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REVIEW ARTICLE

Molecular Portrait of the Normal Human Breast Tissue and Its Influence on Breast Carcinogenesis

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Normal human breast tissue consists of epithelial and nonepithelial cells with different molecular profiles and differentiation grades. This molecular heterogeneity is known to yield abnormal clones that may contribute to the development of breast carcinomas. Stem cells that are found in developing and mature breast tissue are either positive or negative for cytokeratin 19 depending on their subtype. These cells are able to generate carcinogenesis along with mature cells. However, scientific data remains controversial regarding the monoclonal or polyclonal origin of breast carcinomas. The majority of breast carcinomas originate from epithelial cells that normally express *BRCA1*. The consecutive loss of the *BRCA1* gene leads to various abnormalities in epithelial cells. Normal breast epithelial cells also express hypoxia inducible factor (HIF) 1α and HIF-2α that are associated with a high metastatic rate and a poor prognosis for malignant lesions. The nuclear expression of estrogen receptor (ER) and progesterone receptor (PR) in normal human breast tissue is maintained in malignant tissue as well. Several controversies regarding the ability of ER and PR status to predict breast cancer outcome remain. Both ER and PR act as modulators of cell activity in normal human breast tissue. Ki-67 positivity is strongly correlated with tumor grade although its specific role in applied therapy requires further studies. Human epidermal growth factor receptor 2 (HER2) oncoprotein is less expressed in normal human breast specimens but is highly expressed in certain malignant lesions of the breast. Unlike HER2, epidermal growth factor receptor expression is similar in both normal and malignant tissues. Molecular heterogeneity is not only found in breast carcinomas but also in normal breast tissue. Therefore, the molecular mapping of normal human breast tissue might represent a key research area to fully elucidate the mechanisms of breast carcinogenesis.

Key Words: *Breast neoplasms, Carcinogenesis, Normal mammary gland, Transcriptome*

MOLECULAR ROADMAP, FROM PROGENITOR TO MATURE CELLS, IN THE NORMAL HUMAN MAMMARY GLAND

Recent studies have shown that normal breast tissue is composed of epithelial and nonepithelial cells with different profiles reflecting their maturation and differentiation [1]. In addition, cells of normal mammary tissue are known to yield abnormal clones that may contribute to the development of both preneoplastic and tumor lesions [2-4]. It appears that epithelial cells of the normal breast exhibit a heterogeneous profile depending on their differentiation stage. Considering these facts, several reports have referred to the existence of two luminal phenotypes and two basal phenotypes, based on differential immunohistochemical profiles [1]. These cells are known to express CD24, CD49f, and the epithelial cell adhesion molecule Epithelial cell adhesion molecule (EpCAM) [1]. Luminal progenitor cells, of normal breast tissue, express both CD49f and EpCAM while their mature variants do not express CD49f [1]. Myoepithelial cells, along with basal progenitor cells, lack EpCAM expression [1]. Both mature luminal cells and progenitor cells express CD44 and CD24 [1].

Based on cytokeratin (CK) 14 and CK19 expression in normal breast tissue, it appears that the normal mammary gland contains multipotent cells. These cells are located in the ducts,

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lobules, and various other regions, wherein a certain hierarchy is present [5-7]. In previous studies, stem cells observed in the developing and mature human mammary gland have been described as having lower self-renewal ability compared to stem cells located in other organs or compared to those belonging to other species such as mice [5,8]. To identify the stem cells of normal breast tissue, and various benign and malignant lesions, various subclass-specific keratins must be taken into consideration [9]. According to their keratin expression and their regenerative capacity, stem cells of the normal human breast have been split into two groups. The first, CK19+, is characterized as having great regenerative capacity and might form lobular units. The second group, CK19–, is able to form acinus-like structures [5]. However, other data suggest the existence of three main stem cell populations in normal human breast tissue, namely luminal-restricted, myoepithelial-restricted, and bipotent progenitor cells. The first two types are thought to be oriented cell types [6,7]. The cells that compose the luminal-restricted compartment are positive for both CK19 and CK18/8, EpCAM, and mucin 1 cell surface associated protein (MUC1). These bipotent colonies are surrounded by myoepithelial cells that are positive for CK14 [6]. It seems that a high level of α6 integrin and low MUC1 positivity are specific for basal localized stem cells [6]. In contrast to previously mentioned data, Smith and Chepko [10] identified five normal clonal populations of potent progenitor cells that are present in both the human and the rodent mammary tissue.

