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Laboratory Testing in Breast Cancer: Transforming Early Detection and Prognosis

Fatima Mohammed Alghamdi [1], Nasser Mohammad Almohsen [2], Abdul Aziz Saad ALmotairi [3], Ashwag Abdou Hazazi [4], Bader faleh Al-Haqbani [5], Haifa Khalaf Alkhaibary [6], Sarah Masfer Obed AlMalki [7], Sarah khaluf Abdulmajeed Abomrad [8], Reem Nuwayfia Alharbi [9].

1. *Lab Technician, Al-Iman Hospital, Ministry of health, kingdom of Saudi Arabia. fato.mohd35@gmail.com*
2. *Lab Technician, Rweidah General Hospital, Ministry of Health, kingdom of Saudi Arabia. nmalmohsen@moh.gov.sa*
3. *Abdul Aziz Saad ALmotairi, Laboratory Technician, Department of Death Affairs Services, Ministry of Health, Kingdom of Saudi Arabia. azoozsaad020@gmail.com*
4. *Ashwag Abdou Hazazi, Laboratory Specialist, King Khalid hospital in Alkharj, Ministry of Health, Kingdom of Saudi Arabia. Hazazi-a-a@outlook.sa*
5. *Bader faleh Al-Haqbani, Laboratory, King Khalid Hospital Al Kharj, Ministry of Health, Kingdom of Saudi Arabia. r.yy2019@hotmail.com*
6. *Haifa Khalaf Alkhaibary, Laboratory, King Khaled hospital in Alkharj, Ministry of Health, Kingdom of Saudi Arabia. halkabrai@moh.gov.sa*
7. *Sarah Masfer Obed AlMalki, Lab Technician, Department of Laboratories and Blood Banks, Ministry of Health, Kingdom of Saudi Arabia. samaalmalki@moh.gov.sa*
8. *Medical Laboratory, Maternity and children Hospital in Tabuk, Ministry of Health, Kingdom of Saudi Arabia.Kingdom of Saudi Arabia. i_sa@outlook.com*
9. *BSN, Madinah Health Cluster. reem2alharbi@hotmail.com*

Abstract

Breast cancer is the most common cancer globally, with an estimated 2.26 million new cases in 2020. Early detection significantly improves prognosis, but current imaging techniques have limitations. Molecular biomarkers have emerged as valuable tools for enhancing diagnosis, prognosis, and treatment strategies. Established biomarkers such as estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67 are routinely used for breast cancer subtyping and guiding therapy decisions. However, there is a need for more specific and personalized biomarkers, particularly for aggressive subtypes like triple-negative breast cancer. Novel biomarkers under investigation include death-associated protein kinase 1 (DPK1), carnitine palmitoyltransferase-1A (CPT-1A), tumor immune microenvironment markers, and methylated gene panels. Understanding signaling pathways like PI3K/AKT/mTOR and NF-κB also provides opportunities for identifying therapeutic targets and biomarkers. The clinical implementation of biomarkers involves a rigorous multi-phase process, from discovery to validation and clinical utility assessment. Emerging biomarkers such as long non-coding RNAs and circulating tumor DNA show promise for non-invasive monitoring of tumor dynamics. Collaboration between pathologists and physicians, along with evidence-based implementation in clinical trials, is crucial for advancing personalized medicine in breast

cancer management. Continued research and integration of laboratory testing innovations will be essential for improving early detection, precise diagnosis, and targeted treatment of breast cancer.

Keywords: Breast Cancer, Laboratory Testing

1. Introduction

According to the Global Cancer Observatory, the estimated number of new breast cancer cases worldwide in 2020 was 2.26 million, making it the most common form of cancer globally (Ferlay et al., 2021). Projections for the coming years indicate a rising trend, with an estimated 3.19 million new cases anticipated by 2040. This highlights the urgent need for improvements in early detection methods, as early diagnosis substantially enhances prognosis.

For diagnostic purposes, imaging techniques such as mammography, ultrasound, and magnetic resonance imaging are widely employed. While these modalities allow for the visualization of tumor morphology and localization, they are associated with limitations, including the use of contrast agents and exposure to high-energy radiation (He et al., 2020). Additionally, mammography has reduced sensitivity for detecting small tumors and is less accurate in women with dense breast tissue. Moreover, breast cancer is a heterogeneous neoplasm, involving complex genetic processes. Therefore, molecular-level analyses can contribute significantly to diagnosis and to understanding the disease's characteristics.

