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Tumor Markers of Breast Cancer: Role in Early Diagnosis, Monitoring Response to Therapy and Determination of Prognosis

Ahmed M. Kabel[1,2,*], Aisha H. Al-shehri[3], Batool S. Madani[3], Sheemah I. Al-shafie[3], Shroog A. Amasha[3]

[1]Department of Clinical Pharmacy, College of Pharmacy, Taif University, Taif, KSA
[2]Department of Pharmacology, Faculty of Medicine, Tanta University, Tanta, Egypt
[3]Final year student, College of Pharmacy, Taif University, Taif, KSA
*Corresponding author: drakabel@gmail.com

Abstract A tumor marker is a substance that is produced by the body in response to malignant tumors, or produced by the cancer itself. Some of these markers are specific to one cancer, while others are seen in several types of cancer. These markers are generally used to evaluate the patient's response to treatment or to monitor the presence of metastasis or recurrence. Breast cancer is one of the most common malignancies in females worldwide. Tumor markers may play a role in early detection, and hence favourable prognosis of breast cancer. The CA 27-29 is a tumor marker that is most often used in people with breast cancer. Also, CA 15-3, BR 27.29 (CA27.29), carcinoembryonic antigen (CEA), tissue polypeptide specific antigen, p53, cathepsin D, cyclin E, nestin and HER-2 are widely used for diagnosis, monitoring response to therapy, early detection of metastasis and determination of recurrence of breast cancer.

Keywords: *tumor, markers, breast, cancer, prognosis*

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1. Introduction

A tumor marker is a substance produced by a tumor, or by the host in the response to a tumor, that is used to differentiate a tumor from normal tissue or to determine the presence of a tumor [1]. The most important characteristics of an ideal tumor marker include being highly specific to a given tumor type, should provide a lead-time over clinical diagnosis and being highly sensitive to avoid false positive results. Moreover, the levels of the marker should correlate reliably with the tumor burden and should reflect accurately any tumor progression or regression, along with a short half-life allowing frequent serial measurements. Also, the test used for detection of the ideal tumor marker should be cheap for screening application at mass level and should be of such nature as to be acceptable to the target population [2]. Few markers are specific for a certain type of tumors. Most tumor markers are found with various types of tumors of the same tissue type. They are present in higher quantities in cancer tissue or in blood from cancer patients than the case in benign tumors or in the blood of normal subjects. Tumor markers are most useful in evaluating the progression of disease status after the initial therapy and monitoring subsequent treatment strategies [3].

Breast cancer is the second most common type of cancer after lung cancer (10.4% of all cancer incidence, both sexes counted) and the fifth most common cause of cancer death [4]. It is a disease caused by a combination of genetic and environmental factors. Numerous risk factors that may be associated with breast cancer have been recognized. Not all breast cancer patients have the same clinical picture. Some factors increase a woman's risk of breast cancer more than others [5].

Early detection of breast cancer both primary and recurrent, is of considerable clinical importance, and it can be used to make treatment decisions while tumor burden is low, and when patients are most likely to respond to adjuvant therapy [6]. In recent decades, the serum concentartion of tumor markers has been used to detect tumor activity .Tumor markers provide a cost-effective source of data that can be valuable for monitoring disease course, determining prognosis, and helping in treatment planning. An understanding of the individual test characteristics and limitations is important for optimal use and accurate interpretation of results [7]. The real usefulness of tumor markers in the management of breast cancer has been questioned because of the low diagnostic sensitivity for early disease [8].

The American Society of Clinical Oncology (ASCO) has updated its recommendations for use of tumor markers in prevention, screening, treatment and surveillance of breast cancer. 13 categories of breast tumor markers were considered. The tumor markers that showed evidence of clinical utility and were recommended for use in practice

include CA 15-3, CA 27.29, Carcmoembryonic antigen (CEA), Estrogen receptor (ER), Progesterone receptor (PR), Human epidermal growth factor receptor 2 (HER2), Urokinase plasminogen activator (uPA), Plasminogen activator inhibitor 1 (PAI-1) and multiparameter assays for gene expression [9]. However, other categories are also used in screening of breast cancer but they demonstrated insufficient evidence support routine use in clinical practice including P53, cathepsin D, cyclin E and nestin [8].

