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Expression of xenobiotic and steroid hormone metabolizing enzymes in human breast carcinomas

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The potential to metabolize endogenous and exogenous substances may influence breast cancer development and tumor growth. Therefore, the authors investigated the protein expression of Glutathione S-transferase (GST) isoforms and cytochrome P450 (CYP) known to be involved in the metabolism of steroid hormones and endogenous as well as exogenous carcinogens in breast cancer tissue to obtain new information on their possible role in tumor progression. Expression of GST pi, mu, alpha and CYP1A1/2, 1A2, 3A4/5, 1B1, 2E1 was assessed by immunohistochemistry for primary breast carcinomas of 393 patients from the German GENICA breast cancer collection. The percentages of positive tumors were 50.1 and 44.5% for GST mu and CYP2E1, and ranged from 13 to 24.7% for CYP1A2, GST pi, CYP1A1/2, CYP3A4/5, CYP1B1. GST alpha was expressed in 1.8% of tumors. The authors observed the following associations between strong protein expression and histopathological characteristics: GST expression was associated with a better tumor differentiation (GST mu, $p = 0.018$) and with reduced lymph node metastasis (GST pi, $p = 0.02$). In addition, GST mu expression was associated with a positive estrogen receptor and progesterone receptor status ($p < 0.001$). CYP3A4/5 expression was associated with a positive nodal status ($p = 0.018$). Expression of CYP1B1 was associated with poor tumor differentiation ($p = 0.049$). Our results demonstrate that the majority of breast carcinomas expressed xenobiotic and drug metabolizing enzymes. They particularly suggest that GST mu and pi expression may indicate a better prognosis and that strong CYP3A4/5 and CYP1B1 expression may be key features of nonfavourable prognosis.

Key words: breast carcinoma; GST; CYP; immunohistochemistry; estrogen receptor; progesterone receptor

The influence of exogenous and endogenous factors on tumor growth partly depends on the individual potential to metabolize these substances. Phase I and phase II enzymes are key players in the metabolism of a huge number of exogenous and endogenous compounds including steroid hormones. Usually, phase I and phase II are detoxification processes, but this metabolism can also lead to highly reactive electrophiles that can bind to macromolecules, e.g., proteins and DNA. Moreover, these enzymes are highly polymorphic, giving rise to variations in enzymatic activity.[1] This may influence predisposition to cancer as well as tumor development and progression.[2] Therefore, these enzymes are candidates for the investigation of a potential role in breast cancer.

Glutathione S-transferases (GSTs) are phase II metabolizing isoenzymes. They facilitate clearance of endogenous hydrophobic compounds such as hormones, steroids, haem, bilirubin and bile acids. Furthermore, they are essential for metabolism of environmental carcinogens, drugs and pesticides by catalyzing the conjunction of reactive chemical intermediates to soluble glutathione conjugates.[3] Seven classes of cytosolic GSTs are recognized in mammalian tissues (alpha, mu, pi, sigma, omega, theta and zeta).[4] In breast cancer, polymorphic GST isoenzymes may play a role in tumorigenesis and resistance to chemotherapy.[5] By biochemical measurements, components of the glutathione pathway were found to be increased in breast tumor cells.[6] However, immunohistochemical studies investigating the clinical relevance of GST expression in breast cancers showed inconsistent results. Absence of GST pi expression in tumors was found to be associated with poor tumor differentiation.[7] Another study found an inverse association between GST pi expression with estrogen receptor (ER) and progesterone receptor (PR) status.[8] Expression of GST pi in node negative breast cancers was related to early recurrence of disease and shortened long-term survival.[8] No relationship was found for GST pi, alpha and mu expression with response to mitoxantrone chemotherapy in advanced breast cancer.[9]

Cytochrome P450 (CYPs) enzymes are a multigene family of phase I detoxification enzymes. They are involved in the metabolism of several promutagens and procarcinogens including polycyclic aromatic hydrocarbons and aryl amines to mutagenic or carcinogenic metabolites.[10,11] The conversion of 17beta-estradiol (E_2) to 2- hydroxymetabolites by CYP1A1 and CYP3A4, to 4-hydroxymetabolites by CYP1B1, as well as to 16alpha-hydroxyestrone by CYP3A4 may result in potentially genotoxic products.[12–14] Activities of CYP1A1 and CYP1B1 may be altered because of the induction by environmental factors like cigarette smoke or local pollutants[15,16] and by 17beta estradiol itself via the ER alpha.[17] CYP 3A4 comprises the largest proportion of CYP liver proteins.[18] There are well-known differences with respect to the *in vitro* clearance of CYP3A substrates in men and women,[19] but no apparent differences in substrate specifity are known.[20] The main organ for CYP expression is the liver, but CYP1A1, 3A4, 3A5, 1B1 and 2E1 were also expressed in nonneoplastic and neoplastic breast tissue on the mRNA expression and protein levels, pointing to a possible local activation of estrogen to potentially carcinogenic metabolites.[21–25] However, as of today, the role of CYP expression in breast tumors is unknown.

