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MOLECULAR INSIGHTS ON BASAL-LIKE BREAST CANCER

ADVANCES ON BASAL LIKE BREAST CANCER

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ABSTRACT

Molecular classification of breast cancer (BC) identified diverse subgroups that encompass distinct biological behaviour and clinical implications, in particular in relation to prognosis, spread, and incidence of recurrence. Basal like breast cancers (BLBC) compose up to 15% of BC and are characterized by lack of estrogen receptor (ER), progesterone receptor (PR), and HER-2 amplification with expression of basal cytokeratins 5/6, 14, 17, epidermal growth factor receptor (EGFR), and/or c-KIT. There is an overlap in definition between triple-negative BC and BLBC due to the triple-negative profile of BLBC. Also, most BRCA1-associated BCs are BLBC, triple negative, and express basal cytokeratins (5/6, 14, 17) and EGFR. There is a link between sporadic BLBC (occurring in women without germline BRCA1 mutations) with dysfunction of the BRCA1 pathway. Despite the molecular and clinical similarities, these subtypes respond differently to neoadjuvant therapy. BLBCs are associated with an aggressive phenotype, high histological grade, poor clinical behavior, and high rates of recurrences and/or metastasis. Their molecular features render these tumors especially refractory to anti-hormonal-based therapies and the overall prognosis of this subset remains poor. In this article the molecular profile, genomic and epigenetic characteristics as well as BRCA1 pathway dysfunction, clinicopathological behavior, and therapeutic options in BLBC are presented, with emphasis on the discordant findings in current literature.

KEYWORDS:

Breast cancer; basal-like breast cancer; triple negative; BRCA1; transcriptional profiling; prognosis.
1. INTRODUCTION

Breast cancer (BC) is one of the most common human malignancies, accounting for 22% of all cancers in women worldwide. The incidence rate is higher in North America, Europe, and Australia compared to other regions including Africa and Southern and Eastern Asia [1]. Although the incidence remains high, the decrease of the overall mortality has been attributed to advances in early detection and therapeutic modalities [2]. BC represents a complex and heterogeneous disease that comprises distinct pathologies, histological features, and clinical outcome. Current knowledge of BC etio-pathology, biology, and treatment protocols has benefited from the simultaneous analysis of multiple biomarkers. In particular, the status of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor type 2 (HER2) are the predictive markers utilized to identify a high-risk phenotype and for selection of the most efficient therapies [3,4].

Gene microarray profiling of human breast carcinomas has categorized invasive breast carcinomas into five distinct subtypes; luminal A, luminal B, normal breast like, human epithelial growth factor receptor-2 (HER2) overexpressing, and basal-like breast cancer (BLBC) [1]. The unfavorable prognosis as well as the lack of effective targeted therapy makes BLBC the subject of intensive research. The present review summarizes current knowledge in molecular profiling, genomic and epigenetic characteristics, BRCA1 pathway dysfunction, clinicopathological behavior, and therapeutic options in BLBC (Figure 1). Emphasis is given to the discordant findings in the literature.

2. CLASSIFICATION OF BC

The striking heterogeneity of BC in terms of tumor histology, clinical presentation, and response to treatment has been analyzed at the molecular level by gene-expression profiling, which has revealed that each breast tumor has its own unique molecular portrait, providing the basis for an improved molecular taxonomy of this disease [5,6]. BC is classified into major BC subtype signatures: ER-positive and ER-negative groups, which can be further subdivided into additional subgroups with distinct biological and clinical significance [7] (Figure 2).
Approximately 75% of BCs are ER and/or PR positive [8]. The ER-positive tumors express ER, PR, ER-responsive genes, and other genes that encode typical proteins of luminal epithelial cells so they are termed “luminal group”. This group is subdivided in luminal A and B tumors, depending on the level of proliferation-related genes and/or HER2/ERBB2 [7,8]. Luminal A subgroup is characterized by the high expression of ERα gene, GATA binding protein 3 (GATA3), B-cell CLL/lymphoma 2 (BCL2), luminal cytokeratin 8 (CK8), CK18, X-box binding protein, trefoil factor 3, hepatocyte nuclear factor 3α, estrogen-regulated LIV-1, ERBB3, and ERBB4, whereas luminal B group showed low to moderate expression of the luminal-specific genes including ER-clusters (Figure 2) [7,8].

