Clinica Chimica Acta 425 (2013) 128–138

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

Invited critical review

Electrochemical immunosensors in breast and ovarian cancer

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ARTICLE INFO

Article history:
Received 10 April 2013
Received in revised form 19 July 2013
Accepted 20 July 2013
Available online 6 August 2013

Keywords:
Electrochemical immunosensors
Breast and ovarian cancer
Biomarkers

ABSTRACT

During the last decades the incidence of cancer increased dramatically especially in developed countries. In spite of the fact that the immunochemical methods allowed the diagnosis in early stages, the biopsies are generally invasive methods that create discomfort to patients. The need for fast, sensitive, easy to use and noninvasive diagnosis tools is actually of great interest for many research groups all over the world.
Immunosensors (ISs) are miniaturized measuring devices, which selectively detect their targets by means of antibodies (Abs) and provide concentration-dependent signals. Ab binding leads to a variation in electric charge, mass, heat or optical properties, which can be detected directly or indirectly by a variety of transducers.
A great number of proteins could be considered as recognition element. In this review the attention was focused on main cancer biomarkers, currently used by immunological methods (immunohistochemistry, ELISA, flow cytometry, Western blot, immunofluorescence etc) and in the development of electrochemical immunoassays that could be used in cancer diagnosis, prognosis and therapy monitoring.

Contents

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http://dx.doi.org/10.1016/j.cca.2013.07.017

1. Introduction

Cancer must be defined as a disease which is characterized by abnormal growth and development of normal cells beyond their natural boundaries. In the last 50 years despite the global efforts to limit the incidence of this disease, cancer has become the leading cause of death. The breast cancer is the most common malignancy in women and the second most common cause of cancer-related mortality [1]. For this reason, the diagnosis at early stage needs specific and sensitive biomarkers and analytical tools [1]. Because diagnoses based on symptoms are not acceptable for cancer, considering the fact that symptoms usually appear when tumors are sufficiently large, other useful diagnostic tools must be developed. By consequence, a screening for the early detection of cancer is needed. This early detection in asymptomatic populations requires a non-invasive (or a minimally invasive) procedure for the assay, which has to be performed using a small amount of samples (physiological fluids or tissues). Furthermore, an optimal screening assay has to be site-specific (able to detect cancer in different organs and establish site of tumor formation). In addition, the analytical assay needs to operate at the level of differential diagnosis and to be sufficiently specific to produce an acceptable limit of false-positive results, depending on the prevalence of the disease in the population [1,2].

Case studies revealed that the early detection still remains the most promising approach to improve long-term survival of cancer patients [2]. The precise diagnosis of cancer is nowadays relied on histological evaluation of tissues using immunohistochemistry, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunofluorescence (IF), Western blot etc. All these techniques use biomarkers. A biomarker could be defined as a quantifiable laboratory measure of a disease specific biologically relevant molecule that can act as an indicator of a current or future disease state [1]. The ideal cancer biomarker would be a protein or protein fragment, which can be easily detected in the patient's blood or urine, but not detected, in healthy patient. Today, the most common use of cancer biomarkers is for the detection of the recurrent disease and monitoring the cancer therapy [2].

In the last decade, immunology and cancer research has identified several molecules potentially involved in well-known biological mechanisms affected by cancer. These new biomarkers present a striking dissimilarity from the classical “tumor markers”. While the latter were discovered through their prevalent expression in cancer and are therefore more indicators of tumor extension, the former are being discovered via their relation to the known biological mechanisms of cancer cells, which are potentially related to the clinical behavior of the malignancy (i.e., aggressiveness, expression of metabolic pathways). These pathways may be targets for diverse anticancer agents [3,4].

As an example, the early stage ovarian cancer has an excellent prognosis if treated, but advanced stage ovarian cancer, which is diagnosed in approximately 70% of patients, is associated with a poor survival rate of only 10–30% [5]. Given the limitations of treatment for advanced ovarian cancer and the success of treatment for early stage disease, a screening test is demanded. The ability to accurately detect early stage disease would potentially improve ovarian cancer survival. However, the low prevalence of ovarian cancer (30–50 cases/100,000 women) limits the achievable sensitivity and specificity of any screening test [6]. Therefore, clinical outcome and possibly survival may be significantly improved by the identification of stage I disease without the need to change surgical or chemotherapeutic approaches.

