Prognostic Value of Gene Signatures and Tumorbiological Characteristics in Breast Cancer Patients Treated with Anthracycline-containing Chemotherapy

Die prognostische Wertigkeit von Gensignaturen und tumorbiologischer Charakteristika bei Mammakarzinompatientinnen mit adjuvanter, anthrazyklinhaltiger Chemotherapie

Authors A. Rody[1]*, T. Karn[1]*, F. Pobitschka[1], E. Ruckhäberle[1], C. Solbach[1], M. Gehrmann[2], A. Ahr[1], L. Hanker[1], R. Gaetje[1], U. Holtrich[1], M. Kaufmann[1]

Affiliations
[1] Department of Obstetrics and Gynecology, J.W. Goethe-University, Frankfurt, Germany
[2] Siemens Medical Solutions Diagnostics GmbH, Cologne, Germany

Schlüsselwörter
- Gensignatur
- Mikroarrayanalyse
- Anthrazyklin
- Brustkrebs

Key words
- prognostic and predictive multigene signatures
- breast cancer
- anthracycline chemotherapy
- microarray analysis

Abstract

Background: Many prognostic and predictive multigene signatures have been established in breast cancer patients. For treatment decision the assessment of individual prognosis is essential. The choice of specific therapy is basically driven by empirical data although several predictive gene signatures already exist. In this context it would be valuable if specific signatures could be used for estimation of prognosis and prediction of therapy concurrently.

Material and Methods: Microarray data (Affymetrix HG U133A) of a small samples set of n = 48 breast cancer patients who received an anthracycline-based adjuvant chemotherapy were analyzed. Tumor samples were classified according to four prognostic and two predictive previously described gene signatures and compared with standard parameters as histologic subtype, tumor size, nodal status, pathohistological grading as well as estrogen receptor and Her-2 status.

Results: The gene expression values of ER, PR and Her-2 from microarray revealed a high concordance with protein expression assessed by means of immunohistochemistry. The determination of proliferative state of the tumors using gene expression of Ki67 showed a significant correlation with ER-status (p = 0.040, Mann-Whitney U-test) and pathohistological grading (p = 0.005, Kruskal-Wallis test). Neither of the six different signatures was able to predict event status of patients sufficiently. The main discriminatory power of the signatures was related to the ER status and to some extent to pathohistological grading.

Conclusion: In a small cohort of uniformly treated patients prognostic and predictive gene signatures are incapable to predict disease outcome unambiguously. The main driving force of

Zusammenfasung

Hintergrund: Zahlreiche prognostische und prädiktive Multigensignaturen sind bisher für Mammakarzinompatientinnen generiert worden. Die Einschätzung der individuellen Prognose ist für eine optimale Therapieentscheidung wesentlich. Die Auswahl einer spezifischen Therapie ist grundsätzlich durch die empirische Datenlage bestimmt, obwohl bereits zahlreiche prädiktive Gensignaturen existent sind. In diesem Zusammenhang wäre es hilfreich, wenn spezifische Signaturen sowohl zur Abschätzung der Prognose als auch zur Prädiktion des Therapieansprechens gleichzeitig genutzt werden könnten.

Material und Methoden: Genexpressionsdaten (Affymetrix HgU133A) eines kleinen Probenkollektivs von n = 48 Mammakarzinompatientinnen, die eine adjuvante, anthrazyklinhaltige Chemotherapie erhalten haben, wurde analysiert. Die Tumorproben wurden nach 4 prognostischen und 2 prädiktiven bereits publizierten Gensignaturen eingeteilt und mit den Standardparametern wie histologischer Subtyp, Tumorgröße, Nodalstatus, pathohistologisches Grading, sowie dem Östrogenrezeptor- und Her-2-Status verglichen.

Ergebnisse: Die Genexpressionswerte bezüglich ER, PR und Her-2 zeigten im Vergleich zur immunhistochemisch bestimmten Proteinexpression eine hohe Konkordanz. Die Bestimmung des Proliferationszustands mittels Genexpression von Ki67 zeigte eine signifikante Korrelation mit ER-Status (p = 0,040, Mann-Whitney-U-Test) und pathohistologischem Grading (p = 0,005, Kruskal-Wallis-Test). Keine der 6 verschiedenen Signaturen war in der Lage, den Ereignisstatus der Patienten ausreichend vorherzusagen. Die hauptsächlich diskriminierenden Eigenschaften der Signaturen basieren auf dem ER-Status und zu einem gewissen Maße auf dem pathohistologischen Grading.

received 21.10.2008
accepted 24.10.2008

Bibliography
DOI 10.1055/s-2008-1039168
Geburtsh Frauenheilk 2008; 68: 1171–1177 © Georg Thieme Verlag KG Stuttgart · New York · ISSN 0016-5751

Correspondence
Achim Rody, MD
Department of Obstetrics and Gynecology
J.W. Goethe-University
Theodor-Stern-Kai 7
60590 Frankfurt
Germany
achim.rody@em.uni-frankfurt.de

* both authors contributed equally

all signatures are ER-status and proliferation. The value of the individual signatures may be restricted to the specific setting from which they were derived.

