

Basics of DNA biosensors and cancer diagnosis

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Abstract

The human genome is exposed to mutations during the life cycle because of many types of changes in the DNA. Viruses, radiation, transposons, mutagenic chemicals, or any errors that happen during DNA replication or the meiotic process in the cell, may cause the mutation. Many mutations have no effect on phenotype or health, while some mutations cause crucial diseases such as cancer or cardiac diseases; therefore, a better understanding of the effects of mutation on phenotype is a very important part of genetic studies. Biosensors based on DNA, RNA, and peptide nucleic acids are the most sensitive tools, due to a strong pairing of lined up nucleotide strands between bases in their complementary parts. These methods can provide information to assist clinicians in making successful treatment decisions and increase the patient survival rate. In this review, we discuss DNA biosensors based on peptide nucleic acids that have an important role in cancer diagnosis.

Keywords: cancer, DNA, DNA biosensor, gene mutation, peptide nucleic acids

Abbreviations: PCR: polymerase chain reaction; PNA: peptide nucleic acids; POCT: point of care testing; RAPD: random amplified polymorphic DNA; RFLP: restriction fragment length polymorphism

Introduction

Recent processes in the expansion of DNA biosensors for nucleotide sequence-specific DNA hybridization and the detection of DNA damage are briefly reviewed (Palecek et al. 1998). With the discovery of the double helix structure of deoxyribonucleic acid (DNA) in 1953, identifying and sequencing of DNA molecules got more attention (Crick and Watson 1954). DNA is the carrier of genetic information, and the base material of biological heredity. Nucleic-acid sequences, which are different in every living organism, virus or pathogen, provide practical ways to recognize and

discern diverse diseases (Liu et al. 2012). After the recognition of DNA, DNA-based diagnostics have evolved, which include the isolation of DNA polymerase, RAPD, RFLP, and PCR techniques (Singh et al. 2010).

As new genes are discovered and proved to be involved in disease, primary patient diagnosis, carrier detection, and prenatal diagnosis will continue to increase progressively (Chin et al. 2005). The human genome is exposed to mutations during the life cycle, owing to many types of changes in the DNA. The mutation may be created by viruses, radiations, transposons, mutagenic chemicals, or any errors that happen during DNA replication or the meiotic process in the cell. Many mutations have no effect on phenotype or health, while some mutations cause crucial diseases such as cancer or cardiac diseases; therefore, a better understanding of the effects of mutation on phenotype is a very important part of genetic studies (Altintas and Tothill 2012, Bertram 2000, Aminetzach et al. 2005, Sawyer et al. 2007, Nna et al. 2010).

About 5%–10% of cancers are hereditary and due to single gene mutations. In the last decade, a number of different hereditary cancer syndromes and their causative genes have been determined, including the hereditary breast-ovarian cancer syndrome, due to the BRCA1/2 genes (Chin et al. 2005, Wooster et al. 1994). Evaluation of the genetic risk of cancer in clinics demonstrates the primary arm of prevention in oncology, in order to recognize high-risk patients for screening and prevention (Chin et al. 2005).

To improve patient care, molecular diagnostic laboratories have been involved in creating new tests that are dependable, affordable and low-cost. They are also used to optimize the existing protocols, by making them quicker and more economical (Lucarelli et al. 2004). According to the analysis of genomic sequences, molecular diagnostics have suggested a highly sensitive and quantitative method for the detection of infectious disease pathogens and genetic diversity (Lucarelli et al. 2004). Through the efforts of researchers, many DNA-testing technologies were created a few years ago (Beaudet and Belmont 2008, Ding et al. 2009).

Although these conventional technologies provide the gold standard for laboratory-based DNA diagnostics, they cannot meet the needs of point-of-care clinical diagnostics (Liu et al. 2012, Tost and Gut 2005). The identification of DNA sequences and their sequence discriminations is difficult and time-consuming, and has low hybridization efficiency. To overcome these problems, DNA sensors/arrays/chips appeared in high throughput analysis, which in turn indicates a considerable decrease in effort, time and cost (Seeman 1999). These biorecognition elements, in combination with different transduction methods, have assisted rapid extension in the fields of bioanalysis and related technologies, and are known as biosensors and biochips (Vo-Dinh and Cullum 2000).

