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Current Perspective

Predictive signatures for chemotherapy sensitivity in breast cancer: Are they ready for use in the clinic?

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ABSTRACT

Markers that predict the sensitivity of tumours to chemotherapy must address two questions: (a) which tumours are more likely to respond to chemotherapy? and (b) what is the optimal chemotherapy regimen for a specific tumour or group of tumours? To answer these questions will require markers of general chemosensitivity and drug-specific chemosensitivity, respectively. Beyond these fundamental questions lies an important practical question: are the predictive markers in the current literature ready for routine clinical use? The focus of this paper is to address this practical question. We will first review retrospective trials that have reported promising chemotherapy signatures, presenting in a comprehensive manner for the non bio-informatician the different methods used so far. In addition, we will summarise prospective trials (either ongoing or under development) designed to test the multigene classifiers currently thought to predict chemosensitivity. Finally, we will discuss why microarray studies have so far failed to identify new targets, and how we might be able to improve on these results through large-scale genotyping of tumours.

1. Introduction

Identification of markers that predict chemosensitivity is a research priority. The aim is to answer two very different questions: (a) can we use gene signatures to identify tumours which are more likely to respond to chemotherapy? and (b) when chemotherapy is indicated, what is the optimal chemotherapy regimen for a specific tumour or group of tumours? The predictive markers which answer these questions are likely to be different; for the sake of simplicity we will describe them in this review in two categories: markers predicting general chemosensitivity (meaning that a tumour is sensitive to any chemotherapy or to a wide range of chemotherapeutic drugs) and markers predicting drug-specific chemosensitivity (meaning that a tumour is sensitive to a specific class of agents). Of note, the identification of 'molecular features that indicate the optimal chemotherapy regimen' was considered a top priority in a recent internet-based consultation of 420 breast cancer researchers (clinicians, scientists, academics and pathologists).[1]

To date, predictive markers have been analysed either as single markers (for example, proliferation markers, hormone receptors, *HER2* and *p53*) or in groups, commonly referred to as gene signatures, metagenes, multigene biomarkers, multigene predictors or multigene classifiers. If we take oestrogen receptor (ER) status as an example of a single marker, several

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studies have published provocative, but often contradictory, results regarding the value of ER status in predicting the benefit of adding taxanes to or after an anthracycline-based chemotherapy regimen.[2–6] Likely explanations for these apparently contradictory results are the heterogeneity of the ER-positive tumours included in these trials and the use of different cut-offs to define ER status. Multigene classifiers are more robust than single gene classifiers because random variation may negate the predictive information in some of the samples tested with only a single marker. Thus we have chosen in this review to concentrate on the literature assessing the predictive value of multigene classifiers. Two recent publications have comprehensively reviewed gene expression signatures in breast cancer but none has focused on the complex issue of their potential role in predicting chemotherapy sensitivity.[7,8]

Firstly, we will review retrospective trials that have identified promising multigene classifiers of chemotherapy sensitivity. Secondly, we will summarise prospective trials (either ongoing or under development) aiming to test the ability of multigene classifiers to predict chemosensitivity. Simon emphasised that 'a multigene biomarker can be a function that provides a continuous risk score rather than a class identifier'.[9] This difference is particularly important when aiming to prospectively validate a marker since the cut-off thresholds defining different classes (for example, good and bad responders) must be chosen in advance. As suggested by Simon, we prefer the phrase 'multigene classifier' rather than 'multigene biomarker' particularly when discussing prospective trials. Thirdly, we will discuss why, to date, gene expression signatures have failed to identify new targets and how we might be able to improve on these results.

