Cancer Chemother Pharmacol (2001) 47: 255–262
DOI 10.1007/s002800000208

ORIGINAL ARTICLE

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Primary breast tumor levels of suspected molecular determinants of cellular sensitivity to cyclophosphamide, ifosfamide, and certain other anticancer agents as predictors of paired metastatic tumor levels of these determinants

Rational individualization of cancer chemotherapeutic regimens

Received: 29 March 2000 / Accepted: 31 August 2000 / Published online: 24 January 2001

Abstract *Purpose*: Cyclophosphamide is one of the most frequently used agents in the neoadjuvant, adjuvant, and high-dose chemotherapeutic treatment of breast cancers. Preclinical models indicate that cellular sensitivity to cyclophosphamide and other oxazaphosphorines, e.g., ifosfamide, is inversely related to the cellular content of two aldehyde dehydrogenases, viz ALDH1A1 and ALDH3A1, and glutathione. Breast tumor levels of these ''determinants of cellular sensitivity to the oxazaphosphorines'' are known to vary widely, and the decision as to whether or not to use an oxazaphosphorine as part of the therapeutic strategy to treat breast cancer in any given patient is likely to depend, in large part, on the levels of these determinants in that cancer. ALDH1A1, ALDH3A1, and glutathione levels can be easily quantified in primary breast tumors and in detectable metastatic breast tumors present in axillary lymph nodes because the amounts of tissue required for the desired analysis can be readily obtained, whereas these levels cannot be quantified in residual metastatic breast cancer cell populations, i.e., those that escape detection and/or that are inaccessible to surgical harvest. The inability to directly quantify residual metastatic breast cancer cell ALDH1A1, ALDH3A1, and glutathione levels would not preclude a rational decision with regard to the inclusion/exclusion of an oxazaphosphorine as part of the chemotherapeutic strategy intended to eradicate residual metastatic breast cancer cells if primary breast tumor levels of these determinants reliably predicted those in metastatic breast cancer cells. *Methods*: ELISAs and spectrophotometric assays were used to quantify enzyme and glutathione levels in paired human primary and locally advanced metastatic breast tumor samples. *Results*: Primary breast tumor ALDH1A1 and ALDH3A1 levels were highly predictive of their respective levels in paired metastatic breast tumors present in axillary lymph nodes ($r^2 = 0.80$ and 0.85, respectively). On the other hand, those of glutathione were relatively poorly predictive of its levels in paired metastatic offshoots ($r^2 = 0.35$). Primary breast tumor levels of some additional enzymes known to catalyze the detoxification/toxification of various anticancer agents, though not of cyclophosphamide, were poorly predictive (DT-diaphorase and glutathione *S*-transferases α, μ, and π) or not predictive (cytochrome P450 1A1) of their respective levels in paired metastatic offshoots. *Conclusion*: Since ALDH1A1, ALDH3A1 and, to a lesser extent, glutathione levels in primary breast tumors reliably predicted those in detectable and easily accessible metastatic breast cancer cell populations, viz those in axillary lymph nodes, they are also likely to be predictive of these levels in undetectable and/or relatively inaccessible metastatic breast cancer cell populations. Thus, quantification of primary breast tumor ALDH1A1, ALDH3A1 and, to a lesser extent, glutathione levels prior to the initiation of not only neoadjuvant but also adjuvant and high-dose breast cancer chemotherapy is likely to be of value in the rational design of individualized chemotherapeutic regimens intended to eradicate breast cancer cells with a minimum of untoward effects.

Key words Aldehyde dehydrogenase · Glutathione *S*-transferase · DT-diaphorase · Cytochrome P450 · Glutathione · Drug metabolism · Breast cancer ·

Supported by USPHS DOA DAMD 17-94-J-4057.

