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Early detection of lung cancer biomarkers through biosensor technology: a review

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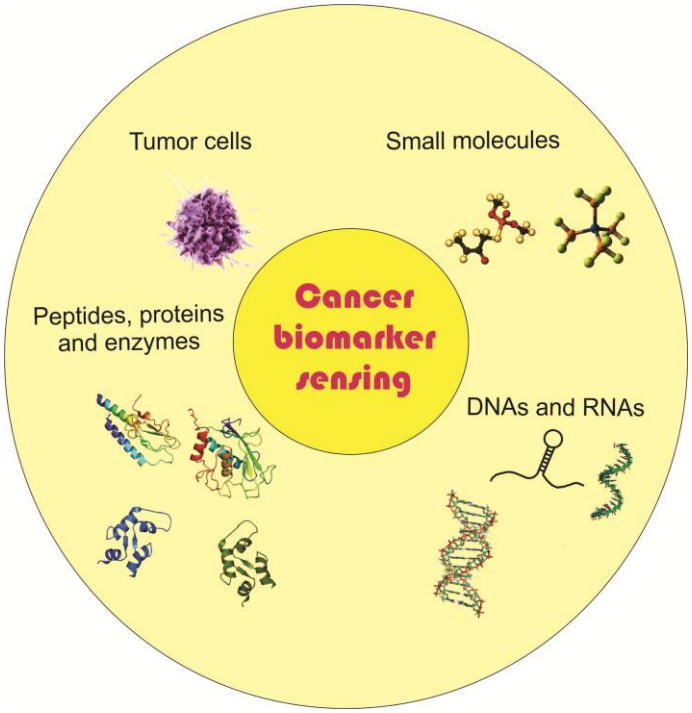
Graphical abstract



Highlights

- This review focuses on the early detection of lung cancer biomarkers.
- Latest biosensing approaches for lung cancer detection studies are addressed.
- Conventional methods for lung cancer biomarkers detection are also summarized.

Abstract

Lung cancer is undoubtedly one of the most serious health issues of the 21st century. It is the second leading cause of cancer-related deaths in both men and women worldwide, accounting for about 1.5 million deaths annually. Despite advances in the treatment of lung cancer with new pharmaceutical products and technological improvements, morbidity and mortality rates remains a significant challenge for the cancer biologists and oncologists. The vast majority of lung cancer patients present with advanced-stage of pathological process that ultimately leads to poor prognosis and a five-year survival rate less than 20%. Early and accurate screening and analysis using cost-effective means are urgently needed to effectively diagnose the disease, improve the survival rate or to reduce mortality and morbidity associated with lung cancer patients. Thus, the only hope for early recognition of risk factors and timely diagnosis and treatment of lung cancer is biosensors technology. Novel biosensing based diagnostics approaches for predicting metastatic risks are likely to have significant

therapeutic and clinical impact in the near future. This article systematically provides a brief overview of various biosensing platforms for identification of lung cancer disease biomarkers, with a specific focus on recent advancements in electrochemical and optical biosensors, analytical performances of different biosensors, challenges and further research opportunities for routine clinical analysis.

Keywords: Lung cancer, biomarkers, detection, diagnosis, electrochemical and optical biosensors

Contents

1. Introduction

Cancer is intrinsically a complex and highly heterogeneous disease usually characterized by rapid and uncontrolled division and proliferation of cells. This complex disease is considered as the leading cause of deaths throughout the globe, accounting for more than 14 million new cancer cases and 8.2 million deaths annually. For this reason, cancer has been the subject of intense scientific research investigation over the past decades due to its widespread occurrence, recurrence after treatment, high morbidity and mortality rate etc.[1-4]. Among the most common and fatal kinds of cancer diagnosed with the greatest frequency, lung cancer (also called pulmonary carcinoma) is considered as a major global public health concern. It is a most prevalent neoplasm and the second deadly cause of cancer-related deaths in both men and women. Each year, lung cancer causes approximately 1.5 million deaths globally[5-9]. The predominant variables associated with the development and progression of lung cancer disease include smoking (use of tobacco products), high exposure to environmental pollutants (e.g airborne genotoxic carcinogens), and multiple epigenetic and genetic alterations. Cough (including coughing up blood), hemoptysis, dyspnea, weight loss, fatigue and anorexia are the most common symptom of lung cancer.

Based on histological features, lung cancer can be classified into small cell lung carcinomas (SCLC) and non-small cell lung carcinomas (NSCLC) [1-9]. Small cell lung carcinomas (SCLC) is one of the most malignant and aggressive subtype of lung tumors, representing approximately 15–20% of all lung cancers. Although patients suffering from the SCLC are highly responsive to both chemotherapy and radiation therapy but it is often extended beyond its origin by the time of diagnosis, and therefore the overall long-term prognosis remains dismal and disease recurrence occurs more frequently in almost all patients. According to the American Cancer Society, non-small-cell lung cancer is one of the

two most common forms of pulmonary carcinoma constituting approximately 80% of cases of the total lung malignancies. The most predominant NSCLC subtypes include adenocarcinoma, squamous-cell carcinoma and large-cell carcinoma) NSCLC primarily develops from the epithelial cell lining and is insensitive to chemotherapy in comparison to small cell carcinoma. Patients associated with this fatal disease have a poor prognosis because of the high relapse rate, early formation of micrometastases, late detection, and problems in diagnostic modalities. Regardless of lung cancer subtypes, the overall 5-year survival rate for lung cancer is among the lowest of all cancers (only 18%) compared to colorectal cancer (65%), breast cancer (90%) and prostate cancer (99%).