In recent decades, significant advancements have been made in disease detection technologies. The application of molecular indicators, known as biomarkers, has proven invaluable in optimizing clinical decision-making. These molecules enhance the accuracy of diagnosis, prognosis, and therapeutic strategies, making them crucial in clinical practice. In breast cancer, biomarkers such as alterations in the breast cancer gene (BRCA), human epidermal growth factor receptor 2 (HER2), and hormonal receptors are extensively used for clinical molecular diagnosis (Gamble et al., 2021).

The incorporation of biomarkers is essential for delivering high-quality oncological care. While current biomarkers provide valuable insights, there remains a need to identify more specific and personalized biomarkers for monitoring disease progression. This requirement is particularly significant in cases of triple-negative breast cancer, which presents treatment challenges due to the lack of viable biological targets.

Research has been focusing on various biomarkers that can be analyzed through material obtained from biopsies or non-invasive methods, such as blood, urine, breath volatiles, breast secretions, tears, and sweat. This review explores different types of biomarkers used for diagnostic and prognostic purposes, aiming to advance personalized approaches to patient treatment.

2. Breast Cancer: Classification and Current Therapy Approaches

Breast cancer is one of the most frequently diagnosed malignancies and the leading cause of cancer-related deaths among women globally. The International Agency for Research on Cancer (IARC) estimates that by 2025, there will be approximately 2.5 million new cases of breast cancer worldwide, resulting in 769,000 deaths related to the disease. While being female is the primary risk factor, other elements such as lifestyle and environmental factors also contribute to the likelihood of developing breast cancer. These factors include advanced age, excessive alcohol consumption, a family history of breast cancer, tobacco use, obesity, physical inactivity, and exposure to radiation. Furthermore, hereditary genetic mutations in genes such as BRCA1, BRCA2, and PALB2 are associated with an increased risk of breast cancer.

Breast carcinoma originates in the epithelial tissue of the mammary gland ducts or lobules and is characterized by the uncontrolled proliferation of malignant cells in these tissues. These tumors can be classified either histologically or molecularly. The primary molecular subtypes include Luminal A, Luminal B, HER2-positive (human epidermal growth factor receptor 2

positive), and triple-negative/basal-like (TNBC). Histologically, breast cancer can be categorized into in situ tumors, which develop in the epithelial lining cells of the ducts (85%) or the lobules within the glandular tissue (15%), and invasive tumors, which possess metastatic potential.

Carcinomas in situ (CIS) are classified into lobular carcinoma in situ (LCIS) and ductal carcinoma in situ (DCIS). LCIS is characterized by the proliferation of small, loosely cohesive cells within one or more ducts and terminal alveoli (Logan et al., 2015). The most common form of breast cancer is invasive ductal carcinoma, which originates in the mammary ducts and is further classified into grades (1, 2, or 3) based on morphology. The second most common type is invasive lobular carcinoma, where cancer cells spread from the mammary lobules to other parts of the body and may also develop metastases. Beyond histological classifications, breast cancer is categorized into distinct biological subtypes that reflect diverse molecular profiles and unique clinical-pathological features (Pandit et al., 2020).

Luminal subtype tumors are characterized by the overexpression of estrogen and/or progesterone hormone receptors. Luminal A tumors are classified by being positive for estrogen receptor (ER) and/or progesterone receptor (PR) and negative for HER2 amplification and/or overexpression. In contrast, Luminal B subtypes also exhibit positivity for these receptors but are associated with higher HER2 gene expression and increased cell proliferation, indicating a worse prognosis than Luminal A tumors. HER2-positive tumors are defined by high levels of HER2 oncoprotein expression and negativity for hormonal receptors. This subtype has the second-poorest prognosis, surpassed only by the triple-negative breast cancer (TNBC) subtype. TNBC is characterized by the absence of ER, PR, and HER2 expression (Lebert et al., 2018).

