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ORIGINAL PAPER

Prognostic relevance of glucosylceramide synthase (GCS) expression in breast cancer

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Abstract
Purpose Multidrug resistance (MDR) has been linked to sphingolipid metabolism and preclinical data ascribe glucosylceramide synthase (GCS) a major role for MDR especially in breast cancer cells but no profound data are available on the expression of this potential therapeutic target in clinical breast cancer specimens.
Methods We analyzed microarray data of GCS expression in a large cohort of 1,681 breast tumors.
Results Expression of GCS was associated with a positive estrogen receptor (ER) status, lower histological grading, low Ki67 levels and ErbB2 negativity ($P < 0.001$ for all). In univariate analysis there was a benefit for disease free survival for patients with tumors displaying low levels of GCS expression but this significance was lost in multivariate Cox regression.
Conclusions Our results suggest ER positive tumors may be the most promising candidates for a potential therapeutic application of GCS inhibitors.

Keywords Multidrug resistance · Breast cancer · Ceramide · Sphingolipids · Microarray

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Introduction

Breast cancer is the most frequent cancer entity worldwide. Beside surgical methods chemotherapy and endocrine therapy are fundamentals of breast cancer treatment. In particular response to chemotherapy in advanced disease is limited by the multidrug resistance (MDR) phenomenon. Several mechanisms have been suggested to be responsible for this resistance with increased expression of drug resistance genes like MDR 1 and bcl 2 to be the most important (Makin and Dive 2001; Shabbits et al. 2001). Knowledge about chemotherapy induced drug resistance is increasing. Recent research focused on the role of glucosylceramide in MDR (Lucci et al. 1998; Gouaze-Andersson and Cabot 2006; Liu et al. 2008). Glucosylceramide belongs to the sphingolipids, a family of membrane lipids that play key roles in apoptosis, senescence, proliferation, MDR, and neo angiogenesis (Ogretmen and Hannun 2004; Hannun and Obeid 2008, see Fig. 1). Accumulation of glucosylceramide is a characteristic finding in multi drug resistant ovarian, breast, colon, and epitheloid cells in vitro (Lucci et al. 1998; Kok et al. 2000; Lavie et al. 1996; Nicholson et al. 1999). Glucosylceramide synthase (GCS) transfers a glucose residue from UDP-glucose to ceramide to synthesize glucosylceramide (Liu et al. 2008; Jeckel et al. 1992). In several in vitro studies (Liu et al. 2008; Ogretmen and Hannun 2004; Reynolds et al. 2004; Gouaze et al. 2004; Liu et al. 1999a, b) glycosylation of ceramide was combined with resistance to drugs like adriamycin and paclitaxel. Transfection with GCS genes conferred resistance to daunorubicin, doxorubicin and TNF-α to breast cancer cells (Liu et al. 1999a, b; Ogretmen et al. 2001). Inhibition of GCS resensitized tumor cells to chemotherapy (Radin et al. 1993; Norris-Cervetto et al. 2004; Liu et al. 2004). In addition it has been proposed that drugs that reverse drug

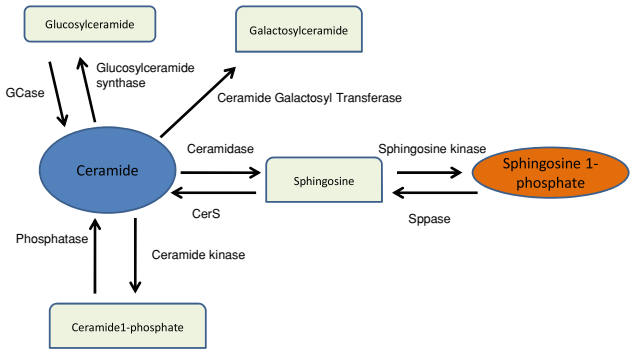


Fig. 1 Glucosylceramide synthase and sphingolipid metabolism

resistance like verapamil, tamoxifen, and cyclosporin A might act via inhibition of ceramide glycosylation (Lavie et al. 1997).

Only very little data is available about the expression of GCS in clinical breast cancer samples and it is not known, whether differences exist between levels of GCS expression in clinical subgroups of breast cancer, for example, according to the estrogen receptor (ER) or ErbB2 status. Since we could previously demonstrate a prognostic impact of sphingolipid metabolism in breast cancer (Ruckhäberle et al. 2007) we set out to analyze possible prognostic effects of GCS. Here we present results of microarray analysis of 1,681 breast cancer samples according to GCS expression.

Materials and methods

A database of 1,681 Affymetrix microarray experiments from primary breast cancer patients was created. 220 samples from our own institutions were included (datasets Frankfurt and Hamburg) which have been described previously (Rody et al. 2007; Ruckhäberle et al. 2007; Rody et al. 2006; ASCO, Ahr et al. 2002) as well as 1,461 samples from nine different publicly available datasets (Table 1): Uppsala (Miller et al. 2005), Stockholm (Pawitan et al. 2005), Rotterdam (Wang et al. 2005; Minn et al. 2007), Oxford-Untreated (Sotiriou et al. 2006), Oxford-Tamoxifen and London (Loi et al. 2007), NewYork (Minn et al. 2005), Villejuif (Desmedt et al. 2007), and expO (http://www.intgen.org). For comparability only data from Affymetrix HG-U133A microarrays were used. The clinical characteristics of the patients in the different datasets are given in Table 1. For 1,363 of the 1,681 patients follow up information was available (no follow up data has been reported for dataset expO). The median follow-up time was 76 months. 1,200 of the 1,681 samples (71.4%) were ER positive. Treatment information could be obtained for 878 ER positive and 262 ER negative patients. Since methods of Affymetrix microarray normalization can have significant effects on the levels for individual probe sets, several uniform normalization methods (Li and wong 2001; Irizarry et al. 2003) of CEL file data has been developed to allow the analysis of sets of multiple arrays. However, important discrepancies between different datasets depend on the dynamics of the measurements originating from different hybridization efficiencies and even uniform normalization methods are incapable in compensating those experimental differences. In addition, no CEL files are available for some studies (e.g., the Rotterdam dataset). Therefore, we used a conservative strategy for dataset stratification which relies on a ranking of samples in each cohort. Each dataset of microarrays was normalized separately using the originally proposed method in the respective study (see Table 1). Log transformed expression values were median centered over each array. For genes the normalization, ranking of expression values and median splits were done separately in each dataset.

