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Guest Editorial

Moving biosensors to point-of-care cancer diagnostics

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This special section of biosensors and bioelectronics contains reports presented at a recent symposium, "Moving Biosensors to Point-of-Care Cancer Diagnostics," which was sponsored by the Cancer Diagnostics Program of the National Cancer Institute (NCI) in June 2005. One major goal of the NCI is to conduct and support research on the causes, diagnosis, prevention, and treatment of cancer. Cancer is a group of about 200 diseases affecting various organs characterized by abnormal and uncontrolled growth, invasion of nearby tissues and spread to distant organs. Cancer is the second largest cause of death in developed countries, accounting for nearly one in five of all deaths in the U.S.

Currently, the most important cancer diagnostic and prognostic indicators are morphological and histological characteristics of tumors or single biomarkers, such as prostate-specific antigen (PSA). However, there is a new trend to use molecular tools, both genomic and proteomic, to profile tumors and produce "molecular signatures" based on numerous variables. Since tumor development involves many biological changes, such signatures are very complex. Correlation of these molecular signatures with clinical parameters may develop information that can be used to enhance patient care.

The challenge to the research community is to measure these complex molecular signatures in clinical settings, and biosensors appear to be an appropriate tool. One of the most promising applications of biosensors is for point-of-care testing (POCT), diagnostic testing that is performed on site, providing results that may impact patient care almost immediately. POCT offers the potential for faster and cheaper diagnostics in all settings, allowing increased testing in traditionally underserved populations both in the U.S. and internationally.

The symposium presentations and companion articles appearing in this issue discuss aspects of cancer biology in terms of their relevance for the development of diagnostic biosensors for cancer. As almost all biosensors are based on a two-component system, with a biological recognition element (ligand) that facilitates specific binding or biochemical reaction of a target, and a signal conversion unit (transducer) (Clark and Lyons, 1962), several types of ligands and transducers with potential use for cancer diagnostics are discussed. In addition, this journal issue includes a white paper produced by a working group convened by the NCI that outlines the research gaps that need to be filled in order to move biosensors to point-of-care cancer diagnostics.

1. Ligands for cancer diagnostics biosensors

The most important characteristics for ligands are affinity and specificity. Biosensors that use antibodies as recognition elements (immunosensors) are common, because antibodies are highly specific, versatile, and bind strongly and stably to the antigen. However, the complexity of cancer detection based on molecular signatures will require multiple ligands, and it is difficult to produce large quantities of uniform antibodies for numerous antigens. The lack of easily synthesized high-affinity ligands is a major limiting factor for development of high throughput biosensor-based detection systems for cancer diagnostics. Therefore, researchers are attempting to develop new types of ligands more suited for high-throughput screening and chemical synthesis including synthetic peptides and aptamers.

One example is peptides isolated from phage display library, which is a large collection of phages expressing numerous peptides or proteins on their coats (Smith, 1985, Brown, 2000). As described here by Kathlynn Brown and colleagues, because it is unlikely that one cell-binding ligand will provide sufficient biological information, multiple ligands for a given cancer type will be needed for reliable clinical diagnosis. The articles describes the use of biopanning of phage displayed peptide libraries for isolation of specific peptides that bind to the large cell lung carcinoma cell line, H1299. Peptides have relatively low affinity (K_d's $\sim 10^{-6}$ to 10^{-7} M) compared to antibodies or aptamers, so multiple cell-targeting peptides from different peptide libraries are needed to create effective ligands. Another use of peptides for biodetection was demonstrated by Homola and co-workers, who used synthetic Epstein-Barr virus peptides as a recognition element for detection of anti-EBNA human serum with a surface plasmon resonance biosensor.

Other potential ligands are aptamers, protein-binding nucleic acids (DNA or RNA molecules) selected from random pools

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based on their ability to bind other molecules (Ellington and Szostak, 1992). Aptamers have previously been selected against a variety of targets ranging from small molecules to proteins to cell surface with high affinity (K_d's $\sim 10^{-8}$ to 10^{-9} M) and have specificities comparable to those of monoclonal antibodies. Aptamers can be designed to be highly resistant to nuclease digestion, and can be chemically synthesized in bulk. Ellington describes work by his lab in which conjugated 70-mer modified RNA aptamers were identified that bind prostate-specific membrane antigen (PSMA) to luminescent CdSe and CdTe nanocrystals for detection.

