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Gene expression profiling of ATP-binding cassette (ABC) transporters as a predictor of the pathologic response to neoadjuvant chemotherapy in breast cancer patients

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Summary

Drug resistance is a major obstacle to the successful chemotherapy. Several ATP-binding cassette (ABC) transporters including ABCB1, ABCC1 and ABCG2 have been known to be important mediators of chemoresistance. Using oligonucleotide microarrays (HG-U133 Plus 2.0; Affymetrix), we analyzed the ABC transporter gene expression profiles in breast cancer patients who underwent sequential weekly paclitaxel/FEC (5-fluorouracil, epirubicin and cyclophosphamide) neoadjuvant chemotherapy. We compared the ABC transporter expression profile between two classes of pretreatment tumor samples divided by the patients' pathological response to neoadjuvant chemotherapy (residual disease [RD] versus pathologic complete response [pCR]) ABCB3, ABCC7 and ABCF2 showed significantly high expression in the pCR. Several ABC transporters including ABCC5, ABCA12, ABCA1 ABCC13, ABCB6 and ABCC11 showed significantly increased expression in the RD ($p < 0.05$). We evaluated the feasibility of developing a multigene predictor model of pathologic response to neoadjuvant chemotherapy using gene expression profiles of ABC transporters. The prediction error was evaluated by leave-one-out cross-validation (LOOCV). A multigene predictor model with the ABC transporters differentially expressed between the two classes ($p \leq 0.003$) showed an average 92.8% of predictive accuracy (95% CI, 88.0–97.4%) with a 93.2% (95% CI, 85.2–100%) positive predictive value for pCR, a 93.6% (95% CI, 87.8–99.4%) negative predictive value, a sensitivity of 88.1%(95% CI, 76.8–99.4%), and a specificity of 95.9% (91.1% CI, 87.8–100%). Our results suggest that several ABC transporters in human breast cancer cells may affect the clinical response to neoadjuvant chemotherapy, and transcriptional profiling of these genes may be useful to predict the pathologic response to sequential weekly paclitaxel/FEC in breast cancer patients.

Introduction

Resistance to chemotherapy is a significant obstacle to appropriate treatment of cancer patients. Various cellular pathways may play a role in drug resistance and ATP-binding cassette (ABC) transporters are one of the most well known mediators leading to drug resistance and treatment failure. To date 49 ABC transporter genes have been identified and classified into seven groups, ABCA, ABCB, ABCC, ABCD, ABCE, ABCF, and ABCG (database of ABC transporters available at http://nutrigene.4t.com/humanabc.htm).

Extensive studies have been conducted on the individual proteins or genes of ABC transporter members regarding their role in chemoresistance. ABCB1 (MDR1-P-gp) [1,2], ABCC1 (MRP1) [3], and ABCG2 (MXR) [4] are particularly well known as mediators leading to resistance to several chemotherapeutic agents including paclitaxel [5], topoisomerase inhibitors [6], anthracyclin [7] and tyrosine kinase inhibitors [8]. Although little has been known about most of ABC transporter members, other members of this family sharing sequence and structural homology may play roles in absorption, distribution, and excretion of chemotherapeutic agents and probably influence the response to chemotherapy.

Recently, using ABC transporter gene expression profiling, studies on the relationship of drug resistance and ABC transporter were performed in cancer cell lines [9,10].

The characterization of the comprehensive expression of these genes in relation to the clinical response to chemotherapy may be useful to determine on an individual basis the patient's underlying risk and choose the optimal therapeutic regimen to which the individual cancer patient is most likely to respond. We studied the relationship between ABC transporter gene expression and the responsiveness to chemotherapy in early breast cancer patients who underwent sequential weekly paclitaxel/FEC (5-fluorouracil, epirubicin and cyclophosphamide) neoadjuvant chemotherapy and evaluated the feasibility of developing a multigene predictor model of pathologic response using differentially expressed ABC transporters on the basis of microarray data.

Materials and methods

Patient and sample preparation

This study was performed at the National Cancer Center Hospital, Tokyo, Japan. This study was approved by the institutional review boards of the National Cancer Center. Twenty-one pretreatment samples were obtained from breast cancer patients who underwent neoadjuvant chemotherapy from 2002 to 2004. All patients underwent pretreatment core needle biopsy (CNB) of the primary tumor tissue before starting neoadjuvant chemotherapy. The core needle biopsy was done using 14–16 gauge needles.

