A Quest for New Cancer Diagnosis, Prognosis and Prediction Biomarkers and Their Use in Biosensors Development

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Abstract

Traditional techniques for cancer diagnosis, such as nuclear magnetic resonance, ultrasound and tissue analysis, require sophisticated devices and highly trained personnel, which are characterized by elevated operation costs. The use of biomarkers has emerged as an alternative for cancer diagnosis, prognosis and prediction because their measurement in tissues or fluids, such as blood, urine or saliva, is characterized by shorter processing times. However, the biomarkers used currently, and the techniques used for their measurement, including ELISA, western-blot, polymerase chain reaction (PCR) or immunohistochemistry, possess low sensitivity and specificity. Therefore, the search for new proteomic, genomic or immunological biomarkers and the development of new noninvasive, easier and cheaper techniques that meet the sensitivity and specificity criteria for the diagnosis, prognosis and prediction of this disease has become a relevant topic. The purpose of this review is to provide an overview about the search for new cancer biomarkers, including the strategies that must be followed to identify them, as well as presenting the latest advances in the development of biosensors that possess a high potential for cancer diagnosis, prognosis and prediction, mainly focusing on their relevance in lung, prostate and breast cancers.

Introduction

Cancer is a disease that represents an enormous public health issue in both developed and developing countries. For 2018, it was estimated 18.1 million new cancer cases and 9.6 million cancer deaths worldwide. The 3 main cancers are lung, breast and prostate cancer.[1] The development of new diagnostic tools for cancer detection is mainly justified because if cancer is detected early, it can be cured. A biomarker is an important tool in the detection and monitoring of cancer; these biomarkers include gene mutations, alterations in transcription or translation of genes, and/or protein products modifications.[2] An advantage of biomarkers is that they can be used in the evaluation of the disease at different stages, serving as diagnosis, prognosis and prediction tools. Currently, the number of biomarkers in clinical use is limited. Examples of these biomarkers include prostate-specific antigen (PSA) for prostate

cancer, carcinoembryonic antigen (CEA) for lung cancer, cancer antigen 125 (CA-125) for ovarian cancer and cancer antigen 15-3 (CA15-3) for breast cancer.[3] Nevertheless, none of these biomarkers provides enough sensitivity and specificity information for an accurate diagnosis in the entire population. Thus, identify new biomarkers that enable the development of new diagnostic tools with high sensitivity and specificity is imperative. Current tools for cancer diagnosis using

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biomarkers are mainly based on immunohistochemistry, serological or PCR tests. A great disadvantage of immunohistochemistry is that it represents an invasive technique that depends on tissue samples, and, biomarker heterogeneity can exist inside the same tumor. Among serological tests such as western-blot, ELISA or PCR-based tests, the main disadvantage is that they suffer from technological limitations such as low detection and the use of expensive reagents in each assay.[4] For these reasons, at present, there is a growing interest on biosensors development for early cancer diagnosis and prognosis, as well as for the prediction of cancer treatment responses, as they have shown a higher sensitivity. Furthermore, given that the measurements can be performed in real-time, the wait time for the result can be reduced.

Strategies for the Search of New Biomarkers

The following are the 5 different stages involved in understanding biomarker development: 1) an exploratory preclinical study aimed at identifying potential biomarkers; 2) the development of a test to identify and quantify the biomarker in the sample; 3) assays that evaluate the correlation with the disease; 4) prospective screening studies and 5) clinical impact studies.[5-7] The first stages are critical because they allow the identification of potential biomarkers and discriminate those that do not meet the required characteristics. In the quest for new biomarkers, new -omics technologies have provided advances for the development of these markers. These technologies, including proteomics technologies such as 2D electrophoresis (2D-PAGE) and mass spectrometry, have facilitated the discovery of new biomarkers.[3] Related to genomic technologies, whole-exome sequencing has allowed the comparison of gene mutations from different cancer types, enabling the identification of associations between gene mutations and the different kinds of tumors.[8] The immune system plays a key role in cancer development and progression; therefore, the polarization to certain kinds of responses through different cytokine profiles or cell subpopulations can discriminate between healthy patients and cancer patients.[9]

Proteomics Approach and Potential Biomarkers

Differential expression of proteins can be used for the discovery and validation of new biomarkers.[10] In conventional proteomics analysis, 2D-PAGE followed by mass spectrometry has been used as a primary technique for new biomarkers discovery during many years.[2] Following this approach, many researches carried out in the last 2 decades has found proteins that could be used as potential biomarkers.[11-14] Ummani et al,[15] through 2D immunoblotting and mass spectrometry, found that PRDX6 and ANXA11 were strongly recognized in prostate cancer patient's serum. Noteworthy, this group developed a test capable of discriminating between healthy and cancer patients with 90% and 100% sensitivity and specificity, respectively. A different method based on 2D-PAGE is 2D differential gel electrophoresis (2D-DIGE).[16] In this method, different

samples are labeled with fluorescent dyes, mixed and separated by 2D-PAGE. Then, laser scanning is used to observe the gel. Two advantages that 2D-DIGE offers over traditional 2D-PAGE are that 2D-DIGE reduces gel-gel variations and does not require a staining step. Xiao et al,[17] using 2D-DIGE and mass spectrometry strategy, identified ANX1, HP, AZGP1 and calprotectin proteins as potential biomarkers in saliva samples from healthy controls and lung cancer patients. Even though the 2D-PAGE approach is effective, it has certain substantial limitations including being slow and laborious and showing limited sensitivity due to the high amounts of proteins needed for visualization on a gel.[18] Latter problems associated with gel-based technologies originated the development of gel-free technologies such as labeling and targeted shotgun proteomics based on mass spectrometry analysis without the need for a previous gel electrophoresis step.[19] An important step in such gel-free methods is the identification of accurate mass tags (AMTs) for protein determination,[20] this step starts with a biological sample, a total lysate or a fractionated biochemical product. The general procedure to identify a protein begins with the tryptic digestion of complex protein mixtures. The tryptic peptides are separated by reverse high-pressure liquid chromatography (HPLC) and then analyzed by LC-MS/MS to obtain partial amino acid sequences. This information sequence is used to establish the identity of the parental protein from which all the peptides originate by searching for MS/MS spectral coincidences in an appropriate database.[18] In a recent study, Kwon et at,[21] using LC-MS/MS to identify AMT, investigated the differences in protein expression patterns between normal, prostate cancer and advanced prostate cancer tissues. Although finding 3 proteins differentially expressed; spermidine synthase (SMR), nucleolar and coiled-body phosphoprotein 1 (NOLC1) and protein prostacyclin synthase (PTGIS) as unknown candidate potential biomarkers, only spermidine synthase was increased in more advanced stages of prostate cancer, which make this protein a promising biomarker for prostate cancer prediction. A recent work, through LC-MS/MS, developed a multimarker test detecting apolipoprotein C-1 (APOCH1), carbonic anhydrase 1 (CAH1) and neural cell adhesion molecule L1-like protein (NCHL1) present in human plasma of breast cancer patients, such test showed diagnostic sensitivity, specificity and accuracy of 71.6%, 85.3%, and 77% respectively. Remarkably, this protein assay was able to discriminate other types of malignancies, suggesting that this assay was organ-specific.[22]

Sokolowska et al,[23] through nanoLC-MS/MS, identified receptors for the tumor differentiation factor (TDF) expressed in human breast and prostate cancer cells, noteworthy, the receptors involved belonged to the heat shock 70-kDa family of proteins (HSP70), showing a relation between this family of proteins and cancer presence. Taking advantage that once a biomarker is found, it is possible to transfer to more simplified instrumentation, including western blot, ELISA, multiplex assays, immunohistochemistry and microarrays,[24] heat shock proteins (HSPs) have gained attention as a promising tool in the diagnosis of cancer including prostate, breast, lung, ovarian and

Table 1. Cancer Associated Proteins With Diagnostic Potential.

