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CROSSTALK BETWEEN EPIDERMAL GROWTH FACTOR RECEPTOR AND P-GLYCOPROTEIN IN ACTINOMYCIN D-RESISTANT CHINESE HAMSTER LUNG CELLS

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Abstract—Multidrug-resistant cells can manifest an increase in epidermal growth factor (EGF) receptor number along with increased P-glycoprotein (Pgp) synthesis. An interrelationship of the two membrane proteins in actinomycin D-resistant Chinese hamster lung cells (DC-3F/AD X) in terms of the effect of EGF on Pgp phosphorylation was investigated. EGF was not a mitogen for the resistant cells, nor was it mitogenic for DC-3F, the parental drug-sensitive line. Brief treatment of DC-3F/AD X cells with EGF resulted in a 30–50% decrease in the level of Pgp phosphorylation, and treatment of the cells with okadaic acid, a specific inhibitor of protein phosphatases-1 and -2A (PP1 and 2A), increased Pgp phosphorylation. Okadaic acid also increased phosphorylation of Pgp in plasma membranes isolated from DC-3F/AD X cells by 30–40%. Protein phosphatase activity in extracts of cells grown in EGF-containing medium was greater by 30% than that of cells grown in standard medium, and okadaic acid inhibited the increases. The results suggested that EGF activated PP1 and PP2A in DC-3F/AD X cells and that Pgp was a substrate for the phosphatases. The properties of Pgp may be modulated by the signalling system transduced by ligand-activated EGF receptor.

P-Glycoprotein (Pgp)‖ is a membrane glycoprotein frequently overexpressed in cells selected for resistance to such natural product cancer drugs as vincristine, vinblastine, and actinomycin D. The cells are termed multidrug-resistant because development of resistance to a single chemotherapeutic agent is coincident with cross-resistance to other drugs in this category [1–4]. Development of resistance to these drugs can occur in cancer patients being treated with chemotherapy, with resultant treatment failure. The need to overcome or circumvent this problem has stimulated the study of multidrug resistance and Pgp. Homology of Pgp with known bacterial transporters suggest that the membrane ion channel-like protein functions as a transporter, mediating drug or substrate efflux in an energy-dependent process [5–7].

Pgp is phosphorylated on serine and threonine residues [8–12] and regulation of the suggested efflux function of Pgp may be controlled, at least in part, by phosphorylation. Verapamil and trifluoperazone have been shown to enhance phosphorylation of Pgp, increase drug accumulation in resistant cells, and restore drug sensitivity in multidrug-resistant cells [13, 14]. Phorbol esters, activators of protein kinase C, a kinase shown to phosphorylate Pgp [12], increase drug resistance of multidrug-resistance human breast cancer cells [15].

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‖ Abbreviations: EGF, epidermal growth factor; MEM, Eagle's Minimum Essential Medium; $^{32}P_i$, ^{32}P-labeled inorganic phosphate; PP1, protein phosphatase-1; PP2A, protein phosphatase-2A; Pgp, P-glycoprotein; and RIPA, radioimmunoprecipitation assay.

A characteristic of many multidrug-resistant cells is increased expression of epidermal growth factor (EGF) receptor [16, 17], an observation which suggested investigation of a role for the EGF receptor in Pgp-mediated multidrug resistance. Known functions of the EGF/EGF receptor, e.g. mitogenic action of EGF [18] and ligand-activated tyrosine kinase activity of the receptor [19], were starting points for the investigation.

DC-3F/AD X, a multidrug-resistant subline of Chinese hamster lung (DC-3F), was used for these studies. DC-3F/AD X cells are 10,000-fold resistant to actinomycin D, have 16 times more Pgp mRNA, about 17 times more Pgp protein, and 3 times more EGF receptor (K_d = 1 nm), as compared with DC-3F [16, 20]. Investigations of the effect of EGF on cell proliferation, Pgp phosphorylation and *in vitro* phosphatase activity are described in this report. Some of these reports have been presented in abstract form [21].

