Molecular and Cellular Probes 30 (2016) 106–112

Contents lists available at ScienceDirect

Molecular and Cellular Probes

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

Review

Application of immuno-PCR for the detection of early stage cancer

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ARTICLE INFO

Article history:
Received 5 December 2015
Received in revised form
29 January 2016
Accepted 29 January 2016
Available online 2 February 2016

Keywords:
Early cancer detection
Biomarker
Cancer screening
Immuno-PCR
Immuno-polymerase chain reaction

ABSTRACT

Cancer detection in premalignant stage is directly related with increase survival rate. Several biomarkers have been investigated and characterized for monitoring changes inside the cancerous cells. Although enzyme-linked immunosorbent assay (ELISA) is the method of choice in clinical practice for detecting biomarkers in serum/urine samples. However, in certain malignancies the amount of biomarkers before reaching metastasis are too low to be detected by conventional ELISA. The seminal work of Sano et al. led to the development of highly sensitive and powerful detection method, the immuno-PCR (iPCR), which can detect very small amount of antigens/biomarkers. In spite of, several publications on iPCR sensitivity, it has not been recommended for clinical use and is limited to the scientific community only. In order to evaluate the importance of iPCR, we have made an effort to collect published studies, supporting the use of iPCR in detecting premalignant cancer.

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1. Introduction

According to the National Cancer Institute, North American Association of Central Cancer Registries, and National Centre for Health Statistics, cancer is the leading cause of death throughout the world [1]. Cell division, growth, and differentiation get out of control in malignancy, resulting in the development of mass of cells called tumor, except in some types of leukemia. Sometime cancerous cells disseminate from the neoplasm and spread in blood stream, thereby, leading to the formation of secondary tumors, known as metastasis. Numerous FDA approved therapeutic antibodies are available on the market for addressing diverse

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http://dx.doi.org/10.1016/j.mcp.2016.01.010
0890-8508/

malignancies. However, saying that the available antibody-based therapeutics are completely safe is still under debate [2]. Therefore, patients receiving treatment need thorough surveillance and follow-up for monitoring recurrence of the disease or adverse events associated with therapy. Compromised physiological activity of cancerous cells produce certain protein antigens that are used as biomarkers in detecting malignancy. But in some types of malignancy biomarkers are produced in trace amount and more than 60% cancer patients do not show any clinical manifestations prior metastasis. After invading the surrounding cells, tissues, and/or organs; even the most effective therapeutics become least effective. Consequently, cancer diagnosis at the preliminary stage is challenging, entailing sophisticated diagnostic methods. Mutant genes sequence, their expression level, and protein structure or function is associated with malignancies. During malignancy tumor cells discharge their nucleic acid into the blood stream following apoptosis, resulting in elevated levels of circulating DNA, mRNA, and microRNA in patient's blood. Hence, circulating cell-free DNA could be used for diagnosing early stage cancer [3,4]. Despite of a number of reported genomic methods for cancer detection, very few of them are reliable and used in clinical settings [5]. On the other hand, protein biomarkers which are used more frequently, because scores of detection approaches have expanded their use in research laboratories. Likewise production of antigen specific antibodies has further aided in their use. Approximately, 1261 malignancy-specific protein biomarkers have been reported that express differentially in diverse types of cancer [6]. Nevertheless, very few of them are used in detecting cancer in premalignant stage. Only 9 of these proteins have been approved by FDA as “tumor associated antigens”. Discovery and validation of new biomarker candidates would help in filling up the gap between basic research and clinical use of advanced diagnostics.

A large number of analytical and clinical studies have used immunoassays and now they have become the most powerful and sensitive diagnostic methods both in research and diagnostic laboratories [7]. Enzyme-linked immunosorbent assay (ELISA), is the most frequently used technique amongst immunoassays. It is reliable, effective, and sensitive for the detection and screening of target biomarkers and other antigens. Yet, some biomarkers are expressed at very small quantity that's why their quantification is beyond the detection limit of ELISA. To obviate this major left over impediment, Sano et al., in 1992 [8], developed and introduced a fast and elegant assay, known as immuno-PCR (iPCR), in which a detection antibody is coupled with a reporter DNA. Detection antibody recognizes and binds the target biomarker, after which the conjugated-DNA fragment is amplified through PCR. This technique is 1000-fold more sensitive than the conventional ELISA and can detect even a single antigen molecule [9]. Furthermore, it has also been shown that iPCR is valuable for the detection of target antigens at large quantitative differences while ELISA, which gives a linear amplification and end point detection, is more suitable for detection of smaller differences at lower concentrations [10].