Besides stem cells, normal human mammary tissue contains mature epithelial cells. The MCF-10 cell line originates from fibrocystic mammary tissue and is characterized by minimal karyotype rearrangements and immortality [11,12]. Epithelial cells are characterized by a proliferative capacity that could contribute to carcinogenesis. Holst et al. [12] showed that some epithelial cells might represent precursors for different breast cancers.

DOES HISTOLOGICALLY "NORMAL" TUMOR-ASSOCIATED BREAST TISSUE HAVE THE SAME MOLECULAR PROFILE AS HEALTHY CONTROL BREAST TISSUE?

The aim of a recent study published by Santagata et al. [13] was to provide some kind of classification for the epithelial cells of the normal breast epithelium. After probing for a large set of breast epithelial markers, four major subtypes (HR0–HR3) emerged. These subtypes were differentiated by vitamin D, androgen, and estrogen hormone receptor (HR) expression. This classification is distinct from the official guidelines for breast cancer classification that rely on estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status. Patient outcomes were best when tumors expressed each of the three hormone receptors (subtype HR3) and worst when they expressed none of the receptors (subtype HR0). Graham et al. [14] focused on examining gene expression in the histologically normal breast epithelium. Their study clearly showed a contrast in gene expression between the normal epithelium of breast cancer cases and that of controls. Schummer et al. [15] attempted to identify biomarkers with potential value for early detection of breast cancer with poor-outcome. It appeared that some histologically normal breast tissue specimens removed from distant sites of breasts with cancer displayed a cancer-like expression profile, whereas others were genetically similar to the control group. Tripathi et al. [16] compared global gene expression between normal breast epithelium of breast cancer patients and cancer-free controls. A large number of genes were differentially expressed in the two groups included in the study, and some of these had been previously implicated in carcinogenesis. Zubor et al. [17] aimed to analyze gene expression in histologically normal epithelium and in breast cancer specimens to establish the value of expression profiles as a potential diagnostic marker for cancer development. It appears that gene expression was increased in both A and B luminal types of breast cancer but also in the surrounding histologically normal epithelium. Thus, the normal epithelium was not "normal" as it could be associated with different molecular abnormalities that might contribute to carcinogenesis.

DOES THE NORMAL PHENOTYPE INFLUENCE CARCINOGENESIS?

Carcinogenesis in the human breast is a complex process that involves a great number of genetic mutations usually occurring in epithelial cells. However, its mechanisms are not yet fully understood. Some authors have demonstrated that tumors derived from transformed mammary epithelial cells, resulting from the introduction of specific genes, present a low differentiation grade, and exhibit the tendency to infiltrate the normal adjacent mammary tissue [18]. These aspects partially explain the molecular mechanism of carcinogenesis in the human breast through the amplification of the c-myc oncogene [18]. Currently, insights into tumorigenesis in the mammary gland are focused on the molecular changes that affect various cells types such as luminal and myoepithelial cells [19]. Some molecular changes that lead to the transformation of luminal and myoepithelial cells seem to occur at a chromosomal level and result in gene inactivation [19]. One

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of these molecular changes is a *BRCA1* gene mutation, specifically occurring through chromosomal loss [19-21]. Histological and molecular abnormalities are not only found in mammary cancer specimens, but also in post-reduction mammoplasty specimens and in benign diseases of the breast [22]. It is important to note that certain histological changes, found in mammary tissue with benign lesions or after surgical interventions, are associated with a higher risk for cancer development.