So far, a wide range of investigations from radiation therapies to different chemotherapies have been done for improving the treatment efficiency of lung cancer patients [1-9]. However, its early diagnosis obviously is of utmost importance for increasing the chance of treatment. The conventional methods, including chemotherapy, X-ray, computed positron emission topographies, optical coherence, spiral and virtual bronchoscopy are inefficient at detecting early lung neoplasms because most of these techniques are based on morphological criteria and show corresponding drawbacks; for instance, they are relatively slow, labor intensive, time consuming, need sophisticated apparatus, and diagnostic tests have a high cost and low yield [10,11]. In its early stages, lung cancer is usually symptomless. There are several challenging issues associated with lung cancer treatment and management. For example, patients suffering from this life threatening disease are often get misdiagnosed as pulmonary tuberculosis for varying periods. Consequently, misdiagnosis not only lead to undue delay in the initiation of right medical diagnosis but also expose the patient to inappropriate, unnecessary or rather harmful medication, which further worsen the health outcome. Incorrect diagnosis makes it maddeningly tough to treat patients experiencing lung cancer sickness. Given the seriousness and incurable nature of lung carcinoma and the growing number of individuals with a heightening risk for developing several serious complications with pulmonary functions, there is a serious need for design and development of highly sensitive biosensing materials and sensing devices for identifying people with premalignant and pre-metastatic malignant tumors.

A biosensor is commonly defined as a self-contained small analytical device that combines biological recognition system and a physiochemical transducer for the detection of target molecules by converting recognition signal into detectable output signal [12-14]. Majority of biosensing platforms generate measurable signal when a sensing element or

probe in the detector interacts accurately with an analyte of biological interest (DNA, biomarkers, enzymes, antibodies, proteins, saccharides, drugs or toxins, cell receptors, whole cells, tissues, etc) [15]. Development of such sensing systems needs a multidisciplinary investigation in biology, chemistry, and engineering. So far, different biosensors including optical biosensors, thermal biosensors, piezoelectric biosensors, bacterial biosensors, magnetic biosensors, DNA based biosensors, etc., have been developed for detection of various markers and diseases [12-16]. In case of lung cancer, biosensors can provide a fast, cheap and high sensitive way for diagnosis of lung cancer, especially in its early stages.

2. Lung cancer biomarkers

Genetic or epigenetic alteration is the most specified character of cancer cells. A cancer biomarker refers to a remarkable change of a protein, metabolite, nucleic acid or a hormone in cancer cells. Such changes are characterized as diagnostic and prognostic, which can be informative in tumor diagnosis and its course of reappearance, respectively. The other types of cancer biomarkers are predictive, which are useful in prediction of treatment response [17-19]. Currently detection of biomarkers is one of the favorite tactics in screening and tracking lung cancer. Their strong linkage to tumor progression will help to understand time and type of a right intervention [20]. Among different methods, although the genetic based techniques can give precise and direct information, they seem to be laborious and time consuming [20,21]. Further, even with recognition of some DNA-based biomarkers in lung cancer, such markers have been shown no sufficient specificity and sensitivity [22]. On the other hand, proteomic techniques attract many attentions as they can provide worthy information on lung cancer pathogenesis with less difficulty [23-27]. However, biosensing techniques can be accounted as the newfangled apparatuses in detection of cancer biomarkers. Some investigated lung cancer biomarkers and their common detection methods are summarized in Table 1. These biomarkers can be obtained directly from tumor cells or in a noninvasive manner from body fluids including blood, urea and sputum.

3.1 Genetics and epigenetic biomarkers

Lung cancer genetic biomarkers can be classified in two chromosomal and gene based alterations. In both categories, genetic materials undergo deletions, amplifications, rearrangements or any other changes. Microsatellite instability (MSI), loss of heterozygosity (LOH) and genetic hyper methylations are the other recognized genetic variations in lung cancer which can be considered as a biomarker [28,29]. The word epigenetics is known as

some alterations on genomes, which affect gene expression profile of cells. Such functional changes are not involved in DNA sequence but mostly on the chromatin packaging proteins [30]. Nowadays epigenetics is considered as an emerging field to investigate normal and pathological conditions. DNA methylation and histone modifications are two important epigenetic biomarkers, which can affect gene expression profile in cancer cells. The other epigenetic mechanism, which can play a remarkable role in gene expression, is the presence of microRNAs (miRNAs) [31]. This small non-coding RNAs can stop protein production by interruption of their expression in a specific manner [32-34]. Among all this mechanisms, DNA-methylation is highly investigated as a tumor biomarker. Hyper-methylation of specific sites of promotors known as CpG islands can change gene expression or lead to gene suppression. Such process has been shown to occur commonly in many of cancer cells and can be an ideal choice for their early detection [35,36]. Until now, many studies have shown the association of lung cancer and DNA methylation in various gene promoters [37-42]. Detection of such methylations in cancer related genes not only has a diagnostic importance but also can provide valuable information for early detection of lung cancer. In following, several most common genetic based lung cancer biomarkers are briefly discussed.

3.1.1 P53

P53 gene is responsible for encoding a tumor suppression protein involved in different cell processes including, apoptosis, DNA repair and cell-cycle control [43]. The association of mutant P53 with survival and recurrence of NSCLC was exhibited in several experiments [44-46]. Moreover, accumulation of mutant P53 was seen in lung cancer cells. Several P53 point mutations were found and evaluated as prognostic factors in NSCLC cases [47,48]. Based on experiments, more than half of the lung cancer cases showed a deviated form of P53 expression with a histological subtype dependency [49].

3.1.2 Epidermal growth factor receptors

The epidermal growth factor receptors (EGFR) are transmembrane receptors from receptor tyrosine kinase superfamily. Upon binding an extracellular ligand to EGFR, they form homo/heterodimers and start signaling cascades, which finally result in cell growth and metastasis [50]. Mutated forms of EGFRs lead to their constant activation and consequently uncontrolled cell growth. Researches have shown the presence and overexpression of mutated

forms of EGFR in about 62% of NSCLC cases [51,52]. Moreover, in East Asia, a linkage was found between EGFR mutations and about 50%-70% of lung cancers cases [53]. Currently, searching for EGFR mutations is carrying out to isolate stage IV of NSCLC in order to guide the drug administration [54].