Early detection of breast cancer significantly increases survival rates. Population-based mammographic screening programs have been implemented as strategies to mitigate tumor progression and improve patient outcomes. Integrating risk models, which incorporate standard risk factors such as family history and hormonal/reproductive factors along with mammographic density and DNA testing, enables more accurate population-based risk stratification. Preventative measures, including lifestyle modifications, risk-reducing medications, and preventive surgeries, also play a crucial role in reducing breast cancer incidence. Breast cancer treatment has evolved significantly, transitioning from a predominantly surgical approach to a coordinated strategy encompassing local and systemic therapies (Agostinetto et al., 2022).

Neoadjuvant endocrine therapy is an effective systemic treatment option for ER-positive breast cancer patients. Although less commonly utilized, this approach is particularly valuable for patients with non-metastatic breast cancer, aiming to reduce tumor size to facilitate breast-conserving surgery and to enable early evaluation of systemic treatment responses. Neoadjuvant chemotherapy is now a standard of care for high-risk breast cancer. For TNBC patients, who are ineligible for endocrine or HER2-targeted therapies due to the absence of ER, PR, and HER markers, chemotherapy remains the primary treatment modality. Chemotherapy regimens incorporating anthracycline and taxane are frequently employed as adjuvant or neoadjuvant therapies, with approximately 50% of patients achieving pathological complete response following treatment.

Immunotherapy is another key modality in breast cancer treatment, aiming to restore and enhance the immune system's capacity to combat diseases. This approach reprograms the immune system to target and eradicate tumor cells. Immunotherapy is approved for advanced-stage TNBC and is being evaluated in clinical trials for its potential role in early-stage TNBC in the neoadjuvant setting. Advances in screening, early detection, and therapeutic strategies continue to significantly improve prognoses and survival outcomes for breast cancer patients (Ferris et al., 2023).

3. Molecular Biomarkers in Disease Diagnosis and Prognosis

The "BEST (Biomarkers, EndpointS, and other Tools) glossary," developed by the National Institutes of Health and the Food and Drug Administration, classifies biomarkers based on their applications, including susceptibility, predictive monitoring, pharmacodynamics, safety, and diagnostic and prognostic purposes. These biomarkers can also be categorized based on their molecular properties, biological origins, and therapeutic implications.

Molecular biomarkers are specific molecules found in tissues, biological fluids, or cells that serve as indicators of biological processes or conditions. These may include proteins, nucleic acids, lipids, or small molecules. The utility of biomarkers in clinical practice is determined by their ability to assist in disease diagnosis, staging, and therapeutic decision-making. Biomarkers such as genetic, protein, and epigenetic markers are supported by strong scientific evidence and can reliably predict clinical outcomes.

3.1. Biomarkers for Breast Cancer: Paving the Path to Prognosis

Biomarkers play a pivotal role in the clinical management of breast cancer, serving as tools for diagnosis and guiding therapeutic strategies. Among the available options, histological and molecular biomarkers are central to breast cancer monitoring, offering reliable and precise parameters. Moreover, advancements in cancer biology have contributed to the development of molecular detection techniques, facilitating the identification of more specific biomarkers tailored to each subtype. These advancements enable a more refined stratification of the disease (Höller et al., 2023).

In addition to their diagnostic applications, biomarkers are instrumental in determining the disease stage, thereby improving the efficiency of subgroup classification. Through biomarker analysis, alterations can be accurately defined and correlated with established patterns. Furthermore, biomarkers provide valuable insights into malignancy progression and help predict responses to therapy, guiding clinicians in selecting the most appropriate treatment options.

3.1.1 Hormone Receptors as Biomarkers

3.1.1.1 Estrogen Receptor (ER)

The estrogen receptor is regarded as one of the most critical biomarkers associated with breast cancer, being overexpressed in approximately 80% of cases. ER expression plays a significant predictive role, with ER-negative tumors generally exhibiting more aggressive characteristics compared to ER-positive tumors. Additionally, ER serves a dual purpose: it indicates prognosis, predicting patient outcomes, and contributes to treatment guidance and response, particularly in endocrine therapy (ET). The combined analysis of ER expression with other hormonal receptors is essential for accurately classifying and diagnosing breast cancer subtypes (Yip & Rhodes, 2014).

