Breast Cancer comprises a remarkably diverse group of diseases in terms of presentation, morphology, biologic characteristics and clinical behavior. Currently, decisions about breast cancer treatment are based on certain clinicopathologic parameters that have been clinically validated and serve as a guide for the use of systemic therapy and prognostication. These include tumor size, lymph node stage and histologic grade, vascular invasion, histologic type, and the patients’ age and menopausal status. In addition, a few molecular markers, namely, the estrogen receptor (ER), the progesterone receptor (PR), and the human epidermal growth factor receptor 2 gene (HER2), which have been proven to provide therapeutic predictive and prognostic value, are currently of central importance for the routine clinical management of breast cancer. For example, it is widely recognized that ER-positive (ER+; 60%–75% of tumors) and ER-negative (ER−) breast cancers are 2 different disease entities. Generally, ER− tumors tend to be of high grade, more frequently have TP53 mutations and HER2 amplifications, and have worse prognoses than ER+ disease (reviewed in Shao and Brown2 and in Lacroix et al3). Most important, ER− disease is not sensitive to endocrine therapies, such as tamoxifen and aromatase inhibitors, but more often shows a complete pathologic response following neoadjuvant chemotherapy.4 HER2 is amplified in approximately 18% to 20% of breast cancers, and this amplification is associated with a worse prognosis (higher rates of recurrence and mortality) in patients with newly diagnosed breast cancer who do not receive any adjuvant systemic therapy. HER2 status is also predictive for response to several systemic therapies, particularly to agents that target HER2.5

All these routinely assessed parameters are currently used to assign patients into risk groups to determine their requirement for local and systemic therapy, such as radiotherapy, hormone and/or chemotherapy, and HER2-targeted therapy. Despite the overall association of these variables with prognosis, outcome, and response to specific therapy,6 these parameters are insufficient to capture the nuances of individual breast cancer cases and fail to provide a level of accuracy that allows tailoring of the therapy to the individual patients.1,3 In fact, it is clear that tumors with remarkably similar morphologic and immunohistochemical features may still vary in response to therapy and have distinct outcomes,7 which may reflect the great variation in biologic characteristics and genetic profiles of breast cancer.

Conclusions.—Basal-like breast cancers constitute a distinct, yet heterogeneous, class of neoplasms associated with specific histologic features and poor prognosis despite high response rates to neoadjuvant chemotherapy. Basal-like breast cancers have features that recapitulate those of tumors arising in BRCA1 carrier mutations, and the majority of patients with BRCA1 germline mutations develop basal-like breast cancers. At the molecular level, basal-like cancers harbor a transcriptome that is distinct from that of hormone-receptor–positive or HER2-amplified tumors, being characterized by the expression of genes usually found in basal/myoepithelial cells of the breast. However, translating the new concepts about basal-like cancer into clinical practice has proven a Herculean task, given the lack of an internationally accepted definition for these tumors and for the method of identification in routine practice.

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Several individual biologic markers have been evaluated in breast cancer, and some have shown association with tumor behavior and patient outcome; however, the level of certainty about the clinical value of these markers is not sufficient enough to incorporate any of them into routine practice. In addition, the degree of cellular and molecular heterogeneity in breast cancer and the large number of molecular events involved in breast cancer progression emphasize the importance of studying multiple molecular alterations in concert. Microarray-based global gene expression profiling (GEP) opened new avenues for classifying breast cancer into biologically and clinically distinct groups based on gene expression patterns. Microarray-based class-discovery studies pioneered by Perou and colleagues have established for the first time that breast cancer could be classified into molecularly distinct groups based on global gene expression profiles and their similarity to those of normal cell counterparts. Multiple independent studies have confirmed and expanded the original results. One of these biologically and clinically distinct subgroups, which has recently attracted the attention of pathologists, oncologists, and scientists as a molecularly distinct subtype of breast cancer with poor prognosis, is basal-like cancer.