Proteomic marker	Cancer type	Clinical use	Detection method	References
PRDX6 and ANXA11	Prostate	Diagnosis	2D-PAGE	Ummani et al., 2015[15]
ANX1, HP, AZGP1 and calprotectin	Lung	Diagnosis	2D-DIGE	Xiao et al., 2012[17]
SMR	Prostate	Prognosis	Mass spectrometry	Kwon et al., 2020[21]
APOCH1, CAH1 and NCHL1	Breast	Diagnosis and prognosis	Mass spectrometry	Kim et al., 2019[22]
HSP70	Breast	Prediction	ELISA	Rothammer et al., 2019[28]
HSP90α	Pan cancer	Diagnosis	ELISA and western blot	Liu et al., 2019[30]
CLDN2, CLDN6, CLDN11 and CLDN14	Breast	Prognosis	Western blot	Jia et al., 2019[34]
Netrin-1	Breast	Diagnosis	Western blot	El-Gamal et al., 2020[36]
CRP, prolactin, HGF and autoantigen NY-ESO-1	Lung	Diagnosis	Multiplexed ELISA	Ma et al., 2016[37]
AMACR	Prostate	Diagnosis	ELISA	Etheridge et al., 2018[38]
Urinary plasminogen and fibrinogen gamma	Prostate	Diagnosis	ELISA	Zhang et al., 2020[39]
HE4 and TTR	Ovarian	Diagnosis	ELISA	Zheng et al., 2018[40]
ZNF71	Lung	Prognosis	Immunohistochemistry	Guo et al., 2018[41]
TTF1, p40, PD-L1	Lung	Diagnosis and prognosis	Immunohistochemistry	Llie et al., 2018[42]

colorectal cancer.[25] HSPs are classified in various families based on their molecular weight including HSP110, HSP90, HSP70, HSP60 and the small heat shock proteins groups.[26] HSP70 has been considered a damage-associated molecular pattern that can stimulate a chronic inflammatory response after radiation-induced tumor cell death. That chronic inflammatory response has been shown to be related to tumor growth.[27] In a recent research, serum levels of HSP70 were correlated with an unfavorable prognosis in breast cancer, since breast cancer patients with distant metastasis or recurrence showed higher serum levels than patients who remained free of the disease.[28] HSP90α has recently been found to localize outside various cancer cells and, clinical trials have demonstrated that plasma HSP90α is a more accurate biomarker than CEA and CYFRA21 -1 in lung cancer.[29] In that sense, Liu et al[30] through a large-scale clinical study, validated HSP90α as a pan-cancer biomarker, showing a sensitivity of 81.33% and a sensitivity of 81.65% in a test cohort, and a sensitivity and sensibility of 81.72% and 81.03% respectively in a validation cohort.

Occludin and claudin proteins family (CLDN) have been associated with cell proliferation and differentiation.[31] The expression of these proteins has been found altered in different tumor types.[32,33] According to these, CLDN2, CLDN6, CLDN11, and CLDN14 have shown a prognostic potential in breast cancer, since the expression of these proteins in breast carcinoma evaluated by western blot and immunohistochemistry, has been found downregulated.[34] Netrin-1 is a protein-related to tumorigenesis in bladder cancer (BC) which inhibits apoptotic pathways.[35] El-Gamal et al[36] through western blot, have shown that Netrin-1 levels in BC tissue could be a potential prediction marker since this biomarker predicts muscle invasion with 96% of sensibility. Something to highlight of this work, urinary levels of Netrin-1 were strongly correlated with tissue levels, showing the possibility for a non-invasive test for BC diagnosis. Based on the fact that detection of biomarkers in body fluids offers a huge advantage over the determination of these biomarkers in tumor tissue samples, Ma et al[37] through a multiplexed assay for the determination of C-reactive protein (CRP), prolactin, hepatocyte growth factor (HGF) and autoantigen NY-ESO-1 in serum, was able to discriminate between healthy patients and those with different types of lung cancer. This panel of 4 biomarkers showed higher sensitivity and specificity compared with CEA.

Etheridge et al[38] using seminal ejaculate, were able to detect the protein AMACR in clinically significant prostate cancer tumors by quantifying the protein in ELISA tests. Zhang et al,[39] measuring urinary plasminogen and fibrinogen gamma chain levels in NSCLC patients and controls detected by ELISA, found that tissue levels and urinary levels were significantly elevated compared with controls, with an area under the ROC curve ranging from 0.827 to 0.947. Continuing with serological tests, by means of ELISA, Zheng et al[40] used human epididymis 4 (HE4) and transthyretin (TTR) and found that HE4 exhibits a better performance in ovarian cancer diagnosis, even better than the performance of CA-125. Using immunohistochemistry, Guo et al[41] analyzed samples from patients with non-small cell lung cancer (NSCLC) and associated the expression of the zinc finger protein ZNF71 with the response to chemotherapy, which showed enormous potential as a predictive biomarker; furthermore, given that the group that expressed this antigen at a higher concentration showed a higher survival rate, this protein has potential as a prognosis biomarker. Llie et al[42] performed a multiplexed immunohistochemical assay on NSCLC samples and demonstrated that a panel of biomarkers, including TTF1, p40, PD-L1, and pan-keratin, as well as an additional panel focused on the molecular profile, including anti-ALK, anti-ROS1, and anti-BRAFV600E antibodies, were able to classify the tissues in different histotypes for diagnosis and immunophenotyping, helping to choose the therapeutic strategy.

Thus, gathering all these proteomic approaches, the biomarkers' search has yielded potential cancer biomarkers that could be used in the development of new and more efficient diagnosis tools. Table 1 summarizes different protein biomarkers with potential in cancer diagnosis, prognosis, and prediction.

Genomics Approach

The advances in new DNA sequencing technologies have allowed the processing of thousands of different cancer types samples for the discovery of systemic mutations. This expanded aim, coupled with noteworthy progress in algorithms,[43,44] has directly contributed to the characterization of significant functional mutations, genes, and pathways.[45] Even though genetic mutations maintain a high frequency, most genetic mutations occur at an intermediate frequency (2-20%). Single nucleotide polymorphisms (SNPs) are important genetic biomarkers in humans that have functions in the beginning and progression of cancer, noteworthy, in addition to identifying mutations, classical procedures for detecting changes in the basal levels of expression or epigenetics features of different genes remain being used for the analysis of new candidates to evaluate their potential as a biomarker for cancer detection, or the announcement of prognosis of the diagnosed patient. Table 2 summarizes a set of different genes with biomarker properties for cancer diagnosis and prognosis reported recently.

