Journal of Pharmaceutical and Biomedical Analysis 129 (2016) 288–293

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Rapid endoglin determination in serum samples using an amperometric magneto-actuated disposable immunosensing platform

Rebeca M. Torrente-Rodríguez[a], Susana Campuzano[a,*], Víctor Ruiz-Valdepeñas-Montiel[a], María Pedrero[a], M. Jesús Fernández-Aceñero[b], Rodrigo Barderas[c], José M. Pingarrón[a,*]

[a] Departamento de Química Analítica, Facultad de CC. Químicas, Universidad Complutense de Madrid, E-28040 Madrid, Spain
[b] Department of Pathology, Hospital Clínico San Carlos, 28040 Madrid, Spain
[c] Departamento de Bioquímica y Biología Molecular, Facultad de CC. Químicas, Universidad Complutense de Madrid, Madrid, Spain

ARTICLE INFO

Article history:
Received 6 June 2016
Received in revised form 12 July 2016
Accepted 13 July 2016
Available online 15 July 2016

Keywords:
CD105
Magnetic beads
Screen-printed electrodes
Amperometric immunosensor
Serum samples

ABSTRACT

A sensitive and rapid method for the determination of the clinically relevant biomarker human endoglin (CD105) in serum samples is presented, involving a magneto-actuated immunoassay and amperometric detection at disposable screen-printed carbon electrodes (SPCEs). Micro-sized magnetic particles were modified with a specific antibody to selectively capture the target protein which was further sandwiched with a secondary HRP-labeled antibody. The immunocomplexes attached to the magnetic carriers were amperometrically detected at SPCEs using the hydroquinone (HQ)/H_2O_2/HRP system. The magneto-actuated immunosensing platform was able to detect 5 pmoles of endoglin (in 25 μL of sample, 0.2 μM) in 30 min providing statistically similar results to those obtained using a commercial ELISA kit for the determination of endogenous content of endoglin in human serum samples.

1. Introduction

Survival and progressive growth of solid cancers requires the continuous formation of new blood vessels, which is known as angiogenesis, resulting from the imbalance between pro- and anti-angiogenic factors produced by both the malignant and normal cells [1]. Increase of tumor size and metastasis are considered as direct consequences of this process, so that detection of potential biomarkers related to angiogenesis can provide relevant information on the behavior and treatment of solid cancers [2]. Human endoglin (also known as CD105 and EDG) is a 180 kDa homodimeric hypoxia-inducible transmembrane glycoprotein that behaves as an auxiliary receptor for the transforming growth factor-β family of cytokines and plays an important role in hematopoiesis, cardiovascular development, vascular remodeling and angiogenesis [3]. Indeed endoglin has been reported to be an angiogenesis inducer that may play a part in rheumatoid arthritis (RA) [4,5]. Recently, several reports have shown also that endoglin may be a good tumor metastasis-related marker since this glycoprotein is abundantly expressed in angiogenic endothelial cells of tumor tissues, showing elevated levels in serum of cancer patients and correlating positively with tumor metastasis [6]. Also several studies demonstrated that CD105 is up-regulated in a wide variety of tumor endothelia, including those in the head and neck, esophagus, colon, liver, breast, brain, lung, kidney, prostate, ovary and endometrium [7–9]. Due to the cleaving of the extracellular endoglin domain by the action of metalloproteases, high levels of soluble endoglin (Sol-Eng), correlating with tumor progression and metastasis have been detected in serum of patients suffering from preeclampsia, hypercholesterolemia, atherosclerosis and different types of cancer such as breast, prostate, lung and colorectal [3].

Roles of endoglin in cancer can be classified according to two different levels: in tumor cells, it acts as modulator of malignancy whereas it acts as a regulator of tumor angiogenesis in endothelial cells. Therefore, circulating endoglin can be considered as a potential and promising biomarker for diagnosis, prognosis and therapy of cancer and other relevant illnesses.