2. Carcinoembrvonic Antigen (CEA)

CEA is a large family of related cell surface glycoproteins that is the most widely used tumor marker in the clinical practice. It is a tumor marker for colorectal, gastrointestinal, lung and breast cancer [10]. CEA was first identified as a tumor specific antigen found in extracts of tumor tissue. It is also found in normal foetal gastrointestinal tract epithelial cells. It is a glycoprotein that contains 45-50 % carbohydrates. It is a single polypeptide chain consisting of 641 aminoacids, with lysine at its N-terminal position [11].

The human carcinoembryonic antigen (CEA) family is composed of 29 genes arranged on chromosome 19ql3.2, of which 18 are expressed. These genes are classified into two major subfamilies, the CEA cellular adhesion molecule (CEACAM) and the pregnancy-specific glycoprotein subgroups [12]. The CEACAM family belongs to the immunoglobulin superfamily. The CEACAM proteins can interact homophilically (CEA binding to CEA) and heterophilically (CEA binding to non CEA molecules) with each other, suggesting that CEA might act as an adhesion molecule. Because alternations in cell adhesions are involved in cancer invasion and metastasis, it was further suggested that CEA may play a roles in these processes [13].

Continuous rising level of CEA in breast cancer may explain either cancer not responding to treatment or recurrence after treatment. As steadily rising CEA may be the first sign that cancer has come back after treatment, the lead time from CEA elevation to clinical recurrence is about 5 months [14]. Also, patients with advanced cancer or metastatic cancer may have high CEA levels rather than in patients with localized diseases [10]. Because CEA lacks disease sensitivity and specificity, it cannot be used for screening the general asymptomatic population, a subpopulation with a high risk for malignancies, or for independently diagnosing cancer. However, CEA can be used to help diagnosis, clinical staging, to detect recurrence in patients who have undergone surgery, and to monitor the therapeutic response in patients undergoing chemotherapy or radiotherapy [15].

In breast cancer, elevated CEA is associated with metastatic disease. Preoperative CEA measurements have been shown to correlate with pathological stage and tumor extent and is stage dependent. Circulating levels of CEA in breast cancer patients are directly dependable on the size of both primary and metastatic tumor. CEA is a tumor antigen of less differentiated cancer cells. For breast cancer, CEA is being replaced by other more specific markers, such as CA 15-3 [16].

3. Cancer Antigen (CA) 15-3

The name of this marker is derived from a combination of the molecular structure and the assays developed for its detection. The numbers 15-3 refer to the antibodies used in immunoassays for these antigens [10]. CA 15-3 is a carbohydrate-containing protein antigen called mucin (MUC). Mucins are large transmembrane glycoproteins with extracellular domains formed of a highly O-linked glycosylated protein core consisting of a variable number of highly conserved 20-amino acid repeat units, classified into 7 families, MUC1 to MUC7, according to their genetic and biomolecular characteristics [17]. CA 15-3 belongs to the MUC1 family. Although the MUC1 gene is found in several tissues, it produces an apparently identical core protein. The variation in the extent of glycosylation (carbohydrate content) is the distinguishing feature between different tissue sources. In breast tissue, the carbohydrate content is approximately 50%. The exact physiological functions of MUC1 proteins are not completely known, but it appears to reduce cell-to-cell interaction and may also inhibit tumor cell lysis [18].

The MUC1 gene is overexpressed in malignant breast tumors, allowing use of gene product CA 15-3 as tumor marker for breast cancer [17]. CA 15-3 concentrations in blood can be used for screening, not onlyfor breast cancer but also for other malignancies, includi pancreatic, lung, ovarian, colon and liver cancer. However, it was also reported to be elevated in benign liver and benign breast diseases (False positive results) [19]. It is more useful in determining the prognosis of breast cancer and to monitor the efficacy of therapy as it was shown that the serum concentration and the proportion of patients with elevated values of this marker tend to increase with the severity of the disease and/or size of the tumor [10].