In an ambitious effort, Cyriac et al[46] carried on an *in silico* analysis on 3281 tumors of 12 different types of cancer and identified 127 genes involved in a wide range of processes, including regulatory mechanisms or transcriptional factors, histone modifiers, genome integrity, tyrosine/kinase receptor signaling, cell cycle, mitogen-activated protein kinases signaling (MAPK), phosphatidylinositol-3-OH kinase (PI(3)K) signaling, Wnt/β-catenin signaling, histones, ubiquitin-mediated proteolysis and splicing. Moreover, Lawrence et al[47] used an algorithm with the ability to analyzing the cancer genomes of 21 different tumor types and found the association of 33 genes not previously reported as mutated genes in cancer that encode proteins with different functions such as anti-proliferation, proliferation, pro-apoptosis and chromatin regulation. Notably, Arg107 mutation in *STX2* gene has been detected as a recurrent mutation in lung cancer patients,[47] and upregulation of this gene has recently been ligated to colorectal cancer.[48] Another mutation detected by exome sequencing with potential properties as clinical biomarker is the rs1052667 polymorphism in ARHGAP35 gene, whose product is a repressor of glucocorticoid receptor transcription,[47,49] mutations determined in this gene by SNP genotyping and RNAseq have been associated with lung cancer and osteosarcoma.[50,51]

In a recent study using the bioinformatics platform tool BioXm, were identified 506 differentially expressed genes in breast cancer, including the genes *BRCA1*, *BRCA2* and *ERBB2*; however, the *PIP* gene showed higher downregulation in cancer cell lines.[69] DNA mismatching repairing (MMR) genes are key factors in genomic stability.[70] As one of the most important MMR genes in humans, mut-S-homolog (*MSH2*) maintains genomic stability by repairing base pair mismatches. In that sense, Zhu et al[52] found a relationship between SNP RR2303428 on *MSH2* and hepatocellular cancer progression using peripheral blood. Interestingly, the same gene is also associated with lung cancer development.[53] Moreover, in

breast cancer, a report shows that the SNPs rs1682111 and rs10439478 in the *ACYP2* gene are associated with an increased risk of breast cancer. Of note, the SNP-SNP interactions between both polymorphisms further increase the risk of breast cancer.[54] The nucleotide excision repair pathway (NER) is one of the most important repairing mechanisms; polymorphisms harbored for genes in this pathway, such as *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC5*, *RAD23B*, *XPA*, *XPC,* and *XPE*, affected the survival of Chinese patients receiving chemotherapy as treatment for esophageal cancer,[55] therefore, showing potential as prognostic biomarkers. By studying one-nucleotide changes in the translesion synthesis DNA polymerases from the Y family associated with breast cancer, Anctzak et al[56] found that these polymerases showed different activities in the presence of damaged DNA, which can inhibit protein expression or lead to the expression of dysfunctional proteins; thus, these polymorphisms can be exploited as potential biomarkers. In prostate cancer, polymorphisms in the repair genes *XRCC1*, *ERCC2*, *ERCC1*, *LIG4,* and *TP53* have been related with clinical variables such as tumor size and Gleason score, demonstrating that these polymorphisms could be potential prognostic biomarkers.[57]

Marshall et al[58] correlated mutations in the repair genes *ATM*, *BRCA1/2*, *CDK12*, *CHEK1/2*, *FANCA*, *FANCD2*, *FANCL*, *GEN1*, *NBN*, *PALB2*, *RAD51* and *RAD51C* with clinical factors for the purpose of obtaining a tool for a better stratification of patients to allow their oncologist to better choose prostate cancer treatments. Mutations in genes associated with the immune response, such as *VTCN1*, *IL2RA*, *ULBP2*, *TREM1*, *MSR1*, *TNFSF9,* and *TNFRSF12A*, are potential biomarkers for the response predictions to treatment with antibodies to anti-immune-check-point-proteins, such as programmed death-1 (PD-1), in different types of cancer.[59] In breast cancer, *AKT1*, *PIK3CA*, *PTEN,* and *TP53* gene mutations have shown potential for the development of a predictive tool based on the results of Li et al,[60] whose deep mutational characterization of these genes showed valuable implications for the clinical handling and the design of tests. The *RUNX1* gene is the most frequently mutated gene in human leukemia, and several studies have focused on its tumor-suppressive function.[71] In triple-negative cancer, which represents between 15 and 20% of total breast cancers, tumors that express the *RUNX1* gene constitutes a group of tumors with the poorest prognosis, suggesting that this gene could contribute in tumor progression[61] and indicating its potential as a prognosis biomarker. Certain overexpressed genes could also be related to the risk of this disease. In that sense, in lung cancer, a 7 genes panel (*ABCC4*, *CCL19*, *SLC39A8*, *CD27*, *FUT7*, *DAG1,* and *ZNF71*) was able to predict the chemotherapy response and the course of the disease in NSCLC, exhibiting its potential as a predictive and prognostic tool. This panel also showed predictive potential for immunotherapy responses.[41] MicroRNAs (miRNAs) are small, 18-25-nucleotide-long, non-coding RNA molecules that down-regulate the target mRNAs.[72] Several types of research have been focusing on the expression profiles of microRNAs and their role in cancer diagnosis and prognosis.[73,74] Some methods are nowadays available to profile the

Table 2. Analysis of Cancer-Associated Genes Used as Biomarkers and With Potential Use in Biosensors.

Genomic marker	Cancer type	Clinical use	Detection method	References
PD-L1	Lung	Selection of patients for therapy	NGS-TMB determination	Cyriac and Gandhi, 2018[46]
STX2	Lung, colorectal		Exome sequencing, qRT-PCR	Lawrence et al., 2014;[47] Wang et al., 2018[48]
ARHGAP35	Lung, osteosarcoma	Risk associated to rs1052667 polymorphism detection	Exome sequencing, RNA sequencing, SNP Genotyping	Kandoth et al., 2013[49]; Lawrence et al., 2014[47]; Zhao et al., 2014[50]; Campbell et al., 2016[51]
PIP	Breast		qRT-PCR	Gangadharan et al., 2018[51]
MSH2	Hepatocellular carcinoma, lung	Risk associated to rs2303428 polymorphism detection	SNP Genotyping	Zhu et al., 2018[52]; Lo et al., 2011[53]
ACYP2	Breast	Risk associated to rs1682111 and rs10439478 polymorphisms detection	SNP Genotyping	Wu et al., 2018[54]
NER genes ERCC1-5 RAD23B XPA XPC XPE	Esophagus	Prognosis-associated to detection of rs3759497, rs3731054, rs2097215 and rs3916788 polymorphisms	SNP Genotyping	Zhang et al.,2018[55]
POLK	Prostate, melanoma, lung, large intestine	Risk associated to detection of 9 different polymorphisms	SNP Genotyping and site directed mutagenesis	Antczak et al., 2018[56]
ERCC1	Prostate	Cancer aggressiveness associated to detection of rs11615 polymorphism	SNP Genotyping	Henríquez-Hernández et al., 2014[57]
ATM	Prostate	Cancer aggressiveness associated to detection of rs17503908 polymorphism; risk associated to specific mutation	SNP Genotyping, Genomic data analysis	Henríquez-Hernández et al., 2014[57]; Marshall et al., 2018[58]
BRCA1/2	Prostate	Risk associated to specific mutation	SNP Genotyping	Marshall et al., 2018[58]
VTCN1	Breast, lung		Microarray analysis	Akiyama et al., 2016[59]
AKT1 PIK3CA PTEN TP53	Breast	Risk associated to specific mutation	NGS	Li et al., 2018[60]
RUNX1	Breast	Poor prognosis	Tissue microarray/IHC	Ferrari et al., 2014[61]
miR-21	Breast	Poor prognosis	Meta-analysis	Adhami et al., 2017[62]
miR-210				
miR-15b-5p miR-16-5p miR-20a-5p	Lung	Diagnostic	qRT-PCR/ Fluorescence quantum dots liquid bead array	Fan et al., 2015[63]
miR-30b-5p miR-96-5p miR-182-5p miR-374b-5p miR-942-5p	Breast	Diagnostic	Microarray/qRT-PCR	Zhang et al., 2017[64]
SCGB2A2	Prostate	Risk associated to differentially methylated detection from biological samples	Bisulfite-converted sample DNA assay	Sherlock et al., 2014[65]
ZNF154	Prostate	Prognosis	RNAseq/ NGS methylation detection	Zhang et al., 2018[66]
ADAMTS1 BNC1	Pancreas	Early detection	Bisulfite-converted sample DNA assay	Eissa et al., 2019[67]
CX43	Lung	Poor prognosis	Tissue microarray/IHC	Aasen et al., 2019[68]