The second broad group, the ER-negative tumors, comprises 20-25% of BC and is further subdivided into three subgroups: HER2-positive, BLBC, and normal breast-like (Figure 2) [7,8,9]. HER2 positive tumors express high levels of HER2 and genes related to the HER2 amplicon [2,7]. The normal breast-like signature defines a group of tumors with high expression of genes of adipose cells and other non-epithelial cell types, as well as low levels of luminal markers [5]. However, molecular classification of this group subtypes remains partially understood and subject of debates. Finally, tumors belonging to the basal-like subgroup express high levels of basal/myoepithelial markers, such as CK 5/14/17 and laminin, and do not express ER, PR, and HER2 and hence they are referred to as triple negative (TN) [4].

BLBC is a distinct group of tumors. They represent from 8% up to 37% of all BC cases, depending on the proportion of grade III cases included in the populations studied [10]. BLBC presents frequent mutations in the TP53 gene, evidence of genomic instability, and inactivation of the Rb pathway [11]. Notably, it was initially assumed that the cell of origin of this tumor subtype was found in the stem cells of the basal compartment. Recent gene expression profiling of the different subpopulations in human normal mammary gland and analysis of tumors with basal-like features showed that BLBC phenotype appears to be more similar to the gene signature derived from the luminal progenitor population [12].

All of these BC subtypes were named to reflect the gene-expression patterns of two principal epithelial cell types of the normal adult breast, namely the luminal epithelial cells, which form a single cell layer lining in the lumen of the duct or lobule, and surface or basal myoepithelial cells, which form a
second cell layer surrounding the luminal cells and are in direct contact with the basement membrane [8]. They are associated with markedly different clinical outcomes, ranging from the good-prognosis ER-positive luminal A tumors to the poor-prognosis ER-negative HER2 and BLBC tumors; these could be used as prognostic marker with respect to overall and relapse-free survival in a subset of patients that had received uniform therapy [6,7].

Herschkowitz et al. [13] described a potential new subtype, referred as “claudin-low”. Claudin-low group are TN. This subtype is characterized by low expression of genes involved in tight junctions and cell-cell adhesion, including Claudins 3, 4, 7, Occludin, and E-cadherin [9,13,14] and shows high expression of epithelial-to-mesenchymal transition (EMT) genes and stem cell features [15,16]. Currently it has been reported that patients with claudin-low tumors have poor clinical outcomes and some studies are focusing on their association with BLBC to identify treatment sensitivity to specific chemotherapeutics and/or targeted agents.

A new class of BC called “molecular apocrine tumors” has been suggested for BC based on increased expression of androgen receptor (AR) [17,18]. These tumors have some morphological hallmarks of apocrine tumors but there are no strict pathological criteria for diagnosis as classical apocrine carcinomas such as overexpression but not amplification of HER2 [17]. Immunohistochemically, these tumors are ER- and PR-negative and AR-positive. It was observed that almost all ER-positive tumors also express AR; however, the expression of AR in ER-negative group is predominantly observed in the HER2-positive subtype. On the other hand, a few TN tumors can also express AR and its expression seems to be related to apocrine differentiation. Indeed, AR-related targeted therapy was proposed for BC, especially for ER-negative/AR-positive tumors [2,8].

3. MOLECULAR PROFILE OF BLBC

BLBC express genes characteristic of basal/myoepithelial cells [2]. They showed no expression of ER- and PR-responsive genes, and other genes characteristic of luminal epithelial cells of the normal
breast as well as genes located on the HER2 amplicon [11]. Moreover, BLBC tumors show an overexpression of EGFR, CK-5, -6, -14, and -17, vimentin, p-cadherin, fascin, caveolins 1 and 2, αβ-crystallin, and EGFR [2,19]. There are also other potentially relevant features including mutated TP53 and BRCA1 genes and deregulated immune response genes [11]. Manié et al 2009 demonstrated that TP53 was frequently mutated in both BRCA1 (97%) and sporadic BLBC (92%). However the rate of complex mutations, such as insertion/deletion was found higher in BRCA1-BLBC than in sporadic BLBC (42% and 9%, respectively). c-KIT expression is also higher in BLBC [9,19,20]. Nielsen et al [19] observed that c-KIT expression was more common in basal-like tumors than in other BC but did not influence prognosis [19]. These authors suggested an immunohistochemical panel of four antibodies (ER, HER1, HER2, and CK-5/6) that could identify BLBC with 100% specificity and 76% sensitivity. However, other studies in BLBC have found different staining patterns of the basal keratins (CK-5/6 and especially CK-17, 8/-18) in part due to difficulty to detect by immunohistochemical methods focal and often weak reactivity [21,22].