A biosensor is an analytical device that incorporates a biological sensing element with a transducer, to produce a signal used for the analyte concentration (Figure 1) (Lei et al. 2006, Clark 1987). In particular, DNA hybridization biosensors suggest significant promise for getting sequence-specific information in a faster, simpler, and cheaper method, in contrast to traditional hybridization assays (Wang 1998). The development of systems permitting DNA detection is stimulated by utilization in many areas like DNA diagnostics, gene analysis, fast detection of biological warfare agents, and legal applications. Detection of genetic mutations at the molecular level results in the possibility of performing accurate diagnostics even before any sign of a disease occurs (Sassolas et al. 2008). Depending on the nature of the biological process, biosensing devices may be divided into two general classes: biocatalytic sensors (based primarily on immobilized enzymes), and affinity devices (using antibodies, receptors and nucleic acids) (Wang 1999). Recent understanding of the structure-function of nucleic acids, specifically RNA, has created a new landscape in the development of new analytical and diagnostic methods (Mascini et al. 2005). With the increasing number of cancer cases that are growing throughout the world and the increased number of mortalities because of delayed detection, biosensors have an important role in the early diagnosis of cancer (Tothill 2009).

Biosensor structures

There are many possible classification designs. Biosensors and biochips are categorized either by their bioreceptor or their transducer type. The sampling part of a biosensor

consists of a biosensitive layer, and this layer can either have bioreceptors or be made of bioreceptors covalently joined to the transducer (Vo-Dinh and Cullum 2000, Koyun et al. 2008, Thévenot et al. 2001, Vo-Dinh 2007). A biosensor is a simple device that is made up of two elements:

- 1) A bioreceptor that is an immobilized sensitive biological component (e.g. enzyme, DNA probe, antibody) recognizing the analyte (e.g. enzyme substrate, complementary DNA, antigen). However, though antibodies and oligonucleotides are widely engaged, enzymes are still the most commonly used biosensing elements in biosensors.
- 2) A transducer is used to turn a (bio) chemical signal, caused by the interplay of the analyte with the bioreceptor, into an electronic one. The intensity of the generated signal is directly or inversely proportional to the analyte concentration.

Biosensors contain three parts:

- 1) The sensitive elements (biologically derived material).
- 2) The transducer or detector element, to transform the detected signal into a readable, quantified output.
- 3) The signal processor that represents the transformed signal in a user-friendly way (Liu et al. 2012).

Classification of biosensors

Classification of transducers

In biosensors, distinct biological elements may be associated with different kinds of transducers, if the reaction of the biological element with the substrate can be monitored (Paddle 1996). Electrochemical transducers are the most widely used transducers in sensor technology, but optical and QCM type of sensors have gained much attention, and their application is expanding in practical uses (Tothill 2009).

- a) Optical measurements (Balslev et al. 2005, Kovacs 1998, Brecht and Gauglitz 1997) (i.e. luminescence, absorption, surface plasmon resonance) (Pattnaik 2005, Liedberg et al. 1983):

Optical transducers used in biosensors include fluorescence, interferometry, and spectroscopy of optical wave guides and surface plasmon resonances (SPR) (Tothill 2009). SPR is used to detect nanomolar levels of PCR products from genetically modified organisms, to resolve human gene mutations (Vercoutere and Akeson 2002).

- b) Electrochemical (Junge et al. 1997, Pividori et al. 2000):

Electrochemical transducers have recently received significant attention in relation to the detection of DNA hybridization (Wang 2002, Wang et al. 2004). They use DNA probe molecules connected to an electrically active surface, and measure current or resistance changes created by the hybridization of target DNA (Vercoutere and Akeson 2002). They have been developed based on DNA hybridization, and are used to detect cancer gene mutation. The electrochemical devices used with the biosensors have been miniaturized to small pocket

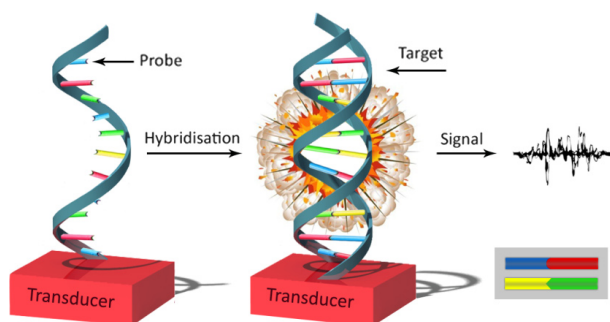


Figure 1. General design of a DNA biosensor.

size instruments which make them applicable for the home use or the doctor's surgery. A range of electrochemical transducers are applied, and these include amperometric, potentiometric, and impedimetric/conductivity instruments (Tothill 2009). New reviews in the application of electrochemical biosensors for cancer diagnosis have been reported by Lin, Ju, and Wang (Tothill 2009, Wang 2006, Lin and Ju 2005).

- c) Mass-sensitive measurements (Lange et al. 2002, Dickert et al. 1999) (i.e. surface acoustic wave, microbalance, etc.) (Vo-Dinh and Cullum 2000, Koyun et al. 2008, Thévenot et al. 2001, Vo-Dinh 2007):

This type of transducer is also classified as being a label-free method. Piezoelectric sensors comprise a quartz crystal coated with a gold electrode, and are applied as microbalances (QCM) sensitive to changes in the mass on the sensor surface (Tothill 2009, Lin and Ju 2005).

