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Application of enzyme-linked immunosorbent assay in cancer detection

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Abstract. The enzyme-linked immunosorbent assay (ELISA) has emerged as an indispensable method in the realm of cancer detection., serving as a significant factor in the timely identification, prediction, and surveillance of many forms of cancer. ELISA as a highly sensitive immunological assay, utilizes the binding affinity between antibodies and antigens to detect and quantify cancer-associated biomolecules, such as tumor markers, oncogenic proteins, and specific antibodies. The recently adopted methodology facilitates the identification of cancer-associated biomarkers in various types of samples, such as plasma, urine, and tissue extracts. In the context of cancer diagnosis, ELISA aids in the early detection of malignancies by detecting cancer-specific biomarkers at low concentrations, allowing for timely intervention and improved patient outcomes. This research provides a concise overview of the multifaceted applications of ELISA in the field of oncology, especially in electrochemical ELISA and gold nanoparticle–based ELISA. It discusses the diverse biomarkers employed, and elucidates its significance in cancer research and clinical practice.

Keywords: ELISA; cancer detection; electrochemical ELISA; nanoparticle-based ELISA.

1. Introduction

Cancer continues to be a prominent and significant health issue in contemporary society. The disorders in question are defined by the uncontrolled proliferation and dissemination of atypical cells, presenting a complicated and multifaceted nature. Globally, the incidence and mortality of cancer are rising as a result of population expansion, aging, and numerous socioeconomic factors [1]. With a multitude of subtypes, cancer has garnered significant attention from the medical and scientific communities. Traditional cancer treatment modalities, such as surgery, chemotherapy, and radiation therapy, have undoubtedly made significant strides in extending patient survival rates and improving their quality of life. However, these approaches often face inherent limitations. They can be invasive, non-specific, and fraught with adverse side effects. Moreover, these treatments may not be effective in cases of late-stage or metastatic cancers, leaving patients with limited therapeutic options.

In light of these challenges, the exploration of novel and targeted approaches to cancer detection and treatment has become paramount. Newer methods aim to enhance the precision of cancer diagnosis and therapy, while minimizing harm to healthy tissues. One potential area of exploration involves the utilization of enzyme-linked immunosorbent assay (ELISA) for the purpose of cancer detection. ELISA, with its exceptional sensitivity and specificity, holds the potential to revolutionize the way we identify and manage various cancer types. ELISA is a well-established and versatile immunological technique. Its fundamental principle involves the specific binding of antibodies to antigens, often yielding highly accurate results. In the context of cancer detection, ELISA offers several distinct advantages. This technology enables the identification of minuscule amounts of cancer-associated biomarkers in diverse physiological fluids, providing a non-intrusive and potentially timely method of diagnosis. ELISA's ability to differentiate between specific cancer types adds a layer of precision that can aid in treatment planning. One often employed protein for cancer detection in diagnostic applications is p53, a tumor suppressor protein. In instances of cancer formation, the body exhibits an elevation in the concentration of p53. The heightened level of concentration can be observed as an indicator for inferring the progression of malignancy [2].

The utilization of ELISA in cancer detection is a burgeoning field of research. Ongoing studies seek to refine assay methodologies, identify novel biomarkers, and expand the applicability of ELISA to a wider spectrum of cancer types. Accurately determining and quantifying the proteins exclusively made and secreted by cancer cells is a task of significant importance, albeit one that presents considerable challenges. The difficulty occurs as a result of a significant proportion of proteins secreted by cancer cells being synthesized in minuscule quantities, occasionally eluding detection by conventional protein identification methodologies. Hence, there is a requirement for a measurement technique capable of identifying minuscule quantities of protein. The ultrasensitive ELISA technique, as devised by Watanabe and Ito, integrates the principles of the sandwich ELISA technique, coupled with thio-NAD cycling, is employed to enhance the accuracy of quantifying small amounts of proteins [3]. This article delves into the current research regarding the application of ELISA in cancer detection, presenting recent findings, challenges encountered, and potential future directions. In the current era of personalized medicine, the use of ELISA as a robust diagnostic tool within the field of oncology represents a significant advancement to combat cancer with more efficacy. This article aims to provide a comprehensive analysis of the notable advancements achieved in utilizing the capabilities of ELISA for the purpose of cancer diagnosis.