3.1.3 Ras genes

K-ras, H-ras and N-ras are three members of a gene family known as Ras, responsible for encoding proteins, which play significant roles in signal transduction and cell proliferation. With considering their role in transporting exterior signals to nucleus, mutated forms of Ras genes can cause an uncontrolled signal transporting into nucleus which finally lead to an increase in cell growth and proliferation [55]. Ras gene mutations are present in around 25% of all tumors and about 30% of NSCLC cases [56]. A great majority of mutations are occurring in codon 12 or 13 of exon number 2 in K-ras gene [57]. Other investigations displayed a strong link between K-ras mutations and 78% of lung cancer cases. Additionally, circulating K-ras mutant proteins were shown to be important prognostic and predictive markers in lung cancers [58].

3.1.4 Micro RNAs

MicroRNAs are 19 to 25 nucleotides RNA molecules with important roles in gene silencing. The action of these small molecules is to block or degrade mRNA molecules and finally suppress their expression [59]. These molecules have been shown to be significant players in cell growth as well as cancer prognosis [60,61]. Their association with different pathological parameters have been revealed in various cancers, suggesting the potential of these small molecules as cancer biomarkers [62,63]. Up and down regulation of different microRNAs in lung cancer cases were identified by several meta-analyses. For instance, in one investigation a higher expression of miR-21 was observed in NSCLC cases with 100% specificity and 70% sensitivity [64]. Other experiments assessing different microRNAs also showed 92 to 100% specificity and 75 to 85% sensitivity in distinction between lung cancer and normal cases [65,66]. The presence of microRNA molecules in body fluids indicates their potential as biomarkers for cancer diagnosis.

3.2 Protein based biomarkers

Although there are various recognized protein biomarkers in lung cancer, their presence and particularly their level of expressions showed a significant fluctuation among different lung cancer subtypes. For instance, carcinoembryonic antigen (CEA) seems to be more specific in NSCLC while other biomarkers such as neuron-specific enolase (NSE) revealed a high specificity for SCLC [49]. Such dependency on histological subtypes is also shown for other biomarkers in lung cancer cases [67]. Among this category, both CEA and NSE are the important investigated lung cancer biomarkers [68,69]. Other highly expressing proteins in lung cancer are including haptoglobin (Hp), annexin II, cytokeratin 19 fragment and enolase 1 (ENO1) [70-72]. Regardless of such highly expressed proteins, each of them merely cannot be a specific indicator of lung cancer and their detection in a combinatorial manner would be a superior choice in lung cancer diagnosis [70]. To be exemplified, by considering a combination of biomarkers including CEA, R1-antitrypsin (AAT), retinol-binding protein (RBP) and squamous cell carcinoma (SCC) researchers successfully detected lung cancer cases with a high sensitivity and specificity [73].

3.2.1 Carcinoembryonic antigen (CEA)

CEA is a glycoprotein with a 2.5 to 5 ng/ml^{-1} concentration in healthy individuals. Higher levels of CEA were shown to be ideal indicators of lung cancer [74-76]. In one comparative study in lung cancer cases the level of CEA was 7 ng/ml^{-1} while in normal cases it was around 3.8 ng/ml^{-1} [77]. In addition, CEA level was exhibited to be much higher in heavy smokers. Some other investigations revealed a strong connection between survival time in NSCLC and CEA levels. According to other investigations, the higher serum level of CEA can be a good indicator for poor post-surgery prognosis. In one experiment, serum amount of CEA was found to be more than its standard levels in 61.9% and 75 % of NSCLC and stage IV lung cancer patients, respectively [78]. Currently for a better prediction, CEA levels is considered along with other markers such as CA 15-3, CCAT2 and AFP [79,80].

3.2.2 Neuron-specific enolase (NSE)

Neuron-specific enolase (NSE) as the other common lung cancer biomarker was found to be higher in serum of individuals suffering from SCLC. This 78 kDa glycolytic enzyme is a dominant enzyme found in neuronal tissues. The presence of this enzyme in serum was found

to be associated with some disorders comprising neuronal destruction [81]. Despite of neurodegenerative disorders, tracking the presence and amounts of NSE in serum also can help for diagnosis of SCLC. Currently NSE is considered as one of the most reliable biomarkers with a high diagnosis ability in SCLC cases [81]. Overexpression and enhanced levels of NSE (over than 9 ng/mL^{-1}) was observed in 70% of SCLC cases. Similarly, in advanced levels of SCLC, about 90% of patients demonstrated higher levels of NSE at diagnosis stage. Although tracking of the NSE as a marker in SCLC is sufficient for diagnosis, with the intention of a reliable detection in NSCLC, it is better to consider other biomarkers such as CEA and CYFRA 21-1 along with this enzyme [82].

3.2.3 CYFRA 21-1 (Cytokeratin 19)

Cytokeratines, particularly fragment 19, as an epithelial tissue-protein, is considered as an ideal candidate in carcinoma detections. This 40 kDa human cytokeratin is found in almost all tested adenocarcinomas and showed more sensitivity and specificity compared to other biomarkers [83]. The role of this protein as a marker for diagnosis of NSCLC was also shown in a recent experiment [84]. The normal serum level of cytokeratin 19 is approximately 3.3 ng/ml^{-1} and in lung cancer diagnosis, it is usually checked along with some other biomarkers [85].

3.2.4 Annexin A2 (ANXA2)

Annexin A2, a 36 kDa protein, is identified as a novel and efficient biomarker in lung cancer. The role of this protein which is usually expressed in macrophages and endothelial cells is to control membrane trafficking and endocytosis. Lately, overexpression of this protein was observed in different cancers especially lung and liver. Its significant increasing in early stages of lung cancer led to its reputation as a lung cancer biomarker. In one recent investigation, a higher level of ANXA2 was seen in serum of lung cancer patients when compared to healthy individuals [86]. In another experiment, combinatorial detection of ANXA2 and heat shock protein 60 (HSP60) as the other new lung cancer biomarker exhibited a significant improvement in early diagnosis of lung cancer [87].