The patients received 4 cycles of FEC (5-Fluorouracil 500 mg/m^2, Epirubicin 100 mg/m^2 and Cyclophosphamide 500 mg/m^2) every three weeks followed by 12 cycles of weekly paclitaxel (80 mg/m^2). Additionally, in the case of HER2 positive determined by immunohistochemical staining (IHC), the specific inhibitory antibody of HER2 receptor, Trastuzumab (Herceptin®) was added in the course of the paclitaxel (Herceptin 4 mg/kg on day1 then 2 mg/kg weekly). Samples that showed 3+ IHC staining were considered as HER2 positive.

Every patient underwent surgery on the completion of the neoadjuvant chemotherapy, and histopathologic examination was performed. As described previously [11], pathologic complete response (pCR) was defined as no pathologic evidence of any residual invasive cancer cells in the breast and axillary lymph nodes, and residual disease (RD) was defined as any residual cancer cells on the histopathologic examination. Informed consent was obtained from all patients for voluntary participation in the study.

Tissue preparation and microarray

Samples for the microarray were collected into tubes containing Isogen (Nippon gene, Toyama) and stored at −80 °C. Total RNA was extracted by the single step method of Chomczynski et al. [12] with acid guanidinium thiocyanate phenol chloroform after homogenizing the tissue using a high speed homogenizer. The mean yield of RNA was 23.1 μg (ranged from 12.3 to 31.6 μg) from each collected samples. RNA that had distinct ribosomal RNA band by electrophoresis and had A_{260}/A_{280} absorbance ratio ranging from 1.8 to 2.1 was used for cDNA synthesis. Gene expression profiles were analyzed on a high-density oligonucleotide microarray (GeneChip® HG-U133 Plus 2.0; Affymetrix, Santa Clara, CA) containing 54,675 probe sets. The oligonucleotide microarray procedure for generation of the biotin-labeled cyclic RNA (cRNA) by *in vitro* transcription, hybridization to the array and scanning were performed according to the manufacturer's instructions. The amplification cycle of RNA to cDNA and cDNA to cRNA was performed using the GeneChip® 3′-Amplification Reagents One-Cycle cDNA Synthesis Kit including SuperScript II reverse transcriptase and a T7-$(dT)_{24}$ primer (Affymetrix). The synthesized cRNA was biotinylated using GeneChip 3′-amplification reagents for IVT labeling. The labeled cRNA was then purified and chemically fragmented at 94 °C for 35 min using the GeneChip Sample Cleanup Module (Affymetrix). The labeled fragmented cRNA was next hybridized to the GeneChip® at 45 °C for 16 h according to the manufacturer's instructions. The hybridized probe array was washed and stained with streptavidin-phycoerythrin. The stained probe array was scanned with a GeneChip® Scanner3000 (Affymetrix) at 570 nm. The signal intensity of the gene expression level was calculated by GeneChip Operating Software, Ver.1 (Affymetrix).

Data analysis

Microarray data analyses were performed with BRB ArrayTools developed by Dr. Richard Simon and Amy Peng Lam. (http://linus.nci.nih.gov/BRB-ArrayTools.html) which provides a variety of tools for the analysis of gene expression profile. Gene expression data were log transformed (base 2) and normalized to the median expression value of all genes on each array. Any genes in which the expression levels did not differ by at least by 1.5 fold from the median in at least 20% of the arrays were filtered out, for the exclusion of the genes showing minimal variation across the set of arrays. In addition, if an expression value was missing or filtered out in more than 50%, these data were excluded. The final data set included 50,508 clones, and contained all 49 ABC transporter genes. The list of transcripts on ABC transporters was obtained using GeneSprings software (http://www.silicongenetics.com/cgi/SiG.cgi/index.smf) from Agilent Technologies (Waldbronn, Germany). (Supplementary data).

