Treatment tailoring based on molecular characterizations

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introduction
Breast cancer is a heterogeneous disease associated with a variety of pathological features and clinical behaviour. Historically, classification of breast cancers has been based on histological type, grade and expression of hormone receptors, but the advent of microarray technology has since demonstrated significant heterogeneity occurring also at the transcriptome level. Through its ability to interrogate thousands of genes simultaneously, microarray studies have allowed for a comprehensive molecular and genetic profiling of tumours. Not only has it changed the way in which we have traditionally classified breast cancer, the results of these studies have also yielded molecular signatures with the potential to significantly impact on clinical care by providing a molecular basis for treatment tailoring.

microarray technology
Gene expression profiling, using microarray technology, relies on the accurate binding, or hybridization of DNA strands with their precise complementary copies where one sequence is bound onto a solid-state substrate. These are hybridized to probes of fluorescent cDNAs or genomic sequences from normal or tumour tissue. By analysing the intensity of the fluorescence on the microarray chip, direct comparison of the expression of all genes in normal and tumour cells can be made [1]. At present, there are multiple microarray platforms that either use cDNA-based or oligonucleotide-based microarrays.

In the past, there has been much scepticism regarding the reliability and reproducibility of microarray technology. Much of the divergent results from these studies have been extensively explored elsewhere, including problems with inconsistent sequence fidelity and annotation; the low specificity of the spotted cDNA microarrays; the lack of probe specificity for different isoforms; as well as differences that exist either with the hybridization conditions, the fluorescence measurement, the normalization strategies or with analytical algorithms applied [2–6]. In addressing the issues of reproducibility, the US Food and Drug Administration (FDA) conducted the MicroArray Quality Control (MAQC) project [7] involving 137 participants from 51 academic and industry partners. Not only did they find good reproducibility across and between different microarray platforms, they found that these platforms detected, in fact, similar changes in gene abundance. When comparing this new microarray technology with other diagnostic techniques, the reproducibility is surprisingly comparable, for example, to that of immunohistochemical analysis for hormone receptors in breast cancer [8, 9].

molecular classification of breast cancer
One of the most important discoveries stemming directly from microarray studies has been the reclassification of breast cancer into molecular subtypes. Four main molecular classes of breast cancers have been consistently distinguished on the basis of gene expression profiling. Based on the original classification described by the seminal study by Perou et al. [10], these subtypes are (i) basal-like breast cancers, (ii) HER2+ breast cancers, (iii) luminal-A breast cancers and (iv) luminal-B breast cancers.

In the basal-like subtype, there is a high expression of basal cytokeratins 5/6 and 17 and proliferation-related genes, as well as laminin and fatty-acid binding protein 7. In the HER2+ subtype, there is a high expression of genes in the erbB2 amplicon such as GRB7. The luminal cancers are estrogen receptor (ER)-positive and luminal A is characterized by a higher expression of ER, GATA3 and X-box binding protein trefoil factor 3, hepatocyte nuclear factor 3 alpha and LIV-1; and luminal B is generally characterized by a lower expression of luminal-specific genes.

Beyond the differing gene expressions, these molecular subtypes also have distinct clinical outcomes and responses to therapy that seem to be reproducible from one study to another. The basal-like and HER2+ subtypes are likely to be more aggressive with a higher proportion of TP53 mutations [11, 12] and a markedly higher likelihood of being grade III (P <0.0001 and P = 0.0002, respectively) than luminal A tumours. Despite a poorer prognosis, they tend to respond better to chemotherapy including higher pathologic complete response rates after neo-adjuvant therapy [13]. Conversely, fewer than 20% of luminal subtypes have mutations in TP53, and these tumours are often grade I [14]. They tend to be more sensitive to endocrine therapy, be less responsive to conventional chemotherapy but, overall, have better clinical outcome.

This identification of distinct expression patterns amongst breast cancer subtypes has provided intriguing insights into the
tumour biology of this disease. It has altered the way that physicians and clinical investigators conceptually regard breast cancer—not as one disease, but as a collection of several biologically different diseases—as well as the way they design clinical trials in order to better target specific subpopulations.

