# A Review on Invitro Methods of Detection of Breast Cancers; Cell Lines Studies

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***Abstract:*** **Breast cancer remains one of the leading causes of cancer-related mortality in women worldwide, necessitating the development of precise and efficient detection methods. In vitro studies using breast cancer cell lines have provided valuable insights into biomarker expression, molecular classification, and diagnostic advancements. This review explores various in vitro detection methods, focusing on Cytokeratin 19 (CK19) as a biomarker for circulating tumor cells, the role for of claudin 1 and E-cadherin in tumor development, and the application of tumor-targeting nanoprobes rapid and sensitive breast cancer detection. The findings highlight the significance of molecular markers in classifying breast cancer subtypes and optimizing targeted therapies. Further research in this domain can enhance diagnostic accuracy and improve treatment strategies.**

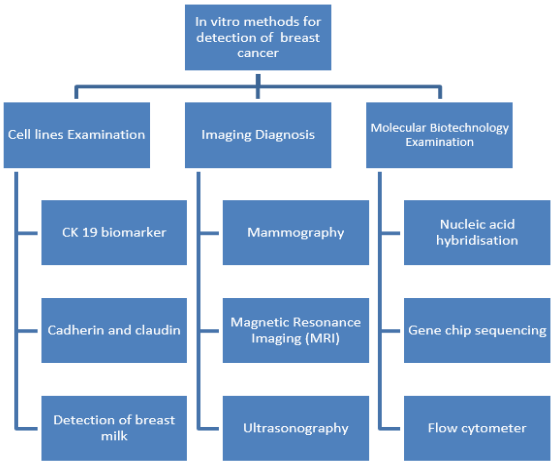
## INTRODUCTION

Breast cancer ranks among the most prevalent cancers affecting women globally. The primary cause of this cancer is the malignant proliferation of the epithelial cells that line the ducts or glands of the breast tissue. The incidence rate of this disease is on a steady rise every year, taking into account the population growth. Experts anticipate that the annual count of new BC cases worldwide will reach approximately 3.2 million by 2050. Several factors, including age, family history, and lifestyle, contribute to this alarming trend. Therefore, early detection of BC is crucial to enhance the chances of patient survival.Breast cancer cell lines are commonly used and reproducible sources for investigating biological and clinical functions, such as exploring tumors, signal transduction pathways, and modern therapeutic targets. Initially, they were utilized as experimental models for breast cancer research in various cancer studies. There are several cell lines commonly used in breast cancer detection studies, including:

1. MCF-7: A human breast cancer cell line derived from a metastatic site in a patient with breast adenocarcinoma.
2. T47D: A human breast cancer cell line derived from a ductal carcinoma in situ.
3. SK-BR-3: A human breast cancer cell line derived from a metastatic site in a patient with breast adenocarcinoma.
4. MDA-MB-231: A human breast cancer cell line derived from a metastatic site in a patient with breast adenocarcinoma.
5. BT-474: A human breast cancer cell line derived from a metastatic site in a patient with breast adenocarcinoma.

These cell lines have been extensively characterized and are widely used for in vitro studies of breast cancer, including drug discovery, biomarker identification, and mechanistic studies.

Several diagnostic methods have been developed based on imaging and molecular biotechnology to screen BC rapidly and accurately. It is crucial to summarize and evaluate these methods to provide valuable information for clinical diagnosis.




In this review, various methods to detect breast cancer are explained in brief emphasizing mostly on detection methods by cell lines studies. Biomarkers like CK19, Claudin I and E- Cadherin are studied in depth and a review of other in-vitro methods by imaging diagnosis like Mammography (MG), Ultrasonography (UG), Magnetic Resonance Imaging (MRI) and Molecular Biotechnology Examination is also given.

