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IN-VITRO METHODS FOR DETECTION OF BREAST CANCER

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Abstract: **The physical and emotional health of women around the world has been significantly threatened by breast cancer, and over time. India has also clearly seen an increase in both its morbidity and mortality rates. Research is necessary to swiftly diagnose breast cancer. Women with early-stage breast cancer have a considerably higher chance of surviving than those with middle- and late-stage breast cancer. Numerous techniques for detecting breast cancer have been created up to this point, primarily based on cell line, imaging, and molecular biotechnology analysis. These techniques significantly aid in the detection and confirmation of breast cancer. We explain and discuss the improvements of such techniques. 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, Ultrasonography, Magnetic Resonance Imaging and Molecular Biotechnology Examination is also given**

Keywords: **Breast cancer, Cytokeratin 19, Mammography, Ultrasonography, Flow cytometer**

I. INTRODUCTION:

Breast cancer (BC) ranks among the most prevalent cancers affecting women globally [1]. The primary cause of this cancer is the malignant proliferation of the epithelial cells that line the ducts or glands of the breast tissue [2]. 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 [3]. 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 [4, 5]

BC has been classified into five distinct subtypes based on gene expression analysis: Luminal A, Luminal B, HER2 positive, Basal-like, and Normal-like/Claudin-low [6, 7]. Each subtype is associated with different prognosis, metastasis patterns, and treatment responses[8]. Luminal A subtype expresses estrogen (ER) and progesterone (PR) receptors and does not express HER2 (ER+/PR+/HER2–), along with Cytokeratin (CK) markers such as CK7, CK8, CK18, and CK19 [9] Luminal B subtype expresses ER, PR, and HER2 receptors (ER+/PR+/HER2+), along with CK7, CK8, CK18, CK19, and up-regulation of genes related to cell proliferation [10]. HER2 positive subtype is negative for ER and PR receptors, but expresses HER2 (ER–/PR–/HER2+), along with markers such as CK5, CK8, CK18, and CK19 [11]. Basal-like subtype is negative for ER, PR, and HER2 (ER–/PR–/HER2–) and expresses CK5, CK6, CK14, CK17, and is also called triple-negative as it does not express PR, ER, and HER2 [12]. Normal-like subtype is negative for ER, PR, and HER2 (ER–/PR–/HER2–) and does not express CK8, CK18, and CK19, similar to non-cancerous breast tissues, and is responsive to treatment [13]. The Claudin-low subtype is negative for ER, PR, and HER2 (ER–/PR–/HER2–)[14]. Recently, new biomarkers have been identified for BC subtypes, and more research is being conducted to develop effective diagnosis and treatment strategies for each subtype[15].

BC 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 BC research in various cancer studies. There are several cell lines commonly used in BC detection studies, including:

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

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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 BCare 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.

Various In-vitro methods for detection of breast cancer

1. Cell line examination

1.1 Cytokeratin 19 biomarker:

Cytokeratins (CKs) are intermediate filaments and are the primary structural proteins in epithelial cells[16]. They normally play a crucial role in organizing the cytoskeleton, but abnormal expression can lead to the development of cancer [17]. CK19 is the smallest member of the CK family and was first identified in squamous cell carcinoma[18]. Unlike other CKs that form heterodimer structures, CK19 is a simple CK that does not heterodimerize with any other CKs [19, 20]. CK19 is highly expressed in metastatic cancers such as breast, liver, lung, pancreas, and esophageal cancers[21]. In addition to its role in maintaining cell structure, CK19 has been shown to play a role in cellular communication [22], apoptosis[23], and regulating protein synthesis and transport. The identification of circulating tumor cells is facilitated by the expression of CKs, which are major structural proteins in epithelial cells [24]. 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 [25]. The data suggest that detecting CK19 in the blood of patients could be a potential marker for detecting BC.

In specific cell lines, CK19 was found to be the most closely associated marker for circulating tumor cells. MCF7, SKBR3, T47D, and MDA-MB-231 cell lines, with HeLa as a negative control, when used to assess CK19 expression, CK19 was not detected in the MDA-MB-231 cell line. The expression of CK19 when compared among T47D, MCF7, and SKBR3 cell lines, T47D and MCF7 belong to the luminal subtype of BC, and CK19 expression is regulated by an ER marker. SKBR3 belongs to the HER2-positive subtype of BC. On the other hand, MDA-MB-231 belongs to the claudin-low subtype of BC, which is strongly related to negative ER, PR, and HER2 and lacks CK19 expression. 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 [26]

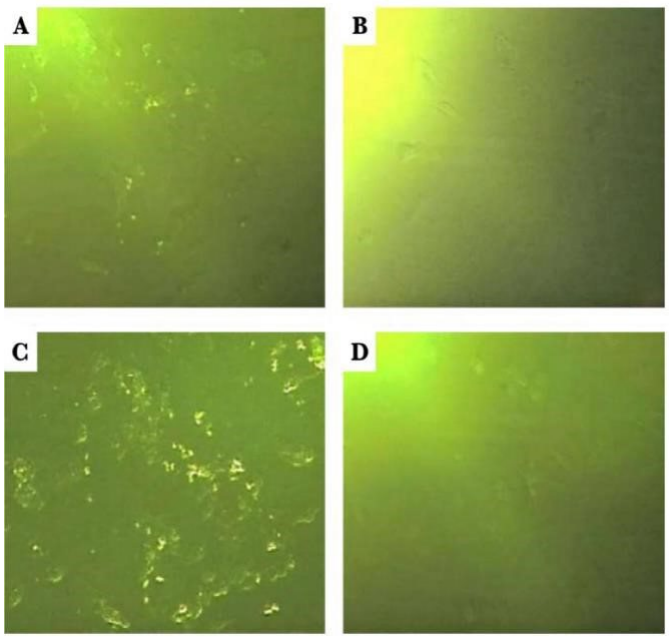

Figure 1. CK19 expression in the MCF7 and T47D cell lines was demonstrated by immunofluorescence microscopy.

