Gene Expression Profiling to Predict Chemotherapy Response in Primary Breast Cancer

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Key Words

Gene expression signature · Pathologic complete response · Response prediction · Neoadjuvant chemotherapy · Primary breast cancer

Summary

Predictive factors to guide selection of the optimal chemotherapy for the individual patient with primary breast cancer are lacking. Neoadjuvant systemic treatment (NST) provides the opportunity to directly assess tumor response to therapy. As only the achievement of pathologic complete response (pCR) is associated with improved survival, it can serve as a surrogate for optimal chemotherapy efficacy. The direct assessment of the achievement of pCR to a particular treatment enables researchers to rapidly evaluate and validate new predictive factors. Recent technological advances have enabled researchers to scan the expression pattern of thousands of genes, i.e. thousands of possible predictive factors, at once. Thus far, 2 independent trials have been successful in identifying and validating gene expression signatures which predict the achievement of pCR to NST with high overall accuracy. The predictive value of these signatures outperformed single conventional parameters in multivariate analysis. Confirmation trials are ongoing to evaluate whether gene expression signatures can discriminate between likelihoods of response to different chemotherapy regimens. This would allow optimal treatment selection, thereby protecting patients from useless toxicity as well as dramatically reducing treatment costs. Gene expression profiling has the potential to help tailoring systemic breast cancer treatment, which is a major goal of current translational research.

Schlüsselwörter

Genexpressionssignatur · Pathologische Komplettremission · Vorhersage des Ansprechverhaltens · Neoadjuvante Chemotherapie · Primäres Mammakarzinom

Zusammenfassung

Prädiktive Faktoren zur individuellen Auswahl der optimalen Chemotherapie für Patientinnen mit primärem Mammakarzinom fehlen. Die neoadjuvante, systemische Therapie (NST) ermöglicht es, das Therapieansprechverhalten eines Tumors direkt zu beurteilen. Da nur das Erreichen der pathologischen Komplettremission (pCR) mit verbessertem Überleben einhergeht, dient die pCR als Surrogatmarker für eine optimale Chemotherapie-Effektivität. Indem es Wissenschaftlern möglich ist, das Erreichen der pCR für eine spezifische Therapie direkt zu erfassen, können neue prädiktive Faktoren rasch evaluiert und validiert werden. Der jüngste technologische Fortschritt ermöglicht es nun, das Expressionsmuster tausender Gene, d.h. tausender möglicher prädiktiver Faktoren, gleichzeitig zu beurteilen. In 2 Studien konnten bisher Gen-Expressions-Signaturen erfolgreich identifiziert und validiert werden, welche das Erreichen einer pCR durch eine NST mit hoher Genauigkeit vorhersagen. In der multivariaten Analyse übertraf der prädiktive Wert dieser Signaturen den Wert einzelner konventioneller Parameter. Derzeit durchgeführte Validierungsstudien sollen klären, inwieweit Genexpressionssignaturen das Ansprechverhalten auf unterschiedliche Chemotherapieprotokolle vorhersagen und unterscheiden können. Hierdurch würde eine optimale Therapieauswahl ermöglicht, welche sowohl Patienten vor unnötiger Toxizität bewahren als auch die Behandlungskosten dramatisch reduzieren könnte. Genexpressionsprofile könnten es zukünftig ermöglichen, die systemische Therapie des Mammakarzinoms individuell zuzuschneiden, ein Hauptziel der aktuellen translationalen Forschung.

Introduction

Since the mid 1990s, breast cancer mortality is decreasing in Western countries due to several factors, including survival benefits from adjuvant systemic treatment [1]. According to current results of the Early Breast Cancer Trialists' Collaborative Group meta analyses, however, only a small proportion of patients do benefit from such adjuvant systemic therapy [2]. In particular, the benefits of adjuvant chemotherapy must be balanced against its toxicities which include both psychosocial and physical effects. Most of these adverse events are short-term, but long-term effects are still being discovered. So far, chemotherapy in early breast cancer is applied empirically. Established predictive factors show only weak association with response, and better predictive markers are urgently needed [3]. Thus far, there is no prospectively tested, clinically useful predictor of response to any cytotoxic drug available [4].