There are several reported biomarkers associated with BLBC as well as putative candidates suitable for immunohistochemical screening (Table 1) [10,11,23], however, currently, there is no specific international consensus on complement biomarkers that can define BLBC.

Deregulated integrin expression has also been detected in BLBC and may contribute to aggressive cell behaviors and progression seen in this subtype. Several basal-like gene products are important structural elements of basal epithelial cell such as the extracellular matrix (ECM) receptor α6β4 integrin, subunits of laminin-5 (an ECM ligand of α6β4 integrin), and bullous pemphigoid antigen (BPAG1). These proteins are components of hemidesmosomes specialized adhesive structures that anchor basal epithelial cells to the ECM via basal CK intermediate filament network (Table 2) [2,6]. These alterations can be related to the biologically aggressive phenotype of these TN tumors although this remains to be established in order to better guides current efforts to develop meaningful targeted approaches.

Several genes related to BLBC have been implicated in promoting cellular proliferation, cell survival, and cell migration and invasion [8]. Despite the wide diversity of signaling pathways involved in these processes, signaling molecules such as the mitogen activated protein kinase (MAPK),
phosphatidylinositol 3 kinase (PI3-kinase)-AKT, and nuclear factor-kB (NF-kB) are commonly deregulated as seen in other BC subtypes [2,6]. A representative subset of gene regulation and function in BLBC are indicated in Table 1.

Other alterations such as Wnt pathway activation has been observed in BLBC [24]. This study reported cytoplasmic and nuclear accumulation of β-catenin in BLBC, and suggested that β-catenin could be a valuable therapeutic target for this subtype [24]. Nevertheless there is strong evidence of stabilization of β-catenin protein in a majority of human breast tumors, and mouse model systems clearly demonstrate that activated Wnt signaling can promote mammary tumorigenesis [25].

Even though BLBC has similar characteristic with others breast tumors subgroups, several large studies provide evidence that BLBC, per se, is an independent adverse prognostic factor, in spite of the fact that approximately 10% of BLBC patients have a good prognosis [26]. Clearly more studies are required to establish how common and often overlapping cell signaling pathways can contribute to histological and biological heterogeneity and progression to metastasis. Nevertheless, gene expression profiling of BLBC provides a myriad of candidate genes that might selectively contribute to the aggressive phenotype of these tumors and emerging evidence strongly support a breast stem-like cell as a precursor for these tumors [2,6,9,27].

Potential biomarkers were presented in this table only if the positivity percentage in BLBC was above 30% and at least twice as high as in non-BLBC (Table 2).

4. GENOMIC PROFILING OF BLBC

It was by the advent and use of high-throughput molecular profiling methods for the study of BC that was brought to the forefront the existence of the so-called BLBC, which has distinct and aggressive clinicopathological characteristics. This subgroup present a greater genetic complexity compared with other BC subtypes, suggesting a greater degree of genetic instability [28,29]. Bergamaschi [30] found that BLBC show the highest frequency of DNA losses and gains compared with others subtypes and also reported that despite of the highest prevalence of genomic aberrations, BLBC show less genomic
amplifications than tumors pertaining to other molecular subgroups [10,28,30].