1.2 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 [27]. Despite the majority of invasive BC showing decreased

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levels of claudin 1, some BCof the basal-like molecular subtype and the luminal-like human BC;cell line MCF-7 display elevated levels of this protein [28, 29]. 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 [30]. This protein is down regulated 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 [31]. Basal-like BC subtype is believed to involve collaborative interactions between tight and adherens junctions, where claudin 1 over expression is associated with the down regulation of E-cadherin. Since the expression of claudin 1 and E-cadherin in BC progression can both be down regulated 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.

Immunofluorescence is the most common and easy technique to detect this. Its first step is fixation which can be done with different solvents like formaldehyde, methanol and ethanol. Studies have found that the most efficient technique is by using the formaldehyde fixation method 1. The study findings also suggest that the use of methanol fixation is more precise and dependable than formaldehyde fixation when examining the distribution of claudin 1 and Ecadherin through immunofluorescence microscopy in human BCcell lines. Results obtained through methanol fixation were consistent with Western blot results, strengthening the trustworthiness of methanol fixation emphasizing the importance of considering expression levels and cell type variations when interpreting the cellular localization of these intercellular junction proteins.

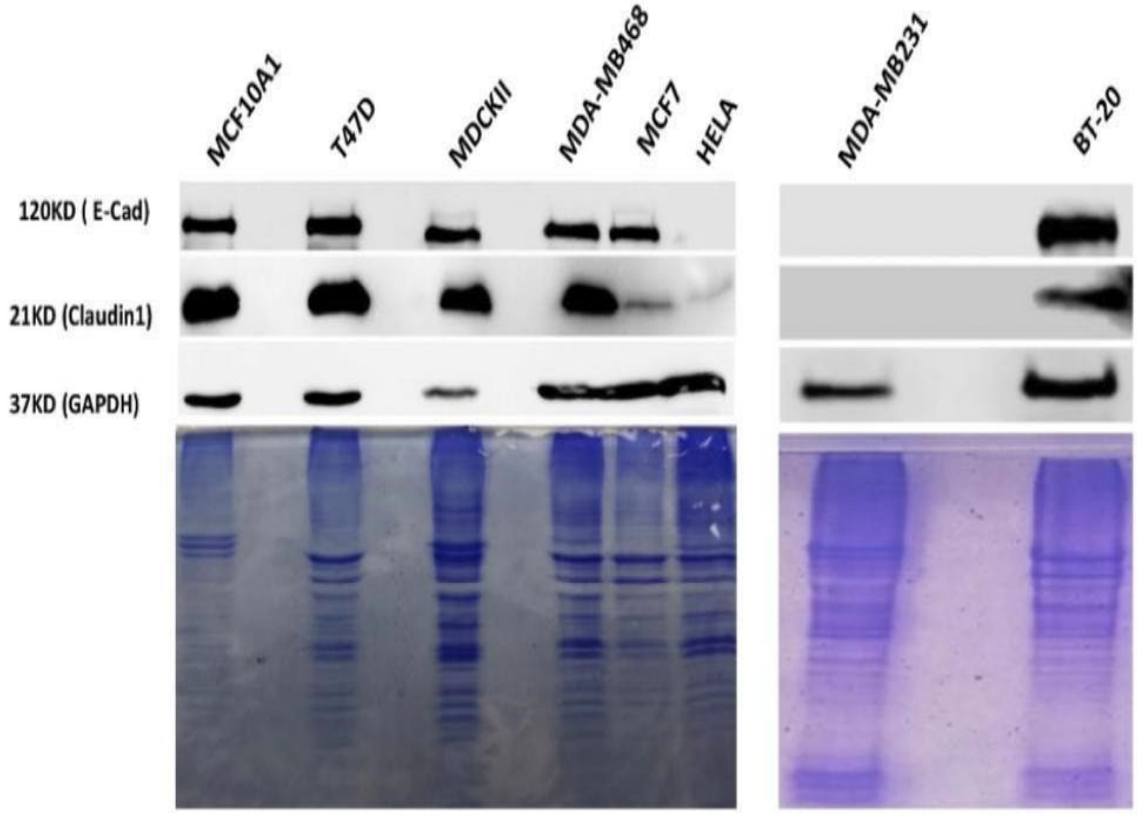

Figure 2. Detection of E-cadherin and claudin 1 in different cell types by Western blot analysis.

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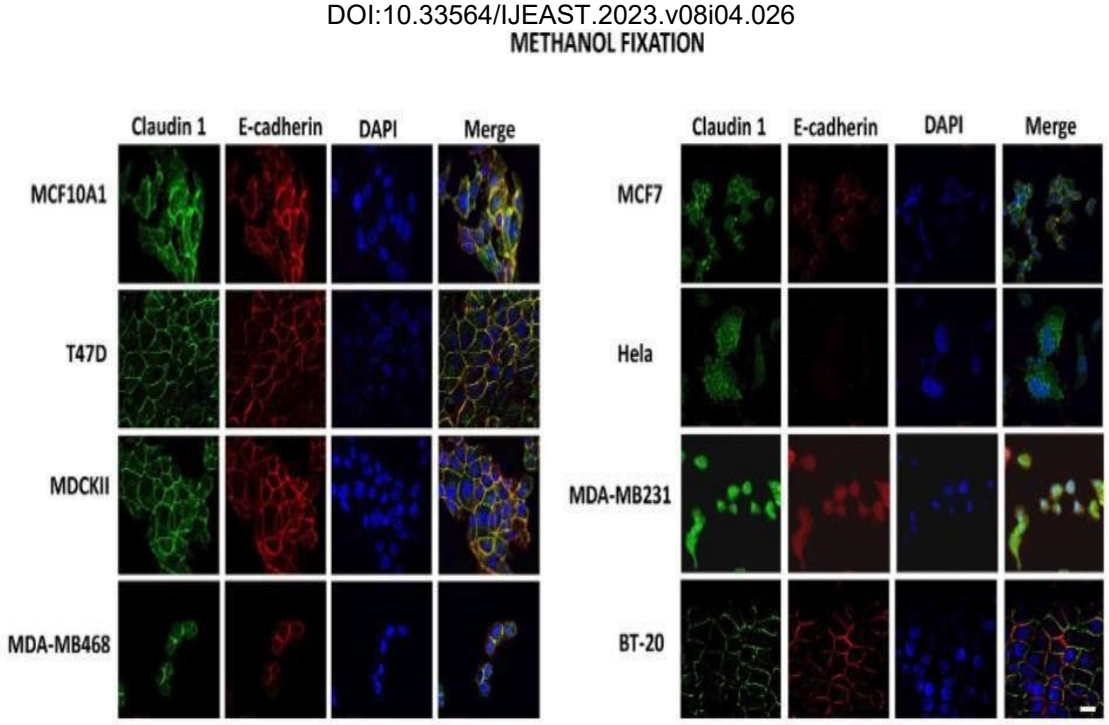

