REVIEW ARTICLE

Immuno-PCR in cancer and non-cancer related diseases: a review

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ABSTRACT
Polymerase chain reaction-amplified immunoassay (immuno-PCR, iPCR) is a method that combines the specificity of an immunological detection method and the sensitivity of a nucleic acid amplification method. In this way, immuno-PCR uses a minimum amount of sample, and allows the detection of rare diseases and those diseases in very early stage (i.e. infectious diseases, degenerative disorders, or neoplastic diseases). The present review was aimed to describe this new methodology and applications to the early detection of cancer and non-cancer related diseases, and discuss about the possibility to detect diverse biomarkers of oncology disorders, such as breast, gastric, colorectal and nasopharynx cancer, and other factors related to the growth of the neoplastic disease.

1. Introduction
The detection and quantitation of analytes, i.e. hormones, toxins, cytokines, and pathogens are major activities of biomedical research and clinical laboratories. The technologies used for this purpose often rely on the ability to detect molecules or ligands that will bind very specifically to the analytes under investigation. Then, a more generic read-out system detects the analyte (Shan & Toye 2009). For many years, the enzyme-linked immunosorbent assay (ELISA) has been used for this purpose. It is a simple, highly sensitive, and easy standardization method; however, ELISA is not suitable for small molecule or trace substance detection (Janssen et al. 2013; Chen et al. 2014), or some antigens at low concentration (Malou & Raoult 2011). In this way, a new methodology that combines the specificity of the immunological methods, i.e. ELISA, with the exponential amplification of the molecular methods, i.e. polymerase chain reaction (PCR), named polymerase chain reaction-amplified immunoassay (immuno-PCR, iPCR) was introduced by Sano et al. (1992). This technique is based on chimeric conjugates of specific antibodies and nucleic acid molecules; the latter molecules are used as markers and amplified by PCR for signal generation (Niemeyer et al. 2005; Deng et al. 2011). So, instead of an enzyme marker, i.e. alkaline phosphatase, immuno-PCR amplifies the attached DNA for signal generation (Adler et al. 2008).

The enormous efficiency of nucleic acid amplification typically leads to a 10–100,000-fold increase in sensitivity of immuno-PCR when compared to ELISA (Zhou et al. 1993; Joerger et al. 1995; Niemeyer et al. 2005; Allen et al. 2006; Kuczis et al. 2012).

The evolution of immuno-PCR included the development of efficient reagents, the design of assay formats, and the maintenance of the functionality, even within complex biological matrices. Nowadays, this technique is a flexible and versatile method. It allows for the detection of protein antigens and respective antibodies, and it is compatible with a large variety of biologically complex matrices (e.g. sera, blood, urine, saliva, stools, cell culture, food, and plant extracts), which facilitates its application to a wide range of areas (Adler et al. 2008; Malou & Raoult 2011). This technique can be useful to detect a wide range of antigens in very low amount of sample, such as small particles of tissue or volumes of fluids, at which ELISA would not be able to do due to the low detection limit of the former technique. Also, immuno-PCR is capable to detect minimal amount of biological macromolecules, i.e. proteins, carbohydrates, and lipids, which is very useful for the clinical diagnosis and biological research (Cao et al. 2000).

The immuno-PCR is considered one of the most sensitive detection methods for tracing proteins (Allen et al. 2006; Deng et al. 2011), and a powerful tool for the detection of small amounts of proteins, including cytokines (Potuckova et al. 2011), hormones (Joerger et al. 1995), toxins (Kwon et al. 2014), viral antigens (McKie et al. 2002; Deng et al. 2011; Matsushita et al. 2013), bacterial antigens (Kakizaki et al. 1996; Allen et al. 2006), and tumor markers (Zhou et al. 1993;
Malou & Raoul 2011), using a reporter DNA molecule that can be amplified by an antibody-conjugated enzyme (Deng et al. 2011). The large majority of the immuno-PCR targets reported so far are disease-associated antigens, i.e. infectious diseases (Kakizaki et al. 1996; Barletta 2006; Deng et al. 2011; Deng et al. 2014), tumors, and other types of disease (Zhang et al. 1998; Wang et al. 2008; Singer et al. 2009; Zhang et al. 2010; Kuczius et al. 2012; Meng et al. 2015; Wu & Qu 2015). In addition, the sensitivity and specificity are very critical factors for immuno-PCR, and insufficient blocking or nonspecific binding may influence the sensitivity and specificity of immuno-PCR assay and the final diagnosis of the disease (Allen et al. 2006; Deng et al. 2011).

