Talanta 150 (2016) 286–295

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Label-free and dynamic evaluation of cell-surface epidermal growth factor receptor expression via an electrochemiluminescence cytosensor

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ARTICLE INFO

Article history:
Received 20 August 2015
Received in revised form
3 December 2015
Accepted 10 December 2015
Available online 18 December 2015

Keywords:
Label-free
Receptor molecules
Electrochemiluminescence cytosensor
Epidermal growth factor
Resveratrol
Silymarin

ABSTRACT

A label-free electrochemiluminescence (ECL) cytosensor was developed for dynamically evaluating of epidermal growth factor receptor (EGFR) expression on MCF-7 cancer cells based on the specific recognition of epidermal growth factor (EGF) with its receptor (EGFR). EGF-cytosensor was fabricated by in-situ electro-polymerization of polyaniline as substrate, using CdS quantum dots (CdS QDs) as ECL probe and gold nanoparticles (AuNPs) as a carrier for loading of EGF. AuNPs and CdS QDs were jointly attached on polyaniline surface to provide a sensitive and stable sensing interface, as well as a simple and label-free mode for ECL assay. Electron microscopy, atomic force microscopy (AFM) and electrochemical methods were employed to characterize the multilayer construction process of the sensing interface. The proposed EGF-cytosensor exhibited excellent analytical performance for MCF-7 cancer cells, ranging from 12 to 1.2×10^6 cells mL^{-1}, with a low detection limit of 12 cells mL^{-1}. Also, it was successfully applied in evaluating EGFR expression of cells surface, which was stimulated by some inhibitors or activator, and the results were confirmed by using flow cytometry and laser scanning confocal microscopy analysis. The proposed ECL cytosensor has potential applications in monitoring the dynamic variation of receptor molecules expression on cell surfaces in response to external stimulation by drugs and screening anti-cancer therapeutic agents.

1. Introduction

Protein phosphorylation by kinase plays a vital regulatory role in many significant biological processes involving cellular signal communications, metabolic pathways, and so on [1]. Receptor tyrosine kinases (RTKs), which are regulated by kinase, have emerged as promising targets for cancer therapy [2]. As a member of RTKs family, epidermal growth factor receptor (EGFR) has been caused attention as a target for screening anti-cancer therapeutic agents [3–5]. It is a cellular transmembrane protein that is activated through binding to its set of growth factors such as the epidermal growth factor (EGF) and the fibroblast growth factor (FGF), etc. [6]. The activation of EGFR initiates a series of intracellular signaling pathways that control critical cell processes such as proliferation, adhesion, migration and apoptosis [7]. Overexpression of EGFR can lead to deregulation of these cell processes and initiation of some human cancers, making it a promising target for screening for likelihood of cancers [8,9]. Thus, there has been interest in investigating EGFR expression levels and as a target for drug-screening.

Conventional biology techniques have been used for the detection of EGFR and still remain the gold standard for protein assay, such as Western Blotting [10], immunofluorescence assay [11] and enzyme-linked immunosorbant assay [12]. However, they are time-consuming, with complicated sample preparation and sophisticated instrumentation. Recently, several biosensing platforms were developed for detection of EGFR protein, including those based on quartz crystal microbalance immunosensors [13], Atomic Force Microscopy probing molecular interaction [14], surface plasmon resonance using gold nanoparticles [15], microfluidic nano-biochip [16] and cytosensor with lab-on-a-chip [19]. These detecting platforms revealed the each with its own special advantages, but need complicated procedures or require advanced instruments. As an alternative analytical technique, electrochemical methods have attracted considerable attention due to rapid analysis speed, satisfactory sensitivity, simple instrumentation, low-cost and nondestructive analysis of living cells. Vasudev

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http://dx.doi.org/10.1016/j.talanta.2015.12.019
0039-9140/

et al. [17] constructed an electrochemical immunosensor via immobilizing anti-EGFR antibody onto assemble of monolayer Au electrode and detected EGFR with a detection limit of 1 pg mL^{-1} by using cyclic voltammetry. Elshafey et al. [18] reported an electrochemical impedance immunosensor based on gold nanoparticles–proteinG as scaffold for oriented anti-EGFR antibody immobilization and detection of EGFR with a detection limit of 0.34 pg mL^{-1} in PBS and 0.88 pg mL^{-1} in human plasma. The electrochemical immunosensors incorporating anti-EGFR specific recognization EGFR have greatly improved the detection limits. However, there are few reports concerning in-situ and real-time detecting EGFR expression level on cell surfaces induced by drugs. EGFR expression on the cell surface has been observed to be associated with advanced tumor stages. Therefore, sensitive analysis of EGFR expression on living cell surfaces is important for understanding EGFR roles in cancer development and provides suitable diagnostic tools.