**BASALNESS IN BREAST CANCER: HISTORICAL BACKGROUND**

Given the importance of basal and luminal concepts in recent molecular classifications and the relationship among features of different classes of breast cancer and different types and features of normal parenchymal cells of the breast, a review of the origin of basal in breast cancer literature is warranted. There is a great deal of confusion about what basal means in the context of breast pathology. In fact, this term has been used to refer to myoepithelial cells, which are basally located, as well as to a subgroup of luminal cells that express high molecular weight basal keratins.

Normal human breast has ducts and lobules lined with 2 cell layers, an inner luminal cell population and a distinct outer cell layer, which is juxtaposed to the basement membrane. These 2 cell populations can be distinguished by their location, by their immunophenotype, and even by their global gene expression profile. The outer, abluminal cell layer exhibits features of both epithelial and smooth muscle cells, and therefore, they are called myoepithelial (ME) cells. These basally, abuminally located ME cells have distinct transcriptomes and proteomes, when compared with luminal epithelial cells, and express high molecular weight cytokeratins (ie, basal CKs), including CK5 (or CK5/6), CK14, and CK17, in addition to other markers related to the myoid apparatus.

In addition to these basally located ME cells, basal CKs are also expressed in a variable subpopulation of cells in the luminal compartment of both large ducts and the terminal duct lobular unit, although there is considerable variation in their expression pattern. These basal marker-positive, non-ME cells usually lack features of myoid differentiation. In these circumstances, these basal CK-positive cells are called basal cells. Therefore, in normal breast tissue, the term basal has been applied to the well-defined ME (contractile) cells and to a specific subpopulation of basal CK-expressing cells that may be found in either a luminal or basal location regardless of smooth muscle marker expression.

In breast cancer, the class of tumors showing basal differentiation was described more than 30 years ago. The expression of basal CKs in breast cancer was first described in 1982, which was followed by several other studies that confirmed the expression of different basal CKs in breast cancer, with a variable percentage of cases from 0% to 17.5%. The potential prognostic significance of the expression of basal CKs was suggested by Dairkee et al and was confirmed in several independent studies. In addition, the poor prognostic impact of other basal markers, such as vimentin, has been reported. Despite this previous recognition of the association between basal marker expression and poor outcome for patients with breast cancer, no specific designation was used to describe this class of tumors, and it did not receive much attention until after its identification in GEP studies as a molecularly distinct subtype of breast cancer. It would be fair to credit microarray-based GEP class-discovery studies with bringing this class of tumors to the forefront of breast cancer research, although such studies were not the first to recognize it. Since their recognition by Perou et al, basal-like breast cancers have been referred to as basal, basal-type, basaloid, basal phenotype, basal cell, basal-like, or basal-like, which is our preferred term for these tumors.

**BASAL-LIKE BREAST CANCER: DEFINITIONS**

It is currently accepted that GEP analysis is the gold standard for the identification of basal-like cancers. Most GEP studies have shown that breast cancer can be grouped into at least 3 main subgroups (1) a luminal subgroup, which encompasses tumors that express ER and genes related to activation of the ER pathway; (2) a HER2 subgroup, which is characterized by overexpression of HER2 and by genes pertaining to HER2 amplicon; and (3) a subgroup of tumors that do not cluster with either hormone receptors or HER2-positive tumors (triple-negative tumors). This latter subgroup of tumors has been shown to be remarkably heterogeneous and to encompass at least 2 subgroups: basal-like cancers, which are largely characterized by positive expression of basal CKs and other genes that are characteristic of basal-like cells of the breast and by high proliferative activity. The other subgroup, the so-called normal breastlike cancers, although also displaying a triple-negative phenotype, does not cluster with the basal-like centroid and is characterized by expression profiles similar to those found in normal breast tissue and samples of fibroadenomas. At the DNA level, basal-like tumors show the most frequent chromosomal gains and losses, less-frequent DNA amplification, and a higher rate of loss of heterozygosity than other subtypes. These tumors seem to harbor a dysfunctional breast cancer 1, early onset (BRCA1) pathway, and tumors arising in BRCA1 mutation carriers often display a basal-like phenotype. The existence of a basal-like subtype of ductal carcinoma in situ and the association of these tumors with characteristic morphologic and immunophenotypic features provide further evidence that this subtype is a distinct disease entity. Taken together, these findings suggest that different biologic mechanisms underlie basal-like tumors. Moreover, GEP studies consistently showed an association between basal-like cancer and poor prognosis, although the number of cases included in most of these studies was limited.