Recently, Meng et al. (2015) proposed the ultrasensitive application of real-time immuno-PCR (quantitative real-time immuno-PCR, qPCR) for the detection of pyrene and other homologous polycyclic aromatic hydrocarbons in drinking and environmental water samples. These substances are hydrophobic molecules normally present in low levels in the environment (water, soil, and air) (Willis & Oris 2014), and some of them are considered as powerful or potent carcinogens (Li et al. 2014).

According to Zhang et al. (2001), Niemeyer et al. (2001), Niemeyer et al. (2005), Wacker et al. (2007), Adler et al. (2008), and Janssen et al. (2013), there are different types of immuno-PCR, i.e. original immuno-PCR (streptavidin (STV)-protein A chimeric fusion protein tags the detection antibody with biotinylated DNA), universal immuno-PCR (the signal generating complex is assembled ‘in situ’ by subsequent incubation steps of biotinylated detection antibody, STV and biotinylated DNA, either using a non-biotinylated primary and a species specific secondary antibody, or a directly biotinylated primary antibody), qPCR (sequential, modular, and direct qPCR), immuno-PCR by proximity ligation, competitive immuno-PCR, direct and indirect sandwich immuno-PCR (‘in situ’; by capture) (Figure 1), and magneto-immuno-PCR (similar to the two-sided, sandwich, immunoassay, but with antibody-functionalized magnetosome conjugates; based on the chemically modified magnetosome nanoparticles bearing STV molecules at the magnetosome membrane). There are also those techniques related to immuno-PCR by PCR signal amplification, i.e. Bio-barcode (immuno-PCR on chip) (Figure 2), and non-PCR signal amplification, i.e. immuno-RCA (immuno-rolling circle amplification; a single primer generates hundreds of tandemly linked copies of the circular template, and the 5’ end of this primer, attached to an antibody, will produce a DNA molecule with multiple copies of the circle DNA sequence attached to the antibody after the rolling circle reaction) and immuno detection amplified by T7 RNA polymerase (IDAT; a double-

![Figure 1: Different strategies of immuno-PCR. (A) Original immuno-PCR. (B) Universal direct immuno-PCR (biotinylated primary antibody). (C) Universal indirect immuno-PCR (primary antibody-specific for target, and a secondary biotinylated antibody species-specific). (D) Direct sandwich immuno-PCR. (E) Indirect sandwich immuno-PCR (adapted from Adler et al. 2008). (F) Immuno-PCR by proximity ligation (sample is incubated with biotinylated antibodies, streptavidin molecule linked to oligonucleotides and linker oligonucleotides (splint); antibodies will bind pairwise to epitopes on target proteins; each streptavidin-oligonucleotides bind to the biotin of each antibody, forming proximity probes; the extremity of the probes are binded by the linker oligonucleotides; when bound to the specific target, it generates an amplicon that can be amplified using different methodologies, i.e. PCR and qPCR) (adapted from Janssen et al. 2013 and Greenwood et al. 2015).](image-url)
stranded oligo contains T7 RNA polymerase promoter that, under isothermal conditions, is bound by the polymerase to repeatedly synthesize RNA molecules, resulting in amplification dependent on the number of original templates.