However, this molecular classification is not without its inherent limitations, with up to 30% of breast cancers not falling into any of the four molecular categories [15]. Exactly how many true molecular subclasses of breast cancer exist remains uncertain, and it is plausible that the molecular classification will evolve, with new technological platforms, with availability of larger datasets as well as improved understanding of tumour biology.

gene expression signatures to predict prognosis (Table 1)

Traditional prognostic factors based on clinical and pathological variables are unable to fully capture the heterogeneity of breast cancer patients. Guidelines such as those produced by the National Comprehensive Cancer Network (NCCN; v2. 2007, www.nccn.org), used in the USA, and the International St Gallen Expert Consensus [16], used in Europe, are useful in providing information regarding risk of relapse for a patient based on ‘average’ results. However, these guidelines cannot allow for the substantial variability that can exist between patients with similar stages and grades of disease. Using microarray technology, several independent groups have conducted comprehensive gene expression profiling studies with the aim of improving upon traditional prognostic markers used in the clinic. Three conceptually different supervised predictor strategies for the development of gene expression prognostic signatures have been used so far: (i) the ‘top-down’ approach, which compares gene expression data from cohorts of cases with known clinical outcomes to identify genes that are associated with prognosis without any a priori biological assumption; (ii) the hypothesis driven or ‘bottom-up’ approach, which first identifies gene expression patterns associated with a specific biological phenotype or a deregulated molecular pathway, and then subsequently correlates these findings with clinical outcome; (iii) the candidate gene approach combines selected genes of interest on the basis of existing biological knowledge into a multivariate predictive model.

Using the ‘top-down’ approach, where gene expression data are correlated with clinical outcome without prior biological assumption, a group of researchers from Amsterdam identified a 70-gene prognostic signature, using the Agilent platform, in a series of 78 systemically untreated node-negative breast cancer patients under 55 years of age [17]. This signature included mainly genes involved in the cell cycle, invasion, metastasis, angiogenesis and signal transduction. Validated on a larger set of 295 young patients [18], including both node-negative and node-positive breast cancer patients, as well as treated and untreated patients, the 70-gene signature was found to be the strongest predictor for distant metastases-free survival, independent of adjuvant treatment, tumour size, histological grade and age. At 5 years, the probability of remaining free of distant metastases was 96% in the good-signature group and 83% in the poor-signature group. At 10 years, the probability was 66% and 55%, respectively.

Using the same top-down approach, another group in Rotterdam identified a 76-gene signature [19], using the Affymetrix technology, which considered ER-positive patients separately from ER-negative patients. These 76 genes were mainly associated with cell cycle and cell death, DNA replication and repair and immune response. In a training set of 115 patients and a multi-centric validation set of 180 patients [20], they were able to demonstrate comparable discriminative power in predicting the development of distant metastases in untreated patients in all age groups with node-negative breast cancer.

Both the Amsterdam and Rotterdam gene signatures have been independently validated by TRANSBIG, the translational research network of the Breast International Group (BIG) [21, 22]. Despite having only three genes in overlap, both signatures were able to outperform the best validated tools to assess clinical risk. In particular, these signatures were both superior in correctly identifying the ‘low-risk’ patients, but were limited in identifying the ‘high-risk’ patients as half of those identified in this category did not, in fact, relapse. This suggests that the highest clinical utility for these molecular signatures may be in potentially reducing over-treatment of low-risk patients.

Also using the top-down approach, Paik et al. [23], in collaboration with Genomic Health Inc. have developed a recurrence score (RS) based on 21 genes, which appears to predict accurately the likelihood of distant recurrence in tamoxifen-treated patients with node-negative, ER-positive

Table 1. Gene expression signatures related to prognostication in early breast cancer

<table>
<thead>
<tr>
<th>Gene expression signatures</th>
<th>Biological hypothesis</th>
<th>Microarray platform</th>
<th>Number of genes in the signature</th>
<th>Independent validation</th>
</tr>
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<tbody>
<tr>
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<td>Affymetrix (oligonucleotides)</td>
<td>186</td>
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</tr>
</tbody>
</table>
breast cancer. A final panel of 16 cancer-related genes and five reference genes forms the basis for the Oncotype DX™ Breast Cancer Assay.

