Deconstructing Breast Cancer Heterogeneity: Clinical Implications for Women With Basal-Like Tumors

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One in eight women in the United States will develop breast cancer in her lifetime, and breast cancer is the second leading cause of cancer death among women (DeSantis, Ma, Bryan, & Jemal, 2013). Breast cancer embodies several clinically distinct diseases that result from the interaction of varied genetic and environmental influences, many of which are not yet well understood. The inherent clinical and molecular heterogeneity of breast cancer poses a challenge for researchers and clinicians. Breast tumors consist of several pathologic subtypes with different clinical presentations and outcomes, and patients show a diverse range of responses to a given treatment (Sorlie, 2004). Because of the aggressive and treatment refractory nature of basal-like breast cancer (BLBC), the goal of the current article is to investigate BLBC in depth, with a particular focus on genetic and environmental risk factors and current clinical targets for this tumor subtype. A brief overview of the five main breast cancer subtypes will also be provided to understand BLBC within the broader context of breast cancer heterogeneity.

Historically, breast tumors were classified via immunohistochemical (IHC) protein staining for estrogen receptor (ER), progesterone receptor (PR), or human epidermal growth factor receptor 2 (HER2), but the advent of gene expression microarrays has made a more comprehensive molecular assessment possible (Litsas, 2013). Major breakthroughs in the understanding of breast cancer heterogeneity have been made by Perou et al. (2000) and Parker et al. (2009) by showing that multiple types of breast tumors exist, each with distinct prognosis and risk indicators defined by differential gene expression (see Table 1). The five main subtypes of breast cancer that reflect distinct gene-expression patterns are luminal A, luminal B, normal-like, HER2-enriched, and basal-like (Yehiely, Moyano, Evans, Nielsen, & Cryns, 2006). More than 95% of all breast cancers arise within the milk ducts of the breast (Tomaskovic-Crook, Thompson, & Thiery, 2009). The origin of the terms luminal and basal refer to the location of either secretory (inner lumen) or basal (outer lumen) epithelial cell types, which have distinct hormone responsiveness and gene expression patterns (Creighton, 2012; Millikan et al., 2008).
Gene expression refers to the process in which DNA is transcribed to RNA and ultimately translated to its final protein product (see Figure 1). What define any tissue are the genes that are differentially expressed. For example, the gene coding for adult hemoglobin is present in every human cell; however, it is only expressed, or made into its functional protein, in red blood cells and some epithelial cell types (Newton, Rao, Dluhy, & Baatz, 2006). Microarray technology allows for the identification and categorization of breast cancers based on differential gene expression. Careful analysis of these gene expression signatures is expected to reveal new targets for therapy, allow for further personalization of treatment strategies, and improve outcomes for individual patients.

Of the five major molecular subtypes, BLBC is particularly aggressive, with the highest chance of disease recurrence and the poorest survival rates. Basal-like tumors represent 10%–20% of all cases and typically lack ER, PR, and HER2 on their cell surfaces. Therefore, triple-negative tumors (ER−, PR−, and HER2−), are not expected to benefit from receptor-targeted therapies such as tamoxifen or herceptin (Cheang et al., 2008). Basal-like tumors are associated with the shortest survival of the five breast cancer subtypes because of increased rates of early relapse within the first five years. In particular, basal-like tumors are typically poorly differentiated, high-grade invasive ductal carcinomas with a high mitotic index (e.g., rapidly dividing cells) and an increased likelihood for metastases to the brain and lungs (Yehiely et al., 2006).

**Basal-Like Versus Triple-Negative Breast Cancer**

Distinguishing between BLBC (defined by genetic analysis) and triple-negative breast cancer (TNBC) (defined by protein staining) is important because these terms are often incorrectly used interchangeably. Although significant overlap exists between these classifications, the terms are not synonymous. Bertucci et al. (2008) showed by protein staining that about 70% of TNBCs were of the basal-like subtype as defined by gene expression profiling, and about 80% of molecularly defined basal-like tumors were triple-negative by IHC staining. Although the term TNBC is used widely as a surrogate for genetically defined BLBC, caution should be exercised because up to 30% discordance exists between the two definitions (Bertucci, Finetti, & Birnbaum, 2012).