The immuno-PCR has high sensitivity and an adaptable design. In this way, it can be used as a screening test for the routine of the veterinary facilities, clinical laboratories, and food industry (Nikitina et al. 2014; Sun & Zhuang 2015; Wu & Qu 2015). Even that, the adaptation and standardization are required for the use in different laboratories and facilities. This standard immuno-PCR approach should detect very low amounts of proteins of host and parasite proteins in body fluids, and tumors. In addition, the early diagnosis of the malignancies is crucial for the success of the treatment and a better prognosis of the patient, and can be the major difference between life and death in these patients.

Some studies have compared different methodologies of immuno-PCR, e.g. tagged-protein detection in yeast. In those studies, immuno-PCR resulted as a specific and sensitive method, being a good alternative to western blot, ELISA, and flow cytometry (Niemeyer et al. 2005; Lind & Norbeck 2007; Askin & Schaeffer 2012). It can also be combined to the phage display technique for the detection of viral antigens (Monjezi et al. 2013) or antibodies (Yu et al. 2007), as an alternative to ELISA.

Another approach is the combination of immuno-PCR and the in situ immunoassay, which presents high sensitivity, detects low concentration of proteins, and represents an alternative to the immunohistochemistry.

An important drawback of immuno-PCR to be considered is that the high amount of protein can cause false-positive results. Background signal is a critical factor, primarily when using complex protein suspensions. In this process, Kuczius et al. (2012) observed a dramatic reduction of the background noise by including two heating steps, the first for protein denaturation and the second for detachment of the immunocomplexed DNA, enabling optimal DNA amplification. In addition, the background value can be easily determined using the negative control sample in immuno-PCR (Lind & Norbeck 2007).

2. Methodology

The requirements for immuno-PCR are much more stringent than for conventional ELISA, due to the PCR amplification step. It is necessary for the synthesis of antibody-DNA conjugate reagents, and the method for the readout of the amplified nucleic acid fragments. These two steps are the keys that make ELISA and immuno-PCR methods to be different, and give the high sensitivity to immuno-PCR (Niemeyer et al. 2005).

Below, the reader can find the information on the method step-by-step, the linker-system, and quantitative applications of immuno-PCR.

2.1. Immunoassay system for immuno-PCR

The immunoassay step of the immuno-PCR resembles the conventional ELISA. It is possible to use the antigen or antibody as attesting sample. Nevertheless, the antigen is absorbed on the solid phase; concerning the PCR, this antigen should be distributed uniformly in the solid phase. It is important that during the PCR, the solid phase should be heat conducting, thermostable, and hard to volatilize. The specificity and affinity of the antibodies are crucial in this step, since they will affect...
the final specificity and sensitivity of the immuno-PCR. For this, it is highly recommended to use monoclonal antibodies. Usually, the antibodies and DNA used for immuno-PCR are tagged with biotin or avidin. In DNA, biotin or avidin will link to a DNA fragment. Additionally, this assay can be classified in direct quantitative immuno-PCR, competitive quantitative immuno-PCR, and sandwich quantitative immuno-PCR (Singer et al. 2009; Deng et al. 2011; Chen et al. 2014).

Nowadays, antigens coated on the Top Yield strips are used for immuno-PCR. These antigens are caught by specific-conjugated immunocomplex (primary antibodies with biotin-conjugated secondary antibodies). In this protocol, the biotinylated reporter DNA uses STV-biotin (streptavidin-biotin) bridges to connect to the antibody. After this reaction, the DNA bound is detached from the immunocomplex, and amplified by conventional PCR. The amplified DNA is used as corresponding signal to the immunocomplex, and it is visualized in agarose gel. Densitometry analyses of DNA signals are used to discriminate between positive results and background noise (Kuczius et al. 2012).

