Transcriptional profile and response to neoadjuvant chemotherapy in breast cancer

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SUMMARY

Objective: To improve the accuracy predictive models of response to neoadjuvant chemotherapy in breast cancer, cDNA microarray technology was used to study tumor transcriptional profile. Gene signatures associated with predicting the response to neoadjuvant chemotherapy are the subject of this review. **Methods:** The data base http://www.ncbi.nlm.nih.gov/pubmed/ search was conducted by using the words "breast cancer" AND "neoadjuvant/primary chemotherapy" AND "gene expression profile/microarray". After excluding the repeats and selecting the publications considered most relevant by the authors to be presented, 279 publications were retrieved. **Results:** The number of publications regarding this subject has been increasing over the years, reaching over 50 in 2010, including the response to different chemotherapeutic drugs, such as anthracyclines and taxanes either alone or in combination. The first studies are from early last decade and used microarray platforms produced by the investigators. Recent studies have used commercial microarray platforms whose data have been stored in public databases, allowing for the analysis of a higher number of samples. Several transcriptional profiles associated with the complete pathological response were identified. Other authors used the clinical response to treatment as an endpoint, and, in this case, a predictive panel of resistance to the chemotherapeutic regimen at issue was determined. This is also a key issue, as it can contribute to individualize treatment, allowing patients resistant to a certain chemotherapeutic agent to be offered another therapeutic regimen. **Conclusion:** Identifying patients responsive to chemotherapy is of essential interest and despite major steps have been taken, the issue warrants further studies in view of its complexity.

Keywords: Breast neoplasms; neoadjuvant therapy; drug therapy; prognosis; molecular biology.

Introduction

Adjuvant chemotherapy reduces the mortality from breast cancer, being indicated according to the patient and tumor characteristics. This includes age, menopause status, tumor size, node involvement, differentiation grade, estrogen and HER2/neu receptor expression. A challenge to be equated is that survival advantage associated with adjuvant chemotherapy is described in a group of patients, being evaluated in each individual particularly just as a probability. Thus, we should consider an individual patient risk for unnecessary toxicity, since she would be cured after the surgical procedure alone or, on the other hand, she would not benefit from chemotherapy, having a relapse even if it is used. Another issue is whether there are advantages in using certain classes of chemotherapeutic agents, such as taxanes and anthracyclines in each patient. Therefore, it is highly desirable to identify highly accurate predictive markers of chemotherapy benefit.

Thus, neoadjuvant chemotherapy is an excellent opportunity to study biomarkers, as pathological complete response (pCR) is an intermediate endpoint having high correlation with a long survival and, therefore, a good prognosis[1-3]. This makes translational study conduction easier, as patients do not need to be followed by long periods. Neoadjuvant chemotherapy is an option for unresectable locally advanced disease, breast inflammatory carcinoma and also an initial stage disease. In patients with a resectable disease, T1-T3 and N0-2, neoadjuvant chemotherapy regimens including four anthracycline cycles have been associated with a high objective clinical response rate (complete and partial response), ranging between 49% and 85%, but a low pathological complete response rate (4%-13%) and low disease progression rate (1%-3% of patients)[1-3]. The sequential use of anthracyclines and taxanes (paclitaxel or docetaxel) rises the objective response rate, as well as the pathological complete response rate, with the latter reaching 26%-34%, associating with the lowest percentage of patients with a node involvement compared with an anthracycline-based regimen[2,4,5]. Also the concomitant administration of either anthracycline and taxane (doxorubicin and paclitaxel) or taxane and non-anthracyclic agent (paclitaxel and cisplatin) induces high objective clinical response rate (89%-91%) and pathological complete response rate in 14% and 24% of cases, respectively[6,7].

Isolated tumor markers non-predictive of a pathological complete response are rare. Among them, we can mention the HER2 tumor expression in the case of trastuzumab therapy. In tumors with HER2/neu overexpression, the combination of trastuzumab with neoadjuvant chemotherapy is observed to translate into a high pathological complete response rate, i.e., 65%[8]. Further markers are associated with the pathological complete response (pCR) rate, such as no ER expression, anaplastic histology, high proliferation index and reduced tumor size[5]. The pCR to neoadjuvant therapy with doxorubicin and cyclophosphamide (AC) was found to be correlated to the specific subtype of breast cancer and occurs more frequently in HER2(+)- (36%) and basal-like (27%) tumors, in contrast with a luminal B (15%) and an luminal A (0%). The objective clinical response (complete response and partial response) rate is also variable according to the subtypes and in ER(+)-tumors (estrogen receptor-positive) ranges from 39% (luminal A) to 58% (luminal B) and, in ER(-) tumors, from 70% (HER2-positive) to 85% (basal-like)[9].