Assessment of ER, ErbB2, proliferative status, and GCS expression of the samples

Since standard pathology for ER and ErbB2 was not available for all samples and to allow comparison of different datasets, receptor status was determined based on Affymetrix expression data as previously described (Foekens et al. 2006; Gong et al. 2007; Bonnefoi et al. 2007; Alexe et al. 2007). ER status was based on Affymetrix ProbeSet 205225_at, the ErbB2 status on ProbeSet 216836_s_at. A specificity of 86.1% and a sensitivity of 92.2% was observed when the chip based ER status was compared to immunohistochemical obtained ER status (available for 1,333 samples), while the specificity and sensitivity of chip based ErbB2 status was 98.6 and 45.8%, respectively, compared to 3+ staining in immunohistochemistry with HER2 antibody (data available for 206 samples). As a surrogate marker for cellular proliferation we used the expression of the proliferation marker Ki67 (ProbeSets 212020-212023_s_at). Appropriate cut off values that distinguish between high and low proliferative activity in a clinically relevant manner using Ki67 immunohistochemistry in breast cancer have not been universally established (de Azambuja et al. 2007). Thus, a conservative median split according to Ki67 gene expression was applied which corresponds to a percentage of MIB-1 positive cells of 16–17% (Spyratos et al. 2002). To allow comparison of GCS expression between different datasets we used a median split of each dataset according to GCS (UGCG) using the ProbeSet 204881_s_at from the Affymetrix HG-U133A array. Samples were characterized as high or low expressing based on a median split of the cohorts according to this ProbeSet.

Table 1 Clinical characteristics of breast cancer patients from Affymetrix microarray datasets used in this study

Dataset	Data source	Array	Norm. method	No. of samples	% of samples					System treatment	Median follow up months	No. of relapses	References
					Age ≤50	Tumor size ≤2 cm	LNN	ER pos.	G3				
Frankfurt	This study	U133A	MAS5	120	54	50	57	66	47	Chemotherapy	39	29	Rody et al. (2007), Ruckhäberle et al. (2007)
Hamburg	This study	U133A	MAS5	100	46	24	59	65	59	Chemotherapy	57	31	Rody et al. (2006)
Rotterdam	GSE2034, GSE5327	U133A	MAS5	344	NA	NA	NA	61	NA	286 untreated, 58 NA	86	118	Wang et al. (2005), Minn et al. (2007)
Uppsala	GSE3494	U133A	MAS5	251	22	51	65	80	22	Yes/no	118	91	Miller et al. (2005)
Stockholm	GSE1456	U133A	MAS5	159	NA	Na	NA	82	42	Yes/no	85	40	Pawitan et al. (2005)
Oxford-Untreated	GSE2990	U133A	RMA	61	44	64	100	69	41	Untreated	121	29	Sotiriou et al. (2006)
Oxford-Tamoxifen	GSE6532	U133A	RMA	109	14	34	64	95	19	Endocrine	61	30	Loi et al. (2007)
London	GSE6532	U133+	RMA	87	6	35	33	98	23	Endocrine	137	28	Loi et al. (2007)
New York	GSE2603	U133A	MAS5	99	37	9	34	58	NA	NA	65	27	Minn et al. (2005)
Villejuif	GSE7390	U133A	RMA	50	80	26	100	72	38	Untreated	108	22	Desmedt et al. (2007)
expO	GSE2109	U133A	MAS5	301	31	32	47	65	49	NA	NA	NA	http://www.intgen.org
Total				1,681	33	38	69	71	37		76	445	

Statistical analysis

All reported P values are two sided and P values of less than 0.05 were considered to indicate a significant result. Subjects with missing values were excluded from the analyses. Chi-square test was used for categorical parameters. Survival intervals were measured from the time of surgery to the time of death from disease or of the first clinical or radiographic evidence of disease recurrence. Data for women in whom the envisaged end point was not reached were censored as of the last follow-up date or at 120 months. We constructed Kaplan-Meier curves and used the log rank test to determine the univariate significance of the variables. A Cox proportional-hazards regression model was used to examine simultaneously the effects of multiple covariates on survival. The effect of each variable was assessed with the use of the Wald test and described by the hazard ratio, with a 95% confidence interval. The model included age, tumor size, lymph node status, ER, ErbB2, Ki67 as well as GCS expression. All analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL).

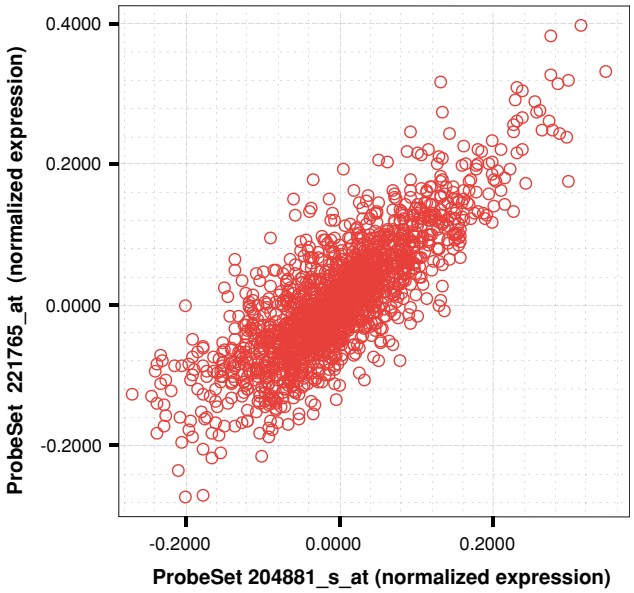


Fig. 2 Validation of consistency of GCS expression data from the Affymetrix microarray. A scatter plot of the normalized expression value from two different ProbeSets for glucosylceramide synthase (GCS) present on the Affymetrix HG U133A microarray (ProbeSet 221765_at and 204881_s_at) is shown for all $n = 1{,}681$ samples