4. CA 27.29

CA27.29 is a tumor marker for breast cancer, also called breast carcinoma-associated antigen [20]. It is a carbohydrate-containing protein antigen that is produced by the MUC-1 gene. CA 27.29 is highly associated with breast cancer, as 80 % of women with breast cancer have an increased CA 27-29 levels. However, CA 27.29 can also be found in patients with other malignancies or with benign disorders of the breast, liver, and kidney, and in patiens with ovarian cysts. Therefore, elevation of this marker is not organ specific [21].

CA 27.29 has clinical performance similar to that of CA 15.3 in patients with breast cancer. Evidence showed that CA 27.29 may be a more sensitive but less specific marker than CA 15-3, but this has not bee definitively demonstrated and it is generally felt that they are essentially equivalent for most clinical purposes [22]. The low sensitivity and lack of specificity make this assay to be no recommended for screening for breast cancer. It appears to be more useful to detect the disease progression and explore the presence of distant metastasis. CA27.29 appears to be more sensitive and specific than CEA, but it is similar to CA15-3 for metastatic breast cancer detection and monitoring [23].

5. Estrogen Receptor (ER)

ER is one of the successful tumor markers in breast cancer. The ER has a role in cellular growth, proliferation and differentiation [24]. In addition to prognostic value, ER is the most important biologic marker of response to treatment in breast cancer. It is a member of the family of nuclear steroid receptors and functions as a transcriptional regulator, which is controlled by the hormone 17p-estradiol estrogen (E2) [25]. Hormone activated estrogen receptors form dimers, and since the two are coexpressed in many cell types, the receptors may form ERa homodimers or ERp heterodimers. ERa is localized on human chromosome 6, in contrast to ERp, which i chromosome 14 [26].

Measurments of ER levels in breast tumor tissue is useful as a prognostic indicator and in determining the probability of hormonal resistant breast cancer [27]. It was recommended that ER should be measured on every invasive breast cancer and on metastatic lesions if the results would influence the treatment plan. In both pre- and postmenopausal patients, steroid hormone status should be used to identify patients most likely to benefit from endocrine therapy such as tamoxifen, and raloxifene in both the early breast cancer and metastatic disease [5]. Clinically, a positive ER-a status correlates with favorable prognostic features, including a lower rate of cell proliferation and histological evidence of tumor differentiation. ER-a status is also prognostic for the site of gross metastasis [27].

The greater the ER content of the tumor, the higher the response rate to endocreine therapy Women with systemically untreated ER-positive/Progesterone (PR)-positive tumors have better clinical outcomes compared with women with ER-negative/PR-negative tumors, confirming the prognostic significance of the receptor-positive phenotype [28].

The potential role of ER determination in the mangement of Carcinoma In-Situ (CIS), which is a complex group of diseases that diverse outcomes and account for approximately 20% to 30% of breast cancer patients, has attracted a particular. As ER negativity is associated with a worse outcome in patients with CIS, it is not an independent predictor in the context of high nuclear grade and necrosis [29].

False-positive results of ER assays (ER-positive tumors but no response to endocrine therapy) are more common than are false-negative results. The most frequent explanation is heterogeneity of tumor with biopsy of a site that is not representative of the other tumor deposits. In addition to this problem, evidence exists that some tumor cells have receptor defects distal to the initial binding steps (e.g., varient cell are able to bind steriod in the cytoplasm but not trasport the receptor to the nucleus) [30].

6. Progesterone Receptors (PR)

PR is one of the successful tumor markers in breast cancer that effectivel predict the hormonal responsiveness [6]. It is a member of the family of nuclear hormone receptors that specifically binds to progesterone. PR is encoded by single gene PGR presenting on chromosome 1 Iq22. Human PR proteins are of two isoforms, termed PR-A and PR-B, that are transcribed from a certain gene under the control of separate promoters [31].