Unlike luminal and HER2-positive classes, which al-
ready have targeted therapy available (ie, endocrine therapy for luminal cancers and monoclonal antibodies or tyrosine kinase inhibitors for HER2 cancers), basal-like cancers do not have specific tailored therapies. Although a subgroup of basal-like breast cancers shows an exquisite sensitivity to neoadjuvant chemotherapy, and patients in the subgroup have an excellent prognosis following pathologic complete response, most patients with basal-like breast cancers have a poor outcome.

**IMMUNOHISTOCHEMICAL DEFINITION**

Although GEP is still the gold standard for the identification of basal-like breast cancer, the use of microarrays is still costly, is largely limited to fresh or frozen samples, and is cumbersome to implement in routine diagnostic practice. To translate this approach into a platform more readily applicable to formalin-fixed, paraffin-embedded samples, several groups have endeavored to define surrogate immunohistochemical or quantitative real-time reverse transcriptase polymerase chain reaction–based signatures for the identification of the molecular subgroups identified by microarray-based expression analysis. Despite active research in this field and the validation of a 53 gene quantitative real-time reverse transcriptase polymerase chain reaction–based intrinsic gene list, which has 93% concordance with microarray-based assignments, only one immunohistochemical panel that recapitulates the intrinsic gene list has been validated by expression arrays. This panel comprises 4 markers (ER, HER2, CK5/6, and epidermal growth factor receptor [EGFR]) and identifies basal-like cancers with 100% specificity and 76% sensitivity. Although its accuracy for the other molecular subgroups remains to be determined, this immunohistochemical surrogate has been shown to be of prognostic significance. The use of this immunohistochemical panel has led to the identification of the morphologic spectrum of basal-like cancer, which encompasses grade III invasive ductal carcinomas, (atypical) medullary carcinomas, and metaplastic breast cancers. However, currently, there is no international consensus on the precise complement of markers that defines a basal-like cancer.

Based on the frequent expression of basal CKs in basal-like cancer and the recognized association between basal CK expression and poor prognosis, the definition proposed by Nielsen et al includes 5 markers, and basal-like breast cancers are defined as those lacking ER, PR, and HER2 expression (the triple-negative phenotype). This new definition identifies 2 distinct basal-like phenotypes. Of the triple-negative phenotype, approximately 75% of cases have expression of CK5/6 and/or EGFR. Although CK14 was not included in the original GEP studies as a basal marker, some authors believe that addition of CK14 to the panel can improve the definition of basal-like cancer (6 markers: ER-negative, PR-negative, and HER2-negative and CK5/6-positive with CK17 staining because it showed focal and weak reactivity and was often difficult to score (only 4.6% of the tumors were considered positive). Livasy et al demonstrated that 61% (11/18) of basal-like tumors express CK5/6. However, the authors did not include CK14 or CK17 in their study. Interestingly, in that study, 61% of the tumors expressed HER2 and were expressed in 8% (1/12) of the HER2 class of tumors. Although these results emphasize the relationship between basal-like cancer and the IHC of basal CK expression, they demonstrate the difficulty in exactly identifying the same tumors. In addition, distinct clones for basal keratins have been used in different studies, adding another level of complexity to translating the results of GEP into immunohistochemical panels to identify basal-like cancer. Moreover, it has been shown that the staining patterns of these basal CKs can be highly variable and heterogeneous.

