Khazar Journal of Science and Technology Volume 5 №1 2021, 29-46
© Khazar University Press 2021 DOI: 10.5782/2520-6133.2021.5.1.29

Approaches to Breast Cancer Diagnosis

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Abstract

Breast cancer is the most common cancer in women, accounting for about one-third of cancer cases in women and approximately 14% of cancer-related death in females in the worldwide. It is of great importance to detect breast cancer in the early stage and avoiding overtreatment in patients who only receive a modest benefit, while suffering from toxic side effects, so aiming early detection, diagnostic strategies are important. Biomarkers that are found in blood, urine or body tissues are mostly useful in evaluating the progression of the disease status after initial chemotherapy and radiotherapy to monitor subsequent treatment strategies. In this review, we discuss the importance of established prognostic factors and predictive biomarkers as well as some emerging biomarkers that are currently undergoing testing for technical validity and clinical utility.

Keywords: Breast cancer, Biomarkers, diagnosis, prognosis,

Introduction

Breast cancer is the most common cancer in women, accounting for about one-third of cancer cases in women and approximately 14% of cancer-related death in females in the worldwide (Duffy et al., 2015). Breast cancer includes a heterogeneous group of tumors with a wide spectrum of morphologically and molecularly different subtypes which display different risk factors, clinical and histopathological features and response to systemic treatments (Dai et al., 2016, Duffy et al., 2015). Therefore, It is of great importance to detect breast cancer in the early stage and avoiding overtreatment in patients who only receive a modest benefit, while suffering from toxic side effects (Dai et al., 2016, Weigel and Dowsett, 2010).

Biomarkers that are found in blood, urine or body tissues are mostly useful in evaluating the progression of the disease status after initial chemotherapy and radiotherapy to monitor subsequent treatment strategies (Kabel, 2017). Based on the clinical role of biomarkers, they are including prognostic, predictive and

pharmacodynamics biomarkers. Gene expression signatures that are related to the risk for recurrence and tumor stage can be considered prognostic biomarkers in breast cancer. While prognostic markers do not predict weather a particular therapy will be successful or not predictive biomarkers including, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) associated with the optimal therapies for patient care. Pharmacodynamics biomarkers measure the proximal effect of a drug on its target. The demonstration of declined phosphorylation of a protein substrate immediately downstream from a target kinase is example of proximal pharmacodynamics effect. Peripheral blood mononuclear cells and skin that are easy-access tissues have used for measurement of pharmacodynamics biomarkers.

Therefore, biomarker analysis in breast cancer not only provides additional information about classical clinical factors, but also enables patients with a more favorable benefit-risk balance to receive certain treatments (Colomer et al., 2018, Ern Ang et al., 2012, Ulaner et al., 2016). In this review, we discuss the importance of established prognostic factors and predictive biomarkers as well as some emerging biomarkers that are currently undergoing testing for technical validity and clinical utility.

1- Biomarkers and Breast cancer

The term “biomarker” refers to a broad subcategory of medical signs – that is, objective indications of medical state observed from outside the patient – which can be measured accurately and reproducibly. The WHO has defined that biomarkers include “almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction. Examples of biomarkers include everything from pulse and blood pressure through basic chemistries to more complex laboratory tests of blood and other tissues (Organization, 1993, Strimbu and Tavel, 2010). Biomarkers are often protein markers, such as prostate-specific antigen for the detection of prostate cancer, and genomic markers, such as epidermal growth factor receptor (EGFR) kinase mutations in non–small cell lung cancer, which predict response to EGFR kinase inhibitors (Ulaner et al., 2016). Biomarkers currently play an indispensable role in the management of patients with breast cancer, especially in deciding the type of systemic therapy to be administered (Duffy et al., 2017). Among the molecular markers associated with breast cancer, the estrogen receptor (ER), the progesterone receptor (PR), the human epidermal growth factor receptor (HER2) and

the Mib1/Ki-67 proliferation index are the most important ones and are firmly established in the standard care of all primary, recurrent, and metastatic breast cancer patients (Beenken and Bland, 2002).