CROSSTALK BETWEEN EPITHELIAL CELLS AND FIBROBLASTS

The molecular heterogeneity of breast cancers seems to be strongly linked to transformed luminal epithelial cells and myoepithelial cells. The genetic differences found in these cells might represent important diagnostic and prognostic factors [23]. Some studies revealed that luminal progenitor cells are more susceptible to carcinogenesis compared to basal stem cells [24]. The differences between the various types of mammary stem cells that generate carcinomas of the breast are related to both their location and their immunohistochemical profile. Stem cells that undergo oncogenic changes may exhibit either a mesenchymal-like or epithelial-like profile [25]. Cells undergoing epithelial-to-mesenchymal transition are typically situated at the invasive front of the tumor and are CD44+/CD24- [25]. Unlike mesenchymal-like cells, mesenchymal-to-epithelial transitioning cells express aldehyde dehydrogenase, have proliferative characteristics, and are located more centrally in the tumor [25]. Considering the fact that the majority of breast cancers originate from epithelial cells, some studies focused on the difference between immortalization and tumorigenicity of this cell population; it has been suggested that immortalization precedes carcinogenesis [26]. Moreover, the loss of the *BRCA1* gene leads to abnormalities in epithelial cell lines and is associated with an accumulation of luminal progenitor cells during pregnancy [27]. In addition, the fact that both *BRCA1* and c-kit are expressed during epithelial cell differentiation [27] further supports the implication of stem cells in mammary carcinogenesis. Besides epithelial cells, fibroblasts are also an important cell population in both the normal breast tissue and in breast cancer. As fibroblasts of normal mammary tissue are known to possess antitumor activity, their cancerous counterparts present tumorigenic properties [28]. In addition, fibroblasts located at the surgical margins seem to exhibit invasive capacities due to their specific genetic profile and may play an important role in tumor recurrence [28]. Although fibroblasts are strongly involved in tumor progression due to their interactions with tumor cells, the main cell populations that are involved in breast carcinogenesis are epithelial and the myoepithelial cell lineages. While epithelial cells represent the starting point for cancer development, myoepithelial cells appear to act as tumor regulators by controlling the invasive potential of tumor cells [29].

THE SWITCH FROM NORMAL DEVELOPMENT TO LOCAL INVASION AND A METASTATIC PHENOTYPE

In addition to the expression of *BRCA1* and c-myc, the epithelial cells of the normal mammary gland also express hypoxia inducible factor (HIF) 1 and 2, which are associated with a high metastatic rate and a poor prognosis. HIF-1α is expressed before epithelial cells gain functional polarity and HIF-2α is expressed in the latter stages of the mammary gland cycle [30]. In addition, it appears that the behavior of epithelial cells, belonging to both normal and tumor breast tissue, is dependent on the expression of estrogen and progesterone receptors and on soluble factors derived from fat tissue. A study performed by Pistone et al. [30] demonstrated that microenvironment changes greatly influence the behavior and phenotype of mammary epithelial cells. Upon exposure to conditioned media, cells are able to undergo various changes, altering motility and metalloprotease activity [31].

There are several controversies regarding the prognostic ability of estrogen and progesterone receptors. Estrogen and progesterone are modulators of cell activity in the human breast. Both hormones bind to specific receptors located in the cell nucleus. Normal epithelial cells from the breast tissue are implicated in estrogen metabolism, with differences depending on the cell population [32]. Currently, it is accepted that high levels of estrogen are implicated in mammary gland carcinogenesis. After receptor binding, estradiol induces DNA synthesis, stimulates the secretion of growth factors, and induces cell division [33,34]. The fact that estrogen may be associated with a higher risk of cancer development in the mammary gland is supported by abnormal changes that occur in hormone signaling pathways and by gene polymorphism that encode these protein products [33]. Normal breast tissue and the majority of ductal invasive neoplasms express estrogen and progesterone receptors [34]. Invasive carcinomas mostly have an ER+/PR+ immunohistochemical profile [34]. Few cases of invasive ductal carcinoma exhibit an ER-/PR- profile [34]. Some differences related to estrogen and progesterone expression during the developmental and physiological state of the mammary gland have been pointed out [35]. Unlike normal mammary tissues belonging to other species, that of human seems to exhibit constant temporal estrogen expres-