Based on protein staining, multiple IHC markers have been used to attempt to distinguish between basal-like and other breast cancers; however, no internationally accepted definition exists for BLBC based on protein staining alone, and no specific hallmark morphologic feature can identify basal-like tumors reliably in routine practice (Rakha, Reis-Filho, & Ellis, 2008). In addition to often being ER−, PR−, and HER2−, basal-like tumors frequently

<table>
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<tr>
<th>Subtype</th>
<th>Approximate Prevalence</th>
<th>Risk Factors</th>
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<tr>
<td>Basal-like</td>
<td>10%–20%</td>
<td>Premenopausal, African American, increased breastfeeding, increasing abdominal adiposity, increasing parity, and younger age at menarche</td>
<td>ER−, PR−, HER2−</td>
<td>Inhibitors of PARP, PI3K, and EGFR pathways; neoadjuvant and platinum-based chemotherapy</td>
</tr>
<tr>
<td>HER2−-enriched</td>
<td>15%–20%</td>
<td>Older age</td>
<td>ER−, PR−, HER2−</td>
<td>Inhibitors of HER2 growth factor (e.g., trastuzumab, herceptin), neoadjuvant chemotherapy</td>
</tr>
<tr>
<td>Luminal A</td>
<td>50%–60%</td>
<td>Older age, postmenopausal, Caucasian, nulliparity, older age at first birth, and hormone-replacement therapy use</td>
<td>ER+, PR+, HER2−</td>
<td>Third-generation AI and SERM hormone inhibitors (e.g., tamoxifen)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>10%–20%</td>
<td>Younger, Caucasian, nulliparity, and older age at first full-term pregnancy</td>
<td>ER+, PR−, HER2+</td>
<td>Tamoxifen, AI, neoadjuvant chemotherapy, trastuzumab, herceptin</td>
</tr>
<tr>
<td>Normal-like</td>
<td>5%–10%</td>
<td>Does not typically vary</td>
<td>ER−, PR−, HER2−</td>
<td>Neoadjuvant chemotherapy</td>
</tr>
</tbody>
</table>

*Not all tumors within this molecular subtype express these features. Variation also occurs based on the particular IHC protocol.*

AI—aromatase inhibitor; EGFR—epidermal growth factor receptor; ER—estrogen receptor; HER2—human epidermal growth factor receptor 2; IHC—immunohistochemical; PARP—poly ADP ribose polymerase; PI3K—phosphoinositide 3-kinase; PR—progesterone receptor; SERM—selective estrogen receptor modulator

**Table 1. Recognized Subtypes of Breast Cancer and Corresponding IHC Classification, Risk Factors, and Selected Clinical Therapies**
express, or IHC stain positive for, vimentin, epidermal growth factor receptor (EGFR), and cytokeratin 5 (CK5) and cytokeratin 6 (CK6) proteins (Yehiely et al., 2006). One study found that a panel of four antibodies comprised of ER, HER1, HER2, and CK5/6 could accurately identify basal-like tumors with high specificity using standard available clinical tools in pathology laboratory settings (Nielsen et al., 2004). When examining the data available on BLBC, clarifying the triple-negative status and specific markers used to identify the subtype is important to obtain an accurate understanding of the complexity and corresponding clinical implications.

Because microarrays are not yet routinely available in clinical practice, IHC staining of breast tumor cell surface proteins like ER and PR has been the most accessible surrogate assay for determining tumor subtypes, particularly in population-based studies. Other potentially confounding problems with IHC are that targeted antibodies must be able to efficiently stain protein receptors in varied locations. For example, the target receptor may be located either inside or on the cell surface; therefore, a tumor may be ER+ and ER− depending on the IHC stain. As with all IHC markers, factors, such as the threshold for interpretation, tissue fixation, and the choice of antibody, can dramatically affect test accuracy and reproducibility, leading to variable results (Gown, 2008).

Such staining heterogeneity presents a challenge when using TNBC as a surrogate marker of BLBC to establish treatment options and effective therapies, and it further highlights that the genes responsible for this aggressive phenotype are not well understood. The predictive analysis of microarray (PAM) 50 is the gold standard for categorizing the five main breast tumor molecular subtypes described previously by measuring differential expression of 50 classifier genes (including the genes coding for ER, PR, and HER2) and five control genes (Parker et al., 2009). In so doing, the PAM 50 provides a risk of recurrence score to estimate the probability of relapse at five years; therefore, genetically defined subtypes are not only descriptive, but also prognostic (Goncalves & Bose, 2013). The PAM 50 assay has been approved by the U.S. Food and Drug Administration (FDA) because of its superiority to IHC tumor protein staining classification for prognosis across the full spectrum of breast cancer subtypes (Chia et al., 2012). Throughout the current article, TNBC will refer to tumors identified to be ER−, PR−, and HER2− by protein staining alone, and BLBC will refer to tumors characterized by gene expression PAM 50 analysis based on microarray data.