2-1 Imaging and Emission-Based Systems

2-1-1 PET scan

A positron emission tomography (PET) scan is an imaging test that uses a radioactive substance (called a tracer) to look for potential spread of breast cancer. This tracer can help identify areas of cancer that an MRI or CT scan may not show. PET has the ability to demonstrate abnormal metabolic activity, and 18F-2-deoxy-D-glucose (FDG) PET provides important tumor-related qualitative and quantitative metabolic information that may be critical for the diagnosis and follow-up (Kubota et al., 1989, Minn and Soini, 1989, Wahl et al., 1991). The ability of PET to detect breast cancer depends on the tumor's size and histology. The sensitivity of PET has been reported to be 68% for small (< 2 cm) tumors and 92% for larger (2 5 cm) tumors (Avril et al., 2000), and its reported overall accuracy for detecting in situ carcinomas is low (sensitivity: 2 25%). The major limitation of PET or PET/CT for breast imaging is its poor detection rate for small breast carcinomas and non-invasive breast cancers (Noh et al., 1999, Schirrmeister et al., 2001).

2-1-2 Computed Tomography (CT scan)

Computed tomography (CT) imaging based on the variable absorption of x rays by different tissues, also known as "CAT scanning" (Computerized Axial Tomography), provides a different form of imaging known as cross-sectional imaging, this types of images (Figure 2) are used for a variety of diagnostic and therapeutic purposes(Administration, 2020). CT imaging is reliable for Breast cancer diagnosis because it can disclose every suspected and unsuspected cancer nodules(Gindi et al., 2014). However, variance of intensity in CT scan images and anatomical structure misjudgment by doctors and radiologists might cause difficulty in marking the cancerous cell (Suzuki et al., 2006). Imaging modalities like CT rely on detecting anatomic changes for the diagnosis, staging and follow-up of cancer patients (Yang et al., 2007).

2-1-3 Magnetic Resonance Imaging (MRI)

The basis of magnetic resonance (MR) techniques is the measurement of radiofrequency radiation resulting from transitions induced between nuclear spin

states of tissue hydrogen atoms (protons) in the presence of a strong external magnetic field(Khoo et al., 1997).

MRI is used widely both for screening women who are at increased risk of breast cancer and for treatment selection. MRI may prove useful in screening younger women with dense breasts who are at a special high risk of developing breast cancer (Morrow et al., 2011). MRI has a higher sensitivity for the detection of breast cancer and is not affected by breast density (Sardanelli et al., 2004). Regarding all mentioned above MRI precision also relies on detecting anatomic changes for the diagnosis, staging and follow-up of cancer patients (Yang et al., 2007).

2-1-4 Mammography

Screening with mammography has the ability to detect breast cancer at an early stage. Subsequent effective diagnostic pathways and treatment regimens can reduce the burden of disease of breast cancer, most importantly mortality in women aged 50 to 70 years (US, 2009). Randomized trials have shown that mammographic screening of all women who are between 50 and 70 years of age can reduce mortality from breast cancer by about 25 percent (Kriege et al., 2004). In women with breast cancer, disease burden is the main determinant of the selection of local therapy, and women selected for breast conserving surgery with mammography successfully complete the procedure in more than 85% of cases. (Morrow et al., 2011). Although the benefits of mammography are proven, not all cancers can be visualized on screening mammograms. The sensitivity of mammography is related to the age, ethnicity, personal history, and technique quality. The sensitivity of mammography is decreased in women with dense breast tissue, and some women who seem to have localized cancer mammographically are found to have extensive disease necessitating mastectomy (Wang, 2017).

2-2 Elisa-based Markers

2-2-1 CA 15-3

Along with the traditional pathological factors and molecular markers, serum tumor markers have an important role in monitoring therapy, early diagnosis of recurrence, determining prognosis, and treatment of many malignancies. The most widely used serum markers in breast cancer are carcinoembryonic antigen (CEA) and cancer antigen 15–3 (CA15-3) (Shao et al., 2015).