MATERIALS AND METHODS

Materials. Media components and the Protein Phosphatase Assay System were purchased from GIBCO/BRL (Gaithersburg, MD). $^{32}P_i$, [^{3}H]-thymidine, and [γ-^{32}P]ATP were purchased from New England Nuclear (Boston, MA). Okadaic acid, a specific inhibitor of serine/threonine protein phosphatases-1 (PPI) and -2A (PP2A), was obtained from Moana Bioproducts, Inc. (Honolulu, HI), EGF from Collaborative Research (Waltham, MA), Protein IgSorb from The Enzyme Center (Malden, MA), C219 antibody from Centocor (Malvern, MA), and the Betascope radioimage analyzer from Betagen, Inc. (Waltham, MA). All other supplies and chemicals were from commercial sources.

Cells. DC-3F/AD X (10,000-fold resistant to actinomycin D as compared with DC-3F parental cells and cross-resistant to such drugs as vincristine, vinblastine, and daunorubicin) and DC-3F cells were maintained in monolayer culture in a mixture of Eagle's Minimum Essential Medium (MEM) and Ham's F12 (1:1) supplemented with non-essential amino acids and 5% (or as noted) fetal bovine serum and had generation times of about 16 hr [23–25]. The maintenance concentration of actinomycin D for DC-3F/AD X was 10 μg/mL. Cells in mid-exponential growth were examined and resistant cells were grown in the absence of drug for 10–14 days before use in experiments.

Flow cytometry and thymidine incorporation studies. DC-3F and DC-3F/AD X cells were grown in the presence or absence of 20 nM EGF for periods of time from 20 hr to 4 days before analysis by flow cytometry. Suspensions of cells (6–10 × 10^6 cells/time point) were prepared and stained with propidium iodide as previously described [26] to determine the percentage of cells in compartments of the cell cycle. Thymidine incorporation studies were carried out with cells growing in 24-well plates in the presence or absence of 20 nM EGF. On days 1 through 5 after plating, growth medium was removed and replaced by medium containing 1 μCi/mL [^{3}H]thymidine. After 24 hr, medium was removed, cells were washed, and trichloroacetic acid-precipitable counts were determined.

Phosphorylation of Pgp in intact cells in the presence and absence of EGF or okadaic acid. DC-3F/AD X cells were grown in 24-well plates in phosphate-free culture medium containing 50 μCi/mL $^{32}P_i$ for 4 hr. For EGF studies, EGF was added to cultures to a final concentration of 20 nM for periods of time ranging from 1 to 120 min before the end of the labeling period. Cells were then washed with phosphate-buffered saline, lysed in 20 mM HEPES buffer (pH 7.4) containing 1% Triton X-100, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, 20 mM sodium fluoride, and 10 μM sodium vanadate, and the homogenates were centrifuged at 12,000 *g*. ^{35}P-labeled Pgp in supernatant aliquots containing 1–2 × 10^5 cpm was detected by immunoprecipitation with C219, a monoclonal antibody recognizing a cytoplasmic epitope of Pgp, as previously described [27]. Briefly, aliquots of supernatants were added to 1 μg of C219 in 0.25 mL of radioimmunoprecipitation assay (RIPA) buffer [10 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% sodium dodecyl sulfate] containing 1 mM EDTA, 20 mM sodium fluoride, and 10 μM sodium vanadate. After incubation for 16 hr at 4° and precipitation of the complexes with Protein A (IgSorb), antigen was solubilized from washed Protein A beads by heating at 100° for 2 min and examined by electrophoresis on 7.5% acrylamide gels [28]. Quantitation of Pgp was accomplished by densitometric tracing of radioautograms. Automatic integration of the plots provided areas under peak curves corresponding to amounts of phosphorylated Pgp. Eight experiments (separate harvests) were evaluated.

For the okadaic acid studies, aliquots of a 0.125 mM stock solution of okadaic acid in ethanol were added to the wells 20 min before the end of the 4-hr labeling period to final concentrations of 0.1, 1, or 4 μM. Aliquots of ethanol were added to some wells with no observable effects on protein phosphorylation. In addition, $^{32}P_i$-containing medium was removed from some wells after the 4-hr incubation and replaced by unlabeled standard medium with or without 1 μM okadaic acid for 1 hr. After experimental conditions were fulfilled, cells were washed and Pgp was immunoprecipitated with C219 antibody and examined by gel electrophoresis as described above.