Results

A database of Affymetrix microarray hybridizations from 1,681 primary breast cancer samples was established which was derived from eleven microarray datasets as given in Table 1. All experiments enclosed in this database were performed using the Affymetrix HG-U133A microarray. The GCS mRNA is represented by two different ProbeSets on this array. Consistency of normalized expression values obtained from these two ProbeSets was verified as shown in Fig. 2. Subsequently, all samples were stratified according to GCS expression based on a median split in each dataset. The clinical parameters of tumors with high and low expression of GCS, respectively, are presented in Table 2. There were no significant differences according to age and lymph node status of the patients when comparing the two groups with high and low GCS expression. In contrast, the analysis revealed a higher GCS expression in smaller tumors ($P < 0.001$ for tumors smaller than 2 cm vs. larger tumors). Tumors with lobular histology were preferentially found in the group with low GCS expression (72%) but the overall difference in histological subtypes between tumors with low and high GCS expression was not statistically significant. Interestingly, high expression of GCS was associated with a lower histological grade ($P < 0.001$) as well as low Ki67 expression ($P < 0.001$). On the other hand, low GCS expression was clearly associated with a negative ER status with 79.4% of ER the negative tumors stratified to the group with low GCS expression ($P < 0.001$). Breast cancer samples with ErbB2 overexpression were associated with lower GCS levels (66.7 vs. 33.3%, $P < 0.001$). The strong correlation of ER status with higher GCS expression is demonstrated in Fig. 3 ($P < 0.001$ for the combined data in Fig. 3a as well as for all individual datasets in Fig. 3b; Mann Whitney test).

The method of Kaplan-Meier was used to analyze the prognostic value of high GCS expression. For 1,363 of the 1,681 patients follow up data were available. As shown in Fig. 4 Kaplan-Meier estimates of disease free survival (DFS) revealed a significant better prognosis for breast cancer patients with high GCS expression ($75.4 \pm 1.7\%$ vs. $70.0 \pm 1.8\%$, $P = 0.005$ for 5 year DFS; and $65.9 \pm 2.1\%$ vs. $63.1 \pm 2.1\%$, $P = 0.047$ for 10 year DFS). Next we analyzed the simultaneous influence of GCS expression and standard parameters on the prognosis of the patients in a multivariate Cox regression model using $n = 699$ patients for which all parameters were available. As presented in Table 3, however, only tumor size and ErbB2 status remained significant factors for disease recurrence in this multivariate analysis but the contribution of GCS expression was no more significant. Since GCS expression of breast cancers displayed a strong correlation with the ER status of the tumor it could be hypothesized that the prognostic value of GCS expression observed in the univariate analysis above (see Fig. 4) and the loss of this effect in the multivariate analysis might result from a confounding effect of the ER status between the two groups. Thus we additionally analyzed the univariate prognostic value of GCS by Kaplan-Meier analysis separately for ER positive and ER

Table 2 Clinical characteristics of patient with high and low GCS expression

Parameter		n = 1,681	Low GCS (n = 840)	High GCS (n = 841)	P value
Age[++]	≤50 year	374	179 (47.9%)	195 (52.1%)	0.45
	>50 year	773	389 (50.3%)	384 (49.7%)	
Lymph node status**	LNN	957	469 (49.0%)	488 (51.0%)	0.4
	N1	439	219 (49.9%)	220 (50.1%)	
Tumor size*	≤2 cm	401	180 (44.9%)	221 (55.1%)	0.032
	<2 cm	665	344 (51.7%)	321 (48.3%)	
Tumor grade[+]	Low grade (G1 and G2)	663	286 (43.1%)	377 (56.9%)	<0.001
	High grade (G3)	387	239 (61.8%)	148 (38.2%)	
Ki67 expression	Below median	839	351 (41.8%)	488 (58.2%)	<0.001
	≥ median	842	489 (58.1%)	353 (41.9%)	
ER status	Positive	1,200	458 (38.2%)	742 (61.8%)	<0.001
	Negative	481	382 (79.4%)	99 (20.6%)	
ErbB2 status	Negative	1,432	674 (47.1%)	758 (52.9%)	<0.001
	Positive	249	166 (66.7%)	83 (33.3%)	
Histological subtype	Ductal	357	185 (51.8%)	172 (48.2%)	0.34
	Lobular	25	7 (28.0%)	18 (72.0%)	
	Mixed	3	0 (0%)	3 (100%)	
	Others	61	28 (45.9%)	33 (54.1%)	

* information on tumor size was not available for n = 615 patients

** information on lymph node status was not available for n = 285 patients

[+] information on tumor grade was not available for n = 631 patients

[++] information on age was not available for n = 534

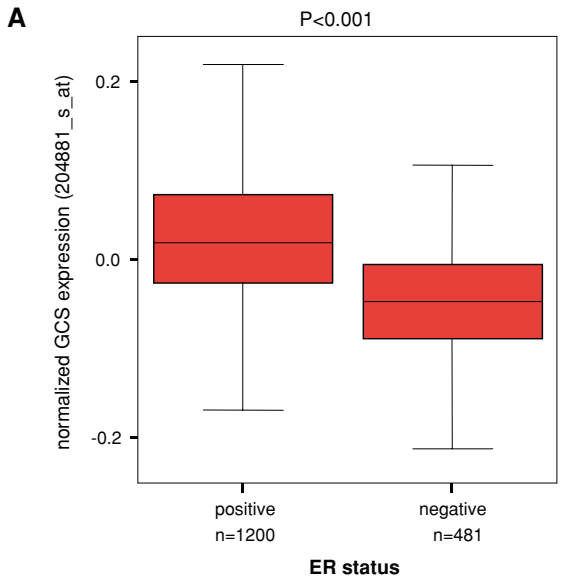
negative subgroups of cancers. As presented in Fig. 5 tumors with high GCS expression seem to have a better survival in both subgroups but this benefit is no more significant.

