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Mini-review

Lectin-based biosensors as analytical tools for clinical oncology

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ABSTRACT

The review focus on the use of lectin-based biosensors in the oncology field, and ponders the potentialities of using these devices as analytical tools to monitor the levels of cancer glycobiomarkers in biological fluids, helping in the diagnosis, prognosis and treatment assessment.

Several examples of lectin-based biosensors directed for cancer biomarkers are described and discussed, and their potential application in the clinic is considered, taking into account their analytical features, advantages and performance in sample analysis.

Technical and practical aspects in the construction process, which are specific for lectin biosensors, are debated, as well as the requirements in sample collection and processing, and biosensor validation.

Today's challenges for real implementation of these devices in the clinic are presented, along with the future trends in the field.

1. Introduction

1.1. Definition and features of a biosensor

Biosensors are chemical sensors in which the recognition system is based on a biochemical or biological mechanism [1–3]. Recognition is the process of selective interaction between the biosensing element, which is immobilized on the sensor surface, and the analyte, present in the sample. The sensing element may be of biological origin or a synthetic material that biomimetizes a natural component.

The recognition event is characterized by two features: reversibility and selectivity [4]. The interaction is based on the establishment of noncovalent chemical bonds, such as ionic bonds, hydrogen bonds or van der Waals interactions, between the analyte and the sensing agent. The constant of this equilibrium reflects the affinity of the sensing element towards the analyte. If the biosensor response depends on the product concentration, then it will be affected by the analyte concentration in the sample [4]. Selectivity of the biosensor is the ability to respond preferentially to the presence of the analyte, and not to other sample components. Ideally, a biosensor should be specific (100% selective) for a particular analyte, so that the response would be exclusively correlated to the analyte concentration in the sample.

Different sensing elements may be used in biosensors, namely nucleic acids, enzymes or substrates, cells or biological tissues, and proteins or glycoproteins of diverse families (antibodies, lectins) that show affinity towards particular molecules (antigens, glycans), forming association complexes. In this case, the interaction could be more or less specific (for example, an antibody-antigen interaction is much more selective than a lectin-glycan interaction).

As for the transduction methods used in biosensors, they can be thermometric, optical, mechanical and electrochemical. Electrochemical-based methods are the most common transduction mechanisms employed in biosensors.

Since the final objective of a biosensor is to be commercialized and applied in real life, it should present some attractive features, namely: a) simple to operate, so it can be easily used; b) portable, to allow *in vivo* analysis; c) miniaturized, to reduce the amount of sample needed and to allow integration of several biosensors in arrays, increasing the throughput; d) free of sample preparation, to simplify the procedure and reduce assay time; e) disposable, to avoid cross-contamination between samples, repetitive calibrations and conditioning after each measurement; f) robust and with reproducible manufacturing, so that it is reliable and does not require calibration; g) sensitive and with low limits of detection, since the majority of biological analytes of interest are in very low concentrations in samples; h) highly selective, to avoid or minimize interference errors; i) quantitative, so that the biosensor response may be correlated to the analyte concentration or amount in the sample; j) compatible with established readout systems, which should be miniaturized as well, to enable the point-of-care (POC) or *in vivo* measurement; k) cheap, so that it can be not reusable without

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increasing the assay cost.

1.2. Advantages of biosensors in point-of-care analysis and precision medicine

Among the several uses of biosensors in real life context, healthcare applications are one of the most significant. Biosensors can be applied for *in vitro* or *in vivo* determinations of particular analytes with physiological or pathological relevance [5–15], in particular biomarkers of physiological or pathological states (in cancer, cardiovascular diseases and hormone-related health problems) [6,8,11,13,16–19].

The developments in biosensor research allow presenting, at this moment, assays with short and separation-free procedures, with the possibility of performing the measurement *in situ*, which has advantages with respect to common enzyme-linked immunosorbent assays (ELISA) [20,21]. Also, the possibility to construct label-free biosensors increases simplicity in the biosensing process and enables to reduce the assay time, which is advantageous for POC analysis [22,23].

The possibility of miniaturization of the biosensing devices allows, on one hand, to decentralize the analysis and, on the other hand, to work in the micro-scale, reducing the volume of reagents and samples required to perform the analysis. This makes the operation cheaper (allowing mass production) and less hazardous, when compared to the macro and semimicro-scale techniques [24]. Furthermore, the automation allowed by the use of biosensors enables screening of populations in a simple and fast way. Also, there is the option to immobilize more than one biorecognition agent on the biosensor, creating thus a multi-analyte biosensor that may be employed to simultaneously determine a series of analytes. This may have advantages in the profiling of samples, since in many pathological situations a profile of markers provides more accurate information than the monitorization of a single marker [7,25–30].

The use of biosensors in clinical testing is more practical, faster, more user-friendly, less expensive and less technically demanding than microarray or proteomic analysis [7]. The later approaches are not practical for routine testing due to their complexity and cumbersome and lengthy protocols, which may affect reproducibility and must be performed by experienced technicians.