3.2.5 Serum amyloid A1 (SAA1)

This protein is considered as one of the acute phase proteins with high expression levels during injuries, infections and different harsh situations. Since this protein is the main precursor of amyloid A, different experiments confirmed the biomarker potential of SAA1 in inflammation situations. Other experiments showed higher levels of SAA1 in patients with lung cancer. In one experiment after evaluation of 350 healthy and patient's sera and plasma samples, the levels of SAA1 were found to be 14 times more in samples of patients with lung cancer [88]. High levels of this protein were also shown in different cancers including breast [89], endometrial [90], prostate [91] and uterine serous papillary[92] cancers which indicate the great potential of SAA1 in early detection of such disorders.

4. Biosensors for lung cancer biomarker detection

Currently different biochemical and immunological based methodologies are developing for detection of cancer biomarkers especially in the early stage of the disease. Some of these conventional approaches have no sufficient sensitivity in detection of biomarkers. Other methods such as optical immunoassay techniques, which are dominant in biomarker detection area can provide a reasonable selectivity and sensitivity; however they are usually time consuming, complex and expensive [93-97]. In this regard, developing a potent strategy to detect slight amounts of biomarkers, especially in early stages, is of utmost importance in cancer diagnosis and treatment. Biosensor is a system applying a biological element, from a small molecule such as an enzyme to a whole living cell, to detect the presence of a specific compound [98-102]. In a biosensor, upon interaction of the biological element with the target molecule in sample, transducer part will convert the interaction response to a quantifiable signal. The signals then will be amplified, processed, and measured via electronic part. Sensitivity, selectivity and reliable results using a time saving and flexible platform made biosensors of interest in biomarkers detection and cancer diagnosis. Generally, biosensors can be categorized as optical, electrochemical and mass-based [103,104]. Various types of biological agents, matrices and transducers can be employed in each type of biosensors. Figure 1 shows a schematic representation of different biosensors and their different parts. Among biosensors, electrochemical ones with a volumetric transducer displayed a great potential in detection of lung cancer biomarkers [13]. Their ability to detect multiple markers simultaneously as well as having a high sensitivity and lower detection limit placed them at the top of the biosensors' list [105,108]. In the following, some recent developed biosensors for detection of various lung cancer biomarkers are mentioned.

4.1 Electrochemical biosensors

In electrochemical biosensors, signal transduction is based on an electrochemical reaction, which is happening at an electrode surface. Occurrence of an electrochemical reaction at the electrode surface upon interaction with target molecule, will results in a change in the electrical signal and the monitoring of such changes is the basic procedure of an electrochemical biosensor. So far, different electrochemical biosensors have been fabricated and improved for sensitive detection of various lung cancer biomarkers [96,109]. Recently Huinan Ye et al. developed an aptameric electrochemical biosensor (Aptasensor) with aim of vascular endothelial growth factor (VEGF165) detection in lung cancer samples. In this biosensor, using an Au-Pd (AP) alloy/ionic liquid, specific DNA aptamer molecules were immobilized on a glassy carbon electrode for direct transformation of target-aptamer induced conformational changes to measurable signals [110]. The developed system successfully showed a linear relationship with different concentrations of VEGF ranging from 5 to 200 pM. Successful determination of VEGF in serum of patients with lung cancer indicated the potential of this aptasensor for lung cancer diagnosis. According to the authors, utilizing AP with combination of an ionic liquid led to an enhancement in sensitivity and stability of the biosensor. In another attempt for detection of VEGF 165 in lung cancer samples, a research group developed an aptasensor using an electrode, which was modified by gold and mesoporous nanoparticles [111]. The strategy was to detect the changes in interfacial properties of the electrode upon interaction between immobilized anti-$VEGF_{165}$ aptamer and $VEGF_{165}$ in samples (Figure 2). The developed aptasensor showed a great performance in ultra-trace level detection of VEGF in lung cancer serum samples.

Another interesting target for detection of lung cancer via electrochemical biosensors is EGFR. Checking the mutation status in EGFR exon 19, in samples of NSCLC patients who are receiving therapies based on tyrosine kinase inhibitors, can be helpful in guiding their treatment. In a recent experiment, a sandwich-type electrochemical biosensor was developed for detection of such mutations in EGFR gene [112]. DNA probes as the bio recognition elements were prepared based on nucleotides around exon 19 deletion parts in EGFR gene. In this experiment λ exonuclease was used for conversion of dsDNA to ssDNA molecules in real NSCLC samples. Employing this enzyme was shown to be far efficient than other sample denaturalization strategies using high temperature or urea. The developed λ

exonuclease assisted DNA biosensor was able to detect and distinguish EGFR mutations in PCR amplified samples (Figure 3).

Since last decade, MicroRNAs attract many attentions as fascinated biomarkers in early diagnosis of many cancers [113]. MicroRNAs aberrant expression in lung cancer cells is the reason for development of various biosensors for their detection in early stage of the disease [114]. For instance, Lin et al. recognized 11 miRNAs in plasma of individuals, which showed a remarkable alteration among patients with benign and malignant pulmonary nodules [115]. In a very recent experiment, Liu et al. developed an electrochemical based biosensor employing a 3D DNA origami for detection of mir-21 in lung cancer samples [116]. The developed biosensor was composed of a top and a bottom part. At the top, there was a ferrocene tagged DNA with a stem-loop structure responsible for hybridization with the target microRNA and at the bottom a thiolated tetrahedron DNA assembled on a gold modified electrode surface. Having a good linearity with different concentrations of microRNA and a good detection limit (10 pM) represent the potential of this biosensor for its clinical application in cancer diagnosis.

