Basal-like Breast Cancer—Characteristics, Risks, and Associations

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Abstract

Breast cancer is a heterogeneous disease. Gene expression profiling has demonstrated the existence of at least five 'intrinsic' subtypes: luminal A, luminal B, human epidermal growth factor-2 receptor (HER2), normal-like, and basal-like. While the estrogen receptor (ER), progesterone receptor (PR), and HER2 remain the most important prognostic markers and predictors of response to therapy, interrogating thousands of genetic transcripts more accurately captures the biological complexity and clinical heterogeneity of this disease. Target identification through massively parallel sequencing is likely to bear fruit in the coming years. Attention to basal-like breast cancers has grown substantially due to their generally poor prognosis, lack of targeted therapies, and connection with *BRCA1*-related cancers. In this article, we discuss the basal-like subgroup with respect to its cellular origins, relationship to *BRCA1*, and associated epidemiological risk factors.

Keywords

Gene expression profiling, basal-like breast cancer, triple-negative breast cancer, *BRCA1*-related breast cancers, cell of origin, epidemiological risk factors, next-generation sequencing

Breast cancer is a heterogeneous disease with varied morphological appearances, natural histories, and treatment outcomes. From an oncologist's perspective, management relies primarily on chemotherapy and drugs that target the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor-2 receptor (HER2). For this reason, clinicopathological examination and immunohistochemical (IHC) staining for hormone receptors and HER2, in addition to cytogenetic testing for HER2 amplification, are the mainstays of evaluation for determining treatment choice. However, much residual intertumoral heterogeneity remains among seemingly homogeneous tumors, reflected by our inability to accurately predict clinical outcome. In the adjuvant setting, traditional prognostic factors—such as patient age, histological grade, proliferative index, tumor size, lymph node involvement, and receptor status—only crudely identify those at low or high risk of relapse. Indeed, 70–80 % of patients with ER-positive, node-negative tumors who are given adjuvant chemotherapy and/or hormonal therapy would survive without it.[1,2]

To refine prognostication, researchers have turned to gene expression profiling on a genome-wide scale.[3,4] By interrogating thousands of gene transcripts, a molecular portrait of breast cancer can be formed that more accurately captures its complex biological programming and heterogeneity. Similarities in gene expression among tumors can be grouped together by unsupervised hierarchical clustering, which has

demonstrated the existence of at least five 'intrinsic' subtypes: luminal A, luminal B, HER2, normal-like, and basal-like. To some degree, they correspond to ER, PR, and HER2 phenotypes, corroborating their central role in breast cancer. Luminal tumors segregate with ER-dependent genes, while HER2 cancers cluster with genes activated by HER2. Basal-like breast cancers (BLBCs), on the other hand, tend to be negative for all three markers, a clinical phenotype known as 'triple-negative.'

Much progress has been made in treating ER-positive and HER2-overexpressing cancers; survival for these patients has improved considerably with the introduction of hormonal and HER2-blocking agents, respectively.[5] By contrast, basal-like cancers, owing to their frequent triple-negative status, cannot normally be treated with targeted therapies. However, the discovery that BLBCs and *BRCA1*-related breast cancers share a similar biology may provide new therapeutic avenues.[6] Since its discovery by gene expression profiling, BLBC, and its close cousin triple-negative breast cancer (TNBC), have received much attention among oncologists, pathologists, and geneticists alike. Here we review BLBC and discuss its cellular origins, relationship to *BRCA1*, and associated epidemiological risk factors.

Basaloid, Basal, Basal-like, Basal Epithelial?

Many terms have been used to describe BLBC, a consequence of the ambiguous nature of this entity. To clarify where the term 'basal-like'

originated, we review the hierarchical lineage of the normal mammary epithelium[7] from which the majority of breast tumors arise (see *Figure 1*). The ducts and lobules of the human breast are lined by two cell layers: an inner luminal and an outer basal layer. Luminal progenitor cells differentiate to give rise to specialized epithelial cells that compose the luminal compartment: ductal cells and milk-secreting alveolar cells. Myoepithelial (ME) cells, the predominant cells of the basal layer, contract in response to oxytocin, and these cells are derived from ME progenitors. Differentiated ME cells typically express high-molecular-weight basal cytokeratins (CKs), such as CK5/6, CK14, and CK17, in addition to smooth-muscle actin (SMA), neutral endopeptidase (CD10), vimentin, and p63, among others.[8] The term 'basal' has in fact acquired two meanings: it can either refer to cells lying adjacent to the basement membrane, which are primarily ME cells, or it can refer to cells that express basal CKs, which, at a minimum, require positivity for CK5/6 and CK14. It is this latter definition that is most often used to describe BLBCs; that is, cancers that stain for basal CKs by IHC staining or cluster around the basal-like centroid by gene expression. The problem here is that the definition is circular, and it provides no insight as to cell of origin, or behavior. Moreover, in humans, basal markers such as CK5/6, CK14, and CK17 can also be found in the luminal layer[8] and therefore describing BLBCs as originating in 'basal cells' on the basis of positivity for CK5/6 and CK17[3] is not accurate. To confuse matters further, most basal-like cancers stain positively for luminal markers CK8/18, while ME markers, such as SMA, CD10, and p63, are infrequently seen, which argues against a true basal derivation for these tumors.[9]