Bioreceptor classification

The recognition element is an important component of biosensors. Primary biosensors use naturally occurring recognition elements which are extracted from biological or environmental systems. With progress in technology and synthetic chemistry, lots of biosensor recognition elements that are used today are made in the laboratories, to provide an improved stability and reproducibility of biosensor action. For instance, recognition elements including receptor proteins, antigens, antibodies, enzymes, and nucleic acids (Bohunicky and Mousa 2011). There are various biological interactions of ligand/bioreceptor, such as:

- 1) Antibody/antigen interactions (Vo-Dinh and Cullum 2000, Thévenot et al. 2001).
- 2) Nucleic acid interactions (Vo-Dinh and Cullum 2000, Vo-Dinh et al. 2001).
- 3) Enzymatic interactions (Vo-Dinh and Cullum 2000, Vo-Dinh et al. 2001).
- 4) Cellular interactions (Vo-Dinh and Cullum 2000, Vo-Dinh et al. 2001) (i.e. microorganisms, proteins).
- 5) Interactions using biomimetic materials (i.e. synthetic bioreceptors (Vo-Dinh and Cullum 2000, Vo-Dinh et al. 2001)).

Basic of DNA biosensors

The necessity for selectivity of the sensors for their designated analytes in a matrix of other chemical or biological elements is probably the main reason for the interest in biosensors. This high selectivity, when compared to the selectivity of currently used chemical sensors, is obtained by the usage of naturally occurring biomolecules (enzymes, receptors, antibodies, or nucleic acids) as the sensory element (Paddle 1996). They are good candidates for cheap diagnosis of genetic disease, and for the detection of pathogenic biological species in clinical research. The detection of specific DNA sequences related to genetically modified organisms is also monitored as an emerging task (Lucarelli et al. 2004). DNA biosensors apply immobilized DNA as diagnostic elements

(Lucarelli et al. 2004). They convert the event of recognition of the Watson-Crick base pair into a readable analytical signal. The DNA duplex on the electrode surface is identified as a hybrid (Kerman et al. 2003).

Biosensors based on DNA, RNA and peptide nucleic acids are the most sensitive tools due to nucleotide strands lined up with strong pairing between bases and their complementary parts (Monošík et al. 2012). Researchers from different fields such as physics, chemistry, biology, engineering, and medicine, want to develop, invent and manufacture novel sensing devices to gain more efficient and dependable information (Garipcan et al. 2017). Some biosensors are either commercially available or under development now (Paddle 1996). Alongside the possibility of being very sensitive and selective, such nucleic acid-based sensors have some advantages over antibody-based biosensors. They are more stable and can be stored for longer periods, and the probe can be frequently regenerated for greater use by immersing in hot buffer, within a short time (Paddle 1996).

Probe design

Immobilization of the probe is a basic step in the development of DNA biosensors (Mascini et al. 2005). As the properties of the hybridization reaction are essentially related to the properties of biorecognition of the captured oligonucleotide, planning the capture probe is surely the most important pre-analytical step. Therefore, many probes, with varying chemical composition and conformational arrangement, have been used to make DNA biosensors (Lucarelli et al. 2008). Extremely high sensibility and selectivity is needed to maximize the hybridization efficiency and minimize the non-specific binding. Probes are routinely short oligonucleotides that are capable of hybridization with individual areas of the target nucleotide sequence (Wang 2002). The amplicons obtained from PCR, and the DNA pieces obtained from enzymatic digestion, have a double helix structure, and the double strands should be denatured to permit the hybridization with the probe immobilized on the surface of the sensor (Mascini et al. 2005).

The planning of probes to analyze samples sensitive to degradation (such as RNA) needs much attention, if a sandwich hybridization scheme is selected (Lucarelli et al. 2008). The design of linear probes has now gained many advantages during the years of experience, which has led to much commercially available software. However, a special challenge is still posed in the planning of a complete series of arrayed probes for the screening and identification of closely related and unrelated pathogens (Lucarelli et al. 2008, Tombelli et al. 2005, Barlaan et al. 2005). The utilization of capture probes in the order of 18–25 nucleotides usually displays higher levels of property to the hybridization reaction (Lucarelli et al. 2008). However, excessively long capture oligonucleotides often show particularly unfavorable hybridization features and yields. Although a single or a few mismatches are impossible to markedly destabilize even a 30-mer probe–target duplex over a wide range of experimental conditions, the general hybridization efficiency of similar or longer probes might be particularly low, due to intra-molecular hydrogen bonding and consequent formation of non-reactive hairpin structures (Lucarelli et al. 2008).