Recent in vitro data suggested a possible co activation of the genes for GCS and MDR1 in breast cancer cells (Gouaze-Andersson et al. 2007). Thus we analyzed if a correlation of these two parameters might also be apparent in our clinical sample collective. However, as demonstrated in Fig. 6 there was no clear relationship of MDR1 and GCS expression, suggesting that in clinical samples such co activation might only be observable after pretreatment with chemotherapeutic agents and not in our treatment naïve cohort.

Discussion

Anticancer chemotherapy is mainly limited by the MDR phenomenon. Multidrug resistance can be caused by several mechanisms like an increase in cellular gluthathione S-transferase (Morrow and Cowan 1990), a decrease in topoisomerase II alpha activity (Deffie et al. 1989), increased expression of proteins of the bcl 2 family (Reed 1995) as well as loss of the tumor suppressor protein p53 (Mueller and Eppenberger 1996). Of particular importance is the transporter P-glycoprotein (P-gp, the product of the MDR1 gene) which reduces the intracellular concentration of antitumor agents as well as certain glycosylated sphingolipids.

Several recent reports have demonstrated an association between glycosylated sphingolipids and MDR for various types of cancer (Liu et al. 1999b; Gouaze-Andersson and Cabot 2006). Accumulation of glucosylceramide and increased activity of GCS is a characteristic finding in multi drug resistant ovarian, breast, colon, and epitheloid cells in vitro (Nicholson et al. 1999; Kok et al. 2000; Lavie et al. 1996; Morjani et al. 2001). An elevated GCS activity might prevent the accumulation of ceramide, which is thought to precede, and trigger apoptosis in response to at least some cytotoxic drugs (Simstein et al. 2003; Gomez-Munoz et al. 2006). Liu and colleagues could demonstrate, that doxorubicin enhances GCS gene expression (Liu et al. 2008) mediated by ceramide as second messenger. Furthermore, MDR reversing drugs like tamoxifen, verapamil, and cyclosporin A were shown to act by inhibition of ceramide glucosylation (Lucci et al. 1998; Lavie et al. 1997). Overexpression of GCS in MCF 7 cells conferred MDR to adriamycin (Liu et al. 1999b). Several authors have demonstrated that transfection of antisense GCS DNA depresses GCS expression, reduces drug resistance in breast cancer cells (Liu et al. 2000), inhibits neuroepithelioma cell growth (Di Sano et al. 2002), and inhibits melanoma growth in mice (Deng et al.



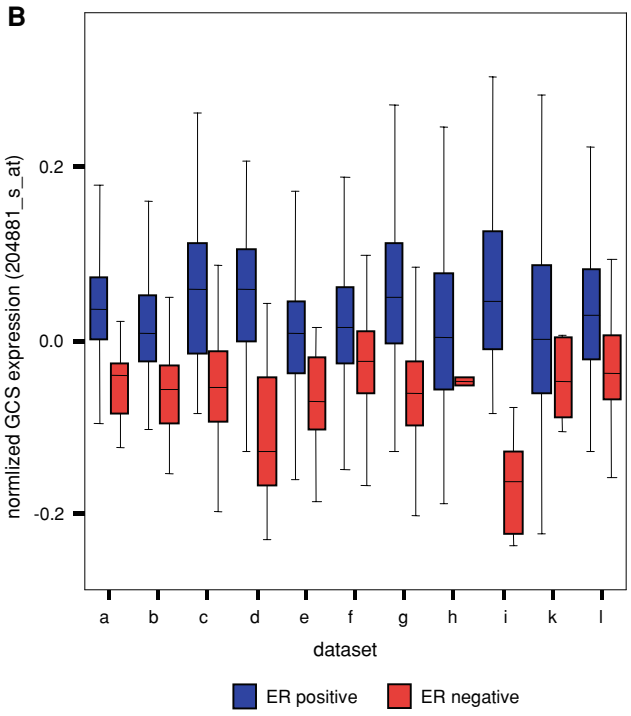


Fig. 3 Correlation of normalized GCS expression with the ER status of the tumor. **a** Box plots of normalized GCS expression values of breast cancers stratified by their ER status from a combined analysis of all $n = 1{,}681$ samples. **b** Results from individual datasets (*a* Frankfurt, *b* Uppsala, *c* Hamburg, *d* Oxford-Untreated, *e* Stockholm, *f* expO, *g* New York, *h* London, *i* Villejuif, *k* Oxford-Tam, *l* Rotterdam)

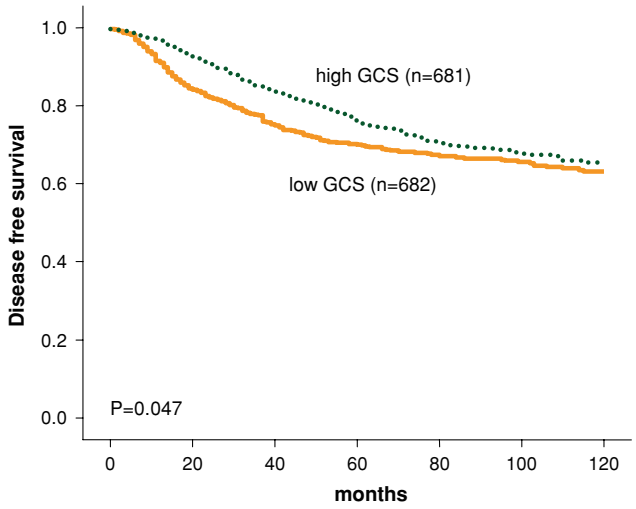


Fig. 4 Prognostic value of GCS expression among breast cancer. Kaplan-Meier analysis of disease free survival of all $n = 1{,}363$ samples with follow up information stratified according to GCS expression

2002; Weiss et al. 2003). In adriamycin resistant MCF 7 cells selective antisense oligodeoxyribonucleotides (ODNs) to GCS were able to substantially reverse the MDR phenotype (Liu et al. 2000). GCS antisense substantially restored cellular sensitivity to many anticancer drugs, including anthracyclines, taxanes, vinca alkaloids, and actinomycin D. All of these agents are substrates for the transporter protein P-gp, whereas 5-flurouracil and cisplatin, for which toxicities were not substantially modified by GCS antisense, are not classified as pump efflux drugs.

