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Application of molecular findings to the diagnosis and management of breast disease: recent advances and challenges

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Summary Established criteria are used in the diagnosis and management of patients with breast cancer. Although there are indicators of long-term survival (eg, the Nottingham Prognostic Index), it is clear that patients with very similar tumors may have quite different clinical behaviors (eg, response to treatment or outcome). This variability is partly explained by the underlying molecular composition of a tumor, encompassing genomic, transcriptomic, and proteomic alterations. Our quest to define this molecular basis of breast cancer has driven the amazing development of high-throughput technologies seen in recent years and that are now revolutionizing cancer biology. It is clear that core molecular pathways that are dysregulated in cancer, including oncogenic signaling and the DNA-damage response, are intimately linked to tumor behavior and hence will make useful targets for therapy. The integration of these multiple layers of new knowledge will have a major impact in clinical oncology, leading to significant breakthroughs in diagnosis and treatment.

1. Molecular biomarkers to predict breast cancer behavior

Due to the heterogeneous nature of breast cancer [1,2], molecular biomarkers have been introduced to improve the clinical management of patients. Their use in clinical practice is to predict prognosis and response to treatment, thereby helping to stratify patients into clinically relevant subgroups (reviewed in Rakha et al [3]). Hormone receptors (estrogen [ER] and progesterone [PR] receptors) and epidermal growth factor receptor 2 (HER2) are currently the only biomarkers routinely used in clinical practice to aid the histopathological classification of breast cancer and to predict response to specific targeted therapeutic agents. Importantly, these markers have good negative predictive value, in that they

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indicate patients who will not benefit from a particular targeted treatment, namely, endocrine therapy or anti–HER2-targeted therapy (trastuzumab). However, they are less robust as positive predictors of response to therapy with a significant proportion of patients not responding as expected.

Improving the power to predict response is therefore a significant challenge to managing patients appropriately. For example, the coamplification of the gene for topoisomerase IIa, *TOP2A* with *HER2*, predicts a significantly better response to anthracycline-based chemotherapy than *HER2* amplification alone [4,5]. The use of a proliferation index, measured by mitotic index (MI) or Ki67 staining, is also frequently discussed as an indicator of tumor aggressiveness and a predictor of response to chemotherapy and endocrine therapy, since highly proliferative tumors are more likely to respond than low proliferative tumors [6-8]. MI or Ki67 would be a practical inclusion in most clinical practices, provided consensus could be obtained for stratifying high versus low proliferative tumors. Furthermore, recent data suggests that a high Ki67 level in combination with high levels of the cellular stress marker p16 might be a useful predictor of recurrence in patients with ductal carcinoma in situ [9,10].

Many markers have also been shown to predict outcome, yet few are used routinely. Urokinase-type plasminogen activator and plasminogen activator inhibitor type-1 remains the strongest prognostic markers for disease-free survival and overall survival and the strongest predictor of metastasis [11,12]. Significant work has also focused on triple-negative breast cancers (negative for ER, PR, and HER2) to stratify this heterogeneous disease into clinically relevant subgroups and to identify appropriate therapeutic targets. In this respect, the additional expression of the basal markers epidermal growth factor receptor (EGFR) and/or Ck5/6 in tumors identifies a proportion of triple-negative tumors with basal phenotype that are associated with a worse outcome than triple-negative, nonbasal tumors [13]. A major challenge in incorporating these types of findings into clinical practice is achieving standardization and consensus for technical aspects of performing the test and in defining the most appropriate scoring cutoffs.

2. Gene expression profiling of breast cancers

Gene expression profiling of breast cancers has also contributed significantly to our understanding of tumor biology, classification, and predicting behavior. Patterns of gene expression and hierarchical clustering dissect the heterogeneity of breast cancer to the luminal A, luminal B, basal-like, HER-2, and normal-like subtypes that relate to histologic properties and clinical outcomes [14-18]. Several gene signatures have also been developed to predict outcome or response to treatment and are now available commercially as prognostic tests. These include the following: MammaPrint [19,20] (the 70-gene signature; Agendia, Amsterdam, The Netherlands), Oncotype DX [21] (the 21-gene recurrence score assay; Genomic Health, Redwood City, CA), Theros H/I, and Theros breast cancer index (the 2-gene assay: *HOXB13:IL17BR* ratio and the 5-gene molecular grade index; both from AvariaDX, Carlsbad, CA) [22-26]. The latter 3 assays, which aim to predict recurrence in ER-positive breast cancer patients treated with tamoxifan, are quantitative reverse transcriptase polymerase chain reaction diagnostic tests that are applicable to formalin-fixed paraffin-embedded clinical samples, and hence may be useful for clinical pathology laboratories. The Theros breast cancer index is a combination of Theros H/I and a molecular grade index (a 5-gene assay developed to refine the classification of tumor grading, which, for histologic grade II cancers, can be quite subjective) and identifies a subgroup of patients with very aggressive disease [23]. Such tests are extremely promising for predicting level of benefit from endocrine and/or chemotherapy but are not yet performed routinely in clinical practice, and their true value to the diagnostic pathologist will become clearer from large prospective clinical trials (eg, TAILORx, MINDACT, I-SPY 1) being performed in Europe and North America.

2.1. "Functionalization" of gene expression profiles

Despite the enormity in cancer-related gene expression profiling, assays that characterize both the biological significance of signatures and the function of specific proteins are also vital to understand the disease and to guide therapeutic options. One method to link gene expression patterns to the underlying pathway activity has been developed based on "metagenes": combination of individual gene expression values obtained as a consequence of introducing oncogene expression into quiescent cells, such as human primary mammary epithelial cell cultures [27,28]. The use of these oncogenic pathway signatures in a large meta-analysis of breast cancer gene expression data (n = 1143) revealed added complexity to the previously defined intrinsic molecular subtypes, leading to further subdivision within each subtype and a total of 17 molecular subgroups [29]. For example, in basal-like tumors, 2 subgroups have low EGFR activity but high v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (SRC) activity, whereas a third subgroup has high EGFR activity but low SRC activity. Moreover, the oncogenic pathway signatures were exploited to predict lymph node status, 3-year survival risks [27], and sensitivity to specific therapeutic agents [28,30]. Refining the heterogeneity of breast cancer in this way, by defining apparently functional pathways, would not only provide better prognostic evaluation of disease progression, but also identify opportunities for rational and personalized combination therapies [29].

2.2. Metastatic gene signatures

The prediction of the metastatic potential of a primary tumor would be of considerable clinical benefit, leading to aggressive treatment and surveillance in high-risk patients.