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Multi-marker Enrichment Enhances the Sensitivity of Electrochemical Sensing Technology for Breast Cancer Detection

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Abstract. Breast cancer is one of the most common cancers, and early detection is crucial for improving patient survival. However, conventional imaging and serum-based diagnostic methods still suffer from limited sensitivity or limited applicability in early-stage detection. In recent years, biomarker-based detection approaches have been widely applied in breast cancer diagnosis and have improved detection accuracy to some extent. Nevertheless, single-biomarker detection is often insufficient to reflect the high heterogeneity of breast cancer, which may lead to inaccurate diagnostic results. In this article, the diagnostic accuracy of single-biomarker detection and multi-biomarker detection is compared. The results show that multi-biomarker detection can significantly increase detection accuracy by providing more comprehensive molecular information. By analyzing multiple biomarkers simultaneously, diagnostic reliability can be improved and the risk of false results can be reduced. In order to achieve highly sensitive and low-cost detection, electrochemical biosensing technology is introduced for multi-biomarker detection. Due to its high sensitivity, fast response, simple operation, and low cost, electrochemical biosensing technology shows great potential for efficient and accurate breast cancer detection.

Keywords: Breast cancer, multi-biomarker, electrochemical biosensing technology

1. Introduction

Breast cancer, as one of the most common cancers in the world, has a major impact on people' s health and daily life. According to the World Health Organization, breast cancer occurs in almost every country globally, and about 670000 people died from it in 2022 [1]. Furthermore, breast cancer mainly affects women, with the female gender being the strongest known risk factor [1]. Breast cancers occur in women about 99%, while occurrences in men are rare, representing only 0.5-1% of all cases [1]. In order to reduce mortality from breast cancer, early detection and treatment are necessary. Many countries use mammograms as the common detection technology to detect breast cancer. However, only using mammograms to verify breast cancer cells has a high risk to misdiagnosis. Scanning breast density is the main step for mammograms, so breast density is a key determinant of mammographic sensitivity. Fatty tissue appears gray on mammograms, glandular tissue appears white, and cancer cells also appear white, which reduces lesion accuracy [2]. To

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improve accuracy, researchers are now using biosensor technologies to convert breast-cancer-related molecules into specific and easily identifiable signals, helping achieve more reliable detection. Current biosensor technologies, the electrochemical biosensing technologies, achieve rapid, sensitive and portable ways to detect cancer cells. These methods identify redox reactions at the electrode or membrane surface through monitoring changes in parameters. And then convert these redox reactions into clear diagnostic signals [3]. The core of electrochemical biosensing is the use of biological markers, including aptamers, antibodies, enzymes, or proteins. When recognition molecules bind to cancer biomarkers through their three-dimensional structures, some measurable changes will occur at the electrode [3]. Although more effective than mammography, many electrochemical are designed to interact with only one biomolecule at a time, which lowers the accuracy of detection results [3]. In this context, by comparing the results of using single marker and multi-marker to detect cancer, multi-marker enrichment enhances the sensitivity of breast cancer detection. This process enhances the accuracy of diagnosis. So in this paragraph, the limitations of traditional breast cancer detection will be discussed, and why they are being gradually replaced will be mentioned. By comparing single-biomarker detection with multi-biomarker detection, the advantages of enriching biomarkers are highlighted. Furthermore, this article will explore the integration of electrochemical biosensors with multi-biomarker detection, emphasizing its potential to improve diagnostic sensitivity and accuracy.