To increase the efficacy and sensitivity of this technique, iPCR protocol was assembled in different formats by bringing some improvements in the classical design (Fig. 1). For example, DNA-directed immobilization of proteins was carried out in order to enhance the sensitivity of iPCR [11]. This modified immunoassay can be performed in a single step, thereby, reducing handling time and cost of analysis. Further advancement in iPCR technique for making the signal detection process more convenient, led to the development of real-time iPCR. This detection method is superior over iPCR, because it quantify the target antigen and interpret results soon, as the PCR reaction proceeds. Although real-time iPCR was more accurate and precise, but it is relatively infantile as compared to the real-time PCR and iPCR, entailing validation and standardization. However, it has been used for the detection of a wide range of analytes including, viral antigens and pathologic proteins [12]. Applications of iPCR for the detection of infection have extensively been discussed and tabularized [13]. Another fascinating and worth noticing format is the phage-based open-sandwich iPCR, which was devised and for the detection of small antigen molecules i.e. human osteocalcin fragment peptide and

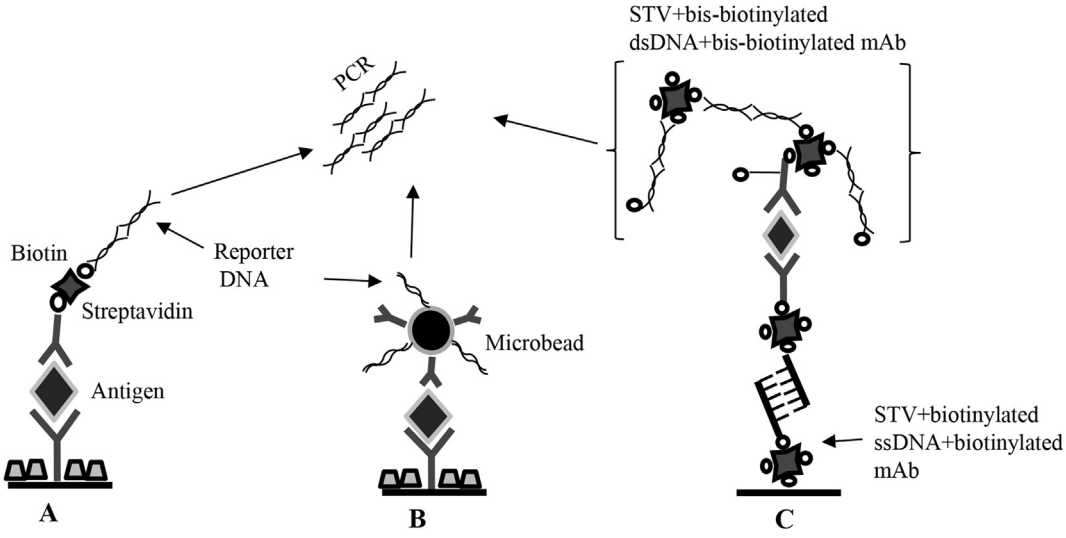

Fig. 1. A. Classical iPCR format: An antigen specific mAb is immobilized on the surface of microtiter plate and free binding sites on the plate are blocked with blocking reagents. Antigen is added to the well which binds to the captured mAb. Streptavidin (STV) conjugated with biotinylated oligonucleotide and biotinylated detection mAb is added. mAb binds with the target antigen after which the signal is generated by amplifying the conjugated oligonucleotide through PCR. B. Microbeads based-iPCR: mAb is captured on the surface of microtiter plate and blocking is done. After adding antigen, then microbeads to which the detection antibody and reporter DNA are attached are added. Finally, oligonucleotide is amplified using PCR. C. DNA directed immobilization: In this format STV which contains a biotinylated ssDNA is coated on the surface of microtiter plate. A preconjugate of STV, containing a biotinylated ssDNA that is complementary to the surface-immobilized ssDNA and biotinylated mAb is added. Complimentary ssDNAs hybridize with each other. Immobilized mAb then binds with the antigen and finally the signal is generated by iPCR, using oligomeric conjugates of STV bis-biotinylated dsDNA and biotinylated mAb. The read-out of iPCR is carried out using real-time PCR [11].