ER exists in two forms, ER-α and ER-β, encoded by the ESR1 and ESR2 genes, respectively. ER-α is expressed at higher levels than ER-β, making it more clinically relevant for managing both early-stage and metastatic tumors. However, the role of ER-β remains poorly understood, which limits its clinical characterization. Both receptors are regulated by endogenous estrogen. The development and proliferation of certain breast carcinomas are closely linked to excessive ER activity, where estrogen binding and ER activation stimulate tumor growth. Estrogen's interaction with genomic regulatory elements enhances the transcription of oncogenes such as MYC and Cyclin D. Consequently, hormonal modulation therapies have been employed, including selective estrogen receptor modulators (SERMs), third-generation aromatase inhibitors, LH-RH agonists, and pure estrogen receptor downregulators (SERDs).

3.1.1.2 Progesterone Receptor (PR)

The progesterone receptor is a biomarker with both prognostic and predictive value. It exists in two isoforms, PR-A and PR-B, which are derived from the same gene (PGR) but differ due

to PR-A truncation. PR is regulated by estrogen, making it a transcriptional target of ER. Approximately 70% of ER-positive breast cancer cases also express PR. Consequently, PR's primary utility lies in monitoring functional ER activity.

PR assessment is commonly performed using immunohistochemistry (IHC), where positivity is defined as ≥1% of tumor cell nuclei exhibiting immunoreactivity. Sequencing methods may also be employed for PR evaluation. From a diagnostic perspective, findings suggest that patients with ER-positive and PR-positive tumors, whether in early or advanced stages, are more likely to respond to endocrine therapy than those with ER-positive but PR-negative tumors (Nicolini et al., 2018).

Therapeutic approaches targeting PR involve natural formulations or synthetic hormones, such as megestrol acetate (Megace) and MPA. These treatments are often used alongside therapies targeting estrogen or ER, as described by Horwitz and Sartorius. However, significant challenges remain due to limited understanding of PR expression levels and how they can be modulated. Further research on PR modulation pathways could yield new discoveries and assist in identifying markers for cancers associated with these receptor activations.

3.1.1.3 Androgen Receptor (AR)

Although research on hormonal receptors in breast cancer primarily focuses on ER and PR, the androgen receptor (AR) has also been identified as a relevant molecule in all stages of tumor proliferation. AR acts as a transcription factor and is expressed in over 60% of breast tumors. Various clinical findings are associated with AR expression levels, complicating its precise characterization. However, it is established that AR functionality is influenced by ER expression levels (Cruz-Tapias et al., 2021).

IHC findings suggest that ER+/HER2- (Luminal A) tumors frequently express AR, making it a potential positive biomarker for this subtype. Overall, evidence indicates a better prognosis when AR is expressed, regardless of whether the tumors are ER-positive or across all subtypes.

3.1.2 Human Epidermal Growth Factor Receptor 2 (HER2) as a Biomarker

The human epidermal growth factor receptor 2 (HER2) is a transmembrane tyrosine kinase protein that belongs to the epidermal growth factor receptor family, which includes EGFR, HER2, HER3, and HER4. HER2 activation does not require a known ligand but occurs through heterodimerization with other receptors in the same family. Furthermore, its overexpression promotes the activation of other HER family receptors, thereby enhancing the activity of the MAPK and PI3K/AKT/mTOR pathways. This activation subsequently leads to increased cell proliferation, migration, invasion, and survival.

Approximately 15% to 20% of breast carcinomas exhibit HER2 positivity. HER2 is used as a biomarker for poor prognosis and as a strong predictor of response to therapies involving anti-HER2 agents, including pertuzumab, trastuzumab, lapatinib, and T-DM1. For diagnostic purposes, the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) recommend employing in situ hybridization (ISH) and immunohistochemistry (IHC) to evaluate HER2 protein expression and the nuclear-level expression of the transmembrane tyrosine kinase receptor ERRBB2 (Wolff et al., 2018). Studies have shown that HER2 overexpression correlates with tumor size, lymph node metastasis, a high percentage of cells in the S phase, and aneuploidy in breast cancer patients. Assessment of estrogen receptor (ER), progesterone receptor (PR), and HER2 status using IHC allows for the classification of breast cancer into three phenotypes: ER-positive (ER+:PR+/ER-:PR+), HER2-positive, and Triple-Negative (HER2-/Hormones-) according to the World Health Organization. The specific marker combinations within a tumor determine the response to hormonal or antibody-based treatments, indicating whether the prognosis is favorable or poor and whether the tumor is highly aggressive. HER2 expression analysis, in conjunction with ISH results from histological sections, further classifies HER2 status into HER2-negative

(HER2-zero/IHC0), HER2-low (IHC1+ or 2+/ISHneg), and HER2-positive (IHC3+ or 2+/ISHpos) (Denkert et al., 2022).