Figure 3. Immunofluorescent detection of E-cadherin and claudin 1 in different cell lines following methanol fixation.

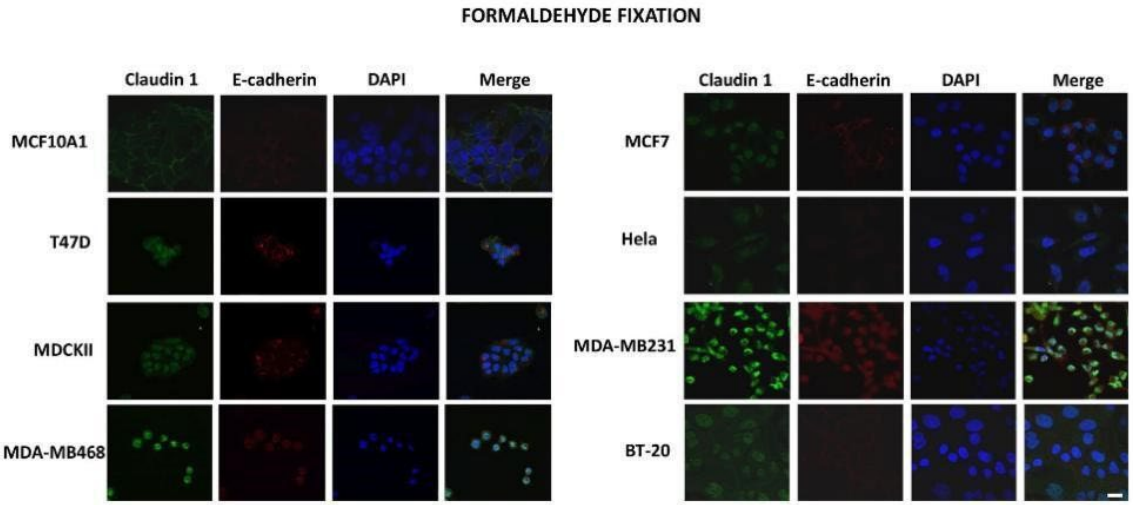

Figure 4. Immunofluorescent detection of E-cadherin and claudin 1 in different cell lines following formaldehyde fixation

1.3 Detection of breast milk:

BC 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 BC. 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 BC by identifying these resistant cells and developing more effective targeted therapies.

Enriched cells from breast milk of a donor with BC are found to contain CSCs and mutations in genes associated with BC initiation and/or progression. BC diagnosed during pregnancy or post-partum is relatively rare, affecting approximately one in 3000 women, and is often associated with high metastatic potential due to the organ remodelling that occurs during lactation, which provides a favourable environment for metastatic cells to thrive [32].

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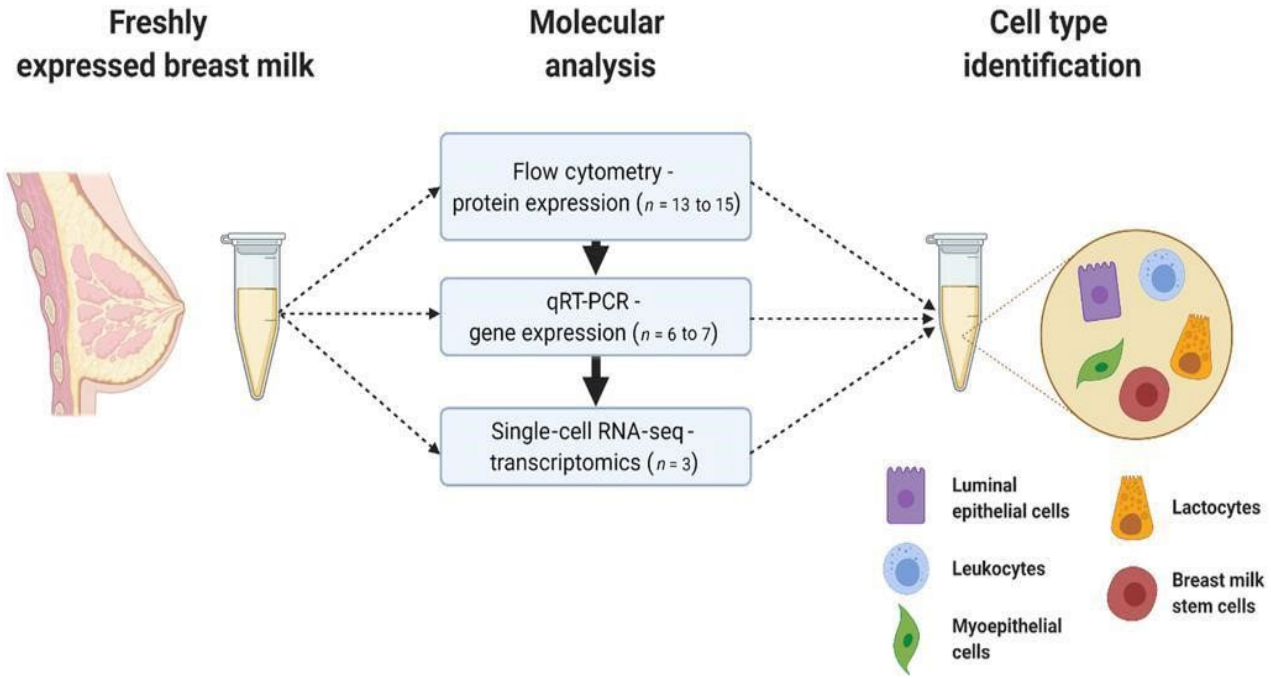