Peptide nucleic acids

A peptide nucleic acid (PNA) is different from DNA, because the deoxyribose-phosphate backbone of DNA is replaced by a pseudo-peptide backbone, *N*-(2-aminoethyl) glycine (Figure 2). The expansion of DNA sensors based on the sequence-specific hybridization between single-stranded nucleic acid probes tethered to solid substrates and their complementary DNA analytes in solution, has gained popularity over the past few years. In particular, the methods that can be followed in the absence of labels, such as electrochemical (Dharuman and Hahn 2007, Fang et al. 2008), optical (Su et al. 2005, Niu et al. 2007, Vikholm-Lundin et al. 2007), and piezoelectric (Tombelli et al. 2005, Duman et al. 2003, Skládal et al. 2004) methods, have gained significant attention (Ananthanawat et al. 2009).

PNA forms complexes with DNA according to the Watson--Crick base-pairing rules. The PNA-DNA complexes exhibit high thermal stability and mismatch sensitivity (Hyrup and Nielsen 1996). The high stability of these hybrids has been illustrated by the absence of negative charges along the PNA backbone. Recently, many new PNA systems with modified structures have been developed, in efforts to improve the binding properties such as directional preference (parallel/antiparallel), selectivity between DNA and RNA, and binding specificity for different applications (Ananthanawat et al. 2009, Ahn et al. 2003, Govindaraju et al. 2003, Huang et al. 2004, Tan et al. 2004, Suparpprom et al. 2005, Vilaivan and Srisuwannaket 2006). PNA is a DNA analog with a neutral peptide backbone (Lucarelli et al. 2004). PNAs demonstrate unique features such as chemical stability, resistance to enzymatic degradation inside a living cell, recognition of specific nucleic acid sequences, formation of stable hybrid complexes, superior thermal stability and ionic strength, strand invasion, and unique hybridization relative to nucleic acids. These excellent physicochemical and biochemical features of PNA can provide a novel method of detection that is rapid, easy, and more dependable as an analytical process, and can be utilized in the field of molecular diagnostics (Singh et al. 2010). High specificity of DNA binding can be achieved using new probes based on PNAs (Palecek et al. 1998). PNAs are DNA mimics, in which the nucleobases

are connected to a neutral *N*-(2-aminoethyl)-glycine pseudopeptide backbone. According to the Watson-Crick rules, PNA probes form stable hybrids by hydrogen bonding and base stacking. The backbone of PNA is neutral, due to which PNA/DNA hybrids demonstrate melting temperatures more than temperatures related to DNA/DNA duplexes, stability against nucleases and proteases, partial insensitivity to ionic strength, and generally higher selectivity against single base mismatches (Tost and Gut 2005). When compared with the conventional oligonucleotide probes, PNAs have received special interest in the development of electrochemical genosensing concepts, which is the major reason for the drastically different electrical characteristics of their molecular backbone (Lucarelli et al. 2008). Due to their neutral charge and appropriate interbase spacing, this synthetic DNA mimic leads to stronger attraction to complementary DNA sequences, and therefore promotes differentiation between complete matches and mismatches (Wang et al. 1997). The followers of PNAs contend that these probes are capable of binding their complementary sequence with comparably more affinity and specificity than the usual oligonucleotide probes, while discriminating mismatched targets to a larger extent. In spite of careful control of the hybridization conditions, many authors have observed partly strong non-specific signals for a number of SNP-containing targets (Lucarelli et al. 2008, Liu et al. 2005, Steichen et al. 2007).

The markedly better features of the PNA probe extensively improve the detection of single point mutation, found in several types of cancer (Wang et al. 1997). The capability to detect point mutations could be its most important advantage. PNA displays superior hybridization specifications and promotes chemical and enzymatic stability relative to nucleic acids. Its different molecular structure is also capable of novel ways of detection, particularly methods that avoid the introduction of a label (Brandt and Hoheisel 2004).

The rearrangement of the molecules of PNA occur under alkaline conditions (Schmidt et al. 1996). For more information about the affinity and characteristics, as well as the chemical and biophysical features of PNA molecules, we offer three views (Brandt and Hoheisel 2004, Demidov and Frank-Kamenetskii 2004, Uhlmann et al. 1998).