Class comparison

To identify informative genes differentially expressed between the two classes of patients grouped by their pathologic response, we used supervised classification methods applying the random variance *t*-test to data using the BRB Array Tools and was accompanied by multivariate permutation tests in order to minimize false-positives with the maximum allowed number of

false positives set at 10, a false discovery rate of 0.1, and confidence 90%. Genes with a parametric *p*-value less than 0.05 were considered statistically significant.

Class prediction

To develop a prediction model of pathologic response using the ABC transporter gene expression profiles, we used the class prediction tools of BRB ArrayTools in which six multivariate classification methods were available including a compound covariate predictor [13], a *K*-nearest neighbor analysis ($K = 1, 3$), a nearest centroid analysis, a support vector machine [14] and a diagonal linear discriminate analysis.

For the evaluation of the feasibility of developing a multigene predictor model of response to neoadjuvant chemotherapy using differentially expressed ABC transporters, six different multivariate classification models were examined. Firstly, we determined the number of genes that were included in the classifier model using a paired *t*-test applying multiple univariate parametric significance thresholds, and developed a classifier model based on these selected genes at the univariate parametric significance thresholds. With changes in the parametric significance thresholds, the multivariate classification algorithms were performed iteratively evaluating the classification error and the classifier *p*-value to identify the best classifier, and the processes were iteratively performed for each number of genes included in the classifier (determined by the significance threshold). The prediction error of each model was evaluated by leave-one-out cross-validation (LOOCV) [15]. This validation procedure was performed in a manner that removed the left-out sample before selecting the discriminate genes [15,16]. The classifier *p*-value, the probability that similar low error rate happen by chance, was obtained by a random permutation test performed 2000 times.

Results

The patient characteristics

All the patients received 4 courses of FEC (5-fluorouracil, epirubicin and cyclophosphamide) combination chemotherapy followed by 12 courses of weekly paclitaxel. In those patients who were HER-2 positive by IHC, Trastuzumab (Herceptin®) was added in the course of the treatment. We divided the patients into two groups from the results of the histopathologic examination performed after the completion of the neoadjuvant chemotherapy. Pathologic data were available for nineteen patients. Patients with no pathologic evidence of any residual invasive cancer cells in breast were classified as 'pCR', and if any residual cancer cells were found in the histopathologic study, these patients were classified as 'RD' group. Thirty-six point eight percent (7) of the nineteen patients showed no pathologic evidence of any residual invasive cancer cells in the breast and were classified as pCR and 63.2% (12) of patients were classified as RD.

Gene expression profiling of differentially expressed ABC transporters

Using gene expression data of the pretreatment tumor sample, we compared the ABC transporter gene expression profile between the two groups (RD versus pCR). A probe set on all of the 49 human ABC transporters genes known so far was contained in the microarray chip we used (HG-U133 Plus 2.0; Affymetrix). To identify differentially expressed ABC transporter genes potentially associated with the clinical response to neoadjuvant chemotherapy, a supervised class comparison analysis was performed. The random variance model *t*-test was used to discover differentially expressed genes and was accompanied by a multivariate 1000 permutation tests in order to minimize false–positives with the maximum allowed number of false positives set at 10, a false discovery rate of 0.1 and 90% confidence.

By comparing the average expression level of each transcript on ABC transporters between the two classes of patients, the median expression level in the RD group was 107.8 (range 15.8–6009.1) and 104.4 in the pCR group (range 17.9–5690.6). The median of fold difference (RD: pCR) of transcripts on the ABC transporters was 1.0, ranging from 0.3 to 7.6. Several ABC transporters showed prominently high expression at over 50 fold of the median value although the tumor samples were all from the pretreatment chemotherapy-naïve patients. The highest average expression level in the RD group, 6009.1, was observed in ABCC5 (AF146074, RD: pCR = 6009.1:2427.5, fold ratio 2.48) and the highest expression level in the pCR group, 5690.6, was observed in TAP1 (ABCB2, NM_000593, RD: pCR = 4551.4:5690.6, fold ratio 0.8), the transporter associated with antigen processing (Table 1).

The ABC transporters, which were significantly differentially expressed with a parametric *p*-value of less than 0.05, are listed in Table 2. Several transcripts (ABCC5, TAP2/ABCB3) selected overlapped for the microarray chip (HG-U133 Plus 2.0) containing 54,675 probe sets, more than 30,000 human transcripts were detected, derived from more than 20,000 loci within the human genome and some transcripts represented the same human gene.

