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Smart applications of bionanosensors for BCR/ABL fusion gene detection in leukemia

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ABSTRACT

The present review discuss the relevance and innovative state-of-the-art on molecular diagnosis of leukemia. This morbidity is one of the most common types of cancer with an annual incidence of 250,000 new cases. The prevalence of leukemia among children up to 15 years of age is 30% on all cases of cancer reported in the childhood. The BCR/ABL fusion gene is one of the most important biomarkers in leukemia, being found in all cases of chronic myeloid leukemia and up to 40% of cases of acute lymphoblastic leukemia. Genosensors are considered smart devices for identification of BCR/ABL fusion gene in clinical samples. Molecular techniques can contribute to early diagnosis of cancer, monitoring of minimal residual disease and implementation of effective drug therapies. The present review presents the scientific advances in the last decades on DNA biosensors constructed for BCR/ABL fusion gene detection. The assembly of nanostructured platforms, molecular immobilization strategies and analytical performances of the biodevices are discussed. The present review assesses the potential of electrochemical and optical techniques for BCR/ABL fusion gene detection. Electrochemical genosensors based on engineered nanomaterials at the transduction interface are useful to obtain high levels of sensitivity (up to 10^{-18} M). On the other hand, optical genosensors had higher detection limits (10^{-15} M). The analytical response time and reusability of genosensors for BCR/ABL fusion gene identification in small sample volumes (in the order of μL) were discussed. The present review highlighted nanostructured platforms as promising tool for diagnosis and monitoring of BCR/ABL fusion gene in leukemia patients.

Contents

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1. Background

Leukemia is one of the most common cancer worldwide, presenting 250,000 cases annually (Ferlay et al., 2012). Leukemia is described as a malignant disease caused by abnormal white blood cells produced in bone marrow. An exacerbated and uncontrolled production of abnormal blood cells occur, leading to a decreased production of healthy blood cells, promoting the rise of bleeding, several infections and severe anemia (Inamdar and Bueso-Ramos, 2007). In addition, leukemic cells can also spread to other organs such as spleen, brain, lymph nodes and other tissues (Inamdar and Bueso-Ramos, 2007).

The clinical practice and scientific research are a support to define the specific hematologic disease (Vardiman, 2010). World Health Organization (WHO) elaborates a differentiated hematopoietic and lymphoid tumors classification based on morphological, clinical, immunophenotypic and genotypic parameters. WHO classified hematopoietic neoplasms according to the lineage of the neoplastic cells as myeloid, lymphoid, histiocytic/dendritic, neutrophilic, eosinophilic, mastocytic, basophilic, monoblastic, monocytic or ambiguous. In addition, leukemias are subclassified according to their clinical evolution in acute or chronic (Arber et al., 2016).

Genetic abnormalities are characteristic of human malignancies (Guarnerio et al., 2016) and characterized by variations of the number of DNA copies and aberrations in the chromosome structure resulting from mutations or gene fusions (Bochtler et al., 2015). Gene fusion is a DNA recombination that involves the exchange of genetic material between chromosomes or between distinct regions of the same chromosome (van Gent et al., 2001). The molecular mechanisms responsible for obtaining a hybrid gene are translocation (t), deletion, insertion and chromosomal inversion (Feuk et al., 2006). Oncogenic fusion occurs in neoplastic cells and includes at least one proto-oncogene during DNA recombination process. Proteins derived from oncogenic fusion have abnormal activities and contribute to the development of cancer, such as leukemia (Mitelman et al., 2007; Nero et al., 2014).