sion [36]. Accordingly, ER overexpression is associated with a high risk for cancer development [36-38]. Shoker et al. [36] showed that the molecular mechanism through which estrogen receptors generate malignant diseases in the human breast are related to aberrant ER expression and to the constitutive activation of ER positive cell division. ER levels seem to increase with age in normal mammary tissue without an association with breast cancer. The high percentage of ER expressing cells is correlated with a high risk of cancer development [37]. Some authors suggest that ER may be useful as a marker for detecting the risk of developing breast cancer [38]. However, a great number of difficulties occur when attempting to perform a comparative study of ER expression in the normal female breast, in the breast tissue postmammoplasty, and in benign and malignant lesions. Normal breast tissue is difficult to obtain and usually the specimens are not "normal" from a molecular perspective. Data suggest that ER-α is strongly related to breast cancer development and is associated with Ki-67 overexpression, especially in advanced lobular carcinoma [39-41]. However, in the early stages of lobular carcinoma, both α and β receptors are overexpressed but the proliferation rate has been determined to be at a much lower level [40]. Ki-67 is regarded as an important prognostic parameter in breast cancer evaluation and appears to be strongly correlated with tumor grade [42-44]. Despite its role as a predictive marker, Ki-67 is not easily determined in all malignant cases, especially those that present an extensive heterogenic profile [44]. Currently, besides Ki-67 and ER-α, ER-β is also in need of further studies [45]. Green et al. [45] demonstrated that there are no significant differences regarding ER-α and -β expression between pure lobular *in situ* carcinomas and invasive breast carcinomas [46]. Other studies showed no correlation between ER-β, cell line invasiveness, and tumor clinical parameters [47]. Both PR and ER play an important role in the development of the mammary gland and are detected at high or low levels in the normal mammary tissue under physiological conditions [48,49]. Haslam and Shyamala [47] demonstrated a decrease in the level of PR in the normal mammary tissue during pregnancy and lactation while responsiveness to estrogen decreased during lactation. The expression of ER and PR in normal conditions is highly differential depending on the physiological status of the mammary tissue. The pubertal mammary gland is less responsive to progesterone in comparison to the mature gland [50]. Estrogen-dependent progesterone receptors appear to be highly implicated in modulating the mitogenic effects of progesterone but are acquired only in the pubertal stage [50]. It is well known that estrogen-inducible progesterone receptors are located in the mammary epithelium while estrogen-independent progesterone receptors are located in the stroma and are less abundant [51].

HER2 AND EGFR: SAME FAMILY BUT NOT RELATIVES

The expression of HER2 and epidermal growth factor receptor 1 (HER1 or EGFR) is found in both malignant and nonmalignant breast tissue. The levels of HER2 expression are higher in malignant samples, whereas the levels of EGFR appear to be similar in both normal and malignant cases [52]. HER2 is a proto-oncogene related to EGFR and is expressed in the genital, gastrointestinal, and respiratory tracts. HER2 displays a membranous expression pattern on epithelial cells [53]. In normal conditions, the levels of HER2 are considerably higher in the fetal tissue compared to normal mature tissue [53]. HER1 to HER4 receptors are expressed in both normal and the malignant mammary tissue. However, HER3 in particular is mostly overexpressed in ER positive tumors [54]. The EGFR/HER1 axis is expressed at higher level in the normal breast [54] but its interaction with estradiol is quite controversial. Data suggest that there is a strong positive correlation between the EGFR/HER1 axis and estradiol only in postmenopausal women who were diagnosed with ER positive tumors [54]. In addition, no positive correlation has been found between estradiol and HER2 in patients diagnosed with ER positive tumors [54]. HER2 is associated with aggressive types of breast cancer and its overexpression is an indicator of poor prognosis [55]. Despite the aggressive phenotype of HER2 positive breast cancers, Camp et al. [54] have shown that breast tumors with normal HER2 expression may have a similar aggressive behavior. Immunohistochemical assessment of HER2 plays an important role in both the diagnosis and prediction of outcome in patients diagnosed with breast cancer; proper treatment application is thus ensured [56,57]. Numerous studies suggest within the same breast cancer molecular subtype, heterogeneity might exist. This, for instance, is the case for HER2 positive tumors, the expression of which is variable status in primary and metastatic tumors [57].

