Classification and prognosis of invasive breast cancer: from morphology to molecular taxonomy

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For many years, patient age, axillary lymph node status, tumor size, histological features (especially histological grade and lymphovascular invasion), hormone receptor status, and HER2 status have been the major factors used to categorize patients with breast cancer in order to assess prognosis and determine the appropriate therapy. These factors are most often viewed in combination to group patients into various risk categories. Although these risk categories are useful for assessing prognosis and risk in groups of patients with breast cancer, their role in determining prognosis and evaluating risk in an individual patient is more limited. Therefore, better methods are required to help assess prognosis and determine the most appropriate treatment for patients on an individual basis. Recently, various molecular techniques, particular gene expression profiling, have been increasingly used to help refine breast cancer classification and to assess prognosis and response to therapy. Although the precise role of these newer techniques in the daily management of patients with breast cancer continues to evolve, it is clear that they have the potential to provide value above and beyond that provided by the traditional clinical and pathological prognostic and predictive factors.

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A variety of clinical and pathological factors are routinely used to categorize patients with breast cancer in order to assess prognosis and determine the appropriate therapy. These include patient age, axillary lymph node status, tumor size, histological features (especially histological grade and lymphovascular invasion), hormone receptor status, and HER2 status. Considering these factors in combination is of greater clinical value than viewing each in isolation, and the combined approach forms the basis of a number of schema used to group patients into various risk categories such as the St Gallen criteria,^{1,2} the NIH consensus criteria,³ the Nottingham Prognostic Index,⁴ and Adjuvant!Online (www.adjuvantonline.com). Although these risk categories have been of great value for assessing prognosis and risk in groups of patients, their role in determining

prognosis and evaluating risk in an individual patient with breast cancer is more limited, as patients with similar combinations of features may have very different clinical outcomes. Better methods, therefore, are required to help assess prognosis and determine the most appropriate treatment for patients on an individual basis.

In recent years, various molecular techniques, particularly gene expression profiling, have been used increasingly to help refine breast cancer classification and to assess prognosis and response to therapy. Although the precise role of these newer techniques in the daily management of patients with breast cancer continues to evolve, it is clear that they have the potential to provide value above and beyond that provided by the traditional clinical and pathological prognostic and predictive factors and, in turn, to have a major impact on the management of patients with breast cancer. This review focuses on the emerging role of molecular techniques in providing new insights into breast cancer classification and in assessing prognosis of patients with breast cancer.

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Molecular classification

Studies of breast cancers using gene expression profiling have identified several major breast cancer subtypes beyond the traditional hormone receptor-positive and hormone receptor-negative types. The most reproducibly identified molecular subtypes among the hormone receptor-positive cancers are the luminal A and luminal B groups. The HER2 and basal-like groups are the major molecular subtypes identified among hormone receptor-negative breast cancers. Other molecular subtypes such as luminal C and normal breast-like groups have also been identified in some studies, but are less well-characterized than the luminal A, luminal B, HER2, and basal-like types.^{5–11} These breast cancer molecular subtypes differ with regard to their patterns of gene expression, clinical features, response to treatment, and prognosis, as summarized in Table 1.

One of the most novel findings to have emerged from these studies has been the characterization of basal-like breast cancers as a distinct molecular group. It should be noted, however, that breast cancers with a 'basal' phenotype were not 'discovered' by gene expression profiling experiments. These tumors were described by pathologists about 30 years ago.¹² Most basal-like carcinomas are invasive ductal carcinomas that feature high histological grade, solid architecture, absence of tubule formation, high mitotic rate, a stromal lymphocytic infiltrate, a pushing border, geographic zones of necrosis and/or a central fibrotic focus, and little or no associated ductal carcinoma *in situ*.^{13–16} Moreover, they are most often estrogen receptor (ER)

negative, progesterone receptor (PR) negative, lack HER2 protein overexpression and gene amplification ('triple negative'), and show expression of basal cytokeratins, epidermal growth factor receptor, and other basal-related genes. Basal-like carcinomas are especially common in African-American women and are generally considered to be associated with a poor prognosis. However, recent studies have emphasized that basal-like carcinomas are a heterogeneous group with regard to their histological features, biomarker expression profile, and clinical course, and that the prognosis of patients with these tumors is not uniformly poor.^{17–20} Of note, ~80% of *BRCA1*-associated breast cancers cluster with the basal-like group in gene expression profiling studies. In addition, many sporadic basal-like breast cancers show *BRCA1* dysfunction.^{21,22} The reader is referred to several excellent recent reviews of basal-like breast cancer for more details about this breast cancer subtype.^{12,15,16,22–27}

