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Review

(Nano)-materials and methods of signal enhancement for genosensing of p53 tumor suppressor protein: Novel research overview

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ABSTRACT

p53 is a gene that codes for a protein that regulates the cell cycle and hence functions as a tumor suppression. p53 plays an important role in cell cycle control and apoptosis. Defective p53 could allow abnormal cells to proliferate, resulting in cancer. Recently, much attention has been devoted to developing procedures to detect the presence of p53 protein at very low concentrations in a physiological environment. This article reviews DNA biosensors (genosensors) reported for the qualitative and quantitative determination of p53. We discuss critical aspects of genosensor design with particular emphasis on analytical characteristics and analysis of real samples. We assess the design and the construction of electrochemical, electrochemiluminescence, optical, and photoelectrochemical genosensors, describing the contributions so far in the p53 field and the analytical challenges involved in this research area.

Contents

1. Introduction to genosensing of p53

Genosensor technology has demonstrated to be a powerful tool for clinical diagnosis, biomedical research, food quality assurance, and environmental monitoring [1,2]. For this reason, the design of original, simple, and cost-effective analytical methods for the sensitive and accurate detection and quantification of specific nucleic acid sequences through hybridization mechanisms constitutes a main research topic in bioanalytical chemistry. During last decades, a great variety of genosensors have been developed by using electrochemical, optical, calorimetric, and piezoelectric transduction strategies [3–6]. Although optical systems have been the most widely employed, electrochemical methods have great prospective due to the simplicity, easy preparation,

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relative low cost, and possibility to be miniaturized in portable point-of-care devices [7]. The assembly of original genosensors has been largely benefited by the use of novel nanomaterials and nano-hybrids as advanced transducer elements. In this sense, a large number of electrochemical genosensors with improved analytical properties has been developed by using carbon nanotubes [8], graphene [9], metal and metal oxide nanoparticles [10–12], and other nanomaterials as constituents of the sensing interface. In general, these electrochemical genosensors involve the use of organic and metalorganic redox indicators [13,14], enzymes [15,16], or nanoparticles [17] as labeling elements. However, such labeling strategies imply tedious and relative complex detection protocols, which limit the further application of these genosensors in portable, patient-oriented, and patient-operated point-of-care devices [18].

An ever-growing number of publications have also reported an overexpressed level of alteration of the p53 protein, wild-type forms as well as structural mutants (non-null), *i.e.*, the arginine 249 to serine (R249S) and cysteine 135 to valine (C135V) point mutations, as a consequence of neoplastic cell formation, tumor invasiveness, and genotoxic stresses [19–21]. These reports indicate that the level of p53 in human serum can be reasonably accurate in reflecting tissue alterations in p53 at the gene and/or protein level, thus leading to a potential convenient and noninvasive tumor screening approach. This article reviews the state of the art in genosensors reported for the detection of p53 tumor suppressor protein. We review the basic elements present in a genosensing, and give a brief description of the various transducers, followed by classification of the analytical methods for genosensing of p53 in terms of specificity. We assess the design and the construction of optical, electrochemical, and electrochemiluminescence genosensors, describing the contributions so far in the detection of p53 and the analytical challenges involved in analyzing real samples.

2. Types of p53 genosensors

2.1. Electrochemical genosensing of p53

Nucleic acid-based electrochemical detection involves the generation of an electrical signal mediated by nucleic acid hybridization and serves as the basis for the DNA detection technology for which the detection of DNA hybridization by means of biosensors (genosensors) is a topic of major scientific and technological interest [22].

Electrochemical transduction based genosensors, rely on the detection of the hybridization event by change in electrochemical signal [23,24]. These genosensors have been reported by changes in the electrochemical response of the transition metal complexes [25] or dyes [26] that are intercalated or electrostatically attracted to the double stranded assembly (label based) and by direct electrical response of the DNA bases (label-free) [27–29]. Such electrochemical methods in genosensing greatly reduce the assay time and simplify the protocol in pathogen identification, mutation detection and genomic sequencing [30]. The electrochemical transduction detections are performed following probe immobilization and hybridization steps. The achievement of high sensitivity and selectivity of the genosensor requires minimization of nonspecific adsorption and the stability of the immobilized DNA probes. Therefore, probe binding strategy is the key factor that affects the designed biosensor's efficiency and quality. The construction of an electrochemical genosensor usually involves the following stages: (**i**) immobilization of the DNA probe onto the electrode surface; (**ii**) hybridization with the target sequence; (**iii**) labeling and electrochemical readout. Optimization of each step is required to improve the overall performance of the device. The probe immobilization step plays a major role in determining the performance of an electrochemical DNA biosensor. Many methods have been employed for the immobilization of DNA, including adsorption, covalent binding, self-assembled monolayers (SAMs), and electropolymerization.