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miRNAs in body fluids, i. e., qRT-PCR, miRNAs microarrays and deep sequencing.[75] From numerous candidate miRNAs, one strategy to specify the most important is to identify intersections between miRNAs reported in not related researches. In that sense, Adhami et al,[62] based on a PubMed search, performed a general systematic review regarding the published miRNA profiling studies, comparing the expression level between breast cancer and normal tissues. Noteworthy, they found 30 miRNAs differentially expressed, the most consistent differentially expressed miRNAs were miR-21, miR-210 and miR-145, showing potential as candidate biomarkers for breast cancer. Fan et al,[63] using serum samples from a cohort of 94 NSCLC patients and 58 healthy volunteers for a miRNA profile training study through qRT-PCR, identified 7 miRNAs (miR-15b-5p, miR-16-5p, miR-17b-5p, miR-19-3p, miR-20a-5p, miR-28-3p y miR-92-3p) differentially expressed. Furthermore, to confirm the accuracy and specificity of these miRNAs, they carried out a validation study through nano-quantum dots microarray finding 5 (miR-16-5p, miR-17b-5p, miR-19-3p, miR-20a-5p y miR-92-3p) from the 7 miRNAs identified in the training study, showing that serum miRNA expression profile could serve as a non-invasive biomarker for NSCLC. In breast cancer, Zhang et al,[64] based on a microarray screening for blood miRNA profile, followed by a validation qRT-PCR, found 5 upregulated miRNAs (miR-30b-5p, miR-96-5p, miR-182-5p, miR-374b-5p and miR-942-5p) in breast cancer patients compared with healthy controls. The detection of these 5 miRNA expression levels could significantly distinguish between breast cancer patients and healthy controls as shown by ROC curves, even in very early stages of the disease, showing its potential as early breast cancer diagnosis biomarker.

CpG island methylation is a common epigenetic modification in tumors that leads to the inhibition of gene expression and functional loss.[76] In that sense, a patent to detect methylations in SCGB2A2 and other genes was developed recently,[65] leading to predict associated risks to the prostate cancer diagnosis. In a new study,[66] differences between the methylation levels in genomic DNA from a prostate cancer tumor (PCa) and from a benign prostate tumor (BP) were analyzed. Using 1706 differentially expressed genes, they demonstrated that hypermethylation in the *ZNF154* gene inhibits its expression and that this repression is associated with cancer progression. Also, it was recently suggested that methylated promoter detection of ADAMTS1 and BNC1 genes is useful for early detection of pancreatic cancer, greatly contributing to the early diagnosis of this aggressive cancer.[67] Likewise, a novel biomarker detected in lung cancer patients is the expression of CX43, a gap junction protein without precedents in cancer implications, and whose presence is strongly related to a poor prognosis in those patients.[68]

Altogether, the genes and their specific characteristics and behaviors reviewed here may count to set a new group of cancer biomarkers with a high potential of use in biosensors development.

Immunological Approach and Biomarkers for cancer Disease

The release of proteins from tumors triggers an immune response in cancer patients. Responses to most tumor antigens are rarely observed in healthy individuals, making the humoral and cellular responses themselves a code that betrays the presence of underlying cancer. Immune responses show potential as clinical biomarkers because are easy to measure and are obtained in blood samples.[77]

Macrophages can be classified into the following 2 main groups: classic pathway-activated macrophages (M1) and alternative pathway-activated macrophages.[78] Within M2 macrophages, there is a subpopulation known as M2d or tumor-associated macrophages (TAMs) that promotes tumor malignancy by facilitating angiogenesis and tumor growth. Therefore, the presence of these subpopulations could represent a discriminatory tool between healthy people and patients with cancer. Hegab et al[79] used a murine model for lung adenocarcinoma to show that M2 macrophages support tumor growth by increasing tumor angiogenesis and proliferative capabilities. Of note, M2 macrophages showed immunosuppressive properties, as they were able to suppress the release of IFN-γ and to increase the production of L-arginase by T cells. However, infiltrating tumoral sites must be considered during the evaluation of M2 macrophages in lung cancer, since macrophages of different origins could show different biological and prognostic properties.[80]

Even though most research has been focused on the tumor-infiltrating cells, peripheral blood cell measurements could also serve as prognostic biomarkers. Zhang et al[81] and Phan et al[82] showed that the neutrophil-to-lymphocyte ratio (NLR) was an independent prognostic biomarker in NSCLC patients, where an elevated NLR was correlated with lower progression-free survival (PFS) and lower overall survival. Furthermore, not only cell populations but cytokines profiles, secreted chemokines and the receptors of both could also serve as diagnosis elements.[83] Chemokines cover a group of approximately 50 small secreted proteins (8-14 kDa) that regulate cell traffic and are structurally like cytokines. In lung cancer, the chemokine CCL18 ligand is highly expressed in M2 macrophages. Schmid et al[84] showed that a higher proportion of TAMs positives to CCL18 was related to a shorter survival rate by survival analysis; thus, this chemokine could be used as a prognosis biomarker. Schmid et al[85] showed that elevated CCL18 levels are related to tumor size and poor prognosis in the NSCLC tumor microenvironment; moreover, this work also highlighted that serum levels of CCL18 were not related to either tumor size or prognosis. In contrast, Plönes et al[86] measured and used CCL18 serum levels to discriminate between healthy controls and NSCLC patients, obtaining an area under the ROC curve of 0.968. It has recently been shown that there is an association between the chemokine CXCL10 and the recruitment of populations of T CD8$^+$ CXCR3$^+$ cells to the tumor site, which increases anti-tumor activities.[87] Given that elevated levels of this chemokine are associated with a higher survival rate, this

chemokine represents a potential prognosis biomarker. Other chemokines have been reported as potential biomarkers, Fujimoto et al[88] analyzed the expression of CCL5 and CXCL9 in serum, stromal and cancer cells and found that serum levels of these chemokines might contribute to determine patient prognosis. High CXCL13 has also been associated with improved outcomes in the luminal-hEGFR2 subtype.[89]

IL-10 and IL-2, together with their receptor (IL-2 R), have been used in breast cancer to discriminate between malignant and benign tumors, showing great potential as diagnostic tools given the values obtained using ROC curves.[90] Notably, these biomarkers were tested together with immune-check-point proteins such as PD-1 and cytotoxic T lymphocyte antigen 4 (CTLA-4). The PD-1 signaling pathway is an evasive strategy used by tumor cells that inhibits immunity by preventing chemotaxis, cell proliferation and release of cytokines by T cells. Dudnik et al[91] evaluated the expression of the PD-1 ligand (PD-L1) in NSCLC containing a mutated *BRAF* gene. Together with the total mutational burden (TMB) and microsatellite instability status (MSI), this cancer ligand was associated with the response to treatment with immune-checkpoint inhibitors (ICPis). However, the high expression levels of PD-L1 were not a reliable predictive biomarker for ICPis.