3.1.3 Ki-67 as a Biomarker

Ki-67, a nuclear antigen encoded by the MKI67 gene, is associated with tumor aggressiveness and cellular proliferation, making it a valuable prognostic biomarker for various cancer types. In cancer cells, Ki-67 overexpression occurs exclusively during the S, G2, and M phases of the cell cycle, reflecting the proliferative activity of the tumor. In breast cancer, elevated Ki-67 expression correlates with tumor aggressiveness, serving as a prognostic indicator for survival and recurrence risk. However, while patients with high Ki-67 expression tend to respond to neoadjuvant chemotherapy, residual tumors with elevated Ki-67 levels after treatment are linked to higher mortality rates (Yoshioka et al., 2015).

For diagnostic purposes, factors such as the age of paraffin-embedded tissue blocks, the use of formalin, and sample type (cytological or histological) can influence the Ki-67 score and result in signal loss during tissue staining. Moreover, the International Ki-67 in Breast Cancer Working Group (IKWG) emphasizes that pre-analytical factors (e.g., collection, processing, storage), analytical factors (e.g., staining and evaluation), and post-analytical factors (e.g., scoring and interpretation) all contribute to variability in Ki-67 scoring (Faragalla et al., 2023). IHC staining for Ki-67, a widely used method, aids in prognosis estimation for early-stage disease, predicts chemotherapy efficacy, and facilitates patient monitoring. At the molecular level, Ki-67 helps distinguish the Luminal A subtype (low Ki-67 expression) from HER2-negative, Luminal B-like subtypes (high Ki-67 expression). To enhance breast cancer management, standardizing Ki-67 antibodies, harmonizing staining protocols, utilizing the Oncotype DX multigene assay (ODX), and applying the Magee Equations TM algorithm are proposed measures to improve diagnostic accuracy.

3.1.4 Multigene Signatures

Multigene signatures provide critical prognostic information regarding therapy and staging in breast cancer patients. One widely used test is Oncotype DX (Genomic Health, Redwood, CA), a 21-gene signature test incorporated into the staging guidelines of the 8th edition of the American Joint Committee on Cancer (AJCC). Similarly, the AJCC guidelines include MammaPrint, a 70-gene signature that serves as a prognostic test in breast cancer. Additionally, Prosigna/PAM50 (Prosigna Breast Cancer Prognostic Gene Signature Assay; NanoString Technologies, Seattle, WA) is a 50-gene molecular signature specifically designed for pre- and postmenopausal women not treated with adjuvant systemic therapy. BluePrint, an 80-gene molecular classification system, enables breast cancer subtyping (Krijgsman et al., 2012).

Among multigene signature tests, Oncotype DX, MammaPrint, BluePrint, and Prosigna/PAM50 are the most employed for breast cancer biomarker analysis. However, these tests do not provide a complete molecular description of breast tumors, particularly in HER2+ and triple-negative subtypes. Therefore, the discovery of novel markers is essential to advancing personalized medicine for breast cancer patients.

4. Exploring Possibilities for the Identification of New Biomarkers for Breast Cancer

Mammography remains the most widely employed method for breast cancer screening, offering a sensitivity and specificity of approximately 91% and 82%, respectively. However, this technique has notable limitations, including false-positive results and reduced sensitivity in cases of high breast density. False positives can necessitate further diagnostic tests and invasive procedures, causing patient discomfort and anxiety. To address these challenges, several biomarkers are under investigation for breast cancer diagnosis to reduce the need for unnecessary diagnostic procedures. Although the clinical utility of currently available biomarkers is well established, novel markers are being identified and analyzed for their potential in diagnosis, prognosis, and risk classification of breast cancer. These new markers,

when combined with traditional biomarkers, can provide medical teams with improved tools to tailor treatments to individual patients (Neves Rebello Alves et al., 2023).