Most of the research described in this issue uses antibodies as ligands, the most common approach. These ligands include Cy5-labeled IgG antibodies for detection of anti-toxin human serum by an array biosensor (Frances Ligler), anti-HER2 antibodies for fluorescent nanoparticle-based detection of breast cancer (Zeev Rosenzweig) and monoclonal anti-cytokeratin antibodies labeled with fluorescent dye to recognize human cytokeratins in circulating tumor cells in blood (Richard Bruce).

2. Biosensors transducers for cancer diagnostics

The transducer converts the biochemical interactions into a measurable electronic signal. Electrochemical, electrooptical, acoustical and mechanical transducers are among the many types found in biosensors. The transducer works either directly or indirectly.

2.1. Direct detection biosensors

Direct recognition sensors, in which the biological interaction is directly measured typically use non-catalytic ligands such as cell receptors or antibodies. The most common direct detection biosensor systems employ evanescent wave mainly surface plasmon resonance (SPR) (Szabo et al., 1995) technology which measures resonant oscillation of electrons on the surface of a metal, as discussed by Homola and co-workers. Spectral surface plasmon resonance (Piliarik et al., 2005) has been used for direct label-free detection of antibodies against human Epstein-Barr virus (anti-EBNA), based on immunoreaction with synthetic peptides immobilized on the sensor surface. Epstein-Barr virus has been associated with certain cancers, including Burkitt's lymphoma, immunoblastic lymphoma, and nasopharyngeal carcinoma.

Interferometric biosensors, which use self-referencing surface-normal interferometry to detect molecules bound to the surface of a spinning disc, is discussed by Nolte and co-workers. The biological compact disc (BioCD) (Varma et al., 2004) allows high-speed sensing of sub-nanometer surface displacements, and has capacity for more than a million spots on a single disc using antibodies as ligands.

Another type of direct recognition sensor is a quartz resonator transducer that measures changes in acoustic resonance. Mascini and co-workers (Tombelli et al., 2005) used this technology for the detection of the *TP53* mutation common in many cancers using synthetic oligonucleotides to analyze PCR amplicons.

2.2. Indirect detection biosensors

The second class of transducers, indirect detection sensors, relies on secondary labeled elements that are often fluorescently tagged antibodies or catalytic elements such as enzymes. Some examples of secondary elements are the enzyme alkaline phosphatase and fluorescently tagged antibodies that enhance detection of a sandwich complex.

One commonly used indirect detection biosensor technology optically measures fluorescence of the secondary ligand. Ligler and co-workers have used optical fluorescence to develop a multi-analyte indirect detection array biosensor (Rowe et al., 1999). The presence of human antibodies against four different antigens were measured simultaneously in sera from eight different donors in a single assay.

Another approach for utilization of optical fluorescence for biomolecule detection is presented by Rosenzweig and co-workers (Rossi et al., 2004). They developed a competitive assay for HER2 (neu) based on the inhibition of interactions between anti-HER2-coated fluorescent nanoparticles and glass slides modified with HER2. The free HER2 molecules compete with the bound molecules on the slides for binding to the antibody particles, which are detected by digital fluorescence imaging microscopy.

Bruce and co-workers describe immunofluorescent labeling in combination with flow cytometery to detect rare cancer cells in blood (Krivacic et al., 2004), using fiber-optic array scanning technology (FAST). The ligand is antibodies labeled with fluorescent dye.

Electrochemical transducers are also commonly used for indirect biosensor detection. Articles from the Gau and Wang labs describe the use of electrochemical transducers to measure the oxidation or reduction of an electroactive compound on the secondary ligand in one common type of indirect detection sensor. Wang and co-workers have combined electrochemical transducers with nanoscale materials to produce a unique coding capability for simultaneous measurement of multiple cancer markers. Gau et al. (2005) focus on the manufacturing of electrochemical biosensors using plastic rather than silicon substrates, and then using this platform for analysis of IL-8 protein in saliva. IL-8 in saliva is being investigated as a biomarker for head and neck cancers.

2.3. Integrated biosensors and lab-on-a-chip

Along with the development of better ligands and alternative transducer technologies, there is significant research on manufacturing to produce advanced integrated devices. "Lab-on-a-chip" biosensors contain integrated microfabricated fluidics systems and are designed to perform multi-step high-resolution biological or chemical assays. These devices can contain many channels, allowing for massively parallel biochemical processes and multi-analyte detection. Many of these devices are fabricated using molding or photolithographic processes developed in the microelectronics industry to create circuits of chambers and channels using composite materials, quartz, silica, or glass chip.