In vitro *phosphorylation of Pgp in plasma membranes isolated from DC-3F/AD X cells.* Aliquots of isolated plasma membranes [29] containing 40 μg of protein (as determined by the procedure described by Lowry *et al.* [30]) were incubated for 1 min at 30° in 20 mM HEPES (pH 7.4) containing 10 mM $MgCl_2$ and 1 mM dithiothreitol in a total volume of 25 μL and in the presence or absence of 1.5 mM $CaCl_2$, or 100 nM EGF, or 100 nM EGF and 1.5 mM $CaCl_2$, or 1 μM okadaic acid, or 1 μM okadaic acid (1 min) followed by a 1-min incubation with 100 nM EGF, or 100 nM EGF (1 min) followed by a 1-min incubation with 1 μM okadaic acid. These preincubations were followed by the addition of 20 μM [γ-^{32}P]ATP at 5 × 10^5 cpm/reaction mixture and an additional 10-min incubation at 30°. Reactions were stopped by the addition of 0.25 mL of RIPA buffer containing EDTA, sodium fluoride and sodium vanadate, and ^{32}P-labeled Pgp was identified by immunoprecipitation with C219 as described above. Quantitation of the labeled Pgp was accomplished by the use of a Betascope radioimage analyzer.

Protein phosphatase assay. DC-3F and DC-3F/AD X cells, harvested before and after a 10- or 30-min exposure to 20 nM EGF in standard medium, were lysed by Dounce homogenization in 50 mM Tris buffer (pH 7.4) containing 0.1 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetra-acetic acid (EGTA) (6–10 × 10^6 cells/sample). Aliquots (10 μg) of supernatants, obtained after a 30-sec centrifugation of homogenates at 12,000 *g*, were assayed for phosphatase activity. ^{32}P-Labeled phosphorylase *a* was used as substrate according to instructions provided with the GIBCO Protein Phosphatase Assay System. Briefly, extracts and aliquots of magnesium-free assay buffer (pH 7.63) were mixed with or without 0.1 or 1 nM or 1 μM okadaic acid followed by the addition of purified ^{32}P-labeled phosphorylase *a* and incubation for 10 min at 30°. The described conditions produced reaction velocities within a linear range. Released $^{32}P_i$ was measured by liquid scintillation counting. Duplicate or triplicate reactions of extracts from each of three separate cell harvests for each line were evaluated and results were reported in picomoles of phosphate released per minute per milligram of protein.

RESULTS

Effect of EGF on cell proliferation. Flow cytometric measurements of DNA in DC-3F/AD X or DC-3F

cells grown in standard medium with 5 or 0.1% serum and in the absence or presence of EGF revealed no significant effect of EGF on cell cycles of either line at 20 hr, or 3 or 4 days after plating. For example, after 20 hr in medium with 5% serum, 35% (−EGF) or 36% (+EGF) of DC-3F/AD X cells were in S phase; after 3 days, 24% (−EGF) or 23% (+EGF) were in S phase; and after 4 days, 28% (+ or −EGF) of cells were in S phase. There was also no significant effect of EGF on the rate of proliferation for either line as measured by thymidine incorporation.

EGF-stimulated dephosphorylation of Pgp. The level of $^{32}P_i$ metabolically incorporated into Pgp was measured after exposure of DC-3F/AD X cells to 20 nM EGF for periods of time up to 120 min. Figure 1 displays a radioautogram representative of eight experiments. Automated analysis of densitometric tracings of the radioautogram showed an approximate 50% decrease in ^{32}P-incorporation in Pgp within 10 min of exposure of cultures to EGF (Fig. 1, bar graph) with return to control levels of phosphorylation by 30 min. In these experiments the levels of decrease of ^{32}P-labeled Pgp within 10 min of EGF exposure ranged from 30 to 50% of control. In every case, levels of Pgp phosphorylation returned to, or slightly exceeded, control values within 20–30 min of incubation with EGF. The timing of the EGF effect on Pgp, as seen in Fig. 1, was very similar, if not identical, to the timing reported for EGF activation of PP1 in 3T3 cells with phosphorylase *a* as substrate [31].

Pgp bands visualized on these gels did not include phosphorylated EGF receptor because antibody C219 did not cross-react with EGF receptor. In addition, hamster EGF receptor, immunoprecipitated with A4 IgG (monoclonal antibody to mouse EGF receptor [32]) from DC-3F/AD X cells solubilized in buffer containing sodium dodecyl sulfate and a mixture of protease inhibitors, did not co-migrate with Pgp on gels (data not shown).