2. Electrochemical ELISA for cancer detection

Electrochemical ELISA is an ingenious amalgamation of electrochemical sensors and the classic ELISA technique. It leverages the specificity of antigen-antibody interactions while capitalizing on the electrochemical signal generated upon binding events. The standard ELISA detection limit falls marginally within the range of concentrations below the nanomolar threshold, which is insufficient to meet clinical threshold for the majority of protein biomarkers, especially during the first phases of illnesses [4]. By virtue of its unique features, electrochemical ELISA exhibits several advantages over traditional ELISA, such as enhanced sensitivity, rapid response times, minimal sample requirements, and the potential for miniaturization. These attributes make it an enticing option for cancer detection, holding the promise of earlier and more accurate diagnoses.

The scientific community has witnessed a surge in research dedicated to the development and refinement of electrochemical ELISA platforms for cancer detection. Researchers worldwide have explored a myriad of approaches, encompassing the selection of suitable biomarkers, design of electrode materials, and optimization of detection methodologies. Promising results have emerged, with numerous studies showcasing the potential of electrochemical ELISA in detecting various types of cancer, ranging from breast and prostate to lung and ovarian cancer.

Nowadays, a plethora of novel biomolecules have been put forth as potential biomarkers for various types of malignancies. As a result, there has been a proliferation of innovative biosensors designed specifically for the identification and measurement of these biomarkers [5]. The utilization of aptamers as a replacement for antibodies in the context of electrochemical biosensors, also known as electrochemical immunoassay, is observed in these biosensors. The schematic diagram depicted in Figure 1 provides visualisation for the possible mechanisms that are implicated in the electronic interaction between enzyme labels and electrodes. In enzyme-electrode systems, there are several possible electron transfer mechanisms. Firstly, direct ET happens between the enzyme's active center and the electrode. Additionally, redox molecules can aid ET by serving as enzyme substrates and undergoing electrochemical recycling at the electrode. Furthermore, facilitated ET can occur within enzyme cascades. Lastly, altering the ET signal due to hydrolysis of electrode layers is a distinct scenario worth considering [6].

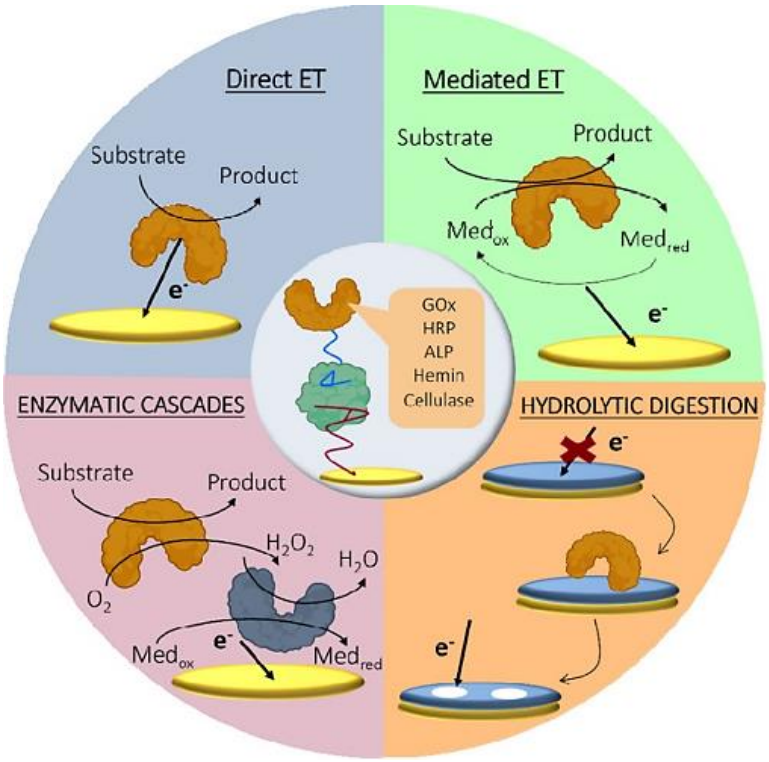

Figure 1. The various potential forms of communication that can occur between enzymes and electrodes [6].

The ELASA is a diagnostic technique that utilizes a traditional sandwich assay format, for target recognition and utilizes an electrochemical technique for detection [5]. The immunoassays are typically composed of three distinct layers: an immobilised biorecognition molecule (called a probe), a target analyte specifically attached to the biorecognition molecule, and a secondary recognition molecule that binds to the target analyte and is labelled with an electrochemically active signal. In the context of measurement, the electrochemical signal tag serves the purpose of either immediately generating the signal or inducing a subsequent interaction with a substrate [6].