BCR/ABL translocation can be observed in myeloproliferative neoplasms, acute leukemia of ambiguous lineage and precursor lymphoid neoplasms class as chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) (Vardiman, 2010). Philadelphia chromosome (Ph chromosome) is a shortened chromosome 22, resulting from translocation between long arms of chromosome 9 and 22 in hematopoietic stem cells, presented as t(9;22)(q34;q11). In addition, Ph chromosome is found in over 90% of patients with CML (Rowley, 1973). Molecular consequences are formation of BCR/ABL fusion gene on chromosome 22 and a reciprocal ABL-BCR on chromosome 9 (Chandra et al., 2011; Liu et al., 2014a,b). The resulting BCR/ABL protein is located in the cytoplasm with a deregulated tyrosine kinase activity (Ben-Neriah et al., 1986). Three main types of hybrid genes BCR/ABL are observed, producing three isoforms of tyrosine kinase protein with abnormal activity: $p230^{BCR/ABL}$, $p210^{BCR/ABL}$, $p190^{BCR/ABL}$ (Melo, 1996). $p190^{BCR/ABL}$ is an uncommon isoform in cases of CML and often observed in children with ALL. $p210^{BCR/ABL}$ is present in most patients with CML in stable phase and some cases of ALL and AML (Li and Du, 1998; Maurer et al., 1991; Winter et al., 1999) (Fig. 1).

1.1. Conventional diagnostic methods

Chromosome banding techniques (CBT) triggered the development and improvement of several others techniques for molecular mechanisms studies. Detection of chromosomal abnormalities was helpful to assist diagnosis, prognosis and monitoring the effectiveness of treatment. The methods used for gene fusion identification are mainly molecular techniques. Fluorescent *in situ* hybridization (FISH) is a technique that allows the narrowing of breakpoint regions for hundreds of Kb. Additionally, real-time polymerase chain reaction (PCR) and flow cytometry immunophenotyping can be used as new alternatives, offering good sensibility and specificity as compared to chromosomal banding (Bennour et al., 2012; Branford et al., 2004; Mertens et al., 2015). However, these are high-cost techniques and require specialized technicians (Craig and Foon, 2008; Weir and Borowitz, 2001). Both techniques exhibit obstacles such as difficulty in obtaining metaphase chromosome spreads for specific types of tumors and growth problems of some lineage cells *in vitro* (Arber et al., 2016). It is required the use of refined culture conditions, aiming to create a sufficient number of mitosis to obtain enough material (e.g. DNA, RNA, proteins) (Guarnerio et al., 2016). Complex karyotypes and chromosomal

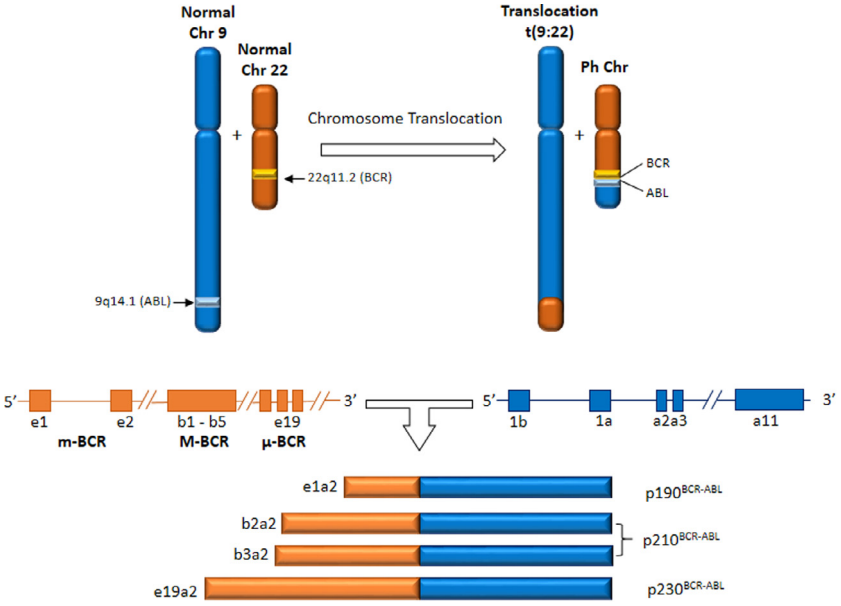

Fig. 1. Translocation (9;22) and BCR/ABL transcripts associated to CML, AML and ALL.