However, all of these data on GCS and chemotherapy resistance were so far obtained either in vitro using cultured cells. There are no firm data on GCS in clinical specimens especially for breast cancer. Therefore, we analyzed microarray data of a cohort of 1,681 clinical breast cancer samples and investigated GCS expression in subtypes of breast cancer and its prognostic relevance for disease recurrence. To our knowledge, this is the first report of clinical data from a large cohort on the expression of GCS in breast cancer and its impact on the course of disease. Expression of GCS was higher in the ER positive subgroup while ErbB2 positive tumors displayed lower GCS expression. In addition significant higher expression was revealed in smaller tumors ($P < 0.001$), breast cancers with low grading ($P < 0.001$) as well as low Ki67 levels ($P < 0.001$). The DFS of patients with tumors displaying low levels of GCS expression was significantly higher in univariate analysis. In multivariate Cox regression analysis, however, this benefit for survival was no more significant. This is in line with separate analysis of the univariate prognostic values of GCS in ER positive and ER negative subgroups, which were also not significant.

There seems to be a link between glycosphingolipids and multi drug resistance caused by P-gp. It is well known that doxorubicin, vinblastine, etoposide, cytarabine, methotrexate, and paclitaxel can induce MDR through direct activation of the MDR1 gene, which codes for the P-gp protein (Chaudhary and Roninson 1993; Kohno et al. 1989). More recently Anderson-Gouaze et al. could demonstrate that elevated levels of ceramide and GCS enhance expression of

Table 3 Multivariate Cox regression analysis of standard parameters and GCS expression in relation to disease free survival among breast cancers patients

Parameter		*n*	*P* value	Hazard ratio	(95% CI)
GCS expression	Low vs. high	354 vs. 345	0. 90	0.98	(0.73–1.32)
Age	>50 vs. ≤50	456 vs. 243	0. 58	0.92	(0.69–1.23)
Lymph node status	LNN vs. N1	447 vs. 252	0.17	0.82	(0.63–1.09)
Tumor size	≤2 cm vs. >2 cm	296 vs. 403	**<0.001**	0.50	(0.36–0.68)
Histological grading	Poor vs. well/intermediate	230 vs. 469	0.43	1.14	(0.83f–1.58)
ER status	Positive vs. negative	541 vs. 158	0.62	0.91	(0.62–1.33)
ErbB2 status	High vs. low	104 vs. 595	**0.044**	1.44	(1.01–2.05)
Ki67 expression	Low vs. high	346 vs. 353	**0.001**	0.59	(0.43–0.80)

Data were available from $n = 699$ patients, significant P values are given in bold

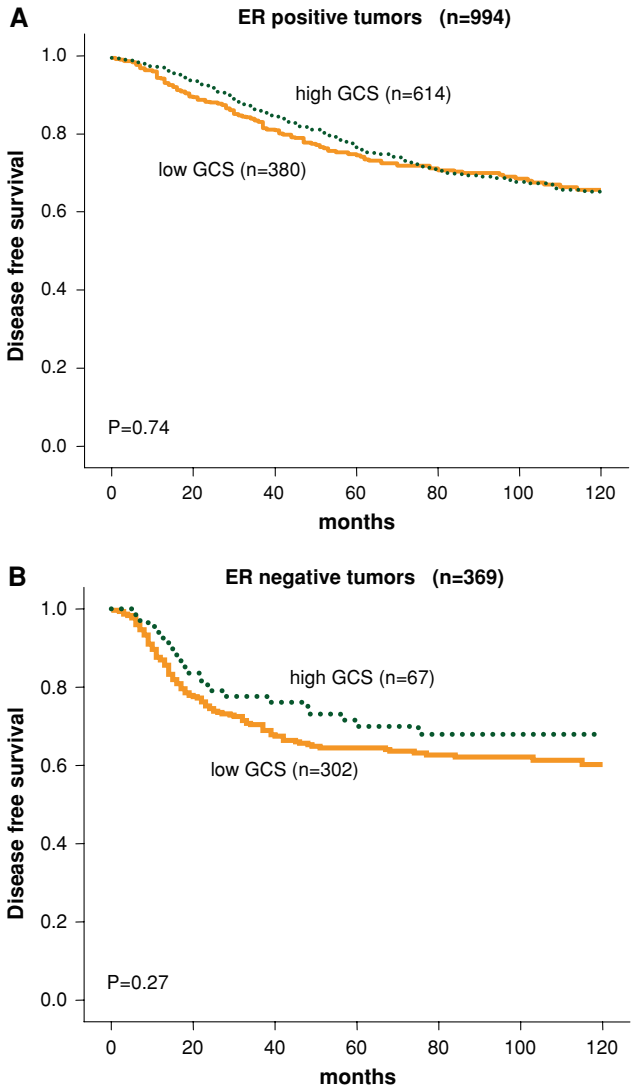


Fig. 5 Prognostic value of GCS expression among ER positive and negative breast cancers. Kaplan-Meier analysis of disease free survival of breast cancers stratified according to GCS expression in the subgroups of **a** ER positive and **b** ER negative tumors