Moreover, Krieger et al[92] reported than TMB status and PD-L1 expression can be used for prediction response to checkpoint inhibitors and/or anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antibodies in NSCLC and melanoma, while a clear predictive trend was not identified in renal, breast, gastro or Merkel cell cancer. PD-1 can be expressed in activated T cells and is usually considered a marker for exhausted T cells. When this molecule is compromised by its ligand PD-L1, a ligand that can be expressed in tumor and immune cells, the function of T cells is inhibited.[46] A different study performed after a vaccination cycle showed that the relatively high concentrations of PD-1$^+$ and CD4$^+$ cells obtained before and after the vaccination cycle were correlated with a higher survival rate in lung cancer[93]; thus, monitoring of these cell subpopulations also showed prognosis potential. El-Guindy et al[94] measured PD-L1 expression and tumor-infiltrating lymphocyte (TIL) populations in NSCLC tumors and showed that elevated levels of PD-L1 correlated with low TILs, and in turn, both were associated with a lower survival rate, strengthening the potential of PD-L1 and cell populations as prognostic tools. Recently, Gonzalez-Ericcson et al[95] reported the use of these biomarkers for optimal patient selection for immunotherapeutic approaches. Morgan et al[96] worked with metaplastic breast cancer samples and demonstrated a greater amount of CD163 in the stroma and PD-L1 in the tumor than TNBC, although more TNBC samples were positive for CD8 in the tumor than metaplastic breast cancer. In the same sense, PD-1 blockade in NSCLC led to enhanced IFN-γ production by CD8$^+$ CD103$^+$ TILs, suggesting that this immune cell subpopulation could be a predictive biomarker for immunotherapy based on PD-1 blockade[97] and has been reported that cytokine signaling may be dysregulated in peripheral blood monocytes.[97] Last year Li et al[98] reported 4 immune-related genes

(*APOD*, *CXCL14*, *IL33*, and *LIFR*) identified as biomarkers correlated with breast cancer prognosis. Their findings may provide different insights into prognostic monitoring of immune-related targets for breast cancer or can be served as a reference for further research and validation of biomarkers.

C-reactive protein (CRP) is an inflammatory biomarker that plays a key role in the innate immune response in humans; it is produced in response to inflammation, infection and tissue damage.[99] Kaur et al[100] measured the concentration of this biomarker in peripheral blood from breast cancer patients and found that elevated levels of CRP are associated with increased risk, recurrence, and mortality, thus showing its potential as a prognosis biomarker. Butyrophilin subfamily 3 member A2 (BTN3A2) was positively associated with better prognosis and could be served as a special diagnostic and independent prognostic marker for TNBC by regulating the T-cell receptor interaction and NF-κB signaling pathways.[101] CCL20 and FOXP3 tumor-infiltrating lymphocytes may have synergistic effects, and their upregulated expressions may lead to immune evasion in breast cancer. Combinatorial immunotherapeutic approaches aiming at blocking CCL20 and depleting FOXP3 might improve therapeutic efficacy in breast cancer patients.[102] Cerbelli et al[103] showed that CD73 expression better predicts the response to neoadjuvant chemotherapy than stromal tumor-infiltrating lymphocytes in TNBC. The authors suggest than the characterization of both TILs and microenvironments could be a promising approach to personalize treatment.

Recently were reported mutations of immune players as biomarkers, analyzed for specific human populations, Ahmad et al[104] reported the TNF-a308 G/A change and its significant association with breast cancer patients from north India and over the major histocompatibility complex class I-related chain A (MICA). Ouni et al[105] reported the MICA-129 Met/Val change as an inherited genetic biomarker contributing to an increased breast cancer risk in Tunisian women. Tumors are a heterogenous mix of different cells, with a wide array of metabolic, phenotypic and stemness-like properties, the immune responses or immune evasion that tumor cells induce at the host reflects these spectra. To select the most accurate immunological biomarker scheme for cancer diagnosis, recent research efforts focus on test combinations of immune markers, some of which are arranged in Table 3.

Biosensors in the Diagnosis, Prognosis and Prediction of Cancer

Conventional methods for cancer diagnosis include ultrasound, nuclear magnetic resonance and, biopsy, but these techniques require expensive equipment and highly qualified personnel, and, in the case of biopsy, is an invasive technique that represents a risk for the patient. Additionally, these techniques are inefficient in the early diagnosis of cancer since they depend on tumor phenotypic properties.[106] Additionally, these techniques have a limit of detection close to 10^9 cells, growing as a single mass, thus being incapable of detecting cancer at early stages of the disease, since primary tumors are small.[107] Biomarkers in

Table 3. Recent Published Immune Biomarkers With Clinical Use.

| Immune marker	Cancer type	Clinical use	Detection method	References
M2 macrophages	Lung adenocarcinoma	Diagnosis and prognosis	Immunohistochemistry	Martínez F 2008[78], Hegab A 2018[79], Li Z 2018[80]
Neutrophil-to-lymphocyte ratio (NLR)	NSCLC	Prognosis and survival prediction	Blood cell count, Kaplan-Meier analysis	Zhang Y 2018[81], Phan T 2018[82]
CCL18	NSCLC	Prognosis and survival prediction	ELISA	Plönes T 2012[86], Schmid S 2018[84]
IL-10, IL-2, IL-2R	Breast	Diagnosis	qRT-PCR	Liu C 2018[90]
CRP, TILs and PD-L1	Breast, Breast, NSCLC, Lung	Diagnosis, Diagnosis	ELISA, Immunohistochemistry, H&E staining	Kaur R 2019[100], Gonzalez 2020[95], El-Guindy 2018[94]
TNF-a308 G/A	Breast	Significant association with breast cancer patients from north India.	PCR-RFLP	Ahmad MM 2020[104]
MICA-129 Met/Val	Breast	An inherited genetic biomarker contributing to an increased breast cancer risk in Tunisian women.	Genotyped	Ouni N 2020[105]
TMB status and PD-L1 expression	NSCLC, Melanoma	Prediction response to ICPis	Sequencing	Krieger T 2020[92]
IFNg, CD163, PD-L1 and CD8	Breast, Breast, TNBC	Cytokine signaling dysregulated, Breast cancer classification	Phosphoflow cytometry, Immunohistochemistry	Wang L 2020[97], Morgan E 2020[96]
CD73, CCL20 and FOXP3	TNBC, Breast	Neoadjuvant chemotherapy response, Immune evasion in breast cancer.	Immunohistochemistry, Immunohistochemistry, qRT-PCR	Cerbelli B 2019[103], Zhao X 2019[102]
APOD, CXCL14, IL33 and LIFR	Breast	As biomarkers correlated with breast cancer prognosis	Weighted gene co-expression network analysis (WGCNA), single-sample gene set enrichment analysis (ssGSEA), multivariate COX analysis, least absolute shrinkage, and selection operator (LASSO), and support vector machine-recursive feature elimination (SVM-RFE) algorithm	Li J 2019[98]
CCL5	Breast	Prognosis	Cytometric bead-based immunoassay, Immunohistochemistry	Fujimoto Y 2020[88]
CXCL9 and CXCL13	Breast, TNBC	Prognosis	qRT-PCR	Razis E 2020[89]
BTN3A2	TNBC	Prognosis	Expression in extensive cancers were analyzed with Oncomine and TIMER databases.	Cai P 2020[101]
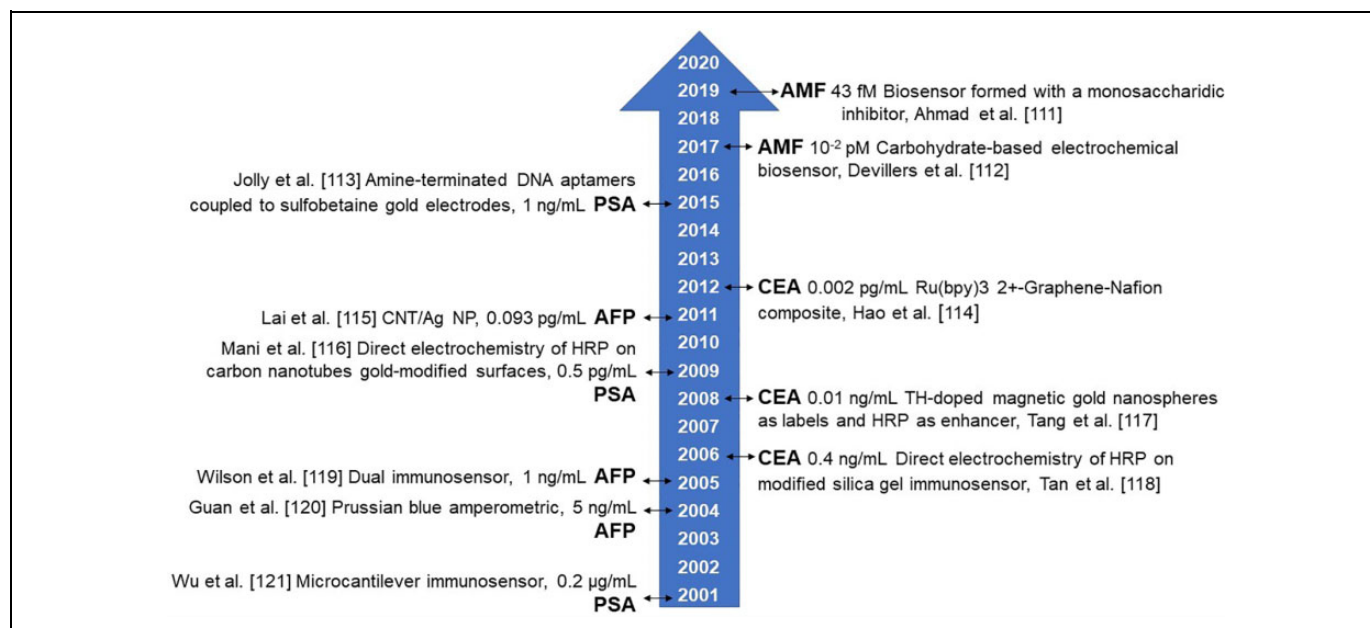
Figure 1. Biosensors sensitivity timeline from 2001 to date for selected biomarkers, as alfa-fetoprotein (AFP), Autocrine motility factor (AMF), carcinoembryonic antigen (CEA) and Prostate-specific antigen (PSA). Abbreviations used: CNT/Ag NT, silver-nanoparticle enriched carbon nanotube and HRP Horseradish peroxidase.

