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Electrochemical Aptasensor for Detection of Lung Cancer Tumor Markers in Blood Plasma

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Abstract. The study investigates the possibility of diagnosing lung cancer through the simultaneous detection of six tumor biomarkers of this disease (CYFRA21-1, EpCAM, PLAUR, LIFR, ProGRP, and CEA) in blood plasma. The levels of these proteins in blood plasma were determined using square-wave voltammetry with multiplexed electrochemical chips modified with DNA aptamers. The results obtained in the study demonstrated the capability of the multiplex electrochemical aptasensor to simultaneously detect in blood plasma such traditionally used lung cancer tumor markers as CYFRA21-1, EpCAM, PLAUR, LIFR, ProGRP, and CEA.

Keywords: aptamers, lung cancer, electrochemical aptasensor, blood plasma tumor biomarkers.

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Introduction

Lung cancer (LC) ranks among the leading causes of cancer-related death globally, accounting for approximately 28% of all cancer-related deaths [1, 2]. With the limited effectiveness of existing LC diagnostic methods, the task of improving overall patient survival remains highly challenging [3, 4]. Currently, most diagnoses are made at stage IV, making the prognosis extremely unfavorable. Only 26.5% of cases are detected at stages I-II, while stages III-IV are diagnosed in 66.4%. The 5-year survival rate after treatment for stage I lung cancer is 70%, while for stage IV it drops below 5% [1]. Therefore, early diagnosis is a crucial factor in reducing mortality and improving survival for LC patients [5]. The application of low-dose computed tomography (LDCT) has reduced LC mortality by 20% [6]. However, the high false-positive rate, reaching 90%, necessitates the use of additional methods to confirm the diagnosis [7]. Current methods include biopsy and repeated LDCT, each placing an additional burden on both financial resources and the patient's body [4]. During the development of cancer, various markers appear in the patient's blood — tumor breakdown products, tumor cells and microemboli, signaling molecules, etc., indicating the appearance and growth of a malignant neoplasm in the body. Assessing the levels of such circulating biomarkers is one of the most sought-after areas of modern diagnostic oncology, as it does not require invasive methods of intervention in the human body. Therefore, blood is one of the most convenient materials for cancer diagnosis [8]. Unfortunately, most tumor biomarkers used in clinical practice have low diagnostic significance. To date, no cancer marker has been found with 100% sensitivity and specificity [9]. Moreover, sometimes people without cancer exhibit levels of tumor markers that are typical for cancer [10, 11, 12, 13]. Therefore, the simultaneous determination of multiple tumor markers is crucial for LC diagnosis.

Recently, DNA aptamers have emerged as recognition molecules for tumor markers. These aptamers are single-stranded RNA or DNA fragments (ranging in length from 30 to 100 nucleotides) that can form three-dimensional structures through interactions between complementary regions of the chain. The spatial organization of charged phosphate groups and unpaired bases within the oligonucleotide creates a unique distribution of functional groups. These groups are capable of electrostatic and van der Waals interactions, as well as hydrogen bonding with functional groups of the molecular target, leading to the high specificity of aptamer binding [14].

This study employed a multiplex electrochemical sensor based on DNA aptamers for lung cancer diagnosis. This sensor enables the simultaneous detection of six proteins associated with lung cancer in blood plasma: cytokeratin (CYFRA21-1), epithelial cell adhesion molecule (EpCAM), urokinase plasminogen activator receptor (PLAUR), leukemia inhibitory factor receptor (LIFR), pro-gastrin-releasing peptide (ProGRP), and carcinoembryonic antigen (CEA). According to literature [13], the determination of these onco-markers in lung cancer patients allows for the assessment of tumor progression and metastasis levels. This study aimed to evaluate the ability of the multiplex electrochemical sensor based on DNA aptamers to simultaneously detect six biomarker proteins of lung cancer (CYFRA21-1, EpCAM, PLAUR, LIFR, ProGRP, and CEA) in the blood plasma of patients and identify the most sensitive markers.

Materials and methods

The aptamer to EpCAM was previously obtained by our team [15], while the aptamers targeting the biomarker proteins CYFRA21-1, PLAUR, LIFR, ProGRP, and CEA were developed by the authors of this article within the framework of the Federal Target Program "Research and Development in Priority Areas of Scientific and Technological Complex Development for

2014-2020," using a modified SELEX method [16].