ABC transporters, the expression of which in the RD group was significantly increased, included ABCC5 (fold ratio 2.48, $p = 0.000368$), ABCA12 (fold ratio 7.64, $p = 0.000795$), ABCA1 (fold ratio 3.30, $p = 0.000859$), ABCC13 (fold ratio 7.54, $p = 0.0194$), ABCB6 (fold ratio 2.17, $p = 0.0271$), and ABCC11 (fold ratio 2.71, $p = 0.0486$) (Table 2). These genes all showed over 2 fold increases in RD compared with pCR tumors. ABCC5 was recently reported to confer resistance to

Table 1. Clinical characteristics of the patients

	No. of patients
Age, years	
Median	51
Range	30–61
Menstruation status	
Pre menopause	12
Post menopause	7
TNM stage	
IIA	8
IIB	7
IIIA	2
IIIB	2
Histology	
Invasive ductal	17
Mixed ductal/lobular	
Invasive lobular	1
Invasive mucinous	1
Nuclear grade	
1	1
2	9
3	9
HER2 status	
HER2-positive	4
HER2-negative	15
ER status	
ER-positive*	5
ER-negative	14
Pathologic response	
Pathologic complete response	7
Residual disease	12
Treatment arm	
A[a]	15
B[b]	4

*Cases in which more than 10% of tumor cells stained positive for ER by IHC classified as ER positive.
[a]Treatment arm A; 4 courses of FEC* followed by 12 courses of weekly paclitaxel.
[b]Treatment arm B; 4 courses of FEC* followed by 12 courses of weekly paclitaxel with Trastuzumab.
*FEC combination chemotherapy (5-fluorouracil, epirubicin and cyclophosphamide).

5-fluorouracil [17] selected with the lowest p-value and it showed the highest gene expression level in tumors with decreased response. (AF146074, expression level RD: pCR = 6009.1: 2427.5, fold ratio 2.48).

CFTR (NM_000492, ABCC7, fold ratio 0.27, p = 0.007030), ABCF2 (NM_005692, fold ratio 0.32, p = 0.015901) and ABCB3 (M74447, TAP2, fold ratio 0.54, p = 0.019345), the transporter associated with antigen processing, showed increased expression in the pCR group but the biological significance concerning responsiveness to chemotherapy remains to be elucidated. The differentially expressed ABC transporter genes are shown in Figure 1 in hierarchical clustering view.

Development of multigene predictor model using the ABC transporter gene expression profile

To evaluate the feasibility of developing a multigene predictor model of response to neoadjuvant chemotherapy using the ABC transporter expression profile, six different multivariate classification models were examined.

Firstly, we determined the number of discriminate genes that were included in the classifier model by applying multiple univariate parametric significance thresholds, and developed a classifier model based on these selected genes at the significance thresholds. With changes in the parametric significance thresholds, the classification error and classifier p-value for each multivariate classification algorithms were evaluated iteratively by LOOCV (leave one out cross validation) [15] and the random permutation test to identify the best classifier model. The classifier p-value, the probability that a similar low error rate could happen by chance, was calculated by 2000 random permutation tests. We calculated the average of the classification error and the classifier p-value of six classifier models at each significance threshold. Figure 2 shows the change in the average classifier p-value for six multivariate classification models from the permutation test and the average of the classification error rate relative to multiple univariate parametric significance thresholds.

During this iterative process, the average estimated misclassification error and classifier p-value also dropped as the significance threshold decreased to 0.003, but applying further stringent significance thresholds caused a steep increase in the classification error. When the ABC transporters differentially expressed between the two classes at a significance threshold level of 0.003 were used for class prediction, the average of the classification error was minimal, 0.072 (92.8% of predictive accuracy, 95% CI, 88.0–97.4%), with the classifier p = 0.012, 93.2% (95% CI, 85.2–100%) positive predictive value for the pCR group, 93.6% (95% CI, 87.8–99.4%) negative predictive value, sensitivity for the pCR group 88.1% (95% CI, 76.8–99.4%), and a specificity of 95.9% (91.1% CI, 87.8–100%). The respective values for each model are represented in Table 3. On applying the compound covariate predictor classifier model, the predictive accuracy reached 100% with a classifier p-value of 0.0005. The ABC transporters selected as the best classifiers are presented in Table 4. The list included ABCA1, ABCA12 and ABCC5, recently reported to confer resistance to cyclic nucleotides including 5-fluorouracil [17].