2. Retrospective trials

2.1. *Predictive multigene classifiers of general chemosensitivity*

2.1.1. *The '21-gene recurrence score' (Oncotype DX™)*

Paik and collaborators developed a 21-gene recurrence score (RS) as a prognostic tool for predicting 10-year survival in a population of patients with early breast cancer.[10] Multiple quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) assays were used to quantify gene expression in formalin-fixed paraffin-embedded tissue. Initially, the correlation of gene expression with the likelihood of distant recurrence was studied in a series of 447 patients with node-negative, hormone receptor-positive breast cancer treated with tamoxifen in the National Surgical Adjuvant Breast and Bowel Project (NSABP) trial B-20. A panel of 16 cancer-related genes and 5 reference genes was selected and used to create a 21-gene recurrence score which increases smoothly from low, through intermediate, to high values. The recurrence score was then tested (some would say validated) in a series of 668 node-negative patients treated with tamoxifen alone in NSABP trial B-14. The rate of distant recurrence was 6.8% in the low recurrence score group, 14.3% in the intermediate score group and 30.5% in the high recurrence score group ($p < 0.001$).

Subsequently, two retrospective studies have reported that this same 21-gene recurrence score has predictive value for chemosensitivity.[11,12] The predictive value of the 21-gene recurrence score was assessed in 651 patients with node-negative, hormone receptor-positive tumours in NSABP trial B-20 randomised to tamoxifen alone ($n = 227$) or tamoxifen plus chemotherapy (methotrexate–fluorouracil or methotrexate–fluorouracil–cyclophosphamide) ($n = 424$).[11] A high recurrence score predicted benefit from chemotherapy (hazard ratio (HR) = 0.26; 95% confidence interval (CI) = 0.13–0.53), with little or no benefit from chemotherapy in the low and intermediate recurrence score groups.

The predictive value of the 21-gene recurrence score was also assessed in a subset of patients more than 50 years old with node-positive hormone receptor-positive tumours included in the SWOG 8814 trial.[12] In the SWOG 8814 trial, patients were randomised to receive either tamoxifen alone ($n = 361$); fluorouracil, doxorubicin and cyclophosphamide (CAF) followed by tamoxifen for 5 years ($n = 566$); or concurrent CAF and tamoxifen ($n = 550$). The 21-gene recurrence score was assessed in 367 of these patients. The score was low (<18) in 40%, intermediate (18–30) in 28% and high (>31) in 32% of patients. The addition of chemotherapy to tamoxifen resulted in no difference in disease-free survival (DFS) or overall survival (OS) in the low recurrence score group, but a clear benefit in DFS and OS in the high recurrence score group. There appeared to be a benefit as well for patients in the intermediate recurrence score group, but the confidence intervals were wide because of the small sample size. The results of this study, not yet fully published, suggest that the 21-gene recurrence score can identify one group of patients with node-positive, hormone receptor-positive disease who may derive no benefit from chemotherapy and another group who derive a larger benefit from chemotherapy than previously thought. If confirmed in further series of node-positive patients, this would seriously challenge the contemporary paradigm that all node-positive patients should receive chemotherapy.

2.1.2. *The '70-gene signature' (Mammaprint™)*

The '70-gene signature' developed by the Amsterdam group stratifies patients into poor prognosis and good prognosis groups.[13] Its prognostic value has been validated in cohorts of patients with node-negative[14,15] and node-positive tumours.[14,16] In a pooled multivariate analysis of two series of patients with node-positive tumours, the prognostic value of the gene signature was confirmed in a multivariate analysis (HR 5.50, 95% CI 1.47–20.62, $p = 0.01$).[16]

In addition, one retrospective study has suggested that the 70-gene signature might also predict the response to neoadjuvant chemotherapy. The signature was assessed in a series of 167 patients with tumours greater than 5 cm or clinically positive nodes.[17] Pathological complete response (pCR) after neoadjuvant chemotherapy was used as a surrogate for chemosensitivity. None of the patients with a good signature ($n = 144$) achieved a pCR (0/23), whereas 20% (29/144) of patients with a bad signature had a pCR. Consequently, the authors concluded that patients with a good signature would be unlikely to respond to chemotherapy.