Electrochemical immunosensors, utilizing an immunological agent such as an antibody as the bio-recognition agent, also are of interest in cancer biomarker detection. Taking advantages of specificity and sensitivity of antigen-antibody reactions make immune-based biosensors attractive in biomarker detection. Recently Yu Li et al. developed an immunosensor using an electrode surface with presence of AuNPs and modified graphene (GR) [117]. The immunological part of the biosensor was a monoclonal antibody aimed for capturing the CEA (anti-CEA) which is one of the recognizable biomarker not only in lung cancer but also in other types such as breast and pancreatic cancers [69]. Employing the Au was due to its unique ability in electron transfer enhancement and increasing the amounts of immobilized antibodies on electron surface. On the other hand, for improving the AuNPs immobilization as well as increasing in graphene hydrophilicity, they modified GR using poly ethylene glycol (PEG) molecules. The modified GR sheets displayed a good electrical character as shown by electrochemical determination. Having a linear relationship for different CEA concentrations in the range of 0.1 to 1000 ng mL^{-1}, and 0.06 ng mL^{-1} as the limit of detection, proposed the meaningfulness of this impedimetric immunobiosensor for detection of CEA in clinical samples. There are various significant reports in design and development of electrochemical biosensor in lung cancer biomarker detection. Some of the new reports are summarized in Table 1. One important factor in fabrication of such

biosensors might be the electrode surface and its stable functionalization. For stabilization of biological agents on electrode surfaces which bring some difficulties, researchers are employing different methodologies. However, regardless of some shortcomings and fabrication complications, electrochemical biosensors still considered as preferred ones in cancer biomarker detection.

4.2 Optical biosensors

Apart from electrochemical biosensors, various types of optical biosensors have been fabricated for detection of biomarkers in lung cancer. In an optical biosensor, the presence of analyte will tested using a bio recognition element along with an optical transducer [118]. As the most investigated type of optical biosensor, surface plasmon resonance (SPR) based biosensors are responding to refractive index changes caused by analyte-biological agent interaction at the surface of sensor [119]. Fluorescence based detection techniques (FRET), chemiluminescence resonance energy transfer (CRET), chemiluminescence (CL) and surface-enhanced Raman scattering (SERS) are other techniques which exploited in fabrication of optical biosensors. There are many reports in using optical biosensors in biomarker detection in lung cancer samples. Recently Lee et al. described a localized SPR (LSPR) biosensor, in which a nanoporous anodic aluminium oxide (AAO) chip was designed to detect serum amyloid A1 (SAA1) as a lung cancer biomarker [120]. In this experiment, anodic aluminium oxide was used as the sensing platform because of its stability, biosafety, chemical resistance and having a large surface area. Upon entrance of antigen into chip pores and its interaction with immobilized antibodies, changes in refractive index of the microenvironment sensed via a sensor chip. In this sensor, the detection limit was 100 ag/mL and AAO chips showed a great clinical potential through highly sensitive detection of SAA1 in samples [120]. More recently, Chiu et al. demonstrated a carboxyl-functionalized graphene oxide based surface plasmon resonance sensing chip system for the label free determination of lung cancer (non-small cell lung carcinoma) via the detection of cytokeratin 19 (CK19) biomarker in spiked human plasma sample [121]. In this immunosensor, the authors immobilized an extremely low amount of cytokeratin 19 specific antibody on sensing chip. The reported sensor showed high sensitivity and specificity (Figure 4).

In another recent report, an ultrasensitive FRET based biosensor was developed using upconverting nanoparticles (UCPs) and palladium NPs (PdNPs) for detection of CEA in lung cancer serum samples [122]. In this biosensor, CTA aptamer molecules were conjugated into

modified UCPs and then a close proximity was obtained between UCPs and PdNPs via interactions of nitrogen groups in aptamer molecules and PdNPs. This event led to fluorescence quenching of UCPs. With presence of CEA in environment, preferential interactions between aptamer and CEA molecules resulted in an interruption in aptamer and PdNPs interactions. Finally, this led to fluorescence recovery of UCPs with a CEA concentration dependent manner. This system demonstrated a good linear relationship in range of 4 pg/mL to 100 pg/mL of CEA concentration in human serum with detection limit of 1.7 pg/mL [122].

Detection of biomarkers in soft matters such as biopsied cancer samples remains as a big challenge. Biosensors can display a great performance in this context, too. In a recent interesting report, an SPR based biosensor was developed for detection of cytokeratin 17 protein (CK17) as a biomarker in lung cancer biopsy samples [123]. In this biosensor, anti-CK17 antibody molecules were immobilized on a biocompatible polymer optical fiber. Afterward, to check the penetration ability of immunosensor into soft matter, experiment was performed on a CK17 containing gel matrix as a mimic for soft tissue. The recorded SPR signal out of gel matrix confirmed the ability of fabricated immunosensor in penetration and detection of CK17 in soft tissue samples. Further, for more validation, with testing the immunosensor in real lung cancer biopsy samples, the results again proved the ability of this biosensor in ex-vivo detection of CK17 in real tissue samples. Such investigations can be an optimistic start for developing biocompatible biosensors for non-invasive in vivo detection of cancer biomarkers. Other recent developed optical biosensors for detection of lung cancer biomarkers are listed in Table 2.

4.3 Mass based (piezoelectric biosensors)

Identification of an analyte with detection of changes in mass is the basic of mass based biosensors. The piezoelectric crystal in these biosensors has a specific frequency oscillation in an applied electric field. This frequency can be changed by any variation in crystal mass or electrical frequency. In these biosensors, upon interaction of an immobilized biorecognition molecule on the crystal with the target analyte, mass of the crystal and consequently the oscillation frequency will be changed. Afterwards, the changes will be measured to provide an exact concentration of an analyte in samples. This principle is built on Quartz crystal microbalance (QCM) which provides a sensitive biosensing method for detection of even one point mutation in a specific gene [124]. Among employed crystals, quartz crystals are the most sensitive and thus the most common piezoelectric crystals in development of mass