**Risk Factors**

The heterogeneity within breast cancer subtypes is further underscored by recent findings indicating that each tumor subtype may have unique associated risk and protective factors. Although a positive family history indicates increased risk for all subtypes, the magnitude of relative risk is highest for basal-like tumors (Yang et al., 2007). Millikan et al. (2008) studied the predominance of specific subtypes by race and found the prevalence of BLBC was highest among premenopausal African American women, whereas postmenopausal Caucasian women showed the highest prevalence of the luminal A subtype. This further highlights that the luminal A and basal-like subtypes are biologically distinct, particularly with regard to hormone receptor status. Lund et al. (2009) replicated these findings by showing TNBCs were the most common breast
Epigenetics and Basal-Like Breast Cancer

The term epigenetics, coined by Conrad Waddington (2012) in the 1940s, was broadly defined as the interaction between an organism’s environment and genetics that ultimately influences phenotype. Epigenetics is often conceptualized as changes in gene function that are not a result of genetic mutations or changes in the DNA sequence (Rodriguez-Paredes & Esteller, 2011). Epigenetic mechanisms are of particular interest because they are heritable and reversible. Unlike genetic mutations, aberrant epigenetic changes can be reversed through pharmacologic interventions and behavioral modifications such as changes in diet and lifestyle (Feinberg, 2008). Epigenetic modifications can occur at the DNA level through methylation of C–G sequences and at the chromatin level through modifications such as methylation or acetylation of histone proteins that collectively comprise chromatin. Chromatin packages DNA so that it can fit into the cell. Epigenetic modifications, such as methylation, can change the structure of chromatin, making the DNA inaccessible to transcription. Such epigenetic changes can result in silencing of critical tumor-suppressor genes and activation of oncogenes involved in breast cancer development (Hinshelwood & Clark, 2008). Different subtypes of cancer demonstrate different methylation profiles, with basal-like tumors having the most distinctive methylation patterns overall (Cancer Genome Atlas Network [CGAN], 2012; Holm et al., 2010; Ulirsch et al., 2013). For example, BRCA1 gene promoter methylation is found almost exclusively in BLBC (Bardowell et al., 2013; Grushko et al., 2010).

BRCA1 is an example of a tumor suppressor gene that can be deficient as a result of genetic mutations, but it can also be silenced epigenetically through methylation (Turner et al., 2007; Yehiely et al., 2006). Because BRCA1 methylation is found predominantly in BLBC, epigenetically induced BRCA1 dysfunction may provide a promising avenue for treatment of BLBC. BLBCs display methylation patterns for many additional genes involved in breast carcinogenesis, including RARB, CDH1, MIA, and APC1, that are distinct when compared to luminal and HER2 subtypes (Bardowell et al., 2013; Lee et al., 2010). These methylation signatures are of particular interest for their potential use as markers for early detection and risk assessment and may be attractive targets for clinical therapies.

The clinical significance of BRCA1 inactivation in BLBC, whether through genetic or epigenetic silencing, is that this alteration increases the sensitivity of basal-like tumors to DNA-damaging agents. Poly ADP ribose polymerase (PARP) inhibitors are a type of DNA-damaging chemotherapy in clinical trials for the treatment of patients with BLBC, and researchers hope that patients with hypermethylation of BRCA1 could serve as a predictor of increased PARP therapeutic response. In addition to BRCA1 inactivation, an important hallmark of BLBC is genetic inactivation of the tumor suppressor gene p53 (CGAN, 2012; Jiang et al., 2011). Genetic mutations in p53 are associated with downstream epigenetic changes that, in a cascade effect, can result in global genome-wide alterations, leading to cancer (D’Anello et al., 2010). Such epigenetic modifications specific to BLBC may help to identify new targets for therapy. Because of the reversible nature of histone acetylation and DNA methylation, epigenetic therapies hold promise.
Current Clinical Targets

What makes any cancer aggressive is its inability to repair DNA mutations and subsequent failure to undergo apoptosis (programmed cell death). In that scenario, mutated cells continuously grow and divide in an unregulated manner, ultimately resulting in the formation of a tumor. DNA-damaging agents are designed to take advantage of this feature because many aggressive cancer cell types entirely lack DNA-repair capability. Therefore, by inducing massive DNA damage, platinum- and anthracycline-based chemotherapies are able to induce widespread cancer cell death that normal cells with functioning DNA-repair mechanisms would otherwise be able to combat.

