



State-of-the-art Hepatocellular Carcinoma Biomarker Detection by Biosensor Technology—a Review

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Accepted: 24 July 2021 / Published online: 4 August 2021
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Abstract

Biosensors have become a very interesting field in recent years, thanks to their potential to be converted into self-diagnostic technologies. Being able to analyze a biomarker with different biosensor systems has great potential. This potential is important in terms of time, cost, and practicality with its use in the analysis of difficult-to-diagnose diseases in clinical studies. In this review, the biosensors used in the determination of hepatocellular carcinoma (HCC) biomarkers were compiled. Electrochemical, optical, and piezoelectric biosensors used in the analysis of proteins, DNA, RNA, and cells with varying concentrations in HCC can be designed based on antibodies, nanocomposites, DNA, and aptamers.

Keywords Hepatocellular carcinoma · Biosensor · Liquid biopsy · Biorecognition receptor

Introduction

Cancer is the second most deadly disease after heart diseases [1]. Cancer formation can be observed in the presence of mechanisms that trigger genetic changes in cells in a particular tissue. Cells that lose primary functions grow involuntarily and show abnormal biochemical activities. It is possible to develop a preliminary diagnosis of cancer by measuring the biomarkers that increase in these biochemical activities. Cancer biomarkers include proteins, metabolites, and in the “liquid biopsy” content, which has been a subject of interest in recent years, circulating tumor DNA (ctDNA), cell-free DNA (cfDNA), exosomes, and circulating cancer cells (CTC) [2]. With the analysis of these biomarkers, the stages of cancer monitoring can be performed.

The biggest disadvantage of biomarkers is their sensitivity and specificity. This is a handicap in making a clear diagnosis and they can mostly increase with external factors (smoking, nutrition).

Besides, it is known that conventional methods bring additional costs due to the determination of these biomarkers, the use of consumables, the need for technical personnel, and time disadvantages. However, it is worth mentioning in

measurements whose reliability is questioned. In particular, the development of standardized methods such as cancer is the most important point in the fight against this disease. Liquid biopsy, which is an important technique in recent years about the origin of cancer and the state of cancer, is a method that can provide a clear diagnosis. However, the most important handicap of this technique is the lack of a standardized analysis method. Especially for the analysis of CTCs, the concentration of 1–10 cells per milliliter also reduces the possibility of encountering these cells. Therefore, their analysis becomes difficult. In order to analyze all these biomarkers, PCR, mass-based techniques, and spectrophotometric techniques are available in advanced laboratories and professional personnel must perform these processes. This adds cost to the measurements. Biosensor technology, which offers high sensitivity, low cost, and fast analysis and features that do not require expert personnel, provides many advantages for biomarker analysis.

Biosensors are combination of a biologically active molecule that immobilized on a transducer [3]. The biologically active molecule, that is, the biorecognition receptor, interacts with the target analyte. As a result of this interaction, according to the principle of the biochemical reaction, electrochemical, optical, or piezoelectric signals are sent to the analyzer with a transducer, which is converted into meaningful signals and the measurement is performed. In this way, the measurement of the desired analyte is performed. According to the activity of the biorecognition receptor

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on the biosensors, they can be called catalytic or affinity-based. Catalytic biosensors also perform a catalytic reaction between the biorecognition receptor and analyte; analysis can be performed by measuring the released product or electron. In affinity-based measurement, on the other hand, the biorecognition receptor and the analyte molecule are interacted without a biochemical reaction. In these measurements, the method can be derivatized by changes in the secondary molecule or biorecognition receptor of the analyte. One of the most important steps in biosensor systems is the immobilization layer, that is, the properties of the layer on which the biorecognition agent is placed on the transducer. Especially for the analysis of low amounts of biorecognition agents, the immobilization layer can be strengthened with nanomaterials or dendrimers, and the sensitivity can be increased both by increasing the surface area and by increasing the conductivity.

Biomolecules used as biorecognition receptor are selected to show affinity for the target analyte. These are enzymes, antibodies, proteins, DNA, RNA, aptamer, cell surface receptors, and cells. The selection of these molecules is also selected according to the target analyte.

Hepatocellular Carcinoma Biosensors

One of the most common malignant cancer types is hepatocellular carcinoma (HCC). It is the leading cause of cancer-related deaths. Although different external factors affect the formation of the disease, the recurrence rate after surgery in the early stage is 50% [4]. Inadequate therapeutic options are associated with disease progression. Therefore, early diagnosis is important. It is important to determine biomarkers in cancers that can still be detected at the cellular level. Analysis of biomarkers in body fluids enables early diagnosis of HCC. Depending on the characteristics of the target biomarkers, PCR, immunoassays, protein markers, ELISA, mass sensitive methods, and many biotechnological methods can be used. Pre-diagnosis of HCC seems possible with the development of new and more sensitive methods.