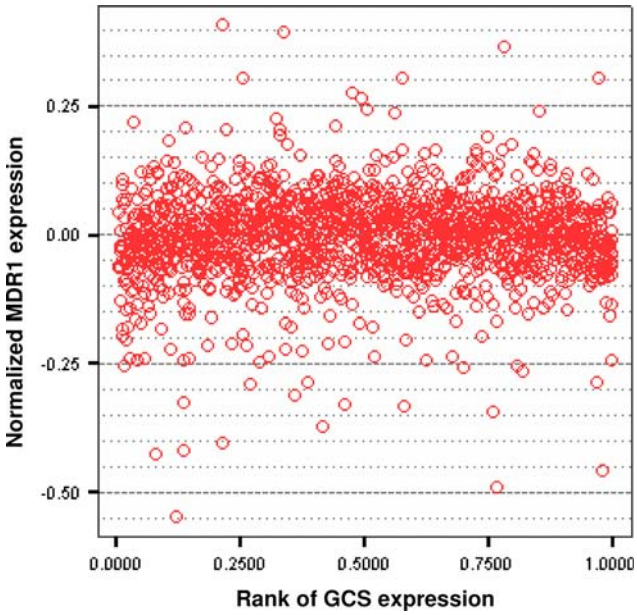


Fig. 6 Relationship of MDR1 and GCS gene expression among breast cancers. Scatter plot of MDR1 and GCS gene expression values from Affymetrix microarrays

the MDR1 gene (Gouaze-Andersson et al. 2007). On the other hand P-gp was shown to modulate ceramide mediated sensitivity to paclitaxel and vincristin (Shabbits and Mayer 2002). Even if those data were obtained in vitro a correlation of GCS and MDR1 expression might also be observable in clinical samples. In our treatment naïve collective we found no evidence for such correlation, but pretreatment with chemotherapy might well induce a co activation of both genes in patients. Thus further analysis of tumors from neoadjuvant treated patients could give important insights in this regard.

Inhibition of GCS is possible by specific inhibitors like PDMP and Miglustat or selective antisense oligideoxyribonucleotides (Weiss et al. 2003). Inhibition of GCS activity

is evaluated as a possible medical treatment for several lipid-storage diseases and proven in vitro for some types of cancer (Butters 2007; Tifft and Proia 2000; Lachmann 2003; Jeyakumar et al. 2002). Glucosyl ceramide synthase inhibitors have already been approved for treatment of type I Gaucher disease and used in trials for Fabry disease, the GM2 gangliosidoses and Niemann-Pick disease (Patterson et al. 2007). They have been proven in principle in Tay-Sachs and Sandhoff mice models (Jeyakumar et al. 2002) and investigated in a clinical setting recently (Bembi et al. 2006). These clinical as well as the in vitro data might also propose a possible application of these drugs in breast cancer. In this context it will be crucial to define those subgroups of patients which might benefit from such treatment. According to our data, patients with ER positive and Her-2-neu negative tumors would be the most adequate starting point for trials of GCS inhibiting drugs since those tumors display high GCS expression.

In conclusion our results suggest that there seems to be no prognostic value of GCS expression in a treatment naïve cohort but ER positive tumors may be the most promising candidates for a potential therapeutic application of GCS inhibitors.

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Conflicts of interest statement There are no conflicts of interest to declare.

Role of the funding source The funding sources were not involved in the study design, in the collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

Ethical approval Ethical approval was not required.

References

Ahr A, Karn T, Solbach C, Seiter T, Strebhardt K, Holtrich U et al (2002) Identification of high risk breast-cancer patients by gene expression profiling. Lancet 359(9301):131–132. doi:10.1016/S0140-6736(02)07337-3

Alexe G, Dalgin GS, Scanfeld D, Tamayo P, Mesirov JP, DeLisi C et al (2007) High expression of lymphocyte-associated genes in node-negative HER2+ breast cancers correlates with lower recurrence rates. Cancer Res 67(22):10669–10676. doi:10.1158/0008-5472.CAN-07-0539

Bembi B, Marchetti F, Guerci VI, Ciana G, Addobbati R, Grasso D et al (2006) Substrate reduction therapy in the infantile form of Tay-Sachs disease. Neurology 66(2):278–280. doi:10.1212/01.wnl.0000194225.78917.de

Bonnefoi H, Potti A, Delorenzi M, Mauriac L, Campone M, Tubiana-Hulin M et al (2007) Validation of gene signatures that predict the response of breast cancer to neoadjuvant chemotherapy: a substudy of the EORTC 10994/BIG 00–01 clinical trial. Lancet Oncol 8(12):1071–1078. doi:10.1016/S1470-2045(07)70345-5

Butters TD (2007) Pharmacotherapeutic strategies using small molecules for the treatment of glycolipid lysosomal storage disorders. Expert Opin Pharmacother 8(4):427–435. doi:10.1517/14656566.8.4.427

Chaudhary PM, Roninson IB (1993) Induction of multidrug resistance in human cells by transient exposure to different chemotherapeutic drugs. J Natl Cancer Inst 85:632–639. doi:10.1093/jnci/85.8.632

de Azambuja E, Cardoso F, de Castro G Jr, Colozza M, Mano MS, Durbecq V et al (2007) Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients. Br J Cancer 96(10):1504–1513. doi:10.1038/sj.bjc.6603756

Deffie AM, Batra JK, Goldenberg GJ (1989) Direct correlation between DNA topoisomerase II activity and ytotoxicity in Adriamycin-sensitive and -resistant P388 leukemia cell lines. Cancer Res 49:58–62

Deng W, Li R, Guerrera M, Liu Y, Ladisch S (2002) Transfection of glucosylceramide synthase antisense inhibits mouse melanoma formation. Glycobiology 12:145–152. doi:10.1093/glycob/12.3.145

Desmedt C, Piette F, Loi S, Wang Y, Lallemand F, Haibe-Kains B, et al. (2007) TRANSBIG Consortium. Strong time dependence of the 76-gene prognostic signature for node-negative breast cancer patients in the TRANSBIG multicenter independent validation series. Clin Cancer Res 13(11):3207–3214. doi:10.1158/1078-0432.CCR-06-2765

Di Sano F, Di Bartolomeo S, Fiorentini C, Matarrese P, Spinedi A, Piacentini M (2002) Antisense to glucosylceramide synthase in human neuroepithelioma affects cell growth but not apoptosis. Cell Death Differ 9:693–695. doi:10.1038/sj.cdd.4401040