## CELL LINES EXAMINATION

Cytokeratin 19 biomarker detection

CKs are intermediate filaments and are the primary structural proteins in epithelial cells. They normally play a crucial role in organizing the cytoskeleton, but abnormal expression can lead to the development of cancer. CK19 is the smallest member of the CK family and was first identified in squamous cell carcinoma. Unlike other CKs that form heterodimer structures, CK19 is a simple CK that does not heterodimerize with any other CKs. CK19 is highly expressed in metastatic cancers such as breast, liver, lung, pancreas, and esophageal cancers. In addition to its role in maintaining cell structure, CK19 has been shown to play a role in cellular communication, apoptosis , and regulating protein synthesis and transport. The identification of circulating tumor cells (CTC) is facilitated by the expression of CKs, which are major structural proteins in epithelial cells. Among the CKs, CK19 is recognized as a sensitive marker for detecting early metastasis and predicting cancer prognosis in tumor cells with an epithelial origin in the bloodstream. The data suggest that detecting CK19 in the blood of patients could be a potential marker for detecting breast cancer.

Hence, there are not only quantitative differences in CK19 expression but also links between its expression and other BC markers that should be considered in the molecular classification of breast carcinoma.

Cadherin and Claudin

The claudin and cadherin families are vital elements of the tight and adherens junctions in epithelial cells. This loosening of intercellular junctions plays a significant role in the mechanisms involved in tumor development. Despite the majority of invasive breast cancers showing decreased levels of claudin 1, some breast cancers of the basal-like molecular subtype and the luminal-like human breast cancer cell line MCF-7 display elevated levels of this protein. Claudin 1, besides its presence in the cell membrane, has also been found in the cytoplasm of certain tumor cells or in cells undergoing epithelial to mesenchymal transition (EMT), a process that promotes cancer invasion and metastasis. This protein is downregulated during EMT. Similarly, E-cadherin has been reported to exist in alternate cellular locations, including the cytoplasm and nucleus, suggesting potential roles beyond the cell membrane. Basal-like breast cancer subtype is believed to involve collaborative interactions between tight and adherens junctions, where claudin 1 overexpression is associated with the downregulation of E-cadherin. Since the expression of claudin 1 and E-cadherin in breast cancer progression can both be downregulated or upregulated, it is crucial to determine their precise location within the cell. This is because their cellular distribution can affect their specific functions within the cell.

Detection of breast milk

Breast cancer cell detection and characterization from breast milk-derived cells is a method used to identify and study cancer stem-like cells (CSC) in breast milk samples from women with breast cancer. These CSCs carry specific mutations within genes related to cell growth and division, which make them more resistant to chemotherapy and other treatments. By enriching for specific markers on the surface of these cells, such as CD49f+/EpCAM−, CD44+/CD24−, and CD271+, researchers can isolate and study the CSCs. This method has the potential to improve early detection and treatment of breast cancer by identifying these resistant cells and developing more effective targeted therapies.

## IMAGING DIAGNOSIS

Mammography

Mammography (MG) is the preferred approach to screen and diagnose breast cancer, providing clinicians with vital information about BC patients. According to research, early MG screening can potentially reduce the mortality rate of BC patients by 30%-40%. In the meantime, only 4%-10% of breast cancer patients receive a positive diagnosis based on the results of mammography. Over time, advancements in mammography technology have led to the development of new diagnostic techniques. Currently, the two primary strategies used for diagnosing breast cancer patients in clinical settings are contrast-enhanced mammography (CEM) and digital breast tomosynthesis (DBT). Research indicates that in terms of diagnostic accuracy and assessment of disease extent, contrast-enhanced mammography (CEM) is comparable to breast MRI and outperforms full-field digital mammography (FFDM).