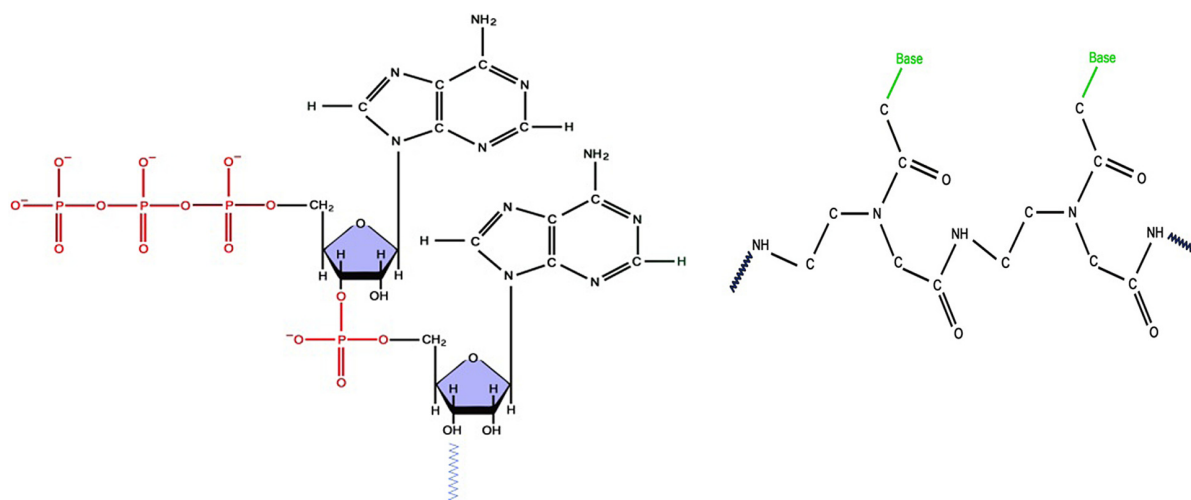


Figure 2. The structures of DNA (left) and PNA (right).

Biosensors for DNA sequence detection

The use of biosensors dates back to 1962, when Clark, known as the father of the biosensor concept, released an assay in which glucose oxidase (GOX) was trapped at a Clark oxygen electrode using a dialysis membrane (Clark Jr. and Lyons 1962) (Monošík et al. 2012). The development of the Human Genome Project has had significant interest in the utilization of nucleic acid hybridization technologies to detect and recognize organisms and mutations. As known, cancer is a genetic disease caused by changes or modification of DNA sequences of key genes, and DNA analysis has a great potential for utilization in cancer diagnostics (Wang 2006). In order to identify the differences between a cancerous cell and a normal cell, it is necessary to assess a complicated network of pathways including gene regulation, signaling, cell metabolism, and changes in dynamics caused by various kinds of mutations which result in malignancy. For example, changes in the structure of the network through mutations or epigenetic effects, can result in changes in the dynamics that lead to different physiological features (Laubenbacher et al. 2009). Since the network is normally complex, with multiple connections between pathways and important feedback loops, it is crucial to show it in the form of a computational model that can be used for a detailed analysis (Laubenbacher et al. 2009). It is well appreciated that many different types of mutations must happen in a multi stage process before a cell becomes malignant, thereby altering different molecular pathways involving the affected genes (Laubenbacher et al. 2009).

Microarray chips that are based on detection of hybridization/interaction in short strands of nucleic acids propose operating systems for applications such as screening of genomes, detection of pathogenic organisms, and effective search in compound libraries to detect and survey potential therapeutic agents (Mascini et al. 2005). Such methods must provide early detection and selection of the treatment of diseases and result in increased patient survival rates (Wang 2006). DNA sequence detection is now a usual method, and is done using DNA chip microarrays. This class of tools allows researchers to measure global genome mRNA expression levels (Brown and Botstein 1999, Wu et al. 2001, Vercoutere and Akeson 2002), and to characterize single nucleotide polymorphisms (SNPs) within individual organisms and populations (Vercoutere and Akeson 2002, Wang et al. 1998, Syvanen 2001). Sample size and multiple preliminary steps hamper microarrays from easily assessing tissue-specific or cell-specific polynucleotide sequences, or providing real-time results (Vercoutere and Akeson 2002, Meldrum 2000, Wang 2000, Eberwine et al. 2001, Gut 2001). These limitations also prevent the quick search for the effect of DNA or RNA levels, which are the techniques to diagnose bacterial food contamination, the genetically modified (transgene) organisms, or the tendency of biological warfare agents. An existing test, called the Enzyme Linked Immunosorbent Assay (ELISA) test, is not sensitive enough to detect proteins at levels associated with advanced stage of the disease. Therefore, smaller, simpler, faster, and much cheaper (one-step) devices are highly favorable for replacing time-consuming laboratory analyses (Wang 2006). Other technologies that