Schlussfolgerung: In einem kleinen, einheitlich behandelten Patientenkollektiv sind prognostische und prädiktive Gensignaturen nicht in der Lage, den Krankheitsverlauf unzweifelhaft vorherzusagen. Die wesentlichen Einflussgrößen für alle Signaturen sind der ER-Status und die Proliferation. Die Wertigkeit der jeweiligen Signaturen ist offenbar ausschließlich auf die spezifische Situation beschränkt, für die sie identifiziert wurden.

Introduction

Proliferation and differentiation are the basic principles of organogenesis and maintenance of integrity. Destabilization of this balance might result in the development of cancer. Breast cancer is a paradigm for the interaction of hormonal influences on proliferation and differentiation. In vitro and in vivo data demonstrate that the expression of estrogen receptor (ER) in breast cancer is associated with low proliferation and favorable prognosis. To date many efforts have been undertaken to detect specific marker genes for predicting tumor response and disease outcome. Global gene expression profiling by microarrays has been used as a valuable tool for the identification of prognostic marker genes. Perou et al. reported that gene expression profiling by DNA microarray analysis of breast tumors is feasible and allows to distinguish different tumor subtypes [1]. Van't Veer et al. could demonstrate that tumor clustering by gene expression profile is able to discriminate breast cancers with poor or good prognosis [2]. This prognostic gene signature set has been validated in a larger cohort of 295 patients with primary breast carcinomas [3]. Sorlie et al. classified breast carcinomas based on gene expression patterns in luminal A and B, basal-like, erbB2+ and normal breast-like subtypes and correlated these groups with overall and disease-free survival [4]. Our group also published a 41 gene signature set [5] which allowed to identify patients with an unfavorable prognosis [6].

Many clinical trials reported a higher response to chemotherapy in patients with rapidly proliferating tumors which could be further highlighted by demonstrating that histological grading [7] and several markers associated with proliferation are predictive for tumor response [8–10]. Despite the employment of new unified methods for the assessment of histological grading as described by Elston and Ellis [11], the interobserver reproducibility is still poor ranging from 50 to 85% [12–14]. Furthermore there is a substantial proportion of tumors with intermediate grading (30–60%) which is not helpful in clinical decision making and predicting of response to neoadjuvant chemotherapy. Sotiriou et al. recently demonstrated that assessment of genomic grading by using gene expression profiling allows to re-classify patients with intermediate grade tumors into two groups with high versus low risk of recurrence [15]. Interestingly approximately one half of all intermediate grade tumors could be attributed to high and low genomic grading respectively. Importantly, a large number of genes is co-regulated with ER-status and proliferation [16, 17]. Thus differences in these two variables may influence the derivation of prognostic and predictive signatures.

Here we analyzed 48 breast cancers with Affymetrix expression data and compared supervised clustering results using genes of previously described prognostic and predictive signatures with clinic-pathological informations. The goal of this study was to investigate whether the so far described prognostic or predictive gene signatures have the power to unambiguously identify patients with a worse prognosis in such a small cohort of similar treated patients or whether they mainly correlate with known clinico-pathological parameters.

Material and Methods

Patients and tissue samples

Tissue samples were obtained from consecutive patients undergoing surgical resection between November 1997 and June 2003 at the Department of Obstetrics and Gynecology at the J. W. Goethe-University in Frankfurt with IRB approval and informed consent of the patients. Patients were selected for this study if they had received adjuvant chemotherapy consisting of anthracycline-based regimens and if sufficient follow-up data of at least 24 months and frozen tissue samples with more than 80% tumor cells were available (n = 48). Patients with positive hormone receptor status received additional tamoxifen for five years. Clinical characteristics of the patients are given in **Table 1**. Tumor samples were snap frozen in liquid nitrogen and part of the removed tumor tissue was used for diagnostic purposes. Tumors were characterized according to standard pathology including immunohistochemistry (IHC) of ER and PR and HER2.

