Clinical and therapeutic perspectives of gene expression profiling for breast cancer

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The standard method for defining prognosis for patients with breast cancer is an integrated model including clinicopathological features, such as tumour size, histological grade, nodal involvement, hormone receptor status and HER-2 overexpression. Nowadays, two multigene prognostic models can stratify patients in new categories of risk. Notably, clinicopathological prognostic prediction and genomic signatures are discordant in at least 30% of cases. For this reason, two trials are going on, aiming to validate clinical utility of gene profiling. As regards the predictive value of genomic assays, many models have been carried out, demonstrating the capacity to identify with high sensitivity and specificity resistant and non-resistant tumours, differently from the traditional markers. These predictors, however, need to be validated by prospective clinical trials.

**Key words:** breast cancer, gene expression profiling, predictive, prognostic

**introduction**

Although a morphological taxonomy has been created and employed in breast cancer management, tumours classified under the same descriptive term may have distinct biological features and clinical behaviour [1].

The most commonly used prognostic and predictive factors are tumour diameter, histological grade, hormonal receptor status, HER-2 amplification and axillary nodal involvement [2–4]. These factors have been combined into outcome prediction models, the most used being The Nottingham Prognostic Index and Adjuvant!Online [5, 6]. However, these models are not useful in selecting one chemotherapy regimen over another. Furthermore, there is a variability in disease course within each prediction category, this different outcome of clinically and pathologically similar tumours being probably due to underlying molecular differences.

After the completion of the human genome sequencing, gene profiling of tumours has become possible through complementary DNA (cDNA) microarrays and RT-PCR, which have ameliorated the understanding of breast cancer taxonomy and provided 'signatures' (groups of genes that can separate tumours into classes with prognostic and predictive implications), showing the potentiality to improve the traditional prognostic and predictive models.

**gene expression profiling as prognostic factor**

A pioneering work has demonstrated that breast tumours can be classified according to the resemblance between the genetic profiles of cancer and normal cells. An 'intrinsic gene set' was developed, representing a subset of genes with significant variation between different tumours than between samples from the same cancer specimen. Using hierarchical clustering analysis, tumours have been classified into four groups: luminal cell like [oestrogen receptor (ER)-positive, with a genetic profile similar to normal luminal cells, expressing cytokeratins 8 and 18]; basal cell like ('triple negative', resembling to normal basal/myoepithelial cells, expressing cytokeratins 5 and 17); HER-2 (overexpressing HER-2) and normal breast like (clustering together with normal breast and fibroadenoma cells) [7]. Adding new cases to the original set of tumours, the luminal group has been divided into three subclasses: luminal A, B and C (the last one eliminated after supplementary analyses) [7–9]. The comparison of the prognosis of these subtypes has demonstrated that luminal-like tumours showed a better evolution than the basal-like and HER-2 tumours [8]. A limitation of this approach is the reorganisation of dendrograms when new samples are added [10].

Another profile has been constructed through the analysis of 78 tumours using a 24 479-oligonucleotide array, obtaining a '70-gene signature' able to classify young (<55 years) node-negative (N0) patients into two groups: good prognosis (without recurrence during a 5-year follow-up) and poor prognosis (recurrence/metastasis in a 5-year follow-up). This classification has demonstrated an accuracy of 83% in predicting the outcome and a sensitivity of 91% in defining patients with poor prognosis. Compared with the National...
Institutes of Health (NIH) and St Gallen criteria, this model has been more accurate in avoiding unnecessary chemotherapy, although it has shown a lower sensitivity in detecting poor prognosis patients (91% versus 97% and 94%, respectively) [11]. Subsequently, a more numerous and heterogeneous cohort of tumours (234 cases including stage I–II and N0–N1 disease) has been studied, confirming this outperformance [12]. These results have led to the development of the prognostic signature MammaPrint® (Agendia, Amsterdam, The Netherlands), which represents the basis of an ongoing European clinical trial called ‘MINDACT’ (Microarray In Node negative Disease may Avoid ChemoTherapy) [13]. It compares a gene-signature-based treatment decision with an Adjuvant!Online-based treatment decision for those cases in which the two models conflict. On the basis of molecular or clinical prognosis assessment, patients are randomly assigned to receive chemotherapy or not. The purpose is to confirm that patients with genomic low risk and clinical high risk can be spared chemotherapy without influencing the distant-metastasis-free survival (DMFS). This study has also secondary objectives: a sub-randomisation in the chemotherapy arm between a docetaxel/capcitabine regimen and an anthracycline-based one, plus another one in the endocrine therapy section between tamoxifen for 2 years followed by letrozole for 5 years versus letrozole alone for 7 years [14].