2.2. Linker system for immuno-PCR

The linker molecule is the key of immuno-PCR. Today, most of the research studies on immuno-PCR shifted focus to the use of multivalent linkers to achieve the coupling of the DNA label and the antibody (Janssen et al. 2013). In the beginning, Sano et al. (1992) reported a recombinant protein chimera composed of polypeptide chains of protein A and STV used as the linker. Protein A selectively binds to the fragment crystallizable region of the immunoglobulin G (IgG), and STV binds to the small molecule biotin with a high specificity and affinity, forming a protein chimera, named A-STV. This protein chimera can link the antibody with the biotinylated double-stranded DNA by mixing a stoichiometric ratio of the three components. The downfall of the system is that protein A can be overloaded with biotinylated substance. DNA molecules should come from different species; however, the purity and homogeneity should be guaranteed. The origin of the DNA tag is generally from plasmids or bacterium DNA. Biotinylated DNA is obtained by labeling biotinylated deoxyadenosine triphosphate (dATP) or deoxyuridine triphosphate (dUTP) onto DNA molecules via DNA polymerase. The ratio of DNA molecules to biotin should be 1:2 to achieve 100% labeling. Overloading the biotinylated DNA will lead to nonspecific background, while insufficient labeling will reduce the sensitivity (Chen et al. 2014).

2.3. Quantitative real-time immuno-PCR (qPCR)

Real-time PCR (quantitative PCR, qPCR) is a very sensitive technique to measure DNA concentrations, and can be highly specific by the use of probes. The qPCR is used for quantification of proteins (Lind & Kubista 2005; Janssen et al. 2013), and it was first described by Sims et al. (2000). This quantitative system combines the advantages of immunoassays (flexibility and robustness) with the exponential signal amplification power and larger dynamic range of PCR, compared to ELISA (Table 1) (Lind & Kubista 2005; Niemeyer et al. 2007; Chen et al. 2014). It can be carried out by a gel electrophoresis or qPCR. The gel electrophoresis is usually the conventional semi-quantitative method due its lower accuracy, and time-consuming. The qPCR method, by non-probe detection system, detects the fluorescence signal produced in every cycle of PCR, and uses it to analyze the amount of DNA template of the initial sample (McDermed et al. 2012). In another way, the use of probes also increases the stringency of the reaction and, consequently the specificity.

Increased use of qPCR has shown that this method displays improved statistical validation of accuracy over immuno-PCR. Inter-assay error is typically 5%—10% versus 15%—20% for immuno-PCR. The main advantage of qPCR is the immediate analysis and quantification of positive data (Barletta 2006).

3. Application to the oncology research

The standard methods usually detect malignancies in advanced stages. Hence, the patient can lose the best chance of treatment. There are some specific known substances, called biomarkers, which are generated during the pathogenesis of the disease, and are not present in the healthy population. It is also known that the surface of cancer cells possesses multiple biomarkers, and a good biomarker can distinguish patients from healthy or benign diseases. Cancer biomarkers can be used in early cancer diagnosis, anticancer therapy development, and monitoring the responses to treatments (Table 2). Protein-based
cancer biomarkers are major forms in use, as they are much easier to be monitored in body fluids or tissues (Hu et al. 2015). In addition, biomarkers can be monitored in blood or on the cell surface (Chen et al. 2014). They reflect the sheer number, complexity, and dynamics of the tumor pathophysiology, and thus are extremely valuable sources for the discovery of cancer biomarkers (Hu et al. 2015). In this way, the sensitivity of the assay used to monitor the biomarker is essential to control the disease.

In oncology research, the sensitivity of immuno-PCRs has ranged from 10-fold (EBNA1; nasopharyngeal carcinoma) by universal immuno-PCR (Wang et al. 2008) and 100-fold (prostate-specific antigen [PSA]) by qPCR (Nam et al. 2003) to 100,000-fold (human proto-oncogene ETS1) by universal immuno-PCR (Zhou et al. 1993) higher than ELISA. Nam et al. (2003) used a nanoparticle probe that carries a large number of oligonucleotides per protein binding event, resulting in a substantial amplification and PSA being detected at 30 attomolar concentration, compared to just 3 picomolar of the comparable clinically accepted conventional assays.