address these issues by combining biological sensor molecules with signal transducers, are being developed (Vercoutere and Akeson 2002). In DNA biosensors, the sensitive element typically contains single-stranded DNA (ssDNA) molecules that permit hybridization of complementary single-stranded molecules (Gill et al. 2005). They have been expanded as alternatives to conventional DNA microarrays. Despite the wide variety of biosensors and biosensor-related techniques that have been introduced, the agreed definition for these devices has remained fairly constant – an analytical device made of a biological recognition element straightly jointed to a signal transducer, which together relate the concentration of an analyte (or group of related analytes) to a measurable response (Turner 1989, Marco and Barceló 1999, Lopez-Avila and Hill 1997, Rogers and Mascini 2004). The biological parts of biosensors are responsible for the selective recognition of the analyte, the generation of the physicochemical signal on the transducer, and finally, the sensitivity of the final device (Paddle 1996). These devices pair with signal transduction directly to sequence recognition. Some of the most sensitive and functional technologies utilize fiber optics or electrochemical sensors in relation to DNA hybridization (Vercoutere and Akeson 2002).

It is essential to develop a DNA biosensor with low cost, high sensitivity and good selectivity (Bo et al. 2011). Expansion of DNA biosensors and DNA microarrays have increased greatly in the past few years, as illustrated by the great number of scientific studies in this area (Sassolas et al. 2008). Biosensor reader devices connected with electronic or signal processors, are initially responsible for showing the results in a user-friendly manner (Cavalcanti et al. 2008). Recently, a hybrid configuration of biosensors, which includes the features of both the high affinity (irreversible) binding of an antibody or DNA/RNA probe by amplifying the qualities of an enzyme, has been introduced (Paddle 1996).

According to Downs (1991), electrochemical methods that can make use of the different redox behaviors of single- and double-stranded DNA to detect the hybridization of DNA, lack sensitivity and are prone to interference (Paddle 1996). The basis of biodiversity is a particular chain of bases along a strand of DNA, and the special complementary nature of the pairing between the base pairs of neighboring strands in the double helix. The capability of a single-stranded nucleic acid molecule to find and adjoin itself to its complementary partner in a sample, has been used in genetic analyses and in a biosensor (Paddle 1996).

Cancer biology

Cancer is a complex disease, due to the disruption of tissue architecture. Thus, tissues, and not individual cells, are the suitable levels of observation for the study of carcinogenesis (Bizzarri et al. 2011). Cancer has also been identified as the second major cause of death in most developing countries (Tothill 2009). A number of factors can cause cancer, including genetic or environmental factors such as the exposure to carcinogenic chemicals and radiations, or microbiological causes like bacterial (e.g. stomach cancer) or viral infections (e.g. cervical cancer) (Tothill 2009). Studies about cancer

have been concentrated on the molecular entities (genes, enzymatic reactions, intracellular pathways), with the indirect assumption that molecules are autonomous and act like a biological system; that is, a collection of entities that work together to do a certain task. In many disease conditions, finding specific and sensitive indicators that are associated with just one type of the disease can be difficult. Moreover, based on different disease conditions and phases, the level of biomarkers in biological fluids will be different. Novel and emerging molecular techniques are used to study cancer and its results, with a better understanding of the disease, as well as the discovery of potential new genomic and proteomic biomarkers. Multi-analyte analysis based on lab-on-a-chip point-of-care devices (POC) are needed to overcome the challenges in cancer diagnosis. A more sensitive and rapid technology platform is needed to perform rapid diagnosis in cancer detection. Rapid diagnosis will help in providing better health care and reducing the waiting time for results, which can be highly stressful to the patients (Tothill 2009, Ahn et al. 2004). Thus, many of the molecular markers usually assess cancer diagnosis, and these can include proteins, peptides, over/under expression of gene markers, and gene mutations (Tothill 2009). However, a system is not simply a collection of genes and proteins, and we cannot understand its qualities completely just by drawing diagrams of their interconnections (Bizzarri et al. 2011). Highly sensitive methods are immediately needed for measuring cancer diagnosis that markers show at ultra-low levels during early stages of the disease (Wang 2006). Development in molecular biology leads to much attention to potential biomarkers that can be used for cancer diagnosis (Wang 2006). For example, DNA point mutations of the *p53* gene, which are engaged in cell cycle control and regulation of apoptosis, were checked using piezoelectric biosensor detection of PCR amplicons, as described by Mascini and co-workers. PCR point mutation analysis of *K-ras*, which is related to colorectal cancers and uses a PCR microfluidics microchip, has been described by Soper and co-workers. Wide-scale POC diagnostic systems offer major promise for early detection of cancer at a curable stage of the disease (Wang 2006). A variety of biosensor platforms have been stated in research on cancer diagnosis. However, introducing this technology to commercial devices may need more time and investment (Tothill 2009). Modern electrochemical bioaffinity sensors, such as DNA-sensors or immunosensors, offer significant sensitivity necessary for rapid testing of cancer (Wang 2006). During the past three decades, we have observed an enormous amount of effort in the enhancing the scope of biosensors (Wang 2006).