Gene expression analysis and statistical methods

Only biopsies with more than 80% tumor cells were considered for analyses. RNA was isolated with Qiagen RNeasy reagents. Quality control analysis of extracted total RNA was performed with Agilent Bioanalyzer 2100 (capillary gel electrophoresis) and photometric quantification of the isolated total RNA was determined by NanoDrop ND-1000. Expression profiling was done using Affymetrix Human Genome U133A GeneChip platform containing 22 283 probes according to the protocols of the manufacturer as described elsewhere [18,19]. Hybridization intensity data were automatically acquired and processed by Affymetrix Microarray Suite 5.0 software. The expression level of each gene was determined by calculating the average of differences in intensity (perfect match-mismatch) between its probe pairs with global scaling to a target intensity of 500. Scans were rejected if the scaling factor exceeded 2 or "chip surface scan" revealed scratches, specks or gradients affecting overall data quality (Refiner, GeneData AG, Basle, Switzerland). The data were subsequently analyzed by using the Cluster and Treeview software package [20], SPSS 15.0 (SPSS Inc., Chicago, IL) and R statistical software package (www.r-project.org). Gene chip expression values were adjusted by $\log_2$ transformation and median centering of the arrays. Prior to cluster analysis an additional median centering of the specific gene set was performed. For receptor status based on microarray the Affymetrix Probe Set 205225_at corresponding to the estrogen receptor gene (ESR1) was used for ER status, Probe Set 208305_at for progesteron receptor status and Probe Set 216836_s_at for Her2 status (see results section). As a surrogate marker for cellular proliferation we

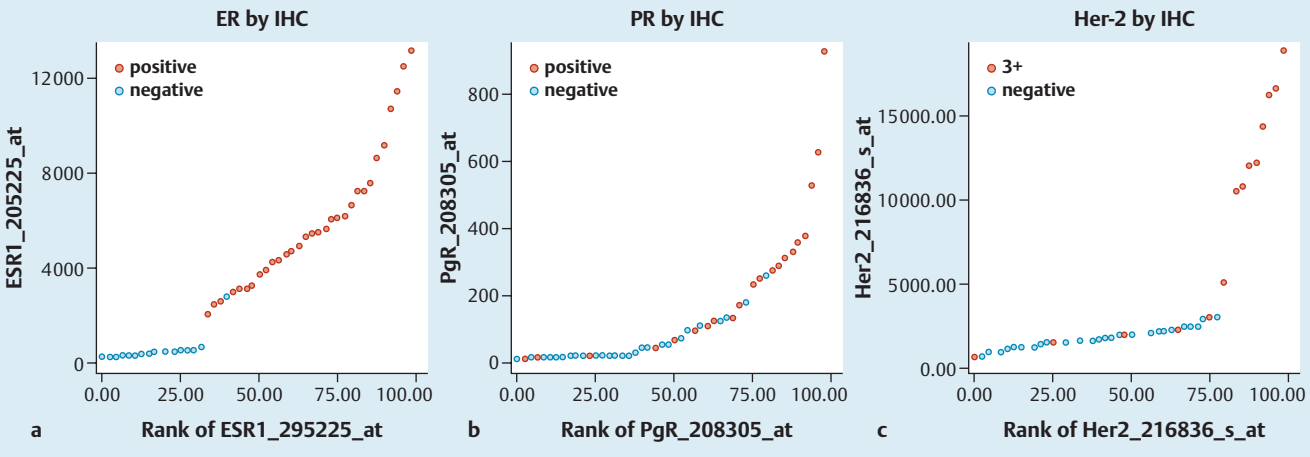


Fig. 1 a to **c** Consistency of Affymetrix microarray and immunohistochemistry measurements of hormone receptor and Her-2 expression. Affymetrix (MAS5) expression values of ER (**a**), PR (**b**), and Her-2 (**c**) of the samples are presented in scatter plots compared to their relative ranks. The results from immunohistochemical analysis is represented by red (positive) and blue (negative) colour.

used the expression of the proliferation marker Ki67 (ProbeSets 212020–212023_s_at). Affymetrix Probe Sets of the gene signatures of Wang et al. [21], Sotiriou et al. [22], Hess et al. [23], and Rody et al. [24] were obtained from the respective publications. The gene signatures of Sorlie et al. 2001 [4] and van't Veer et al. [2] were mapped to Affymetrix Probe Sets by utilizing Unigene annotation and genomic sequence information. 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. The non-parametric Mann-Whitney U-test and Kruskal Wallis H test were applied to study the association of Ki67 gene expression with ER status and histological grading, respectively. For categorical variables χ^2 test or Fisher's exact test were used. Disease free 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. Patients without event were excluded from the study when the follow-up time was less than 24 months. Statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL) and R statistical software package (www.r-project.org).