In the attempt to improve response predictive model accuracy to neoadjuvant chemotherapy, analyses using cDNA microarray technology were conducted. The prospect is this methodology, which allows a concomitant analysis of the tumor overall gene expression, in contrast with standard immunohistochemical tests, in which the expression of only a number of proteins could be analyzed, gives rise to advances in the identification of patients responsive to chemotherapy. The evaluation of gene signatures associated with prediction of response to neoadjuvant chemotherapy is this review target.

Methods

A search in the database http://www.ncbi.nlm.nih.gov/pubmed/ was performed by using the keywords: 1) "breast cancer" AND "neoadjuvant chemotherapy" AND "gene expression profile"; 2) "breast cancer" AND "neoadjuvant chemotherapy" AND "microarray"; 3) "breast cancer" AND "primary chemotherapy" AND "microarray"; 4) "breast cancer" AND "primary chemotherapy" AND "gene expression profile". A total of 279 publications were retrieved in this search, excluded the repeats, with those considered more relevant by the authors being chosen for exposition. Interestingly, several publications concerning chemotherapy other than neoadjuvant chemotherapy were retrieved, but they were not considered in this report.

Results

The number of publications on the considered subject has been increasing over the years (Figure 1), reaching over 50 in 2010. The first studies are from early last decade and attempted to identify transcriptional patterns which were predictive of response to isolated drugs (anthracycline or taxane) or in combinations [AC; 5-fluorouracil, epirubicin and cyclophosphamide, FEC; paclitaxel + FAC (5-fluorouracil, doxorubicin and cyclophosphamide); gemcitabine, epirubicin an docetaxel] (Table 1). Tumor samples obtained before the beginning of chemotherapy were used to analyze the gene expression by using customized microarray platforms, i.e., samples produced by the investigators. In these studies, samples were analyzed in a training group and they were used to identify a gene expression pattern. Next, this transcriptional pattern was tested in a second group named validation group, with the model accuracy
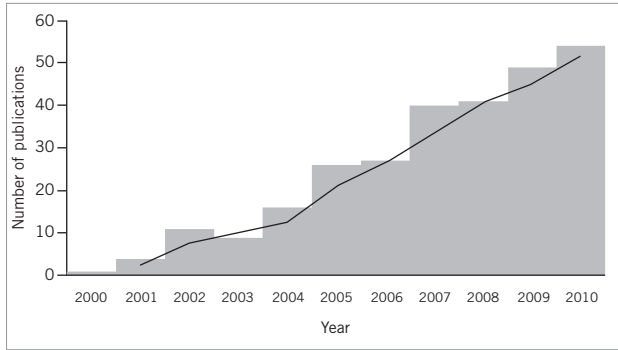
Figure 1 – Number of yearly publications related to breast cancer, gene expression profile (microarray) and primary/neoadjuvant chemotherapy (PubMed database).

in predicting the response being evaluated. In this setting, we can mention pioneering studies by Sotiriou[10], Chang[11], Zembutsu[12], Ayers[13], Hess[14], Thuerigen[15], including 10-81 patients in training groups and 6-51 patients in validation groups, and they identified different transcriptional profiles associated with the response with a 78%-88% accuracy.

In the Brazilian population, our group tried to identify gene expression patterns allowing the tumors to be classified according to their aggressiveness[16] and their response to neoadjuvant chemotherapy[17]. In this recent study, 44 patients with a disease in clinical stage II/III receiving 4 AC cycles were included. The clinical response determination followed RECIST criteria[18] and 35 patients were classified as responsive and nine (7 in training group and 2 in validation group) as nonresponsive. Ten transcript triplets separating the samples with high accuracy were identified. Among them, PRSS11 (serine protease 11 or ligand protein protease 5 of the insulin-like growth factor), MTSS1 (metastasis suppressor 1) and CLPTM1 (cleft lip and palate-associated transmembrane protein), whose expression sorted correctly all samples out in the training group. As an extended study, we tried to determine whether this gene triplet expression could be assessed by quantitative RT-PCR, a method much more available, maintaining the treatment response predictive power. The expression of nine genes included in five response rater gene triplets was

Table 1 – Transcriptional patterns studies as predictive of response to neoadjuvant chemotherapy

