Short communication

Adjuvant treatment: the contribution of expression microarrays

Per Eystein Lønning[1,2], Ranjan Chrisanthar[1,3], Vidar Staalesen[1,3], Stian Knappskog[1,3] and Johan Lillehaug[3]

[1]Section of Oncology, Institute of Medicine, University of Bergen, Bergen N-5020, Norway
[2]Department of Oncology, Haukeland University Hospital, Bergen N-5020, Norway
[3]Department of Molecular Biology, University of Bergen, Bergen N-5020, Norway

Corresponding author: Per Eystein Lønning, per.lonning@helse-bergen.no

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Introduction

Although gene expression microarrays provide novel tools and hold great promise in cancer research, achievements thus far in terms of improved prognostication and, in particular, prediction of drug sensitivity have been moderate. To improve clinical therapy, we believe that it is imperative to integrate gene expression arrays with other laboratory methods based on functional concepts [1,2].

Breast cancer taxonomy

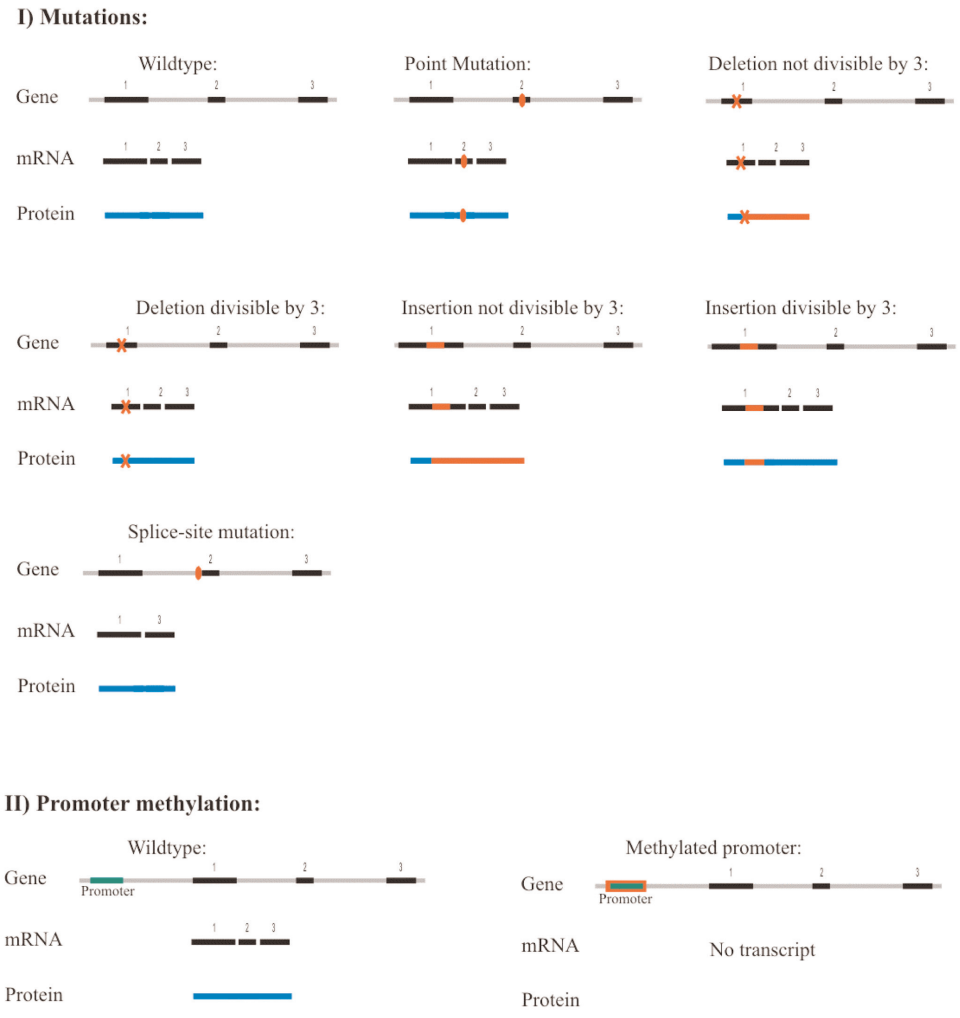
The first study to explore human breast cancer biology applying gene expression signatures was that reported by Perou and coworkers [3] in 2000. Here, oestrogen receptor (ER)-positive breast cancers (designated luminal class, based on cytokeratin expression) were found to be associated with particular gene expression profiles. Moreover, the gene expression signatures revealed (at least) two distinct subclasses among the ER-positive tumours, termed luminal A and luminal B. This subclassification provided novel prognostic information. Thus, among patients with locally advanced breast cancers undergoing primary chemotherapy with either doxorubicin monotherapy [4] or 5-fluorouracil and mitomycin given in concert [5] to be followed by tamoxifen adjuvant for 5 years, a poor prognosis was identified among patients with tumours expressing a luminal B profile as opposed to the luminal A group [6]. Interestingly, when the classification was applied to a second cohort of patients with early stage breast cancers who had not received adjuvant endocrine therapy [7], again the luminal A and B classes were associated with different prognosis; the relative difference, however, was much less than that in patients receiving tamoxifen treatment. Although this could indicate a predictive component (higher sensitivity for luminal A class tumours to tamoxifen treatment compared with luminal B ones), such conclusions should not be inferred from indirect comparison.

