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Tumor markers of breast cancer: New prospectives

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ABSTRACT

Tumor markers are substances produced by the tumors or by other cells of the body in response to cancer or certain benign conditions. Although most of these markers are made by the normal cells as well as by cancer cells, they are produced at much higher levels in cancerous conditions. These markers are used to evaluate the patient's response to treatment and to detect the presence of metastasis or recurrence. Breast cancer is one of the most common malignancies in females worldwide. The CA 27-29, CA 15-3, CA27.29, carcinoembryonic antigen, tissue polypeptide specific antigen, p53, cathepsin D, cyclin E, nestin and HER-2 are tumor markers that are often expressed in people with breast cancer. They play a crucial role in diagnosis, monitoring response to therapy, early detection of metastasis and determination of recurrence in patients with breast cancer.

1. Introduction

A tumor marker is a biomarker that is found in blood, urine or body tissues that can be elevated by the presence of one or more types of cancer. It is produced either by the tumor itself or by the host in the response to a tumor.[1] The ideal tumor marker should be both specific and sensitive to detect small tumors to allow early diagnosis or help in screening. Few markers are specific for a single tumor. Most markers are produced by different tumors of the same tissue type. They are present in higher quantities in cancer tissue or in blood from cancer patients more than in the blood of normal subjects. Tumor markers are mostly useful in evaluating the progression of the disease status after initial chemotherapy and radiotherapy to monitor subsequent treatment strategies.[2]

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.[3] 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.[4]

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.[5] In recent decades, the serum concentration of tumor markers has been used to detect tumor activity. Tumor markers provide a minimally invasive cost-effective source of data 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.[6] The real usefulness of tumor markers in the management of breast cancer has been questioned because of the low diagnostic sensitivity for early disease.[7]

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, Carcinoembryonic 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.[8] 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.[7]

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2. Carcinoembrvonic antigen (CEA)

Carcinoembryonic antigen (CEA), which belongs to a family of related cell surface glycoproteins, is the most widely used tumor marker in the clinical practice. It is a tumor marker for colorectal, gastrointestinal, lung and breast cancer.[9] 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.[10]

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.[11] 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 crucial role in these processes.[12]

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 of cancer recurrence after treatment, the lead time from CEA elevation to clinical recurrence is about 5 months.[13] Also, patients with advanced cancer or metastatic cancer may have higher CEA levels rather than in patients with localized diseases.[9] 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.[14]

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. For breast cancer, CEA is being replaced by other more specific markers, such as CA 15-3.[15] Sławicki et al.[16] reported that CEA alone is non specific for diagnosis of breast cancer. Geng et al.[17] suggested that there should be an association between CEA, CA 15-3 and the clinicopathological parameters for proper diagnosis in patients with metastatic breast cancer.

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.[9] 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.[18] 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.[19]

The MUC1 gene is overexpressed in malignant breast tumors, allowing use of gene product CA 15-3 as tumor marker for breast cancer.[18] CA 15-3 concentrations in blood can be used for screening, not only for 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).[20] 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 (stage) of the disease and/or size of the tumor.[9] Lumachi et al.[21] suggested that CEA and CA 15-3 should be considered complementary in detecting recurrence of breast cancer but their sensitivity is low and independent of the majority of the prognostic parameters that may be considered before relapse. Darlix et al.[22] reported that serum CA 15-3 level is independent prognostic factor in metastatic breast cancer patients.

4. CA 27.29

CA27.29 is a carbohydrate-containing protein antigen that serves as a tumor marker for breast cancer. It is also called breast carcinoma-associated antigen.[23] It 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 patients with ovarian cysts. Therefore, elevation of this marker is not organ specific.[24]

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.[25] The low sensitivity and lack of specificity preclude the use of this assay for screening for breast cancer. It appears to be more useful in detecting the disease progression and metastatic involvement. CA27.29 appears to be more sensitive and specific than CEA, but it performs similarly as compared to CA 153 for earlier detection of metastatic disease during follow-up screening.[26] Gion et al.[27] reported that CA27.29 provides comparable results to CA15.3. They found that CA27.29 seems to be more sensitive than CA15.3 to limited variations of tumor extension. However, it cannot help clinicians in distinguishing stage I patients from stage II patients. Rack et al.[28] indicated that there is a close relationship between CA27.29 levels and tumor mass. They attributed the increased values after completion of chemotherapy to treatment effects and suggested that these values should be considered with caution.

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.[29] 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).[30] 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.[31]

Measurement of ER levels in breast tumor tissue is useful as a prognostic indicator and in determining the probability of hormonal resistant breast cancer.[32] It was recommended that ER should be measured in every invasive breast cancer as well as in 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 setting and metastatic disease.[4] 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.[32] Han et al.[33] found that ER-a status predicts late-onset skeletal metastasis in breast cancer patients. However, Beije et al.[34] reported that discordances regarding ER status between circulating tumor cells and the primary tumor occurred frequently but had no prognostic impact in metastatic breast cancer patients.