Foekens JA, Atkins D, Zhang Y, Sweep FC, Harbeck N, Paradiso A et al (2006) Multicenter validation of a gene expression-based prognostic signature in lymph node-negative primary breast cancer. J Clin Oncol 24(11):1665–1671. doi:10.1200/JCO.2005.03.9115

Gomez-Munoz A (2006) Ceramide 1-phosphate/ceramide, a switch between life and death. Biochim Biophys Acta 1758:2049–2056

Gong Y, Yan K, Lin F, Anderson K, Sotiriou C, Andre F et al (2007) Determination of oestrogen-receptor status and ERBB2 status of breast carcinoma: a gene-expression profiling study. Lancet Oncol 8(3):203–211. doi:10.1016/S1470-2045(07)70042-6

Gouaze V, Yu JY, Bleicher RJ, Han TY, Liu YY, Wang H et al (2004) Overexpression of glucosylceramide synthase and P-glycoprotein in cancer cells selected for resistance to natural product chemotherapy. Mol Cancer Ther 3:633–639

Gouaze-Andersson V, Cabot MC (2006) Glycosphingolipids and drug resistance. Biochim Biophys Acta 1758:2096–2103

Gouaze-Andersson V, Yu JY, Kreitenberg AJ, Bielawska A, Giuliano AE, Cabot MC (2007) Ceramide and glucosylceramide upregulate expression of the multidrug resistance gene MDR1 in cancer cells. Biochim Biophys Acta 1771:1407–1417

Hannun YA, Obeid LM (2008) Principles of bioactive lipid signalling: lessons from sphingolipids. Nat Rev Mol Cell Biol 9:139–150. doi:10.1038/nrm2329

Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP (2003) Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res 31(4):e15. doi:10.1093/nar/gng015

Jeckel D, Karrenbauer A, Burger KN, van Meer G, Wieland F (1992) Glucosylceramide is synthesized at the cytosolic surface of various Golgi subfractions. J Cell Biol 117:259–267. doi:10.1083/jcb.117.2.259

Jeyakumar M, Butters TD, Dwek RA, Platt FM (2002) Glycosphingolipid lysosomal storage diseases: therapy and pathogenesis.

Neuropathol Appl Neurobiol 28(5):343–357. doi:10.1046/j.1365-2990.2002.00422.x

Kohno K, Sato S, Takano H, Matsuo K, Kuwano M (1989) The direct activation of human multidrug resistance gene (MDR1) by anticancer agents. Biochem Biophys Res Commun 165:1415–1421. doi:10.1016/0006-291X(89)92761-7

Kok JW, Veldman RJ, Klappe K, Koning H, Filipeanu CM, Muller M (2000) Differential expression of sphingolipids in MRP1 overexpressing HT29 cells. Int J Cancer 87:172–178. doi:10.1002/1097-0215(20000715)87:2<172::AID-IJC3>3.0.CO;2-K

Lachmann RH (2003) Miglustat Oxford glycoSciences/actelion. Curr Opin Investig Drugs 4:472–479

Lavie Y, Cao H, Bursten SL, Giuliano AE, Cabot MC (1996) Accumulation of glucosylceramides in multidrug-resistant cancer cells. J Biol Chem 271:19530–19536. doi:10.1074/jbc.271.32.19530

Lavie Y, Cao H, Volner A, Lucci A, Han TY, Geffen V et al (1997) Agents that reverse multidrug resistance, tamoxifen, verapamil, and cyclosporin A, block glycosphingolipid metabolism by inhibiting ceramide glycosylation in human cancer cells. J Biol Chem 272:1682–1687. doi:10.1074/jbc.272.3.1682

Li C, Wong WH (2001) Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection. Proc Natl Acad Sci USA 98(1):31–36. doi:10.1073/pnas.011404098

Liu YY, Han TY, Giuliano AE, Cabot MC (1999a) Expression of glucosylceramide synthase, converting ceramide to glucosylceramide, confers adriamycin resistance in human breast cancer cells. J Biol Chem 274:1140–1146. doi:10.1074/jbc.274.2.1140

Liu YY, Han TY, Giuliano AE, Ichikawa S, Hirabayashi Y, Cabot MC (1999b) Glycosylation of ceramide potentiates cellular resistance to tumor necrosis factor-alpha-induced apoptosis. Exp Cell Res 252:464–470. doi:10.1006/excr.1999.4649

Liu YY, Han TY, Giuliano AE, Hansen N, Cabot MC (2000) Uncoupling ceramide glycosylation by transfection of glucosylceramide synthase antisense reverses adriamycin resistance. J Biol Chem 275:7138–7143. doi:10.1074/jbc.275.10.7138

Liu Y-Y, Han TY, Yu JY, Bitterman A, Le A, Giuliano AE et al (2004) Oligonucleotides blocking glucosylceramide synthase expression selectively reverse drug resistance in cancer cells. J Lipid Res 45:933–940. doi:10.1194/jlr.M300486-JLR200

Liu YY,, Yin D,, Gupta V, Hirabayashi Y, Holleran WM, Giuliano AE, Jazwinski SM, Gouaze-Andersson V, Consoli DP, Cabot MC. (2008) A role for ceramide in driving cancer cell resistance to doxorubicin. FASEB J [Epub ahead of print]

Loi S, Haibe-Kains B, Desmedt C, Lallemand F, Tutt AM, Gillet C et al (2007) Definition of clinically distinct molecular subtypes in estrogen receptor-positive breast carcinomas through genomic grade. J Clin Oncol 25(10):1239–1246. doi:10.1200/JCO.2006.07.1522

Lucci A, Cho WI, Han TY, Giuliano AE, Morton DL, Cabot MC (1998) Glucosylceramide: a marker for multiple-drug resistant cancers. Anticancer Res 18:475–480

Makin G, Dive C (2001) Apoptosis and cancer chemotherapy. Trends Cell Biol 11:S22–S26

Miller LD, Smeds J, George J, Vega VB, Vergara L, Ploner A et al (2005) An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. Proc Natl Acad Sci USA 102(38):13550–13555. doi:10.1073/pnas.0506230102