The second major achievement was further subclassification within the group of ER-negative tumours. This led to identification of the so-called 'triple negative' class (tumours negative with respect to expression of ER and progesterone receptor that, in addition, lack over-expression and/or amplification of HER2) as a distinct subclass. These triple negative tumours expressed keratin markers that are strongly suggestive of a basal cell origin (for which reason they are frequently referred to as 'basal cell class' tumours), contrasting with the luminal origin of breast cancers in general.

Prognostication

Subsequently, several studies [8-13] have identified different gene expression profiles as being associated with prognostication in breast cancer [8-13]. Notably, however, the various profiles identified differ considerably with respect to genes included, and the extraction of multiple signatures from the same dataset questions the specificity of such signatures [14]. Others have argued that the information provided may not be superior to what is achieved by optimal use of conventional factors [15]. Moreover, because these studies in general were conducted retrospectively in unselected patient cohorts, meaning that the patients were exposed to various drug regimens, the issue of potentially predictive components may not be excluded. Notably, a main reason why lymph node status may be used as a single marker to select high-risk patients for adjuvant therapy based on risk for having a relapse is due to the fact that it is a 'pure' prognostic factor; patients defined as having a poor prognosis are not more likely to be therapy resistant than those having a better prognosis. This underlines a general principle. When looking for novel prognostic factors, it is mandatory to keep in mind that no prognostic factor may be defined and implemented for clinical use without detailed knowledge regarding its potential predictive effect for the therapy applied [16,17]. Considering a factor such as TP53, mutations that affect the DNA-binding part of the protein are associated with a poor

Figure 1



Different types of gene mutations and mechanisms of epigenetic silencing. Reproduced with permission from [16].

prognosis [18]; however, they also confer poor sensitivity to chemotherapeutics such as anthracyclines and mitomycin [5,19].

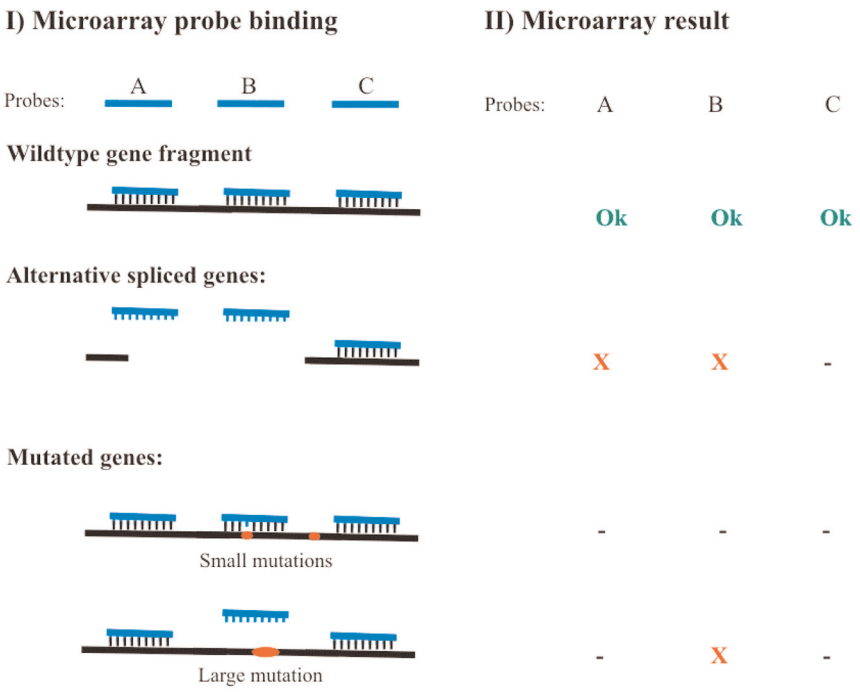
Predicting response to therapy

Primary medical therapy (previously termed 'neoadjuvant treatment') represents an optimal setting in which to study drug effects on tumours directly [20]. Thus, several studies have explored gene expression profiles that predict responsiveness to different chemotherapeutic regimens, including taxane monotherapy [21,22] or anthracycline- or mitomycin-containing regimens administered as monotherapy [23] or in combination with other drugs [23-25], including taxanes [24,26-29]. The general conclusion from these studies may be summarized as follows. First, independent of regimen and the statistical approach (supervised or unsupervised), there is in general a correlation between gene expression profiles and responsiveness to therapy. Second, for none of the signatures identified has the combined sensitivity and specificity reached a level that allows its implementation for clinical use outside trials. Third, in none of these signatures has the value repeatedly been corroborated by other investigators.