Effect of okadaic acid on phosphorylation of Pgp in isolated plasma membranes and intact cells. Phosphorylation of Pgp in isolated DC-3F/AD X cell plasma membranes in the presence and absence of modulating factors was examined. A sequence of assays, representative of three qualitatively identical studies carried out with different plasma membrane preparations, is shown in Fig. 2. Quantitation of Pgp in the radioautogram is shown in the bar graph, and relative values in three identical experiments agreed with these data to within 10%. Addition of 1.5 mM $CaCl_2$ to assay mixtures resulted in 30–40% increased phosphorylation of Pgp (Fig. 2, lane 2) as compared with controls (Fig. 2, lane 1); 100 nM EGF promoted 10–15% decreased phosphorylation (Fig. 2, lane 3); $CaCl_2$ and EGF together did not appreciably change the effect of EGF alone (Fig. 2, lane 4). One micromolar okadaic acid increased the level of Pgp phosphorylation by about 30–40% over controls (Fig. 2, lane 5). EGF added after okadaic acid resulted in an apparent neutralization of the okadaic acid effect, with a return to near control levels of phosphorylation (Fig. 2, lane 6). However, EGF added before okadaic acid (Fig. 2, lane 7) resulted in an approximately 15% reduction of Pgp phosphorylation, as compared with controls, and about a 40% decreased phosphorylation, as compared with okadaic acid alone. This latter result represented a reversal of the effect seen in Fig. 2, lane 5, to a level of phosphorylation comparable to that imposed by EGF alone (Fig. 2, lane 3). The demonstration of phosphorylation modulation in isolated plasma membranes suggested that certain kinases (perhaps calcium-mediated) and phosphatases and/or enzyme activation components were endogenous to the plasma membrane preparation. That EGF had an effect on Pgp in the isolated membranes suggested that the mechanism whereby EGF affects phosphatase activity is intact, at least in part, in the isolated membranes.

The response to okadaic acid was also assessed in intact cells. Okadaic acid increased the level of Pgp phosphorylation by 20–40% in DC-3F/AD X cells within 20 min of exposure to the inhibitor (Fig. 3). In that time frame, cell morphology and viability were not disrupted at concentrations of okadaic acid up to 4 μM. Figure 3 also provides evidence of a rapid rate of phosphate turnover on Pgp. There was an approximately 50% decrease in level of ^{32}P-labeled Pgp during a 1-hr chase period both in the presence and absence of okadaic acid (Fig. 3, 1-hr chase lanes), suggesting the rapid action of phosphatases on Pgp.

EGF-stimulated cellular protein phosphatase-1/-2A (PP1/PP2A) activity. The effects of EGF and okadaic acid on Pgp phosphorylation prompted an investigation of the effect of EGF on cellular phosphatase activity. A phosphatase assay specific for PP1/PP2A was deemed appropriate because: (a) stimulation of PP1 by EGF has been demonstrated in 3T3 and A431 cells [31, 33] and (b) okadaic acid, a specific inhibitor of PP1 and PP2A [22], enhanced phosphorylation levels of Pgp. Dephosphorylation of Pgp is likely to be catalyzed by serine/threonine phosphatases because only serine or threonine residues are phosphorylated on Pgp in various cell types [8–12] or on Pgp in EGF-treated or -untreated DC-3F/AD X cells (data not shown). and PP1/PP2A are two of the four known types of serine/threonine phosphatases [34, 35]. The phosphatase activity measured in extracts of DC-3F and DC-3F/AD X cells with phosphorylase *a* as substrate was considered to be PP1 and/or PP2A because PP1 and PP2A are the only enzymes with significant ability to dephosphorylate phosphorylase *a* [34, 36]. Phosphatase activities in picomoles $^{32}P_i$ released from phosphorylase per minute per milligram of protein in DC-3F and DC-3F/AD X cells before and after 10-min exposures of intact cells to 20 nM EGF are given in Table 1. The 10-min exposure time period was chosen to correspond with the period of maximum dephosphorylation of Pgp. EGF increased activity in both cell lines by 30%, and activity measured in resistant cells was 40% higher than that in DC-3F. Okadaic acid, added to cell extracts before addition of the substrate [^{32}P]phosphorylase *a*, inhibited phosphatase activity in extracts from both cell lines by 22% at 0.1 nM, by 56% at 1 nM, and completely at 1 μM. DC-3F cells were not evaluated in the other studies in this investigation having to do with Pgp effects or measurements