Earlier reports suggested that the three biomarkers that are routinely used to assess breast cancers in clinical practice, that is, ER, PR, and HER2, can be used to approximate the molecular category of breast cancer, as defined by gene expression profiling.⁷ Using this approach, tumors that are ER positive and/or PR positive, and HER2 negative are most likely luminal A, those that are ER positive and/or PR positive, and HER2 positive are most likely luminal B, those that are ER negative, PR negative, and HER2 positive are most likely HER2 type, and those that are ER negative, PR negative, and HER2 negative are most likely basal-like. However, more recent studies have indicated that other markers in addition to ER, PR, and HER2 are required to more

Table 1 Major molecular subtypes of breast cancer determined by gene expression profiling.

	Molecular subtype		
	Luminal	HER2	Basal
Gene expression pattern	High expression of hormone receptors and associated genes (luminal A > luminal B)	High expression of HER2 and other genes in amplicon Low expression of ER and associated genes	High expression of basal epithelial genes, basal cytokeratins Low expression of ER and associated genes Low expression of HER2
Clinical features	~70% of invasive breast cancers ER/PR positive Luminal B tend to be higher histological grade than luminal A Some overexpress HER2 (luminal B)	~15% of invasive breast cancers ER/PR negative More likely to be high grade and node positive	~15% of invasive breast cancers Most ER/PR/HER2 negative ('triple negative') <i>BRCA1</i> dysfunction (germline, sporadic) Particularly common in African-American women
Treatment response and outcome	Respond to endocrine therapy (but response to tamoxifen and aromatase inhibitors may be different for luminal A and luminal B) Response to chemotherapy variable (greater in luminal B than in luminal A) Prognosis better for luminal A than luminal B	Respond to trastuzumab (Herceptin) Respond to anthracycline-based chemotherapy Generally poor prognosis	No response to endocrine therapy or trastuzumab (Herceptin) Appear to be sensitive to platinum-based chemotherapy and PARP inhibitors Generally poor prognosis (but not uniformly poor)

Table 2 Immunophenotyping to approximate molecular subtype using six biomarkers^a

Molecular subtype	Biomarker profile
Luminal A	ER+ and/or PR+, HER2−, and low Ki67 (<14%)
Luminal B	ER+ and/or PR+ and HER2+ (luminal-HER2 group)
HER2	ER+ and/or PR+, HER2−, and high Ki67 (>14%)
Basal-like	ER−, PR−, and HER2+ ER−, PR−, HER2−, and CK5/6 and/or EGFR+

Abbreviations: CK, cytokeratin; EGFR, epidermal growth factor receptor; ER, estrogen receptor; PR, progesterone receptor.

^aBased on data from references Cheang *et al*²⁹ and Cheang *et al*.³¹

accurately approximate the molecular subtype, particularly the basal-like group (only about 70–80% of which are ‘triple negative’)²⁸ and the luminal B group (only about 30% of which are HER2 positive).²⁹ For example, the basal-like group can be defined more precisely using, in addition to ER, PR, and HER2, antibodies to CK5/6 and epidermal growth factor receptor (EGFR) as basal-like cancers are most often ‘triple negative’ and also express CK5/6 and/or EGFR. In fact, using this combination of markers by immunohistochemistry, cancers that cluster in the basal-like group in gene expression profiling experiments can be identified with a sensitivity of 100% and a specificity of 76%.^{30,31} Further, assessing the proliferative rate as determined by Ki67 expression helps to identify cancers in the luminal B group more precisely than does knowledge of the ER, PR and HER2 status alone.²⁹ Thus, at the present time, a six biomarker profile seems to be the most useful panel for approximating the molecular subtype of breast cancers as determined by gene expression profiling (Table 2).