BRCA1 pathway-deficient cells found in a subset of basal-like tumors are susceptible to platinum-based DNA-damaging agents, such as carboplatin and cisplatin, and have shown improved clinical outcomes for women with this subtype. These agents induce DNA damage that would normally be repaired by a functioning BRCA1 or BRCA2 pathway. This is an elegant way to target a natural vulnerability in a rogue cancer cell because BRCA1- and BRCA2-deficient cells are highly sensitive to apoptosis by DNA-damaging agents compared to normal cells (Quinn et al., 2003). For example, one study showed that BRCA2 is required for normal functioning of a repair protein called RAD51. In healthy cells, BRCA2, RAD51, and other repair proteins would normally be able to respond and repair DNA damage caused by radiation therapy. It makes sense that BRCA1-deficient tumor cells are five times more sensitive to the DNA-damaging agent cisplatin compared to normal cells (Bhattacharyya, Ear, Koller, Weichselbaum, & Bishop, 2000). Gronwald et al.’s (2009) study supported the biologic rationale for these therapies because 72% of BRCA1-deficient women were observed to have complete pathologic response when treated with a cisplatin regimen.

Similarly, PARP inhibitors target tumors with a BRCA1-deficient pathway because these cells no longer have any ability to repair double-stranded breaks in DNA (Farmer et al., 2005; Toft & Cryns, 2011). PARP inhibitors have been studied as combination therapy in conjunction with platinum-based chemotherapies and have been shown to improve targeted cancer cell toxicity in animal models, making them candidates for human clinical evaluation (Donawho et al., 2007). Other clinical targets specific to BLBCs include phosphoinositide 3-kinase (PI3K) and EGFR/HER1 inhibitors. Many of these drugs have been found to be beneficial when used as neoadjuvant therapies or in combination. The PI3K pathway is responsible for promoting cell survival and growth and is activated in many cancers through somatic mutations or receptor tyrosine kinases (Engelman, 2009). An association between an activated enzyme PI3K pathway and the basal-like subtype suggests that inhibitors of enzyme pathways like PI3K may be potentially therapeutic targets in treating the basal-like subtype of cancer (Hoeflich et al., 2009; López-Knowles et al., 2009; Wong, Engelman, & Cantley, 2010). For example, Moestue et al. (2013) found that long-term treatment with PI3K inhibitors resulted in significant growth inhibition in basal-like, but not luminal-like, mouse models.

Although BLBCs often lack hormone receptors that are used as clinical targets, a subset of basal-like cancers express EGFR/HER1 or c-KIT genes, which may be attractive targets alone or in combination with standard chemotherapy (Kashiwagi et al., 2013; Nielsen et al., 2004). EGFR is a member of the HER family of transmembrane receptor kinases that is associated with cell division, migration, adhesion, differentiation, and apoptosis (Yarden & Sliwkowski, 2001). Studies suggest EGFR inhibitors may be a viable treatment option because they are effective in a subset of basal-like tumors and have the potential to increase the effectiveness of chemotherapeutic agents such as cisplatin (Hoadley et al., 2007; Oliveras-Ferraros et al., 2008; Siziopikou & Cobleigh, 2007).

Neoadjuvant chemotherapies are a promising treatment option for individuals diagnosed with BLBC. The basal-like and HER2 subtypes of breast cancer were found to be more sensitive to preoperative chemotherapies because they showed higher percentages of complete response to paclitaxel and doxorubicin than luminal and normal-like tumors. Although rates of pathologic complete response for preoperative chemotherapy are higher for TNBC, the majority of women will have residual disease and a higher risk for relapse and death within the first two to five years of diagnosis (Carey et al., 2007; Liedtke et al., 2008; Rody et al., 2007; Rouzier et al., 2005). Based on the evidence, reasonable expectation exists that a combination of chemotherapeutic agents targeting the genetic vulnerability of BLBC and epigenetic therapies may be most effective in treating the aggressive basal-like subtype. Although many of the FDA-approved epigenetic therapies target blood cancers, such as leukemia and lymphoma, these therapies are on the horizon for solid tumors such as breast cancer (Rodríguez-Paredes & Esteller, 2011). Given the unique methylation profiles of BLBCs (Bardowell et al., 2013), specific epigenetic therapies may be designed in the future to improve clinical outcomes.