2.1. The utilization of horseradish peroxidase (HRP) as a labelling agent

Horseradish peroxidase (HRP), which is obtained from the root of the horseradish plant, is classified as an oxidoreductase and has gained significant usage in ELISA and biosensor development [7]. This is primarily attributed to its notable attributes, including high activity, selectivity, and proficiency in catalysing the process of oxidizing diverse organic and inorganic substrates [8]. Hydrogen peroxide reductase (HRP) efficiently catalyzes the oxidation of compounds like 3,3',5,5'-tetramethyl benzidine (TMB) and hydroquinone (HQ) by reducing hydrogen peroxide [9]. In the study, a sensor was employed, featuring Mn_3O_4/Pd@Pt nanoezyme labels enhanced with HRP and a secondary aptamer. Additionally, gold electrodes are immobilised with tetrahedral DNA nanostructure-aptamer recognition probes. This sensor is specifically designed for the detection of HER2 and demonstrates a linear operational range that extends from 0.5 femtomolar (fM) to 0.5 nanomolar (nM), accompanied by a LOD of 0.5 fM. The assay exhibits favorable selectivity and storage stability, effectively identifying the presence of HER2 in serum samples for the purpose of breast cancer detection [10].

2.2. The utilization of glucose oxidase as a labelling agent

Glucose oxidase (GOx) catalyses the oxidation of glucose to D-glucono1,5-lactone by the utilisation of oxygen as an electron acceptor, afterwards undergoing reduction to H_2O_2 [11]. The utilisation of GOx was employed in the identification of thrombin for the purpose of detecting various types of cancers through the implementation of a sandwich aptasensor. Furthermore, the secondary aptamer

was immobilized on a substrate consisting of reduced graphene oxide coated with platinum nanoparticles (PtNPs), alongside the process of GOx. The oxidation of GOx was catalysed by PtNPs, resulting in the formation of H_2O_2. The reaction was later examined by the utilization of differential pulse voltammetry (DPV). The sensor exhibits a linear operational span ranging from 0.3 picomolar (pM) to 35 nanomolar (nM), accompanied by a limit of detection (LOD) of 0.21 picomolar (pM). Furthermore, the sensor demonstrates high selectivity and repeatability [12].

Contemporary research focuses on advancing electrochemical aptasensors, known as ELASAs, as a promising method for detecting cancer biomarkers, challenging traditional ELISA. However, the transition of ELASAs from labs to commercial use faces hurdles, primarily the need for practical, point-of-care platforms and clinical validation of these biosensors [13].

3. Electrochemical ELISA for cancer detection

The concentrations of protein biomarkers in the blood that are linked to different forms of cancer exhibit a range spanning from 10^{-16} to 10^{-12} M. The limited abundance of a specific biomarker renders ELISA a suitable technique for the identification of cancer proteins. Nanotechnology has ushered in novel prospects across various domains of scientific inquiry. The advancements in the field of nanotechnology have led to significant development in its biological applications, particularly in the context of enhancing human health. Nanocarriers offer a theragnostic platform for the diagnosis of cancer [14]. Nanocarriers possess unique attributes in relation to their capacities in chemical solubilization, encapsulation, and disease marker detection. Gold nanoparticles (AuNPs) have gained significant attention in current scientific research due to their abundance and diverse use as nanocarriers.

The unique optical features of AuNPs have garnered significant attention within the domain of biomedical research. The fabrication of AuNPs is a straightforward process, rendering them highly suitable for use in various applications. Moreover, these nanoparticles exhibit biocompatibility, enabling their safe interaction with biological systems. Additionally, their surface can be effectively manipulated, allowing for precise control over their features. Furthermore, AuNPs possess remarkable stability, ensuring their long-term viability. Lastly, they exhibit surface plasmonic capabilities, which contribute to their unique optical characteristics. The incorporation of AuNPs in optical biosensors has promise for greatly improving the specificity, resolution, sensitivity and other performance characteristics of these devices [15].