In early breast cancer, neoadjuvant (preoperative) and adjuvant (postoperative) chemotherapy offers patients a similar chance of survival [5]. Neoadjuvant systemic treatment (NST), however, has advantages over adjuvant therapy. Besides the increased ability to perform breast-conserving surgery, the rapid assessment of response to a particular treatment regimen enables researchers to rapidly evaluate and validate new predictive factors for response and resistance. This is of particular importance, as pathologic complete response (pCR), defined as disappearance of all invasive cancer cells in the breast after NST, strongly correlates with improved long-term survival [6–8]. pCR is currently considered the best surrogate parameter for cure after NST [9]. On the other hand, patients not achieving pCR might not generally benefit, as they experience the same long-term outcome as those who do not respond [10].

Gene Expression Signatures as Prognostic and Predictive Factors

Recent technological advances have enabled researchers to scan the expression pattern of thousands of genes, i.e. thousands of possible prognostic or predictive factors, in individual tumors at once. In retrospective analyses, microarray gene expression profiling has indicated that there may be complex molecular gene expression signatures that are predictive of outcome. The results of a reverse transcriptase polymerase chain reaction (RT-PCR) assay of 21 prospectively selected genes in paraffin-embedded tumor tissue, i.e. the 21-gene recurrence score (RS), has been shown to be associated with prognosis in patients who have estrogen receptor (ER) positive, node negative breast cancer and are treated with tamoxifen [11]. Independent of age and tumor size, patients with a high RS had the highest distant recurrence rate. In addition, a further analysis finds that patients with the high-RS tumors, who are treated with chemotherapy, have a lower recurrence

Fig. 1. Treatment protocol to discover and validate a gene expression signature predictive for the achievement of pathologic complete response (pCR) following neoadjuvant chemotherapy (NST) with epirubicin (E), docetaxel (Doc) and gemcitabine (G).

rate compared with those not treated with chemotherapy. Conversely, patients with low RS do not benefit from chemotherapy [12]. Using RNA microarray analysis, 2 gene expression signatures containing 70 and 76 genes, respectively, have been identified, which represented by far the strongest predictors of the likelihood of distant recurrence in multivariate analysis, considering all established prognostic factors [13, 14]. Finally, Sotiriou et al. [15] created a 97 genes containing gene expression grade index that appeared to reclassify patients with grade 2 tumors into 2 groups with high versus low risk of recurrence.

Gene Expression Profiling to Predict pCR to NST

In order to assess the utility of gene expression signatures as predictors of pCR following NST in primary breast cancer, we launched a study in January 2002. Patients were eligible if they had a biopsy-proven T2–4 N0–2 M0 primary breast cancer with sufficient amount of snap-frozen tissue collected for microarray analysis, were 18–65 years old, had an Eastern Cooperative Oncology Group performance status of 0–2, had not received any prior therapy for breast cancer, had adequate organ function and gave written informed consent. Following core-cut biopsy for diagnosis and tissue collection, a group of 52 patients received 5 cycles of gemcitabine and epirubicin, sequentially followed by 4 cycles of docetaxel (GE→Doc) administered every 2 (q2) weeks before surgery [16]. This group was used to determine the pCR predictive gene expression signature (training set). A second independent cohort of 48 patients was used to validate the signature. These patients received gemcitabine, epirubicin and docetaxel as a triplet q3 weeks, gemcitabine was repeated on day 8 of each cycle

Fig. 2. Validation of the pCR predictive gene expression signature on the validation set of 48 patients who received triple therapy with gemcitabine, epirubicin, and docetaxel. Performance of the gene expression signature shown for both patient groups (pCR and non-pCR) and in total (CI = confidence interval; pCR = pathologic complete response; PPV = positive predictive value; NPV = negative predictive value).

Table 1. Multivariate analysis of predictive factors on the validation set (48 patients who received triple therapy with gemcitabine, epirubicin, and docetaxel)

Predictive factor	Odds ratio (95% CI)	p value
Signature positive vs. negative	38.3 (2.4–6,560)	0.01
HER2 3+/2+(FISH+) vs. 0/1+/2+(FISH-)	10.5 (1.3–151)	0.03
Tumor size ≤ 5 vs. > 5 cm	6.9 (0.3–1128)	0.22
Histological grade 3 vs. 1/2	5.0 (0.4–∞)	0.24
ER/PgR _{IHC} 0 vs. ≥ 1	2.0 (0.1–33.3)	0.64
PR or CR after 6 weeks of NST vs. NC	1.7 (0.1–50.0)	0.70

CI = Confidence interval; CR = complete remission; ER = estrogen receptor; FISH = fluorescence in situ hybridization; IHC = immunohistochemistry; NC = no change; NST = neoadjuvant systemic treatment; PgR = progesterone receptor; PR = partial remission.