body fluids represent a convenient, noninvasive and cheaper method for cancer diagnosis. Body fluids including serum or plasma, urine, saliva or sputum can be used in order to perform the biomarker detection. There have been considerable efforts in order to develop analytical assays for biomarkers detection. For protein biomarkers, the most common include western blotting, ELISA and mass spectrometry; for nucleic acids qRT-PCR, microarray, and next generation sequencing; and for cell populations flow cytometry and immunohistochemistry. Despite these techniques are in usage, they still have limitations like sophisticated analysis process, time-consuming operations and low sensitivity.[108] New detection technologies need to be at least as sensitive as current technologies. The new methods based on biochemistry, immunology, and molecular biology are verified, developed and used in a continuous way in order to increase the sensibility of the biosensors, because of only trace levels of biomarkers exist in body fluids.[109] In Figure 1 we represented a timeline for improved sensitivity of biosensors used to analyze representative biomarkers, as α-fetoprotein (AFP), Autocrine motility factor (AMF), carcinoembryonic antigen (CEA) and Prostate-specific antigen (PSA).

Currently, there is a growing interest in biosensors development for cancer diagnosis, prognosis, and prediction since these devices have shown real-time measurements and superior analytical performance. Since biosensors can detect minimal amounts of biomarkers in physiological samples, they can contribute to early cancer diagnosis.[4] Biosensors offer flexibility related to biomarkers that can be used for their manufacturing, including antibodies, nucleic acids, or different specific recognizing molecules such as cytokines and chemokines. A biosensor is based on the interaction of the biomarker with a transducer, which converts the biological response produced into a signal that, depending on the transductor type, can be electrochemical, optical or a change in mass. Electrochemical biosensors (Figure 2) work analogous to an electrical circuit, converting the biosensor-biomarker interaction into a signal that can be translated as impedance, conductance, electrical current or potential. Electrochemical biosensors can be used for rapid biosensing and the measurement of key analytes in different cancer types using field-effect transistor (FET) techniques, square wave voltammograms (SWV), square wave stripping voltammetry (SWSV), electrochemical impedance spectroscopy (EIS), and cyclic voltammetry (CV).[110]

Most optical biosensors (Figure 3) for cancer diagnosis are based on phenomena such as fluorescence resonance energy transfer (FRET) or surface-enhanced Raman spectroscopy (SERS).[4]

Biosensors for detecting changes in mass (Figure 4) make use of ultra-sensitive piezoelectric devices for detecting mass changes. These devices are analogous to a spring-mass mechanical system or electrical circuit with impedances, inductances, and capacitances. The changes in frequency within piezoelectric resonance gives a detectable signal due to an increase or decay in mass from biomarker interaction.

Proteomic Biosensors

Proteomic biosensors are based on the recognition of molecular elements like antibodies, enzymes, and peptides. An et al[111] developed a magneto-mediated electrochemical biosensor to sense the proteins MUC1, EpCAM, HER2, and CEA in the exosomes of breast cancer cells, showing potential for the
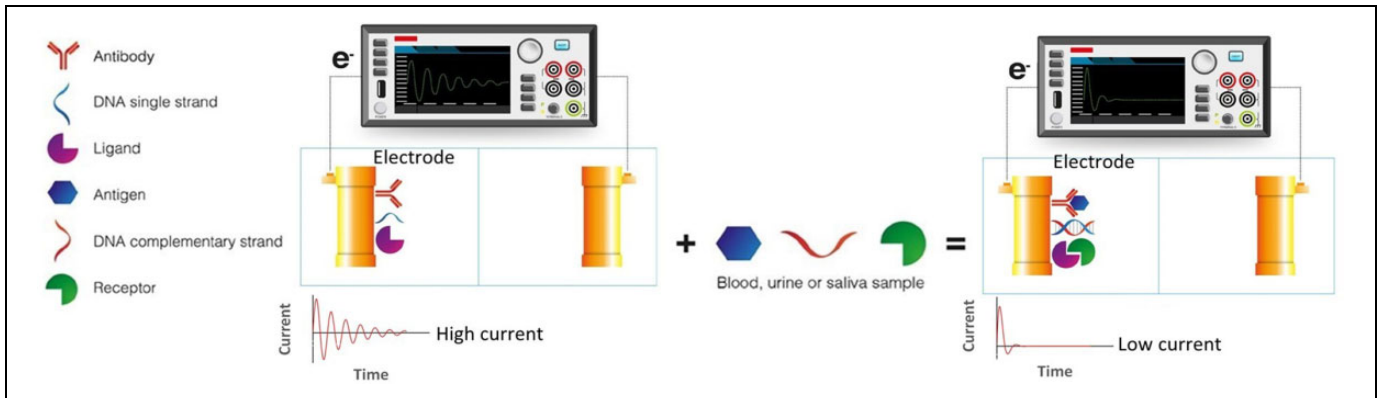
Figure 2. Electrochemical biosensor. An increase in the electrode resistance owed to the interaction between a biomarker present in body fluids (antigen, complementary DNA strand or ligand) and its target molecule (antibody DNA single strand or receptor) attached to the electrode is measured as a change of current.
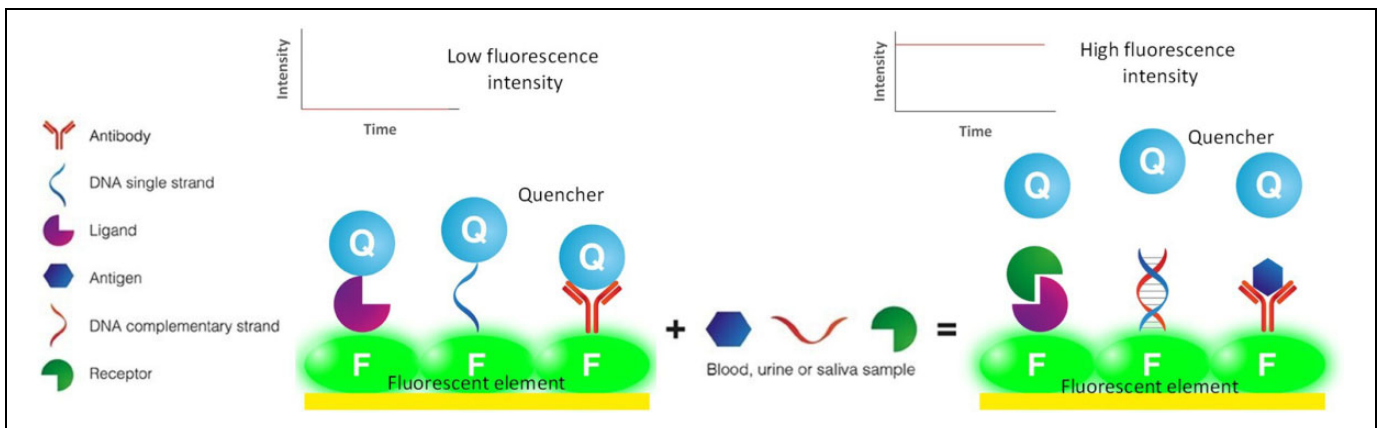