Another approach often used to identify basal-like breast cancer is the use of a triple-negative phenotype because the vast majority of basal-like breast cancers lack ER, PR, and HER2 expression (the triple-negative phenotype). This has led some authors to claim that “triple-negative tumors are synonymous with basal-like tumors.” In our view, this is a misconception, given that not all basal-like breast cancers lack ER and HER2, and not all triple-negative cancers are of basal-like phenotype. It should also be emphasized that triple-negative breast cancer constitutes a more heterogeneous group of tumors than basal-like breast cancer and that the use of a definition based on the lack of markers may lead to misclassification of cases because of technical artifacts (ie, false-negative immunohistochemical results)

Previous studies have reported that only 50% to 80% of triple-negative tumors express basal markers and that this expression identifies a subgroup of tumors that are associated with poor outcomes. Other markers that have also been used as part of the definition of basal-like cancer include EGFR, c-Kit, P-cadherin, nestin, osteonectin, vimentin, and laminin. Conclusively, this variability in the IHC definition has created some degree of discordance and contradictory results among different studies regarding the prognostic significance and behavior of tumors identified using different definitions. However, it is important to mention that the most pragmatic definition of basal-like tumors that can be used in routine practice is the definition that is based on hormone receptors, HER2 negativity, and specific basal marker positivity (CK5/6, CK14, CK17, and EGFR). Consequently, some authors have advocated the definition of basal-like cancers based solely on the expression of basal keratins; it should be noted, however, that the significance of the expression of basal markers in hormone receptor–positive or HER-positive tumors is not known at present. These tumors are likely to be treated by endocrine or anti-HER2 therapy.

More recently, lack of PR expression has been added to the definition proposed by Nielsen et al. This new definition includes 5 markers, and basal-like breast cancers are defined as those lacking ER, PR, and HER2 expression and expressing CK5/6 and/or EGFR. Although it is not identified in the original GEP studies as a basal marker, some authors believe that addition of CK14 to the panel can improve the definition of basal-like cancer (6 markers: ER-negative, PR-negative, and HER2-negative and CK5/6-positive).
positive and/or CK14-positive and/or EGFR-positive tumors), given that CK14 (1) is expressed in basal/ME cells of the breast and forms complexes with CK5, (2) has a staining pattern that is more reliable than that of CK17, (3) stains a proportion of breast cancer cases that overlap with CK5/6 positivity, and (4) is associated with a poor outcome.48,95–97

**EPIDEMIOLOGY**

Basal-like cancer represents from 8% up to 37% of all breast cancer cases, depending on the proportion of grade 3 cases included in the population (mean, 16.7%). The average age of patients with basal-like cancer ranges from 47 to 55 years,73,78,101 and most patients are younger61,73,85,96 and more are premenopausal73 than patients with nonbasal tumors. In the United States, basal-like breast cancer is more common among African American and Hispanic women.73 Some authors have reported that basal-like tumors may achieve extraordinarily rapid clinical growth rates102 and are overrepresented among the so-called interval breast cancers (eg, cancers arising between annual mammograms).39

**CLINICOPATHOLOGIC FEATURES**

Most (68%–86%) basal-like tumors are invasive ductal carcinomas of no special type,78,86,96 but occasionally, the carcinoma is tubular mixed.96 In addition, the most metastatic79 or medullary-like cancers103–105 display a basal-like phenotype. The basal-like phenotype is rare in other specific types of breast cancer, such as tubular or cribriform lobular carcinomas.96,106 However, some low-grade breast carcinomas, such as adenos ductal93,107 secretory carcinomas of the breast108 (J.S.R-F., unpublished data, 2006), and adenomyoepitheliomas, also display a basal-like phenotype. The inclusion of these low-grade lesions in the group of basal-like breast cancers is a rather contentious issue: given their histologic features are distinctive, their molecular findings are different from those of most basal-like breast cancer (eg, they rarely harbor the TP53 gene mutation), and their favorable prognosis, they may warrant a separate classification.

Basal-like carcinomas are usually of high histologic grade; 75% to 100% are grade 3.31,85,86,96 Other important histologic features include pushing, noninfiltrative borders of invasion; large zones of geographic or comedo-type necrosis; stromal lymphocytic infiltrates; scant stromal content; lack of tubule formations; marked cellular pleomorphism; high nuclear–cytoplasmic ratios; vesicular chromatin; prominent nucleoli; high mitotic indices; and frequent apoptotic cells (Figures 1, A through D). Basal-like carcinomas are also characterized by the presence of metastatic elements, in the form of squamous and spindle cells, and glomeruloid microvascular proliferation.52,61,66,96,97,109 Most studies have reported an association between basal-like cancer and both larger primary tumor size61,96 and lymph node negativity.36,60,78,96 However, other authors did not find such an association.66,82,85,86 Basal-like tumors may be associated with high-grade ductal carcinomas in situ, which are usually solid, flat, or micro-papillary types, and demonstrate the same immunoprofile as the invasive component.81

Although these features are generally not specific and individual features can be seen in other high-grade tumors, regardless of their molecular profile, the presence of these features, together with the absence of hormone receptors and HER2, should raise the possibility of basal-like cancer and be stained for specific basal markers.