Cancer markers

Molecular biology and associated technologies lead to much better understanding of human cancer and malignancy, and of potential biomarkers which may be used for diagnosis (Soper et al. 2006). Biomarkers can either be seen inside the cancer cells or be extracellular. If the markers are inside the tumor cells, they need to be collected and enriched with the cells, needing to be lysed in order to release the biomarkers before analysis. Enrichment methods for selecting tumor

cells have been reported, and these methods include immunomagnetic separation using magnetic beads, and centrifugation using a density gradient (Tothill 2009, Ahn et al. 2004, Lara et al. 2004, Choesmel et al. 2004). We show potential biomarkers in Table I (Akbarzadeh et al. 2014, Akbarzadeh et al. 2012b, 2012c, 2012d, Valizadeh et al. 2012,).

Application of biosensors

Arrays of biosensors can be utilized to detect the signature patterns of several proteins and multiple DNA mutations to guide and assist in treatment and screening (Wang 2006). The new solutions will enable the biosensors to perform cancer tests quickly, cheaply and reliably, in a decentralized environment (Wang 2006). The introduction of biosensor technology to the market, and its establishment as a measurement technique, requires a lot of focus on the interactions that occur between industry and academia (Connolly 1995).

To understand the link between cancer and genetics more thoroughly, DNA analysis has become increasingly important in the diagnosis and treatment of cancer [1]. Various techniques such as electrophoresis, hybridization of probes with DNA, and *in vitro* amplification methods, are used to recognize the various cancers. (Wang et al. 1997). Recent studies have shown the hybridization of electrochemical biosensors for the detection of unique DNA sequences (Wang et al. 1997). DNA biosensors have been identified as a screening tool for the bioanalysis of environmental pollution studies and DNA-drug reactions. The changes in the DNA redox features (i.e. the oxidation of the guanine base) were reviewed, to study the interactions between DNA and analytes (Bagni et al. 2006). Several biosensor applications for cancer diagnostics are described. Rasooly and Jacobson reviewed some of the elements of cancer for cancer diagnostics, including proteins and DNA variations. Other useful cancer protein biomarkers are used by several researchers, including prostate-specific membrane antigen (PSMA), HER2/*neu* as a breast cancer marker, and cytokeratins to identify circulating tumor cells in peripheral blood of breast cancer patients. The new ultimate aim for biosensor utilization is point of care testing (POCT). POCT, which is diagnostic testing is done on site, makes it possible to significantly improve the delivery of health services and health care (Rasooly 2006). The POC clinical programs offer great potential for clinical cancer trials. POC systems are considered

Table I. Potential biomarkers for cancer diagnosis (Tothill 2009, Bohunicky and Mousa 2011, Smith et al. 2000, Meyer and Rustin 2000).

Type of cancer disease	Common biomarkers
Breast	BRCA1, BRCA2, CA 15-3, CA 125, CA 27.29, CEA, NY-BR-1, ING-1, HER2/NEU, ER/PR
Colon	CEA, EGF, p53
Liver	AFP, CEA
Esophageal	SCC
Lung	CEA, CA 19-9, SCC, NSE, NY-ESO-1
Ovarian	CA 125, HCG, p53, CEA, CA 549, CASA, CA 19-9, CA 15-3, MCA, MOV-1, TAG72
Melanoma	Tyrosinase, NY-ESO-1
Prostate	PSA

as integrated systems that can process clinical samples for many different types of biomarkers in a diversity of settings, such as clinical laboratories, doctors' offices and finally, at home (Soper et al. 2006). The development of POC methods will provide chances for better screening of patients at risk, tighter surveillance of disease recurrence, and better monitoring of treatment. Moreover, POC technologies are, by their very nature, low cost in their implementation, enabling large scale screening for disease prevention, and are more attractive to health care insurers (Soper et al. 2006). There are several challenges in moving biosensors to POCT for cancer, including:

- Development of reproducible biomarker assays.
- Advances in recognition ligands, including non-antibody recognition ligands.
- Development of multi-channel biosensors.
- Advances in sample preparation and cancer cell enrichment.
- Miniaturization and integration.
- Development of new, more sensitive transducers.
- Microfluidics integration.
- Advanced manufacturing techniques.
- Cost reduction (Rasooly and Jacobson 2006).

Biosensors should be compared with these procedures with regard to assay times, sensitivities, and low prices (Connolly 1995). The following points may help the formative stage of the project:

- Carefully identify target users and required performance.
- Base parameter selection on diagnostic needs.
- Develop prototypes for a panel of parameters: thyroid, fertility, etc.
- Interact with clinicians at an early stage of the project.
- Be realistic in terms of the practical use of the sensor (Connolly 1995).