Figure 5. Detection of breast milk

2 Image diagnosis

2.1. Mammography:

Mammography is the preferred approach to screen and diagnose BC, providing clinicians with vital information about BC patients. According to research, early mammography screening can potentially reduce the mortality rate of BC patients by 30%-40% [33]. In the meantime, only 4%-10% of BC patients receive a positive diagnosis based on the results of mammography [34]. Over time, advancements in mammography technology have led to the development of new diagnostic techniques. Currently, the two primary strategies used for diagnosing BC patients in clinical settings are contrast-enhanced mammography (CEM) and digital breast tom synthesis (DBT)[35]. Research indicates that in terms of diagnostic accuracy and assessment of disease extent, CEM is comparable to breast MRI and outperforms full-field digital mammograph [36]. The development of computer-aided detection (CAD) in 1998 significantly enhanced the sensitivity of instruments from approximately 60% to 100% [37]. Combining CEM with CAD is an effective approach to diagnose BCpatients. This technique enables the classification of breast masses, and the ROC curves for patients can be significantly increased to 0.848 ± 0.038 (P < .01) [38]. Likewise, combining CAD with DBT can enhance the reading time by approximately 29.2%, and the ROC curves for patients can be elevated from 0.841 to 0.850 (95% CI, −0.012 to 0.030) [39].

Overall, mammography and its derivatives are essential tools for screening and diagnosing BC 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 BC. 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[40].

In the future, mammography is expected to become more advanced, with higher resolution and reduced harmful effects. Moreover, with the progress of artificial intelligence (AI) techniques and sensor development, the automation of BC detection and analysis is becoming increasingly feasible.

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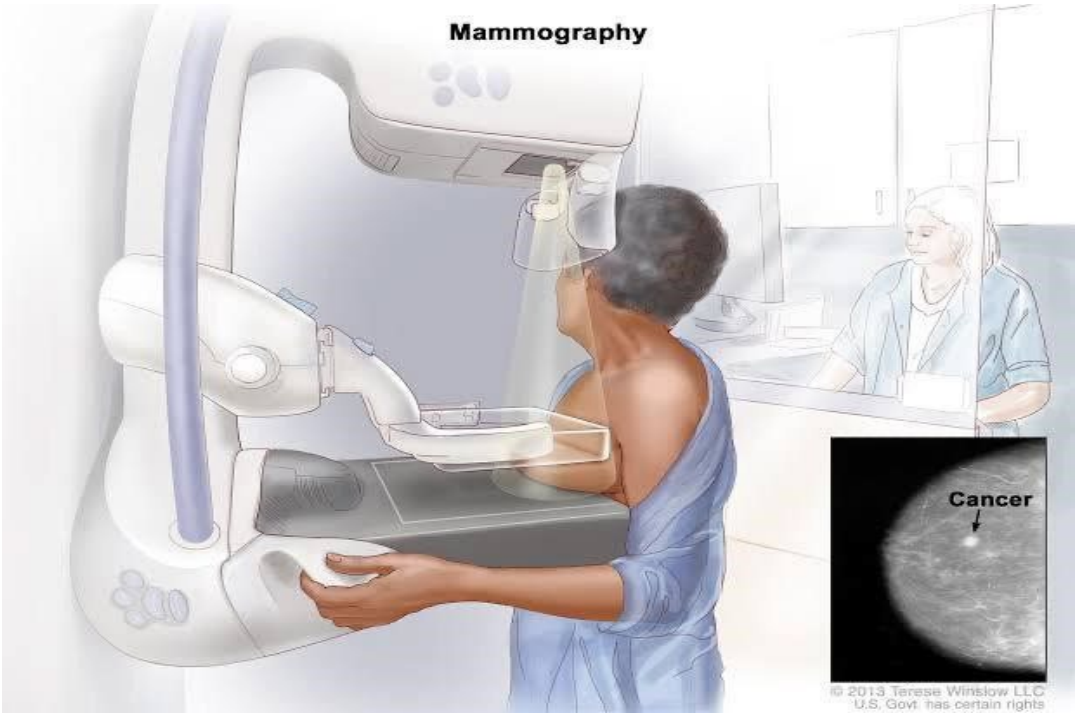

Figure 6. Screening and diagnosis of breast cancer by Mammography

2.2 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 is 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 [41]. 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 [42]. 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[43]. Studies have shown that utilizing elastic US to screen suspected pathological tissues has significantly improved the accuracy of BC diagnosis [44]. However, by incorporating three-dimensional US, elastic US can be utilized to diagnose axillary lymphadenopathy and categorize the state of a patient's tumor [45]. Although mammography is considered the preferred method for detecting calcification in BC, small-sized calcifications can be challenging to detect through mammography or regular ultrasound [46].A novel technique in US image processing called Micro Pure 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[47]

US is advantageous as it requires minimal use of contrast agents, does not emit high-energy rays, and is suitable for all age groups. Moreover, when MG is not feasible, US can be used as an alternative diagnostic method for BC. However, US has certain limitations such as the need for professional operation and lower definition and resolution compared to CT. Additionally, US is not recommended for obese patients or those with metastasis in the parasternal lymph nodes. In the future, intelligent US detection is expected to become a new trend, which will significantly reduce errors resulting from unprofessional judgments and provide doctors with more accurate diagnostic results.