Copy number aberrations (CNAs) are distributed throughout the genome in BLBC resulting in a sawtooth pattern, which is similar to that seen in BRCA1-associated hereditary BC [32], such as a frequent loss of 5q, being that BRCA1-modifier locus for hereditary BC penetrance has been mapped to 5q [30]. Chromosomal regions 8p12, 8q24, 11q13, 17q12, and 20q13 are recurrently amplified in BC in general [31]. But some particular recurrent amplifications described in BLBC are approximately two to three times higher than the other subtypes [10,28] and it includes 7p11.2 involving the region of EGFR, 7q31 affecting caveolin 1, and 12p13 being the amplifications of 8p12 and 17q11.2 associated with poor outcomes [31]. Adélaïde et al [33] observed rare high-level amplifications in basal tumors affecting small regions, including PIK3CA (3q26), IGF1R (15q26), and CCNE1 (19q11-12), but also single genes, such as EGFR (7p11), FGFR2 (10q26), and BCL2L2 (14q11). EGFR, FGFR2, and IGF1R are tyrosine kinase receptors with a broad mitogenic and angiogenesis function and thus can serve as potential therapeutic targets. The existence of these amplifications and such high degree of heterogeneity in BC, even within a given subtype, confirms that molecular profiling will be paramount to select the appropriate treatment. In the same line, specific genomic losses were also detected in basal subtype. The loss of heterozygosity (LOH) at 4p and 5q has been able to define a subclass of BLBC [33,34]. Losses of 4p and 5q associated with BLBC targeted several genes including candidate or known tumor suppressing genes such as SLIT2 (4p15.31), GPR125 (4p15.31), RASA1 (5q14.3), and APC (5q22.2) [33]. So, the aim of these efforts in genomic studies is to understand the function of these markers in mammary oncogenesis and progression and to develop therapeutic approaches against critical markers adapted to various molecular categories of tumors.

5. EPIGENETIC CHANGES OF BLBC

BC development depends on both genetic alterations and epigenetic changes involving DNA methylation and histone modifications [35]. Roll et al. [36] reported a methylation signature in BLBC. BLBC express a hypermethylator phenotype that is characterized by concurrent methylation-dependent silencing of CEACAM6, CDH1, CST6, ESR1, LCN2, and SCNN1A genes that are involved in a wide range of neoplastic processes relating to tumors with poor prognosis [36]. ESR1 (encodes for the estrogen receptor α) and CDH1 (encodes for the E-cadherin) are concurrently methylated in BC and both can
regulate tumor progression [37]. Tumors with CDH1 and ESR1 methylation were associated with significantly lower hormone receptor levels, younger age at diagnosis, and TP53 mutations [38]. Recently Holm et al. [39] showed that ARGDIBI, GRB7, and SEMA3B are also methylated in BLBC [39].

Some authors found equally distributed methylation events at specific genes among different histological subsets of neoplasms suggesting that a CpG island methylator phenotype does not occur in BC [40]. Otherwise, Dumont et al. [41] proposed that DNA methylation profiles observed in BC may reflect the history of environmental exposures based on the induction of p16/Rb pathway and impact on epigenetic changes resulting from methylation of CpG islands associated with tumorigenesis [36,41]. Elsheik et al. [42] described a variation in global levels of histone markers in BC. Moderate to low levels of lysine acetylation (H3K9ac, H3K18ac, and K4K12ac), lysine (H3K4me2 and H4K20me3) and arginine methylation (H4R3me2) were observed in BLBC and HER2-positive tumors and were related with adverse prognosis [42]. Alterations in histone methylation and demethylation are likely critical steps in neoplastic progression by disrupting the normal stem- or progenitor-cell program [35]. Further studies are needed involving BLBC and DNA methylation machinery to fully understand the clinicopathological implications of the hypermethylator phenotype in primary BC and subtypes for better diagnosis and improved treatment strategies.

6. BLBC AND BRCA1

Several large and integrative research studies based on expression and copy number profiling of familial BC demonstrated molecular heterogeneity of these tumors similar to sporadic tumors, as well these studies defined molecular subtypes based on markers other than BRCA1 and BRCA2 germline status [11,43,44]. Microarray or immunohistochemical analyses demonstrated that approximately three quarters of BRCA1-related BC are BLBC whereas BRCA2 tumors generally cluster within the luminal A or B groups [43,44,45,46] and non-BRCA1/2 with luminal A tumors [11,44].

BRCA1-related BLBC are TN and frequently positive for Ki67, basal CKs (CK5/6, CK14), TP53, EGFR, P-cadherin [44,47] and with frequent X-chromosome abnormalities [6]. Interesting, the
clinical outcomes for women with BLBC and BRCA1-related BC are broadly similar in particular for early (within 5 years) relapse and pattern of metastatic spread.