Multiplex electrochemical chips were employed for the construction of the electrochemical aptasensor. The chips consisted of six sensing electrodes and two auxiliary electrodes [17]. These multiplex electrochemical chips were three-layered electrodes made of copper, nickel, and gold, with linear dimensions of 11×35 mm (see Fig. 1).

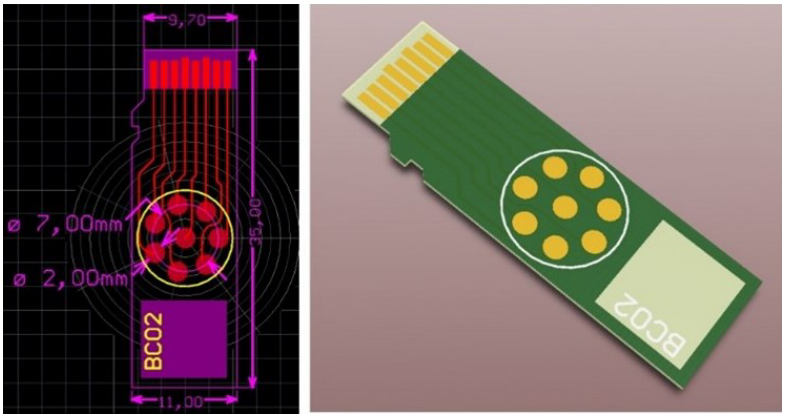

Fig. 1. Schematic of a Multiplex Electrochemical Chip with Immersion Gold

The electrode was fabricated on a surface of textolite, a material used in printed circuit board manufacturing. A gold layer was deposited onto nickel using a chemical method. Nickel served as a barrier to prevent interdiffusion of gold and copper. All electrodes, except for the working surfaces in contact with the blood plasma under investigation, were coated with an electrically insulating layer of varnish. Electrode surface preparation, aptamer modification, incubation with blood plasma samples, and subsequent measurements were performed according to protocols outlined in the article [17]. Electrochemical measurements were conducted using a CH-600 electrochemical workstation (CH Instruments, USA). Studies on blood plasma from lung cancer patients and conventionally healthy individuals were performed using square-wave voltammetry under standard laboratory conditions at room temperature. Each blood plasma sample was analyzed three times, and the data was then averaged.

To obtain samples suitable for investigation, 500 μL of venous blood was collected from each participant and centrifuged to remove cellular components. The determination of biomarker proteins was conducted in the blood plasma. Blood samples were collected from patients with lung adenocarcinoma and conventionally healthy individuals. This study was approved by the Krasnoyarsk State Medical University Local Ethics Committee (Resolution No. 37/2012 of 31.01.2012).

Results and discussion

A panel of various tumor markers commonly used for lung cancer diagnosis was employed in the development of the electrochemical aptasensor. This panel included the markers CYFRA21-1, LIFR, PLAUR, ProGRP, EpCAM, and CEA, all of which play significant roles in tumor progression and metastasis.

Cytokeratins CYFRA 21-1 are protein components of intermediate filaments in epithelial cells [18]. Their levels in the serum of lung cancer (LC) patients are particularly elevated at the metastatic stage of the disease [19].

Leukemia inhibitory factor receptor LIFR (CD118) is a multifunctional protein that influences cell differentiation, survival, and proliferation. It functions as a marker of metastasis [20].

Urokinase plasminogen activator receptor PLAUR (CD87) acts as a biomarker for non-small cell lung cancer and is used to assess patient survival prognosis. The primary biological role of this serine protease lies in the activation of plasminogen, which contributes to the degradation of the extracellular matrix surrounding cells. By participating in the proteolytic degradation of the extracellular matrix, PLAUR initiates processes of tumor invasion and metastasis. The expression of this protein is enhanced by cytokine stimulation [21].

High levels of pro-gastrin-releasing peptide ProGRP are observed in patients with lung cancer (LC), while healthy individuals, those with non-malignant diseases, and those with other types of cancer exhibit negligible levels of this protein in blood plasma. The use of ProGRP as a marker for routine investigations is limited by its instability in blood and the complexity of its isolation [22].