The RS classifies patients into three risk groups, based on cutoff points from the results of the National Surgical Adjuvant Breast and Bowel Project (NSABP) trial B20; high risk of recurrence was assigned if RS >31, intermediate risk with RS 18–30 and low risk if RS <18. Retrospective validation of this predictor in 675 archival samples of the NSABP trial B14 [24] showed that the RS was significantly correlated with distant recurrence, relapse-free interval and overall survival, independent of age and tumour size.

In using a different approach that is hypothesis-driven, otherwise known as the 'bottom-up' approach, Sotiriou et al. [25] looked at whether gene expression patterns associated with histologic grade could improve prognostic capabilities especially within the class of intermediate grade tumours. Accounting for 30–60% of all breast cancers, these intermediate grade tumours display the most heterogeneity in both phenotype and outcome [23].

Of the 97 unique genes that formed the gene-expression grade index (GGI), most were associated with cell-cycle progression and differentiation. These genes were differentially expressed between low- and high-grade breast tumours, without a distinct gene-expression pattern to distinguish the intermediate group. Instead, the intermediate tumours showed expression patterns and clinical outcomes matching those of either low- or high-grade cases. The GGI, therefore, can potentially aid the treatment decisions for these otherwise problematic patients with intermediate grade by reclassifying them into two distinct and clinically relevant subtypes.

In examining genomic grade with ER status, ER-negative tumours with poor clinical outcome were found to be mainly associated with high GGI, although ER-positive tumours were more heterogeneous with a mixture of GGI levels [25]. Thus, these two variables are not entirely independent of each other; with tumour genomic grading capable of providing an extra level of information when stratifying the ER-positive group.

Other prognostic signatures derived from the bottom-up approach include the wound response signature [26], mutant/wild p53 signature [27], invasive gene signature (IGS) [28] and the cancer stem cell signature [29]. These and other prognostic signatures, whether derived with the bottom-up or top-down approach, have only a few genes in common, but seem to offer similar predictive information, with proliferation-related genes being the major driving force. Furthermore, it appears that the prognostic power of many of these signatures is limited to ER-positive patients, and is less informative for ER-negative disease [30].

gene expression signatures to predict response

Improved prognostic tools will help better identify those patients needing or not needing treatment, but there still remains the challenge of knowing which therapy is best to use for the individual patient. Only a proportion of patients will respond to any given treatment but, unfortunately, many will experience the adverse side effects. Currently, only ER and HER2 are used in clinical practice as predictive markers, for the selection of patients likely to respond to hormone therapy and trastuzumab, respectively.

Several investigators have recently applied microarray technology to identify gene expression signatures that could predict for drug sensitivity in breast cancer. In predicting for endocrine therapy resistance, several studies have been performed; including the study with 44 genes by Jansen et al. [31] and the one using the expression ratio of two genes—homeobox B13 and IL17BR, which could predict for disease survival with 80% accuracy [32–34] when treated with adjuvant tamoxifen. Other endocrine sensitivity tests include the estradiol-induced genes by Oh et al. [35] and the 200-gene signature that predicts for recurrence-free survival after 5 years of tamoxifen therapy [36]. The RS, previously discussed, is also often regarded as a predictive tool for endocrine therapy, rather than a pure prognostic tool, because the NSABP B14 and B20 trials enrolled patients who were treated with tamoxifen [23, 24].

In looking at chemotherapy response, fewer studies have been reported so far because these studies ideally require prospective sample collection. Nonetheless, several groups have identified genes associated with response to chemotherapy [37–45], with the majority of the studies using pathologic response rate (pCR) as a surrogate marker for long-term benefit after neo-adjuvant chemotherapy. The largest of these studies generated a 30-gene predictor [46] from 82 patients treated with neo-adjuvant weekly paclitaxel and 5-fluorouracil, doxorubicin, cyclophosphamide (T/FAC) chemotherapy. This predictor performed very well, predicting for pCR with high sensitivity and negative predictive value, compared with clinical variable-based predictors such as age, nuclear grade and ER.