(GEDoc) (validation set) (fig. 1) [17]. Considering all 100 patients, median age was 48 years and median tumor size was 3.5 cm as measured by sonography. Most patients had a ductal invasive breast cancer, approximately 50% were clinically node negative and had a grade 3 carcinoma. 29% of the tumors did not express any hormone receptors as determined by immunohistochemistry (IHC), and 20% overexpressed HER2/neu, defined as 3+ expression by HercepTest™ (Dako Deutschland GmbH, Hamburg, Germany). No viable tumor cells were detected in the breast tissue removed from 17 patients after NST, and another 7 patients had only carcinoma in situ in the breast yielding an overall pCR (ypT0 + ypTis) rate of 24% [18].

Fig. 3. Treatment protocol of H3C-ME-S080.

For gene expression analysis, RNA was extracted from the deep frozen biopsies. As total RNA yield from biopsies was insufficient for direct labeling, RNA was amplified by a protocol developed in our laboratories and labeled using Cy3 or Cy5 linked nucleotides [19]. Human reference RNA was amplified and labeled with the same protocol. Labeled cDNA samples from each biopsy were hybridized against labeled reference cDNA on microarrays containing 21,329 gene-specific 70mer oligonucleotides. The Support Vector Machine algorithm was applied in combination with a recursive feature elimination approach for building a predictive model based on these data. A cross validation of predictive models based on differently sized sets of genes was performed. Cross validation of predictive models using subsets of genes of different sizes showed that the number of genes used for the model can be reduced to 512 without decreasing its performance. The 512 genes that formed our pCR predictive gene expression signature predicted the achievement of pCR in the independent validation set of 48 patients with a sensitivity of 78% and a specificity of 90% for an overall accuracy of 88% (95% confidence interval (CI), 75–95%). The positive and negative predictive value of this gene expression signature for the achievement of pCR was 64 and 95%, respectively (fig. 2) [18]. Overall accuracy and positive predictive value of the signature in predicting pCR was superior to those data of conventional parameters, i.e. ductal invasive histology, histological grade 3, achievement of a partial remission (PR) after 6 weeks of NST or absence of hormone receptors. Only HER2 overexpression achieved similar results in predicting pCR as compared to the gene expression signature. The multivariate logistic regression analysis considering the gene signature, HER2 expression, initial tumor size as measured by ultrasound, histological grade, hormone receptor status, and PR after 6 weeks of NST revealed that only the gene expression signa-

Table 2. Studies to evaluate gene expression signatures as predictive factor for pathologic response to neoadjuvant chemotherapy of primary breast cancer

Reference	Endpoint	Ntot	Nv	NST	Genes, n	Accuracy, % (95% CI)	PPV (NPV), %
[21]	ypT0/is	42	18	Pac→FAC	74	78 (52–94)	100 (73)
[20]	ypT0/is + 'scattered tumor cells'	48		AC, ADoc	no predictive signature		
[18]	ypT0/is	100	48	GE→Doc, GEDoc	512	88 (75–95)	64 (95)
[22] (extension of [21])	ypT0/is plus ypN0	133	51	Pac→FAC	26	76 (62–87)	52 (96)

A = Doxorubicin; C = cyclophosphamide; CI = confidence interval; Doc = docetaxel; E = epirubicin; F = fluorouracil; G = gemcitabine;

N_{tot} = total number of patients; N_v = number of patients within validation set; NPV = negative predictive value; NST = neoadjuvant chemotherapy;

Pac = paclitaxel; PPV = positive predictive value.

ture (hazard ratio (HR) 38.3, $p = 0.01$) and HER2 overexpression (HR 10.5, $p = 0.03$) were independent predictors for the achievement of pCR (table 1) [18].