The PR has an amino and a carboxyl terminal, and between the regulatory domains, a DNA binding domain, the hinge section and and activation function domains (AFs). Detailed molecular dissection has identified two distinct functional domains (AFs) within both isoforms of PRs. AF-1 is located in the N-terminal region and is ligand independent. AF-2, which is ligand dependent, is contained in the ligand-binding domain that is located near the C-terminal region. Furthermore, a unique activation function doma 3, is contained in the upstream segment of PR-B, at the amino acid fraction that is not present in PR-A [32].

The two PR isoforms, PR-A and PR-B, possess different activities, suggesting that in tumors, the ratio of their expression may control hormone responsiveness. PR-B are strong transcriptional activators of some promoters in a variety of cell types in which PR-A have low activity. PR-A, on the other hand, are dominant repressers of PR-B, estrogen receptors (ERs), and other steroid receptors [31]. In breast cancer cells, although some genes are regulated by progesterone through both PR isoforms, most genes are regulated through one or the other isoform, predominantly through PR-B [32].

The mechanisms by which PR regulates hormone-response genes are complex. Progesterone binds PR, inducing a conformational change in PR causing its nuclear translocation, dimerisation and interaction with specific DNA progesterone response elements (PREs) present in the promoter regions of target genes. PR can also mediate its effect independently PREs, through the protein-protein interactions of PR with other specific transcription factors [31].

Protein products from PR target genes are involved in a variety of cellular activities, including transcription, steroid and lipid metabolism, cell growth and apoptosis. Some of these proteins are associated with mammary gland breast cancer development [32]. Clinically, PR are important therapeutic targets. Progestational agents are widely used for oral contraception, menopausal hormone replacement therapy (HRT), and to treat breast cancer and endometrial hyperplasia. Antiprogestins are used for contraception, induction of labor, treatment of meningiomas, endometriosis, and endometrial carcinoma [33].

PR should be measured on every invasive breast cancer and must be measured on metastatic lesions if the results would influence treatment plan. In both pre- and postmenopausal patients, steroid hormone status should be used to identify patients most likely to benefit from endocrine therapy in both early breast cancer and metastatic disease [34]. It was recognized that transcription of the progesterone receptor (PR) gene was regulated by estrogen in breast and reproductive tissues and that estrogen receptor-positive (ER+) breast tumors that lacked PR expression were less responsive to endocrine therapy than those that express PR [31]. During tamoxifen therapy, levels of both PR and ER decrease but PR levels decrease more dramatically than ER levels, with up to half of the tumors completely losing PR expression as they develop tamoxifen resistance. In patients with such tumors, the loss of PR translates into a more aggressive disease and worse overall survival, suggesting that other

alterations in the molecular machinery driving tumor growth accompany the loss of PR receptor expression. Loss of PR in ER+ tumors may be a marker of aberrant growth factor signaling that could contribute to the tamoxifen resistance found in the tumors; i.e., poorer survival in tamoxifen treated women [35].

7. Human Epidermal Growth Factor Receptor (HER)

The activation and overexpression of cellular oncogenes is considered to play an important role in the development of cancer. An important member of the oncogene family is the human epidermal growth factor receptor-2 (HER-2), which referred to as HER-2/neu [8]. HER-2 receptor consists of an extracellular ligand-binding domain (E single transmembrane domain, and an intracellular tyrosine kinase. The extracellular domain undergoes proteolytic cleavage, releasing products into the blood, which can be detected. All are involved in cell proliferation, differentiation and survival. The HER-2/neu gene is localized to a chromosome that encodes a transmembrane tyrosine kinase receptor protein [36].

This family of receptors is involved in cell-cell communication primarily through signal transduction in which external growth factors affect the transcription of genes by phosphorylating or dephosphorylating a series of transmembrane proteins and intracellular signaling intermediates [37]. HER-2/neu gene is normally expressed on the epithelium of numerous organs, including lung, bladder, pancreas, breast, and prostate, and has been found to be elevated in cancer cells [38].