CA 15-3 peptides are shed or soluble forms of MUC- 1, which exists as a transmembrane protein consisting of two subunits that form a stable dimer (Kabel,

2017). CA 15-3 in combination with CEA is the most widely used serum marker in patients with breast cancer (Cristofanilli et al., 2005). Accordingly, the European Group on Tumor Markers has mentioned the CEA and CA15-3 levels can be used for assessing prognosis, the early detection of disease progression, and treatment monitoring in breast cancer (Molina et al., 2005). Using CA 15-3 has its disadvantages, CA 15-3 levels may also be increased in several benign and malignant conditions. This results in low sensitivity, specificity, and positive predictive values, making it difficult to reliably screen, diagnose, or stage breast cancers. CA 15-3 is only elevated in 10% of patients with early-stage breast cancer, and levels of CA 15-3 can also be elevated due to lung and ovarian cancers. Furthermore, the polymorphic, glycoprotein structure of MUC1, detected by CA 15-3 and CA 27.29, presents similar assay problems to those described for other mucins such as CA 19-9(Wild, 2013).

2-2-2 CA 27.29

Cancer antigen (CA) 27.29 is a monoclonal antibody to a glycoprotein (MUC1) that is present on the apical surface of normal epithelial cells (Gion et al., 1999). The molecule exists in a normal, highly glycosylated form, and a cancer-associated, relatively underglycosylated form (Beveridge, 1999). CA 27.29 is highly associated with breast cancer. However, this mucin is not specific to breast cancer and is considered a pan-epithelial marker. It is also expressed on other adenocarcinomas, including lung, colon, pancreas, and ovary (Hayes et al., 1985). The CA 27.29 level is elevated in approximately one third of women with stage I or II breast cancer and in two thirds of women with late-stage disease (Perkins et al., 2003). Dou to the current tumor marker guidelines of the American Society of Clinical Oncology, CA 15-3 and CA 27.29 are not recommended as prognostic markers for routine clinical use because there are no trials available demonstrating a clear benefit regarding improved survival or diminished toxicity resulting from a timely detection of recurrence and early treatment initiation (Rack et al., 2010). Despite these drawbacks, testing for the existence of tumor markers is widely used in disease surveillance and treatment monitoring in daily practice. As non-invasive, reproducible and easily accessible tests are available at any point in time during disease progression for CA 27.29 markers they are a highly suitable measure by which to select patients at risk of recurrence, both at primary diagnosis and during follow-up, and to monitor treatment efficacy (Laessig et al., 2007, Rack et al., 2010).

CA 27.29 has some drawback like CA 15.3, CA 27.29 is not elevated in all patients with breast cancer and also it could be elevated in some noncancerous and cancerous conditions other than breast cancer(Wild, 2013).

2-2-3 CEA

Carcinoembryonic antigen (CEA), an oncofetal glycoprotein and type of cell adhesion molecule, is expressed in normal mucosal cells and overexpressed confirmed (Perkins et al., 2003). CEA was one of the first tumor markers to be studied and characterized as prognostic factors in breast cancer for more than 30 years and the most common tumor markers used in breast cancer. Several studies have showed that an increase or a decrease in the CEA levels may reflect the status of disease progression or regression and correlate with the stage of disease (Guadagni et al., 2001). CEA levels in the blood are usually increased once the cancer has metastasized. However, CEA levels typically return to normal within four to six weeks after successful surgical resection (Wu et al., 2014). Recently, the prognostic value of preoperative CEA andCA15-3 levels in breast cancer has gained much attention. Plasma CEA levels combined with CA15-3 levels may provide useful information for diagnosis and treatment of breast cancer (Ebeling et al., 2002, Park et al., 2007).