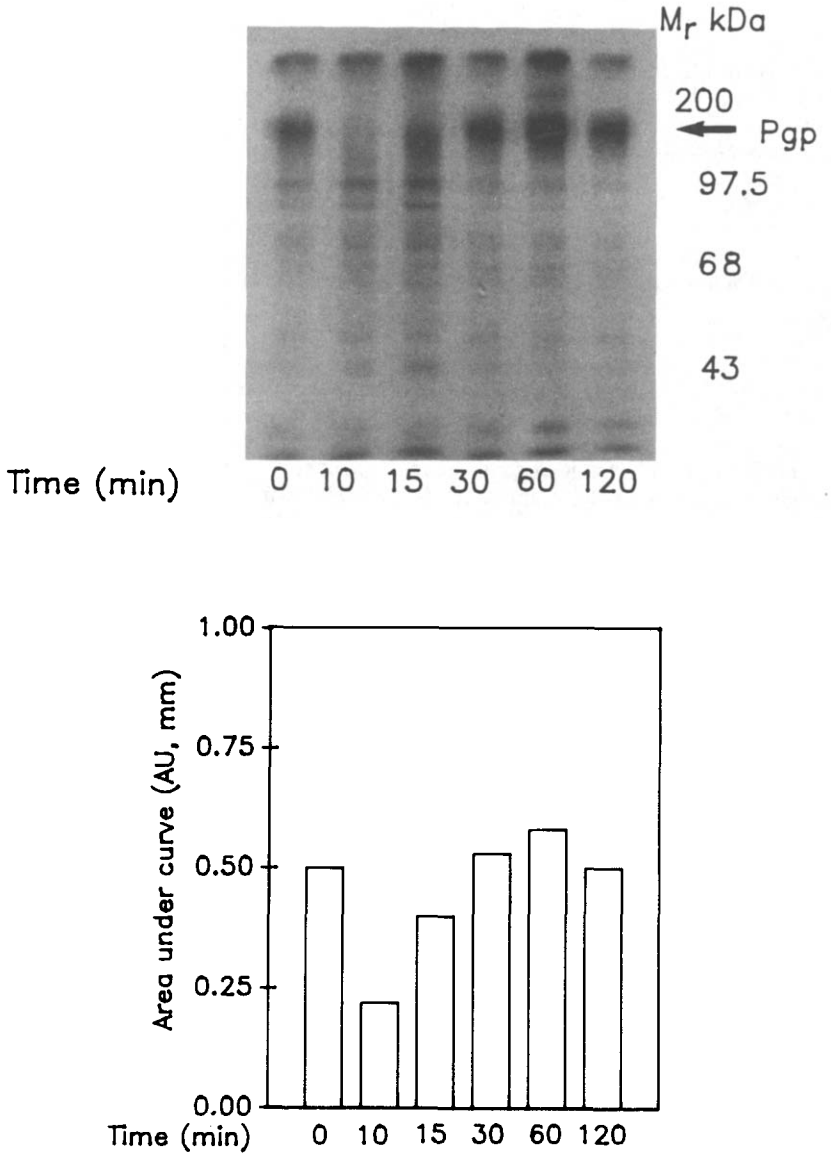
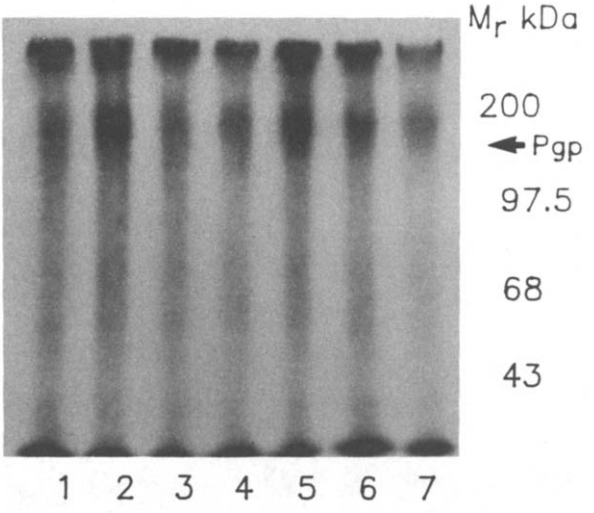