Several investigators have been exploring the role of the BRCA1 pathway in sporadic BLBC, even if not all BC arising in BRCA1 mutation carriers are TN or BLBC [11]. Although it is not clear whether BRCA1 inactivation is the cause or consequence of a BLBC phenotype, Rakha et al. [47] suggested two hypotheses for the similarities between BLBC and tumors arising in BRCA1 mutation carriers: (i) the precursor cells of BLBC may be more tolerant to loss of BRCA1 function than those of other BC subtypes, possibly because of the phenotype of the cell at the initiating event or the concurrent inactivation of other tumor suppressor genes, such as TP53; and alternatively, (ii) BRCA1 may be involved in the differentiation of breast epithelial cells and, therefore, BRCA1 inactivation would lead to tumors with a stem cell-like phenotype. Although the aforementioned hypotheses are attractive, there is no definitive answer at present time. In fact, there are increasingly more coherent data to suggest that BRCA1 pathway dysfunction may play an important role in development of not only familial but also sporadic BC tumors [47].

Decreased BRCA1 transcript levels and nuclear protein expression have indeed been observed in BLBC. In addition, BRCA1 promoter hypermethylation has been reported in metaplastic BC (a rare type of BLBC) and overexpression of ID4 (a negative regulator of BRCA1 expression) was shown in sporadic BLBC [29]. Furthermore, Gorski et al. [48] showed that siRNA mediated inhibition of BRCA1 up-regulates genes associated with the BLBC phenotype, suggesting that loss of BRCA1 expression may contribute to the development of BLBC [48]. The characteristics of hereditary BRCA1-associated BC found in sporadic BLBC cancers have thus been termed “BRCA-ness” with potential clinical implications [11].

More studies are needed to better characterize the profile of BRCA1-mutated BLBC based on genomic, epigenomic and proteomic analyses in order to pinpointing novel candidate cancer genes in this particular BC subtype.

7. CLINICOPATHOLOGICAL FEATURES OF BLBC

BLBC are associated with high histological and nuclear grade, poor tubule formation, the
presence of central necrotic or fibrotic zones, pushing borders, conspicuous lymphocytic infiltrate, and typical/atypical medullary features with exceptionally high mitotic and proliferative indices [1,11]. Most of these tumors are infiltrating ductal tumors with solid growth pattern, aggressive clinical behavior, and high rate of metastasis to the brain and lung. Unlike other BC subtypes, there seems to be no correlation between tumor size and lymph node metastasis in BLBC [1,11,49]. The most common histological type of BLBC is invasive ductal carcinoma, however BLBC also involves some unique histological types including invasive lobular, medullary, metaplastic, myoepithelial, neuroendocrine, apocrine, adenoid cystic, and secretory breast carcinoma [50]. BLBC constitutes a different clinical entity associated with worse clinical outcome [7,11].

Some interesting correlations have been found in the literature. BLBC showed a significantly higher incidence in premenopausal African-American patients (20-27%) compared to Caucasian woman (10-16%) [50,51]. Maybe a large part of the racial difference in the distribution of BLBC may be attributable to different distribution of specific risk factors. The use of oral contraceptives in women <40 years old, younger age at diagnosis, hispanic ethnicity, lower socio-economic status, with abdominal adiposity and metabolic syndrome were also shown to significantly increase risk of BLBC [50,52] (Table 3). Interestingly, as shown on Table 3, some of the principal risk factors of BLBC are opposite to those observed for BC (Luminal A).

8. THERAPEUTIC CONSIDERATIONS

BLBC are particularly enigmatic because the genes that are responsible for their aggressive phenotype are not well understood, and this constitutes a major barrier to develop targeted therapies for this group. The urgent necessity for new therapies is underscored by the fact that BLBC do not express ER or HER2 and thus are typically refractory to endocrine therapy and to trastuzumab, a humanized monoclonal antibody that targets HER2 [6,28].

Nevertheless, as BRCA1 pathway may be deficient in BLBC, these tumors may respond to specific therapeutic regimens, such as the currently available inhibitors of the poly (ADP-ribose)
polymerase (PARP) enzyme. Cells deficient in BRCA1 have indeed a defect in DNA double strand break repair that could render them particularly sensitive to chemotherapy drugs that generate DNA double strand breaks, such as inhibitors of PARP enzyme [53]. However, as stated above, not all BLBC are associated with BRCA1 inactivation [54].