2-3 Tumor-based Markers

2-3-1 Estrogen receptor (ER)

Estrogen receptors (ERs) belong to the subfamily of ligand-regulated transcription factors which transduce hormones signaling into a large variety of physiological responses in various organs. This steroid hormone receptor is one of the successful tumor markers in breast cancer which was first identified in the late 1960s. ER found on nearly 70% of primary breast cancers and plays an important role in tumor progression. ER exists in two main forms, ERα and ERβ. Whereas the original ligand-binding ER assays are likely to have detected both of ER forms, the current immunohistochemistry (IHC) measurements detect only ERα (Duffy et al., 2017, Kabel, 2017). However, for both ERα-positive breast cancer and ERα-negative one, ERβ can be observed, which plays a key role in breast cancer classification and endocrine therapy (Osborne, 1998). ERα is responsible for estrogen-induced mitogenic signaling in epithelial cells in breast, uterine, and ovarian tissues and is prevalently expressed by breast cancer cells, whereas ERβ is usually associated with less aggressive tumors, as it inhibits both ERα-mediated transcription and estradiol-induced proliferation in various types of cancer cells. The ERα/ERβ ratio may play a critical role in the regulation of estradiol activity in breast cancer cells (Matthews and Gustafsson, 2003, Paruthiyil et al., 2004). Five lysines on ERα are reportedly acetylated by p300: Lys266, Lys268, Lys299, Lys302 and Lys303, all localized in the hinge region. The effects of ERα acetylation result from a two-step mechanism:

short exposure of cells to HDAC inhibitor (HDACi) leads to acetylation and stabilization of the receptor, whereas after long exposures, the receptor is delocalized and subsequently degraded by the proteasome (Paruthiyil et al., 2004). While the absence or presence of the ER is used to obtain treatment decisions, little attention has been paid on the value of the quantitative expression levels as a predictive indicator. The Early Breast Cancer Trialists' Collaborative Group reported that higher levels of ER were associated with a lower risk of recurrence when receiving adjuvant tamoxifen (Weigel and Dowsett, 2010). Tamoxifen is a selective ER modulator (SERM) and the most frequently used anti-estrogen adjuvant treatment for ER+ pre-menopausal women Tamoxifen exhibits antagonistic effects in breast tissue, thus has preventive effects on breast cancer development and cytotoxic effects on breast cancer cells (Egeland et al., 2015). The ER has a role in cellular growth, proliferation and differentiation. When ER interacts with estrogen, they will regulate transcription of specific genes, such as PR, TFF1, GREB1 and PDZK (Ern Ang et al., 2012). In addition to prognostic value, ER is the most important biologic marker of response to treatment in breast cancer. Clinically, ER status is a critical index of sensitivity to endocrine therapies because ER-positive breast cancers use estradiol as a main growth stimulus. While endocrine therapy with 5-year tamoxifan as an adjuvant decline the ER-positive breast cancer death rate, ER-negative disease illustrates no significant benefit from this treatment except in the unusual type of tumor; ER-negative but PR-positive tumors (Group, 2005). Multiple clinical studies have demonstrated that the ER-negative breast cancer patients are more likely to achieve a pathological complete response (pCR) with neoadjuvant chemotherapy than the ER-positive patients, with pCR rates of 7–8 vs 21–33% respectively (Colleoni et al., 2004).

2-3-2 Progesterone Receptor (PR)

The progesterone receptor (PR) is a well-known estrogen receptor (ER)-regulated gene that is expressed in over two-thirds of ER-positive breast cancers. Like ER, PR protein exists as two receptor isoforms namely A and, but these forms are the products of the same gene. These isoforms of PR (A is a slightly truncated form of B) bind with one another to create homo- and heterodimers (Hammond et al., 2010). The potential of PR expression as a prognostic biomarker has been appreciated since 1975 when it was first suggested that PR expression could predict outcome and response to ER-directed therapy in advanced disease. PR is more highly expressed in the luminal A breast cancer subtype, and is associated with tumor grade, ER expression as well as negative HER2 status in early breast cancer (Lim et al., 2016, Purdie et al., 2014). In positive ER breast cancers, PR is often used as a positive prognostic marker of disease outcome. There is increasing evidence that substantial crosstalk occurs between ER and PR signaling pathways. Noticeably, when PR is

activated by its native ligand in the presence of estrogen, it interacts with ER in breast cancer cells to redirect ER chromatin binding, signifying the critical role PR plays in modulating ER action (Mohammed et al., 2015). Additionally, high levels of PR associated with decreased metastatic events in early stage disease and administration of a progesterone injection prior to surgery can provide improved clinical benefit (Bardou et al., 2003, PichÃ³n et al., 1980).