Fig. 1. Effect of EGF on the level of phosphorylation of Pgp in DC-3F/AD X cells. Monolayer cultures of DC-3F/AD X cells grown in 24-well tissue culture plates were metabolically labeled with $^{32}P_i$ for 4 hr. At 10, 15, 30, 60, or 120 min before the end of the labeling period (before removal of label), EGF was added to the individual wells to a final concentration of 20 nM. In some experiments EGF was added at periods of time from 1 to 10 min before the end of the labeling period. Decrease in Pgp phosphorylation was not observed with EGF exposure of less than 5 min. At the end of the labeling period cells were washed and lysed. Pgp was immunoprecipitated from aliquots of cell lysates each containing $1–2 \times 10^5$ cpm and subjected to electrophoresis on 7.5% acrylamide gels ($8 \times 9 \times 0.75$ cm). See Materials and Methods for complete procedures. Exposure of dried gels to X-ray film (16–24 hr) produced radioautograms such as the one shown here. Densitometric tracing of the film and automated integration yielded values representing relative amounts of radioactivity in Pgp bands by areas under the curves. The bar graph shows the curve areas pertaining to the depicted radioautogram. Eight experiments with cells from separate harvests were evaluated in this manner with the same result in terms of time frame of maximum dephosphorylation and extent of Pgp dephosphorylation ranging from 30 to 50% of control.

because Pgp is not readily detected in DC-3F membranes or extracts.

DISCUSSION

The study demonstrated that EGF stimulated protein phosphatase activity and dephosphorylation of Pgp in DC-3F/AD X cells. PP1/PP2A are the probable phosphatases involved in these activities because: (a) okadaic acid inhibited the EGF-stimulated phosphatase activity and increased Pgp phosphorylation *in vivo* and *in vitro*; (b) EGF stimulates PP1/PP2A in other cell types [31, 33]; and (c) the enzymes have been shown to be active in dephosphorylating Pgp in KB cells [37].