4.1 Death-Associated Protein Kinase 1 (DPK1)

Protein biomarkers such as Death-Associated Protein Kinase 1 (DPK1) show promise as diagnostic markers for breast cancer. DPK1, a kinase involved in apoptosis induction, is encoded on the long arm of chromosome 9, region 3, subregion 4.1. This protein triggers cell death signaling pathways in response to various stimuli, including apoptotic inducers and oncogenes. Specifically, DPK1 dephosphorylates the tumor suppressor p53, inactivating it via the p14/p19ARF pathway. In cases where p53 is mutated, DPK1 may activate pathways that promote cell proliferation, rendering it pro-proliferative (Arko-Boham et al., 2017).

Research has revealed that DPK1 levels are elevated in the serum of breast cancer patients compared to healthy individuals, particularly in aggressive phenotypes such as triple-negative breast cancer, which is characterized by rapid growth and high metastatic potential. The increased expression of DPK1 represents an effort by the body to induce apoptosis and autophagy in defective breast cells. However, elevated serum DPK1 levels may paradoxically enhance pro-proliferative activities. Immunohistochemistry studies have shown that 88% of healthy breast tissue samples exhibit negative staining for DPK1, compared to only 25% of cancerous tissue samples. This indicates that DPK1 expression in cells and tissue remains relatively low, while serum levels are significantly elevated in patients with breast neoplasms. Consequently, high serum DPK1 expression in breast cancer patients offers potential as a blood biomarker, particularly for aggressive breast cancer subtypes.

4.2 Carnitine Palmitoyltransferase-1A (CPT-1A)

Carnitine Palmitoyltransferase-1A (CPT-1A) is involved in fatty acid oxidation, facilitating the formation of the acyl-carnitine complex that crosses the inner mitochondrial membrane for beta-oxidation. Its role in tumorigenesis is linked to its ability to activate signaling pathways involving cytokines, chemokines, interferons, and NF-κB. Elevated CPT-1A expression in patients with Luminal A and Luminal B breast cancer correlates with poor prognosis. This protein interacts with genes associated with autophagy, cell proliferation, DNA repair, gene overexpression, and angiogenesis while recruiting regulatory, epigenetic, and immune pathways during tumor progression.

Notably, CPT-1A clustering with JAK-STAT signaling, MAPK activation, matrix remodeling, metastasis, and Wnt signaling identifies a subgroup with poor prognosis within mucinous carcinomas, a subtype typically associated with favorable outcomes. A shorter CPT-1A variant, referred to as variant 2, has been identified in the MCF7 cell lineage, interacting with HDAC1 to form a stable complex with significant epigenetic implications. Epigenetic pathways mediated by CPT-1A are observed across carcinomas regardless of tumor stage (Das et al., 2022).

4.3 Tumor Immune Microenvironment (TIME)

The growing use of immunotherapy in breast cancer treatment has spurred interest in understanding the tumor immune microenvironment (TIME) and its regulatory mechanisms. Consequently, new biomarkers have been identified, including CHI3L1 (YKL-40), which polarizes macrophages toward the M2 phenotype, promotes metastasis, and suppresses the tumor immune microenvironment by reducing T-lymphocyte infiltration. Similarly, the adhesion molecule L1CAM modifies TIME by recruiting regulatory T lymphocytes, inhibiting the tumor immune response, and promoting proliferation through NF-κB activation and FAK and Src phosphorylation.

In addition, the chemokine receptor CCR8, a member of the chemokine family, plays a critical role in recruiting regulatory T cells. High CCR8 expression in regulatory T cells is associated with poor survival outcomes in breast cancer patients. Cytochrome P450 1B1 (CYP1B1), which physiologically degrades 17β-estradiol into carcinogenic 4-hydroxyestradiol, is linked

to poor clinical prognosis in inflammatory breast cancer. The combined evaluation of these genes could provide a comprehensive panel of biomarkers for breast cancer prognosis in the future.