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Drug resistance · Cyclophosphamide · Ifosfamide · Oxazaphosphorines · Cancer chemotherapy

Abbreviations *ALDH1A1* (formerly ALDH-1) and *ALDH3A1* (formerly ALDH-3): cytosolic aldehyde dehydrogenases [57] · *GST* glutathione *S*-transferase · *DT-D* DT-diaphorase [NAD(P)H:quinone oxidoreductase-1; NQO1] · *CYP1A1* cytochrome P4501A1 *GSH*: glutathione *mIU* · milli-international unit of enzyme activity [nanomoles of NAD(P)H formed per minute in the case of aldehyde dehydrogenase activity; nanomoles of the conjugate of 1-chloro-2,4-dinitrobenzene and glutathione formed per minute in the case of GST activity; and nanomoles of 2,6-dichlorophenol-indophenol reduced per minute in the case of DT-D activity] · *ELISA* enzyme-linked immunosorbent assay · *Pgp-170* P glycoprotein-170 · *MRP* multidrug resistance-associated protein

Introduction

Whereas the ultimate goal of cancer therapeutics is to identify agents that effect tumor regression or, at a minimum, inhibit tumor growth, without causing serious untoward effects, the interim goal is to individualize, thereby optimize, the use of the currently available, far less than ideal with regard to the ultimate goal, anticancer agents [36]. Individualization of chemotherapeutic regimens is likely to be of particular value in the treatment of breast cancer. This is because: (1) a number of agents, each of some value in the treatment of breast cancers, are already available, (2) none of these agents is without serious untoward effects, and (3) therapeutic responses to any given agent are not uniform and range from tumor progression to cures [reviewed in 22, 23, 28]. The failure of these agents to rid all patients of all malignant cells is because: (1) drug-resistant mutant clones often appear early in the natural history of tumor progression, i.e., even before drug treatment (intrinsic resistance); and/or (2) new drug-resistant clones may develop quite rapidly after the initiation of therapy (acquired resistance).

Cyclophosphamide is one of the most frequently used agents in the conventional treatment of breast cancer, whether pre- or postoperatively (neoadjuvant and adjuvant chemotherapy, respectively) [reviewed in 8, 16, 22, 23, 24]. Further, cyclophosphamide or ifosfamide are almost always part of the high-dose chemotherapy/autologous hematopoietic stem cell rescue strategies that are used to treat advanced breast cancers [reviewed in 55, 61]. Finally, another oxazaphosphorine, viz 4-hydroperoxycyclophosphamide, is used to ''purge'' autologous sources of hematopoietic stem cells, e.g., bone marrow and peripheral blood, of contaminating breast cancer cells [34, 42].

Preclinical models indicate that cellular levels of ALDH1A1, ALDH3A1, and GSH are important determinants of cellular sensitivity to cyclophosphamide and other oxazaphosphorines [reviewed in 15, 43, 44, 45]. Specifically, cellular sensitivity to the oxazaphosphorines was found to be inversely related to the cellular content of ALDH1A1, ALDH3A1, and GSH. This was because ALDH1A1 and ALDH3A1 each catalyze the detoxification of these agents, and GSH, acting as an alternative, noncritical target, sequesters oxazaphosphorine intermediates.

Given the above and that breast tumor ALDH1A1, ALDH3A1, and GSH levels vary widely [reviewed in 15, 12, 20, 35, 45, 50], it follows that, at least in part, the decision as to whether or not to use an oxazaphosphorine as part of the therapeutic strategy to treat breast cancer in any given patient will depend on the levels of ALDH1A1, ALDH3A1, and GSH in that cancer.