Results

48 consecutive patients for which tumor tissue has been collected were enrolled in this study. All patients underwent tumor surgery and received adjuvant anthracycline-based chemotherapy and antiestrogen treatment if patients were classified as endocrine responsive. The clinico-pathological characteristics of the patients are given in **Table 1**. Microarray analysis was performed using Affymetrix HG U133 A chips and high quality data could be obtained for all of the samples. To validate Affymetrix gene expression data we compared ER (ProbeSet 2005225_at) and PR (ProbeSet 208305_at) microarray data with ER and PR status as assessed by immunohistochemistry. As displayed in **Fig. 1 a** and **b** this analysis allows the definition of a cut-off level for the determination of ER and PR status by gene expression analysis (cut-off level for ER: 1000 and for PR: 50). When using these cutoffs we obtained a sensitivity of 100% and a specificity of 94.1% for ER status and a sensitivity of 81.0% and a specificity of 74.1% for PR status, respectively. A corresponding analysis of Her-2 expression (ProbeSet 216836_s_at) and the Her-2 status from immunohistochemistry, using a cut-off value of 4500 resulted in a sensitivity of 64.3% and a specificity of 100% for Her-2 positive ("3+") samples (**Fig. 1 c**). The proliferative status of the samples was analyzed using Ki67 expression as a surrogate marker for tumor proliferation. As shown in **Fig. 2 a** clear correlation of Ki67 expression measured by Affymetrix microarray

Table 1 Patients clinical characteristics.

	Number	Percentage
	48	
Age		
≤ 50	22	45.8%
> 50	26	54.2%
T state		
T1	17	35.4%
T2	25	52.1%
T3	3	6.3%
T4	3	6.3%
Pathohistological grading		
G1	1	2.1%
G2	18	37.5%
G3	29	60.4%
Nodal status		
Nodal positive	29	60.4%
Nodal negative	19	39.6%
Hormone receptor status		
ER positive	31	64.6%
ER negative	17	35.4%
PR positive	21	43.8%
PR negative	27	56.3%
Her-2 status (IHC)*		
Her-2 positive	14	35.0%
Her-2 negative	27	65.0%
Event status		
No event	37	77.1%
Event	11	22.9%

* imunohistochemical values from 8 patients were not available

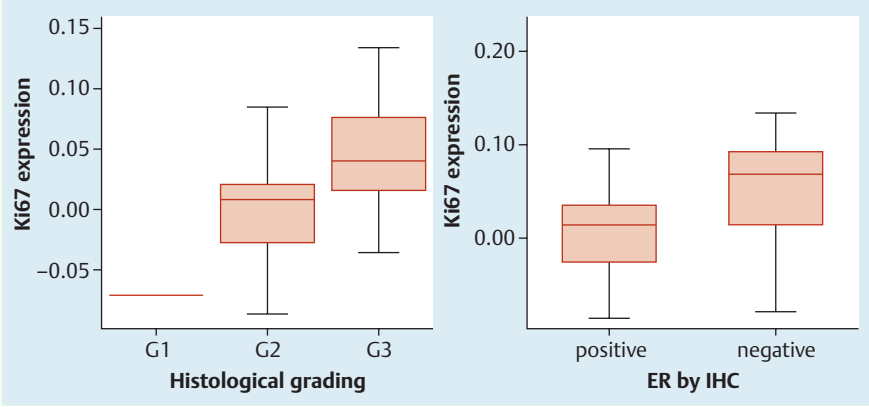
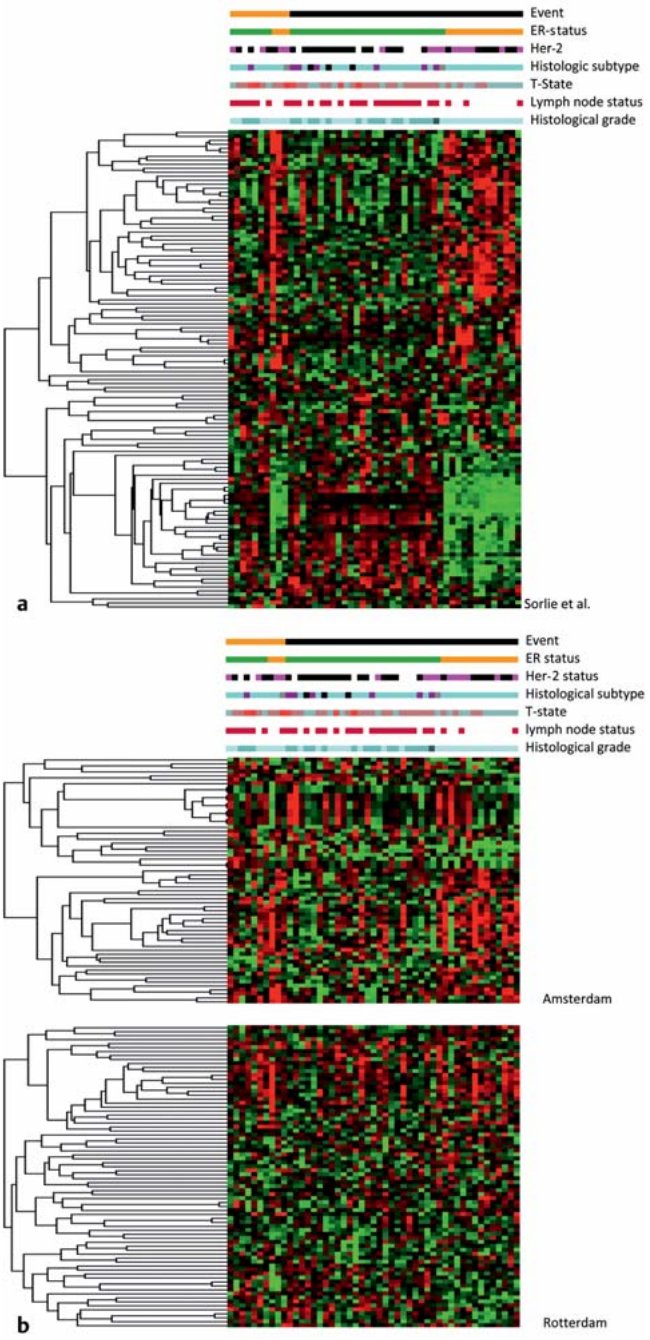