The newly emergent electrochemiluminescence (ECL) technique integrates the advantages of electrochemistry and chemiluminescence, so that it has been widely used in the area of biological analysis, such as DNA analysis [20], immunoassay [21], clinical diagnosis [22], carbohydrate analysis and cytosensing [23,24]. Ju's group [25] developed an ECL cytosensor for detecting of cell surface carbohydrate expression by using CdSe quantum dots as ECL emitting species. Chen et al. [24] reported an sandwich ECL biosensor based on concanavalin A-integrating AuNPs-modified $Ru(bpy_3^{2+})$-doped silica nanoprobe for evaluating cell surface N-glycan expression. Chen et al. [26] demonstrated an electrochemical cytosensing for dynamic evaluation cell surface N-glycan expression by combination of aptamer and horseradish peroxidase-modified AuNPs as nanoprobes. Zhou et al. [27] showed an enhanced iridium complex electrochemiluminescence cytosensing and dynamic evaluation of cell-surface carbohydrate expression. The as-reported biosensors exhibited excellent analytical performance for evaluating cell surface carbohydrate expression with the need to label ECL probes. In our previous work, an ECL cytosensor was designed for evaluating cell surfaces EGFR expression by integrating the specific recognition of cell surfaces EGFR with EGF-funtionalized CdS QDs-capped magnetic bead as nanoprobe and signal amplification [38]. Up till now, only a few references have reported the detection of cell surface EGFR expression via ECL. Compared with our previous work, an simple and efficient label-free mode for in situ and dynamically evaluating cell surface EGFR expression is presented in this work.

Currently, polymers and nanomaterials have been successfully applied to modify and functionalize the surfaces of biosensor for improving the sensitivity and stability of sensing platforms [28,29]. Polyaniline(PANI), a representative of the family of conducting polymers [39], has been widely applied in sensors as an excellent interface material due to its surface microgaps and positive charges, larger surface area, high conductivity and good environmental stability [40]. AuNPs possess high chemical stability, good biocompatibility and electrocatalytic properties [41]. Especially, AuNPs exhibit excellent adsorption for protein and other biomolecules via linking to the nanoparticles with sulfur atoms, thiol linkers or amine groups [42,43], so that it has been used as an ideal immobilized material in electrochemical biosensors [44]. Since the first work on the ECL of silicon QDs in 2002 [45], the ECL sensors have been developed based on highly luminescent semiconductor nanocrystals such as CdTe QDs [34], CdS QDs [46], CdSe QDs [47] in presence of different co-reactants. In this work, CdS QDs and AuNPs were utilized to jointly attach on PANI surface to fabricate a simple, sensitive and label-free ECL sensor for detection of cell surface EGFR expression.

Herein, a label-free electrochemiluminescence cytosensor was developed for in situ and dynamic evaluation of EGFR expression on MCF-7 cells surface in response to drugs stimulation at different incubation time and concentration. PANI was modified onto the glassy carbon electrode by in-situ electro-polymerization to improve the stability and conductivity of sensor interface. AuNPs were anchored on polyaniline via electrostatic interaction for immobilization of EGF. L-cysteine-capped CdS QDs as ECL probe was also coupled to polyaniline via conjugation of carboxylic group of CdS QDs with amino group of PANI, and the connection might be promoted further through ionic interaction between negatively-charged CdS QDs and positively-charged PANI. A suitable amount of AuNPs was optimized to avoid quenching ECL signals of CdS QDs as well as to enhance the loading amount of the EGF. The EGF-functionalized sensing interface can be used to specifically recognize EGFR expression on MCF-7 cancer cells. In fact, we found AuNPs/PANI substrate enhanced reproducibility of the cytosensor assay and exhibited high sensitivity for collecting ECL signals. Therefore, the cytosensor was applied to monitor cell-surface EGFR expression in response to stimulation by activator EGF, inhibitors resveratrol (RVL) [30] and silymarin (SM) [31], which could affect the activity of tyrosine protein kinase (TPK) and regulate the EGFR expression of cells surface. Flow cytometry (FCM), laser scanning confocal microscopy (LSCM) were further utilized to confirm the obtained results. The protocol would be potentially extended to investigate all kinds of receptor molecules on cells surface, and further used for screening anti-cancer therapeutic agents.

2. Experimental section

2.1. Reagents and apparatus

All reagents were of analytical-reagent grade or the highest purity available and directly used for the following experiments without further purification. Epidermal Growth Factor (EGF) was purchased from PEPROTECH Company. Rabbit anti-EGFR/Rhodamin B isothiocyanate (RBITC) was purchased from Beijing Biosynthesis Biotechnology Co., Ltd. Resveratrol (RVL) and Silymarin (SM) ($\geq$ 98% purity) were purchased from Langze Pharmaceutical Technology Co., Ltd. (Nanjing, China). Bovine serum albumin (BSA), chloroauric acid ($HAuCl_4 \cdot 4H_2O$) and trisodium citrate were purchased from Sigma-Aldrich Inc. (USA). Human breast cancer cell lines MCF-7 were donated by Institute of Physiology, Jinan University. Fetal bovine serum (FBS) were purchased from Gibco Co. Double-distilled water was used in all experiments. Phosphate buffer solution (PBS, 0.1 M, pH 7.4) contained 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4 and 0.1 M KCl.

The ECL measurements was conducted on a MPI-E multifunctional electrochemical and chemiluminescent analytical system (Xi'an Remex Electronic Instrument Limited Co., Xi'an, China) at room temperature. Electrochemical measurements were performed with a CHI 660D electrochemical working station (CH Instruments Co., USA) with a conventional three-electrode system composed of platinum wire as the auxiliary electrode, a saturated calomel electrode as the reference electrode, and a 4 mm-diameter glassy carbon electrode (GCE) as the working electrode. The morphology of the EGF-cytosensor surface was visually characterized by scanning electron microscopy (SEM) (JEOL-JSM-6700F), transmission electron microscopy (TEM, Philips) and AutoProbe CP Research atomic force microscopy (AFM, Veeco, USA) in the tapping mode. The EGFR expression on drug-stimulated cells surface was analyzed by flow cytometer (Becton Dickinson, German) and Laser scanning confocal microscopy (LSCM) (Carl Zeiss, Thornwood, NY).