CONTROVERSIAL MARKERS OF NORMAL BREAST TISSUE WITH POTENTIAL IMPACT ON BREAST CARCINOGENESIS

Bcl2 is another important marker of breast cancer, with its utility in both research and diagnosis being highly recognized. *Bcl2* is known to be involved in blocking cell apoptosis and generating immortal cells [58]. *Bcl2* plays an important role in the developmental stages of the mammary gland and is expressed early in prenatal life in the fetal mammary plaque. In

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fetal mammary tissue, Bcl2 appears to regulate the patterning behavior of mammary cells [58]. During adulthood, its expression is identified in all epithelial cells of the normal human breast tissue [58]. In pathological conditions, Bcl2 is expressed in malignant and hyperplastic lesions of the breast [58]. *In situ* and invasive carcinomas of the breast are associated with high expression of Bcl2. However, it appears that Bcl2 expression is not related to lymph node status and tumor stages [58,59]. Moreover, Bcl2 expression in normal and pathological conditions seems to depend on reproductive and hormonal factors [59-62]. Bargou et al. [59] demonstrated that there are no differences between Bcl2 expression in the normal breast epithelium, nonmalignant cell lines, and breast cancer. Yu et al. [58] showed a higher expression of Bcl2 in breast carcinoma tissue compared to expression in malignant specimens of the breast from menopausal women. It was demonstrated that Bcl2 is positively correlated with ER and PR expression [59]. In a study performed on fibroadenomas of the breast, Lima and da Silva [61] have concluded that there were no differences in Bcl2 expression after placebo or raloxifene treatment. Differential expression of Bcl2 found in normal human breast tissue is cell-phenotype related and supports the fact that not all mammary cell types are equally responsive to apoptotic stimuli [63]. Unlike Bcl2, androgens determine the inhibition of breast growth during pubertal and postpubertal stages [64]. They act as inhibitors of ER-α expression and reduce myoepithelial cell proliferation [64]. Androgen receptor (AR) expression is known to be present in the hormone sensitive cell population and in the basal cell population. The basal cell population was reported to retain a higher level of AR expression [64]. Tarulli et al. [63] showed that AR expression in the normal breast tissue is predominant in luminal cells, but fibroblasts and adipose cells often exhibit a strong immunoreactivity for AR. In addition, previous data support the fact that AR is mostly expressed in the luminal cells of mammary ducts and acini, rather than basal cells [64]. Due to its inhibitory effects on stromal cells, a lack of androgens stimulates the development of the mammary gland [64]. The relationship between ER and AR expression in breast cancer is characterized by an inverse correlation. AR is usually expressed in mammary cancer cells that exhibit an ER-/PR- profile [64,65]. Surprisingly however, ER positive malignant cells tend to gain AR positivity [65]. The luminal subtype of breast cancer is ER+/AR+ while the basal subtype is ER-/AR- [65]. Histopathological data has shown an ER-/AR+ immunohistochemical profile in apocrine breast cancer [65]. Triple negative breast cancers, may exhibit an ER-/PR-/HER2-/AR+ profile or may lack AR expression [66].