ALDH1A1, ALDH3A1, and GSH levels can be easily quantified in detectable primary breast tumor tissue, and detectable locally advanced breast cancer, viz metastatic tumors in axillary lymph nodes, because the amounts of tissue required for the desired analysis can be readily obtained. In contrast, these levels cannot be easily quantified in metastatic breast tumor cells found elsewhere in the body, sometimes at substantially more distant sites, e.g., bone, because the amounts of tissue required for the desired analysis cannot be readily obtained. Indeed, the presence of such metastatic cells is generally anticipated (hypothesized) based on the usual history of the disease, rather than physically established. Similarly, the presence of contaminating breast cancer cells in autologous sources of hematopoietic stem cells, e.g., bone marrow and peripheral blood, is routinely anticipated (hypothesized) rather than physically established, because the latter requires a heroic effort and, in those instances where the effort has been made, malignant breast cells have often (up to 70% of cases) been detected in bone marrow and peripheral blood samples obtained from patients diagnosed with advanced breast cancer [3, 7, 9, 25, 34, 37, 39].

The inability to directly quantify ALDH1A1, ALDH3A1, and GSH levels in undetectable and/or relatively inaccessible metastatic breast cancer cell populations seemingly precludes a rational decision with regard to whether or not to include an oxazaphosphorine as part of the chemotherapeutic strategy intended to eradicate such populations. However, if ALDH1A1, ALDH3A1, and GSH levels in detectable and relatively easily accessible breast tumor populations reliably predict those in undetectable and/or relatively easily inaccessible metastatic breast cancer cell populations, a solution to this dilemma would be at hand, i.e., there would be no need to quantify the levels of these determinants of cellular sensitivity to the oxazaphosphorines in the latter, because doing so in the former would suffice.

Whether ALDH1A1, ALDH3A1, and GSH levels in detectable and relatively easily accessible breast tumor populations reliably predict those in undetectable and/or relatively inaccessible metastatic breast cancer cell populations is not known. Hence, an investigation was

initiated to address this question, the premise being that predictive capability of the type referred to and desired is likely to be the case if ALDH1A1, ALDH3A1, and GSH levels in primary breast tumors reliably predicted those in detectable and easily accessible metastatic breast cancer cell populations, viz those in axillary lymph nodes.

Also addressed in this investigation was whether DT-D, GST, and CYP1A1 levels in primary breast tumors reliably predict those in metastatic breast cancer cell populations. These questions, too, have not been addressed previously. Again, they are of potential importance with regard to individualizing cancer chemotherapeutic regimens because: (1) levels of these enzymes in malignant breast tissue also vary widely [20, 27, 30, 31, 41, 50], and (2) preclinical models indicate that cellular levels of these enzymes greatly influence cellular sensitivity to certain other anticancer agents that may be of use in the treatment of breast cancer. For example, cellular sensitivity to melphalan decreases as cellular levels of GST increase, because this enzyme catalyzes the detoxification of this agent [reviewed in 21; 38], and cellular sensitivity to EO9 and ellipticine increases as cellular levels of DT-D and CYP1A1, respectively, increase, because these enzymes catalyze the toxification of EO9 [reviewed in 58; 38] and ellipticine [reviewed in 33; 38], respectively.

Materials and methods

Paired ($n = 17$) human primary and metastatic (16 from axillary lymph nodes and 1 from breast skin) breast tumor tissue samples were obtained from the Cooperative Human Tissue Network, Midwestern Division, Columbus, Ohio. They had been surgically removed [(modified) radical mastectomies] from female patients, snap-frozen in liquid nitrogen (within 6 h after removal), stored at –70 °C (5–30 days), and shipped to us in dry ice. Enzyme proteins and/or activities of interest, *vide infra*, are stable under these storage conditions. Patient age (less than 45 years, 7; 45–60 years, 4; over 60 years, 6) and diagnosis [infiltrating ductal (adeno)carcinomas] were obtained from the pathology reports that accompanied the tissue specimens. As determined by the Cooperative Human Tissue Network's Department of Histology, sample compositions ranged from 30% to 100% tumor in the cases of both primary and metastatic breast tumor tissues; median values were 85% and 80%, respectively. Further, samples were judged to be from 0% to 60% necrotic in both primary and metastatic breast tumor tissues; median values were 30% and 10%, respectively. Still further, samples were judged to be from 0% to 50% and 0% to 40% fibrotic in the cases of primary and metastatic breast tumor tissues, respectively; median values were 0% in both cases. Three of the seventeen subjects were known to have been treated prior to surgery with a chemotherapeutic regimen that included cyclophosphamide. Eleven subjects were not known to have been treated with any chemotherapeutic regimen prior to surgery. Unknown is whether or not the remaining three subjects were treated prior to surgery with a chemotherapeutic regimen that included cyclophosphamide.