Overall, mammography and its derivatives are essential tools for screening and diagnosing breast cancer patients. These techniques offer numerous

advantages, including rapid screening, high accuracy, low cost, and suitability for widespread use. As a result, mammography is an optimal imaging diagnostic method for patients with limited financial resources and can help eliminate the risk of developing breast cancer. However, there are certain limitations that may make mammography unsuitable for some individuals. For example, it requires the use of harmful contrast agents and X-rays for imaging, and cannot be repeated frequently within a short period of time. Additionally, it is not recommended for use in patients under the age of 40

Magnetic resonance imaging (MRI);

MRI enables the detection of familial breast cancer at an early stage, without being affected by factors such as the patient's age, breast density, or risk status. The magnetic resonance diffusion weighted (MRDW) technique is used to observe the movement of water molecules in the body, making it a valuable tool in diagnosing BC patients. Based on research, malignant tumors display restricted water diffusion compared to benign tumors, and this difference can be detected by measuring the apparent diffusion coefficient (ADC) values of the tumors using MRDW.

Magnetic resonance imaging (MRI) is a useful diagnostic tool for BC, but there are several factors that limit its widespread use, such as long imaging time, high cost, and contraindications for patients with metal in their bodies. Therefore, MRI is typically used in cases where the primary BC is small, where more detailed information about the tumor is needed, or for screening high-risk groups. In the future, improvements in MRI technology may lead to higher signal-to-noise ratios, shorter imaging times, and lower costs. Additionally, reducing the use of contrast agents should be a priority in advancing MRI technology for use in all stages of BC.

Ultrasonography

Ultrasonography (US) is a technique used to observe the morphology and variations of tumor tissues, and it can accurately determine the location of lesions. Unlike other imaging techniques, US are non-invasive and safe for all individuals. Throughout its development history, early grayscale US was only able to determine the presence of a tumor at the detection site. However, it was challenging to distinguish between benign and malignant tumors due to its low resolution. The two-dimensional US technique only produces flat images of the tumor, which can sometimes affect physicians' ability to make accurate judgments. As a solution, three-dimensional US technology has been developed to provide a more comprehensive imaging of the tumor morphology and blood vessel distribution, which is displayed during the patient's diagnosis. Among the many types of three-dimensional US, color Doppler US is especially useful as it can provide doctors with valuable clinical information by clearly reflecting the status of the tumor and blood flow, which helps distinguish between benign and malignant tumors. Studies have shown that utilizing elastic US to screen suspected pathological tissues has significantly improved the accuracy of BC diagnosis. However, by incorporating three-dimensional US, elastic US can be utilized to diagnose axillary lymphadenopathy and categorize the state of a patient's tumor. Although mammography is considered the preferred method for detecting calcification in breast cancer, small-sized calcifications can be challenging to detect through mammography or regular ultrasound. A novel technique in US image processing called MicroPure was developed to address the limitations of detecting small calcifications through routine US or MG. This technique is designed to analyze spatial and frequency features of images to reduce speckle and produce images with high tissue uniformity and contrast resolution.

MOLECULAR BIOTECHNOLOGY

Nucleic acid hybridization ;

Fluorescence in situ hybridization (FISH) and aptamer probe hybridization (APH) are nucleic acid hybridization techniques that are used for diagnosing breast cancer. These techniques can identify specific fragments of tumour biomarkers and also help in discovering new biomarkers for breast cancer diagnosis.

Fluorescence in situ hybridization (FISH) is a powerful tool in molecular biology diagnostics, with its principle based on base pairing. Research has found that approximately 25-30% of all BC cases are HER-2 positive BC. FISH has a high response rate (98%) in amplifying the HER-2 gene and determining high HER-2 copies per cell. Thus, FISH is an important factor in determining whether medication (Herceptin) is needed for BC patients,

and is considered the "gold standard" for detecting HER-2 gene activation. Other advantages of FISH include reproducibility, stability, and high sensitivity. However, its promotion is limited by the need for complex probe design and a special fluorescence detector.

