPE were found to act as competitive inhibitors of lysophospholipase activity, thus reducing lysophospholipid hydrolysis.

Table 2. Cell cycle distribution of MB231 cells treated with drugs

<table>
<thead>
<tr>
<th>Phase</th>
<th>Control</th>
<th>Paclitaxel</th>
<th>Nocodazol</th>
<th>Vincristine</th>
<th>Colchicine</th>
<th>Adriamycin</th>
<th>Methotrexate</th>
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<td>24 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Apoptosis</td>
<td>1.7 ± 0.8</td>
<td>10.4 ± 5.4</td>
<td>9.7 ± 3.6</td>
<td>10.2 ± 0.5</td>
<td>9.1 ± 2.2</td>
<td>2.0 ± 1.5</td>
<td>1.7 ± 0.9</td>
</tr>
<tr>
<td>G0/G1</td>
<td>67.9 ± 3.7</td>
<td>19.5 ± 4.0</td>
<td>16.3 ± 3.5</td>
<td>19.6 ± 7.6</td>
<td>24.4 ± 4.3</td>
<td>25.3 ± 4.9</td>
<td>74.6 ± 1.7</td>
</tr>
<tr>
<td>S</td>
<td>11.8 ± 3.3</td>
<td>13.0 ± 4.5</td>
<td>11.7 ± 2.8</td>
<td>15.2 ± 6.6</td>
<td>18.0 ± 1.4</td>
<td>7.0 ± 2.1</td>
<td>7.1 ± 1.4</td>
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<tr>
<td>G2-M</td>
<td>18.8 ± 3.3</td>
<td>57.0 ± 6.9</td>
<td>62.2 ± 4.5</td>
<td>55.1 ± 6.5</td>
<td>48.5 ± 5.0</td>
<td>65.7 ± 6.5</td>
<td>16.4 ± 2.1</td>
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<tr>
<td>48 h</td>
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</tr>
<tr>
<td>Apoptosis</td>
<td>1.7 ± 0.8</td>
<td>22.1 ± 5.0</td>
<td>ND*</td>
<td>14.7 ± 5.6</td>
<td>30.0 ± 1.1</td>
<td>4.1 ± 2.4</td>
<td>2.7 ± 0.5</td>
</tr>
<tr>
<td>G0/G1</td>
<td>74.1 ± 2.4</td>
<td>14.6 ± 2.9</td>
<td>ND</td>
<td>23.3 ± 5.5</td>
<td>14.4 ± 3.4</td>
<td>17.1 ± 5.5</td>
<td>76.6 ± 0.9</td>
</tr>
<tr>
<td>S</td>
<td>8.1 ± 0.0</td>
<td>14.8 ± 3.1</td>
<td>ND</td>
<td>14.3 ± 3.0</td>
<td>11.7 ± 1.0</td>
<td>6.8 ± 1.9</td>
<td>6.9 ± 0.7</td>
</tr>
<tr>
<td>G2-M</td>
<td>16.1 ± 1.7</td>
<td>48.2 ± 7.4</td>
<td>ND</td>
<td>47.7 ± 7.7</td>
<td>43.9 ± 4.4</td>
<td>71.9 ± 1.3</td>
<td>13.7 ± 0.6</td>
</tr>
</tbody>
</table>

* ND, not determined.

Fig. 7. 31P MRS spectra of phospholipid extracts of cells after drug treatment: MB231 cells were incubated for 48 h with 0.1% DMSO (A), with 2.5 × 10−7 M paclitaxel (B), or with 3.4 × 10−6 M Adriamycin (C). Assignments for the signals are as follows: peak 1, PtdC; peak 2, plasmamyl PtdC; peak 3, phosphatidylinosine; peak 4, sphingomyelin; peak 5, phosphatidylerine; peak 6, phosphatidylethanolamine; peak 7, plasmamyl phosphatidylethanolamine; peak 8, phosphatidic acid.
and lowering the rate of membrane phospholipid degradation (49). Changes in microtubule stability may alter local membrane composition and the interaction of membrane proteins with their lipid environment. The resultant degradation of phospholipids causes an elevation in the GPC level that may serve as a "protective" measure of the system against further degradation. As our results indicate, the intracellular level of GPC is indicative of cellular microtubule function and, thus, cell survival and the effectiveness of antimicrobial drugs.

In conclusion, we present here the prospect of using MRS techniques for defining the metastatic potency of tumors and for the prediction of anticancer treatment success.

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Levels of Phospholipid Metabolites in Breast Cancer Cells Treated with Antimitotic Drugs: A 31P-Magnetic Resonance Spectroscopy Study

Marina Sterin, Jack S. Cohen, Yael Mardor, et al.


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