How Do We Define Basal-like Breast Cancers?

A large number of BLBCs wil need to be analyzed to determine their natural history, risk factors, and response to therapy. Moreover, gene expression profiling requires RNA from fresh or frozen material, which is generally not available and its extraction from formalin-fixed, paraffin-embedded tumors remains technically challenging. Investigators have instead turned to IHC staining in order to identify these tumors for study. The association of BLBC with TNBCs, which are routinely identified in clinical diagnostic work-up, has resulted in this convenient phenotype being used in studies as a surrogate for the basal-like entity,[10] with some claiming them to be effectively synonymous.[11] However, only 71 % of TNBCs fall into the basal-like category and approximately 77 % of basal-like cancers are triple-negative; thus the overlap is not complete.[12]

Many studies have attempted to improve the identification of basal-like tumors by IHC staining,[9,13,14] but as yet there is no internationally agreed-upon definition. This has caused much confusion, with many studies defining basal-like on the basis of different IHC criteria, which has led to inconsistent results.[15] For now, the simplest discriminator of basal-like cancers defined by IHC staining is tumors being negative for ER, PR, and HER2 and positive for CK5 and/or epidermal growth factor receptor (EGFR): the so-called 'core basal phenotype.'[14,16]

Tumor subtypes differ significantly in prognosis and have been shown to consistently predict relapse-free and overall survival.[4,17] Luminal ER-positive tumors are generally associated with a good prognosis, while those of the HER2 and basal-like subgroups fare relatively poorly.[18,19] It should be pointed out that there are no data to suggest that 'basalness' (i.e., CK5/6 or CK14) in itself has predictive value.[20,21] Some, therefore, have questioned

Figure 1: Model of the Mammary Epithelial Hierarchy
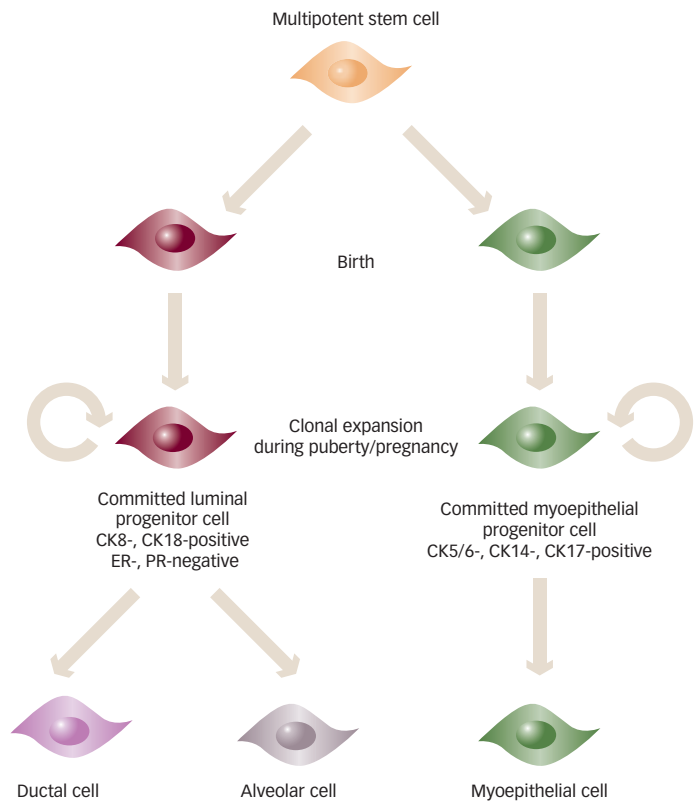
ER = estrogen receptor; PR = progesterone receptor. Source: Van Keymeulen et al., 2011.[29]

the usefulness of subdividing TNBC on the basis of so-called 'basal markers', but it does seem that adding basal markers to TNBC has some prognostic importance.[16,22] Others argue that there are many subdivisions of TNBC and the basal-like group is only one of many such smaller groups within TNBC.[23] Moreover, since there is no consensus on how to best define BLBC, the term's clinical utility is limited. Furthermore, there are no data indicating that basal-like cancers that are ER-positive should be managed any differently from luminal ER-positive cancers, despite the fact that basal-like ER-positive cancers respond poorly to standard treatment.[24]

Where Do Basal-like Breast Cancers Originate?