Another important marker found in normal and malignant breast tissue is human mammaglobin. This protein can be detected in two main forms with different molecular masses [67]. The expression for both forms was higher in malignant lesions, although its presence is associated with a favorable prognosis [67]. The function of human mammaglobin is not yet fully understood, but molecular methods have shown that its expression is restricted to the mammary tissue [67]. Clinical studies point out that the human mammaglobin expression is lower in the normal cells compared to malignant ones [68,69]. Data suggest that human mammaglobin overexpression in breast cancer is associated with a decrease in cancer cell migration and reduction in invasiveness [68]. Human mammaglobin may also be useful in determining the lymph node status in patients diagnosed with breast cancer. It appears that normal lymph nodes lack human mammaglobin compared to pathological nodes [70]. By means of DNA and tissue microarray, Tafreshi et al. [69] have postulated that human mammaglobin in the positive axillary lymph nodes might represent a potential alternative diagnostic and therapeutic approach compared to time consuming surgical excision. It appears that human mammaglobin levels do not depend on tumor size, its expression being equal in both large and small tumors, and may be identified in all three grades of breast cancer [71].

Tumorigenesis of the mammary gland implies not only the disruption of hormone expression but also deterioration in cell-cell adhesion. One of the most important regulators of cell-cell adhesion is E-cadherin. This protein is not expressed or poorly expressed in breast carcinomas. E-cadherin is a member of the cadherin protein superfamily, and is a calcium-dependent adhesion molecule. It is implicated in regulating tissue formation and controls the patterning of epithelial cells [72,73]. E-cadherin is also implicated in cancer suppression [72]. In normal breast tissue, E-cadherin is expressed in luminal epithelial cells. Loss of E-cadherin is found in various types of breast cancers and is associated with an unfavorable prognosis [74]. Along with ER, PR, HER2, and CK5, E-cadherin may become a useful marker for additional differentiation and classification of molecular subtypes of breast cancer [75]. Breast cancers are not homogenous diseases and they do not remain stable throughout their evolution [76]. Certain molecular subtypes could exhibit shifts to other subtypes, thus influencing the patient outcome [76]. Fulga et al. [75] have concluded that E-cadherin expression is not stable during tumor progression and metastasis. Breast cancer is known to produce metastases through the lymphatic system into regional and distant lymph nodes. One common location of metastasis in breast carcinoma is the axillary lymph nodes. Thus, the estimation of lymphatic vascular density to ensure

Table 1. Markers of normal and malignant breast tissue with prognostic and therapeutic role

Marker	Normal breast	Tumor tissue	Epithelial to mesenchymal transition	Cancer metastasis	Targeted therapy	Survival	Reference
EpCam	Luminal progenitor cells	Cancer stem cells, tumor cells	Loss of EpCam	Enhanced bone metastases and influence lymph node metastases	Edrecolomab Scattered use in breast cancer	Negative impact by its overexpression	[87-90]
CD24	Luminal mature and progenitor cells	Suppress malignant phenotype	Regulation through Notch1 signaling in breast cancer cells	Lack of CD24 promote metastasis	None	Lack of CD24 is correlated with low survival	[91-93]
CD44	Luminal and progenitor cells	Predominant in "basal like" and "claudin low" subtypes	CD44 expression-epithelial-mesenchymal transition in basal like type	CD44+/CD24– cells favor metastasis	Salinomycin (cancer stem cells CD44+)	Low survival associated with CD44+ cells in triple negative breast cancer	[94-97]
MUC1	Underglycosylated MUC1 overexpression and syalation, on normal tissue adjacent to tumor	Upregulation and/or underglycosylation of MUC1 associated with higher tumor grade	The oncogenic MUC1-C subunit induces EMT	MUC1/ICAM 1 promote promigratory signal	PankoMab-GEX™	Its downregulation improves survival	[98-102]
BRCA1 and *BRCA2*	Developing breast tissue, highly proliferating cells	*BRCA1* and *BRCA2* mutations	None for breast cancer	*BRCA1* favors brain metastases while *BRCA2* has no influence	None	Low survival	[103,104]
c-myc	Induced by lactation, influenced by steroids, not yet studied in breast development and pubertal mammary gland	Cause malignant transformation by several mechanisms	None data in breast cancer	Promote metastasis	Overexpressed c-myc induces resistance to endocrine therapy in luminal types of breast cancer	Reduced survival especially for basal like subtype	[105-107]
HIFs	Low levels	Overexpress close related to angio and lymphangiogenesis	It acts thorugh COX2 favoring EMT	Promote metastasis	Indirect effects through COX2 inhibition	High levels	[108,109]
ER/PR	Expression in ductal and acinar cells	Luminal types A and B	Involved in EMT and mesenchymal to epithelial transition most intensely studied being ER-α	Involved in discordances between phenotypes of primary tumor and their correspondent metastasis	Aromatase inhibitors or selective estrogen receptors modulators. PI3K inhibitors, insulin-like growth factor receptor (IGF-R) and histone deacetylase (HDAC) inhibitors for endocrine resistance	Dependent by response to therapy	[110-113]
AR	Most of the AR-positive luminal cells are also ER positive, about 10% of the cells are AR-positive only	Highly expressed in HER2 positive/ER negative and triple negative breast cancers	Not yet proved by a direct mechanism	AR expression is kept in metastatic foci.	Enzalutamide (Xtandi®)-new antiandrogen therapy	AR expression associated with lower risk of recurrence in patients with all breast cancer types and better survival in cases with ER positive. Predict decrease survival in triple negative breast cancer	[114-118]