The immunophenotype of basal-like tumors, as defined by GEP or by IHC surrogate markers, is characterized by positive expression of basal CKs, EGFR, p53, P-cadherin, caveolins 1 and 2, cyclin E, Ki-67, c-Kit, fascin, Sox2, moesin, vimentin, nestin, and laminin (Figure 2). Basal-like tumors are predominantly negative for ER, PR, and HER2 (triple-negative), BRCA1, FHIT protein, cyclin D1, p27, and MUC1.1 One misconception often held is that basal-like breast cancer does not express low molecular weight (ie, luminal) keratin. In fact, most basal-like carcinomas do express luminal keratins (CK8/18), albeit at lower levels than those found in luminal cancers.117 Furthermore, basal-like cancers more often express ME markers (eg, p63, SMA, and CD10).31,96

**METASTATIC PATTERN**

In addition to the association with an aggressive clinical history, development of recurrence, distant metastasis, shorter survival, and a relatively high mortality rate,1 basal-like cancer is associated with a specific pattern of distant metastasis. Such cancers show a higher proclivity for visceral metastases to brain (central nervous system) and lung, sites known to be associated with a poorer prognosis,106 and are less likely to metastasize to bones or lymph nodes.82,60,104,113 In previous studies, Gaedcke et al20 and Hicks et al119 found that the majority of breast cancers that develop central nervous system metastases are ER and PR negative and express either a basal-like phenotype or HER2. These findings may suggest that basal-like cancer might also possess a distinct mechanism of metastatic spread or may merely mean that ER-negative diseases favor a hematogenous, rather than a lymphatic, route, with a tropism for visceral organs. Further studies are warranted to clarify this issue.

**BASAL-LIKE BREAST CANCER: THE BRCA1 CONNECTION**

Patients with BRCA1 germline mutations have a substantially higher risk of developing breast cancer than the rest of the population.111 The BRCA1 tumor-suppressor gene is involved in a plethora of cellular functions, including regulation of ER expression,122 cell proliferation, chromosomal stability, and DNA repair via homologous recombination. Therefore, BRCA1-deficient cells lack competent homologous DNA repair mechanisms and are unable to repair DNA double-strand breaks by homologous recombination. This results in genomic instability and a proclivity of BRCA1 tumors to malignant transformation.123,124 There is increasing evidence to suggest that tumors arising in patients harboring BRCA1 mutations share similar pathologic characteristics, gene expressions, and genomic aberrations with basal-like cancers.46–48 In addition, recently engineered mouse models targeting BRCA1 and Trp53, in either luminal epithelial cells125 or basal cells126 of the mouse mammary gland, resulted in the development of tumors that have histologic and immunohis-
tochemical features that recapitulate those of human basal-like breast cancers.

Although somatic BRCA1 gene mutations are rare in sporadic basal-like cancers, there is evidence to suggest that BRCA1 mRNA and protein levels are preferentially down-regulated in these cancers when compared with breast carcinomas pertaining to other phenotypic subgroups. BRCA1 pathway dysfunction in basal-like cancers appears to be mediated by either epigenetic silencing of the BRCA1 gene promoter (ie, BRCA1 gene promoter methylation) or by up-regulation of ID4, a BRCA1-negative regulator. Whereas the former is more often seen in metaplastic and medullary cancers, the latter seems to be more prevalent in invasive ductal carcinomas of a basal-like phenotype. It is, therefore, not surprising that some authors have hypothesized that basal-like cancers not only make phenocopies of tumors arising in BRCA1 mutation carriers but also harbor the same defects in DNA repair mechanisms. This offers the potential for exciting, novel therapeutic strategies for patients with BRCA1 tumors and for a significant subgroup of sporadic basal-like cancers. There are several lines of evidence to suggest that cancer cells harboring a defective mechanism for homologous recombination DNA repair show an exquisite sensitivity to cross-linking agents, such as carboplatin, and to inhibitors of the PARP enzyme. Currently, clinical trials addressing whether platinum salts would be more effective than the standard of care for patients with basal-like breast cancers are underway (reviewed in Rakha et al and Reis-Filho and Tutt).