Circulating HER-2/neu receptor protein levels have predicted the presence and progression of HER-2/neu-positive cells. In breast cancer, circulating HER-2/neu receptor protein levels appear to be useful as prognostic indicator of survival as tumor size or ER and PR expression [36]. HER-2/neu is amplified and overexpressed in 15% to 30% diagnosed breast cancer and is associated with more aggressive types of breast cancer [39].

Several potential clinical applications have been proposed for determination of HER/2 status in breast cancer patients, including determination of prognosis in untreated patients, prediction of resistance to endocrine therapy or of selective resistance to tamoxifen, prediction of relative resistance to certain chemotherapies, such as cyclophosphamide, methtrexate, and fluorouracil regimens and prediction of benefit from athracycline and anti-HER/2 therapies such as trastuzuamb [37].

Reports focusing on the response of HER2-overexpressing breast cancers to either hormonal therapy or chemotherapy are conflicting, some studies suggesting that these tumors have a decreased response to tamoxifen and an increased response to anthracycline-containing chemotherapy. However, these results have not been uniformly observed in all studies [39]. Breast cancers without HER2 over-expression usually metastasize to bone, whereas HER2-overexpressing breast cancers usually spread to visceral organs, such as lung, liver and brain [40].

HER2 was reported to affect the genomic actions mediated by ER. HER2 overexpression was associated with an aggressive phenotypes of breast cancers [41]. The ER can be phosphorylated at the Ser 118 or 167 within the AF-1 domain by MAP kinase and Akt, respectively, which are downstream components of the HER2 signaling pathway. This leads to ligand-independent activation of ER. Some studies demonstrated that ER function is augmented by crosstalk between ER and HER2 signaling and tamoxifen resistance is also associated with this crosstalk. Use of a growth factor receptor kinase inhibitor (RKI) in combination with tamoxifen is suggested as the therapeutic method which can prevent or circumvent tamoxifen resistance [42]. Also, some studies have shown that the antitumor activity of tamoxifen can be restored or delayed when a growth factor RKI is combined in either HER2 overexpressing breast cancer cell lines or xenograft models [43]. Inhibition of a variety of key signal transduction mediators of growth factor signaling such as farnesyl transferase, mTOR, or Raf are also investigated as a measure to delay resistance or resensitize the cellular responsiveness to tamoxifen [44].

Increased crosstalk between ER and HER2 together with high expression of coactivator SRC3 is suggested as one of mechanisms by which cells do not respond to tamoxifen by switching tamoxifen bound ER from an antagonistic form to that of an agonist [45]. In this case, tamoxifen-bound ER do not recruit corepressors but rather co-activator such as SRC3 and silencing the SRC3 or inhibiting the activity of HER2 was able to resensitize cells to tamoxifen treatment [46]. Also, Akt can be activated by 4-OHT in breast cancer cells overexpressing HER2 in a HER2-dependent manner, implying conversion of 4-OHT to an agonist. These data clearly indicate the role of growth factor receptor signaling in tamoxifen-stimulated growth and resistance [47].

One of potential mechanisms for HER2-mediated tamoxifen resistance is that overexpression of HER2 and its downstream MAP kinase may contribute to the loss of ER, which is directly attributed to endocrine resistance. It has been shown that ER levels are negatively correlated with those of HER2 [41]. Other data suggest that increased growth factor signaling induced by receptor-specific ligands such as EGF, IGF-1, transforming growth factor (TGF)-β, and heregulin can downregulate ER protein expression and thus lead to a more hormone-independent phenotype. Overactivity of kinases may be associated with acquired loss of ER via its effect on nuclear factor-kappa B (NF-κB) [48]. However, other clinical data suggest that expression of ER can be converted from ER-negative to ER-positive after treatment with a HER2 inhibitor, trastuzumab, implying that endocrine therapy becomes benefi cial in this case [49]. This ability of inhibition of growth factor kinase receptors to restore ER may become an additional therapeutic opportunity to endocrine therapy of breast cancer [50].