Our results suggest that the ABC transporter genes expression pattern may be useful in predicting the pathologic response to sequential weekly paclitaxel/FEC in breast cancer patients.

Discussion

To determine the optimal therapeutic regimen to which the individual cancer patient is most likely to respond on

Table 2. Differentially expressed ABC transporters ordered by significance

Gene symbol	Genbank	Parametric *p*-value*	% CV support	RD[a]	pCR[b]	Fold difference[c]	Description
ABCC5	AF146074	0.000368	100	6009.1	2427.5	2.48	ABC, sub-family C (CFTR/MRP), member 5
ABCC5	BE550362	0.000463	100	3571.5	1234.4	2.89	ABC, sub-family C (CFTR/MRP), member 5
ABCA12	AL080207	0.000795	100	711.7	93.1	7.64	ABC, sub-family A (ABC1), member 12
ABCA1	AL833227	0.000859	100	166.8	50.5	3.3	ABC, sub-family A (ABC1), member 1
CFTR	NM_000492	0.007030	100	27.7	104.4	0.27	cystic fibrosis transmembrane conductance regulator, ABC (sub-family C, member 7)
ABCF2	NM_005692	0.015901	100	49.4	154.1	0.32	ABC, sub-family F (GCN20), member 2
TAP2	M74447	0.019345	89	543.4	1008.5	0.54	Transporter 2, ABC, sub-family B (MDR/TAP)
ABCC13	NM_172025	0.019377	100	157.5	20.9	7.54	ABC, sub-family C (CFTR/MRP), member 13
ABCB6	NM_005689	0.027077	89	1471.9	677.5	2.17	ABC, sub-family B (MDR/TAP), member 6
TAP2	AA573502	0.042069	58	1740.5	2802	0.62	Transporter 2, ABC, sub-family B (MDR/TAP)
ABCC11	AF352582	0.048626	42	160.9	59.4	2.71	ABC, sub-family C (CFTR/MRP), member 11

Table sorted by *p*-value. * *p* by random variance *t*-test.
[a]Geometric mean of intensities in the RD group.
[b]Geometric mean of intensities in the pCR group.
[c]Fold difference of geometric means RD: pCR.