Figure 3. Optical biosensor. Owed to the interaction between a biomarker (antigen, complementary DNA strand or ligand) present in body fluids and its target molecule (antibody DNA single strand or receptor) attached to a florescence element, a quencher release occurs which translates in higher fluorescence.
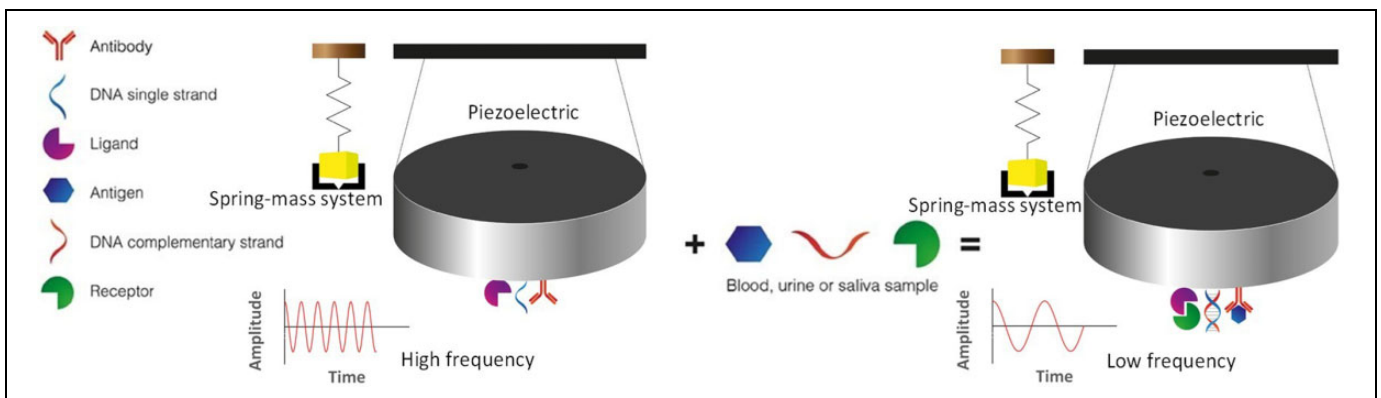

Figure 4. Mass change biosensor. As a result of a change in mass in the piezoelectric device owed to the interaction between a biomarker (antigen, complementary DNA strand or ligand) present in body fluids and its target molecule (antibody DNA single strand or receptor) attached to the piezoelectric device a decay in frequency is recorded.

determination of this proteins in breast cancer serum, which is promising for clinical diagnosis. Autocrine motility factor (AMF) or AMF receptor overexpression is closely related to tumor progression and malignity.[112] By attaching the substrate

for the enzyme glucose-phosphate-isomerase (GPI) in the surface of a gold electrode and given that AMF and GPI are identical and possess the same functions, Devillers et al[113] developed a biosensor for the detection of AMF and its

association with cancer progression that exhibits a minimal detection limit on the order of 10^{-2} pM. Using the same AMF protein, a more recent work reported an electrochemical biosensor capable to detect even lower concentrations, with a lower limit of 43 fM, however, this detection was performed in phosphate buffer and not in serum.[114] The epithelial cell adhesion molecule (EpCAM) has been considered a tumor prognostic biomarker in different types of cancer.[115] Using graphene quantum dots (GQDs) as a fluorescence emissary and molybdenum disulfide (MoS_2) as a fluorescence quencher, Shi et al[116] developed an optical biosensor based on FRET capable of detecting EpCAM in cancer cells with a pM level detection limit. Li et al[117] used up-converting nanoparticles (UCPs) and palladium nanoparticles (PdNps) for fluorescence quenching and made a biosensor for CEA detection through an aptamer linkage on a FRET system. Of note, the detection limit in serum was on the order of 0.8 pg/mL. Su et al[118] designed and constructed a dual biosensor for the detection of PSA and α-fetoprotein using a ceramic piezoelectric device by immobilizing antibodies against these antigens on the surface of gold electrodes. The sensitivity and specificity of this biosensor were on the order of pg/mL and were comparable to ELISA methods. Parathyroid hormone-related peptide has been associated with cancer metastasis in breast and prostate cancer.[119] Crivianu-Gaita et al[120] constructed a device based on acoustic waves by attaching anti-PTHrP antibodies to quartz disks treated with different linkers, achieving a sensitivity of 61 ng/mL. However, despite these results, we must consider that clinically relevant concentrations of this protein oscillate between 120 pg/mL and 14 ng/mL, which is lower than the limit detection of the device.

Sarcosine has been found to activate prostate cancer cells; therefore, its occurrence in blood and urine indicates malignity. Narwal et al[121] constructed a biosensor capable of detecting sarcosine levels on the order of picograms with a chitosan electrode covered with copper nanoparticles (CuNps) and carbon nanotubes. This biosensor has enormous potential for prostate cancer detection and can maintain its stability during a long period, showing storage stability. A biosensor that could be used in order to detect different types of cancer is something desirable and achievable, since Rangel et al,[122] continuing with previous work,[123] developed a biosensor based on impedances capable of detecting the T antigen expression in serum samples of patients with different types of cancer, including breast, prostate and lung cancer, in which an increase in the expression of this antigen is well known.