The GENICA study is a population-based breast cancer case-control study for the assessment of environmental and molecular risks in Germany.[26,27] In addition, the study provides a comprehensive series of incident breast tumors suitable for the investigation of tumor-associated factors potentially relevant in breast tumorigenesis and tumor progression. Here, we describe GST (pi, mu, alpha) and CYP (1A1, 1A2, 3A4, 1B1, 2E1) isoenzyme expression in tumor tissues of this large collection of breast cancer cases and discuss their role in conjunction with known breast cancer prognostic factors.

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TABLE I – PRIMARY ANTIBODIES AND CUT OFF FOR POSITIVE SCORES

	Clonality	Dilution	Staining	Evaluation	Cut off
Mib	Mono	1:1000	Nuclear	Number of cells	>5%
p53	Mono	1:250	Nuclear	Number of cells	>10%
HER2	Poly	1:300	Membranous	DAKO score™	2+, 3+
ER (α)	Mono	1:50	Nuclear	Number of cells × intensity[1]	3–12
PR	Mono	1:50	Nuclear	Number of cells × intensity[1]	3–12
AR	Mono	1:75	Nuclear	Number of cells × intensity[1]	3–12
GSTpi	Poly (rabbit)	1:750	Cytoplasmic	Number of cells × intensity[1]	3–12
GSTmu	Poly (rabbit)	1:400	Cytoplasmic	Number of cells × intensity[1]	3–12
GSTalpha	Poly (rabbit)	1:1500	Cytoplasmic	Number of cells × intensity[1]	3–12
CYP1A1/2	Poly (goat)	1:200	Cytoplasmic	Number of cells × intensity[1]	3–12
CYP3A4/5	Poly (rabbit)	1:1000	Cytoplasmic	Number of cells × intensity[1]	3–12
CYP2E1	Poly (goat)	1:200	Cytoplasmic	Number of cells × intensity[1]	3–12
CYP1A2	Mono (mouse)	1:100	Cytoplasmic	Number of cells × intensity[1]	3–12
CYP1B1	Poly (rabbit)	1:1000	Cytoplasmic	Number of cells × intensity[1]	3–12

[1]German Immuno Reactive Score.[28]

Methods

Patients

Patients and tumor tissues were recruited within the GENICA breast cancer population-based case-control study during the period from 2000 to 2003. Inclusion criteria for study subjects were a histopathologically confirmed diagnosis of first primary breast cancer, Caucasian descent, residency in the greater Bonn area in Germany and age below 80 years. The ethics committee of the University of Bonn approved the GENICA study. All participants gave written informed consent. Our present study refers to the immunohistochemical analysis of protein expression in breast cancers of 393 patients.

Materials

Tissue specimens were fixed in 4% buffered formaldehyde and embedded in paraffin. Immunohistochemical stainings were performed on 4 μm sections with an immunostainer (Techmate 500; DAKO). The antigen–antibody binding was visualized by means of the avidin–biotin complex (ABC-method) using AEC (3-amino-9-ethylcarbazol) as chromogen.

Antibodies against p53, Mib, hormonal receptors and HER2 were purchased from DAKO, Glostrop, Denmark. GST antibodies were purchased from Novo Castra, Newcastle upon Tyne, UK. Antibodies for CYP1A1/2, CYP3A4/5 and CYP2E1 were purchased from Natutec, Frankfurt am Main, Germany (Manufacturer Daiichi Pure Chemicals, Tokyo, Japan) and antibodies for CYP1B1 and 1A2 were purchased from Gentest, Woburn, USA.

According to the manufacturers declarations, specificity of antibodies was determined by immunoblot analysis. This refers to the specifity of polyclonal anti-CYP1B1 and 1A2 as determined by western blot analysis using a panel of cDNA-expressed CYP enzymes including CYP1A1, 2A6, 2B6, 2C8, 2C9, 2D6, 3A4, 3A5, 4A11. This also refers to the specificity of polyclonal antiserum for human CYP2E1 as determined by western blot analysis with CYP1A1, 1A2, 2A6, 2B6, 2D6 and 3A4 proteins. In case of the polyclonal anti-CYP3A antiserum for CYP3A4, the manufacturer states that this antiserum also recognizes CYP3A5, but not CYP3A7 in immunoblots. Accordingly, this antiserum may be used to asses the major CYP3A levels in human tissues. Also, the polyclonal anti-CYP1A1 detects both CYP1A1 and 1A2 according to the manufacturer's immunoblot analysis. For clarity, we will therefore refer to CYP3A4/5 and CYP1A1/2 expression.

