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BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

Development of a Biosensor for Electrochemical Detection of Tumor-Associated Proteins in Blood Plasma of Cancer Patients by Aptamers

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Abstract—A molecular biosensor based on DNA aptamers (aptasensor) for the diagnosis of lung cancer in blood plasma samples was designed. To create the aptasensor, the aptamer 17_80, obtained in the study of postoperative material, was used. The affinity and binding selectivity of the aptamer 17_80 to the lung tumor tissue was confirmed on histological sections of postmortem samples of lung tissue. Using affinity enrichment and mass spectrometry, a possible target molecule of the aptamer 17_80, vimentin, was found.

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Diagnostics based on biosensors, which makes it possible to identify biomarkers of various diseases, including cancer, using biorecognizing molecules is actively developing in medicine. In recent years, aptamers began to be used for these purposes [1]. Biosensors based on aptamers are called aptasensors.

This study is devoted to designing an aptasensor for diagnosing lung cancer.

Aptamers that interact with ligands of lung cancer cells were obtained by tissue-SELEX technology, for which postoperative tumor lung tissue materials were used. Aptamers were selected and their synthetic analogues were obtained as described in [2]. In total, we sequenced and analyzed 17 DNA aptamers, 10 of which were synthesized. To create an aptasensor, we used the aptamer 17_80, whose affinity and specificity of interaction with lung tumor cells was assessed by its binding to the postmortem samples obtained in the Postmortem Bureau of Krasnoyarsk. Tissue samples were stored in formaldehyde solution and used to prepare tissue sections. The latter were treated for 30 min with fluorescently labeled aptamer 17_80 and then washed with phosphate-buffered saline (PBS, Sigma-Aldrich, United States) to remove the unbound aptamers. The results of the study presented in Fig. 1 show that the aptamer 17_80 binds to adenocarcinoma and squamous-cell lung cancer and does not bind to normal lung cells.

The protein target of the aptamer 17_80 from the lung tumor tissue was isolated by affinity enrichment [3]. The target protein of the aptamer 17_80 was determined with an Orbitrap mass spectrometer (Thermo Scientific, United States). The search for the target protein of the aptamer was performed using the method of exclusion of the most common contaminant proteins and proteins present in the control samples. The proteins that were identified by the program by at least five unique peptides, which were found in all three series of independent experiments in each replicate and had a positive correlation coefficient, were considered as candidate targets of the aptamer [3]. Mass spectrometry data, processed using the MaxQuant 1.3 software, showed that a presumable target of the aptamer 17_80 is vimentin, a component of mesenchymal cell filaments that is involved in cell differentiation. High expression of vimentin is an indicator of adverse prognosis in lung cancer [4].

The aptamer 17_80 was used to create an electrochemical aptasensor intended for diagnosing lung cancer. The xCELLigence system (Roche Applied Science, Switzerland), which is commonly used for analyzing cell response in work with cell cultures, was used as a measuring instrument. The principle of operation of the system is based on measuring the resistance on gold electrodes. To create an aptasensor, the gold surface of electrodes was coated with a sensor layer containing the aptamer 17_80 as described in [5].

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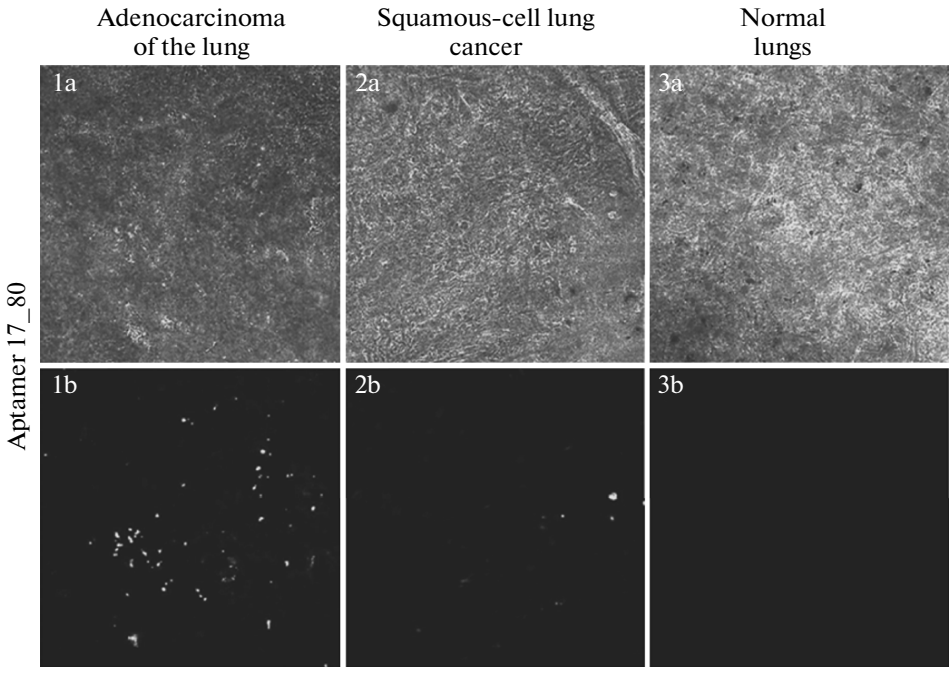

Fig. 1. Histological sections of adenocarcinomas, squamous-cell lung cancer, and normal lungs stained with the aptamer 17_80 fluorescently labeled with FAM: (1a–3a) phase contrast and fluorescence, superposition; (1b–2b) fluorescence. Studies were performed using an Olympus FluoView 10vi confocal fluorescence microscope (Olympus Optical Co., Japan). Magnification, 10×.