Recently Bild et al. [47], using a 'hypothesis-driven' approach, identified several expression patterns associated with the deregulation of a variety of oncogenic pathways that could predict response to different therapeutic agents targeting specific deregulated pathways. Using publicly available drug sensitivity data derived from in vitro experiments, they also developed multiple classifiers of response to a variety of chemotherapy drugs and showed that a combination of these classifiers could accurately predict response to preoperative multi-drug regimen treatments [48].

While the preliminary results are encouraging, the conclusions that can be derived from these predictive studies are limited by the small sample size as well as by heterogeneity in endpoints, treatment regimens, patient populations and statistical analyses [49]. Furthermore, these predictive tests will still need independent validation.

treatment tailoring—the future

There is significant potential for gene expression profiles to aid treatment tailoring of breast cancer patients. Prognostic signatures can differentiate subpopulations based on risk of relapse and, indeed, there is suggestion that these signatures may be most useful in identifying low-risk patients who potentially can be spared adjuvant chemotherapy. Predictive signatures, on the other hand, can aid in the decision of which
therapy to use in each patient to maximize individual benefit and minimize individual toxicity. While the results from these 'first-generation' microarray studies are exciting, there is still a long way to go before these molecular tools can enter routine clinical use. Many of these studies are retrospective, and the gene expression data come from archival material of heterogeneous populations. While level 1 evidence is currently lacking, prospective clinical validation has started for two expression-profiling platforms—OncotypeDx RS with the 21-gene signature in TAILORx; and Mammaprint with the 70-gene signature in MINDACT (Table 2).

TAILORx (Trial Assigning IndividuaLized Options for Treatment) is a large randomized prospective study designed to evaluate whether women with node-negative, ER-positive breast cancer need chemotherapy based on the RS. Patients with RS <11 (low risk) will be given only hormonal therapy. A RS >25 (high risk) will mean that patients receive chemotherapy in addition to hormone therapy. Patients with RS 11–25 (intermediate risk) will be randomly assigned to receive hormone therapy or chemotherapy followed by hormone therapy. This trial is conducted under the auspices of the US Intergroup and is expected to accrue over 10 000 patients.

The MINDACT (Microarray In Node negative Disease may Avoid CheMoTherapy) Trial is an international prospective, randomized study assessing the potential added value of the 70-gene signature classifier to the commonly used clinico-pathological criteria for selecting node-negative breast cancer patients for adjuvant chemotherapy. By hypothesizing that the 70-gene signature will be able to better select appropriate patients for adjuvant treatment, the benefit would be best seen in patients with good prognostic signatures spared from unnecessary chemotherapy.

Approximately 6000 node-negative patients will have their risk assessment made by using common clinico-pathological factors (through a modified version of Adjuvant OnLine) and by the 70-gene signature. Those patients who are classified as high risk by both methods will be offered chemotherapy; those classified as low risk by both methods will not be offered chemotherapy; the discordant group, an estimated 33% (1900 patients) will be randomized between the two methods and will either receive or not receive chemotherapy according to the result of the assigned method. This trial is being conducted within the BIG network under the sponsorship of the European Organization for the Research and Treatment of Cancer (EORTC).

Indeed the technological know-how in this field is rapidly evolving but the criterion for level 1 evidence must remain uncompromised, and the results of TAILORx and MINDACT are eagerly awaited. However, it is probable that even with positive validation in large prospective trials, it is unlikely that gene expression profiles will altogether replace existing clinico-pathological guidelines but, rather, they will become part of an integrative decision-making model based on multiple levels and sources of prognostic data. The best use of these gene-expression signatures may actually be in directing treatment decisions when clinical risk parameters for an individual patient are equivocal. Until there is more certainty regarding the clinical utility of this new molecular technology, treatment guidelines such as those of St Gallen and the NCCN, having withstood the tests of both evidence and time, will continue to be used widely.

disclosures

Christos Sotiropoulos is an inventor of the Genomic Grade Index.

references