based biosensors. A QCM based immunosensor was described by Chen Y. et al. for rapid detection of lung cancer in its early stages [125]. After immobilization of the lung carcinoma cells on the crystal surface, and dripping the serum sample from immunized rabbits on the surface, changes in mass and resonant frequency caused by the cell-antibody interactions were measured. The results provide a rapid and sensitive detection of antibody content at nanogram levels. In another study, the sensitivity of QCM biosensor in biomarker detection was shown to be equal to an SPR based biosensor. In this experiment, both SPR and QCM biosensors were developed for detection of single nucleotide polymorphism (SNP) in TP53 gene in lung cancer samples by means of a specific DNA molecule. The results showed a highly sensitive hybridization reaction in the same range (0.03 – 2 μM) for both biosensors [124]. Other developed QCM immunosensors with a similar procedure also revealed their applicability in this area [126-128]. So far, various mass based biosensors were reported for detection of different cancer biomarkers such as breast cancer 1 gene (BRCA-1) gene mutants [129], hormone-related peptide (PTHrP) [130], ($p16^{INK4a}$) [131], HER-2 [132] and PSA [133]. However, there are just a few reports in literature applying these type of biosensors in recognition of biomarkers in lung cancer samples.

5. Conclusion and future outlook

Advances in comprehensive molecular characterization of cancer carcinomas elucidate that disease biomarkers play crucial role in prognosis, diagnosis and providing better understandings of cancer etiology. The normal ranges of a large variety of tumor markers in body fluids are at ng ml^{-1} and their potential use in healthcare practices depends largely on their detection with enough sensitivity and specificity. Very early stage detection of lung cancer associated biomarkers is very is crucial to better understanding the wide spectrum of pulmonary malignancies and to provide efficient medical care for recuperating and recovery of patients [134-143]. In this review, the rapid progresses of biosensor technology for lung cancer biomarkers detection were summarized. These sensing strategies may have great effect in the near future upon routine clinical analysis of biomarkers of pharmacogenomics and pharmacogenetics interest. Further advancements in nanobiotechnology, with its nanobiocomposite and miniaturization approaches will significantly enhance our current biodiagnostic capacity to detect cancer biomarkers in real biological samples with enough sensitivity, selectivity, robustness and cost effectiveness.

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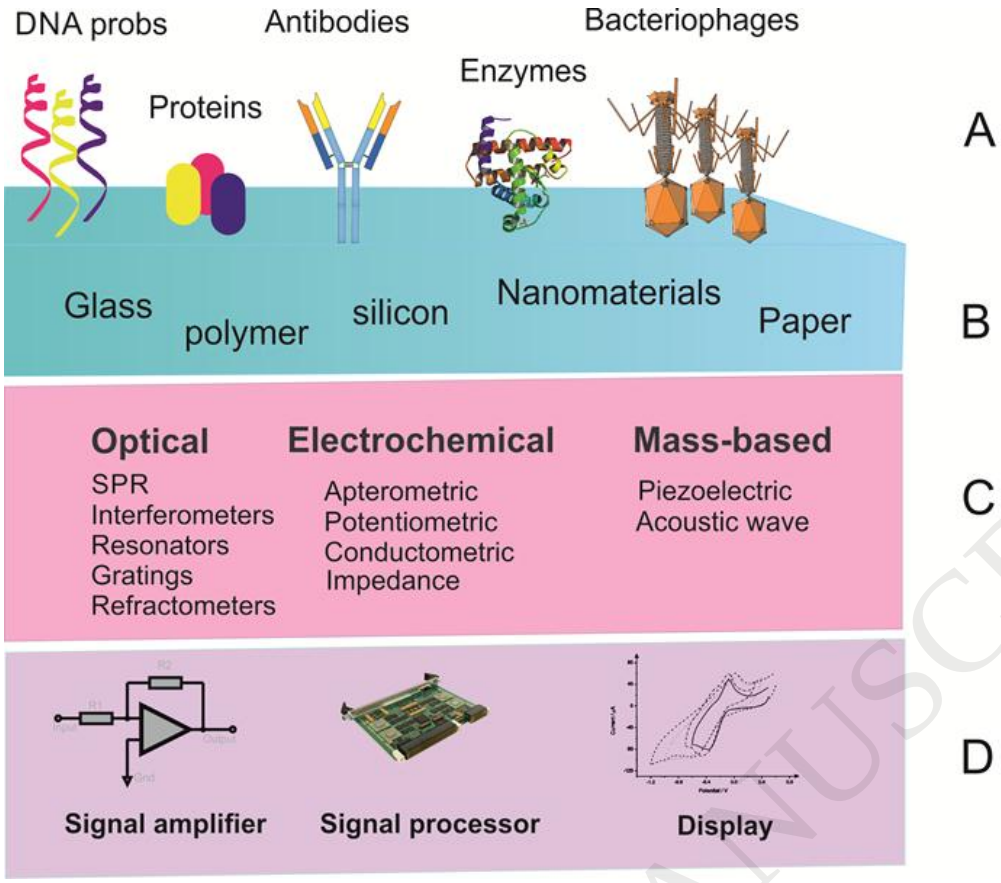
Figure captions

