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E15. Use of microarray analysis to predict outcome and response in breast cancer patients

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Breast cancer is diagnosed one million times every year worldwide. It is a clinically heterogeneous disease, as evidenced by widely variable morphological appearances, distinctive gene expression profiles and different responses to therapies. For breast cancer patients, the accuracy with which the progress of their disease can be mapped will make the difference between whether they are over- or under-treated. Defining which patients will respond to therapy impacts upon treatment outcome and patients' survival.

The current prognostic criteria used for breast cancer include age, tumour size, axillary-node status, histological type, pathological grade and hormone–receptor status. Initially, human breast cancers depend on oestrogens for their development and progression. Approximately 75% express the classical oestrogen receptor (ER-α), which is an important target for therapy. Endocrine strategies that interfere with the action of natural hormones have been developed and given to patients as adjuvant treatment of their primary cancer or for treatment of metastatic breast cancer, as well as for the prevention of breast cancer in women at high-risk. The proven classical selective oestrogen receptor modulator (SERM) is tamoxifen (reviewed by Jordan in Ref. [1]). Nevertheless, in women with metastatic breast cancer, endocrine therapies produce response rates of only 30% in unselected patients and of approximately 50% in women with ER-α-positive tumours. By contrast, only 5–10% of ER-α-negative tumours will respond favourable to hormonal treatments.

In recent years, gene expression investigations have developed from the analysis of a single gene to that of ten of thousands of genes per experiment. This so-called high-throughput gene analysis or gene expression profiling makes use of specific oligonucleotides or cloned cDNA fragments spotted on a glass slide (micro-array). In 2000, Hanahan and Weinberg [2] proposed, "The growth regulation within a tumour can only be explained once we understand the contributions of the ancillary cells present in a tumour – the apparently normal bystanders such as fibroblasts and endothelial cells – which must play key roles in driving tumour cell proliferation". This means that knowledge of the interactions of malignant cells with neighbouring cells within a tumour environment is critical to understand the processes involved in breast cancer metastasis or its progression to therapy-resistance. Current studies of gene expression profiling on clinical samples or model systems show the enormous impact that expression profiling can have on our understanding of various biological processes in well-defined tumour models or selected clinical specimens (for reviews see [3]). The analysis of the overwhelming body of data was initially guided through the identification of "clusters" of genes showing similar patterns of expression by using algorithms and software described by Eisen in [4]. More recently, several other statistical approaches that provide additional information are applied, although rules of evidence for marker discovery and validation have not yet been fully developed [4,5].

Using RNA isolated from human breast tumours and from normal breast tissue, and by comparison with those expression profiles obtained for mammary epithelial cell lines grown *in vitro*, the expression profiles for some of the clusters of co-expressed genes in the tumour samples could be attributed to specific cell types, including stromal cells and B lymphocytes. This means that gene expression patterns in tumours have recognisable counterparts in specific cells, reflecting

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the tumour epithelial, stromal and inflammatory components of the tumour tissue. Unsurprisingly, two main subgroups of breast cancers have been identified by gene expression profiling; the ER-α-positive and -negative subsets [6]. Interestingly, relevant information on tissue specimens of various cancer subtypes can be extracted from the profiles as well. This has led to improved tumour classifications. For example, Hedenfalk and colleagues [7] and our Group [8] were able to distinguish *BRCA1*- and *BRCA2*-mutated tumours from sporadic breast cancer cases. Moreover, specific gene expression patterns were found to be predictive of disease outcome for breast cancer patients [9]. The colleagues at the Dutch Cancer Institute, for example, used a previously established 70-gene prognotic profile to classify patients with primary breast cancer into poor- and good-prognotic groups. They showed that (in young breast cancer patients, i.e. those patients younger than 53 years of age with stage I or II disease) this profile is a more powerful predictor of disease outcome than standard systems based on clinical and histological criteria. They proposed that this more accurate means of prognostication in breast cancer will improve the selection of patients for adjuvant systemic therapy [9,10]. If validated by others and in larger series, the differences in gene expression profiling may become a widespread strategy in the future for predicting clinical outcome.

Another potential application of gene expression profiling involves studies of the expression of particular genes in relation to mechanisms of drug sensitivity and resistance. As described above, there is a clinical necessity to distinguish an individual patient who will benefit or fail from endocrine treatment, whether applied as adjuvant therapy or for advanced disease. Molecular profiling of a panel of breast tumour tissues from a well-defined group patients who received anti-oestrogens as first-line treatment for advanced disease with known clinical follow-up, offers a unique opportunity to link the expression of large numbers of genes to the type of response. Our department has gathered together information on a huge panel of frozen breast tumours from patients who have well-defined responses and clinical follow-up in a computerised database, thereby allowing this type of unique study. For example, we have shown in large sample cohorts that the urokinase plasminogen activator (uPA) pathway, *TP53* and vascular endothelial growth factor (VEGF) are predictors of response to tamoxifen. We also used glass arrays with approximately 18 000 spotted human cDNAs to identify a set of genes whose expression pattern predicts the type of response to anti-oestrogen therapy. At present, we have identified and validated a set of classifier genes that can distinguish primary breast tumours from patients who responded and from those who did not respond to anti-oestrogen treatment [11].

The identification of new genes will also allow mechanistic studies aimed at the development of new effective treatment strategies, such as combination therapy with an anti-oestrogen and a drug interfering with one of the pathways that may have been activated during hormone-independent tumour growth. Moreover, molecular profiling of a breast cancer tissue sample obtained by fine-needle or through-cut biopsy from patients included in prospective trials may be feasible in the near future. These studies could involve patients with advanced breast cancer, but also patients with primary breast cancer who may benefit from adjuvant treatment. Ultimately, micro-arrays will be developed that can be used for future diagnostic purposes: to predict the type of response to therapy on an individual breast cancer patient basis.

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