Details about clonality and dilutions of the primary antibodies are given in Table I.

Except for Mib and p53, all stainings were performed together with positive and negative tissue controls on each slide. For hormonal receptor and HER2 stainings, positive and negative tested breast cancers were used as controls. For GST staining liver (GST alpha, mu, pi), kidney (GST pi) and testis (GST mu) tissue served as positive controls. CYP expression was found to be positive in liver (CYP3A4/5, 1A1/2, 1A2, 2E1) and trophoblast epithelium (1B1).

Evaluation of immunohistochemical stainings

Evaluation took into account the different staining patterns of each antibody. For Mib and p53, the number of positive cells (nuclear staining) was counted. For hormonal receptors (estrogen, progesterone, androgen), the number of positive cells was multiplied by the intensity of nuclear staining. Evaluation followed the German Immuno Reactive Score as it is routinely used for evaluation of hormonal receptor expression for estrogen (ER), progesterone (PR) and androgen (AR).[28] This score was also applied to the staining of GSTs pi, mu, alpha and CYPs 1A1/2, 1A2, 3A4/5, 1B1, 2E1. For evaluation of enzyme expression, only cytoplasmic staining was assessed. The following score levels were used: for percentage of positive cells: 0% = 0, 1–10% = 1, 11–50% = 2, 51–80% = 3, >80% = 4. For intensity of staining: negative = 0, weak = 1, moderate = 2, strong = 3. The maximum score level was 12 for hormonal receptors, GSTs and CYPs. Score levels 3–12 were defined as positive expression and score levels 6–12 were defined as strong expression in the tumors.

For determination of HER2 protein overexpression, the staining intensity of the cellular membrane was evaluated following the guidelines for scoring HercepTest™, DAKO: <10% = 0, faint, incomplete membrane staining >10% = 1+, weak to moderate staining of the complete membrane in >10% = 2+, strong complete membranous staining in >10% = 3+. Zero and 1+ was assessed negative, 2+ and 3+ positive.

For statistical analysis, χ^2 analysis and Fisher's exact test were used, if appropriate.

Results

Staining of GST and CYP in breast cancer tissue

Expression of GST pi was found in 21.1% of tumors, more than half of them exhibiting strong staining intensity (score 6–12, 12.2%). In some GST pi negative invasive carcinomas, the *in situ* component of the tumors showed faint GST pi staining (Figs. 1*a* and 1*b*). In nonneoplastic breast tissue, strong GST pi expression was consistently found in the inner layer of the glandular epithelium (Fig. 1*c*).

GST mu was expressed in 50.1% of the tumors, 30.5% of tumors showed a strong expression. In contrast to GST pi, staining intensity of GST mu in nonneoplastic mammary glands was variable and could be either stronger or weaker than in the tumors (Fig. 1*d*).

GST alpha staining was a very rare finding in nonneoplastic tissue and tumor cells. In the few positive cases, there was specific staining of singular tumor cells (Fig. 1*e*). The nonneoplastic tissue