**BASAL-LIKE CANCER IN ROUTINE PRACTICE**

Despite the different definitions of basal-like cancer, these tumors have common, albeit nonspecific, features that can help to identify them in routine practice. In this respect, the issue of basal-like breast cancer definition is not unique because many other traditional, well-established prognostic variables also suffer from lack of consensus. For example, the subclassification of the luminal class varies widely between different GEP studies; no consensus about the definitions for ER and PR positivity has been reached (ie, different scoring systems and cutoffs are still employed for patient management and clinical trials), and the criteria for HER2 positivity has recently been changed. It is, therefore, important to mention that the basal-like class only garnered attention a few years ago, and research is going on to identify the best criteria to use in identifying these tumors and to provide the most mean-
Figure 2. Immunohistochemical profile of basal-like breast carcinomas. Most, but not all, basal-like cancers are negative for the estrogen receptor (ER), the progesterone receptor (PR), and HER2. Expression of basal keratins is seen in >75% of triple-negative cancers. Epidermal growth factor receptor (EGFR) is found in approximately 50% of these tumors. P53 nuclear expression and/or TP53 gene mutations are found in most of these tumors. Basal-like cancers have the highest Ki-67 (MIB-1) labeling indices of all molecular subtypes of breast cancer (original magnifications ×100).

In conclusion, basal-like breast cancer constitutes a distinct, yet heterogeneous, group of tumors that display distinctive morphologic, genetic, and immunophenotypic features and is associated with poor clinical outcome and specific patterns of distant metastasis and response to chemotherapy. Sporadic, basal-like breast cancers have fea-
tures that recapitulate those of tumors that arise in patients with the BRCA1 mutation, and most tumors in carriers of the BRCA1 mutation have a basal-like phenotype and transcriptomic profile. Although current definitions of basal-like carcinoma vary and no consensus has been achieved on a definition, basal-like cancer can be identified in routine clinical practice based on a constellation of morphologic and immunohistochemical features. Until a panel of basal markers that can accurately identify basal-like cancer with a high degree of specificity and sensitivity is developed and internationally accepted, we recommend using the lack of ER and HER2 and 4 basal markers (CK5/6, CK14, CK17, and EGFR) for the identification of these cancers. Although the diagnosis of basal-like breast cancer is still not routine, this discussion is by no means an academic exercise, given the prognostic implications and the likely development of specific chemotherapy regimens (eg, carboplatin) and/or tailored therapies (eg, PARPi inhibitors) for basal-like breast cancers.

References

Basal-like Breast Cancer—Rakha & Reis-Filho

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20.474–481.
expression is associated with basal-like phenotype in both sporadic and BRCA1-
JAMA.
Vir-
driver of the basal-like phenotype in sporadic breast cancer.
Clin Cancer Res.
2008;26:2568–2581.
Foulkes WD, Brunet JS, Stefanos TM, et al. The prognostic implication of the basal-like (cyclin E high/p27 low/p53 –)/gliomerulin-microwave-prolifera-
Sziopikou KF, Cobleigh M. The basal subtype of breast carcinomas may represent the group of breast tumors that could benefit from EGFR-targeted ther-
expression is associated with basal-like phenotype in both sporadic and BRCA1-
JAMA.
20.474–481.
expression is associated with basal-like phenotype in both sporadic and BRCA1-
JAMA.
867
Basal-like Breast Cancer—Rakha & Reis-Filho


119. Hicks DG, Short SM, Prescott NL, et al. Breast cancers with brain metastases are more likely to be estrogen receptor negative, express the basal cytokeratin CK5/6, and overexpress HER2 or EGFR. Am J Surg Pathol. 2006;30:1097–1104.