As clinical diagnostics and other applications (e.g., environmental screening) do not commonly need the massive gathering of data typical of gene chips, alternative technologies that promise to provide flexible and economical alternatives for utilizations that need relatively fewer measurements are developing. Other methods, such as electrochemical and piezoelectric transductions, are the most interesting methods because of their simplicity, low cost instrumentation, real-time and label-free detection, and commonly high sensitivity (Lucarelli et al. 2008). In the level of basic research, biosensors for clinical diagnostics will profit typically from research in the growing field of bioelectronics, but there is a

special need for contributions in the following areas (Connolly 1995) that are shown in Table II.

Achieving the same accuracy in an *in vitro* technique would create a revolution in *de novo* DNA or RNA sequence detection, so considerable effort has been devoted to develop such a device (Vercoutere and Akeson 2002).

Advantages and disadvantages

Some researchers have tabulated the working features, cost, the advantages and disadvantages of these various technologies (Paddle 1996). The two main prerequisites for a good action of a DNA biosensor are high specificity and high sensitivity (Wang 2002). The time consuming preparative steps in gene probe evaluation make it hard for them to be considered as the basis of biosensors for *in situ* detection of pathogenic microorganisms. Although large effort gone in to develop biosensors, a still relatively small number of analytes, particularly toxic materials, can be measured by commercially available devices (Paddle 1996). Evaluations involving antibody or DNA based on biosensors are time consuming when we have to work in a dangerous environment. Despite this, biosensors are capable of being used for highly sensitive and specific onsite measurements of contamination by specific toxic materials (Paddle 1996). Biosensors have also been offered for application in the detection of fatigue (Dambrot, 1992), and studying vigilance status (Dittmar et al., 1992) (Paddle 1996).

Although the biosensor reduces 65%–85% of its original activity after 3–7 days in buffer, the dried enzyme membranes maintain their original activity even after 3 years of storage at 4°C. The benefit of this method is the low cost and easy preparation and replacement of the enzyme membrane. Therefore, there is no need to reactivate and reuse the membranes after enzyme inhibition; they may be arranged after each use (Paddle 1996). Commercialization of biosensors is another important factor limiting the stability of the biological component during both storage and application. Naturally occurring protein molecules (enzymes, receptors and antibodies) are dependent on denaturation, and commonly need an amount of bound water for integrity of their performance. Low moisture and high ambient temperatures would inappropriately affect biosensors operating in the gas phase (Paddle 1996). In an effort to develop a method considerably more rapid and sensitive than microarrays, most biosensors are at the proof-of-principle stage (Vercoutere and Akeson 2002). The most difficult obstacle in DNA biosensor development is the move to clinical use, but methods such as the nanopar-

Table II. Many areas that need biosensors.

Areas	References
Direct electron transfer in proteins	Bidan (1992), Schalkhammer (2002)
Optimization of molecular interactions in the solid phase of diagnostic devices	Du et al. (2005), Lowery et al. (2005)
Device design for whole blood sensors	Connolly (1995), Lauks (1998)
Biocompatibility studies for <i>in vivo</i> sensors	Quinn et al. (1995)
Packaging for <i>in vivo</i> sensors	Grisel et al. (1989)
Microfabrication techniques for mass fabrication of sensors	Hierlemann and Brand (2003), Suzuki (2000)
Immobilization and protection of biomolecules on multi-analyte devices	Mauriz et al. (2006)
Signal interference in biological samples	Connolly (1995)

ticle-assisted electrochemical detections seem encouraging (Vercoutere and Akeson 2002).

Conclusion and future prospects

Cancer is the second most prominent cause of death in developing countries. At the present, due to the increasing number of cancer patients, it has attracted a lot of attention. Various techniques and markers are used to detect cancer-causing genes. As noted above, one of these new techniques is the use of DNA biosensors. The main features of this technique are simplicity, cheapness, portability and reliability. DNA biosensors are good candidates for cheap diagnosis of genetic diseases and for the detection of pathogenic biological species in clinical search. Their high specificity and sensitivity also led to detection of cancer markers in the early stages of the disease. Several biosensor applications for cancer diagnostics are described. Many researchers have reviewed some of the elements of cancer for cancer diagnostics, including proteins and DNA variations. Biosensors and microarray chips that are based on detection of hybridization/interaction in short strands of nucleic acids propose an operating system for applications such as screening of genomes, detection of pathogenic organisms, and effective search in compound libraries to detect and survey potential therapeutic agents. (Abbasi et al. 2014a, Abbasi et al. 2014b, Ahmadi et al. 2014, Akbarzadeh et al. 2012a, Akbarzadeh et al. 2013a)

Authors' contributions

NS conceived of the study and participated in its design and coordination. AA participated in the sequence alignment and drafted the manuscript. All authors read and approved the final manuscript.

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Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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