Draberova et al. (2013) observed that immuno-PCR presented higher sensitivity (0.086 ng/mL) and wider dynamic range than ELISA plus biotinyl-tyramide amplification (2.5 ng/mL) on α-tubulin quantification. This immuno-PCR shows a > 4 log dynamic range and femtomolar detection limit for tubulin dimer. αβ-tubulin dimers form the microtubules and represent cellular structures that are indispensable for the maintenance of cell morphology and for cell motility generation. In this way, immuno-PCR can be used for quantification and monitoring the changes of tubulins in cytosol after treatment with antimitic drugs (microtubule stabilizing or destabilizing agents), during the cell cycle of various cell types of different cells, and activation or differentiation events.

In another scenario, Yoshida et al. (2009) standardized an immuno-PCR protocol to detect and quantify

| Table 1. List of advantages and disadvantages of ELISA and immuno-PCRs. |
|-----------------------------|-----------------------------|
| **ELISA**                   | **Immuno-PCRs**             |
| **Advantages**              | **Advantages**              |
| Simple method               | Exponential signal amplification power |
| Easy standardization        | 10–100,000-fold more sensitive than ELISA |
| Low cost reagents and equipment | Detect very low amounts of proteins and rare biomarkers in complex biological samples |
| Flexible and robust         | Large dynamic range |
| Duration: 3–6 hours         | Improved statistical validation of accuracy (qPCR) |
|                            | Immediate analysis and quantification of positive data |
|                            | High specificity by the use of probes (qPCR) |
|                            | Duration: 4–7 hours, with only about 3 hours hands-on-time |
|                            | Generic utility for different ligand-analyte combinations |
|                            | Versatile method: large variety of biological samples |
|                            | iaPCR does not require modifications of the antibodies |
|                            | Minimized sample volume requirements |
|                            | DNA and RNA both adhere to simple complementarity rules |
|                            | Flexibility (oligonucleotides easily synthesized and chemically modified) |
|                            | Specific sequences can be used for direct ‘barcoding’ of analytes |
|                            | High tolerances against drug and matrix effects |
|                            | Adaptability for the detection of basically any antigen |
|                            | Require a PCR machine (thermocycler) |
|                            | Complex conjugation chemistries to link the antibody and DNA-markers |
|                            | Need optimization: high background signals prohibit meaningful results |
|                            | Multistep protocol requires experimental expertise in PCR and ELISA |
| **Disadvantages**           | **Disadvantages**           |
| Not suitable for small molecule/trace | More variants to be controlled than ELISA for standardization |
| substance detection         | Require a PCR machine (thermocycler) |
| Analysis and quantification | Complex conjugation chemistries to link the antibody and DNA-markers |
| just at the end             | Need optimization: high background signals prohibit meaningful results |

| Table 2. Applications of immuno-PCR in oncogenesis research, and comparison to ELISA performance when available. |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| **Target**                  | **Cell type**               | **Immuo-PCR enhanced sensitivity compared to ELISA** | **Dynamic order** | **Immuo-PCR** |
| PSA                         | Prostate cancer             | 100-fold                    | 6                           | qPCR (nanoparticle probe)    | Nam et al. (2003) |
| EBNA1                       | Nasopharyngeal carcinoma    | 10-fold                     | >-2                         | Immuno-PCR                   | Wang et al. (2008) |
| ETS1                       | Human proto-oncogene        | 100,000-fold                | 4–5                         | Universal immuno-PCR         | Zhou et al. (1993) |
| VEGF<sup>+</sup>             |                               | 100,000-fold (iaPCR)        | 2–6                         | Immuno-PCR                   | Yoshida et al. (2009) |
| MGC7-Ag<sup>+</sup>         | Gastric carcinoma           | 10,000-fold (immuno-PCR)    | >4 log                      | Universal immuno-PCR         | Draberova et al. (2013) |
| Her2<sup>+</sup>            | Mammary adenocarcinoma      | 100-fold                    | Universal immuno-PCR        |                                 | Zhang et al. (2010) |
| CCR2<sup>+</sup>            | Colorectal carcinoma        | 100-fold                    | Universal immuno-PCR        |                                 | Kazane et al. (2012) |
| α-tubulin<sup>+</sup>       | Mouse bone marrow-derived mast cells | 100-fold                    | Universal immuno-PCR        |                                 | Li et al. (2012) |
| CA 15-3<sup>+</sup>         | Breast cancer               | 100-fold                    | Universal immuno-PCR        |                                 | Draberova et al. (2013) |