2. Evaluation of biomarkers suitable for breast cancer detection

To improve the diagnostic accuracy of liquid biopsy for breast cancer, it is essential to evaluate the commonly used biololgical markers and determine which offers the most reliable diagnostic applications. Circulating tumor cells(CTCs), which are released from primary or metastatic tumors, are shed into the blood and circulate in the peripheral blood [4]. Nowadays, numerous studied have showed that cancer cells is developing associating with the level of CTCs, which let CTCs be an important biomarker [4]. However, because of its low sensitivity and reproducibility, CTCs are not recommended as a biological marker for cancer detection [5]. Circulating tumor cells(ctDNA), a type of circulating extracellular nucleic acids(cfDNA), may also be derived from tumor cells [4]. Some studies have shown that ctDNA is with advanced-stage breast cancer and metastatic disease [5]. Patients who have breast cancer exhibit higher ctDNA levels compared with healthy people [5]. When the ctDNA fraction exceeds a certain threshold, sensitivity and specificity of breast detection will increase [5]. However, the shorter life of ctDNA is not suitable for detection of breast cancer [4]. Exosomes are nanoscale extracellular vesicles released by cells and mediate intercellular communication through transfering molecular cargo [6]. Exosomes are enclosed by a lipid layer membrane which can protect molecular cargo, including nucleic acids and proteins. Because of protection from the lipid layer, the reliability and stability of biomarker detection increase [6]. In addition, the molecular contents carried by exosomes reflect the biological state of their parents cells closely, particularly tumor-specific genetic information and protein expression profiles.These features make exosomes highly valuable for early cancer diagnosis, tumor sub-typing, and therapeutic response monitoring [6]. Although exosomes show great potential as cancer biomarkers, their clinical application is still limited by several technical challenges. In particular, the isolation and purification of exosomes remain difficult. Exosomes coexist with other vesicles, lipoprotein, and proteins in complex biological fluids, making it hard for current methods to achieve high purity [7]. In addition, some diagnostically relevant molecules carried by exosomes are present at a very low concentrations in body fluids [7]. Table 1 summarizes the advantages and disadvantages of commonly used biomarkers, as well as their biological sources.

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Table 1. Summary of different liquid biopsy markers from reference [4,6,7]

Form	Source	Advantages	Disadvantages
CTCs	Blood, cerebrospinal fluid, urine, etc	Complete genetic information	Low sensitivity and reproductivity
ctDNA	Blood, urine, saliva, synovial fluid, etc	High rarity (especially in early-stage tumors)	Short half-life Low heterogeneity
Exosome	Blood,urine, cerebrospinal, etc	High stability Specificity and high sensitivity Non-invasive sampling and patient-friendly	Low-abundance Dependent on special detection techniques

3. A significant disadvantage of

In the previous discussion, various biological factors have shown certain advantages as cancer biomarkers in terms of sensitivity, specificity, and clinical feasibility. However, they also face common challenges, such as limited information content, insufficient stability, or an inability to fully capture tumor heterogeneity. Under these circumstances, a biological carrier that can remain stable in body fluids and carry multiple types of molecular information, which may improve the accuracy of disease detection. Under these conditions, exosomes, owing to their unique membrane structure and multi-molecular cargo capacity, have gradually become a major focus in biomarker research and development. However, due to the low abundance of tumor-derived exosomes and the complex background of biological fluids, the accuracy of detection will decrease.

4. Several cancer-related studies focusing on the comparison between single-biomarker and multi-biomarker detection and the advantages of multi-biomarker detection are recorded

According to previous studies, compared to using a single biomarker, using multimarker panels will provide sufficient biological information [8]. For example, in liquid biopsy studies of pancreatic ductal adenocarcinoma (PDAC), this conclusion has also been consistently validated. In studies of PDAC detection, individual biomarkers were first evaluated in blood samples [8]. Based on these results, multimarker panels were then constructed [8]. These panels combined proteins with other biomarker types, such as circulating DNA or extracellular vesicle–derived molecular cargo [8]. Evaluations show that single protein biomarkers typically yield AUC values in the range of 0.7–0.8 when distinguishing early-stage PDAC patients from healthy controls [8]. On the other hand combinations of multiple proteins or proteins combined with metabolites or nucleic acids can significantly increase AUC values to above 0.9 [8]. In studies focusing on early-stage PDAC, multi-biomarker panels have achieved AUC values ranging from 0.76 to 0.99, with some multi-purpose strategies approaching an AUC of 1.0 [8]. The advantage of using multi-biomarker is not only shown in the detection of PDAC, but also in the detection of colorectal cancer(CRC). In CRC, changes in lipid-related molecules are commonly observed in patient serum [9]. Several lipid-associated proteins show altered levels in CRC patients, among which apolipoprotein A2(ApoA2) has attracted attention [9]. However, if only use ApoA2 as the single biological marker, the diagnostic accuracy was limited [9]. Better performance was achieved when ApoA1, ApoA2, lithocholic acid(LCA), and carcinoembryonic antigen(CEA) were analyzed together [9]. The AUC was evidently greater than that of individual lipid indicators, bile acid, or tumor marker [9]. To sum up, the research in the field of pancreatic ductal adenocarcinoma and colorectal cancer demonstrates