Protein-detecting Biosensors

Among the biomarkers found in body fluids for the diagnosis of HCC, the most commonly used one is alpha-feto protein (AFP) as a standard biomarker. This glycoprotein produced during pregnancy and usually increased during HCC, normal levels are lower than 20 ng/mL. Therefore, although it is a standard marker, the variability in its levels does not constitute a diagnosis of the disease. However, its use above 500 ng/mL and with ultrasound increases its sensitivity [5]. Consequently, low-cost methods such as biosensors are very convenient for their detection. Since AFP

is a particularly popular biomarker, there are different biosensor methods. In particular, it is possible to see different biosensor technologies modified with anti-AFP. Kim et al. [6] developed a field-effect transistor on a graphene surface in a biosensor they developed. They successfully determined AFP in the serum of HCC patients with these transistors. It showed detection between 12.6 and 784.9 ng/mL and the lowest detection limit of 0.1 ng/mL. The linearity and the lower limits are important for this biomarker and above of the 500 ng/mL is important for HCC patients. Li et al. [7] developed a label-free photoelectrochemical biosensor modified with anti-AFP biomolecules immobilized on Au/CsxWO₃ films that provide low background signal and high sensitivity for AFP detection. Surface plasmon resonances of Au and CsxWO₃ nanocrystals improved the photoelectrochemical properties and provided AFP concentration in the range from 0.01 to 500 ng/mL with a low detection limit of 7.0 pg/mL. Electrochemical impedance spectroscopy (EIS) and photoelectric response (PS) were the main detection method for AFP detection. In Amarnath et al. [8], anti-AFP was immobilized on glassy carbon-polyaniline-bovine serum albumin-silver (GCE-PANI-BSA-Ag) modified electrode. A secondary antibody labeled with horseradish peroxidase (HRP) was sent to the AFP connected to the biosensor prepared in this way, and the measurement was performed with the HRP signal that reduces hydrogen peroxide. Biosensors analyzed the AFP by using cyclic voltammetry (CV) and reached linear range from 0.01 to 1 and 1 to 10 ng/mL with detection limit 4.7 pg/mL. As a different study is developed to detect alpha-fetoprotein-L3 (AFP-L3) by Li et al. [9], AFP-L3 is especially important for HCC patients. Previously, multifunctional silver nanoprobe have been synthesized with biotinylated *Lens culinaris* agglutinin. In the preparation of the biosensor, this material was used as an electrical indicator. The single-wave voltammetry (SWV)-based detection system provided 25–15,000 pg/mL with 12 pg/mL limit of detection. There are also quartz crystal microbalance (QCM)-based biosensors for AFP detection by using anti-AFPs [10]. As can be seen, it is possible to develop AFP biosensors by reaching low sensitivities with different methods and different modification methods.

Another HCC biomarker was considered as des-gamma-carboxy prothrombin (DCP), which is nonfunctional prothrombin induced by vitamin K or antagonist 2 [11]. DCP was identified as both an autologous growth factor promoting HCC growth and a paracrine factor involved between HCC and vascular endothelial cells. The biological malignant potential of DCP and its aberrant expression in HCC tissues determine its potential in HCC prediction. Although it is a better biomarker than AFP in large tumors [12], it is not suitable for early diagnosis of HCC [13]. Therefore, this biorecognition receptor has less attention than the others do. One of the DCP-based biosensor studies by Fu et al.

[14] was the Pd@PtCu-WS₂-based immunoassay biosensor system. WS₂ nanosheets were modified by Pd@PtCu core-shell nanomaterials and secondary anti-DCPs, respectively. This structure was used as electrochemical indicator material. Afterwards, the biosensor was constructed the modification of GCE modified by Au-MWCNTs and anti-DCP. DCP binding to the surface is followed by the electrochemical indicator material that previously synthesized with secondary antibodies. Authors proposed ultra-detection range between 100 fg/mL and 100 ng/mL with a low limit of detection of 71.6 fg/mL. The basis of the detection of DCP was carried out by the reduction of hydrogen peroxide on core-shell Pd@PtCu-alloys. Another zinc oxide nanoparticles modified immunosensor study [15] showed that DCP can be detected for HCC diagnosis in a linear working range of 3.125–2000 ng/mL and 0.078 ng/mL lower limit of detection. An optical biosensor for DCP detection was developed based on the electro-chemiluminescence immunoassay (ECLIA) in HCC tissues ranged from 0.7 to 209,862.4 mAU/0.1 g tissue weight [16].