Intra- and intertumoral heterogeneity of breast cancers are thought to be the product of stochastically acquired oncogenic mutations in distinct cells of origin.[25] If the cell of origin imparts a genetic program that dictates how the cell evolves,[26] it could also be hypothesized that different tumor subtypes identified by gene expression are a reflection of their tumor-initiating cells (although it is possible that, during clonal evolution, cells trans- or de-differentiate, acquiring characteristics not resembling their founding cells).[25] For BLBC, the gene expression profile more closely resembles that of normal luminal progenitor cells, which suggests that luminal progenitors are the target population of basal-like cancers.[27] In support of this hypothesis, *in vivo* lineage-tracing studies of *BRCA1* (and *p53*) conditional knockouts targeted at either the luminal or basal progenitor cell population of the murine mammary gland demonstrated that only deletions in luminal progenitor cells recapitulated the basal-like phenotype,[28] providing stronger evidence that BLBCs

originate from the luminal progenitor population. While the debate continues, determining the cells of origin for all breast tumor subtypes should be facilitated by the recent characterization of the entire mammary epithelial hierarchy from multipotent stem cells to the terminally differentiated luminal and ME cells.[29]

BRCA1 in Basal-like Breast Cancers

BRCA1 is most well known for its role in the repair of double-strand breaks by homologous recombination and its regulation of the cell cycle, both critical processes in maintaining genome integrity.[30] Without *BRCA1*, cells are susceptible to gross chromosomal rearrangements during successive rounds of cell division, which increases the mutation rate and thus favors tumorigenesis. This is exemplified by an elevated risk of developing breast and ovarian cancer in women who carry a *BRCA1* mutation.[31] *BRCA2* also performs an important, but distinct, function in homologous recombination,[32] and mutations in *BRCA2* similarly predispose women to breast and ovarian cancer; however, unlike *BRCA2*, *BRCA1* has functions beyond that of DNA repair.

Recently, data have suggested that *BRCA1* functions as a stem cell regulator.[33] According to the current model, in normal circumstances, breast stem cells are 'driven' in the direction of the fully differentiated ER-positive luminal state by the presence of the transcriptional influence of *BRCA1*. Persistent lack of *BRCA1* not only promotes cancer, on account of the DNA repair defects that are present, but the transcriptional effects of a lack of *BRCA1* also result in a block in the normal progression of luminal maturation, creating a bigger population of ER-negative luminal progenitor cells. The presence of genomic instability in an enlarged pool of long-lived stem cells possessing a large proliferative potential significantly heightens their susceptibility to cancer transformation.[27] Interestingly, lack of *BRCA2* promotes cancer, via the DNA repair defects, but the absence of a transcriptional effect of *BRCA2* could result in the cancer proceeding to a more differentiated luminal state, which may explain why these cancers are usually ER- and CK8/18-positive.

BRCA1-related breast cancers are often basal-like, defined both immunohistochemically[34] and by gene expression.[17] In addition, both *BRCA1*-related breast cancers and sporadic BLBCs are characterized by high histological grade, pushing margins, high proliferation rates, frequent TP53 mutations, an attenuated correlation between tumor size and lymph node status, and a peculiar tendency to metastasize to the lungs and brain, findings that suggest they share a similar biology.[35] However, not all *BRCA1*-related breast cancers are basal-like, and this could be because of a genotype–phenotype effect caused by a partially defective *BRCA1* protein that retains luminal differentiation properties but loses DNA repair functions (i.e., behaving more like a *BRCA2* mutation). Conversely, sporadic BLBCs rarely exhibit *BRCA1* mutations,[36] suggesting that *BRCA1* may be downregulated through epigenetic mechanisms[37–39] or, alternatively, the entire luminal differentiation pathway could be inhibited by a defect in *BRCA1*-interacting proteins, such as PALB2[40] and Abraxas.[41]

Do Epidemiological Risk Factors Vary According to Breast Cancer Subtype?

