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Molecular Phenotype, Multigene Assays, and the Locoregional Management of Breast Cancer

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Molecular profiling has revealed that breast cancer is not a single disease entity, but rather a class of heterogeneous subtypes, each with its own inherent biology and natural history. As a result, different treatment approaches have been optimized for the various subtypes and, in turn, the ability to identify subtypes has become a critical element in the management of breast cancer. Comprehensive transcriptional profiling studies have revealed at least 4 principal subtypes that, in practice, are often distinguished by immunohistochemical staining of the estrogen receptor (ER), progesterone receptor (PR), and HER2, along with a determination of histologic grade or Ki-67 staining: luminal A (ER+/HER2−/grade 1 or 2), luminal B (ER+/HER2−/grade 3), HER2 enriched (any HER2+ tumor), and basal like (ER−/PR−/HER2−). Although these immunohistochemically derived subtypes show robust prognostic and predictive ability, there remain many cases that demand profiling that more closely approximates the original transcriptionally derived definitions of the intrinsic subtypes. The need for improved prognostication and risk stratification has led to the development of several multigene assays in breast cancer. Although there is little molecular overlap between current assays, they all rely heavily on quantifying the transcriptional output of ER signaling and proliferation-related genes. These data are typically then used in multivariate prediction models that incorporate other canonical risk factors such as the tumor size, lymph node involvement, and patient demographic parameters, among others. Indeed, the advent of scalable molecular profiling technologies has brought a number of assays into routine clinical use for optimizing risk prediction and treatment assignment. The landscape of these assays and the clinical utility of contemporary molecular profiles are the main focus of this overview. In addition to the clinical advances in transcriptional subtyping, recent reports have characterized the most common genomic and epigenomic alterations that are likely to drive certain breast cancers. The identification of these "driver" lesions has heralded an era of precision medicine in which vulnerable oncogenic pathways may be targeted to disrupt the etiologic lesion(s) of a specific tumor. A number of such early targeted approaches have yielded success in treating breast cancer, demonstrating the critical need for molecular diagnostics in this disease.
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Introduction

Breast cancer is not a single disease entity, but rather a class of several distinct biologic subtypes, each with its own natural history and risk profile. The discovery of the diversity among breast cancers has heralded an era of increasingly effective therapies that now include biologic agents, endocrine modulators, and targeted small molecules, in addition to the systemic drugs that have long been used in routine practice. The importance of estrogen-receptor (ER) and progesterone-receptor (PR) expression in prognosis and prediction of endocrine therapy response was among the first molecular features to be identified in breast cancer.[1] Furthermore, it has been readily apparent that although ER-positive tumors generally have more favorable outcomes,[2] this overall class harbors a subset of tumors that are not readily responsive to

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endocrine therapy.[3] HER2-neu/ERBB2 amplification has been similarly informative as a prognostic factor for nearly 4 decades, and in the past 10 years has become a canonical success story of precision medicine and targeted therapy with the introduction of the anti-HER2 monoclonal antibody, trastuzumab, into routine use for HER2-enriched breast cancers.[4] The application of these fundamental molecular principles to clinical practice is now being facilitated by multigene assays.

In recent years, a growing body of literature has begun to meticulously dissect the underlying genomic, transcriptomic, epigenetic, and proteomic molecular profiles of breast cancer and to correlate these findings with the ultimate metric: clinical outcome.[5,6] The breast oncology community now describes breast cancer in terms of intrinsic biologic subtypes of which there are 4: luminal A (ER positive with low proliferation), luminal B (ER positive with high proliferation), HER2 enriched (with HER2 amplification), and basal (ER negative without HER2 amplification).[7] In practice, some have divided "HER2 enriched" into ER-positive and -negative classes. These subtypes have become increasingly relevant for predicting local control, with numerous studies demonstrating that luminal A tumors appear to have the most favorable outcomes whereas luminal B and HER2-enriched lesions are most likely to exhibit lymph node involvement.[2,8,9] As a result, critical treatment decisions now hinge on these molecular findings. Numerous efforts are now underway to refine our understanding of breast cancer biology and to enhance our ability to individualize therapy. Multigene assays such as the 21-gene Oncotype DX,[10] Prosigna PAM50,[11] and 70-gene Mammaprint[12] all promise to refine the practice of breast oncology, ushering in an era of personalized disease management.