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Table 1. Continued

Marker	Normal breast	Tumor tissue	Epithelial to mesenchymal transition	Cancer metastasis	Targeted therapy	Survival	Reference
HER2	Low or not expressed	Overexpressed in triple positive and HER2 subtypes	Interstitial flow-induced invasion of erbB2-expressing breast cancer cells, dependent on EMT and acting through a CXCR4-PI3K pathway	Positive in 30% of cases with metastases	Trastuzumab, its efficiency being dependent by intratumor heterogeneity of expression	Low survival high rate of recurrences	[119,120]
EGFR	Downregulated in normal tissue compared with tumor tissue	Highly expressed in triple negative breast cancer	EMT decreases EGFR expression	Paradoxical function of EGFR between primary and metastatic tumors	Cetuximab alone or in various combinations. EGFR may be molecular marker for response to Vandetanib Lapatinib with low response in advance breast cancer	Not yet proved significant influence on survival	[121-127]
Bcl2	Rare Bcl2+ cells spotted the tubulo-lobular units of normal resting gland	Controversial	It induces epithelial to mesenchymal transition	Increase metastatic process	Obatoclax and ABT-737 in clinical trials for other tumor types only in preclinical phase	None	[57,128-131]
Mammaglobin	Present in less than 50% of cells from normal breast tissue lobular unit	Increased levels starting from *in situ* carcinoma to invasive carcinoma Predominant in luminal type cancer	Regulate epithelial to mesenchymal transition	High level of mammaglobin reduce invasion and metastasis	Change tumor cell sensitivity to chemotherapy. Phase 1 clinical trial demonstrated the safety of a mammaglobin-A DNA vaccine in metastatic breast cancer	Its elevation was associated with distant recurrences and decreased survival periods	[67,132-136]
E-cadherin	Expressed	Its loss is associated with aggressive behavior of the tumor	Loss of E-cadherin sustains epithelial to mesenchymal transition	Associated with tumor size and lymph node metastasis	Controversial between its restoration and inhibition of its ectodomain by DECMA 1 in HER2+/E-cadherin+ cases	Related to its involvement in tumor invasion and metastasis	[137,138]
PDPN	Myoepithelial cells, myofibroblasts	Cancer associated fibroblasts, tumor cells from invasion front, lymphatic vessels	PDPN induce tumor invasion without E-cadherin loss	PDPN expression in cancer cells induce and sustain nodal metastasis	NZ1 antibodies not yet tested in clinical trials for patients with breast cancer	Close related with invasion and metastasis potential of PDPN	[139-141]
GCDFP-15	Secretory component of the normal mammary gland	Present in HER2 positive and luminal types and usually negative in triple negative breast cancer	Not yet stated any direct evidence of involvement in EMT	Indirect evidence of its involvement in metastatic process especially by activation of AR dependent pathway	No targeted therapy	GCDFP-15 positive tumors are correlated with better survival compared with GCDFP-15 negative tumors	[81,142]