Interestingly, looking at response to therapy across the different breast cancer subclasses [23,25], some differences in responsiveness could be detected. However, these differences were not of sufficient magnitude to allow clinical application to therapy selection.

There are several limitations to the use of microarray analysis as a single method for exploring tumour biology. The spectrum of pathological events that lead to disturbed gene function is huge [16], involving components such as large

Figure 2



Microarray signals are generated by hybridizing a probe to the gene product (complementary DNA [cDNA]). Alternative splicing, as well as different types of mutations, may influence the result but may go undetected as well, depending on the exact type of lesion as well as its location with respect to the area hybridising with the probe. 'X' and '-' indicate potential errors; 'X' indicates wrongly detected transcript levels, while '-' signifies correct levels reported, but with other undetectable lesions present in the gene. Reproduced with permission from [16].

and small deletions or single base substitutions, mutations that affect promoter regions or splice-sites, as well as epigenetic silencing (Figure 1). In addition, the issue of multiple splice variants generated from the same gene has received increasing attention [30]. Alternative splices may be transcribed into protein products with different biological function [31]; whether such splices are detected together with the main transcript on microarrays depends on whether the sequence covered by the probe is included in the splice transcript and how the mutation affects hybridization (Figure 2). Additionally, the stability of the different splice transcripts and encoded proteins may vary considerably. Finally, many signalling pathways, including activation of p53 [32], involve post-translational modifications such as protein phosphorylations, deacetylations, and so on, meaning that information relevant to changes in biological function of specific proteins is not reflected in altered mRNA expression.

It is clear (Figures 1 and 2) that although microarrays may provide information about transcriptional status of individual genes, interactions such as inclusion of alternative splices may confound the biological interpretation. Mutations that affect genes encoding proteins that are involved upstream or downstream in a particular functional cascade may generate different overall gene expression profiles, despite having similar effects on this particular pathway [2]. On the other hand, a single mutation in a critical gene may have profound biological effects despite having a limited effect on the total gene expression profile. Thus, to identify defects in functional pathways that lead to outcomes such as drug resistance, we need a panel of methods that detects different pathological disturbances based on functional hypotheses [2].

Adjuvant therapy

In contrast to the number of studies conducted in the primary medical setting, data with respect to the predictive value of gene expression profiles in the adjuvant setting are scarce. Although studies have addressed genetic factors that determine prognosis in patient cohorts exposed to defined therapies such as tamoxifen [33], the only large study exploring gene expression profile with respect to benefit of chemotherapy was that conducted by the NSABP (National Surgical Adjuvant Breast and Bowel Project). Taking a 21-gene expression signature previously shown to be associated with prognosis in tamoxifen-treated breast cancer patients [34], they reported that the same profiles also predict the likelihood of benefit from adjuvant chemotherapy with a regimen containing cyclophosphamide, methotrexate, and 5-fluorouracil [35]. Notably, they found a high recurrence score to be associated with profound effect of chemotherapy; this

is in contrast to the intermediate and low scores, for which no significant clinical benefit of chemotherapy was achieved. Although this test has been implemented in many centres around the world, independent validation is still awaited.

Testing for chemo-resistance *in vivo*: adjuvant versus primary medical treatment as the optimal setting

Although primary medical therapy is considered to be the optimal way to assess direct antitumour efficacy of drug treatment, this may not automatically imply a correlation with outcome defined as general relapse or cancer death. Pathological complete response to primary medical treatment has clearly been correlated with long-term prognosis [27]; however, several patients achieving a complete response may later relapse. There may be a number of explanations for this observation, such as survival of resistant subclones among micrometastases. However, we should recognize that a number of biological parameters in addition to direct drug sensitivity are involved in the metastatic process, such as blood vessel wall invasion, tumour-host organ interactions and angiogenesis. Notably, gene signatures have been identified that predict organ-specific metastatic propensity in experimental as well as clinical materials [36-38]. Interestingly, Massagué and coworkers [39], in addition, have identified a few key genes from their lung metastases signature associated with growth, invasion and angiogenesis, which play a key role in regulating lung metastases in experimental systems. Although adjuvant studies need larger patient cohorts as well as longer follow up in comparison with studies of primary medical therapy, there is clearly a need for long-term follow up of patients undergoing primary medical as well as adjuvant therapy to address these issues.

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