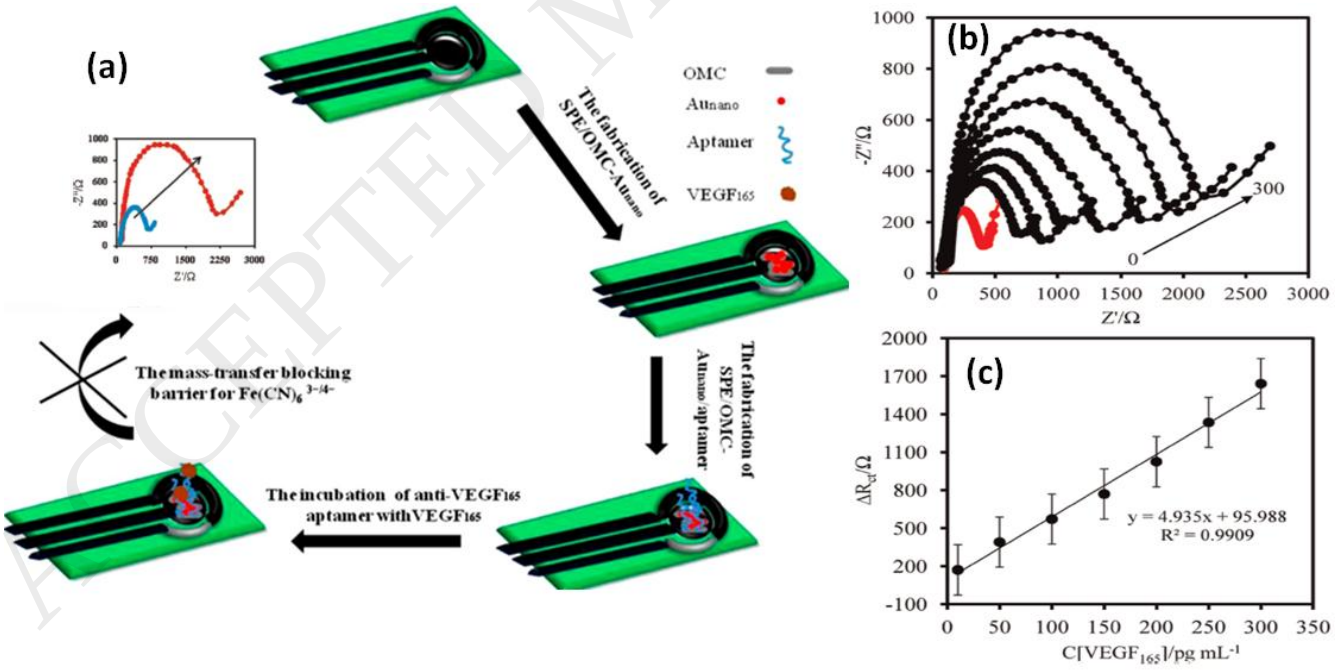
Fig.1 shows schematically the different parts of a biosensor: (a) biorecognition molecules (b) matrixes (c) transducer types and (d) electronic part.

Fig. 2: (a) Fabrication of Electrochemical aptasensor for the determination of vascular endothelial growth factor in serum of lung cancer patient (b) Electrochemical impedance spectra for detection of $VEGF_{165}$ in a concentration dependent manner (c) Calibration curve for ΔRct versus concentration of $VEGF_{165}$. Reproduced from Tabrizi et al. [111] with permission from Elsevier.

Fig. 3: Fabrication of sandwich-type electrochemical DNA biosensor for the determination of Epidermal growth factor receptor in tumor tissue of lung cancer patients. (a) Amperometric response of wild and 6 types of deletion for (b) pair C/R, (c) pair R, and (d) pair C. (e) The signal ratio of deletions to wild-type target with the same concentration of 10 nM. Reproduced from Xu et al. [112] with permission from Elsevier.

Fig. 4: (a) Schematic representation of surface modification of covalent links to carboxylic acid functionalized graphene oxide. (b) NHS/EDC reaction and covalent attachment of cystamine (Cys) as a linker on the GO-COOH sheet and on the surface of gold chip. (c) Immobilization of the biomarker anti-cytolerayin-19 (anti-CK19) antibody on the GO-COOH-based SPR chip. (d) Estimation of the detection of low concentration of CK19 protein in spiked 10% human plasma for the GO-COOH-based SPR chip. (e) Calibration curves for the detection of different concentrations of CK19 protein in PBS buffer and spiked 10% human plasma. Reproduced from Chiu et al. [121] with permission from Elsevier.