8. Urokinase Plasminogen Activator (uPA) & Plasminogen Activator Inhibitor 1 (PAI-1)

Plasminogen activating proteins such as urokinase-type plasminogen activator (uPA), plasminogen activator inhibitor-1 (PAI-1), and uPA receptor (uPAR) represent

reliable tumor markers. High levels of uPA, PAI-1, and uPAR in tumor tissue usually correlate with poor prognosis in many types of human cancers, including breast, endometrial, ovarian, colon, lung, stomach, and renal cancer [51].

uPA is a 53-kDa trypsin-like protease that converts the plasminogen into active plasmin. In vivo, uPA catalytic activity can be inactivated by several inhibitors, including PAI-1, PAI-2, and maspin. PAI-1 was thought to be the primary inhibitor of uPA. In addition to binding to uPA, PAI-1 can also attach itself to the extracellular matrix protein (EMP) allowing PAI-1 to modulate cellular adhesion and migration [52]. uPA was proven to be involved in cancer invasion and metastasis. Antibodies and inhibitors of uPA prevent or reduce metastasis. Prevention of uPA from binding to its receptors decreases the formation of metastases [53]. It was believed that uPA promoted cancer dissemination by degrading the ECM, thus allowing cancer invasion and metastasis. uPA has the ability to stimulate angiogenesis, mitogenesis, and cell migration and to modulate cell adhesion. Moreover, uPA was shown to prevent apoptosis which will increase the survival of malignant cells during the metastatic process, thus increasing the possibility for the establishment of a secondary deposit [54]. PAI-1 is an inhibitor of uPA that is expected to prevent invasion and metastasis. Tumor expression of urokinase-type plasminogen activator (PAI-1), and uPA receptor (uPAR) represent important breast cancer prognostic factors [52].

Because uPA is directly involved in metastasis, it is an ideal candidate for investigation as a prognostic marker. As a marker for breast cancer, the prognostic information reported that uPA is independent of the traditional prognostic factors for this disease, as tumor size, tumor grade, axillary node status, and steroid receptors [53]. High concentrations of PAI-1 predicted an adverse outcome for patients with breast cancer. As with uPA, these early results have confirmed by multiple investigators. Similar to uPA in breast cancer, PAI-1 is also an independent prognostic factor and predicts outcome in node-negative patients [55]. Patients with high uPA and PAI-1 levels benefit from adjuvant chemotherapy than those with low levels. Levels of uPA, PAI-1, and uPAR in breast tumors are now considered by many to be appropriate for the routine assessment of prognosis in patients with newly diagnosed breast cancer [56].

9. P53

P53 (also known as protein 53 or tumor protein 53) is a nuclear protein that plays a crucial role in the regulation of cell cycle and thus functions as a tumor suppressor that is involved in preventing cancer. It has been described as "the guardian of the genome", referring to its role in conserving stability by preventing genome mutation [57]. In humans, p53 is encoded by the TP53 gene located on the short arm of chromosome 17 (17pl3.1). It is a complex, containing 393 amino acids and has seven domains. It is found in very low levels in normal cells. However, in a variety of transformed cell lines, it is expressed in high amounts, and believed to contribute to cellular transformation and malignancy [58].

P53 mutation remains the most common genetic change identified in human tumors. Mutations in the p53 tumor suppressor gene have been detected in a wide variety of human cancers. In breast cancer, p53 mutation is associated with more aggressive disease and worse overall survival. However, the frequency of mutation in p53 is lower in breast cancer than in other solid tumors. In breast cancer, p53 mutations appear to be an early event in the progression of cancer and occur in about 22% of malignant breast tumors [59]. P53 has many anti-tumor mechanisms including activation of DNA repair proteins when DNA has sustained damage, induction of growth arrest by holding the cell cycle on DNA damage recognition, and induction of apoptosis if the DNA damage proves to be irreparable [57].