Molecular techniques have also been used to understand the taxonomy of special type cancers better. For example, using the combination of immunohistochemistry and gene expression profiling, one study recently showed that some special type cancers represent discrete entities (for example, invasive micropapillary carcinoma), but that others are very similar at the transcriptome level despite morphological differences (for example, tubular carcinomas and lobular carcinomas). Further, when classified by expression profiling, lobular carcinomas (similar to ductal carcinomas) are composed of tumors in all molecular categories (luminal, HER2, and basal), whereas most other special type cancers belong to only one molecular category (for example, tubular, mucinous, endocrine, and micropapillary carcinomas are luminal; medullary, adenoid cystic, and metaplastic carcinomas are basal).³²

Another area in which expression array analysis may be of value is in refining the grading of invasive breast cancers. Two recent studies have suggested that, although histological grade I and grade III

tumors have distinct expression signatures, there is no characteristic expression signature for histological grade II tumors. Further, in these studies applying genomic grading to histological grade II tumors was able to separate that group into those which had a favorable prognosis (similar to that of grade I tumors) and those in which the prognosis was poor (similar to that of grade III tumors).^{33–35}

Although tests to determine molecular subtype and genomic grade are or will soon be commercially available,^{10,36} at the present time the clinical value of characterizing invasive breast cancers beyond routine histological type, histological grade, and ER, PR, and HER2 status has not been established, and at most institutions, additional biomarker studies such as CK5/6, EGFR, and Ki67, are not routinely performed to help determine the molecular breast cancer subtype. This, however, may change as treatment regimens that target specific molecular subtypes are developed and validated. In this regard, recent studies have suggested that basal-like breast cancers are particularly sensitive to platinum-based chemotherapy and poly(ADP-ribose) polymerase inhibitors.^{22,37,38} Further, the identification of basal-like subtype using immunohistochemistry for basal cytokeratins and other markers may be of clinical value to help identify women who harbor *BRCA1* germline mutations,^{39,40} although the strength of the association between basal cytokeratin expression and the presence of *BRCA1* mutations has recently been questioned.^{41,42}

Molecular prognostic tests

As noted above, although evaluation of individual prognostic and predictive factors has permitted the identification of clinically distinct subgroups of patients with breast cancer, there is a pressing need to develop a comprehensive profile of the biological and molecular characteristics of a tumor that may aid in the assessment of prognosis and the prediction of response to various therapeutic modalities in individual patients. A number of gene expression profiles and gene sets have been reported to be prognostic and/or predictive for patients with breast cancer.^{11,36,43–45} It is of interest to note that despite similar prognostic or predictive value, there is very little, if any, overlap in the genes included in these profiles.⁴⁶

A recent meta-analysis of gene expression data of almost 3000 breast cancers found that the signatures for ER, HER2, and proliferation could be used to group the tumors into breast cancer subtypes analogous to those described above (that is, luminal A, luminal B, HER2, and basal-like). In addition, proliferation genes were found to be the common driving force behind all of the prognostic signatures. Finally, this analysis showed that clinical indicators of tumor burden (such as tumor size and lymph node status) add independent prognostic informa-

tion to the information obtained from the gene signatures.⁴⁷

Two molecular tests that are now commercially available merit specific comment. OncotypeDX (Genomic Health) is an RT-PCR-based assay that can be performed on formalin-fixed tissue from paraffin blocks. It is based on the analysis of the expression of 21 genes and provides a 'recurrence score' that correlates with outcome, as well as likelihood of response to endocrine therapy and likelihood of response to chemotherapy.^{48–51} Mammprint (Agendia) uses expression array analysis of 70 genes to identify patients with good and poor prognostic signatures.^{52–54} This assay requires fresh frozen tumor tissue. It should be noted that, although both of these tests are already being used in patient management, their ultimate value will be determined by the results of prospective clinical trials that are currently underway, namely, the TAILORx trial to study OncotypeDX⁵¹ and the MINDACT trial to study Mammprint.⁵⁴

Conclusions

Molecular tests, especially gene expression profiling, have great potential to refine breast cancer classification, enhance our understanding of the disease biology, and improve patient management. However, their role in daily clinical practice is continuing to evolve. As indicated in a recent review, 'the additional clinical value of molecular classification is limited by its close association with the status of ER, PR and HER2, along with tumor grade'. However, 'the molecular differences that underlie the phenotypes of breast cancer could reveal new therapeutic targets'.¹⁰

Prospective, randomized trials are currently underway to address the clinical role of two prognostic signatures (OncotypeDX and Mammprint), but it will be years before the results of these trials are available. In the meantime, despite the availability of a growing number of molecular tests, management of patients with breast cancer remains critically dependent on careful pathological evaluation supplemented by ER, PR, and HER2 test results using validated assays.

Disclosure/conflict of interest

The author declares no conflict of interest.

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