2-3-3 HER2

Human epidermal growth factor receptor 2 (HER2) is a transmembrane member of the tyrosine kinase epidermal growth factor receptors, which are normally expressed at low levels in all epithelial cells in normal fetal and adult tissues, but are also essential for cancer proliferation and survival. HER2 gene amplification has been associated with increased levels of expression of HER2 mRNA and protein product, which lead to oncogenic signaling and resultant self-sufficiency in growth signals, uncontrolled proliferation, sustained angiogenesis, enhanced invasion, and metastasis processes, which are drivers of tumor development and progression in a subset of breast cancer (Beenken and Bland, 2002, Schwarzenbach et al., 2012). HER2 enriched breast cancer reports between 20% and 30% of all breast cancer. It is characterized by over expression of HER2/*neu* proliferation genes with low expression of luminal clusters including CK7, CK8, CK18, CK19 and other luminal-associated markers like X-box-binding protein 1, hepatocyte nuclear factor 3, GATA-binding protein 3 and estrogen receptor 1, among other (Kittaneh et al., 2013). Several potential clinical applications have been suggested for determination of HER2 status in breast cancer patients such as determination of prognosis in untreated patients, prediction of resistance to endocrine therapy or of selective resistance to tamoxifen but not aromatase inhibitors, prediction of relative resistance to certain chemotherapies like cyclophosphamide, methotrexate, and fluorouracil (CMF) –like regimens and prediction of benefit from anti-HER2 therapies, in particular trastuzumab (Harris et al., 2007).

Trastuzumab, the most well-known humanized monoclonal antibody against HER2, dramatically improves response rates, time to progression and survival when used both alone and/or with chemotherapy in both early stage and metastatic breast cancer. Other HER2-targeted drugs, including lapatinib as a tyrosine kinase inhibitor, the antibody pertuzumab, and the antibody drug conjugate adotrastuzumab emtansine (T-DM1), improve outcomes in HER2-positive metastatic breast cancer (Geyer et al., 2006, Slamon et al., 2011).

HER2 gene amplification is directly associated with its mRNA expression and protein levels, therefore, overexpression of the HER2 protein product may be

evaluated by Western blotting, ELISA or IHC; overexpression of its mRNA by Northern blotting or RT-PCR, and its gene amplification by fluorescence (FISH), chromogenic (CISH) or silver-enhanced in situ hybridization (SISH) (Hammond et al., 2010). Among of all, IHC has been more widely used as the primary test for HER2 status due to its results quicker, permits parallel viewing of tumor morphological features, and stained tissues do not degrade over time (Penault-Llorca et al., 2009).

2-3-4 Ki-67

Ki67 is associated with biologic breast cancer markers which may have a role in clinical practice as prognostic, predictive factors and possible targets for future therapies (Wiesner et al., 2009). The Ki-67 antigen, a non-histone protein was originally identified by Gerdes and colleagues in the early 1980s, by use of a mouse monoclonal antibody against a nuclear antigen from a Hodgkin's lymphoma-derived cell line (Yerushalmi et al., 2010). The Ki-67 antigen can be identified by immunostaining with a monoclonal antibody in all phases of cell proliferation. Non-existent in the resting (G0) phase, it appears within the nucleus in the S, G1 and G2 phases (Mannell, 2016). Ki-67 score is the most often measured on histological sections by IHC methodology and is defined as the percentage of stained invasive carcinoma cells (Kabel, 2017). It is really characteristic that Ki67 is expressed exclusively in estrogen receptor (ER)-negative cells, which means that ER-positive cells do not proliferate under normal circumstances. This separation does not exist in malignant tissues (Kontzoglou et al., 2013).

Many studies have shown that Ki67 can be used as a prognostic marker for breast cancer.