In the particular case of hepatocellular carcinoma (HCC), it is one of the most common malignant tumors showing a high degree

* Corresponding authors.
E-mail addresses: rebeca.magnolia@gmail.com (R.M. Torrente-Rodríguez), susanacr@quim.ucm.es (S. Campuzano), victor_lega90@hotmail.com (V. Ruiz-Valdepeñas-Montiel), mpedrero@quim.ucm.es (M. Pedrero), jmariajesus.fernandez@salud.madrid.org (M.J. Fernández-Aceñero), rbarderas@quim.ucm.es (R. Barderas), pingarro@quim.ucm.es (J.M. Pingarrón).

http://dx.doi.org/10.1016/j.jpba.2016.07.020
0731-7085/

of mortality and, even after the application of different treatment therapies and surgery, this tumor shows a high percentage of recurrence and metastasis with a short mean survival for patients compared to other major solid tumors. Although alpha-fetoprotein (AFP) detection is commonly used for the clinical early diagnosis of liver cancer, it is known that the specificity and sensitivity of AFP for screening of this type of cancer are not satisfactory, thus highlighting the need for new early detection biomarkers for the accurate diagnosis of HCC [10]. Selim and Mohamed found independently that patients suffering with HCC showed significantly increased level of serum endoglin compared with no-HCC patients or control subjects with cut-off levels of 6.9 [10] and 10.6 [11] ng mL^{-1} endoglin. In this context, circulating serum endoglin is considered as a useful and promising marker, especially if combined with AFP, for diagnosis and follow-up of HCC.

On the other hand, Fujita et al. [12] demonstrated that endoglin levels in both urine and serum may aid in prostate cancer detection and prognostication. While serum endoglin levels appear to correlate with the stage of the disease, high plasma levels of soluble endoglin correlate with metastasis and PSA recurrence after radical prostatectomy [13,14].

Therefore, considering the undeniable role that endoglin plays in the development and progression of various types of cancer and other important diseases, such as RA, the development of new methodologies able to determine this relevant biomarker in an accurate, simple and rapid manner is highly desirable. Currently, clinical analysis of endoglin relies mostly on enzyme-linked immunosorbent assays (ELISA) which have some well-known limitations for successful implementation in *point-of-care* (POC) diagnostics. In this context, electrochemical immunosensors have attracted a wide interest due to their inherent high sensitivity and selectivity, great precision and accuracy, low cost, minimum sample requirement, simplicity of operation and possible integration in compact analytical devices. However, surprisingly, only one electrochemical immunosensor for endoglin has been reported so far [6]. This voltammetric immunosensor achieved a LOD of 0.9 ng mL^{-1} and involved gold electrodes modified with AuNPs and bioconjugates of Pt nanoparticles, thionin acetate and detector antibodies (PtNP-THI-Ab2). However, times required for the immunosensor and PtNP-THI-Ab2 bioconjugates preparation were rather long (30 and 12.5 h, respectively) limiting strongly the immunosensor applicability for routine determinations.

Currently, the use of superparamagnetic iron oxide particles functionalized with different reactive groups, is a well-established methodology for selective, rapid and easy capturing of a specific target molecule from a complex sample, which can be easily coupled with disposable electrochemical sensors [15]. Moreover, these magnetic microparticles enable efficient capture bioreceptor immobilization and minimize non-specific adsorptions occurring in complex samples [16].

Herein, an amperometric immunosensing strategy making use of a sandwich configuration and magnetic micro-carriers to perform the determination of CD105 in real human serum samples is described.

2. Materials and methods

2.1. Materials

2.1.1. Apparatus and electrodes

Amperometric measurements were performed with a CHI812B potentiostat (CH Instruments) controlled by software CHI812B. Screen-printed carbon electrodes (SPCEs) (DRP-110, DropSens), consisting of a 4-mm diameter carbon working electrode, a carbon counter electrode and an Ag pseudo-reference electrode, were employed as transducers. A specific cable connector (ref. DRP-CAC also from DropSens, S.L.) acted as interface between the SPCEs and the potentiostat. All measurements were carried out at room temperature.