Note: PSA, prostate-specific antigen; EBNA1, Epstein–Barr nuclear antigen 1; VEGF, vascular endothelial growth factor; MGC7-Ag, human gastric carcinoma-associated antigen-specific monoclonal antibody; Her2, human epidermal growth factor receptor 2; Cox-2, cyclooxygenase-2; CA 15-3, carbohydrate antigen 15-3.

<sup>a</sup>The research did not focus on a specific neoplasm.

<sup>b</sup>No comparisons were made to ELISA in these papers.

<sup>c</sup>The research did not run immuno-PCR, but compared their iaPCR and ELISA results to the most common immuno-PCR values observed in the literature.
minute amounts \((16 \times 10^{-18} \text{ moles})\) of vascular endothelial growth factor (VEGF), a protein released by the platelets and it seems to be associated with the microvascular density and grading of tumor (Patruno et al. 2009). Yoshida et al. (2009) have used a RNA aptamer (immuno-aptamer PCR, iAPCR) for rabbit IgG instead of the conventional secondary antibody to avoid problems with antibodies that cannot accept the chemical modification (probing antibody must be linked to nucleic acids or biotin, for biotinylated nucleic acids), because of conformational changes or additional modifications in the antigen binding sites. They observed higher sensitivity (100-fold) compared to ELISA, using the same combination of antibodies for both methods, but lower when compared to the original immuno-PCR values (10,000-fold), as reported by Kakizaki et al. (1996) and Adler et al. (2008). Yoshida et al. (2009) also observed that iAPCR has the potential to expand the quantitative dynamic range by about 100- to 1000-fold, with the original ELISA antibody combination and without any modification of the rabbit IgG. In this way, sensitive ELISA assays tend to be usually 10—1000 less sensitive than immuno-PCR, unless very specific procedures are followed, old-fashioned ELISAs are used for comparison, and different protocols or incomparable conditions are used. The sensitivity of both ELISA and immuno-PCR may reflect the quality of the antibodies, reagents, and protocol used. The usefulness of immuno-PCR strongly depends on further development of high quality antibodies (Adler et al. 2008). Another important and critical step is the standardization of the protocol with these antibodies and reagents. Although easy to standardize, any problem on the standardization of the ELISA protocol will affect and reduce much more the sensitivity for the detection of proteins.

According to Battinelli et al. (2011), breast cancer cells activate platelets. In this way, it is possible that the tumor cells cause VEGF release by platelets, considered a pro-angiogenic factor. Moreover, it is crucial to have minimal blood amounts of this protein being measured, since it can influence the prognosis and the treatment of the patient.

Nowadays, several diagnostic methods are used for cancer diagnosis, such as breast examination and image diagnosis (e.g. mammography, ultrasonography, and computerized tomography). These methods present low sensitivity to detect tumors in their early stages, and cannot distinguish between benign and malignant tumors. Hence, they are just considered as screening tools for cancer diagnosis. Therefore, the use of tumor markers in early diagnosis remains a very indispensable tool for the treatment of breast and other types of cancer. Extensive research efforts are focused on the identification and validation of biomarkers to help the early diagnosis of cancer patients. A recent example of that is the Sadhasivam et al. (2014) research. They used immuno-PCR to detect the breast cancer marker, CA 15-3 from the mucin-1 family, in the early stage of the disease. The immuno-PCR technique presented a limit of detection of 0.001 U/mL, which is considered very low when compared to other bioanalytical techniques and essential for the detection of early stages of breast cancer (Niemeyer et al. 2005).