Genomic Biosensors

Some genetic-approaching biosensors used for the detection of several types of cancer biomarkers have been developed recently. miRNAs, of which some examples of potential biomarkers are mentioned in Table 2, are easy to hybridize with specific DNA probes generating a signal that, depending on the method, can be detected and used for discriminating between a healthy or ill patient. Even though the concentration threshold of the biomarker in the biological sample that these biosensors

are capable of detecting could become a limiting factor, devices with better resolution features are now appearing. To depict an important example, minimum concentrations from 10 fM of multiple target miRNAs involved in liver cancer were detected by a sensor equipped with a surface-enhanced Raman scattering (SERS) technology.[124] By using a similar strategy, Ouyang et al[125] reported the construction of an optical biosensor based on SERS able of detecting DNA methylation levels and reported a minimum detection limit of 0.2 pg/μL. Interestingly, this device was reusable, showing its practical relevance. Likewise, a dual-SERS biosensor capable of detect miRNAs from pancreatic cancer from exosomes and plasma is a promissory technology that matters for the early detection of this type of cancer.[126] In this way, an electrochemical biosensor using a graphene electrode covered with gold nanoparticles (AuNps) was recently built,[127] the device was able to detect mutations in the CYFRA-21 gene with a minimal DNA concentration of 10^{-2} pM, showing enormous potential in lung cancer diagnosis. Lung cancer patients with mutations in the gene for the epidermal-growth-factor-receptor (*EGFR*) are treated with molecules that inhibit tyrosine/kinases (TKIs) that promote apoptotic pathways; thus, these mutations could be a predictive biomarker for treatment.[128] Also, Weng et al[129] constructed an electrochemical biosensor capable of discriminating between different kinds of mutations on the *EGFR* gene. Notably, these mutations were corroborated through sequencing, confirming the applicability of this method. More recently, another device useful to detect the lung cancer related PIK3CA gene mutations was developed. By using a DNA probe coupled to an FMNs/MoS_2 nanocomposite, this device detects the self-redox signal loss when the target DNA binds to the DNA probe and changes its conformation with a limit sensitivity of 1.2×10^{-17} mol/L.[130] Purposely, with another strategy, PIK3CA$^{H1047\ R}$ mutation was efficiently detected using a biosensor based in strand displacement amplification mediated by a restriction enzyme and a system of 4-way DNA junction with a low detection limit of mutation from biological samples of human serum.[131]

For breast cancer, an immobilized ssDNA probe attached to a poly (amidoamine) dendrimers matrix and coupled to the surface of an Au electrode was used for the construction of a new biosensor[132]; this device shows high sensitivity for DNA detection in the order of 1nM or less, and specifically is able to differentiate single-base mismatches of cancer biomarker BRAC1 gene. A biosensor with the accuracy to detect quantities from 1 pM of the p53 gene and its mutations was developed by Luo et al.[133] In order to improve selectivity and sensitivity, this device uses a double level of biomarkers (a hairpin and an enzyme) and emits the test result in just 23 min, positioning it at a more acute level of detection compared to other biosensors with a single biomarker level. This apparatus constituting a new, fast and useful strategy for the clinical and non-invasive diagnosis of various types of cancer using body fluids. By the other hand, detection of liver cancer is the goal of other strategy utilized in a strip-style biosensor which combines the detection of SNP on CYP1A1 gene and the expression of the protein biomarker alpha-fetoprotein (AFP) with a sensitivity in the

order of ng/mL.[134] The same strategy for detection of SNPs on the CYP1A1 gene but without the involvement of AFP was published a couple of months later with the premise of detecting any SNP in no more than 10 minutes.[135]

Methylation of MGMT gene as a biomarker in head and neck cancer is the detection objective of a new genosensor built with DNA probes immobilized on a gold surface in combination with 11-mercaptoundecanoic acid (11-MUA), which uses electrochemical impedance spectroscopy; results obtained with this sensor indicated high selectivity for HN13 cells, a high degree MGMT methylation cell line, and good sensitivity in the range of pmol/L of methylated DNA.[136]

Advantageously in many cases, the use of these and other genetic biosensors increases the speed that diagnosis with traditional methods and devices can represent. However, many of these new biosensors include no reusable and expensive components that block the use in the clinic field, especially in countries with limitations to provide basic health services.

Novel Biosensors for Immune Biomarkers

Dysregulation of the chemokine system is implicated in several autoimmune and inflammatory diseases, as well as cancer. In 2015, Chen et al[137] introduced a novel biosensor for simultaneous detection of multiple cytokines (IL-2, IL-4, IL-6, IL-10, TNFα, and interferon γ (IFNγ). Based on a microfluidic surface plasmon resonance (LSPR) chip, this sensor achieved a linear range detection between 5 and 20 pg/mL with only 1 μL of serum sample. Noteworthy, such biosensor showed an increase in sensitivity 10 times higher than conventional LSPR chips. Later, in 2018 Aydin et al[138] constructed an electrochemical biosensor based on the immobilization of anti-IL-1β antibody on an indium tin oxide (ITO) electrode for interleukin 1β (IL-1β) detection in saliva and serum samples. Through EIS, CV and SFI, this electrochemical biosensor showed a detection limit of 7.5 fg/mL.

The use of a labeled secondary antibody (Ab2) specific to the target analyte is an alternative approach to improve the limit of detection for cancer biosensors. In that sense, Peng et al,[139] using a Sandwich Nanoparticles Labeled Electrochemical Immunoassay (sECIA-NP) technique, developed an electrochemical ultrasensitive biosensor for IL-6 detection, reaching a detection limit of 0.1 pg/mL. Something to highlight, the biosensor showed good reproducibility and long term stability, 2 desirable features in this kind of devices.

Continuing with electrochemical biosensors, Chung et al[140] immobilized the chemokine CXCR2 (C-X-C Motif Chemokine Receptor 2) over a nanocomposite film covered with gold nanoparticles (AuNPs). Among 3 ligands tested, CXCL5 (C-X-C Motif Chemokine Ligand 5) showed the strongest affinity to CXCR2, showing a detection limit of 0.078 ng/mL. Remarkably, the biosensor showed high sensitivity and specificity in human serum and colorectal cancer cells samples.

Conclusions

The public health problems presented by cancer requires societies to develop new tools for the detection of this disease during its initial stages when the probabilities of a cure are higher. However, patients who are already suffering from this disease and are following a treatment plan also require tools capable of predicting their responses to therapy and the course or advancement of the disease. In that sense, the search for new diagnostic, prognostic and predictive biomarkers from proteomics, genomic or immunological nature is relevant. On the other hand, the limitations related to the sensitivity and specificity of current methods using biomarkers, in addition to the high operation costs, make new technologies an attractive solution for resolving the many current issues that have been previously cited.

Traditional detection of cancer requires highly trained health personnel able to perform sophisticated and expensive tests, sometimes with very invasive procedures for patients. As an alternative to traditional methods, biosensors have shown enormous potential, especially given their higher sensitivity and specificity compared with traditional methods, achieving limits of detection on the order of pM or even fM. Furthermore, because biosensors are small and easy-to-use devices, they diminish operation costs and the sample processing time, therefore facilitating and streamlining cancer diagnosis, prognosis and prediction. Devices that sense cancer biomarkers from body fluids or from non-invasive-obtained biological samples represent in many cases an advantage, being able to obtain fast and reliable results; however, even though hundreds of biosensors have been built in recent years, their distribution is not cosmopolitan due to various factors such as: i) the cost of materials for their construction; ii) issues associated with copyright; iii) priorities of the health systems in each country; iv) limitations specific to the chosen biomarker, among others. Many of these challenges must be overcome before one of these devices could be used commercially, making cheaper the components for their construction and selecting highly sensitive and specific biomarkers; until now, none of the used biomarkers has gathered both. Nevertheless, the new advances in materials sciences, molecular biology, immunology, and artificial intelligence are continuously increasing the knowledge related with these devices, contributing to its successful development in a not so far future.

The search for cancer biomarkers has been an arduous task for decades by researchers in the area. More and more molecules of different nature are cataloged as markers associated with various types of this disease, and although a flood of data on this can be found in the literature, many new biomarkers are detected at every moment.

Cancer biomarkers can form a haystack where finding a useful "needle" can be difficult. Depending on the type of cancer, the known information on these markers can become more limited and selected to be sensed by new devices capable of diagnosing or generating prognosis. Because of this, the search for proteins, genes or components of the immune system

that contribute to having a better point of care in this disease will continue to be in force.

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