Besides our group, to date only 2 other collaborative groups have aimed at the identification of gene expression signatures predicting pathological response to NST (table 2). Notably, one of these groups did not use the endpoint of pCR which is the only one that has been shown to prospectively correlate with patient survival [8, 10]. Hannemann et al. [20] pooled pCR and near pCR for the endpoint of their trial. Because near pCR is not likely to correlate with patient survival, this might explain why the authors were unable to identify a gene signature within their training set correlating with therapy response. Only Hess et al. [22] (update/extension of Ayers et al. [21]) used the endpoint pCR defined as no invasive tumor residue in breast and axilla. Using microarray gene expression profiling, they identified a gene expression signature containing 26 genes predictive for the achievement of pCR, following NST with paclitaxel followed by fluorouracil, doxorubicin and cyclophosphamide (Pac→FAC). Within an independent validation set of 51 patients, this signature predicted pCR with an overall accuracy of 76% (95% CI, 62–87%). The positive and negative predictive value of this gene expression signature for the achievement of pCR was 52 and 96%, respectively. It showed a significantly higher sensitivity (92 vs. 61%) than a clinical predictor including age, grade, and ER status. The overall accuracy, however, was not improved (76 vs. 78%), and a formal statistical comparison of the 3 distinct receiver operating characteristic (ROC) curves for the genomic predictor, the combined clinical and genomic predictor, and the clinical-only predictor showed no significant difference between the curves [22]. Nevertheless, it is justified to conclude that i) transcriptional profiling can identify gene expression signatures in primary breast cancer that predict pCR to NST with a high overall accuracy, and ii) the predictive value of this gene expression signatures might outperform single conventional predictive parameters like hormone receptor expression and grade.

Why are the Signatures Totally Different?

There are no overlapping genes between the pCR predictive gene signature of Hess et al. [21, 22] and our group. This might be due to different tissue sources (fine-needle aspirates vs. core-cut biopsies) and gene expression-profiling platforms. Gianni et al. [23], however, showed a 30% overlap in the predictive value of single genes across different platforms (microarray vs. RT-PCR) and materials (fresh frozen tissue vs. formalin-fixed paraffin-embedded tissue). In addition, for complex diseases like breast cancer several sets of outcome-predictive genes may exist as an answer to the same scientific question, i.e. achievement of pCR following NST, because many genes can show a tight correlation to patient survival [24]. Furthermore, the genes of a predictive signature are not necessarily the key players involved in the etiology of the investigated disease [25]. The task to exclusively find all genes with biologic importance for a given disease is different from the task of finding a set of genes with discriminative power.

On the other hand, it is currently not possible to determine to which degree the predictive gene signature is discovering response to NST in general or specific effects of the administered drugs. Hess et al. [22] and our group [18] applied different treatment regimens (Pac→FAC vs. GE→Doc/GEDoc), and the non-overlapping signatures might simply indicate that gene signatures predictive for NST response contain drug-specific features. Currently, we are conducting a multicenter international, randomized phase II trial of 2 different sequential regimens of NST for patients with primary, operable breast cancer (trial H3E-MC-S080). One regimen comprises doxorubicin plus pemetrexed followed by docetaxel (AP→Doc), and the other one doxorubicin plus cyclophosphamide followed by docetaxel (AC→Doc). The primary objectives of this study are to assess the pCR rate of the 2 regimens and to identify differentially expressed genes predictive of the achievement of pCR following either AP→Doc or AC→Doc. Secondary clinical objectives include assessments of tumor response by Response Evaluation Criteria In Solid Tumors (RECIST) cri-

teria [26], the rate of histologically negative axillary lymph nodes, disease-free survival, and safety. Secondary objectives for the translation research include the identification and validation of gene expression signatures predictive for the achievement of pCR, chemotherapy resistance, and clinical response following either AP→Doc or AC→Doc. To date, more than half of the 256 patients planned for this study have been enrolled, which is a faster rate than initially expected, and more than 80% of the baseline samples collected are of high quality and evaluable for pharmacogenomic analysis. We anticipate this study will provide more detailed knowledge about both the drug and treatment specificity of either a single gene expression or a gene signature.

Conclusion

If confirmed in such large, prospective, randomized, multicenter studies with different treatment arms, which proved reproducibility, practical robustness, and chemotherapy specificity of the new marker sets in response prediction, gene expression profiling will impact chemotherapy treatment decision making in early breast cancer, leading to more tailored approaches, thereby protecting patients from useless toxicity as well as reducing treatment costs. To individualize therapy is a major goal of current translational research in systemic breast cancer treatment and the common aim of collaborating groups.

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