Li et al. (2012) found a strong correlation between the expression of Cox-2 and colorectal cancer metastasis. According to the authors, Cox-2 is an inducible enzyme that converts arachidonic acid to prostaglandin, and it is hypothesized to induce carcinogenesis and metastasis in colorectal cancer. They observed that the Cox-2 protein is infiltrated into the serum and the knockdown of Cox-2 expression suppressed the proliferation and invasion of colorectal cancer cells both ‘in vitro’ and ‘in vivo’ by using an immunobead real-time PCR method to detect very low concentration of Cox-2 in serum samples. These results can help the early diagnosis of this type of cancer and improve the prognosis of the patients.

In another study, the immuno-PCR was standardized to detect EBNA1, a biomarker used for nasopharyngeal carcinoma. This biomarker is very specific and sensitive, but it does not have high levels in the early stages of the disease. It makes immuno-PCR important for the diagnosis of these patients (Wang et al. 2008). The sensitivity of the immuno-PCR detected by these authors was approximately two orders of magnitude higher than conventional ELISA.

Another point of view is with gastric cancer. Gastric cancer is the second most common type of neoplasm in China and, because of the high cost of endoscopy exams, researchers started to look for a better diagnosis. In this way, Zhang et al. (2010) tested MG7-Ag protein as a good biomarker for human gastric carcinoma. This protein is elevated in gastric cancer tissues compared with normal mucosa and benign lesions. The authors used the immuno-PCR to detect this protein in serum samples of high-risk population from China (samples of 2710 participants of 35—65 years old, during 2002—2003, and observed 5.5% (148/2710) MG7-Ag positive participants. The authors also observed 75% sensitivity (31/40 gastric cancer cases), 95.6% specificity (2553/2670 nongastric cancer subjects), and 73.1% accuracy. In this way, MG7-Ag may have a potential as a biomarker and to be used as good screening method for gastric cancer in high-risk population.

Kazane et al. (2012) have developed a methodology to site-specifically conjugate oligonucleotides to antibodies containing a genetically encoded unnatural amino acid with orthogonal chemical reactivity. In this study, they applied this methodology on Her2 (human epidermal growth factor receptor 2) research in three human mammary adenocarcinoma cell lines using...
immuno-PCR. They observed that the site-specific conjugation generates enhanced positive signal and lower background signal compared to nonspecific conjugation. The conjugation position affects immuno-PCR signal, and Her2 antigen can be detected on cells with high selectivity and sensitivity by oligobodies, both on isolated cells and in a mixed population of cancer cells and WBCs. So, this methodology has higher sensitivity and specificity than nonspecifically coupled fragments, and can detect extremely rare Her2 positive cells in a complex cellular environment.

In this way, immuno-PCR opens a new window for the biomedical analysis of neurodegenerative and cancer diseases, viral infections, and detection of carcinogen substances as well as new tools for the development of novel pharmaceuticals (Niemeyer et al. 2007; Kazane et al. 2012), but more studies are necessary focusing on human and animal cancer diagnosis.

4. Final considerations

The immuno-PCR can detect only a target to which proper antibody is available. Therefore, it is a diagnostic tool only for diseases to which good antibodies are available. This technique has been updated along the years and allows for the detection of proteins in early stages of cancer or non-cancer diseases, being recommend by the use on clinical routine. The wide use of the immuno-PCR and the strategies related to this technique will contribute to improve the quality of the diagnosis of the disease, and, indirectly, the life of each patient.

Disclosure statement

No potential conflict of interest was reported by the authors.

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