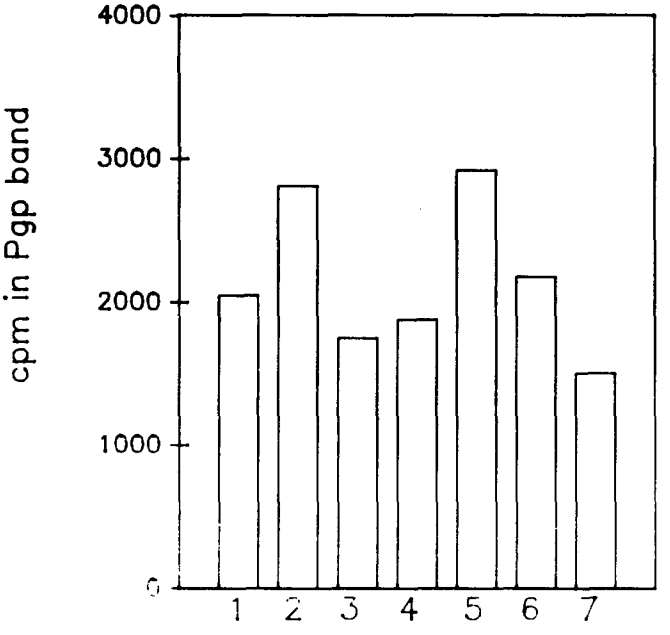


Fig. 2. Phosphorylation of Pgp in plasma membranes isolated from DC-3F/AD X cells. DC-3F/AD X cells were grown to exponential growth phase in flasks and plasma membranes were prepared as previously described [29] by centrifugation of cell homogenates over sucrose cushions. Aliquots of membranes containing 40 μg of protein were incubated for 1 min at 30° in 20 mM HEPES (pH 7.4) containing 10 mM $MgCl_2$ and 1 mM dithiothreitol in a total volume of 25 μL. This was the control reaction mixture (lane 1). To examine parameters of Pgp phosphorylation, the following were added to the control mixtures (final concentrations are given): 1.5 mM $CaCl_2$ (lane 2), or 100 nM EGF (lane 3), or 1.5 mM $CaCl_2$ and 100 nM EGF (lane 4), or 1 μM okadaic acid (lane 5), or 1 μM okadaic acid for 1 min followed by 100 nM EGF for an additional 1 min (lane 6), or 100 nM EGF for 1 min followed by 1 μM okadaic acid for an additional 1 min (lane 7). These incubations were followed by addition of [γ-^{32}P]ATP and a further 10-min incubation at 30°. Reactions were stopped by addition of RIPA buffer. Labeled Pgp was immunoprecipitated from total reaction mixtures as described in Materials and Methods and subjected to electrophoresis on 7.5% gels (8 × 9 × 0.75 cm). Dried gels were exposed to X-ray films for 16 hr (for radioautogram) or analyzed by Betascope for 1 hr. The bar graph provides cpm in [^{32}P]Pgp bands shown in the figure. The depicted experiment is representative of three sets of assays with different plasma membrane preparations. Relative values, with respect to controls, agreed with the data shown here to within 10%.

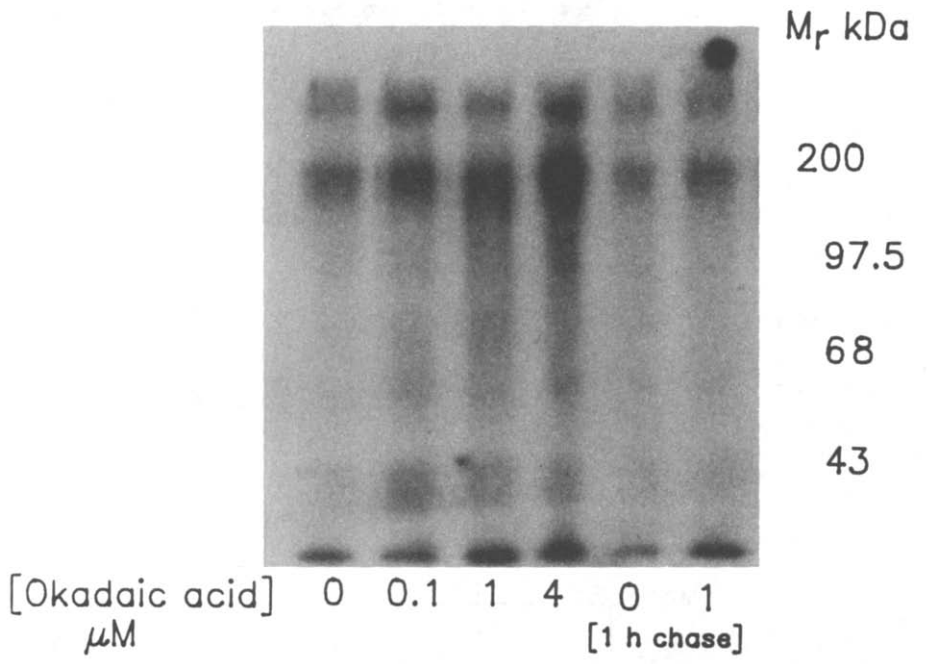


Fig. 3. Effect of okadaic acid on Pgp in intact DC-3F/AD X cells and demonstration of rapid dephosphorylation of Pgp by chase of label. DC-3F/AD X cells, in 24-well plates, were metabolically labeled with $^{32}P_i$ for 4 hr. Twenty minutes before the end of the labeling period okadaic acid was added to separate wells to final concentrations of 0.1, 1, or 4 μM. Labeled medium was removed from some wells (pairs of 0 and 1 μM okadaic acid wells) after 4 hr and replaced with fresh regular medium with or without 1 μM okadaic acid for a 1-hr chase period (lanes labeled 1-hr chase). After all procedures were completed, medium was removed, and cells were washed with phosphate-buffered saline and lysed. Pgp was immunoprecipitated from aliquots of the various lysates each containing 1–2 × 10^5 cpm. Gels (8 × 9 × 0.75 cm) displaying labeled Pgp were exposed to X-ray film for 6–8 hr. The data shown here are representative of three experiments.