EpCam = epithelial cell adhesion molecule; CD = cluster of differentiation; Notch1 = Notch homolog 1, translocation-associated (Drosophila), also known as NOTCH1, is a human gene encoding a single-pass transmembrane receptor; MUC1 = mucin 1 cell surface associated protein; ICAM1 = intercellular adhesion molecule 1; EMT = epithelial-mesenchymal transition; HIFs = hypoxia inducible factors; COX2 = prostaglandin-endoperoxide synthase 2; ER = estrogen receptor; PR = progesterone receptor; AR = androgen receptor; HER2 = human epidermal growth factor receptor 2; CXCR4 = C-X-C chemokine receptor type 4; EGFR = epidermal growth factor receptor; DECMA 1 = anti-E-cadherin antibodies; PDPN = podoplanin; NZ1 = a neutralizing monoclonal antibody (mAb; NZ-1); GCDFP-15 = gross cystic disease fluid protein 15.

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proper management and predict patient outcome is necessary. One of the most important markers used to examine lymphatic vessels in both normal and pathological conditions is D2-40. Normally, D2-40 is expressed in lymphatic endothelial cells. However, some studies performed on breast tissue specimens show that it also stains the glandular myoepithelial cell belonging to the terminal duct lobular unit [77]. This could lead to misinterpretation of lymphovascular invasion in pathological conditions [77]. It seems that D2-40 expression in myoepithelial cells from breast tissue is lower than that in lymphatic vessels [78]. Rabban and Chen [78] demonstrated that D2-40 exhibits a variable degree of expression in myoepithelial cells with a patchy distribution. In the same study, the authors showed that a D2-40 positive reaction occurred more often in the large ducts than in the terminal ducts and in the lobules [79]. D2-40 can be used to examine lymphatic vessels in both intratumoral and peritumoral areas [80,81].

Unlike other markers of breast tissue, gross cystic disease fluid protein 15 (GCDFP-15) is less studied. As a marker of apocrine differentiation, GCDFP-15 was first identified in pathological conditions, namely in the cyst fluid of cystic breast disease [82,83]. GCDFP-15 is found in normal fetal tissue as a marker of glandular differentiation [82]. Expression of this glycoprotein occurs in both fetal and adult normal tissues and both apocrine and eccrine glands [82]. It has been demonstrated that GCDFP-15 exerts a mitogenic effect on breast cancer cell lines and on the normal breast tissue cells [83]. It is well known that the mammary gland is in fact a modified sweat gland, a developmental fact that, along with different experimental studies [84], supports the function of GCDFP-15 in the normal human breast. A more common marker of human breast tissue is CK5, also known as CK5/6. It can be used to achieve a more complex molecular profiling of breast cancer specimens, along with the classical markers ER, PR, and HER2. Some authors have shown that CK5 is more highly expressed in the ducts of normal breast tissue than in the lobules [85]. CK5 appears to play an important role in breast cancer carcinogenesis in association with other molecules that are regarded as potential oncogenes [86]. Moreover, CK5 has been proposed by some authors to be a marker that determines basal-like features of breast cancer, along with ER, PR, HER2, and EGFR [86]. Several of the most important markers with prognostic and therapeutic values in breast cancer are summarized in Table 1 [87-142].

CONCLUSION

Human breast tissue is characterized by heterogeneous histological and molecular features, the recognition of which might contribute to a better understanding of carcinogenesis. The same histopathological type is known to have different patterns of evolution, thus supporting the hypothesis that malignant diseases of the breast are characterized by genetic instability. The heterogeneous profile of human breast cancers may be partially explained by the variable features of normal human breast tissue. Normal breast tissue appears to give rise to abnormal genes that are able to induce the formation of breast carcinomas. The markers used to establish a complete molecular profile of the various types of breast cancers are not yet sufficient. Further investigation is needed, on both tumor and normal specimens, for complete molecular characterization of normal human breast tissue and its pathologic variants.

CONFLICT OF INTEREST

The authors declare that they have no competing interests

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