A Bunsen AGT-9 Vortex was used for the homogenization of the solutions. A Thermomixer MT100 constant temperature incubator shaker (Universal Labortechnik) and a magnetic separator DynaMag^{TM}-2 Magnet (ThermoFisher Scientific) was also employed. Capture of the modified-MBs onto the SPCE surface was controlled by a neodymium magnet (AIMAN GZ) embedded in a homemade Teflon casing. A Magellan V 7.1 (TECAN) ELISA plate reader was also used.

2.2. Reagents and solutions

All reagents were of the highest available grade. Carboxylic acid-modified MBs (HOOC-MBs, 2.7 μm Ø, 10 mg mL^{-1}, Dynabeads^{TM} M-270 Carboxylic Acid, Cat. No: 14305D) were purchased from Invitrogen-Thermo Fisher. Recombinant human endoglin (CD105), mouse anti-CD105 monoclonal antibody (used as capture antibody, AbC), mouse anti-CD105 monoclonal antibody conjugated to horseradish-peroxidase (HRP) (used as detector antibody, AbD) and tetramethylbenzidine (TMB) were purchased as an ELISA Kit (ref. SEK10149-5, Sino Biological Inc., China).

Sodium chloride, potassium chloride, sodium di-hydrogen phosphate, di-sodium hydrogen phosphate, sulfuric acid and Tris-hydroxymethyl aminomethane-HCl (Tris-HCl) were purchased from Scharlab. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) was purchased form Acros Organics. *N*-hydroxysulfosuccinimide (Sulfo-NHS), ethanolamine, hydroquinone (HQ), Tween® 20 and hydrogen peroxide (30%, w/v), sodium monohydrogen carbonate (≥99.7%,) and disodium carbonate (≥99.0%) were purchased from Sigma-Aldrich. Ethylenediaminetetraacetic acid (EDTA) was purchased from Merck (Germany) and commercial blocker casein solution (a ready-to-use, PBS solution of 1% w/v purified casein) was purchased from Thermo Scientific. Bovine serum albumin (BSA Type VH) and 2-(*N*-morpholino)ethanesulfonic acid (MES) were purchased from Gerbu Biotechnik, GmbH. Human hemoglobin (H7379) and IgG from human serum (I2511) were also purchased from Sigma-Aldrich (USA). Recombinant human ErbB2 protein was purchased from Sino Biological Inc. (ref. SEKA10004, China). Recombinant human progesterone receptor (PR) (ref. DYC5415) and recombinant human estrogen receptor α (ERα) (ref. DYC5715) were obtained from R&D Systems Inc. Recombinant human TNFα protein (ref. IM1121) was purchased from Immunotech and N-terminal GST-tagged, recombinant full length human p53 protein (Catalog#14-865) was purchased from EMD Millipore Corporation.

The following solutions, prepared with water from Millipore Milli-Q purification system (18.2 MΩ cm), were employed: 0.05 M phosphate buffer, pH 6.0; 0.1 M phosphate buffer, pH 8.0; phosphate-buffered saline (PBS) consisting of 0.01 M phosphate buffer solution containing 137 mM NaCl and 2.7 mM KCl, pH 7.5; 0.025 M MES buffer, pH 5.0; 0.01 M sodium phosphate buffer consisting of PBS with 0.05% Tween® 20 (pH 7.5, PBST) and 0.1 M Tris-HCl buffer, pH 7.2.

Activation and blocking steps of the HOOC-MBs were carried out using an EDC/sulfo-NHS mixture solution (50 mg mL^{-1} each in MES buffer, pH 5.0) and a 1 M ethanolamine solution (prepared in 0.1 M phosphate buffer solution, pH 8.0), respectively.

2.3. Bio-functionalization of MBs and sandwich immunoassay

Unless otherwise stated, the MBs were placed in the magnetic separator and concentrated for 3 min before removing the supernatant after all the involved steps. All incubation and washing steps