Fig. 2 a and **b** Correlation of Ki67 expression with histological grading and estrogen receptor status. The proliferative status of the tumor was assessed using Affymetrix expression values of the Ki67 proliferation marker. Box plots are given comparing the expression of this gene with histological grading ($p = 0.005$, Kruskal-Wallis test) and ER status ($p = 0.040$, Mann-Whitney U-test) of the tumor.



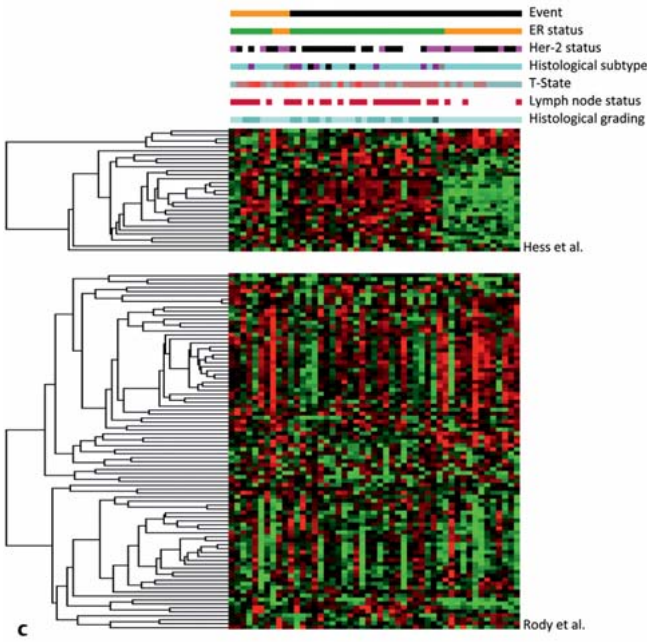


Fig. 3 a to **c** Supervised analysis of gene signatures in the patient cohort. **a** Analysis of the expression of the markers from the "intrinsic subtype signature" of Sorlie and colleagues [4]. Tumor samples were ordered according to outcome (event/no event) and estrogen receptor status. Above the expression matrix the outcome of the patient, ER and Her-2 status as well as histological subtype, T state, lymph node status, and histological grading are given by coloured blocks. **b** Expression of genes from the prognostic signatures from Amsterdam [2] and Rotterdam [21]. Samples are ordered as in **a. c** Expression of genes from the predictive signatures from Hess et al. [23] and Rody et al. [24]. Samples are ordered as in **a**.

with the histological grading of the tumor was observed ($p = 0.005$, Kruskal-Wallis test, ▶ **Fig. 2 a**). Moreover ER positive tumors showed a significant lower Ki67 expression as compared to ER negative breast cancers ($p = 0.040$, Mann-Whitney U-test), suggesting an inverse correlation of ER status and proliferation (▶ **Fig. 2 b**). Next we performed supervised cluster analyses of markers from several described gene signatures. Tumor samples were sorted according to (i) the clinical outcome parameter (event vs. no event) and (ii) the ER status of the tumor. First we applied the signature from Sorlie and colleagues [4] for the "intrinsic subtypes" of breast cancer (▶ **Fig. 3 a**). Since this signature mainly distinguishes the ER positive "luminal" subtype from the

ER negative "basal-like" subtype, a clear correlation with the ER status was observed as expected. However, no strong differences were observed in tumors from patients with an event compared to those without. We then analyzed the markers of the prognostic signatures from Amsterdam [2] and Rotterdam [21]. However, as shown in **Fig. 3b**, we observed no clear correlation of the expression of these genes with the outcome of the patient. In contrast some genes from these signatures seemed also to be associated with the ER status of the tumor. Similar results of no clear association with outcome but a correlation with the ER status were observed when we analyzed two predictive signatures for adjuvant treatment response. **Fig. 3c** presents the cluster analysis of these signatures, which were originally obtained from studies on neoadjuvant treated patients (Hess et al. [23], Rody et al. [24]).