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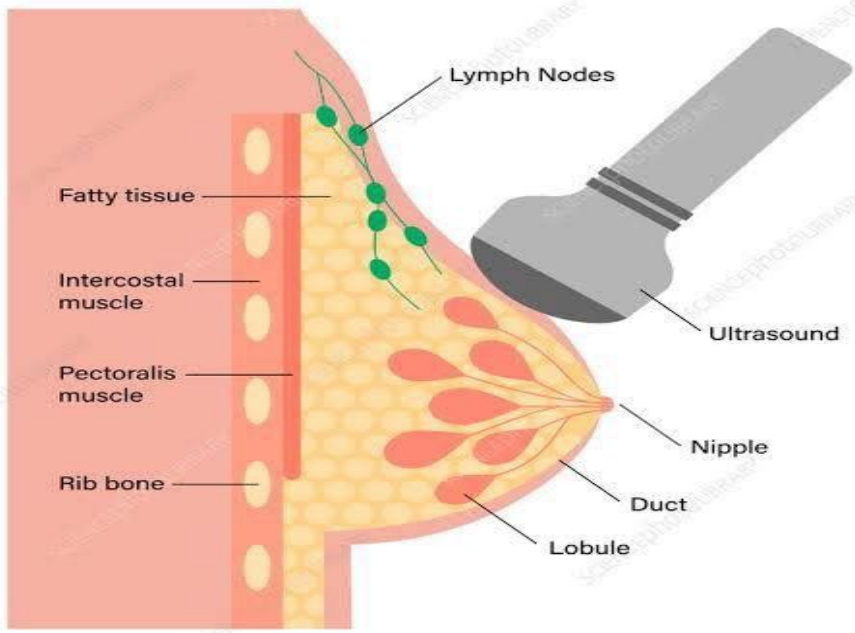

Figure 7. Detection of breast cancer by Ultrasonography

2.3.Magnetic resonance imaging:
Magnetic resonance imaging (MRI) enables the detection of familial BCat an early stage, without being affected by factors such as the patient's age, breast density, or risk status [48. 49]. 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 [50]. A recent review has provided threshold values for ADC, which are considered optimal for differentiating between benign and malignant lesions. These values range from 1.06 × 10−3 mm2/s to 1.10 × 10−3 mm2/s [51]. Dynamic contrast-enhanced MRI (DCE-MRI) provides higher resolution of soft tissues compared to MRDW. This technique can accurately display the morphological and haemodynamic features of lesions in vivo [52]. According to research, the combination of DCE-MRI and MRI alone has a higher positive predictive value (98%) than MRI alone (77%), with a specificity of 97%. In comparison to other diagnostic techniques for BC, such as biannual DCE-MRI and annual MG, this combination has demonstrated low recall rates [53]. Magnetic resonance spectroscopy (MRS) is a non-invasive technique that can enhance the diagnostic accuracy of BC, assess the risk of developing BC, and assist in the treatment of the disease. This has been reported in several studies [54]. Magnetic resonance elastography (MRE) is a unique type of magnetic resonance technology that utilizes mechanical waves transmitted through tissues to provide information on tissue elasticity [55]. PET/MRI, which combines positron emission computed tomography (PET) with MRI, has the ability to display soft tissue structures of the breast and chest wall. With PET's ability to provide molecular-level information in vivo, the PET/MRI combination has been shown to improve the positive predictive rate of patients and has significant value in evaluating BC metastasis, according to research studies [56].
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.

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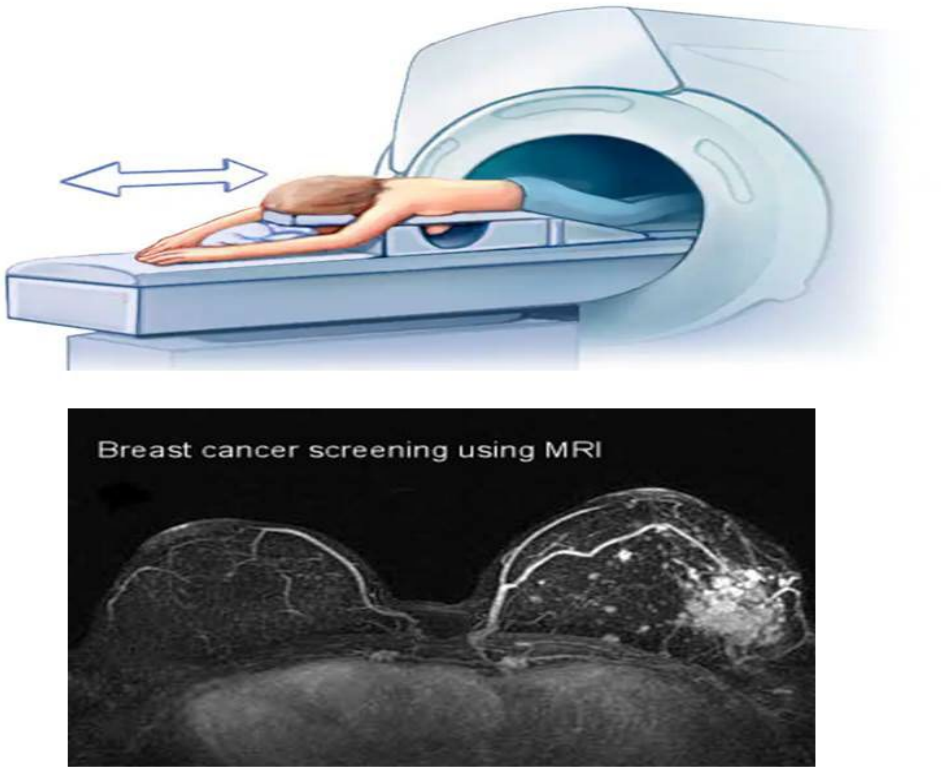

Figure 8. Magnetic resonance imaging for diagnosis of breast cancer

3. Molecular biotechnology examination:

3.1 Nucleic acid hybridization:

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

FISH is a powerful tool in molecular biology diagnostics, with its principle based on base pairing [57]. 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 [58]. 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. Multicolour fluorescence in situ hybridization is a potential future direction to greatly improve throughput when searching genetic sites [59]. Another precise and sensitive technique for diagnosing BC is 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) [60]. Currently, Cell-SELEX is one of the most widely used methods for obtaining high-quality aptamers from tumors [61]. 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% [62].