The greater the ER content of the tumor, the higher the response rate to endocrine 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.[35]

The potential role of ER determination in the management of Carcinoma In-Situ (CIS), which is a complex group of diseases that diverse outcomes and account for approximately 20%–30% of breast cancer patients, has attracted a particular attention. 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.[36]

False-positive results of ER assays (ER-positive tumors but no response to endocrine therapy) are more common than false-negative results. The most frequent explanation is heterogenicity of tumor with biopsy of a site that is not representative of the other tumor deposits. In addition to this problem, there exists an evidence that some tumor cells have functional receptor defects distal to the initial binding steps (e.g., variant cells are able to bind steroids in the cytoplasm but will not transport the receptor to the nucleus).[37]

6. Progesterone receptors (PR)

PR is one of the successful tumor markers in breast cancer that effectively predict the hormonal responsiveness.[5] 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.[38]

The PR has an amino and a carboxyl terminal, and between the regulatory domains, a DNA binding domain, the hinge section 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.[39]

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.[38] 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.[39]

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 of PREs, through the protein-protein interactions of PR with other specific transcription factors.[38]

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.[39] 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.[40]

PR should be analyzed in every invasive breast cancer as well as 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.[41] 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 high levels of PR.[38] 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 leading to a poorer survival in women treated with tamoxifen.[42]

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.[7] 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 are detectable. All domains of the Her-2 receptor 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.[43]

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 phosphorylation or dephosphorylation of a series of transmembrane proteins and intracellular signaling intermediates.[44] HER-2/neu gene is normally expressed on the epithelial cells of numerous organs, including lung, bladder, pancreas, breast, and prostate, and has been found to be overexpressed in cancer cells.[45]

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.[43] Cao et al.[46] found that high level of expression of HER-2/neu receptor protein was associated with significantly decreased survival rate in patients with breast cancer. Reix et al.[47] reported that HER-2/neu receptor protein appears to be a helpful surveillance biomarker for early diagnosis of relapses and to predict the fate of metastases of breast cancer. HER-2/neu is amplified and overexpressed in 15%–30% diagnosed breast cancer and is associated with a more aggressive biologic behavior.[48]

Several potential clinical applications have been proposed for the use of HER/2 status in breast cancer patients, including prognostic estimation in untreated patients, prediction of resistance to endocrine therapy or of selective resistance to tamoxifen, prediction of relative resistance to certain cytotoxic agents, such as cyclophosphamide, methtrexate, and fluorouracil regimens and prediction of benefit from athracycline and anti-HER/2 therapies such as the use of trastuzuamb.[44]

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.[48] 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.[49]

8. Urokinase plasminogen activator (uPA) and 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.[50]

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.[51] 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.[52] 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.[53] 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.[51]

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.[52] 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.[54] 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.[55]

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.[56] 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.[57]

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.[58] 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.[56]

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 an increased metastatic potential.[57] 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.[58] 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 through p-53 dependent apoptosis. 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.[59]

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.[60]

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.[61] 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.[62]

It is 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 stimulates the expression and secretion.[63] 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.[64] 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.[61]

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.[65]

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.[66]

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.[67] 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.[66]

12. Nestin

Researchers have identified a cellular protein called nestin that could help in diagnosis and manage aggressive forms of breast cancer.[68] 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.[69]

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.[70] 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.[71]

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.[70] 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.[72]

13. Human epididymis protein 4

Human epididymal protein 4 (HE4) is a secretory protein initially identified in epithelial cells of the human epididymis.[73] Expression of HE4 has been demonstrated in many types of normal human tissues. Increased HE4 expression has been demonstrated in a range of malignant tumors, particularly those of gynecological, pulmonary and gastrointestinal origin.[74] Galgano et al.[75] reported that HE4 is also expressed in ductal carcinoma of the breast. However, the serum expression levels and their diagnostic and prognostic value in breast cancer remain to be elucidated. Gündüz et al.[73] tried to determine the diagnostic value of serum HE4 for breast cancer. They found that a significant elevation of serum HE4 levels in patients with breast cancer compared with that in the healthy controls was identified. They suggested that HE4 may serve as a novel biomarker for diagnosis of breast cancer.

14. Conclusion

CEA and MUC-1 antigen are the most useful serum tumor markers in patients with breast cancer. Serial determination of these markers may be beneficial in monitoring the response to therapy and for early detection of recurrence or metastasis. The main disadvantages of these markers are lack of sensitivity for low-volume disease and lack of specificity. So, they are of no value in either screening or diagnosing early breast cancer. Steroid receptors and HER-2 are tissue-based markers accepted in clinical practice, having the ability to predict the response of the tumor to hormonal therapy. PAI-1 and uPA are recently validated as prognostic factors for lymph node-negative breast cancer patients and may be used for selecting those patients who may not need to receive adjuvant chemotherapy. Other markers for breast cancer such as HE4, p53, cathepsin D, cyclin E and nestin look promising, but further studies are needed before their clinical utility is well established.

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