P53 mutations are common in breast cancer. Testing for p53 alternations may have a prognostic clinical application. Alternations in the gene lead to loss of its negative regulatory function, and hence to more rapid cell proliferation. Also alternations are more often found in more advanced breast cancer suggesting the possibility that p53 alternations occur more often as a late in the transformation process, or are associated with increased metastasis potentials [58]. For these reasons, p53 mutations could be associated with aggressive tumors or those with distant metastasis, thus, may be a prognostic factor in predicting future recurrence [59]. Also, p53 status might be used as a predictor of response to chemotherapy. It is now established that tumor cell death following exposure to chemotherapy or radiotherapy occurs by apoptosis, and is a p53-dependent event. Thus, chemotherapy and radiotherapy induce DNA damage, p53 detects that damage and, unable to repair it, triggers apoptosis. It has therefore been suggested that reduced levels of functional p53 would prevent chemotherapy- or radiotherapy-induced cell death and that detectable levels of mutant p53 should be a marker of resistance to these therapies [60].

10. Cathepsin D

Cathepsin D is defined as lysosomal aspartyl endopeptidase. It breaks down proteins into several polypeptide fragments that digest other lysosomal endopeptidases and exopeptidases. The cathepsin D gene is located at the end of the short arm of chromosome 11. Its expression is regulated by steroid hormones, growth factors, tumor necrosis factor alpha and retinoic acid [61].

Cathepsin D can be found in nearly all cells, tissues and organs, but not in mature lysosome-free erythrocytes. Cathepsin D takes part in digestion of exhausted and denaturated cell proteins or proteins showing abnormal structure and those which entered the cell via endocytosis. It initiates proteolytic degradation of proteins, cleaving it into large fragments, thus they are further digested [62]. It is proven that the major function of cathepsin D is the intracellular catabolism within the lysosomes. Cathepsin D is also involved in the processing of antigens 32, hormones, and neuropeptides. Procathepsin D was also suggested to take part in apoptosis [63].

It is very well documented that procathepsin D is overexpressed and secreted by many cancer-derived cell lines and in many of them the addition of estrogen and

progesterone are increasing the expression and secretion [64]. In estrogen receptor positive (ER +ve) cell lines, procathepsin D is secreted only after estrogen stimulation. In ER +ve cell lines, estrogen interacts with and regulates the expression of procathepsin D at promoter level [65]. It was reported that cathepsin D can serve as an independent prognostic factor in many types of cancers. A strong predictive value was found for cathepsin D concentrations in breast cancer as well as many other tumor types. Using the monoclonal antibodies specific for the pro-form, it has been shown that the procathepsin D level increases in plasma of patients with metastatic breast cancer. Also, cathepsin D overexpression was associated with an increased risk of recurrence and death [62].

11. Cyclin E

Cyclin E, a regulator of the cell cycle, is a 50-kd protein expressed during the late phase of the cell cycle. Disturbances in the activity of cell cycle regulatory proteins play a key role in cancer. Cyclin E forms active complexes with cyclin-dependent kinase-2 (CDK2) and enable progression through the Gl phase of the cell cycle and control entry into the S phase. The activity of the cyclin E-CDK2 enzyme complex is inhibited by the p21 and p27 proteins. In malignant cells, there is imbalance between cyclins, CDKs and CDKs inhibitors, which leads to uncontrolled cell division [66].

Cyclin E overexpression induced differences in gene expression patterns associated with cell adhesion as well as reduced ability to migrate and invade in functional assays. Cyclin E overexpression has been observed in breast, gastrointestinal and hematological malignancies, lung cancer, genitourinary tract cancers, sarcomas and skin cancers. Cyclin E is present at high levels or is abnormally stable in about 25% of breast tumors as compared to normal human breast cells [67].

In breast cancers, cyclin E is cleaved to lower molecular weight (LMW) fragments by elastase and by calpain 2. These LMW fragments have greater affinity for CDK2 and resist inhibition by p21 and p27. In addition, the LMW fragments confer resistance to tamoxifen and increase genomic instability [68]. Elevated levels of cyclin E protein have been consistently associated with poor prognosis in breast cancer. Also, overexpression of cyclin E was associated with an increased risk of recurrence of breast cancer [67].