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.

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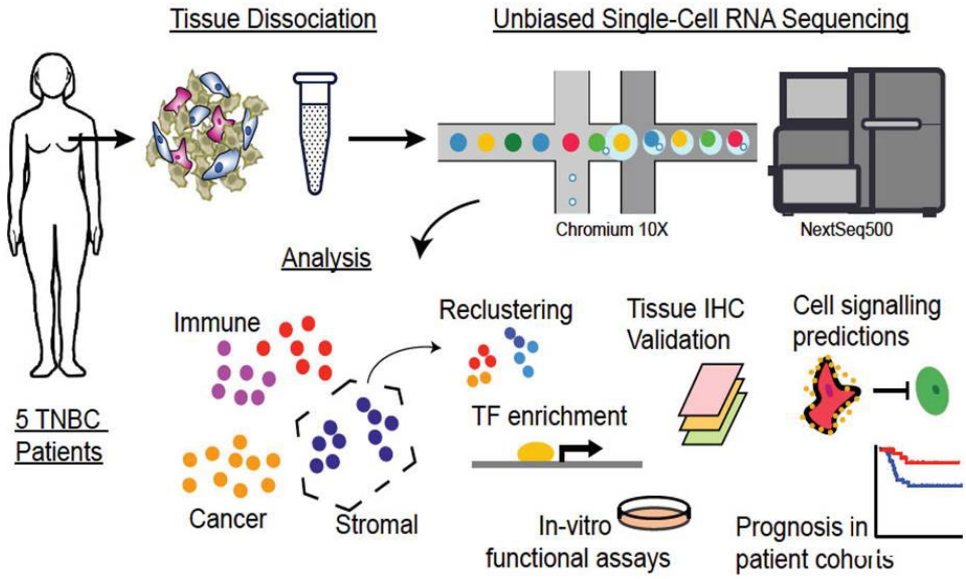

Figure 9. Mechanism fornucleic acid hybridization technique

3.2 Gene chip Sequencing:

A commonly used technique in diagnosing BC 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 BCcells 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 [63, 64]. Two methods are available for preparing gene chips: in situ synthesis and direct point method [65].

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.

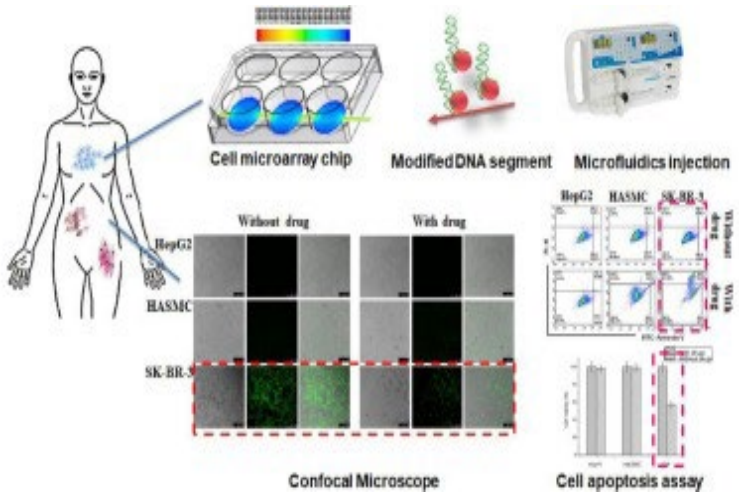

Figure 10. Gene chip Sequencing for detection of breast cancer

3.3 Fluorescence Quantitative PCR system:

The real-time fluorescence quantitative PCR (RT-qPCR) system is a valuable tool in monitoring nucleic acid amplification and predicting protein expression in BC. Although various biomarkers such as cfDNA, ctDNA, lncRNA, circRNA, microRNA, etc. are expressed in BC,

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their low content makes detection difficult using conventional instruments. Hence, the RT-qPCR system is a suitable option as it can predict BC risk by analyzing mRNA expression levels. This method has several advantages, including high sensitivity and specificity, less time consumption, and lower sample requirements compared to other molecular techniques [66]. RTqPCR is considered as the optimal technology to identify the differences in mRNA expression levels between malignant tumors and normal tissues [67]. Matouk et al employed the system to evaluate the expression of the H19 gene in both BC patients and healthy individuals and discovered significant differences in their expression levels, indicating the potential of the H19 gene as a diagnostic biomarker for BC. Nevertheless, obtaining reliable outcomes necessitates the extraction of high-quality Mrna [68]. The occurrence of cancer can also be attributed to DNA methylation in the gene promoter region, which can have similar effects to gene mutations, such as loss or gain of gene function [69].The Methylation-based RT-qPCR system is commonly utilized to analyze genetic methylation patterns. In particular, MethyLight can be employed to investigate the expression of methylated silencing genes in cell lines treated for BC. The methylation of the ESR1 gene may serve as a potential biomarker for liquid biopsy-based risk evaluation of BC. Moreover, MethyLight can assist in identifying chemo-resistance in breast tumors by analyzing methylation genes[70].

Thus, MethyLight has a crucial role in the diagnosis of BC. MethyLight, despite its importance, has some limitations that must be considered. For instance, the nucleic acid used must be specifically treated, either fully methylated or unmethylated. Moreover, designing the required probes can be a complex process, and its operation requires professional expertise.