The study by Cheang and colleagues describes an immunopanel of ER, PgR, HER2, and Ki67 that can segregate the luminal A and B subtypes in a similar manner to that defined by a 50-gene expression profile. Luminal breast cancers with Ki67 levels of at least 14% were assigned to the luminal B category and had a worse prognosis for both breast cancer recurrence and death compared with luminal A subtype with Ki67 levels of less than 14% (Cheang et al., 2009, Yerushalmi et al., 2010).

Apart from the contribution of Ki-67 to prognosis, the Ki-67 index is used on a daily basis in the selection of therapy. Dividing cells have increased sensitivity to cytotoxic drugs, and a high Ki-67 is associated with a good response to neoadjuvant chemotherapy (NAC) (Fasching et al., 2011, Mannell, 2016). The prognostic and predictive value of Ki-67 was evaluated by Luporsi et al. and they concluded that this biomarker could be considered as a prognostic factor for therapeutic decision;

however, standardization of techniques and scoring methods are needed for integration of this biomarker in everyday practice (Luporsi et al., 2012).

2-4 Molecular markers

2-4-1 MicroRNAs

MicroRNAs (miRNAs) are a 21–25 long class of small non-coding RNA, which are capable of altering gene expression post-transcriptionally by inhibiting translation of their target mRNAs. miRNAs have been demonstrated to be involved in cell development, differentiation, proliferation and apoptosis (McGuire et al., 2015). miRNA can exert their action in cancers through both tumour suppression and oncomirs (by oncogenic mechanisms). The first human, disease-related miRNA characterized was from chronic lymphocytic leukaemia and subsequently, circulating miRNA were identified in patients with diffuse large B-cell lymphoma (Shi et al., 2010, Wang and Wang, 2012). The potential of miRNA as biomarker targets is facilitated by their stability in blood as well as formalin-fixed paraffin-embedded tissues and their ability to withstand repeated freezing and thawing cycles (Mitchell et al., 2008). Significantly, as miRNAs have been implicated in cancer metastasis, miRNA signatures are being pursued as novel clinical diagnostic targets to allow further subtyping of breast cancer and for predicting metastasis or therapeutic resistance (Shi et al., 2010). Studies have showed that miRNAs link to all stage along the metastatic cascade in breast cancer (Jang et al., 2014). The first miRNA shown to be highly expressed in metastatic breast cancer was miR-10b, with a clinical correlation in primary breast carcinomas. Surprisingly, a noticeable increase in circulating miR-10b and miR-373 was illustrated in lymph node positive patients, in comparison to patients with no nodal involvement or healthy controls. Admittedly, miR-21 has also been identified as a marker for breast cancer and predictor of stage (Asaga et al., 2011, Ma et al., 2007, Schwarzenbach et al., 2012). MiR-155, the most widely studied circulating miRNA in breast cancer, has been associated with ER/PR/HER2 expression. It is upregulated in the serum of breast cancer patients compared to healthy controls. The expression levels of miR-155 decreases significantly in metastatic breast cancers compared to primary cancer and negative control. As a result of these findings, it has been classed as a stable biomarker for breast cancer, confirmed by a meta-analysis of circulating miRNAs in breast cancer (Mathe et al., 2015, Roth et al., 2010, Wang et al., 2010). Recent studies have investigated that microRNAs namely, miR-210, miR-328, miR-484 and miR-874 have the potential to predict prognosis or risk of breast cancer recurrence (Volinia and Croce, 2013). Additionally, many findings have sowed that miRNAs

play a key role in regulating the sensibility of breast cancer cells to chemotherapy.miR-218 regulates cisplatin chemosensitivity by targeting BRCA1 as well as miR-451 and miR- 326 were found to down-regulate the expression of MDR-1 and MRP-1, respectively, resulted in increased sensitivity of MCF-7 cells to doxorubicin. Some miRNAs can cause breast cancer cells to grow without estrogen and develop resistance to endocrine therapy by down-regulating the expression of ERα (Ji et al., 2019). Identifying circulating miRNA to use as biomarkers for metastatic breast cancer is presently a key priority for many research groups. A noticeable increase in circulating miR-10b and miR-373 was presented in lymph node positive patients, compared to patients with no nodal involvement (Mitchell et al., 2008).