Author/Year	n (training/validation)	Regimen	Gene signature	Accuracy
Sotiriou 2002[10]	10	Anthracycline	37G	–
Chang 2003[11]	24/6	Docetaxel	92G	88%
Ayers 2004[13]	24/18	T + 4 FAC	74G	78%
Bertucci 2004[20]	26	Doxorubicin	–	–
Hannemann 2005[21]	31	AC or AD	–	–
Hess 2006[14]	82/51	T/FAC	30G	95%
Thuerigen 2006[15]	5248	GE or Docetaxel	512G	88%
Folgueira 2006[16]	31/13	4 ac	Triplets	84.6%
Bonnefoi 2007[22]	125	FEC or TET	–	79-80%
Straver 2009[29]	167	–	MammaPrint	–
Farmer 2009[28]	63/51	FEC	Stromal metagene	AUC 0.7
Zembutsu 2009[12]	20	Docetaxel	9G	–
Williams 2009[34]	275	FAC	GEM (gene expression model)	S 71%, Sp 53%, PPV 32%, NPV 85%
Tabchy 2010[35]	138 (T/FAC)	T/FAC	DLDA 30 (30 genes)	PPV 38%, NPV 88%
Korde 2010[36]	21	TX	39 categories	–
Ronde 2010[30]	191	AC/TX or Trastuzumab + T	Melecular subtyping	–
Lin 2010[37]	37/13	ET/Zoledronic Acid	23G	92%
Lee 2010[38]	100	T/FAC	Nomogram/DLDA30/in vitro COXEN/in vivo COXEN	Nomogram//DLDA30 AUC 0.73
Rodriguez 2010[26]	105/28	FEC or AC or TET	69G	AUC 0.79 (AC)
Bauer 2010[39]	14	TE T/radiotherapy	IG (MAP2)	–
Bianchini 2010[25]	233	T/FAC	High MKS (mitosis – associated kinase score)	RR 2.6 (associated with pCR)
Barros Filho 2010[19]	14	4AC	Triplets	71%
Chen 2011[40]	55/55	T Cb	20G	80%
Naoi 2011[41]	50/34	T-FEC	106G	VPN > 90%
Fan 2011[42]	150/75	T/FAC		AUC ~ 0.8

G, genes; N, number of patients; F, 5-fluorouracil; A, doxorubicin; C, cyclophosphamide; T, paclitaxel; G, gemcitabine; E, epirubicin; Cb: carboplatin; X, capecitabina; RR, relative risk, AUC, area under curve; pCR, pathological complete response; NPV, negative predictive value; PPV, positive predictive value; S, sensitivity; Sp, specificity.

analyzed in another sample group with 14 patients treated with neoadjuvant therapy based on AC. The expression of two out of five triplets attributed a correct classification in 71% of samples in the biological validation group[19] (Table 1), indicating these transcripts are associated with a response to chemotherapy.

A number of other authors included patients with specific characteristics; Bertucci *et al.*[20] determined a transcriptional pattern associated with pathological complete response to doxorubicin in patients with inflammatory carcinoma. CDKN1B (p27), a cell cycle progression inhibitor, was observed to have a greater expression in tumors with pathological complete response, as well as genes encoding chemokines, cytokines, and cytokine receptors, such as CSF1R, CCL2, CCL3, MMP9, suggesting a host immune system role in eradicating tumor following chemotherapy[20].

In prior cases, we were concerned to identify a predictive signature of response a specific chemotherapy regimen. Another investigation approach evaluated whether response profiles would be specific or shared by different chemotherapy regimens. With this purpose, Hannemann *et al.*[21] tried to classify the gene profile in 31 tumor samples from AC- or AD- (doxorubicin and docetaxel) treated patients; however, they were not successful. Following this hypothesis, but then in a specific tumor subgroup, i.e., ER-negative (which are believed to have a higher pCR than ER-positive tumors), Bonnefoi *et al.*[22] tried to set a neoadjuvant chemotherapy pCR-predictive gene signature consisting of 6 FEC or TET (e docetaxel cycles followed by 3 epirubicin + docetaxel cycles). One hundred and twenty-five Affymetrix X3P microarray hybridized samples were studied. The analysis was based on the combination of an *in vitro* cell culture sensitivity profile to a specific drug; this data was previously reported[23]. The predictive signature showed an accuracy to predict response of 79% and 80% in FEC and TET groups, respectively. However, a similar study conducted by another group, considering the same hypothesis and using a gene panel identified by its correlation with breast cancer lineage sensitivity to four chemotherapeutic agents alone, did not reproduce the response prediction outcomes in patients treated with a combination of these drugs (paclitaxel followed by FAC)[24].

More recent studies, published in 2009-2011, use commercial microarray platforms, such as Mammaprint, Affymetrix HG-U133A, for gene expression analysis. From results obtained more homogenously and stored in public data bases, studies with bioinformatic analysis of a higher number of samples present in the data bases were generated. Generally, pathways that might be involved in mechanisms associated with proliferation process, DNA repair, chemotherapy resistance, and others are addressed.