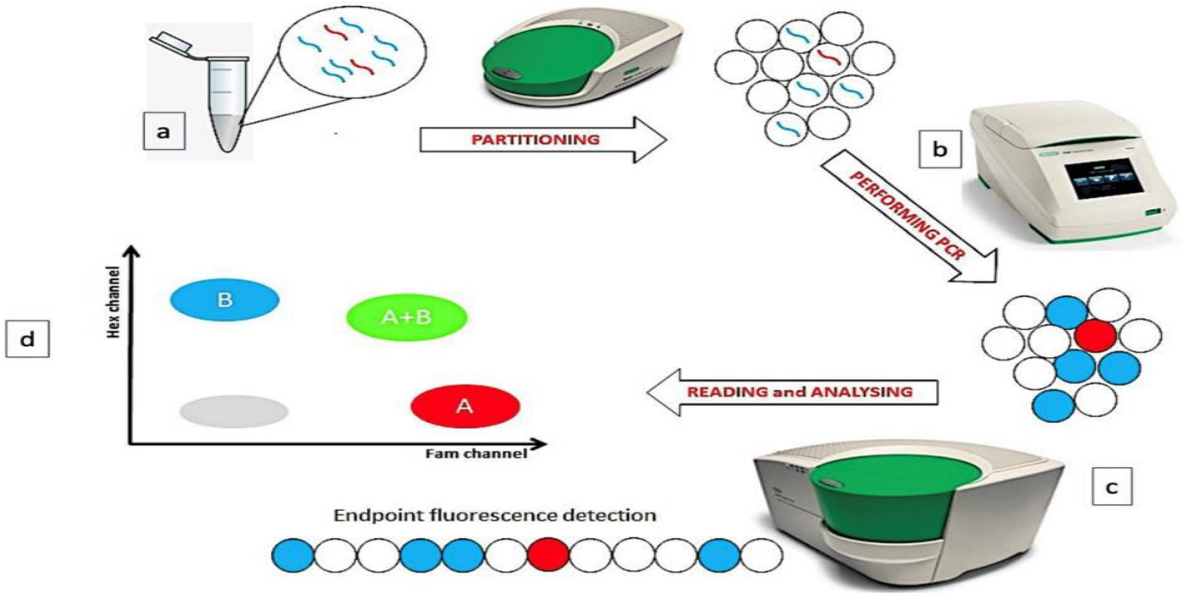

Figure 11. Fluorescence Quantitative PCR system for detection of breast cancer

3.4 Immunochemistry:

Immunostaining (IHC) is a helpful diagnostic tool for accurately identifying the location of tissue organization. There are four main advantages to using IHC analysis in breast tumours: it can distinguish between benign and malignant tumours, assess interstitial infiltration, distinguish between ductal and lobular tumours, and detect protein expression associated with BC treatment and prognosis, helping to guide endocrine therapy and prognosis [71, 72].

IHC is a method that utilizes antigen-specific binding of antibodies labeled with color reagents, such as fluorescein and metal ions, to detect various antigens, proteins, and peptides. It can be used to screen and diagnose BC patients by assessing the levels of marker proteins. Furthermore, IHC is a useful tool for researchers to investigate the correlation between external factors and BC. By analyzing protein levels, IHC can provide insights into the mechanism of breast tumors. However, IHC is a time-consuming process that requires fluorescence labelling, which can be challenging to prepare.

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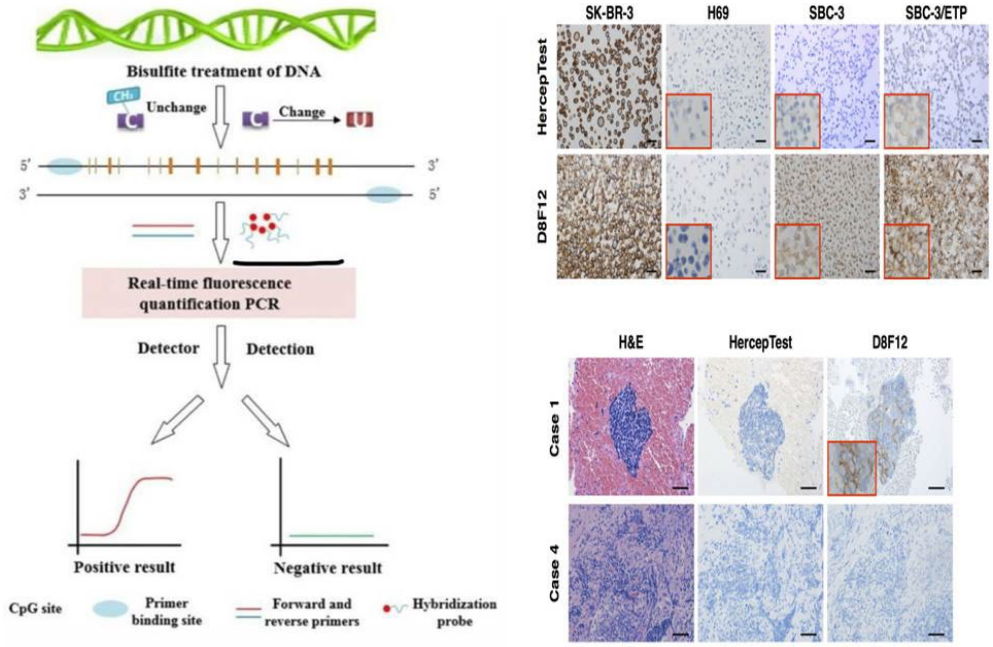

Figure 12. Immunochemistry assay method for detection of breast cancer

3.4 Western blot:

Western blotting also relies on the highly specific antigen-antibody binding characteristic. While Western blotting has a poorer capacity for histological localization than IHC, it has a more accurate capacity for quantitative protein level analysis. In contrast, RT-qPCR and Western blotting both evaluate protein levels quantitatively, but their detection objects differ. RT-qPCR is for nucleic acids, while Western blotting is for proteins [73]. Western blotting can be used not only to determine the expression of proteins, but also to confirm if the protein expression is abnormal [74].