Multigene Assays

Oncotype DX

The Oncotype DX 21-gene assay (Oncotype DX, Genomic Health, CA) was among the first clinically validated molecular tests to offer patients with breast cancer a robust model of risk stratification.[10] Since then, it has adopted an increasingly prominent role in predicting the benefit of adjuvant systemic therapy among patients with early-stage breast cancer and ER+ disease. This assay, among other transcription-based profiling platforms, is based on a landmark study showing that the reverse-transcriptase real-time polymerase chain reaction (RT-PCR) can be used to accurately quantify RNA from formalin-fixed paraffin-embedded (FFPE) tissue samples.[13] A parallel study demonstrated the reproducible nature of this technique on samples that were more than 2 decades old.[14]

The assay itself is based on an RT-PCR analysis of the expression of 21 genes (16 of which are tumor related with 5 reference genes for normalization; Table). These 16 genes were selected following a comprehensive analysis of 250 candidate genes among samples from 3 unrelated clinical studies. The 16 were chosen on the basis of robust statistical correlation between gene expression and distant-recurrence risk. As one might expect, this panel is driven by the analysis of ER, SCUBE2, and PR, in addition to a number of proliferation-related elements, including Ki-67, survivin, serine-threonine kinase 15, cyclin B1, MYBL2, GRB2, HER2, and BCL2. The invasion-related genes stomelysin-3 and cathepsin L2 are included, along with CD68, BAG1, and GSTM1.[10] Notably, the use of 5 reference genes for internal normalization enabled cross-patient comparisons and has since become a standard approach for RT-PCR-based studies.

Tissue for this test is typically derived from either biopsy or resection specimens, submitted to a central facility to be processed for messenger RNA (mRNA), and analyzed by a commercial vendor. Results from the assay yield a trademarked "recurrence score" (RS), which translates to an estimate of the 10-year risk of distant recurrence. The RS is calculated using a regression model of the following form:

$$RS = +0.47 \times \text{HER2 group score} - 0.34 \times \text{estrogen group score} + 1.04 \times \text{proliferation group score} + 0.10 \times \text{invasion group score} + 0.05 \times \text{CD68} - 0.08 \times \text{GSTM1} - 0.07 \times \text{BAG1}.$$

Clinical validation studies have demonstrated that the RS yields information beyond the usual clinical parameters (TNM stage, pathologic parameters, and immunohistochemical (IHC) markers), reliably predicting the likelihood of benefit from chemotherapy in early-stage ER + breast cancer that is either node negative[10] or node positive.[14-16] Although chemotherapy decision-making practices vary widely across institutions, use of the Oncotype DX assay appears to consistently reduce the proportion of patients who receive chemotherapy, while similarly identifying those at high risk who otherwise would likely have received endocrine therapy alone.[17-19]

The RS ranges from 0-100; patients with a low RS (<18) appear to derive minimal benefit from chemotherapy whereas those with high RS (≥ 31) appear to benefit considerably. Those with an intermediate risk score (18-30) likely benefit only marginally, although this stratum likely includes patients who are at high risk and, therefore, should receive adjuvant chemotherapy. A number of studies are ongoing to further inform the risk profiles of those patients with an intermediate RS, including the TailoRx trial (discussed later), which is randomizing 10,000 patients with an RS of 11-25 to endocrine therapy alone vs endocrine plus chemotherapy.

Although Oncotype DX is being widely employed to predict benefit from chemotherapy, an established body of literature has now demonstrated that the assay also accurately predicts distant-recurrence risk.[10,16,20-23] The initial study to validate this observation was based on tumor samples from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14 trial.[10] This trial was designed to assess whether adjuvant tamoxifen could provide a survival benefit for patients with early-stage, ER+, node-negative breast cancer and the tissue was meticulously collected with an eye toward subsequent correlative studies. Indeed, mRNA was successfully extracted from 668 archived FFPE tumor samples and the Oncotype DX assay was employed to yield 3 discrete risk groups (low, intermediate, or high, defined earlier based on RS). Estimates of distant recurrence at 10 years as stratified by these groups revealed that the low-risk group had a significantly more favorable recurrence-free rate (93.2%) than their high-risk