Purified human GSTα, GSTμ, and GSTπ, and affinity-purified polyclonal antibodies specific for each of these isozymes, i.e., anti-GSTα IgG, anti-GSTμ IgG, and anti-GSTπ IgG, respectively [54], were generously provided by Dr. A.J. Townsend, Department of Biochemistry, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, N.C. All other chemicals, reagents, and supplies were purchased from commercial sources or were prepared as described previously [47, 48, 49, 51, 53].

Preparation of purified ALDH1A1 and ALDH3A1 from human stomach mucosa, and chicken polyclonal antibodies specific for these enzymes, i.e., anti-ALDH1A1 IgY and anti-ALDH3A1 IgY, respectively, was as described previously [10, 11, 47].

Soluble (105,000 g supernatant) and Lubrol (0.3%)-solubilized particulate (105,000 g pellet) fractions of paired primary and metastatic breast tumor tissue samples were prepared as described previously for breast tissues [47]. Soluble (105,000 g supernatant) fractions were used when tissue levels of ALDH1A1, ALDH3A1, DT-D, pan-GST, GSTα, GSTμ and GSTπ, were to be quantified [38, 50, 52]. Lubrol-solubilized particulate fractions were used when tissue levels of CYP1A1 were to be quantified [38, 50].

Quantification of protein levels in soluble (105,000 g supernatant) and Lubrol-solubilized particulate (105,000 g pellet) fractions was by a spectrophotometric assay [47].

Quantification of ALDH1A1, ALDH3A1, GSTα, GSTμ, and GSTπ catalytic activities was by ELISAs [38, 50, 51]. Tumor levels (catalytic activities per gram of tissue) of ALDH1A1, ALDH3A1, GSTα, GSTμ, and GSTπ were estimated from standard curves generated with purified enzymes; specific activities of the latter were 2,850, 60,500, 44,600, 24,100, and 56,800 mIU/mg protein, respectively. Substrates and cofactors were: acetaldehyde and nicotinamide adenine dinucleotide (NAD; 4 mM each) in the case of ALDH1A1; benzaldehyde and nicotinamide adenine dinucleotide phosphate (NADP; 4 mM each) in the case of ALDH3A1; and 1-chloro-2,4-dinitrobenzene and GSH (1 mM each) in the cases of GSTα, GSTμ, and GSTπ [38, 50, 51].

Quantification of pan-GST and DT-D catalytic activities was by the spectrophotometric assays described previously [49]. Co-substrates were 1-chloro-2,4-dinitrobenzene and GSH, 1 mM each, when pan-GST activity was quantified. Substrate, cofactor, and inhibitor were 2,6-dichlorophenol-indophenol (40 μM), NADH (160 μM), and dicumarol (10 μM), respectively, when DT-D activity was quantified.

Quantification of CYP1A1 levels was by an ELISA [50]. Tumor levels (picograms per gram of tissue) of CYP1A1 were estimated from standard curves generated with Lubrol-solubilized, CYP1A1-containing microsomes (15 ng CYP1A1/mg microsomal protein). Spectrophotometric quantification of GSH levels was as described by Anderson [1].

The Macintosh-based Statview II (Brainpower, Calabas, Calif.) computer program was used to generate linear regression lines (r^2, Pearson's regression coefficients) and P-values thereof.