An important concept suggested from several recently published analyses is that the proliferative capacity of the tumor which is also described by the histological grading is the major determinant for patient prognosis. In line with this argument proliferation markers represent an important constituent of prognostic gene signatures [22]. Thus we used the recently described method of Sotiriou et al. [22] to determine the "genomic grade" of the tumors. **Fig. 4** presents the results of this analysis where tumor samples are sorted according to the "Genomic Grade Index (GGI)" [22]. The analysis revealed that the "Genomic Grade" is positively associated with higher histological grading and a higher number of ER negative samples in our cohort. However, no association with outcome was observed.

To analyze the impact of proliferation markers on the prognostic and predictive signature described above we performed a further supervised analysis. First, the tumor samples were sorted according to the "Genomic Grade Index" as in **Fig. 4**. Then the clustered markers from the signatures as shown in **Fig. 3b** and **c** were applied to the samples sorted in this way. The results from this analysis are shown in **Fig. 5**. From this analysis it can be concluded that many markers from both the prognostic (**Fig. 5a**) and the predictive signatures (**Fig. 5b**) are clearly correlated to the proliferation of the tumors as measured by the Genomic Grade Index.

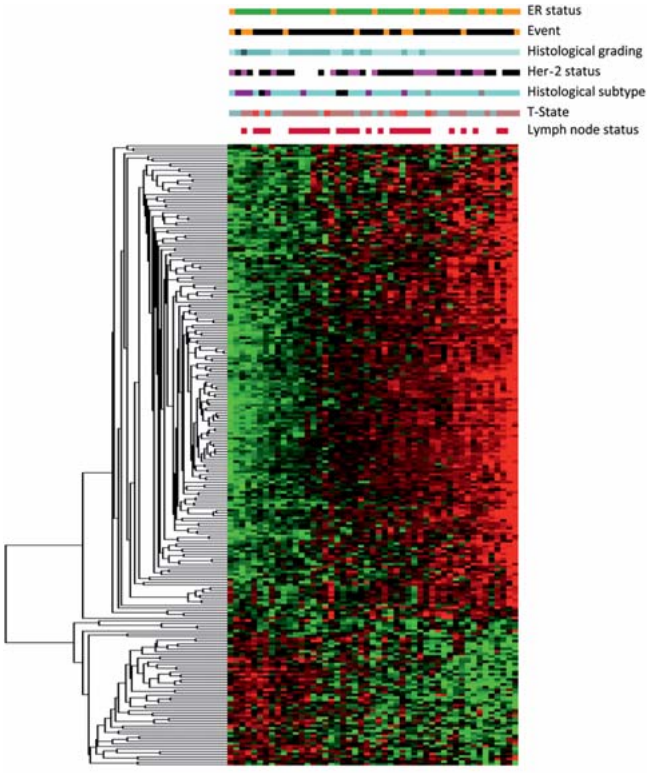


Fig. 4 Analysis of breast cancers according to the Genomic Grade Index (GGI). The "Genomic Grade" gene signature [15] of 242 was applied to the dataset of 48 tumors. Samples were sorted according to this Genomic Grade Index (GGI) from left to right. Clinical and pathological parameters of the tumors are given above the expression matrix.

Discussion

Our data indicate that the power of several prognostic and predictive gene signatures is fairly limited when using our relative small sample set of adjuvant treated tumor samples. On the other hand the analysis of standard parameters (ER, PR, Her-2 and proliferation) revealed a good consistency with the microarray measurements. Thus the technical measurement of gene expression seems to be reliable. Without doubt the comparison of signatures derived from variable treatment settings (adjuvant versus neoadjuvant) and different chip platforms is critical and a major flaw of this study. Moreover, our sample size was very small and the follow-up relatively short since only patients which obtained recently introduced treatment schemes were included in the study. However, our analyses demonstrate that a substantial proportion of the markers from prognostic and predictive signatures is strongly associated with specific standard parameters as e.g., ER status, pathohistological grading or histology. This further emphasizes that estrogen receptor and proliferation are the major determinants for gene regulation in breast cancer patients [16,17] and suggests that the classical parameters might well substitute for the more sophisticated novel methods. On the other hand, coordinated expression of markers with ER or other standard parameters does not necessarily imply that those genes could simply be replaced by respective parameters. A positive correlation of some markers with Her-2 in predictive signatures supports the observation that even Her-2 itself or at least Her-2-dependent genes plays a crucial role in this setting. Pusztai et al. could demonstrate for Her-2 positive breast cancers that Her-2 itself is not the top predictor for response to trastuzumab treatment [25]. But other markers showing a higher predictive value compared to Her-2 are also Her-2-dependent. This is an important issue since new powerful biomarkers are urgently awaited and microarray technology could have important contribution in this regard.