Epithelial cell adhesion molecule (EpCAM), a reliable biomarker for lung cancer (LC), is expressed in epithelial tissues and epithelial neoplasms. The oncogenic potential of EpCAM is realized through the molecular cleavage into domains, leading to the release of the extracellular domain surrounding the cell and the translocation of the intracellular domain to the cytoplasm. There, it forms a complex with FHL2, β-catenin, and Lef proteins in the nucleus, resulting in DNA binding and enhanced transcription of various genes. This promotes the overexpression of c-myc, e-fabr, cyclins A and E, ultimately stimulating tumor growth. The level of EpCAM expression correlates with the proliferative activity of cells [15]. EpCAM plays a crucial role in cell signaling, migration, proliferation, and differentiation. It also regulates epithelial-mesenchymal transition and contributes to tumor metastasis [23].

Carcinoembryonic antigen (CEA) is one of the embryonic antigens associated with embryonic development. High levels of CEA in serum are associated with metastases to the brain and spinal cord [24]. CEA is overexpressed in approximately 70% of non-small cell lung cancer (NSCLC) cases [25]. Therefore, all the proteins considered for LC diagnosis play significant roles in tumor progression and serve as important prognostic markers.

The concentrations of biomarker proteins (CYFRA21-1, LIFR, PLAUR, ProGRP, EpCAM, and CEA) in the blood plasma of lung cancer (LC) patients and conventionally healthy individuals were measured using a multiplex aptasensor and square-wave voltammetry. The graphs of average peak difference values obtained from the electrodes during the determination of these biomarkers are presented in Fig. 2. Analysis of these graphs reveals distinct differences in the voltammetric characteristics of the electrodes when measuring in the blood plasma of LC patients and healthy individuals.

However, it is worth noting that significant differences in the voltammetric characteristics of the electrodes were detected for only two of the six markers – cytokeratin CYFRA21-1 and EpCAM – when detecting tumor markers CYFRA21-1, LIFR, PLAUR, ProGRP, EpCAM, and CEA using DNA aptamer-modified multiplex electrochemical sensors with square-wave voltammetry in the blood plasma of LC patients and healthy individuals (see Tab. 1).

Conclusion

Given the histological heterogeneity of most malignant lung tumors, the European Group on Tumor Markers (EGTM) recommends using at least three different onco-markers for lung cancer diagnosis. For screening and treatment monitoring of non-small cell lung cancer in clin-