In one of these studies, Bianchini *et al.*[25] evaluated the profile of mitosis-associated kinase expression and observed that a high score was associated with a higher pCR probability, but also with a poorer prognosis, in ER-positive tumors. Interestingly, this profile, contrary to expectations, is not related to a pathological complete response and a good prognosis.

Drawing on this *in silico* database analysis strategy, Rodriguez *et al.*[26] analyzed the profile of triple negative tumors regarding the expression profile of BRCA1 mutation-associated DNA repair genes. In this case, patients treated with several chemotherapy regimens (FEC, AC, TET) were included, and an expression defective pattern of this repair gene panel was associated with a response to doxorubicin and resistance to taxanes. Staying on *in silico* analysis and using gene set coordinate expression analysis, Iwamoto *et al.*[27] suggest the profile associated with cell proliferation is correlated to chemotherapy response in ER-positive tumors, but not in ER-negative tumors. In addition, Farmer *et al.*[28] found stroma gene expression is associated with ER-negative tumor resistance to neoadjuvant treatment with two regimens containing different anthracyclines, FEC and T-FAC. Interestingly, this stroma signature did not predict relapse-free survival in patients who did not undergo chemotherapy, indicating this is not a prognostic factor, but a treatment response predictive factor[28].

Another experimentation attack involved the analysis of profiles already established for their relation to disease prognosis. Studies such as that by Straver *et al.*[29] tested the hypothesis that the prognostic signature of 70 Mammaprint genes would also be predictive of a response to chemotherapy. For this purpose, 166 neoadjuvant chemotherapy-treated patients (various regimens) were included, with 86% and 14% being classified as having poor and good prognosis, respectively. No patients with a good prognosis signature (0/23) had a pCR, in contrast with 29/144 patients with a poor prognosis. Thus, Mammaprint signature seems to be predictive of a chemotherapy benefit[29].

Otherwise, Ronde *et al.*[30] analyzed whether transcriptional profile could additionally contribute to chemotherapy response prediction in histological subtypes defined by immunohistochemistry (triple negative tumors; HER2-positive; luminal [ER-positive/HER2-negative] tumors). In this study, 195 tumors were compared regarding subtypes defined by immunohistochemistry and by mRNA expression profile (basal, HER2-positive, luminal A, Luminal B and normal-like) and tested for the power to predict a neoadjuvant chemotherapy complete response. The results found HER2-positive tumors, according to the immunohistochemical test, could not be classified as such by the molecular profile and, when this occurs, the chemotherapy response rate is low (8%) *versus* 54% in HER2-positive tumors, according to the gene profile analysis.

Discussion

We observed the subject "Transcriptional profile and response to neoadjuvant chemotherapy in breast cancer" is a reason for increasingly concern, in view of the number of studies being produced; however, numerous questions remain.

A number of neoadjuvant chemotherapy response-predictive gene expression profiles were identified, probably contributing to a better understanding of mechanisms involved in treatment resistance. However, these transcriptional profiles are heterogeneous and probably reflect the chemotherapy response biological complexity. The question is whether the different panels are superimposable and identify the same tumor regarding treatment response and there are more homogenous patterns at other transcriptional and translational regulation levels. Worth remembering tumor gene signature is strongly linked to disease prognosis[31,32] and most neoadjuvant chemotherapy response-predictive gene expression profiles ever described are based on the pathological complete response, thus, they are partly linked to prognosis. Nevertheless, recent studies indicate pCR-predictive transcriptional signature might not be related to a disease good prognosis[25,28,29].

On the other hand, a number of authors use the treatment clinical response as an endpoint[11,17,28]. As in this case objective response is considered *versus* no response (stable disease and disease progression), a resistance predictive panel to a chemotherapeutic regimen concerned is determined. This issue is also essential, as it can contribute to individualize the treatment regimen, allowing patients resistant to a determined chemotherapeutic agent undergo another therapeutic regimen.

Chemotherapeutic resistance seems complex. A number of resistance mechanisms may be common to several drugs, such as MDR1 e MRP gene expression, encoding a membrane glycoprotein causing drug extrusion. Another example are the apoptosis machinery changes by BCL2 overexpression, BAX lower expression, TP53 mutation, H-RAS oncogene expression, MDM2 expression. Moreover, the medication itself, by interacting with DNA, can cause additional mutations. In order to study intrinsic and acquired tumor resistance in breast cancer, we used the strategy of assessing the differential gene expression between treatment resistant patient samples (stable disease and disease progression) and residual samples considered early responders (partial response). In this case, we observed regulated pathways are JNK and apoptosis, which can contribute to the resistance process, and that a higher CTGF and DSUP1 expression in residual samples may reflect the resistance to additional AC cycles[33].

Conclusion

In short, the identification of chemotherapy responsive patients is of central interest and despite important steps have been taken, the subject deserves additional studies in view of its complexity.

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