Another precise and sensitive technique for diagnosing BC is aptamer probe hybridization (APH). The accuracy of APH largely depends on the appropriate selection of aptamers, which are usually generated by Systematic Evolution of Ligands by Exponential enrichment (SELEX). Currently, Cell-SELEX is oneof the most widely used methods for obtaining high-quality aptamers from tumors. Aptamers that are appropriate can recognize particular fragments that can be utilized for disease diagnosis. A novel fluorescent aptamer (AAI2-5) has been developed, which can detect MCF-7 BC cells and MDA-MB-231 cell lines sensitively and easily from breast cells with an accuracy rate of 90%. Obtaining suitable aptamers or probes through APH is currently a complex and challenging process, which requires significant time and financial resources, making it unsuitable for widespread use in primary hospitals. However, in the future, there is potential for the development of simpler and more efficient screening methods for aptamers, leading to the discovery of new biomarkers for BC using APH.

Gene chip Sequencing:

A commonly used technique in diagnosing breast cancer is gene chip analysis. It allows for the simultaneous analysis of a large number of nucleic acid fragments and is widely utilized in the field. This method is particularly useful in observing and analyzing the nucleic acid condition in breast cancer cells or tissues, and also in identifying new diagnostic biomarkers by screening large sample sizes. The gene chip is essentially a high-density oligonucleotide microarray, as is well known in the field. Two methods are available for preparing gene chips: in situ synthesis and direct point method.

Gene chip technology has some limitations, including the difficulty in synthesizing probes, the possibility of generating false positive signals, and the complexity of nucleic acid extraction. However, with the development of nanotechnology, it is expected that the size of the chip will become smaller, and the throughput of the gene chip will increase in the future.

Flow cytometer

Flow cytometry (FCM) is a technology that can measure multiple physical characteristics of a single cell as it flows in suspension, and it has become an essential tool in the diagnosis of BC. Developed in the 1960s, FCM is a highly interdisciplinary technology that combines cytochemistry, immunology, materials science, molecular biology, spectroscopy, optical systems, fluidic systems, laser technology, and computer technology. In addition to its sorting function for tumor cells, FCM can rapidly detect cells or biological particles through the one-by-one flow state, multi-parameters, or rapid qualitative and quantitative analysis. However, in FCM, cells or biological particles must first be treated and labeled to enable detection by laser. Despite its advantages, FCM has the limitation of requiring pre-treatment and labeling of cells or particles. FCM has been combined with other detecting techniques in recent years to achieve quantitative detection of low-abundance genes. This technology is also an excellent method for diagnosing BC and guiding medication.

FCM can not only detect biomarkers of BC cells but also identify them based on morphology. However, there are some drawbacks of FCM, such as non-specific binding of antigen- antibody, which can affect the signaling pathway of FCM. Another issue is the problem of dye pollution in FCM experiments, and the high cost of the required instruments. In the future, it is important to standardize the diagnostic scheme for FCM and develop high- efficiency and low-cost agents.

## CONCLUSION

In conclusion, in vitro methods for the detection of breast cancer using cell line studies, imaging diagnosis, and molecular biotechnology have provided valuable insights into the pathogenesis of breast cancer. The use of cell line studies has allowed for the examination of the mechanisms underlying breast cancer progression and has been instrumental in identifying potential therapeutic targets. Imaging diagnosis techniques such as mammography, ultrasound, and MRI have greatly improved the early detection of breast cancer and increased patient survival rates. Molecular biotechnology techniques, such as PCR and gene expression profiling, have provided novel biomarkers for the early detection, diagnosis, and

treatment of breast cancer. These biomarkers, such as HER2, estrogen receptor, progesterone receptor, and Ki67, are used clinically to determine the molecular subtype of breast cancer and guide treatment decisions.

Overall, the integration of these various in vitro methods has greatly improved our understanding of breast cancer and has paved the way for personalized medicine approaches. By combining biomarker analysis with imaging diagnosis and cell line studies, clinicians can now develop targeted therapies for individual patients based on the molecular characteristics of their tumors. As new technologies continue to emerge, it is likely that in vitro methods will continue to play a critical role in breast cancer detection and treatment.

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