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that the detection of multi-biomarkers is more effective as compared to single biomarkers. The research tendency also offers valuable implications in the direction of the early detection of breast cancer. Breast cancer exhibits a great level of cellular heterogeneity, and the usage of a single biomarker does not normally lead to the establishment of consistent and reliable diagnostic results. Hence, the simultaneous acquisition and stabilization of several tumor-related molecular expressions in the multifaceted body fluids is a pivotal condition of the effective implementation of multi-biomarker recognition in breast malignancy.

5. Methods of exosome enrichment

Analysis of exosomes detection. Ultracentrifugation, polymer-based precipitation are the most popular traditional exosome isolation strategies [10]. Nevertheless, these methods are time-consuming in most cases and experience the challenge of determining the balance between recovery yield and purity [10]. Besides this, they are also likely to co-isolate huge volumes of free proteins, lipoproteins, or other non-vesicles [10]. These disadvantages exacerbate the background noise, have a pronounced effect on EV isolation quality, and could present bias when running downstream analyses [10]. But in the recent years, microfluidic technology has been applied in enriching the exosomes. The microfluidic devices are capable of working with biofluid samples in extremely small channels [11]. Selective collection of particular exosomes can be achieved by antibody binding, size filtering or fluid flow effects [11]. The microfluidic systems require less time and less sample as compared to the traditional separation techniques [11]. They also are useful in eliminating undesirable constituents, including free proteins and lipoproteins [11]. Consequently, it is possible to reduce the background noise during the detection. Moreover, certain microfluidic systems are able to preserve the structure of exosomes [11]. This aids in preserving the biological data transported by exosomes and enhances the level of detection.

6. Electrochemical sensing platforms for detection of enriched exosome

Biomarkers of cancer cells should be followed after the enrichment of exosomes to detect breast cancer [12]. This can be measured using the electrochemical biosensing, rapid, sensitive, and portable, which monitors the change in the electrochemical parameters [13-16]. Subsequently, redox causes chemical variations at the electrode or the surface of the membrane, which may be recorded and directly evaluated into determining diagnostic findings [17,18]. As an illustration, integrating the independent testing biomarkers, cancer antigen 15-3(CA 15-3) and HER2 extracellular domain(HER2-ECD), is one of the major steps in the diagnosis of high-risk BC [19] diagnosis. Also, the bi-immunisensor was initially subjected to a sample of HER2-ECD with CA15-3, and a layer overlaid, to identify these antibodies [3]. Then the information based on the detection is gathered. The maximum current has a peak rate [3] on a voltammogram. Individual deposition gathers respective, which eliminates undesired overlap of signals [3]. Besides, a sensor board was also placed into an electrical connector which could interface working electrodes and a dual-channel electrochemical reader [19]. With this plan, it is possible that the dream of detecting enriched exosomes can become possible.

7. Conclusion

In this paper, improved detection strategies are compared to traditional detection methods whereby, multi-biomarker testing is superior to single biomarker testing in regard to breast cancer detection.

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The greatest benefit of single-biomarker is that the use of a single biomarker can only give a partial result such that it can be misdiagnosed. Besides this, in case the concentration of the biomarker is low, the biomarker might not be detected at all. Conversely, when we apply several markers then we get more comprehensive information as well as a more accurate conclusion. This conclusion is significant in order to have important practical screening and follow up. When a test is in a position to identify many signals on a single sample, there will be an ability to detect a lot of biological markers simultaneously, hence making it not to be tested repetitively. Electrochemical biosensing can come in handy in such a situation since several markers can be analyzed on a single platform. The electrochemical biosensing could help to make the concept of multi-biomarkers detection more effective and more affordable because of its sensitive signal readout and straightforward design. Although this has been developed, the majority of the research prototypes are yet to be transformed into clinical practice. There are still a lot of studies that include small samples or that are deliberately controlled and no extensive clinical validation has been done yet. How the field of diagnostic technologies will evolve in the future remains yet to be seen but it will be up to future labor whether electrochemical biosensing testing can really help Assist in the detection of breast cancer, through the aspects of standardization, stability testing, and increased clinical trials.

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