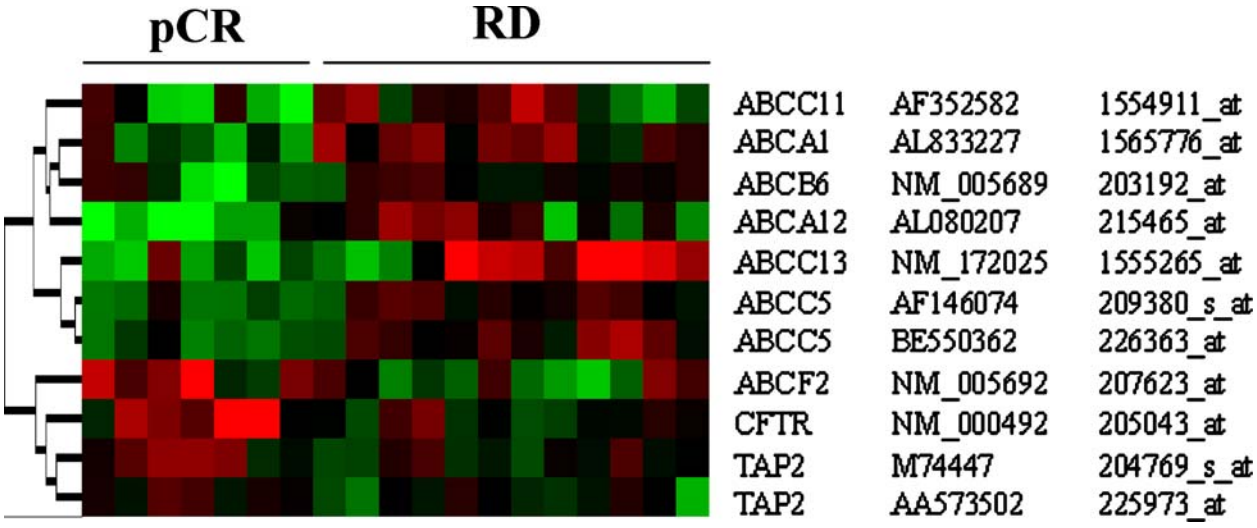

Figure 1. Hierarchical clustering of differentially expressed ABC transporters associated with the response to neoadjuvant chemotherapy in breast cancer patients. The cluster image map shows patterns of differential ABC transporter gene expression in breast cancer patients in respect to the response to neoadjuvant chemotherapy. The hierarchical clustering on each axis was performed using the complete linkage algorithm. Relatively highly expressed genes are shown in red, low expressed genes are shown in green.

an individual basis, there is a real need to develop an appropriate predictor to identify those cancer patients most likely to require or benefit from particular therapies. Resistance to chemotherapy is significant obstacle to appropriate treatment of cancer patients and affects the treatment outcome. Numerous cellular mechanisms exist which are responsible for the treatment failure due to chemoresistance. ABC transporters are the one of the major factors leading to drug resistance. Extensive study has been conducted on the ABC transporters, and ABCB1 (MDR1-P-gp) [1,2], ABCC1-MRP1 [3], and ABCG2-MXR [4] are particularly well known for their role in resistance to several chemotherapeutic agents. Because the members of the ABC transporters are grouped by sequence homology, the remained members may play roles in absorption, distribution, and excretion of chemotherapeutic agent and probably be related to drug resistance although little has been known about most of the functions of these genes. Characterization of the expression of the genes related to chemoresistance is an interesting subject and may lead to clinically useful predictors of response to chemotherapy. The profiling of ABC transporter genes in relation to the clinical response to chemotherapy may also be useful to determine the patient's underlying risk and choose the optimal therapeutic regimen to which the individual cancer patient is most likely to respond.

Focusing on the ABC transporters, we analyzed the gene expression profile in breast cancer patients using microarray data that contain the transcripts of all the

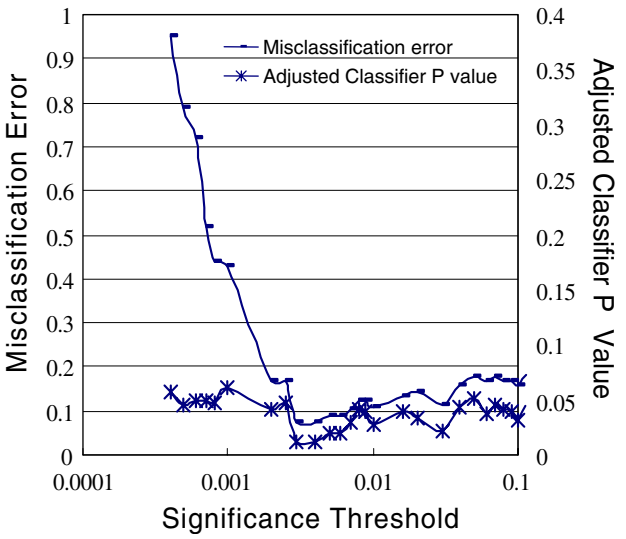

Figure 2. Multivariate predictive classification models in leave-one-out cross-validation and permutation test with an increasing significance threshold at which genes were selected as a classifier. The *x*-axis represents the significance threshold p value used to select the discriminate genes as classifiers. The *y*-axis shows the average of the misclassification error rate determined by leave-one-out cross-validation and the average classifier *p*-value, the probability that a similar low error rate could happen by chance calculated after 2000 permutations. Classifier genes selected as differentials between the 2 classes at a significance threshold $p = 0.003$ level showed the highest discriminate value.

members of ABC transporter family. We compared the expression pattern of the ABC transporters between two classes of pretreatment tumor samples divided by the pathologic response to neoadjuvant chemotherapy (RD versus pCR).