Results

In agreement with our previous findings [reviewed in 45; 50], ALDH1A1 and ALDH3A1 levels in primary and metastatic breast tumor tissue samples varied widely (Table 1). Highest levels of ALDH1A1 and ALDH3A1 in primary breast malignancies were, respectively, ~60- and ~84-fold greater than the lowest levels of these enzymes in these tissue samples. Highest levels of ALDH1A1 and ALDH3A1 in metastatic breast malignancies were, respectively, ~70- and ~119-fold greater than the lowest levels of these enzymes in these tissue samples. As in our previous studies [50], cellular levels of ALDH1A1 and ALDH3A1 were directly related in both primary ($r^2 = 0.67$; $P \leq 0.0001$) and metastatic ($r^2 = 0.73$; $P \leq 0.0001$) breast tumors (data not presented). Importantly, ALDH1A1 and ALDH3A1 levels in the primary breast tumor samples predicted the respective levels of these enzymes in metastatic breast tumor samples taken from the same donors (Fig. 1, Table 1). Interestingly,

Table 1 Enzyme and GSH levels in paired primary and metastatic breast tumor tissue samples: summary and statistical analysis. Preparation of soluble (105,000 g supernatant) and Lubrol-solubilized particulate (105,000 g pellet) fractions from paired human primary and metastatic breast tumor tissue samples, and quantification of enzyme and GSH levels were as described in Materials and methods. Values are means of duplicate determinations made on single primary and metastatic breast tumor tissue samples taken from each of 17 patients, except in the cases of CYP1A1 and GSH, where the single primary and metastatic breast tumor tissue samples were taken from each of 16 patients. Values are expressed as milli-international units of enzyme activity per gram of tissue in all cases except in those of CYP1A1 and GSH, where they are expressed as picograms per gram and nanomoles per gram of tissue, respectively. Some of the primary data are presented in Fig. 1

Enzyme	mIU, pg, or nmol/g breast tumor tissue				Regression analysis[a] (primary vs metastatic)	
	Primary		Metastatic		r^2	P-value
	Mean ± SD	Range	Mean ± SD	Range		
ALDH1A1	26 ± 30	2–119	38 ± 35	2–139	0.80	≤0.0001
ALDH3A1	39 ± 57	3–251	68 ± 84	3–356	0.85	≤0.0001
DT-D	406 ± 315	55–1,030	998 ± 1,080	85–4,280	0.40	0.006
pan-GST	1,600 ± 999	271–3,420	2,970 ± 2,430	470–8,220	0.55	0.001
GSTα[b]	262 ± 403	0–1,310	486 ± 693	0–1,950	0.52	0.001
GSTμ[c]	265 ± 416	0–1,560	313 ± 506	0–2,000	0.46	0.003
GSTπ	1,310 ± 855	180–2,800	2,140 ± 1,630	230–5,400	0.48	0.002
CYP1A1[d]	41 ± 149	0–600	9 ± 10	0–28	0.19	0.096
GSH	265 ± 245	16–750	414 ± 406	80–1,400	0.35	0.015

[a] Unweighted regression analysis was as described in Materials and methods

[b] GSTα values were zero in 4 (24%) of the 17 paired samples. When absent from one of the pairs, it was always absent from the other. Zero values were included in the calculation of mean values and when generating the regression line ($r^2 = 0.45$ and $P = 0.012$ when the 4 paired-zero values were not included in the regression analysis)

[c] GSTμ values were zero in 7 (41%) of the 17 paired samples. When absent from one of the pairs, it was always absent from the other. Zero values were included in the calculation of mean values and when generating the regression line ($r^2 = 0.30$ and $P = 0.105$ when the 7 paired-zero values were not included in the regression analysis)

[d] CYPIA1 values were zero in 10 of 16 (63%) primary and 4 of 16 (25%) metastatic breast tumor tissue samples. When absent in the metastatic tumor, it was always absent from the primary tumor. Zero values were included in the calculation of mean values and when generating the regression line ($r^2 = 0.17$ and $P = 0.185$ when the 4 paired-zero values were not included in the regression analysis)

both the highest ALDH1A1 levels and the highest ALDH3A1 levels were observed in primary (poorly differentiated infiltrating ductal carcinoma) and metastatic (axillary lymph nodes) breast tumor samples originating from one patient, a Caucasian woman, who had not been given cyclophosphamide or any other chemotherapeutic agent prior to tumor resection.