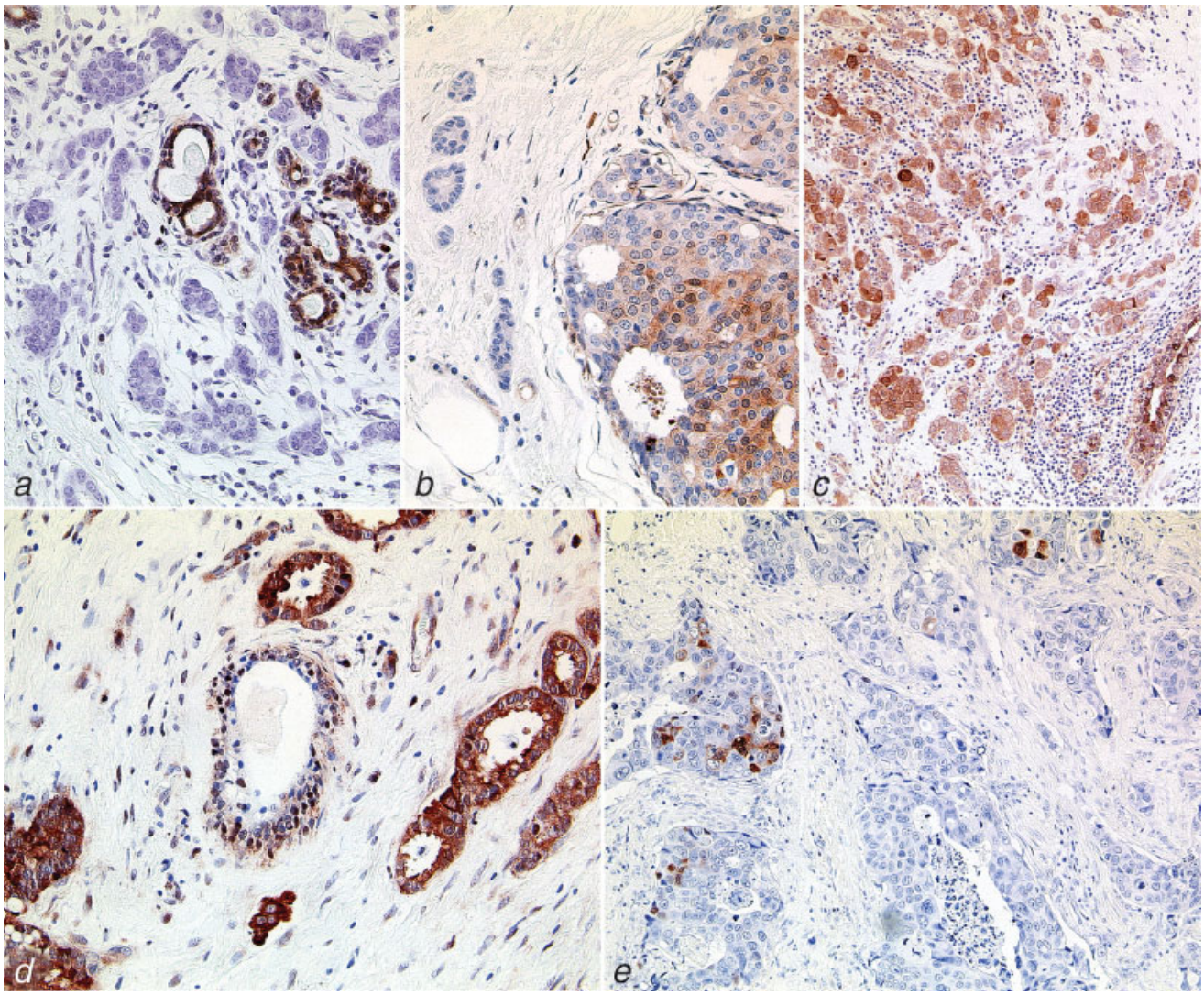

FIGURE 1 – (*a*)A GST pi expression in a nonneoplastic lobule adjacent to a GST pi negative grade II invasive ductal carcinoma. (*b*) Weak GST pi expression in the *in situ* component of the same GST pi negative invasive carcinoma. (*c*) Strong GST pi expression in a grade III invasive ductal carcinoma (score 12), equivalent to that in the nonneoplastic ductulus (right margin). (*d*) Weak GST mu staining in myoepithelial cells of nonneoplastic mammary glands beside a GST mu positive grade II invasive ductal carcinoma. (*e*) Intense GST alpha in singular glandular cells of a grade III invasive ductal carcinoma.

of those tumors also contained sporadic GST alpha positive glandular cells.

Anti-CYP1A1/2 and CYP1A2 showed predominantly faint cytoplasmic staining in 20.6% and 13% of the tumors (Fig. 2*a*). Both enzymes revealed strong expression (score 6–12) in 6.9% of cases. Positive staining of CYP1B1, CYP3A4/5 and CYP1B1 in the tumors ranged from 16.5 to 44.5%. The proportion of tumors with a strong staining intensity (score 6–12) was 21.6% for CYP2E1, 11.9% for CYP3A4/5 and 8.1% for CYP1B1. In nonneoplastic tissue, CYP1B1 occasionally stained myoepithelial cells of mammary glands and smooth muscles of blood vessels (Fig. 2*b*). Expression of CYP2E1 and CYP3A4/5 was most intense in the inner layer of the glandular and duct epithelium (Figs. 2*c* and 2*d*).

GST and CYP expression in relation to tumor characteristics

Tables II and III give an overall view to expression rates of the GST and CYP enzymes and clinical and pathological tumor characteristics. Table II summarizes tumors with a positive immunohistochemical expression (score levels 3–12). For some enzymes, associations became more evident in tumors with strong expression (score 6–12). These data are shown in Table III.

GST pi positive tumors showed a significantly lower rate of lymph note metastasis compared with the GST pi negative tumor group ($p = 0.02$, Table II). In addition, tumors with strong expression of GST pi showed an association with smaller tumor size (T-stages, $p = 0.049$, Table III). GST mu expression in the tumors correlated with a better tumor differentiation ($p = 0.049$, Table II), especially in tumors with strong GST mu staining ($p = 0.018$, Table III).