Table 1. Effect of a 10-min exposure to 20 nM EGF on protein phosphatase activity in DC-3F/AD X and control cells

Cell line	EGF receptor no./cell*	$^{32}P_i$ released† (pmol/min/mg protein)	
		−EGF	+EGF
DC-3F	500	0.46 ± 0.09	0.59 ± 0.04
DC-3F/AD X	1500	0.65 ± 0.06	0.83 ± 0.07

* See Ref. 18.
† Values are means ± SEM of 3 experiments.

Both PP1 and PP2A may have been stimulated by EGF and both may have catalyzed dephosphorylation of Pgp. Okadaic acid is reported to cause half-maximal inhibition of PP2A at 0.1 nM, with complete inhibition at 1 nM, whereas 100-fold higher concentrations are required for inhibition of PP1 [36, 38]. In our studies, nanomolar amounts of okadaic acid inhibited phosphatase activity in cell-free extracts by about 56% (see Results) and increased the level of phosphorylation of Pgp in isolated membranes by 22% (data not shown). Micromolar amounts of the inhibitor completely inhibited *in vitro* phosphatase activity and increased *in vitro* phosphorylation of Pgp by 30–40% (Fig. 2). In addition, EGF reversed the okadaic acid-induced increase in Pgp phosphorylation in isolated plasma membranes (Fig. 2, lanes 6 and 7). The reversal may have been due to activation of PP1 and PP2A by EGF to an extent not inhibitable by okadaic acid. PP1/PP2A are thought to be the chief enzymes involved in reversal of the action of protein kinase C [35], a kinase shown to catalyze phosphorylation of Pgp [12].

Stimulation of phosphatase activity by EGF, *per se*, is not a component of the multidrug-resistant phenotype because stimulation was manifest in DC-3F cells as well as in the resistant subline. Whether the higher basal level of phosphatase activity in the resistant, as compared with the sensitive, cells is associated with the modulation of EGF receptor number or some other aspect of drug resistance is not known.

Considerable information is available suggesting that EGF-transduced signals could modulate regulation of Pgp. According to the literature, c-*fos* and c-*jun* form a complex transcription factor, AP-1, which binds to a specific DNA sequence contained in enhancer regions of genes responsive to 12-*O*-tetradecanoylphorbol-13-acetate [39], a phorbol ester that increases Pgp expression in a variety of cells [40] and modulates EGF receptor function and synthesis [41, 42]. The phorbol ester is an agonist of protein kinase C, and it has been reported that the effect of the ester on Pgp expression demonstrates that expression is regulated by a protein kinase C-mediated pathway [40]. In addition, the kinase is known to participate in modulation of the multidrug resistance phenotype [43]. The Pgp promoter region contains an element that responds to serum starvation [44] and an AP-1 element shown to influence expression of Pgp in hamster and mouse [45]. EGF modulates the AP-1 proteins [46] and PP2A potentiates activity of promoters containing AP-1 binding elements in rodent cells [47]. Together these observations suggest that EGF transduces a signal through its receptor that affects the factors that regulate Pgp expression and/or function. The data contained in this report demonstrating EGF-stimulated Pgp dephosphorylation are consistent with the idea of crosstalk between EGF receptor and Pgp through an intracellular signal pathway. Whether EGF-stimulated dephosphorylation of Pgp directly affects the expression or function of the protein remains to be seen. We have observed that long-term (24 hr) exposure of DC-3F/AD X cells to EGF results in a decreased half-life of Pgp from 17 hr to 12 hr (data not shown); the mechanism by which that is regulated is an open question.

Literature reports show that there is an interaction of EGF receptor and drugs, such as Adriamycin®, which select for the multidrug-resistant phenotype. Adriamycin up-regulates EGF receptor (and TGF-α) in HeLa, 3T3 [48], and A431 [49] cells within hours of drug exposure of EGF increases A431 cell sensitivity to doxorubicin [50]. We have shown that the cytotoxic effect of Adriamycin against well-established athymic mouse xenografts is enhanced when the drug is combined with blocking EGF receptor antibody [49]. Evaluation of whether Pgp is involved in these effects is underway.

Pgp may be linked to the signal transduction pathway initiated by EGF binding to its receptor, a pathway that plays a role in the regulation of cell growth and differentiation [18].

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