On microarray analysis, several ABC transporters showed differential expression between the two groups of tumors. Of interest, several ABC transporters showed increased expression in the pCR group, including CFTR (NM_000492, ABCC7, fold ratio 0.27, $p = 0.007030$), ABCF2 (NM_005692, fold ratio 0.32, $p = 0.015901$) and ABCB3 (M74447, TAP2, fold ratio 0.54, $p = 0.019345$). ABCB3 is known to be involved in antigen presenting by transporting peptides necessary for the assembly of major histocompatibility complex (MHC) class I molecules from the cytoplasm to the endoplasmic reticulum [18]. It is also known that its reduced expression is associated with HLA class I deficient human tumor cell lines [19] and it has been suggested that it is related to the aggressive features of some kinds of tumors [20–22]. Its increased expression has been found to be associated with pathological complete response in our clinical samples, but any clinical significance in the treatment of in breast cancer remains to be elucidated.

Five ABC transporters ABCC5 (AF146074, fold ratio 2.48, $p = 0.000368$), ABCA12 (AL080207, fold ratio 7.64,

Table 3. Performance of the multivariate classifier; the sensitivity, specificity, PPV and NPV for the pCR group of each predictor model at a significance threshold of $p = 0.003$

	CCV[a]	1NNC[b]	3NNC[c]	NCC[d]	SVM[e]	LDD[f]	Average[g]
Sensitivity	100	85.7	85.7	85.7	71.4	100	88.1
Specificity	100	91.7	91.7	100	100	91.7	95.9
PPV	100	85.7	85.7	100	100	87.5	93.2
NPV	100	91.7	91.7	92.3	85.7	100	93.6
Misclassification error	0	0.05	0.11	0.11	0.05	0.11	0.072
Percent correctly classified	100	95	89	89	95	89	92.8
Classifier P	5.00E-04	0.014	0.025	0.006	0.023	0.005	0.01225

[a]Compound covariate predictor classifier.
[b]1-Nearest neighbor classifier.
[c]3-Nearest neighbor classifier.
[d]Nearest centroid classifier.
[e]Support vector machine classifier.
[f]Linear diagonal discriminant analysis classifier.
[g]Average value of six multivariate classifier models.

Table 4. ABC transporters selected as best classifiers at a significance threshold of 0.003

Gene symbol	Genbank	*t*-Value	Parametric *p*-value*	% CV support	RD[a]	pCR[b]	§Fold difference	Description
ABCC5	AF146074	4.43	0.000368	100	6009.1	2427.5	2.48	ABC, sub-family C (CFTR/MRP), member 5
ABCC5	BE550362	4.32	0.000463	100	3571.5	1234.4	2.89	ABC, sub-family C (CFTR/MRP), member 5
ABCA12	AL080207	4.07	0.000795	100	711.7	93.1	7.64	ABC, sub-family A (ABC1), member 12
ABCA1	AL833227	4.04	0.000859	100	166.8	50.5	3.30	ABC, sub-family A (ABC1), member 1

Table sorted by *p* value.
*Parametric *p*-value by random variance *t*-test.
[a]Geometric mean of intensities in the RD group.
[b]Geometric mean of intensities in the pCR group. §Fold difference of geometric means; RD: pCR.

$p = 0.000795$), ABCA1 (AL833227, fold ratio 3.30, $p = 0.000859$), ABCC13 (NM_172025, fold ratio 7.54, $p = 0.0194$), ABCB6 (NM_005689, fold ratio 2.17, $p = 0.0271$) and ABCC11 (AF352582, fold ratio 2.71, $p = 0.0486$) showed significantly increased expression in the RD group associated with a decreased responsiveness to sequential weekly paclitaxel/FEC (5-fluorouracil, epirubicin and cyclophosphamide) neoadjuvant chemotherapy. Of these, ABCC5 was selected with the highest significance ($p = 0.000368$) and the highest expression level (RD: pCR 6009.1: 2427.5) although correlation between the gene expression level and the functional protein level remains to be seen. The ABCC5 (MRP5) transporter on human chromosome 3q27 has been known to be involved in the transport of nucleoside analogs [23] and has been reported to confer resistance to several drugs including methotrexate, GW1843 and ZD1694 (raltitrexed) [24]. Recently, Pratt et al. demonstrated that ABCC5 confers resistance against 5-fluorouracil [17] that was used in our neoadjuvant chemotherapy regimen. These results suggest that ABCC5 mediates transport of several chemotherapeutic agents and may contribute to resistance against 5-fluorouracil which is presently used in neoadjuvant chemotherapy.