Expression of CYP3A4/5 showed an association with poor tumor differentiation ($p = 0.06$) and was significantly correlated with the occurrence of lymph node metastasis ($p = 0.018$, Table II). In tumors with strong expression of CYP3A4/5 (score 6–12), there was additionally an association with advanced T-stages ($p < 0.001$, Table III). Strongly CYP1B1 expressing tumors (score 6–12) revealed an association with poor tumor grading ($p = 0.049$, Table III).

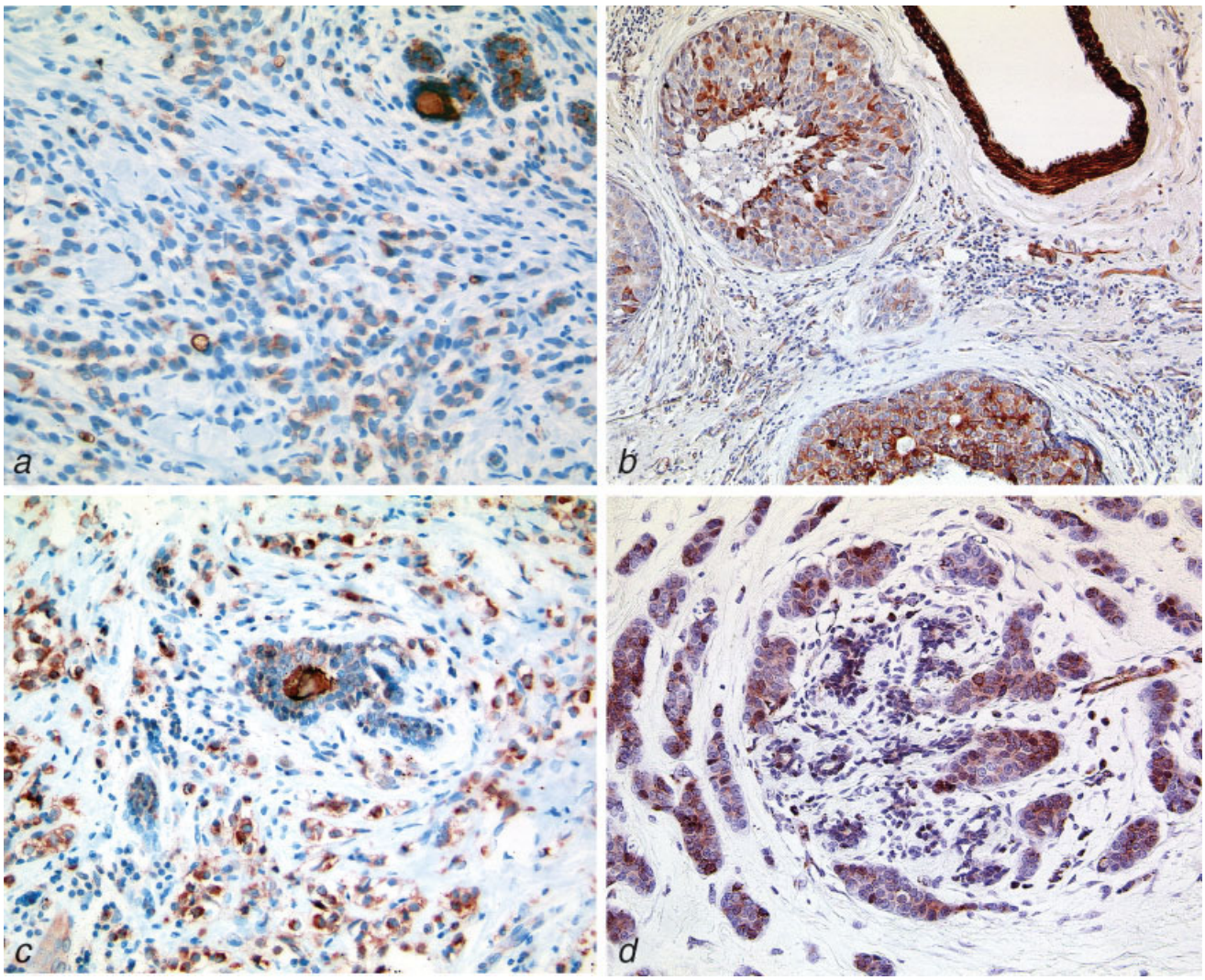

FIGURE 2 – (*a*) only faint positive staining for CYP1A1/2 in this grade III invasive lobular carcinoma (score 3) compared with the stronger expression in nonneoplastic ductular cells. (*b*) CYP1B1 expression in the *in situ* component of a grade III invasive ductal carcinoma and in smooth muscle cells of a small blood vessel. (*c*) CYP2E1 positive grade II invasive ductal carcinoma. (*d*) CYP3A4/5 positive grade II invasive ductal carcinoma surrounding a nonneoplastic lobule.