Glypican 3 (GPC3), a heparan sulfate proteoglycan, is known as an important biomarker in tumor development due to its effect on cell proliferation and differentiation, and is also seen as a potential biomarker in the diagnosis of HCC [17]. It is not yet possible to evaluate GPC3, which is overexpressed compared to normal tissues in HCC formation [18] as a feasible serum biomarker. Chikhaliwala et al. showed that a GPC3 biosensor developed using GCEs modified with PAMAM dendrimers conjugated with magnetites has reached a linear range of 0.02 to 10 ng/mL and a lowest detection range of 70 pg/mL. In this study, the GPC3 analysis was performed by analyzing the reduction of toluidine blue by differential pulse voltammetry [19]. In Dai et al. [20], after modifying the anti-GPC1 biomolecules with S-acetylmercaptosuccinic anhydride (SAMSA), they developed a biosensor system by forming monolayer antibodies. They performed surface modification with EIS and GPC-1 measurement with DPV. Shi et al. [21] developed a different biosensor to detect GPC3 with aptamers other than antibodies. Aptamers are synthetic nucleic acid chains with target biomolecular affinity in recent years. They are also used as a biorecognition agent with affinity for the target biomolecule in biosensor systems. This biosensor was developed by using reduced graphene oxide-chitosan-ferrocene deposition of Pt–Pd bimetallic nanoparticles (RGO-CS-Fc/Pt–Pd BNPs) and GPC3 aptamers. Concentration of GPC3 was detected in the range from 0.001 to 10.0 µg/mL and LOD found as 3.67 ng/mL. Another aptamer-based biosensor was developed for HCC detected by Chen et al. [22]. GPC3 was detected in 0.01 µg/mL to 10.0 µg/mL with a detection limit of 7.9 ng/mL (S/N = 3).

Another biomarker, alpha-I fucosidase (AFU), a lysosomal enzyme, can be used for diagnosis for HCC. It is not

a specific biomarker for the disease as it has been found to be specifically elevated in diseases other than HCC. Therefore, it is only necessary to measure it to give an idea at the disease stage. Han et al. [23] developed a biosensor to measure troponin and AFU levels with an InGaZnO-based chip technology and reached 0.016 U/L levels.

Nucleic Acid-detecting Biosensors

The use of protein-based biomarkers in the diagnosis of HCC brings along non-specific measurements. Besides measuring the target protein, measurement at the nucleic acid level can give clearer results in the diagnosis phase. Determination of HCC at the nucleic acid level is more effective than protein biomarkers like AFP with single-nucleotide polymorphism [24], cfDNA and methylation analysis [25], exosomal DNA [25], and cells. However, it was observed especially that miRNA analyses were in the class of better biomarkers in determining the difference between different infections and HCC, and even evaluated together with AFP [26–29]. It eliminates the disadvantages of PCR by using different methods in the determination of nucleic acids other than PCR, and it also has the potential to be converted into tests suitable for personal use.

In order to detect HCC, nucleic acids were used for direct detection for the biosensor technology. These studies are biosensors that can measure independently from PCR. Analysis in pM array is possible with an electrochemical study that performs analysis with DPV (LOD = 1.0 pM) by depositing circular DNAs, which are HCC indicators, on a surface [30]. In another study, the target lncRNA was determined electrochemically at the level of 0.247 fM by increasing the signal with long non-coding RNA (lncRNA) PtPd nanodendrite/nano-flower-like@GO nanocomposite [31]. With a surface-enhanced Raman scattering (SERS)-based biosensor technology, multiple miRNAs were detected in a multiplex system. In this system, HCC-associated miRNAs were identified as 349 aM for miRNA-122, 374 aM for miRNA-223, and 311 aM for miRNA-21 [1]. As a biorecognition agent for miRNA analysis, DNA sequences were used to target miRNAs.

In the HCC determination, circulating cancer cells are seen in possible metastasis, together with increased nucleic acids in the blood, apart from the biomarkers rising in the blood. Identification of cells is very difficult because the concentrations of CTCs in the blood, called liquid biopsies, are extremely low. Kashefi-Kheyraabadi et al. [32] developed a thiolated TLS11a aptamer on a gold electrode to target HepG2 cell lines. The EIS-based aptasensor has the ability to detect 100 to 1 million cells in per milliliter. LOD is also 6 cells in per milliliter.

Future Remarks

The development of biosensors is increasing dramatically. This increase can be made at lower levels with the discovery of new biorecognition agents and nanomaterials. It is obvious that different measurement methods can also help with these measurements. The determination of HCC is difficult due to the low specificity of protein-based biomarkers. These biomarkers can only provide preliminary information or form an idea about the disease. On the other hand, genetic analyzes can clearly diagnose HCC. Especially with “liquid biopsy” analysis, precautions can be taken against such a dangerous disease at an early stage. Biosensor systems have the capacity to fill this field due to the importance of HCC’ diagnosis and the potential to be miniaturized.

Author Contributions Zihni Onur Uygun (writing, conceptualization).

Declarations

Consent to Participate I consent to participate.

Consent for Publication I consent for publication.

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