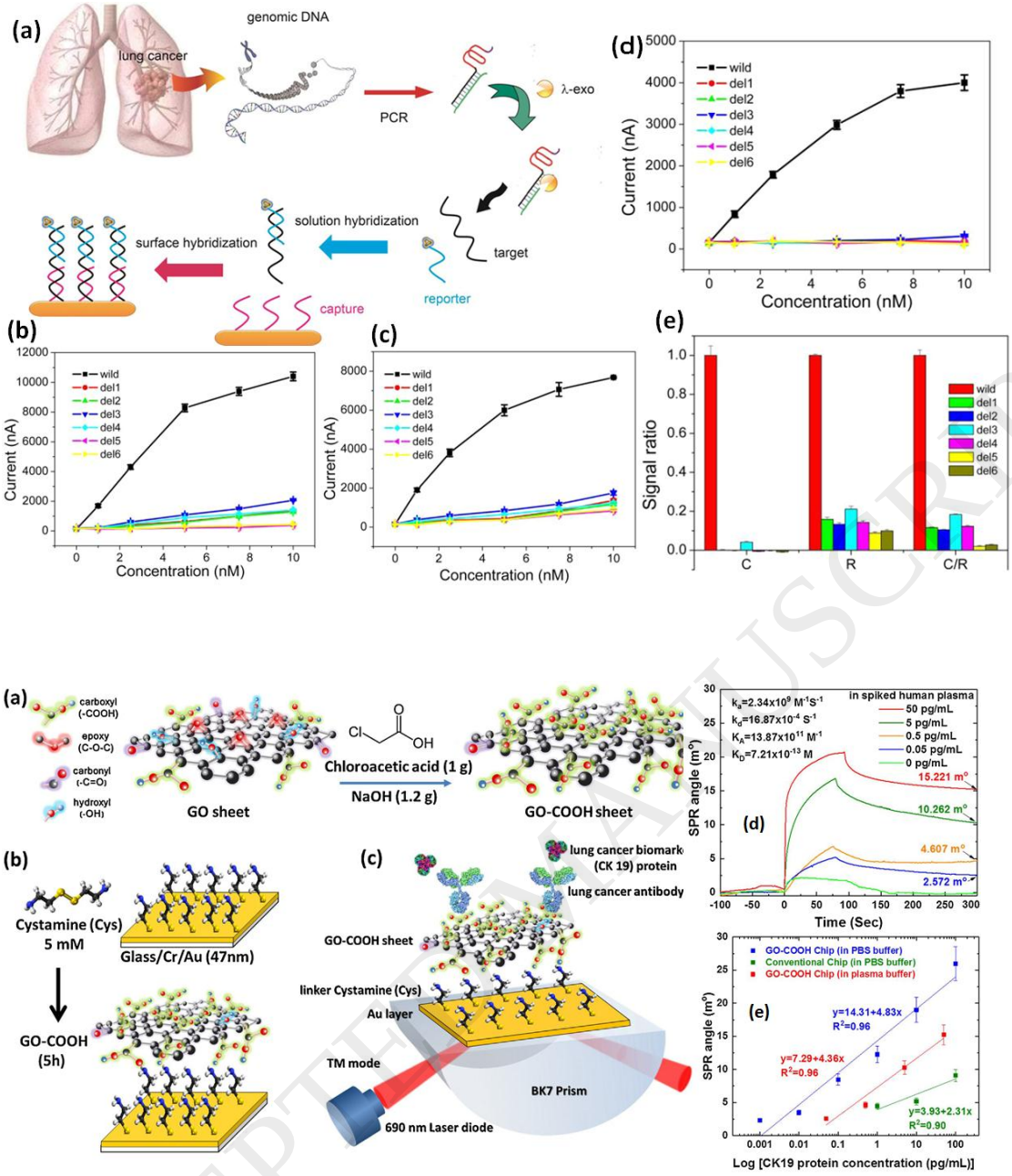


Table captions

Table 1: Lung cancer biomarkers and their conventional detection methods

Table 1

Category	Biomarker	Conventional detection method
Genetics and epigenetics biomarkers	❖ Mutations, rearrangements, amplifications or deletions in genes: Epidermal growth factor receptors (c-ErbB-1, c-ErbB-2), K-ras and p53 mutant, FHIT, COX2, RASSFIA, MET, Her2, BRAF, PIK3CA, RET, PTEN, IL-8 mRNA ❖ DNA hyper-methylation of genes: SHOX2,CDKN2A,RASSF1A,APC,RASSF1A,APC ESR1,HOXA9,CDH13 ❖ miRNAs: miR-205,miR-210,miR-708,miR-486,miR-21,miR-200b, miR-375, miR-137	• Reverse transcription polymerase chain reaction assays (qRT-PCR) • Multiplex ligation-dependent probe amplification (MLPA), • Single-strand conformation polymorphism (SSCP), • Restriction fragment length polymorphism (RFLP), • Denaturing gradient gel electrophoresis (DGGE), • Methylation-sensitive restriction enzyme digestion, • Differential methylation hybridization (DMH), • TaqMan Low Density Arrays (TLDA), • Methylation-specific PCR (MSP), • GeneChip miRNA Array, • Microarray techniques, • Heavy Methyl qPCR • Nested MSP • Pyrosequencing • qRT-PCR
Protein based biomarkers	CEA, NSE, CYFRA 21-1, vascular endothelial growth factor (VEGF), Haptoglobin-R 2, KLKB1, R-enolase (ENO1), APOA1, chromogranin A, TPA, bombesin-like gastrin-releasing peptide, cytokeratin-7, tumor M2-pyruvate kinase, nitrated ceruloplasmin, CD34 and CD59 glycoproteins, transthyretin (TTR), Cytokeratin 17 and 18, GM2 activator protein (GM2AP), ProGRP, carbohydrate antigens 19-9, 125 and 15-3 (CA 19-9, CA 125, CA 15-3), Annexin II, R-1-acid glycoprotein, Protein gene product 9.5 (PGP 9.5),	• SDS-PAGE • Phage Display • Mass spectroscopy, • Protein truncation test (PTT), • Chromatographic techniques, • Immunohistochemistry (IHC), • Protein array-based technology • Enzyme linked immunosorbent assay

Table 2: Recent biosensors for lung cancer biomarker detection.

Table 2.

Biosensor	Transducer type	Biomarker	Ligand	Sample	Linear range	Detection limit	Reference
Electrochemical	Voltammetric	mir-126	DNA probe	Serum	-	0.1 fM	[82]
		CEA	Anti CEA antibody	Serum	-	1 fg/ml	
	Impedimetric	CEA	Anti CEA antibody	Serum	0.1 to 1000 ng mL^{-1}	0.06 ng mL^{-1}	[103]
	Voltammetric	NSE	Anti NSE antibody	Serum	0.1 to 2000 ng mL^{-1}	0.05 ng mL^{-1}	[119]
	Voltammetric	VEGF165	DNA aptamer	Serum	1 to 150 pM	0.5 pM	[99]
	Amperometric	EGFR	DNA probe	saliva	0.5 to 500.0 nM	0.167 nM	[120]
	Amperometric	EGFR	DNA probe	Tissue	-	-	[101]
	Voltammetric	miR-21	2D stem-loop DNA structure combined with 3D DNA origami	Serum	100 pM to 1 µM	10 pM	[102]
	Impedimetric	VEGF165	DNA aptamer	Serum	10.0 to 300.0 pg mL^{-1}	1.0 pg mL^{-1}	[100]
Optical	SPR	CEA, EGFR	Anti-CEA and anti-GFR antibodies	Serum	-	-	[121]
	SPR	CK17	Antibody	Tissue	-	10^{-10} g/mL	[108]
	FRET	CEA	DNA aptamer	Serum	4 to 100 pg mL^{-1}	1.7 pg mL^{-1}	[107]
	localized SPR (LSPR)	Amyloid A1	Antibody	Serum	-	100 ag mL^{-1}	[106]
	SERS	Adenosine	-	Urine	0.5 nM to 5 mM	0.1 µM	[122]
	CL	VEGF	DNA Aptamers	Serum	-	60 pg mL^{-1}	[123]
	CL	CEA	Antibody	Serum	100 pg mL^{-1} to 1,000 ng mL^{-1}	20 pg mL^{-1}	[124]