Minn AJ, Gupta GP, Siegel PM, Bos PD, Shu W, Giri DD et al (2005) Genes that mediate breast cancer metastasis to lung. Nature 436(7050):518–524. doi:10.1038/nature03799

Minn AJ, Gupta GP, Padua D, Bos P, Nguyen DX, Nuyten D et al (2007) Lung metastasis genes couple breast tumor size and metastatic spread. Proc Natl Acad Sci USA 104(16):6740–6745. doi:10.1073/pnas.0701138104

Morjani H, Aouali N, Belhoussine R, Veldman RJ, Levade T, Manfait M (2001) Elevation of glucosylceramide in multidrug-resistant cancer cells and accumulation in cytoplasmic droplets. Int J Cancer 94:157–165. doi:10.1002/ijc.1449

Morrow CS, Cowan KH (1990) Glutathione S-transferases and drug resistance. Cancer Cells 2:15–22

Mueller H, Eppenberger U (1996) The dual role of mutant p53 protein in chemosensitivity of human cancers. Anticancer Res 16:3845–3848

Nicholson KM, Quinn DM, Kellett GL, Warr JR (1999) Preferential killing of multidrug-resistant KB cells by inhibitors of glucosylceramide synthase. Br J Cancer 81:423–430. doi:10.1038/sj.bjc.6690711

Norris-Cervetto E, Callaghan R, Platt FM, Dwek RA, Butters TD (2004) Inhibition of glucosylceramide synthase does not reverse drug resistance in cancer cells. J Biol Chem 279:40412–40418. doi:10.1074/jbc.M404466200

Ogretmen B, Hannun YA (2004) Biologically active sphingolipids in cancer pathogenesis and treatment. Nat Rev Cancer 4:604–616. doi:10.1038/nrc1411

Ogretmen B, Schady D, Usta J, Wood R, Kraveka JM, Luberto C et al (2001) Role of ceramide in mediating the inhibition of telomerase activity in A549 human lung adenocarcinoma cells. J Biol Chem 276:24901–24910. doi:10.1074/jbc.M100314200

Patterson MC, Vecchio D, Prady H, Abel L, Wraith JE (2007) Miglustat for treatment of Niemann-Pick C disease: a randomised controlled study. Lancet Neurol 6(9):765–772. doi:10.1016/S1474-4422(07)70194-1

Pawitan Y, Bjohle J, Amler L, Borg AL, Egyhazi S, Hall P et al (2005) Gene expression profiling spares early breast cancer patients from adjuvant therapy: derived and validated in two population-based cohorts. Breast Cancer Res 7(6):R953–R964. doi:10.1186/bcr1325

Radin NS, Shayman JA, Inokuchi J-I (1993) Metabolic effects of inhibiting glucosylceramide synthesis with PDMP and other substances. Adv Lipid Res 26:183–211

Reed JC (1995) Regulation of apoptosis by bcl-2 family proteins and its role in cancer and chemoresistance. Curr Opin Oncol 7:541–546. doi:10.1097/00001622-199511000-00012

Reynolds CP, Maurer BJ, Kolesnick RN (2004) Ceramide synthesis and metabolism as a target for cancer therapy. Cancer Lett 206:169–180. doi:10.1016/j.canlet.2003.08.034

Rody A, Holtrich U, Muller V, Gaetje R, Diallo R, Gehrmann M et al (2006) c-kit: identification of co-regulated genes by gene expression profiling and clinical relevance of two breast cancer subtypes with stem cell like features. ASCO annual meeting proceedings part I. J Clin Oncol 24:622

Rody A, Holtrich U, Gaetje R, Gehrmann M, Engels K, von Minckwitz G et al (2007) Poor outcome in estrogen receptor-positive breast cancers predicted by loss of plexin B1. Clin Cancer Res 13(4):1115–1122. doi:10.1158/1078-0432.CCR-06-2433

Ruckhäberle E, Rody A, Engels K, Gaetje R, von Minckwitz G, Schiffmann S, Grösch S, Geisslinger G, Holtrich U, Karn T, Kaufmann M (2007) Microarray analysis of altered sphingolipid metabolism reveals prognostic significance of sphingosine kinase 1 in breast cancer. Breast Cancer Res Treat [Epub ahead of print]

Shabbits JA, Mayer LD (2002) P-glycoprotein modulates ceramide-mediated sensitivity of human breast cancer cells to tubulin-binding anticancer drugs. Mol Cancer Ther 1:205–213

Shabbits JA, Krishna R, Mayer LD (2001) Molecular and pharmacological strategies to overcome multidrug resistance. Expert Rev Anticancer Ther 1:585–594. doi:10.1586/14737140.1.4.585

Simstein R, Burow M, Parker A, Weldon C, Beckman B (2003) Apoptosis, chemoresistance, and breast cancer: insights from the MCF-7 cell model system. Exp Biol Med (Maywood) 228:995–1003

Sotiriou C, Wirapati P, Loi S, Harris A, Fox S, Smeds J et al (2006) Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. J Natl Cancer Inst 98(4):262–272

Spyratos F, Ferrero-Poüs M, Trassard M, Hacène K, Phillips E, Tubiana-Hulin M et al (2002) Correlation between MIB-1 and other proliferation markers: clinical implications of the MIB-1 cutoff value. Cancer 94(8):2151–2159. doi:10.1002/cncr.10458

Tifft CJ, Proia RJ (2000) Stemming the tide: glycosphingolipids synthesis inhibitors as therapy for storage diseases. Glycobiology 10:1249–1258. doi:10.1093/glycob/10.12.1249

Wang Y, Klijn JG, Zhang Y, Sieuwerts AM, Look MP, Yang F et al (2005) Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. Lancet 365(9460):671–679

Weiss M, Hettmer S, Smith P, Ladisch S (2003) Inhibition of melanoma tumor growth by a novel inhibitor of glucosylceramide synthase. Cancer Res 63(13):3654–3658