In this sub-section, we comprehensively summarize the selected latest research articles on application of various electrochemical based genosensors for detection of p53. This sub-section is aimed to illustrate the development of a variety of electrochemical genosensors for qualification of detection of p53.

De Ávila and coworkers [31] constructed a disposable electrochemical DNA sensor using reduced graphene/CMC hybrid nanostructured scaffolds and DNA hairpin probes of different length for the detection of intact and SNP-containing TP53 gene sequences. The electrochemical platform consisted of screen-printed carbon electrodes (SPCEs) functionalized with a water-soluble reduced graphene oxide-carboxymethylcellulose (rGO-CMC) hybrid nanomaterial. Two different configurations involving hairpin specific capture probes of different length covalently immobilized through carbodiimide chemistry on the surface of rGO-CMC-modified SPCEs were implemented. Steps involved in the preparation of the DNA biosensor for the detection of the TP53 gene using rGO-CMC hybrid nanomaterial as a scaffold ((a) the Long TP53 capture probe (lcpp53) or (b) the short (spp53) TP53 Capture Probe) and labeling with strep-HRP was illustrated in Scheme 1. Limits of detection of 3 nM were obtained without any target or signal amplification.

Interestingly, a biofuel-cell-based self-powered biosensor for p53 was reported, in which bilirubin oxidase/DNA-modified graphene/platinum nanoparticles hybrid nanosheet worked as a bio-cathode to control the capture of p53, and thus tune the electron transfer process of O_2 reduction for signal amplification [32]. It was observed that the strong interaction between p53 and its consensus DNA sequence on the electrode surface could block the electron transfer from bilirubin oxidase to the electrode, thus reducing the electrocatalytic activity of O_2 reduction at the biocathode. In combination with glucose oxidation at the CNTs/Meldola's blue/glucose dehydrogenase bioanode, a current or power decrease of the biofuel cell was observed in the presence of p53. The designed BFC-based sensor showed a wide linear range extending from 53 ng L^{-1} to 53 mg L^{-1}, with a LOD of 53 ng L^{-1}.

The discovery of the enzyme-assisted DNA recycling strategy can offer new amplification routes for highly sensitive DNA monitoring by integrating other signal enhancement approach to achieve dual or multiple amplifications within one assay protocol. Recently, by coupling NEase-assisted target DNA recycling with rolling circle amplification (RCA), Wang and coworkers [33] prepared a new hybrid signal amplification strategy for highly sensitive electrochemical (EC) detection of the mutant human p53 gene cancer biomarker DNA. RCA, as an advanced DNA amplification technique alternative to PCR, can achieve significant signal amplification via the production of thousands of repeated sequences under mild reaction conditions and with speediness, high efficiency, and specificity [34]. Due to the drastic signal amplification power, RCA has been widely employed in various sensing schemes for the analyses of proteins and nucleic acids [35,36]. According to this approach, the target mutant p53 genes hybridize with the hairpin DNA probes on the senor surface and create nicking sites for NEase to initiate the target DNA recycling amplification process to cleave numerous probes. The cleaved probes on the sensor surface are subject to *in situ* RCA to generate massive long DNA sequences with repeat G-quadruplex units, which associate with hemin to form G-quadruplex/hemin complexes. Direct electron transfer between hemin and the sensing electrode during the potential scan thus can offer amplified current response for highly sensitive detection of the mutant p53 gene (See Scheme 2 for details). With the rationally designed padlock DNA as circular templates, the resulting RCA products contains numerous, tandem-repeat G-quadruplex sequences for the association of a large number of hemin, which generates significantly amplified current response for the detection of the mutant human p53 gene down to 0.25 fM.

Wang et al., [37] develop a high sensitivity electrochemical biosensor for the determination of p53 tumor suppressor based on functional composite nanofibers using methylene blue (MB) as an electrochemical