Nowadays, precision medicine centred in the patient can make the difference in the life quality of patients, by proposing screenings, diagnosing entire families for particular mutations before the disease reveals and offering treatments with more quality (higher efficacy and less adverse effects) by adjusting doses to the patient metabolism. In all these actions, analytical tests based in biosensors may be employed, which selectively detect particular biomarkers that are indicative of the disease presence and progression, and treatment efficacy [31].

2. Application of biosensors in clinical oncology

Cancer incidence is increasing and will continue to increase in the next decades, due to the raise in life expectancy of populations and the difficulty to control some of the stigmas that characterize today's societies, such as sedentarism and obesity [32,33]. This is, obviously, a worrying scenario, and the need to intensify prevention is mandatory. Furthermore, a personalized prevention is becoming a target, which will allow to improve the individual adhesion and the efficacy of interventions [34].

Both in population screening methods and patient stratification, the use of biosensors for biomarkers is, theoretically, an useful option [28,35,36]. Detection of serum cancer biomarkers would permit to carry out screenings in a fast way, helping on the precise diagnosis in an early phase, and this could be performed by using biosensors towards the cancer-associated biomolecules produced and secreted by tumour cells. Unfortunately, the success of cancer biomarkers in screenings has been disappointing, mainly due to insufficient sensitivity. Biomarker amounts secreted by tumour cells are very low and the dilution effect of blood decreases even more its concentration. A solution to this may be to use proximal fluids [36]. In addition, many cancer biomarkers are elevated in benign diseases [28,37], which require further testing to confirm malignancy.

In advanced or metastatic disease, current guidelines recommend the assessment of response to treatment through history, physical-clinical examination, radiological exam and routine blood tests [38]. Among them, tumour biomarkers are quantified, namely CA15-3 and CA27-29 for breast cancer, α-fetoprotein for liver cancer, PSA for prostate cancer, CA125 for ovarian cancer, CA19-9 for pancreatic and ovarian cancer, CEA for colon, gastric, pancreatic, lung and breast cancer, HER2 for breast cancer and hCG for testicular and ovarian cancer [8,37,39]. Although a change in a tumour biomarker alone is not sufficient for treatment decisions, its level is considered helpful in monitoring the response and for diagnosis of distant metastasis [8,37,40]. The use of serum cancer biomarkers in therapy monitoring may reduce the follow-up expenses by up to 50% as compared to the use of imaging techniques [41].

3. Lectin-based biosensors as potentially useful tools in clinical oncology

3.1. Aberrant glycans as cancer biomarkers

Aberrant glycosylation of sphingolipids and proteins is a feature of essentially all types of experimental and human cancers. According to the current knowledge, this is a result of the oncogenic transformation of cells, which induces invasion and metastasis [42,43].

Cancer-associated abnormal glycosylation includes incomplete synthesis of *O*-glycans (namely the formation of sialyl-Tn, Tn and T epitopes), altered glycosylation of mucins (with changes in MUC2 and MUC5AC for colon cancer, MUC1 in several types of cancer and CA125 in ovarian cancer), alterations in the synthesis of histo-blood group-related antigens (A and B glycan epitopes, which are reduced or absent, being replaced by the precursor H epitope, and Lewis antigens, whose profiles are different from that seen in adjacent normal tissues), increased (β1,6) branching of *N*-linked glycans, namely in breast and colon cancers, alterations in sialylation and fucosylation (for the Lewis family antigens and T and Tn antigens), and synthesis of HPA-binding glycans [43–46].

These aberrant epitopes have been identified as tumour-associated antigens and, clinically, their expression is inversely correlated with survival of patients [43], being more obvious in early stages than in later stages of human cancer [47], when other factors may be involved. The antigens may, therefore, be useful as prognostic markers [44,45].

The use of glycan biomarkers instead of protein biomarkers for cancer has several advantages: (1) glycan biosynthesis is more susceptible to be affected by disease than protein biosynthesis, because glycans are metabolic products. Small changes in proteins cause amplified variations in glycans; (2) glycans are synthesized only in the Golgi apparatus or in the endoplasmic reticulum, therefore abnormal glycosylation can potentially impact all glycoproteins produced in the tumour cell; (3) with the current analytical techniques, it is easier to measure oligosaccharide expression than protein expression [48,49]; (4) one protein can carry many copies of an altered glycan, which may also be added to other scaffolds, generating an amplification effect; (5) glycosylation acts to shield the peptide backbone from proteolytic degradation, thus glycan-based biomarkers are, in theory, more stable than unmodified proteins, in a disease setting [50]. Further, glycans are secreted by the cells and are abundant in most biological fluids, such as serum, plasma, saliva and tears. The glycan may be used as a marker by detecting its amount or, in a more deep analysis, by detecting site-specific glycosylation [49]. In conclusion, capitalizing on alterations in cancer-related protein glycoforms enables an increase in diagnostic sensitivity and specificity [50]. For example, the employment of the lectin from *Maackia amurensis* with different affinity for PSA glycoforms