Usually different biomarkers are probably important for different tasks. For instance, CA125 is a useful serum biomarker for monitoring ovarian cancer patients and possibly for prediction of the patient's response to therapy, but has insufficient sensitivity for diagnosis [7]. Unfortunately, CA125, a high molecular weight glycoprotein and serum biomarker [7], is approved for monitoring recurrence of disease [4,5,8]. Clinically significant elevations in CA125 occur in only 80–85% of women diagnosed with early stage disease and 50–60% of late stage disease. Furthermore, CA125 can also be increased in a variety of other conditions, both benign and malignant, such as the first trimester of pregnancy, breast cancer and endometriosis, as well as lesions that promote any type of peritoneal irritation [9].

It is well known that the distinctive characteristic of immune system is its ability to distinguish self from nonself. An extraordinarily selective and versatile reagent is provided by nature in the form of the antibody (Ab). As a part of the immune defense system in animals, antibodies of high specificity can be synthesized by an organism in reasonable quantity within weeks of injecting a foreign species called an antigen (Ag). Organisms recognize the presence of nonself and respond rapidly by synthesizing Ab that exhibits high binding constants for the different surface chemical features of the Ag. This is a remarkable feat of life that can be used to great advantage in analytical chemistry. Immunoreactions are recognized for their high sensitivity and selectivity. This is the main reason to select immunochemical methods for clinical analysis. The use of immunosensors instead of other immunochemical analyses simplifies considerably the analysis, making it rapid and reliable. Molecules generally designed as antibodies (Abs) include a number of classes and subclasses of the immunoglobulin whose physiological sites of action, specificity and even molecular weights vary widely.

Cancer is one of the leading causes of mortality. Given this, early clinical diagnosis is crucial for successful treatment of the disease. Many immunosensors and immunoassay methods have been developed to isolate single tumor marker, whose concentration in human serum to be associated with the stages of tumors [5–7].

To improve the sensitivity and the limit of detection of the immunosensors, researchers started to work with new promising materials like carbon nanotubes (single and multi walls SWCNT, MWCNT), graphene sheets or nanoparticules (magnetic MNP and gold nanoparticles GNP).

Carbon nanotubes have many structures, differing in length, thickness, type of helicity and number of layers. Although they are formed essentially from the same graphite sheet, their electrical characteristics differ depending on these variations, acting either as metals or as semiconductors. Overall, carbon nanotubes show a unique combination of stiffness, strength, and tenacity compared to other fiber materials which usually lack one or more of these properties. Thermal and electrical conductivity are also very high and comparable to other conductive materials. Their electronic properties suggest that carbon nanotubes have the ability to mediate electron-transfer reactions with electroactive species when used as an electrode [8,9]. It has been suggested that the electrocatalytic properties of the CNTs originate from the open ends. The properties of carbon nanotubes also make them extremely attractive for chemical and biochemical sensor assembly.

Belonging to the same carbon family, graphene is the basic structural element of some carbon allotropes including graphite, charcoal, carbon nanotubes and fullerenes. Its structure is one-atom-thick planar sheets of sp^2-bonded carbon atoms that are densely packed in a honeycomb crystal lattice [10].

The magnetic nanoparticles, generally less than 50 nm in diameter, are made up of an iron oxide core stabilized by an organic shell. They are called superparamagnetic iron oxide (SPIO) nanoparticles because of their magnetic properties. Magnetic nanoparticles can be used for the detection of cancer. In this case, the magnetic nanoparticles are coated with antibodies targeting cancer cells or proteins. Patient's blood can be inserted into a microfluidic chip with magnetic nanoparticles in it. These magnetic nanoparticles are trapped inside due to an externally applied magnetic field as the blood is free to flow through. The magnetic nanoparticles can be recovered and the attached cancer-associated molecules can be assayed for their existence [11]. Another configuration of an electrochemical immunoassay using magnetic nanoparticles and carbon based screen printed electrodes is designed to be used as disposable device. At the surface of the nanoparticles an ELISA like sandwich assay can attached a primary antibody recognizing the antigen (like MUC 1 or CA125) that binds to a secondary followed by a third enzyme labeled antibody. The formed complex will transform the substrate into