Another scoring system demonstrated the ability to predict risk of recurrence in ER-positive and N0 breast cancers. Through a real-time RT-PCR, gene expression in sections of fixed, paraffin-embedded tumours was quantified, thus selecting 250 candidate genes. Subsequently, data from three clinical trials were analysed to estimate any relationship between expression of those genes and tumour recurrence. In all, 16 cancer-related and five reference genes were selected to design an algorithm calculating a ‘recurrence score’. In a multivariate analysis, it demonstrated a significant predictive power, independent of age and tumour size (P < 0.001). It was also predictive of overall survival (OS) (P < 0.001) and could be used to predict distant recurrence in single patients [14]. These results, confirmed by a case-control retrospective study [15], represent the basis of the Oncotype DX® (Genomic Health Inc., Redwood City, CA) recurrence score, under evaluation in an ongoing North American clinical trial named ‘TAILORx’. This study randomises patients with intermediate recurrence score to receive hormonal therapy alone or hormonal therapy plus chemotherapy to find out whether adjuvant chemotherapy improves survival in this subset of patients. Patients with low recurrence score will be treated only with tamoxifen, while those with high scores will receive chemotherapy plus hormonal therapy.

Using an 18 400 oligonucleotide array, 286 chemotherapy-naive patients were analysed (80 and 206 randomly assigned to training and testing sets, respectively) and divided into subgroups of ER-positive and ER-negative tumours, obtaining specific signatures for each of them. A 76-gene signature has been created (60 genes for ER-positive and 16 for ER-negative subclass) with a specificity of 58% and a sensitivity of 93% in identifying patients with poor prognosis, being an independent prognostic factor in multivariate analysis for DMFS outperforming the St Gallen and NIH criteria [16].

Considering the similarities between wound healing and cancer, the expression profile of fibroblasts exposed to serum was studied with a 36 000-gene microarray, finding 677 genes constantly induced. By excluding genes directly correlated to cell proliferation, a fibroblast-core-serum-response (CSR) signature was obtained, composed by 312 serum-responsive and cell cycle-independent genes, also expressed in poor prognosis breast, lung and gastric cancers. When it was applied to the patients of the previous study, tumours expressing the CSR signature demonstrated a decreased OS and DMFS in comparison with those having a quiescent profile. It also outperformed the St Gallen and NIH criteria in a cohort of 185 chemotherapy-naive patients [17].

Studying these signatures in a multivariate model, only 70-gene and CSR signature demonstrated an independent prognostic value. Consequently, an attempt to couple the two signatures was made by classifying patients into good and poor prognosis and dividing the poor prognosis tumours according to the CSR signature as wound response or quiescent. Those patients with a poor 70-gene prognosis but a quiescent CSR signature had a risk of metastatic disease similar to baseline, whereas those with both poor prognosis and wound-response signature demonstrated a risk 6.4-fold higher than baseline [18].

Another comparison of the 70-gene, CSR and hypoxia-induced signature [19] (genetic profile derived from the comparison of gene expression of normal cells exposed to hypoxia and cancer cells) was carried out, aiming to predict local recurrence risk after breast-conserving surgery. This analysis has demonstrated that the CSR signature segregates patients with high or low risk of local recurrence at 10 years with a sensitivity of 87.5% and specificity of 75%, being an independent predictor in multivariate analysis [20].

A scoring system for the prediction of post-operative outcome in N0 breast cancers has been developed. Using a 25 344-oligonucleotide microarray, the profile of 24 primary N0 tumours (12 recurrence-free and 12 recurrent within 5 years) was investigated, finding 21 genes overexpressed in the recurrent cases and other 37 genes highly represented in the recurrence-free patients. The model showed an accuracy of 100% [21].

Another model has tried to improve the interpretation of histological grade. Microarray from 189 breast tumours and from published data sets has been analysed, identifying 97 differentially expressed genes through the comparison of grade 3 and grade 1 carcinomas from a training set of 64 ER-positive samples. A ‘gene expression grade index’ has been created, highly related to histological grade 1–3, and reclassifying grade 2 cancers into two subgroups with high versus low risk of recurrence, according to a high or low index score, respectively (P < 0.001) [22].

Starting from the observation that breast cancers contain a subpopulation of cells CD44+/CD24−/low, with higher tumorigenicity, the gene expression profile of these cells has been compared with that of normal breast cells. A 186-gene ‘invasiveness’ gene signature (IGS) has been created, finding a significant association with OS and DMFS (P < 0.001). Comparing the IGS with the NIH criteria, patients with high-risk early breast cancer have been stratified into good and poor
Gene expression profiling as predictive factor

Besides the MINDACT trial, designed as a predictive treatment-decision model in its secondary objectives [13], other studies have been carried out.

As regards hormonal therapy, three models have been evaluated: 44-gene predictor, HOXB13:IL17RB ratio and 200-gene ER reporter index.

A study on 112 ER-positive breast cancers treated with tamoxifen has been carried out, identifying 81 genes with significantly (P < 0.05) different levels of expression between hormone-responsive and -resistant carcinomas. A 44-gene predictive signature has been also defined, being superior to traditional predictive factors in univariate analysis (P = 0.03) and related with longer progression-free survival both in univariate and multivariate analysis (P = 0.03) [25].