In our clinical trial setting, ABCB1, known to confer resistance to several chemotherapeutic agents including paclitaxel, did not significantly increase in tumors with decreased response to neoadjuvant chemotherapy. Samples used in this study were all from chemotherapy-naïve patients and the time of exposure to the drug may not have been sufficient to induce the gene expression of this transporter. Although several ABC transporters showed high expression levels in the pretreatment samples, ABCB1 did not show significantly high expression. ABCB1 may thus play a greater role in resistance to chemotherapy in a secondary chemotherapy clinical setting than in first line chemotherapy when the exposure time is sufficiently long to induce the gene expression of the transporters known to be inducible by exposure to that chemotherapeutic agent [25,26].

But, some ABC transporters may also play significant role in chemoresistance in early breast cancer. Recently, it was reported that ABCC1 expression predict shorter relapse free survival and overall survival and play important role in resistance to chemotherapy in early breast cancer who underwent CMF (cyclophosphamide, methotrexate, and fluorouracil) adjuvant chemotherapy [27].

A variety of compounds are transported by ABC transporters through the lipid bilayer and still little has been known about the function of individual transporters in transport of chemotherapeutic agents. ABCA1 has been implicated in the control of the extrusion of membrane phospholipids and cholesterol toward specific extracellular acceptors [28] and macrophage interleukin-1 beta secretion and apoptosis [29]. ABCC13, highly expressed in the RD group mapped to chromosome 21q11.2 has been suggested that it might be associated with hematopoiesis. It has also been reported that ABCC13 shows decreased expression during cell differentiates [30]. ABCC11, called MRP8 is known to be a cyclic nucleotide efflux pump and a resistance factor for fluoropyrimidines 2′,3′-dideoxycytidine and 9′-(2′-phosphonylmethoxyethyl) adenine [31]. Szakacs et al. [10] suggested ABCC11 mediated resistance may not be confined to nucleoside analog, demonstrating that the ABCC11 transfected cell confers resistance to NSC 671136 by 2–3 fold. ABCB6 is a mitochondrial half transporter that is known to be involved in the transport of a precursor of the Fe/S cluster from mitochondria to the cytosol [32]. A recent report showed that several ABC transporters including ABCB6 amplified drug resistance in a non small cell lung cancer cell line (A549/CPT) in comparison with its parental cell [33].

Although the role in chemoresistant of individual transporters selected in our study to discriminate between the pCR and RD groups remains to be revealed, the transporters may also play roles in response to chemotherapy by influencing absorption, distribution, and excretion of chemotherapeutic agents.

To evaluate the predictive signature of ABC transporters, we examined multigene predictor model of response to neoadjuvant chemotherapy using differentially expressed ABC transporters. Six different multivariate classification models were examined. When the ABC transporters differentially expressed between the two classes at a significance threshold level of 0.003 were used for class prediction, an average 92.8% of predictive accuracy was observed, with a 93.2% positive predictive value for the pCR group, 93.6% negative predictive value, sensitivity for the pCR group of 88.1%, and 95.9% specificity. The classifier p-value, the probability that a similar low error rate could happen by chance, was also low ($p = 0.012$). The optimum classifier model included ABCC5, ABCA1, and ABCA12. These genes all showed high expression in tumors in the RD group.

Of interest, although we developed the class prediction model from a small subset of genes, i.e., genes belonging only to the ABC transporter family, the predictive accuracy reached above 90% with quite a low classifier p-value although these prediction models based on ABC transporter genes need to be validated in future studies by comparing the classification model with all subsets of genes and with larger numbers of samples.

Our result suggest that several ABC transporters in human breast cancer cells may contribute to the clinical response to neoadjuvant chemotherapy and gene expression profiling of these ABC transporters may be useful in prediction of the pathologic response to sequential weekly paclitaxel/FEC in breast cancer patients.

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