3.1. An ELISA device utilizing AuNPs for oral diagnosis

Osteopontin (OPN) is a phosphoglycoprotein rich in sialic acid that has cytokine-like properties and is closely linked to the extracellular matrix (ECM). This particular protein is an individual belonging to the small integrin binding ligand and N-linked glycoprotein (SIBLING) family. The findings of multiple recent research indicate that the osteopontin is synthesized by stromal cells that have undergone tumor-induced reprogramming, hence facilitating the advancement of cancer. [16] The overexpression of OPN in oral tongue cancer was analyzed at the mRNA level using quantitative analysis. The study substantiated the upregulation of OPN in tongue neoplasms in comparison to healthy tissue. The study utilized immunohistochemistry (IHC) analysis as a method to identify the presence of elevated levels of OPN protein in tissue samples obtained from patients diagnosed with oral tongue cancer. The research further confirmed the overexpression of OPN in tongue tumors [17].

An ELISA technique utilizing gold nanoparticles was developed to enable the non-invasive detection of OPN. The utilization of AuNPs was employed to augment the sensitivity and precision of the test. The utilization of improved testing methods including AuNR and AuNS resulted in a noteworthy decrease in the detection limit for the analyte, namely 0.02 ng/mL and 0.03 ng/mL, respectively. This is in contrast to the commercially accessible OPN ELISA kit, which exhibited a detection limit of 0.14 ng/mL. The linear detection range of the modified ELISA assay was wide, ranging from 0.31 to

20 ng/mL [17]. Additionally, it demonstrated favourable reproducibility and specificity when evaluated against interferents present in saliva.

3.2. The detection of cancer

The current ELISA technique is constrained by sensitivity limits arising from the low surface-to-volume ratio of the polystyrene ELISA plate and the non-specific orientation of the adsorbed antibody (Ab) or antigen [18]. The accurate identification of biomarkers holds significant importance in facilitating the prompt diagnosis and treatment of cancers in their initial stages. Therefore, it is crucial to devise innovative approaches that demonstrate improved selectivity, sensitivity, and a lower LOD in order to facilitate routine diagnostic applications.

The utilization for AuNPs, which is applied in bioanalytical field has garnered significant interest, because to its advantageous characteristics, including a substantial loading capacity, a significant surface-to-volume ratio and enduring durability. The manufacture of stable layers of AuNPs was achieved using a chemical reduction procedure conducted on ELISA plates that are readily accessible in the market. The study revealed a significant enhancement in the protein binding ability, while maintaining the biological activity unaltered. The ELISA plates that underwent modification exhibited increased durability and indicated greater efficacy in indirect ELISA as compared to the ELISA plates used in the experiment were not changed and had good binding capacity.

A sandwich format ELISA was created using a GNPL-based platform. The results of this assay shown exceptional performance in terms of its limit of detection and sensitivity for the measurement of rabbit IgG in a buffer solution. Empirical studies that employed plasma infused with carcinoembryonic antigen (CEA) as a representative biomarker have shown that the ELISA method based on GNPL (graphene nanoplatelets) significantly improved signal amplification and lowered the limit of detection when compared to alternative assays, such as commercially accessible CEA ELISA kits. The sandwich ELISA utilizing GNPLs exhibits promise for application in clinical environments owing to its straightforward methodology and economical nature.

The classification of biomarkers has been facilitated by the diverse range of nanotechnology-based methodologies that have been devised. There exist a multitude of applications for each of these, contingent upon the objective and the methodology employed for signal interpretation. The investigation of composites or hybrids involving AuNPs and other nanoparticles holds significant significance in elucidating potential avenues for innovative research and prospects for practical implementation. The utilisation of multifunctional AuNPs and nanohybrids incorporating AuNPs is expected to gain significant popularity in the next years, particularly in the field of multi-analyte imaging.

4. Conclusion

The utilization of ELISA for the purpose of cancer detection has demonstrated its significant utility within the domain of oncology. ELISA's high sensitivity and specificity make it an indispensable method for early cancer diagnosis and monitoring treatment efficacy. The adaptability of this technology enables the identification of a diverse array of cancer biomarkers, thereby providing doctors and researchers with a potent tool to enhance patient outcomes. Nevertheless, it is crucial to recognize that ELISA, similar to any diagnostic methodology, possesses inherent constraints and need complementary clinical and laboratory evaluations. As advancements in technology continue to refine ELISA and expand its capabilities, it holds great promise for further enhancing our ability to detect and manage cancer effectively.

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