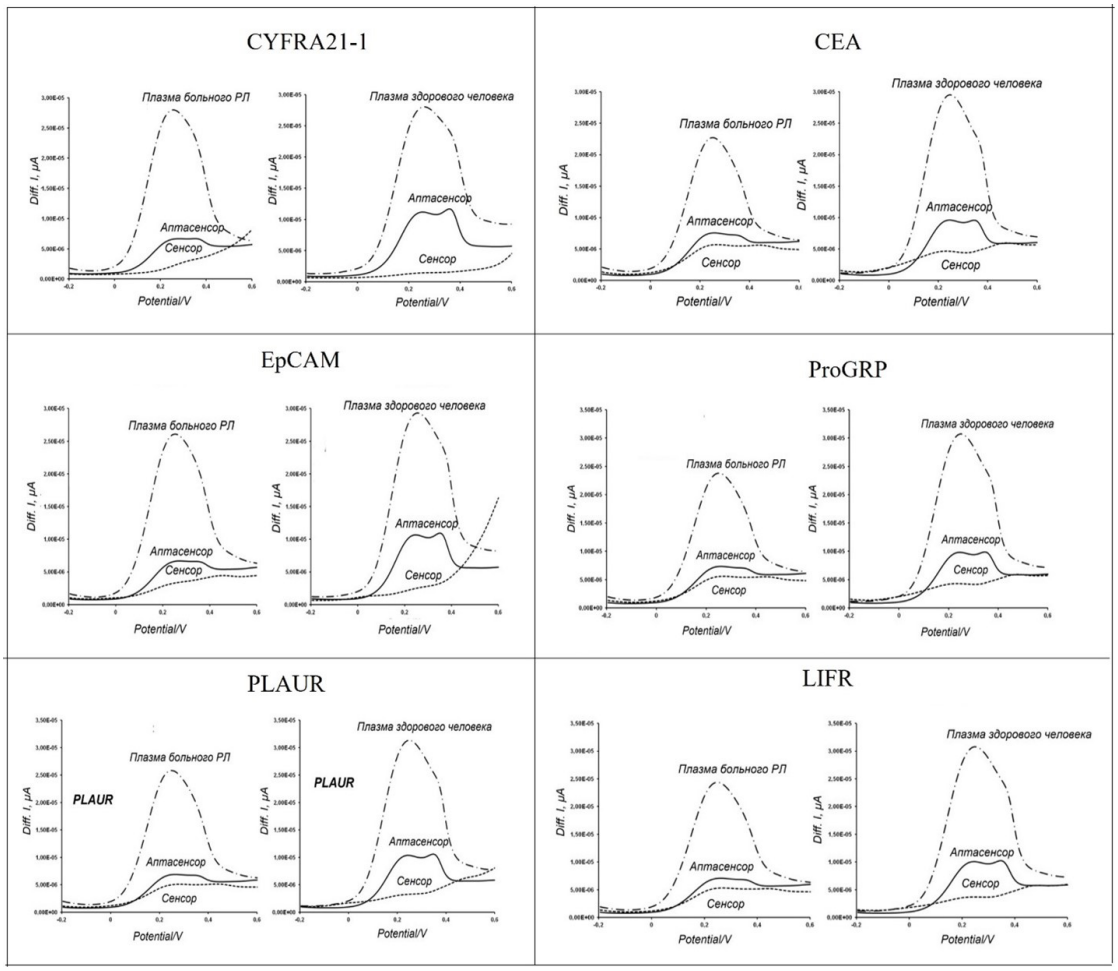

Fig. 2. Square-wave voltammetry measurements on eight-electrode chips functionalized with aptamers to CYFRA21-1, LIFR, PLAUR, ProGRP, EpCAM, and CEA

Table 1. Electrochemical response measurements obtained using a square-wave voltammetry-based electrochemical aptasensor in the blood plasma of lung cancer patients and conventionally healthy individuals

Oncomarker	Electrochemical Signal (μA)		P
	Healthy Plasma (n=3)	LC Plasma (n=3)	
Cyt	0.165 ± 0.020	0.215 ± 0.090∗	<0,05
EpCAM	0.095 ± 0.015	0.17 ± 0.029∗	<0,05
PLAUR	0.185 ± 0.023	0.19 ± 0.032	>0,05
CEA	0.20 ± 0.045	0.155 ± 0.039	>0,05
ProGRP	0.21 ± 0.030	0.165 ± 0.051	>0,05
LIFR	0.21 ± 0.021	0.17 ± 0.049	>0,05

ical laboratories in Russia, one to two biomarkers (CYFRA 21-1, CEA) are used, with their plasma concentrations determined by enzyme-linked immunosorbent assay (ELISA). However, the specificity and information content of such systems is not always high due to the high het-

erogeneity of tumors. Therefore, for improved diagnosis, it is essential to determine the levels of several onco-markers simultaneously. PLAUR, leukemia inhibitory factor receptor (LIFR), pro-gastrin-releasing peptide (ProGRP), and carcinoembryonic antigen (CEA) could be valuable additions to existing markers. The results obtained in this study demonstrated the ability of a multiplex electrochemical aptasensor, employing six DNA aptamers simultaneously, to detect onco-markers in the blood plasma of LC patients. These onco-markers used for diagnosis are reliable indicators and can serve as predictors of lung cancer.

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Электрохимический аптасенсор для определения онкомаркеров рака легкого в плазме крови

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Аннотация. В работе исследована возможность диагностики рака легкого с помощью одновременной детекции в плазме крови шести онкомаркеров этого заболевания (CYFRA21-1, EpCAM, PLAUR, LIFR, ProGRP и CEA). Уровень этих белков в плазме крови определяли методом квадратно-волновой вольтамперометрии с помощью мультиплексных электрохимических чипов, модифицированных ДНК-аптамерами. Полученные в работе результаты продемонстрировали способность мультиплексного электрохимического аптасенсора одновременно находить в плазме крови такие традиционно используемые для диагностики рака легкого онкомаркеры, как CYFRA21-1, EpCAM, PLAUR, LIFR, ProGRP и CEA.

Ключевые слова: аптамеры, рак легкого, электрохимический аптасенсор, онкомаркеры плазмы крови.