4.4 Methylated Genetic Panel

The diagnostic sensitivity of methylated genetic panels increases with decreasing tumor size, making them a potential alternative for identifying small breast tumors. Aberrant methylation profiles in genes such as SFN, P16, hMLH1, HOXD13, PCDHGB7, and RASSF1a, which result in gene inactivation, have been detected in the serum DNA of breast cancer patients compared to healthy individuals and those with benign breast disease. These genes are involved in various cellular processes, including DNA binding, cell cycle control, chromatin binding, and cytokine activity (Shan et al., 2016).

Previous studies have demonstrated that tumor DNA from patients with early-stage or metastatic cancer is released into the serum, enabling the identification of specific methylation alterations in the serum DNA of various cancer types. Understanding critical biological processes such as immune response, tumor suppression, apoptosis, and cell adhesion is essential for identifying potential biomarkers.

5. Signaling Pathways and Identification of Therapeutic Targets in Breast Cancer

Various cellular pathways are under investigation to identify potential therapeutic targets for breast cancer. One example is the use of CDK4/6 inhibitors, such as abemaciclib, ribociclib, and palbociclib, in certain metastatic cases. These inhibitors target CDK4/6, which mediates the phosphorylation of pRb, a process that facilitates cell cycle progression and subsequent cell proliferation. By inhibiting CDK4/6, these compounds can arrest the cell cycle, leading to reduced proliferation of tumor cells.

The PI3K/AKT/mTOR signaling pathway is another prominent source of therapeutic targets in breast cancer. MK-2206, an allosteric inhibitor, has demonstrated significant efficacy in enhancing signaling inhibition and inducing apoptosis. Moreover, the inhibitory activity of MK-2206 against AKT has been observed both as a monotherapy and in combination with other therapeutic strategies, such as chemotherapy (Sangai et al., 2012).

Studies have also identified elevated expression levels of circular RNA (circRNA) in breast cancer patients. CircRNA contributes to tumorigenesis by sequestering microRNAs, thereby reducing their activity, and by positively regulating the transcription factor ZEB2 through vesicles extracted from breast cancer patients. ZEB2 plays a pivotal role in promoting epithelial-mesenchymal transition (EMT), a process characterized by cellular morphological changes, reduced adhesion, and loss of polarity—key mechanisms in cancer cell invasion and metastasis.

Extracellular vesicles have been widely studied in various cancers, including breast cancer. These vesicles, which are organelles released by different tissues, play a crucial role in cell signaling with significant implications for tumorigenesis. They can be detected in normal circulation and other biofluids such as breast milk, saliva, and urine, and they carry a diverse range of molecules, including RNAs, proteins, and metabolites.

Functional enrichment analyses of circRNA host genes have revealed that these RNAs are involved in diverse processes, such as regulation of the JNK pathway and IκB phosphorylation, both of which are associated with cell migration and invasion. This connection highlights the role of dysregulated circRNAs in breast cancer development and underscores their potential as biomarkers. CircRNAs are particularly appealing due to their ease of extraction, high specificity relative to cancer stage, and stability.

A recent study emphasized the significance of the NF-κB pathway in metastasis, noting that CECR2 recruitment enhances chromatin accessibility to genes such as TNC, MMP2, and VEGFA, which are implicated in metastatic progression. This finding suggests that

pharmacological inhibitors targeting CECR2 could be explored to mitigate metastasis. Beyond therapeutic interventions, the understanding of functionality and regulation of these pathways and their members provides additional opportunities for identifying specific biomarkers for breast cancer diagnosis and prognosis.

The advancements in understanding breast cancer biomarkers have significantly enhanced diagnostic and prognostic capabilities. The utilization of biomarkers in this context represents a promising approach for both detection and prognosis. Strategies such as gene hypermethylation, proteins associated with mammary neoplasms, genetic prognostic signatures, signaling pathway-related genes, and extracellular vesicles containing circRNA offer potential for improving cancer detection and stratifying patients for treatment. These approaches enable a more precise and personalized treatment strategy for individuals with breast cancer.

6. Emerging Biomarkers: From Discovery to Clinical Utility

The transition of biomarkers from experimental to clinical status involves a multi-phase process, culminating in reproducible results supported by statistically significant data in a sufficiently large population, ensuring at least 95% confidence. To perform biomarker assays, adherence to the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) guidelines is critical. Moreover, Pepe et al. outlined a timeline for studies aimed at testing and confirming biomarkers for clinical applicability.