The percentage of CYP2E1 expressing tumors was higher in advanced tumor stages (T1/2 vs. T3/4 and N0 vs. N1-3 $p = 0.071$, Table II).

No significant association was found with age and histological tumor type.

Association of hormonal receptor status, HER2, Mib and p53 with tumor characteristics

A strong association of HER2, Mib and p53 expression and negative ER, PR or AR status with poor tumor differentiation was observed. None of these markers correlated with tumor stage and nodal status. Data are shown in Table IV.

Coexpression of GST and CYP and association of GST/CYP expression with hormonal receptor status

Significant coexpression was found for CYP1B1 and 3A4/5 as well as GST pi and mu ($p < 0.001$ each). There was also a significant association between expression of GST mu and a positive ER and PR status ($p < 0.001$). CYP1B1 expression was associated with a negative ER status ($p = 0.031$). No association of GST and CYP expression was found with expression of the androgen receptor status, HER2 and Mib. Data are shown in Table V.

Discussion

We investigated protein expression patterns of GST and CYP isoenzymes in a large collection of incident breast cancer cases from the German GENICA collection. The aim of our study was to demonstrate to which extent breast carcinomas express xenobiotic enzymes and whether enzyme expression was associated with tumor characteristics and progression. Although immunohistochemical expression is not equivalent to the functional status of the enzymes, our results provide important insights into differential expression patterns and possible metabolic activities of the tumor cells at the time of tumor diagnosis.

In our analysis, we first established phase I and II enzyme expression patterns with respect to positive and negative protein expression using a score level of 1–12, with 3–12 referring to positive expression. In a second step, we defined strong expression by

TABLE II – PROPORTION OF CASES SHOWING POSITIVE GST AND CYP EXPRESSION (SCORE 3–12) ACCORDING TO AGE AND TUMOR CHARACTERISTICS

	No of tumors (% of total)	GST pi	GST mu	GST a	CYP1B1	CYP3A4/5	CYP1A1/2	CYP1A2	CYP2E1
Total	393	83	197	7	65	97	81	51	175
%		21.1	50.1	1.8	16.5	24.7	20.6	13.0	44.5
Age (years)									
<50	81 (20.6)	13.6	40.8	1.2	14.8	19.8	21.0	8.6	34.6
50–< 60	112 (28.1)	25.9	54.5	1.8	17.9	25.0	18.8	16.1	45.5
60–< 70	126 (32.1)	19.8	50.0	2.4	16.7	19.8	19.1	11.9	50.0
70+	74 (18.8)	24.3	54.0	1.3	16.2	37.8	25.7	14.9	44.6
Histology									
Ductal	274 (69.7)	19.3	49.6	2.2	17.9	26.6	22.6	15.7	46.3
Lobular	73 (18.6)	26.0	54.8	1.4	15.1	26.0	13.7	8.22	43.8
Others[1]	46 (11.7)	23.9	45.7	0	10.1	10.9	19.6	4.35	34.8
Grading			*0.049*			*0.06*			
Well	31 (7.9)	22.6	51.6	0	25.8	19.4	9.7	6.5	32.3
Moderate	207 (52.7)	21.3	55.6	2.4	12.6	21.7	23.2	15.9	45.4
Poor	155 (39.4)	20.1	42.6	1.3	20.0	29.7	19.4	10.3	45.8
Tumor stage									*0.071*
In situ	10 (2.5)	30.0	40.0	0	20.0	10.0	40.0	10.0	60.0
T1	226 (57.5)	23.5	50.9	2.7	14.6	22.1	18.6	14.6	42.5
T2	121 (30.8)	17.4	47.9	0.8	19.8	28.1	22.3	9.9	43.0
T3/4	36 (9.1)	15.8	52.6	0	15.8	31.6	21.1	13.2	55.3
Nodal status[2]		*0.02*				*0.018*			*0.071*
N0	251 (63.9)	24.7	50.6	2.4	15.5	21.1	19.5	11.6	40.6
N1-3	127 (32.3)	14.1	48.8	5.5	18.9	32.3	22.8	15.0	50.4

For enzyme expression, the percentage of positive cases is related to the variable in column one. *p*-values given in italics.
[1]Including tubular, medullary, papillary, mucinous carcinomas.–[2]Nodal status not specified in 15 cases (including not surgically treated T4 carcinomas).