In conclusion microarray analysis revealed high concordance with standard parameters as e.g., ER, PR and Her-2, thus demonstrating the principal validity of the method. However, the reproducibility of several previously described signatures on one platform is limited even in a homogeneously treated patient cohort. This observation might be due to inter-platform differences as well as specific therapeutic settings for which those signatures have been established originally. Our data demonstrate that estrogen regulation and proliferation are the major driving forces in gene expression and it raises the question if accurate and thoroughly determined standard parameters could have a comparable power in terms of disease outcome and prediction of therapeutic success.

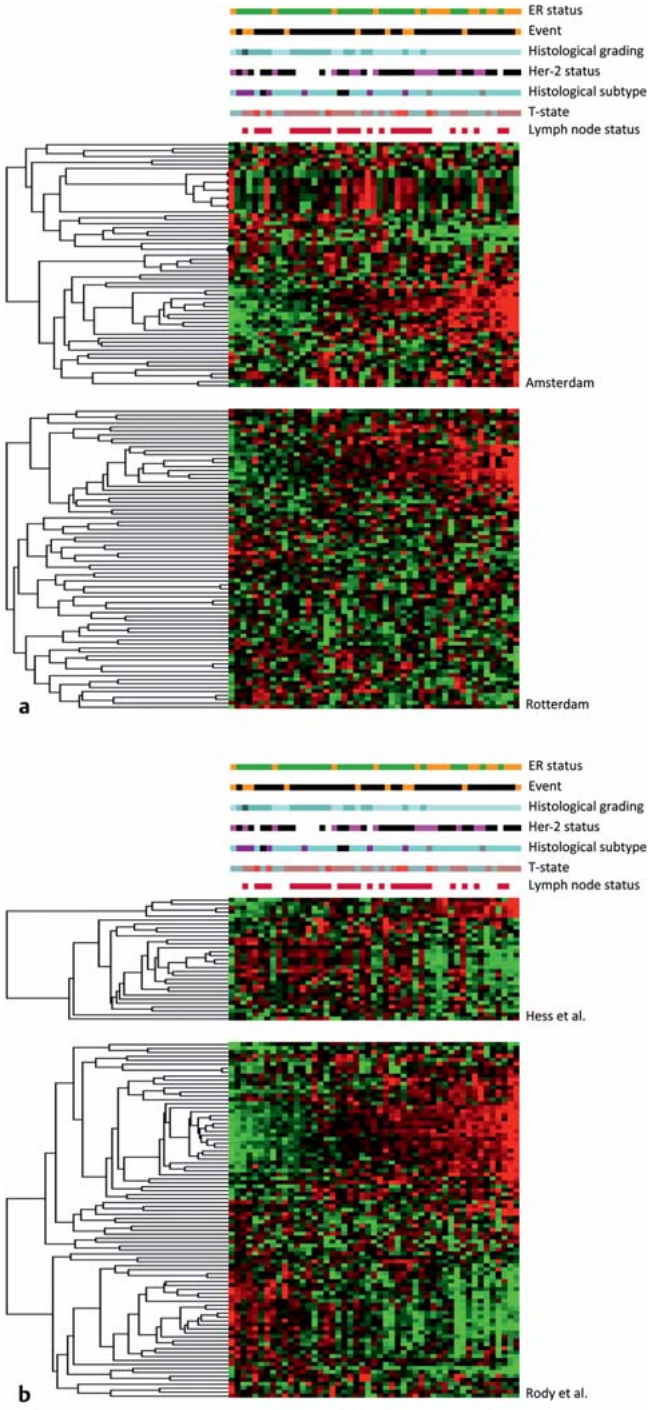


Fig. 5 a and **b** Impact of proliferation on prognostic and predictive gene signatures. Tumor samples were sorted from left to right according to the "Genomic Grade Index (GGI)" [15] as given in ◻ **Fig. 4** above. Subsequently, the prognostic (**a**) and predictive (**b**) gene signatures from ◻ **Fig. 3** were applied to the dataset to reveal the influence of proliferation on the markers in the signatures (clinical and pathological parameters of the tumors are given above the expression matrix).