EGFR could also represent a therapeutic target as it is often overexpressed in BLBC. Recently, Dong et al. [55] identified Notch pathway as one compensatory mechanism leading to resistance to EGFR inhibition in BLBC, providing additional insights and potential strategies to overcome resistance, and rendering dual-pathway inhibition a viable clinical strategy that can be tested in the near term of BLBC [55].

Finally, research on tumor stem cells may guide the search for better therapeutic approaches such as by targeting cell surface markers or signaling pathways activated in cancer-stem cells. These exciting concepts are currently taken a greater priority in therapeutic drug discovery research [1].

9. CONCLUSIONS

Current research on BC molecular profiling and classification has generated exciting impetus to ongoing efforts to deepen our basic understanding of the complex biology of BLBC. The exciting progress is not without challenges owing in part to technology issues. For instance, a more accurate identification of BLBC requires to determine the immunohistochemical sensitivity and specificity of some of biomarkers addressed in this article, including in relation to the size of the study cases, antibody specificity toward protein isoforms. Also, exploring a more comprehensive hypermethylation profile, it can be useful for understanding the expression of genes involved tumorigenesis, hallmarks process and tumor progression of BC, especially BLBC. Currently, BLBC lack any specific targeted therapy and the identification of new markers and therapeutic targets in relevant preclinical models and then in human trials are urgently needed before meaningful therapeutic outcomes could be achieved.
10. ACKNOWLEDGMENTS

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11. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.
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Figure 1. Genomic, epigenetic, and clinicopathological characteristics of BLBC.
Figure 2. A. Molecular classification of BC based on gene expression profiling: ER-positive group is subdivided into Luminal A and B, characterized by high expression of ER, PR, and CK8/18. ER-negative group is subdivided into HER2-positive with high expression of genes located in HER2 amplicon. BLBC (TN and overexpression of CK 5, 6, 14, 17, and EGFR) and other subtypes comprising normal-like, claudin-low and apocrine tumors. B. Distribution of subtypes of BC based on their frequencies [2,5,10,20].
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Frequency among basal-like (%)</th>
<th>Frequency among non basal-like (%)</th>
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<td>Vimentin</td>
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<td>β4 integrin</td>
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Potential biomarkers were presented in this table only if the positivity percentage in BLBC was above 30% and at least twice as high as in non-BLBC.

Source: Adapted from [23]
Table 2. Genes up- and down-regulated in BLEC and their functional implication.

<table>
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<th>GENES</th>
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<th>FUNCTIONAL GROUPS</th>
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<td>Structural elements of basal epithelial cells</td>
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<td>Up-regulated</td>
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<td></td>
<td>transcription</td>
</tr>
<tr>
<td>TGFβ2</td>
<td>Up-regulated</td>
<td>Heat shock protein</td>
</tr>
<tr>
<td>ER alpha, PR, GATA transcription factors (GATA3), basic transcription</td>
<td>Down-regulated</td>
<td>Hormone receptors and</td>
</tr>
<tr>
<td>3, FOXC1, FOXA1, TFF3, X-box binding protein 1, RAB, cyclin D1</td>
<td></td>
<td>transcription factors</td>
</tr>
<tr>
<td>HER2, GRB7, GTPase binding effector protein 1, forkhead box-1, and</td>
<td>Down-regulated</td>
<td>Oncogenes and others</td>
</tr>
<tr>
<td>marxin-1, Rb</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These information were searched in Entrez Gene (http://www.ncbi.nlm.nih.gov) and [6].
Table 3. Specific risk factors of BLBC compared to Luminal A BC

<table>
<thead>
<tr>
<th>RISK FACTORS</th>
<th>BLBC</th>
<th>BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young age at menarche (&lt;13)</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Parity (Yes)</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Young age at first full term pregnancy (&lt;26)</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Breast feeding (Yes)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Abdominal adiposity (WHR&gt;0.77)</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) Positive symbols mean an increase of BC risk (+: risk factor odd ratios between 1.1-1.5; ++: risk factor odd ratios >1.5) whereas (-) negative symbols mean a decrease of BC risk (−: risk factor odd ratios between 0.9 and 0.8; --: risk factor odd ratios <0.7). WHR: waist–hip ratio.