Hormonal factors, such as early age of menarche, nulliparity, late age at first birth, and obesity, have traditionally been associated with an increased risk of breast cancer. However, these risk factors reflect the associated risk for breast cancers as a whole and do not hold true for individual subtypes. Since luminal ER-positive tumors make up the majority of breast cancer, it is not surprising that a woman's reproductive history is strongly associated with her risk of developing luminal cancers. However, some of these associations are lost or may become more pronounced in relation to ER-negative BLBCs. For example, the effects of little to no breastfeeding, obesity, and a positive family history, while linked to breast cancer risk in general, show a stronger association with the BLBC subtype.[42,43] Paradoxically, parity and younger age of a first full-term pregnancy, classically associated with reduced breast cancer risk, appear to be associated with an increased risk of BLBC.[42] Genetic susceptibility alleles also vary by subtype: single nucleotide polymorphisms at the 19p13.1 locus are associated with an increased risk of TNBC,[44–46] the same locus being associated with breast cancer risk among *BRCA1* carriers,[46] implying that perhaps basal-like and *BRCA1*-related breast cancers are linked by some common etiological factor. There is an ethnic predisposition that is apparent: BLBCs are more prevalent among pre-menopausal African-American women compared with other ethnicities.[36] These data support the notion that BLBCs arise through distinct etiological mechanisms compared with tumors of other subtypes. Notably, if the above data are confirmed, one could speculate that three-quarters of BLBCs in young African-American women could be prevented through weight control and breastfeeding alone.[42] Additional epidemiological data stratifying breast cancers by subtype are needed to identify additional risk factors for BLBCs.

Next-generation Sequencing of Basal-like Breast Cancers

Advances in sequencing technology have ushered in a new era of genetic testing for cancer diagnosis and management. The cost and time required to sequence DNA have fallen precipitously and continue to decline. Already numerous tumor genomes, epigenomes, and transcriptomes have been interrogated by next-generation sequencing (NGS) technologies and studies of BLBC genomes are at the forefront of this endeavor. NGS of BLBCs has provided unprecedented insights into tumor initiation and progression. For example, mutational patterns can be discerned that reveal signatures of mutagenesis. TNBCs exhibit frequent tandem duplications, a particular type of rearrangement that is not present among other breast cancer subtypes, including those derived from germline *BRCA1* and *BRCA2* mutations, suggesting that they are not due to defects in homologous recombination.[47] Understanding the mechanism causing these rearrangements, which are often precipitating events of tumorigenesis, might point to cancer prevention measures. In addition, a transcriptome analysis of TNBCs identified recurrent gene fusions involving *NOTCH1* and *NOTCH2* that activate the downstream oncogenes *MYC* and *CCND1*.[48] The tumor cells were characterized by decreased cell–matrix adhesion and the ability to propagate in suspension, a property not present in other breast cancer cells, perhaps explaining why TNBCs have a unique ability to spread hematogenously.[49–51] *In vitro* and *in vivo* inhibition of γ-secretase, the enzyme responsible for activating *NOTCH1*, resulted in reduced tumor proliferation. These studies represent ongoing work which aims to catalog the full repertoire of cancer genes that initiate and drive tumor progression, providing a molecular framework for a targeted approach to cancer therapy.

Not just mutations, but tumor evolution itself can be characterized by NGS. Although intratumoral heterogeneity can be indirectly inferred by determining the frequency of mutations present, studies have sought to more accurately characterize this by sequencing the genome of individual tumor cells.[52] These studies have revealed that clonal evolution of BLBCs is characterized, not by a succession of increasingly cancerous lesions, but as a punctuated clonal expansion with cells from the dominant subpopulation seeding the metastatic tumor. These findings support previous NGS data that suggest metastatic properties arise relatively late in tumor evolution,[53,54] providing hope that, if eliminated early enough, metastasis may be prevented.

Conclusion

BLBCs must be seen as biologically separate from luminal and HER2 subtypes of breast cancer. They represent distinct cancers associated with a different set of epidemiological risk factors than seen in most other subtypes. The associated somatic mutations that dictate their evolutionary path, govern their cellular behavior, and determine their clinical course are likely to be constrained by the as yet underdetermined cell(s) of origin of these cancers. Along with standard histological and IHC analyses, gene expression arrays have revealed considerable heterogeneity in the underlying biology of BLBC. Whole-genome, epigenome, and transcriptome sequencing, as well as proteomics will be required to provide the means necessary to characterize the full spectrum of mutations that drive aberrant cellular networks for individual tumors. Moreover, we need to know much more about the implications of these genetic alterations for treatment. Further subtle subclassification of BLBC is unlikely to help very much in this endeavor. While analysis of *BRCA1*-related breast cancers has provided key insights into BLBC, understanding the key driving forces behind non-*BRCA1*-related BLBC will be essential if we are to make progress in preventing death from this disease. ■

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