Another expression profile of 60 breast tumours treated with tamoxifen has been analysed using a 22 000 oligonucleotide array. The comparison of response with expression profiles has identified two predictive genes: homebox gene HOXB13 and interleukin 17B receptor (IL17BR). The ratio HOXB13:IL17BR significantly predicted recurrence both in univariate (P = 0.0003) and multivariate (P = 0.0022) models [26].

An oestrogen-responsive gene expression profile has been analysed among ER-positive cell lines using a 9182 human cDNAs microarray. Two hundred genes have been selected for custom microarray to investigate pathways of oestrogen signalling, leading to the identification of 11 candidate genes as predictive factors. Their levels of expression in breast cancer tissues have been assessed by RT-PCR and analysed by cluster analysis. Among these genes, the expression of histone deacetylase 6 (HDAC6) and other genes such as IGFBP4, IGFBP5 and EGR3 significantly correlated with disease-free survival rate of tamoxifen-treated patients [27–31].

Conversely, predictive models for chemotherapy response are: 3-gene and 59-gene adriamycin/cyclophosphamide (AC)/epirubicin/cyclophosphamide (EC) predictors, 92-gene and 85-gene docetaxel predictor, 23-gene paclitaxel predictor and 30-gene T-FAC predictor.

Gene profile of 51 breast cancers treated with preoperative doxorubicin-based chemotherapy has been studied using a 692-gene microarray and dividing it into trios. Unsupervised cluster analysis could not segregate responders from non-responders even if a classifier has been identified (EMILIN1, FAM14B and PBEF). Using a larger cDNA platform (4608 genes), samples have been segregated and a new trio has been identified (PRSS11, MTSS1 and CLPTM1), distinguishing doxorubicin-resistant tumours with a robustness 2.5-fold greater than the first classifier [32].

Another research concerning the responsiveness to preoperative EC chemotherapy regimen has permitted the identification of 59 genes differently expressed between responders’ and non-responders’ tumours, from which a ‘favourable outcome signature’ (31 genes) and a ‘poor outcome signature’ (26 genes) have been derived [33].

Gene profile of breast cancers related to sensitivity to preoperative docetaxel has been carried out. Analysing core biopsies from 24 patients, a 92-gene predictor related with response to docetaxel (P = 0.001) [34]. Another study concerning the same argument has been carried out analysing 44 tumours with RT-PCR. Genes differentially expressed between responders’ and non-responders’ cancers have created an 85-gene algorithm, having accuracy >80% in identifying non-responders [35].

Focusing on responsiveness to preoperative paclitaxel, 75 breast cancers have been studied, dividing them into five groups according to the degree of clinical/pathological response. From an initial set of 197 genes differentially expressed between high responders and extremely resistant carcinomas, a predictive set of 23 genes has been selected, with a 100% accuracy in classifying responders and non-responders [36].

The profile of breast tumours receiving neo-adjuvant paclitaxel and doxorubicin has been also analysed. Starting from a group of 384 genes, a 24-gene predictor has been extracted, significantly correlating with pathological complete remission (pCR) in univariate analysis (P < 0.05) [37].

A 30-gene predictor has been selected from a set of 780 distinct classifiers, showing a higher sensitivity (92% versus 61%) than a clinical predictor (including age, grade and ER status) to define resistance to weekly paclitaxel and FAC (T-FAC) preoperative chemotherapy. The negative predictive value (96% versus 86%) and the area under the curve (0.877 versus 0.811) were better, but not statistically significant [38]. Notably, a previous study analysing the patterns of gene expression in T-FAC-treated breast tumours has led to a 74-gene model, showing a sensitivity of 43%, a specificity of 100%, a negative predictive value of 73% and a positive predictive value for pCR of 100% [39]. The combination of genomic and clinical information has yielded the best model, with a sensitivity of 92%, a specificity of 71%, a negative predictive value of 96% and a positive predictive value of 52% [40].

Conclusion

Many studies have been carried out with unsupervised and supervised methods, demonstrating the ability of gene profiling to build new predictors of prognosis and responsiveness. The future trend is that genomic profiling will provide information that will affect clinical decision making. So, the use of supervised techniques rather than cluster analyses has been recommended for class prediction and comparison studies, because the latter are less powerful for distinguishing predefined classes and do not provide valid statistical identification of differentially expressed genes [41].
The studies carried out were characterised by a small dimensioned group of cases and almost were all retrospective analyses, this representing a selection bias and a limitation of the accuracy and predictive power of gene expression-based markers when applied to the heterogeneous population of patients who are treatment-naive facing everyday with physician.

The future direction is represented by two ongoing trials (MINDACT and TAILORx) evaluating the clinical utility of the 70-gene signature and of the 21-gene recurrence score. They are designed to analyse a broader population than that included in the earlier studies, making it possible to estimate the extent to which heterogeneity of patients, treatment protocols and other factors influence the value of expression signature as prognostic and predictive markers.

acknowledgements

Authors have indicated no financial relationships with companies whose products are mentioned in this article.

references