The implementation of biomarkers for clinical use generally follows five key stages: discovery, validation, verification, clinical application, and clinical utility. During the discovery phase, factors such as the expression levels of candidate RNAs and their predictive value are studied, as differential gene expression often correlates with biological functions associated with specific conditions. In instances where sample acquisition poses challenges, public databases enriched with mass sequencing expression data can address these limitations by increasing the population size in such studies.

In essence, the discovery phase entails selecting and testing potential biomarkers using samples obtained from patients with a defined pathology, while the validation phase requires increasing the sample size based on calculations derived from earlier studies. Biomarkers identified during the discovery phase must exhibit specificity and sensitivity levels of 60% to 80%, respectively, which can subsequently be used in additional calculations to determine the optimal population size for validation studies (Ray et al., 2010).

The verification phase involves evaluating biomarkers using clinical laboratory techniques to determine their feasibility in clinical settings. Following this, the application and clinical utility phases focus on establishing sensitivity, specificity, and precision through larger sample studies, which may include clinical trials. The attrition of biomarkers during their progression from discovery to clinical practice is attributed to factors such as the need for unequivocal and statistically robust associations, as well as economic, ethical, and regulatory considerations. To assess the clinical utility of biomarkers, prospective, randomized trials and, where possible, retrospective studies are recommended, particularly using large-scale trials with survival outcomes as endpoints.

Long non-coding RNAs (lncRNAs) have been linked to various clinical conditions, including therapy resistance and metastasis development in breast cancer patients, indicating their potential as biomarkers. Nonetheless, their clinical application remains constrained by the limited number of large-scale validation studies. For example, studies in the validation phase have shown that the lncRNA DSCAM-AS1 serves as a biomarker for tamoxifen resistance in endocrine therapy due to its elevated expression in HER2-positive breast cancer patients (Niknafs et al., 2016).

The advantages of lncRNAs over other biomolecules have been demonstrated in multiple studies. For instance, the lncRNA GATA3-AS1 predicts resistance to neoadjuvant

chemotherapy in luminal B breast cancer patients with a sensitivity of 92% and specificity of 75%. These values indicate slightly lower sensitivity but significantly higher specificity compared to Ki-67, a clinical biomarker for predicting response to neoadjuvant chemotherapy in breast cancer (sensitivity: 95.7%, specificity: 54.3%). Additionally, another study demonstrated that the lncRNA AC10538, in combination with four mRNAs (OR7C1, TBX2, RSPH4A, and C2orf61), surpasses the Oncotype Dx signature test in predicting overall survival in early-stage breast cancer patients (Liu et al., 2020).

In the context of triple-negative breast cancer, treatment with atezolizumab is routinely employed, with PD-L1 being used as a predictive biomarker to identify patients who might benefit from such therapies. While PD-L1 is widely applied across different cancer types, there is growing consensus that relying on a single biomarker may be insufficient to predict clinical responses to immunotherapy. Consequently, studies propose the combined use of PD-L1 and tumor-infiltrating lymphocytes (TILs) as an immuno-oncological marker for selecting triple-negative breast cancer patients eligible for immunotherapies. These combined markers are already undergoing clinical implementation.

Currently, circulating blood biomarkers are being evaluated as a relatively non-invasive means of monitoring tumor dynamics. Although clinical trials utilizing circulating tumor DNA (ctDNA) biomarkers are underway, no such biomarker has yet been approved or regulated for clinical use. Lastly, personalized medicine necessitates the collaboration of pathologists and physicians, alongside evidence-based and regulated implementation of biomarkers in clinical trials and practices, to ensure improved outcomes for patients.

Conclusion

Laboratory testing has revolutionized breast cancer diagnostics and management, offering unprecedented insights through molecular biomarkers. These tests enhance early detection, facilitate precise tumor characterization, and inform personalized treatment strategies. However, challenges persist in developing reliable, non-invasive, and widely applicable biomarkers. Continued advancements in laboratory methodologies and biomarker research are pivotal to bridging gaps in current diagnostic capabilities, improving patient outcomes, and paving the way for next-generation cancer care. The integration of laboratory testing innovations will remain a cornerstone in the fight against breast cancer, enabling clinicians to tailor interventions and optimize therapeutic success.

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