Confirming previous reports [reviewed in 45; 12, 20, 35, 50], GSH levels varied widely in both primary (~47-fold) and metastatic (~18-fold) breast tumors (Table 1). As in our previous studies [50], cellular levels of GSH were not related to those of ALDH1A1 or ALDH3A1 in either primary ($r^2 = 0.14$, $P = 0.147$; and $r^2 = 0.003$, $P = 0.830$, respectively) or metastatic ($r^2 = 0.05$, $P = 0.412$; and $r^2 = 0.001$, $P = 0.895$, respectively) breast tumors (data not presented). Although statistically significant as judged by regression analysis, GSH levels in primary breast tumor samples were a poor predictor of GSH levels in metastatic breast tumor samples taken from the same patients (Fig. 1, Table 1). Perhaps relevant, significant intratumor (melanoma, ovarian carcinoma) differences in GSH levels have been reported [2]. Our determinations were made on single subsamples obtained from each of the primary and metastatic breast cancer samples.

Again confirming previous observations [20, 27, 30, 31, 41, 50], DT-D, pan-GST, GSTα, GSTμ, GSTπ, and CYP1A1 levels also varied widely in both primary and metastatic breast tumors (Table 1). DT-D, pan-GST, GSTα, GSTμ, and GSTπ levels in primary breast tumor samples predicted the respective levels of these enzymes in metastatic breast tumor samples taken from the same patients, but not very well (Table 1). CYP1A1 levels in primary breast tumor samples did not predict CYP1A1 levels in metastatic breast tumor samples taken from the same patients (Table 1).

Both a stable and a transient coordinated elevation of ALDH3A1, DT-D, and GSTπ levels with or without that of CYP1A1 levels have been effected in preclinical breast cancer models [38, 46, 48, 49, 53]. As judged by the criteria that each enzyme level is greater than 1 SD above the respective mean level, coordinated elevation of ALDH3A1, DT-D, and GSTπ levels with or without that of CYP1A1 levels was not observed in any of the primary or metastatic breast tumor samples evaluated in the present investigation (data not shown).

Discussion

Confirming earlier reports [reviewed in 15, 45; 12, 20, 27, 30, 31, 35, 41, 50], ALDH1A1, ALDH3A1, DT-D, pan-GST, GSTα, GSTμ, GSTπ, CYP1A1, and GSH