Implications for Nursing and Conclusions

Great strides are being made in deconstructing the molecular and clinical heterogeneity of breast cancer.
The authors of the current article have demonstrated that the basal-like subtype has distinct genetic and epigenetic alterations and exhibits specialized, targeted responses to clinical therapies. Breast tumors are as unique as the individuals in which they develop; therefore, molecular analysis of each tumor subtype will be essential to identify the best clinical course of action for each patient. Future clinical trials investigating the effect of these novel therapies, alone and in combination, are expected to show improved clinical outcomes for women with BLBC.

Women with a breast cancer diagnosis view their informational needs as key to their treatment decision-making process (Spittler, Pallikathayil, & Bott, 2012). Soliciting understanding of how patients view risk with knowledge of the illness trajectories, clinical trials, and therapeutic options for their breast cancer subtype can allow nurses to meet the informational needs of patients at all phases in the cancer continuum. By communicating this information, nurses can facilitate comprehensive care, act as true patient navigators, and assist patients in making informed and satisfied decisions (Pedersen, Hack, McClement, & Taylor-Brown, 2014; Spittler et al., 2012). By increasing knowledge and understanding about the genetic and epigenetic mechanisms underlying breast cancer, nurses will be equipped to communicate that information as simply or complexly as the patient requests or desires.

Bedside nurses care for women undergoing treatment for aggressive BLBC and must be knowledgeable regarding possible short- and long-term side effects. Studies have shown that women with breast cancer experience an array of treatment-related symptoms that greatly affect quality of life, and many symptoms remain after treatment has ended (Binkley et al., 2012; Shapiro & Recht, 2001). The specific side effects of basal-like therapies can be profoundly challenging. Anti-EGFR therapies can cause skin toxicity (e.g., acneiform eruption), gastrointestinal toxicity (e.g., nausea, vomiting, diarrhea), interstitial lung disease, as well as long-term sequelae (e.g., osteoporosis, cardiotoxicity) (Widakowich, de Castro, De Azambuja, Dinh, & Anda, 2007). PARP inhibitors have mild and reversible adverse side effects, whereas platinum-based chemotherapies have been shown to cause myelosuppression, immunosuppression, nephrotoxicity, cardiotoxicity, neurotoxicity, and hearing loss (Florea & Büsselberg, 2011; Leung, Rosen, Fields, Cesano, & Budman, 2011). Therefore, careful monitoring of blood laboratory values is particularly necessary during aggressive treatment cycles. Nurses could help patients with BLBC to understand genetic pathways that inform treatment options and to be aware of ongoing clinical trials testing new BLBC therapies.

In addition to physical effects of treatment, a diagnosis of breast cancer causes emotional trauma because patients experience feelings of vulnerability, uncertainty, and loss of control. Nurses must continually assess the supportive care needs of these patients and address issues such as quality of life, illness trajectories, and end-of-life care when treatment is no longer a viable option (Schmid-Büchi, Halfens, Müller, Dassen, & van den Borne, 2013). Because women diagnosed with the basal-like subtype are often younger than women diagnosed with other subtypes, nurses must recognize that their coping strategies, family roles, social support systems, and overall experience with the diagnosis will likely be different (Coyne, Wollin, & Creedy, 2012). Nurses are in a position to assist patients coping with an aggressive cancer diagnosis, and they also have the ability to assess the needs of family members and provide crucial support as they adapt to the diagnosis.

A multidisciplinary approach to care is particularly warranted for individuals diagnosed with breast cancer because they require an oncology team devoted to their physical and mental health to create optimal conditions for remission and recovery. Nurses provide the full scope of holistic care. Given the introduction of innovative chemotherapeutic agents into the clinical setting, oncology nurses must be aware of the complex array of treatment options and their underlying mechanisms. This will enable nurses to confidently refer patients to the broad range of contextually appropriate resources including information on clinical trials, genetic counseling, educational and social support, and treatment. Considering the poor prognosis associated with the basal-like subtype, patients will have many concerns. By being active consumers of research and implementing evidence-based practices, oncology nurses achieve the highest standards of patient advocacy and holistic care for patients living with cancer.

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References