TABLE III – PROPORTION OF CASES SHOWING STRONG EXPRESSION (SCORE 6–12)[1] OF SELECTED GSTS AND CYPS AND ASSOCIATION WITH TUMOR CHARACTERISTICS

	No of tumors	GST pi >6	GST mu > 6	CYP3A4/5 >6	CYP1B1 >6
Total	393	48	120	47	32
%		12.2	30.5	11.9	8.1
Grading			*0.018*		*0.049*
Well	31	16.1	41.9	3.2	3.2
Moderate	207	10.6	33.3	11.1	5.8
Poor	155	13.5	21.9	14.8	12.2
Tumor stage		*0.049*		*<0.001*	
In situ	10	30.0	30.0	0	0
T1/2	347	12.7	30.8	10.1	8.1
T3/4	36	2.8	27.8	33.3	11.1
Nodal status		*0.045*		*0.017*	
N0	251	15.1	31.1	9.6	7.2
N1-3	127	7.1	28.3	18.1	11.0
ER			*<0.001*		*0.006*
Positive	290	11.0	35.9	11.4	5.9
Negative	103	15.5	15.5	13.6	15.5
PR			*0.009*		
Positive	265	10.6	34.7	10.9	6.8
Negative	128	15.6	21.9	14.1	10.9

For enzyme expression, the percentage of positive cases is related to the variable in column one. *p*-values given in italics.
[1]Only GSTs and CYPs with significant associations are shown.

score levels of 6–12. The latter enabled us to describe a number of significant associations between protein expression levels and histopathological tumor characteristics.

Expression of GST pi and mu was detected in 20% and 50% of tumors, comparable with former immunohistochemical studies.[7,29–31] Both enzymes exhibited significant coexpression in the tumors, but they showed different expression levels in relation to nonneoplastic breast tissue. GST pi expression was consistently weaker in invasive carcinomas than in nonneoplastic mammary glands. Obviously, the ability to express GST pi was reduced or lost in the majority of breast cancers.[7] In contrast, GST mu expression was frequently found to be stronger in tumors than in nonneoplastic breast tissue, indicating the capacity of the tumor to enhance GST mu expression.

The third isoenzyme GST alpha was detected in only 3% of the tumors and nonneoplastic tissue and thus far less common than otherwise reported.[4,30,31] Therefore, in our study, this enzyme seems to be of minor importance for breast cancer progression.

When we focused on tumors with high expression levels of GST pi and mu (scores 6–12), we observed an association with more favorable prognostic tumor features. In particular, tumors expressing high GST mu levels showed a tendency for better tumor differentiation and tumors expressing high GST pi levels revealed lower rates of lymph node metastasis. It remains unclear whether GST expression in the tumors per se may have affected tumor progression or if this observation is merely a reflection of the better differentiation state of the tumor cells. Information on whether these features of maintenance of detoxification capacities will be of further prognostic or chemotherapy predictive value will become available upon long-term clinical follow-up in the future.

Unexpectedly, we found a strong association of GST mu expression with a positive ER and PR status. This finding contradicts recent reports that explained estrogen-induced cancer development by downregulation of GST expression through estrogenic compounds.[32] Obviously, this hypothesis does not apply to the increased GST mu expression in ER/PR positive breast carcinomas of the present study.

Positive expression of 4 CYP enzymes involved in endogenous estrogen metabolism (CYPs 1A1, 3A4, 1A2 and 1B1) was also analyzed in our study. CYP3A4/5 and CYP1B1 were detected in about 25 to 45% of breast carcinomas. With respect to CYP3A4/5, positive and strong protein expression was significantly associated with a positive nodal status, one of the strongest known prognostic factors in breast cancer.

Since CYP3A4 is involved in the formation of the potentially genotoxic estrogen metabolite 16alpha-hydroxyestrone capable to activate ER alpha,[33,34] it may be plausible that CYP3A4 associated ER alpha signaling may contribute not only to tumor initiation, but also to tumor progression. Given the circumstance that our CYP3A4 detection was not specific but may have included cross reactivity with CYP3A5, we are not able at this point to attribute this association to CYP3A4 alone. There is a considerable discordant debate on the relevance of CYP3A5 in the metabolism of CYP3A substrates.[35,36] The known overlap of CYP3A isoforms