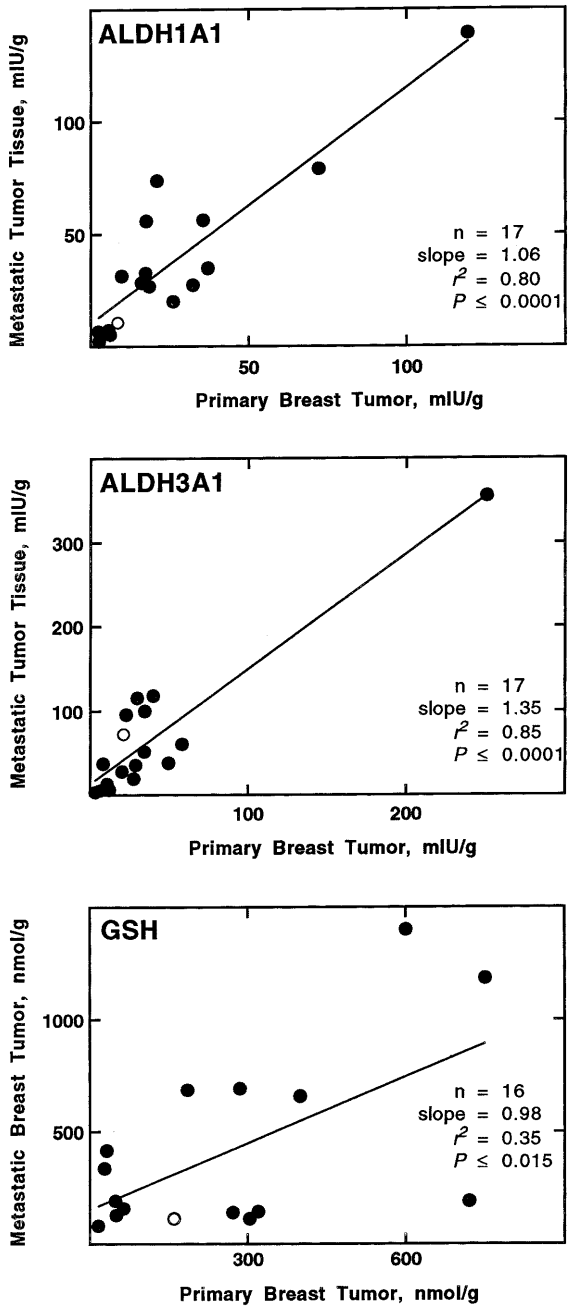

Fig. 1 ALDH1A1, ALDH3A1, and GSH levels in paired human primary and metastatic breast tumor tissues. ALDH1A1, ALDH3A1, and GSH levels were quantified as described in Materials and methods. Points are means of duplicate determinations made on primary and metastatic (● axillary lymph node, ○ skin) breast tumor tissue samples, one of each from any given patient. $r^2 = 0.54$ and $P = 0.001$ when the highest value for ALDH1A1 is excluded; $r^2 = 0.37$ and $P = 0.017$ when the two highest values for ALDH1A1 are excluded; and $r^2 = 0.27$ and $P = 0.038$ when the highest value for ALDH3A1 is excluded

levels were each found to vary widely in breast tumor tissue.

Importantly, primary breast tumor levels of ALDH1A1 and ALDH3A1 were found to be highly predictive of their respective levels in paired metastatic breast cancers present in axillary lymph nodes, despite the fact that, as noted in Materials and methods, the composition of both primary and metastatic breast cancer samples varied substantially with respect to percentage tumor, necrosis, and fibrosis. On the other hand, those of GSH were relatively poorly predictive of its levels in paired metastatic breast cancers present in axillary lymph nodes. These findings are consistent with those of our previous investigations [50], wherein normal breast levels of ALDH1A1 and ALDH3A1, but not those of GSH, were predictive, albeit with somewhat less accuracy, of their respective levels in paired primary, as well as metastatic, breast tumors.

Thus, quantification of primary breast tumor ALDH1A1, ALDH3A1, and, to a lesser extent, GSH levels in primary breast tumors should assist in the making of rational decisions with regard to whether or not to include an oxazaphosphorine as part of not only: (1) preoperative neoadjuvant chemotherapeutic strategies intended to reduce the size of the primary tumor prior to surgical resection, as well as to eradicate any metastatic breast cancer cells that may be present, but also (2) postoperative adjuvant chemotherapeutic strategies, including high-dose chemotherapy, intended to eradicate residual metastatic breast cancer cells following surgical resection. The premise is that, since ALDH1A1 and ALDH3A levels in primary breast tumors reliably predicted those in detectable and easily accessible metastatic breast cancer cell populations, viz those in axillary lymph nodes, they are also likely to be predictive of these levels in undetectable and/or relatively inaccessible metastatic breast cancer cell populations. A further caveat is that, whereas preclinical models indicate that ALDH1A1, ALDH3A1, and GSH are molecular determinants of cellular sensitivity to cyclophosphamide and other oxazaphosphorines [reviewed in 15, 43, 44, 45], whether even the highest breast tumor levels of any of these putative determinants are pharmacologically relevant in the clinic remains to be established, although preliminary findings in our laboratory already indicate an inverse relationship between ALDH1A1 levels and the therapeutic outcome of cyclophosphamide-based chemotherapy of breast cancers.