In the future, there may be a tendency for the price of Western blot agents to decrease and for the process of Western blot operation to be simplified. However, Western blotting still has some deficiencies, such as the use of expensive agents and the potential for false positives, as well as the need for professional operation.

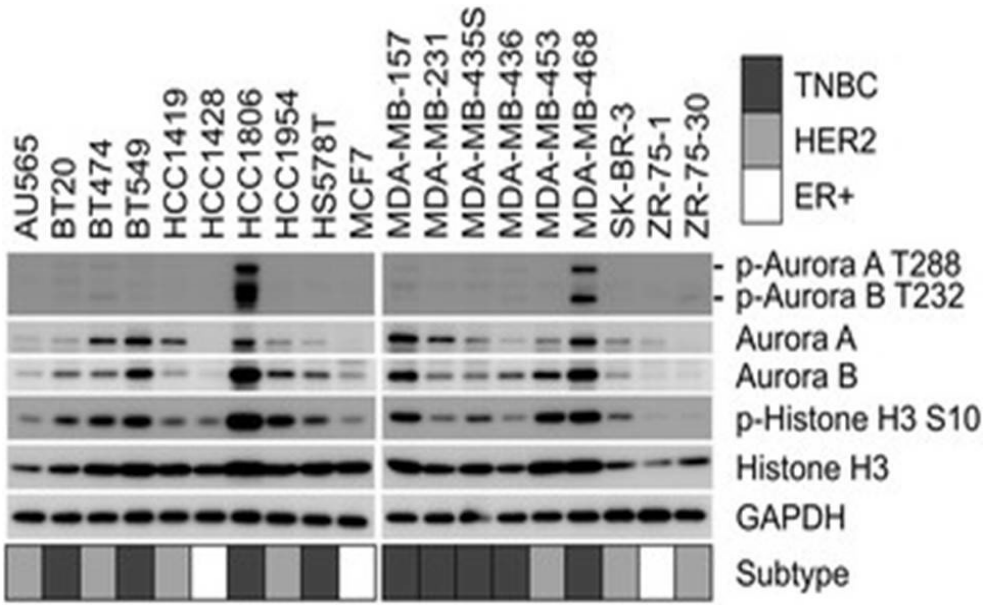

Figure 13. Western blot analysis of breast cancer cell lines

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3.5 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 [75]. Developed in the 1960s, FCM is a highly interdisciplinary technology that combines cytochemistry, immunology, and materials science
e, 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 [76]. However, in FCM, cells or biological particles must first be treated and labelled to enable detection by laser. Despite its advantages, FCM has the limitation of requiring pre-treatment and labelling 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 [77].

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.

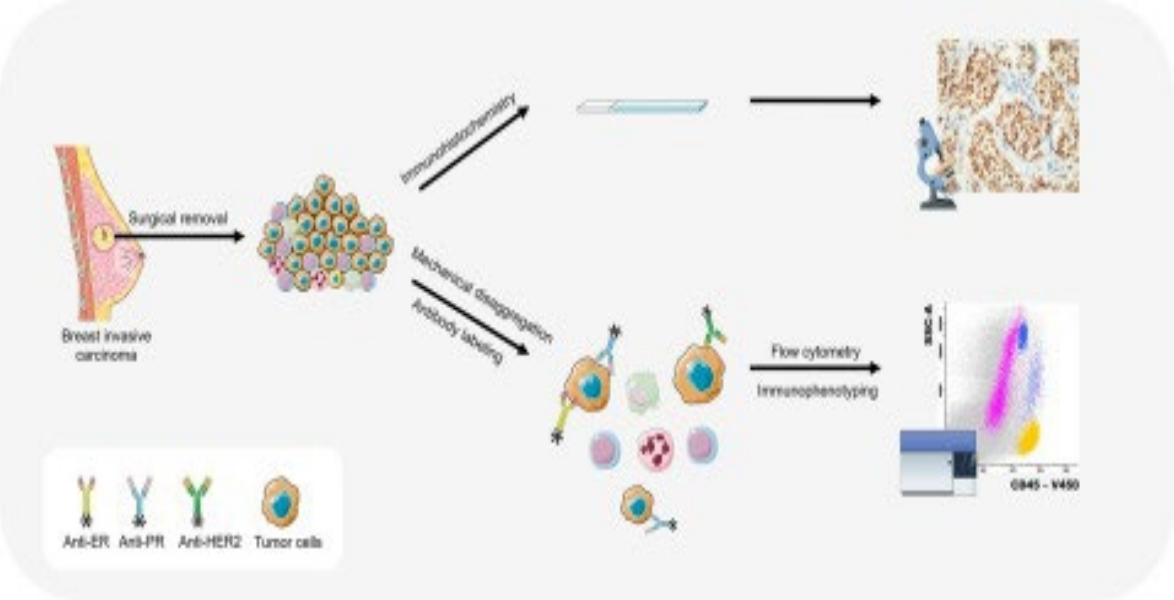

Figure.12 Flow cytometery technique for detection of breast cancer

II. CONCLUSION:

In conclusion, in vitro methods for the detection of BC using cell line studies, imaging diagnosis, and molecular biotechnology have provided valuable insights into the pathogenesis of BC. The use of cell line studies has allowed for the examination of the mechanisms underlying BC 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 BC 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 BC. These biomarkers, such as HER2, estrogen receptor, progesterone receptor, and Ki67, are used clinically to determine the molecular subtype of BC and guide treatment decisions.

Overall, the integration of these various in vitro methods has greatly improved our understanding of BCand 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 BC detection and treatment.

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