12. Nestin

Researchers have identified a cellular protein called nestin that could help in diagnosis and manage aggressive forms of breast cancer [69]. Nestin is an intermediate filament protein that exists in adult stem cells in the central nervous system and other tissues. Nestin has the shortest head domain (N-terminus) and the longest tail domain (C-terminus) of all the intermediate filament proteins and has a high molecular weight. It was thought to have a role in stabilizing the structure of adult stem cells as they regenerate and divide into daughter cells [70].

Nestin has been considered a marker of neural progenitors, and now it is identified in the mammary gland as well, in the basal and myoepithelial layer. Also, nestin is a potential biomarker for basal epithelial breast tumor [71]. Normal basal epithelial tissue produces nestin, but basal epithelial tumors produce a large amount of nestin, which represents an abnormal expansion of the basal epithelium. It is considered as an excellent diagnostic tool for a cancer of regenerative mammary cells [72].

It was reported that the structural protein nestin might help to diagnose and treat basal epithelial breast cancer. This aggressive and deadly form of disease can be elusive because it cannot be identified by estrogen or progesterone receptors and HER2 and, as a result, generally cannot be treated with key therapies designed to target these pathways, as they lack almost all important diagnostic markers [71]. Nestin was exclusively expressed in aggressive breast carcinoma. Nestin-positive tumors displayed high proliferation rates and p53 nuclear expression. Lymph-node positive patients with nestin-positive cancers had a shorter breast cancer survival [73].

Table 1. Common tumor markers of breast cancer

Tumor marker	Required sample for identification	Tumors associated with elevated levels	Non-cancerous reasons for elevated levels
Carcinoembryonic antigen (CEA)	Blood	Colorectal cancers, breast, lung, gastric, pancreatic, bladder, kidney, thyroid, head & neck, cervical, ovarian, liver, lymphoma, melanoma	Cigarette smoking, pancreatitis, hepatitis, inflammatory bowel disease, peptic ulcer disease, hypothyroidism, cirrhosis, COPD, biliary obstruction
CA 15-3	Blood	Breast (often not elevated in early stages of breast cancer), lung, ovarian, endometrial, bladder, gastrointestinal	Liver disease (cirrhosis, hepatitis), lupus, sarcoid, tuberculosis, non-cancerous breast lesions
CA 27.29	Blood	Breast (best used to detect recurrence or metastasis). Colon, gastric, liver, lung, pancreatic, ovarian, prostate cancers	Ovarian cysts, liver and kidney disorders, non-cancerous (benign) breast problems
Estrogen receptor (ER)	Tissue	Breast cancer	Ovarian cysts, benign breast lesions
Progesterone receptor (PR)	Tissue	Breast cancer	Ovarian cysts, benign breast lesions
Human epidermal growth factor receptor 2 (HER2)	Tissue	Breast, gastric, esophageal cancer	Benign breast lesions, benign prostatic hyperplasia
Urokinase plasminogen activator (uPA)	Blood	Gastric cancer, colon cancer, breast cancer and its bone metastases	Fibroadenoma of the breast, benign prostatic hyperplasia
Plasminogen activator inhibitor 1 (PAI-1)	Blood	Gastric, colon, breast cancer and its bone metastases	Fibroadenoma of the breast

13. Conclusion

CA 15-3, CA 27.29, carcinoembryonic antigen, estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, urokinase plasminogen activator, plasminogen activator inhibitor 1, and certain multiparameter gene expression assays may be used as potential markers for breast cancer. Also, the expression of p53, cathepsin D, cyclin E and nestin may be affected in cases of breast cancer. Taken together with the results of the clinical examination and radiological investigations, these markers may help in early detection, staging, monitoring response to therapy and diagnosis of recurrence or metastasis of breast cancer.

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