Acknowledgements

We thank Samira Adel and Katherina Kourtis for expert technical assistance. This work was supported by grants from the Deutsche Krebshilfe, the Margarete Bonifer-Stiftung, Bad Soden, the Banss Stiftung, Biedenkopf, and the Dr. Robert Pfleger-Stiftung, Bamberg. This publication contains parts of the MD thesis of Florian Pobitschka.

Conflict of Interests

None of the authors have competing interests.

References

1 *Perou CM, Jeffrey SS, van de Rijn M et al.* Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. Proc Natl Acad Sci USA 1999; 96: 9212–9217
2 *van't Veer LJ, Dai H, van de Vijver MJ et al. Gene expression profiling predicts clinical outcome of breast cancer.* Nature 2002; 415: 530–536
3 *van de Vijver MJ, He YD, van't Veer LJ et al.* A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002; 347: 1999–2009
4 *Sorlie T, Perou CM, Tibshirani R et al.* Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 2001; 98: 10869–10874
5 *Ahr A, Holtrich U, Solbach C et al.* Molecular classification of breast cancer patients by gene expression profiling. J Pathol 2001; 195: 312–320
6 *Ahr A, Karn T, Solbach C et al.* Identification of high risk breast-cancer patients by gene expression profiling. Lancet 2002; 359: 131–132
7 *von Minckwitz G, Raab G, Caputo A et al.* Doxorubicin with cyclophosphamide followed by docetaxel every 21 days compared with doxorubicin and docetaxel every 14 days as preoperative treatment in operable breast cancer: the GEPARDUO study of the German Breast Group. J Clin Oncol 2005; 23: 2676–2685
8 *Remvikos Y, Beuzeboc P, Zajdela A et al.* Correlation of pretreatment proliferative activity of breast cancer with the response to cytotoxic chemotherapy. J Natl Cancer Inst 1989; 81: 1383–1387
9 *Bottini A, Berruti A, Bersiga A et al.* Effect of neoadjuvant chemotherapy on Ki67 labelling index, c-erbB-2 expression and steroid hormone receptor status in human breast tumours. Anticancer Res 1996; 16: 3105–3110
10 *Collecchi P, Baldini E, Giannessi P et al.* Primary chemotherapy in locally advanced breast cancer (LABC): effects on tumour proliferative activity, bcl-2 expression and the relationship between tumour regression and biological markers. Eur J Cancer 1998; 34: 1701–1704
11 *Elston CW, Ellis IO.* Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 1991; 19: 403–410
12 *Paik S, Shak S, Tang G et al.* A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004; 351: 2817–2826
13 *Robbins P, Pinder S, de Klerk N et al.* Histological grading of breast carcinomas: a study of interobserver agreement. Hum Pathol 1995; 26: 873–879
14 *Hopton DS, Thorogood J, Clayden AD et al.* Observer variation in histological grading of breast cancer. Eur J Surg Oncol 1989; 15: 21–23
15 *Sotiriou C, Wirapati P, Loi S et al.* Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. J Natl Cancer Inst 2006; 98: 262–272
16 *Gruvberger S, Ringnér M, Chen Y et al.* Estrogen receptor status in breast cancer is associated with remarkably distinct gene expression patterns. Cancer Res 2001; 61: 5979–5984
17 *Ein-Dor L, Kela I, Getz G et al.* Outcome signature genes in breast cancer: is there a unique set? Bioinformatics 2005; 21: 171–178
18 *Rody A, Holtrich U, Gaetje R et al.* Poor outcome in estrogen receptor-positive breast cancers predicted by loss of plexin B1. Clin Cancer Res 2007; 13: 1115–1122
19 *Rody A, Karn T, Solbach C et al.* The erbB2+ cluster of the intrinsic gene set predicts tumor response of breast cancer patients receiving neo-

adjuvant chemotherapy with docetaxel, doxorubicin and cyclophosphamide within the GEPARTRIO trial. Breast 2007; 16: 235–240
20 *Eisen MB, Spellman PT, Brown PO et al.* Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 1998; 95: 14863–14868
21 *Wang Y, Klijn JG, Zhang Y et al.* Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. Lancet 2005; 365: 671–679
22 *Sotiriou C, Wirapati P, Loi S et al.* Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. J Natl Cancer Inst 2006; 98: 262–272
23 *Hess KR, Anderson K, Symmans WF et al.* Pharmacogenomic predictor of sensitivity to preoperative chemotherapy with paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide in breast cancer. J Clin Oncol 2006; 24: 4236–4244
24 *Rody A, Karn T, Munnes M et al.* Predictive gene signatures for response to neoadjuvant TACchemotherapy from expression profiling. Breast Cancer Res Treatm 2005; Vol. 94, Suppl. 1, abstract 5046
25 *Pusztai L, Anderson K, Hess KR.* Pharmacogenomic predictor discovery in phase II clinical trials for breast cancer. Clin Cancer Res 2007; 13: 6080–6086