TABLE IV – POSITIVE HORMONAL RECEPTOR STATUS AND EXPRESSION OF HER2, MIB AND P53 IN RELATION TO TUMOR CHARACTERISTICS

	ER	PR	AR	HER2	Mib	p53
No of positive cases (% of total)	290 (73.8)	265 (67.4)	144 (36.6)	88 (22.4)	98 (24.9)	73 (18.6)
Grading	*<0.001*	*<0.001*	*0.044*	*<0.001*	*0.003*	*<0.001*
Well	90.3	77.4	51.6	6.5	12.9	12.9
Moderate	83.1	74.4	39.1	16.9	20.3	11.1
Poor	58.1	56.1	30.3	32.9	33.6	29.7
Tumor stage						
In situ	40.0	20.0	10.0	60.0	20.0	50.0
T1/2	74.9	68.6	38.3	21.6	26.5	17.0
T3/4	72.2	69.4	27.8	19.4	11.1	25.0
Nodal status						
N0	76.9	66.9	38.3	21.1	23.5	17.5
N1-3	67.7	66.9	33.1	25.2	27.6	22.5

For protein expression, the percentage of positive cases is related to the variable in column one. *p*-values given in italics.

TABLE V – EXPRESSION OF HORMONAL RECEPTORS, HER2, MIB AND P53 AND RELATION TO GST- AND CYP EXPRESSION (SCORE 3–12)

	GST pi positive	GST mu positive	CYP1B1 positive	CYP3A4/5 positive	CYP2E1 positive
ER		*<0.001*	*0.031*		
Positive	20.7	56.9	14.1	23.8	45.9
Negative	22.3	31.1	23.3	27.2	40.8
PR		*<0.001*			
Positive	19.6	56.2	16.6	24.2	44.5
Negative	24.2	37.5	16.4	25.8	44.5
AR					
Positive	25.0	54.1	16.0	20.1	47.9
Negative	18.0	47.8	16.9	27.3	42.6
HER2					
Positive	21.6	46.6	21.6	29.6	41.0
Negative	21.0	51.2	15.1	23.3	45.6
Mib					
Positive	27.6	49.0	22.5	29.6	53.1
Negative	19.0	50.5	14.6	23.1	41.7
p53		*0.048*			
Positive	23.3	39.7	12.3	16.4	53.4
Negative	20.6	52.5	17.5	26.6	42.5
GST pi	–	*<0.001*	–	–	–
Positive		69.9			
Negative		44.8			
CYP1B1	–	–	–	*<0.001*	–
Positive				46.1	
Negative				10.7	

For enzyme expression, the percentage of positive cases is related to the variable in column one. *p*-values given in italics.

with respect to substrate specifity,[20,35] however, suggests that both CYP3A4 and CYP3A5 may play a role in breast tumor progression. Within this context, it is of note that within our series CYP3A4/5 expression was not associated with a positive ER/PR status.

High CYP1B1 expression showed a significant association with poor tumor grading which was also not associated with ER/PR positivity. Moreover, among CYP1B1 expressing tumors, there were significantly fewer ER positive cases. This finding corresponds to a previous observation in ER negative breast cancer cell lines showing greater basal CYP1B1 expression.[37] However, CYP1B1 mRNA expression was found to be induced by estrogen in ER positive breast cancer cell lines.[14,17] It follows, that in the primary tumor, increased CYP1B1 levels may contribute to tumor progression; obviously, a direct comparison and interpretation with data obtained from *in vitro* studies is not possible.

Special interest was paid to CYP1A1 for its central role in conversion of estradiol to hydroxylated estrogen and its possible induction by cigarette smoke and environmental pollutants.[38] Expression of CYP1A1/2, as assessed by immunohistochemistry, was detected in a minority of breast cancers only, according to former studies reporting low CYP1A1 mRNA expression levels in neoplastic and nonneoplastic breast tissue.[22,39] Even in this small group of cancers, expression was not associated with any clinical or histochemical features, including ER status. This also applied to CYP1A2.

CYP2E1 showed the highest expression level of all CYPs examined in our study. This finding supports former studies detecting CYP2E1 in the majority of breast cancers and in nonneoplastic tissue samples.[22,39,40] Only one study showed contradictory results.[23] CYP2E1 is involved in metabolic activation of N-nitrosamines and generation of reactive oxygen species in the human liver.[41,42] There was a weak tendency for CYP2E1 expressing tumors toward advanced tumor-stages and lymph node metastasis.

In conclusion, we found GST and CYP enzymes expressed in a substantial number of breast carcinomas, thus implicating a certain biological importance. Particularly, CYP1B1 and 3A4/5 expression may represent key features of unfavorable prognosis. On the other hand, GST mu and pi expression may be indicators of a better tumor outcome. Although our study cannot provide clues on the biological mechanisms associated with these tumor characteristics, the newly identified protein expression features may help to define breast cancer prognosis in addition to ER status. Whether these CYP and GST expression patterns may have influenced a tumor's sensitivity or resistance to systemic treatment will await the joint analysis with long-term clinical follow-up data in the future.

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