Primary breast tumor levels of DT-D, pan-GST, GSTα, GSTμ, and GSTπ were also found to be predictive, albeit relatively poorly so, of their respective levels in paired metastatic breast cancers present in axillary lymph nodes, whereas those of CYP1A1 were not. A previous investigation [50] revealed that levels of these enzymes in normal breast tissue did not predict their respective levels in paired primary or metastatic breast tumors. Thus, whereas knowledge of primary breast tumor levels of these enzymes may be of some value (with caveats analogous to second of those noted above) in the making of a rational decision with regard to whether or not to include anticancer drugs that are bioactivated or bioinactivated by them as part of a preoperative neoadjuvant chemotherapeutic strategies

intended to reduce the size of the primary tumor, such knowledge may be of more dubious value in the making of a rational decision with regard to whether or not to include such agents as part of a postoperative adjuvant chemotherapeutic strategy intended to eradicate residual metastatic breast cancer cells. However, perhaps of pragmatic pharmacological value and consistent with previous observations [50], GSTμ was not found in metastatic breast tumor samples when it was not found in primary breast tumor samples obtained from the same patient, as would be expected if the absence of this enzyme was due to a GSTμ null genotype [reviewed in 21]. GSTα was also not found in metastatic breast tumor samples when it was not found in primary breast tumor samples obtained from the same patient. This finding, however, differed from those made in a previous investigation, viz in two of three cases, detectable GSTα levels were present in primary breast tumor, but not in normal breast, tissue samples obtained from the same patients, and, conversely, in 5 of 26 cases, GSTα was found in normal but not in malignant (primary and metastatic) breast tissue samples obtained from the same patients [50].

Methotrexate, 5-fluorouracil, vincristine, doxorubicin (adriamycin), and, more recently, paclitaxel (taxol) are also used extensively to treat metastatic breast cancer [reviewed in 8, 22, 23, 24, 28]. The additional quantification of breast tumor levels of determinants of cellular sensitivity to these agents would be of value in further individualizing breast cancer chemotherapeutic strategies. For example, preclinical models indicate that cellular levels of two ABC (adenosine triphosphate-binding cassette) cell-surface multidrug transporters, viz Pgp-170 and MRP, are important determinants of cellular sensitivity to doxorubicin [reviewed in 13, 18; 6, 19, 59, 60]. Specifically, cellular sensitivity to this agent was found to be inversely related to the cellular content of Pgp-170 and MRP. This is apparently because doxorubicin is subject to Pgp-170- and MRP-mediated transport out of cells [reviewed in 5, 13, 17, 18; 6]. Variations in malignant breast tissue levels of Pgp-170 and MRP have been reported [4, 14, 32, 40], but whether Pgp-170 or MRP expression influences the therapeutic outcome of doxorubicin-based chemotherapy of clinical breast cancers is uncertain [26, 56]. Intriguingly, an inverse relationship between MRP expression and therapeutic outcome of cyclophosphamide/methotrexate/5-fluorouracil (CMF)-treated breast cancer has been observed [32]; none of these agents is known to be exported out of cells by this transporter, but a modest resistance to "activated cyclophosphamide," i.e., 4-hydroxycyclophosphamide, was observed when a human breast adenocarcinoma cell line was made to express large amounts of MRP by stably transfecting it with the cDNA that encodes this transporter [29]. Whether primary breast tumor Pgp-170 and MRP levels are predictive of their respective levels in metastatic breast tumors present in the same individual has yet to be determined.

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