2-4-2-cfDNA

The presence of circulating, cell-free nucleic acids in the bloodstream was first described by Mandel and Métais in 1948 (Leon et al., 1977). Circulating tumor cells (CTCs) and circulating nucleic acids such as cell-free DNA (cfDNA) have a potential to use for cancer screening, prognosis determination, and monitoring of the efficacy of anticancer therapies (Chimonidou et al., 2013). cfDNAs are considered a promising new diagnostic tool, especially for patients with advanced-stage cancer, in whom the cfDNAs can be used as a "liquid biopsy," allowing physicians to follow cancer changes over time and tailor treatment accordingly (Pantel et al., 2009). The size of cfDNA may indicate its source. For example, apoptotic cells release DNA fragments of 180–200 base pairs whereas higher molecular-weight DNA fragments of over 10,000 bp in size are produced by necrotic cells (Jahr et al., 2001).

The release of cfDNA into the bloodstream occurs by different sources, including the primary tumor, tumor cells that circulate in peripheral blood, metastatic deposits present at distant sites, and normal cell types. Thus, both tumor and normal cfDNA circulate in the bloodstream of patients with cancer (Schwarzenbach and Pantel, 2015). Genetic alterations found in blood from patients with breast cancer include mutations, loss of heterozygosity, and altered methylation patterns. These alterations detected in the primary tumor may also be found in plasma/serum cfDNA of patients with Breast cancer (Li et al., 2012). The detection of tumor-specific DNA alterations in cfDNA provides a less invasive, more easily accessible source of DNA for genetic analysis than tumor biopsies (Skvortsova et al., 2006). Using fluorometry, showed the continuous increase in plasma cfDNA during tumor progression and its decrease after surgery(Tangvarasittichai et al., 2015).

The tumor-derived fraction of this total cfDNA, is under wide investigation as diagnostic and prognostic biomarker in several types of cancer, including breast,

lung and colon cancers(Fernandez-Garcia et al., 2019). This studies are using genomic alterations, such as methylations or tumor-specific mutations, the most valuable factors that allow us to precisely distinguish circulating DNA from normal-cell and tumor circulating ctDNA(Warton et al., 2016). Methylation aberrations are frequent features of many malignant diseases and can be detected in serum/plasma ctDNA when released into the bloodstream. Changes of methylation status usually occur in an early stage of carcinogenesis and hence are considered to be a better diagnostic factor than DNA mutations(Baylin and Jones, 2011). The DNA methylation pattern is often consistent between the cfDNA and the DNA from its tissue origins in cancer. This fact opens many opportunities for applying DNA methylation changes to the field of cancer diagnosis. The use of methylation status as a biomarker for cancer detection has several advantages over the methods established on genetic differences(Gai and Sun, 2019). First, epigenetic alterations are similar between any two tumors of the same type (same tissue origin)(Kundaje et al., 2015). Second, a methylation profile is tissue specific as well as constant between several tissue types among different patients. Consequently, investigating plasma DNA allows one to specify the tumor origin of cfDNA(Gai and Sun, 2019).

Conclusion

This paper reviewed the current most commonly available screening and biomarkers for diagnosing early and late stage breast cancer. The standard and new techniques based on tumor and blood markers, imaging and emission-based systems and molecular markers in diagnosis approaches for breast cancer detection were reviewed. Based on the above, it is clear that biomarkers are currently playing an important role in the management of patients with diagnosed breast cancer. Among all reviewed markers, molecular markers like cfDNA and microRNA have been suggested as a remarkable breast cancer markers duo to the detection of tumor-specific DNA alterations such as mutations and methylation in cfDNA provides a less invasive, more easily accessible source of DNA for genetic analysis than tumor biopsies. In addition, detecting somatic mutations from plasma DNA in advanced cancer patients may be potentially preferable when repeated tumor biopsies are not feasible and genomic analysis of archival tumor is deemed insufficient. However, molecular markers are still not mature and many challenges need to be solved before they can be implemented for clinical trials. A further urgent need is the identification and validation of biomarkers for predicting response to specific forms of chemotherapy.

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