As discussed in the article by Bruno Frazier and his group, microfluidic circuits can be used for magnetophoretic separation of suspended breast cancer cells in peripheral blood and for sorting them based on their electrophysiological characteristics. In combination with a micro-electrical impedance spectroscopy system (μ-EIS) for a downstream cell analysis, the system was used for pathological characterization of the cancer cells. Microfabricated flow-through biochip for the analysis of single base mutations in genomic DNA was described by Soper and co-workers (Wang et al., 2003; Hashimoto et al., 2004). In this microfluidic device, sequential polymerase chain reaction (PCR) and ligation detection reaction (LDR) were preformed in a continuous-flow format. The amplicons were analyzed by hybridization to an olignocleotide array detected by fluorescence scanning.

2.4. Nanotechnology applications for cancer diagnostics

Recent research efforts in the area of biosensing are focused on nanoscale assemblies which can be used for detection and may change their mechanical, optical or electric properties in response to changes on surface. Some of the nanoscale assemblies under development include micro-cantilevers which measure bending because of changes in mass surface, particles like Quantum Dots and metal nanoparticles that can broaden the spectrum of fluorescent dyes and may change their optical properties because of changes on their surface. Nanotransducers offer new potential for miniaturization to nanoscale size, ability to detect large number of molecules simultaneously, reduced cost of production, high throughput and an enormous number of channels. Brown and co-workers utilized Quantum Dot technology for labeling peptide ligands labeling, while Ellington and co-workers used them to label aptamers. The work by Rosenzweig with HER2 is also nanotechnology, in that it uses highly luminescent fluorescent silica nanospheres.

2.5. DNA based cancer diagnostics

Cancer is a genetic disease caused by changes or modification of DNA sequences of key genes and DNA analysis has a great potential for use for cancer diagnostics. Two aspects of cancer genetic analysis are discussed in this special section, point mutations and alteration of telomere elongation. In general biosensors are used for detection and analysis of PCR amplicons. DNA point mutations of the *p53* gene, which is involved in cell cycle control and regulation of apoptosis, was analyzed using piezoelectric biosensor detection of PCR amplicons as described by Mascini and co-workers. PCR point mutation analysis of *K-ras*, which is relevant for colorectal cancers, using a PCR microfluidics microchip is described by Soper and co-workers. Another type of genomic alteration, telomere elongation upregulation that is typical to many malignant tumors, was measured by Gorbovitski and co-workers using a telomerase-extended DNA fragment analysis based on a combination of PCR and capillary electrophoresis (CE) (Alaverdian et al., 2002).

3. Applications of biosensors for cancer diagnostics

As biosensor technology advances, the range of applications broadens. Several biosensor applications for cancer diagnostics are described in this issue. Rasooly and Jacobson review some of the elements of cancer relevant to cancer diagnostics include proteins and DNA alterations. Other useful cancer protein biomarkers are used by various investigators, including prostate-specific membrane antigen (PSMA) (Ellington), HER2/nue as a breast cancer marker (Rosenzweig), and cytokeratins for identification of circulating tumor cells in peripheral blood of breast cancer patients (Bruce). The work by Frazier uses electrophysiological characteristics of cancer cells, rather than protein biomarkers. Finally, nucleic acid biomarkers are used in the cancer-related biosensors described by Mascini (mutations in p53), Soper (mutations in *K-ras*), and Gorbovitski (telomere elongation upregulation).

4. Moving biosensors to point-of-care cancer diagnostics

Point of care testing, which is diagnostic testing performed on site, might significantly improve healthcare and healthcare delivery. As discussed above, POCT may permit rapid and less costly cancer diagnostics, thereby permitting increased testing in underserved populations.

The white paper by Soper and co-workers summarizes the challenges in moving biosensors to POCT cancer diagnostics. These include improved biomarkers, ligand development (especially non-antibody ligands), the need for more sensitive transducers, and for multi-channel biosensors, miniaturization, and integration. Finally, cost reduction is crucial to make this technology widely available and useful.

The articles in this journal issue represent the first steps to meeting many of these challenges, especially in the improved miniaturization and sensitivity of biosensors, and in the development of microfluidic, integrated instruments, with multiple channels. As this work continues and new ligands and cancer biomarkers emerge from the lab, the use of biosensors for POCT cancer diagnostics will become increasingly practical.

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