We studied peripheral blood plasma samples of healthy subjects of both sexes (volunteers 25–65 years old) and lung cancer patients of both sexes (32–65 years old, samples were obtained from the Kryzhanovsky Krasnoyarsk Regional Cancer Clinic) with a verified diagnosis (table). All studies were performed in strict accordance with the documents governing the ethical standards of research using biological material

Correspondence of the electrochemical index (Cell index) values of blood plasma of patients to the histological type and stage of lung cancer

Examined subjects	Cell index, art. units	Diagnosis, histological type	Stage of disease	Age, years
Patients with lung cancer				
1	0.0612	Keratinizing squamous-cell lung cancer	T4N2M0	44
2	0.0739	Keratinizing squamous-cell lung cancer	T3N0M0	58
3	0.0872	Highly differentiated adenocarcinoma of the lung	T1N0M0	58
4	0.0899	Lung cancer (histological type not identified)	–	32
5	0.1211	Moderately differentiated adenocarcinoma of the lung	T2N0M0	65
6	0.0729	Keratinizing squamous-cell lung cancer	T4N2M0	44
7	0.0740	Moderately differentiated adenocarcinoma of the lung	T3N2M0	54
Healthy subjects				
8	0.1401	Apparently healthy	–	25
9	0.1347	Apparently healthy	–	25
10	0.1397	Apparently healthy	–	65
11	0.1091	Apparently healthy	–	34
12	0.1392	Apparently healthy	–	28
13	0.1389	Apparently healthy	–	30
14	0.1592	Apparently healthy	–	30

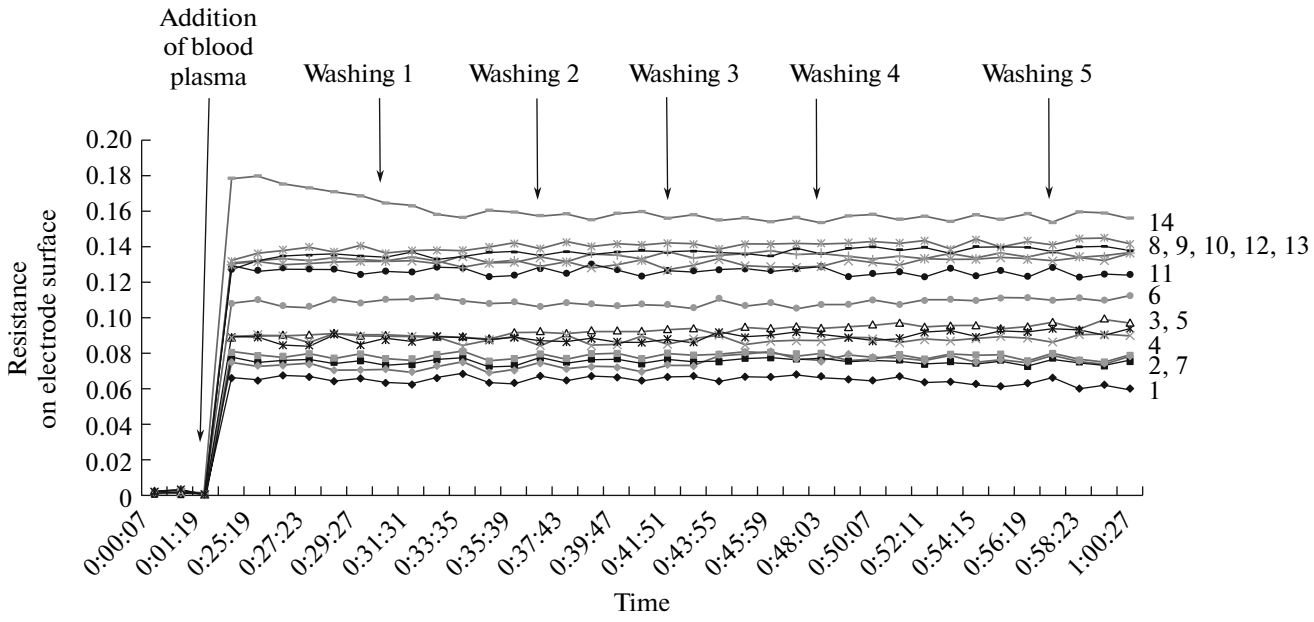

Fig. 2. Resistance on the surface of a gold electrode modified with the aptamer 17_80 after the addition of blood plasma of healthy subjects and lung cancer patients after washing. The numeration of the samples corresponds to that in the table. Curves showing the resistance on the electrodes corresponding to (1–7) lung cancer patients and (8–14) apparently healthy subjects.

of human origin (the decision of the Local Ethics Committee KrasGMU no. 37/2012 from January 31, 2012 and the decision of the KKKOD Local Ethics Committee no. 8/2011 from March 16, 2011).

Before the experiments, blood plasma samples were incubated with yeast RNA (Sigma-Aldrich) for 30 min at room temperature to block the nonspecific binding sites for the aptamer. Thus prepared blood plasma (100 μL) was added to the wells of a plate with gold electrodes modified with the aptamer 17_80 at the bottom and incubated for 30 min in the RTCA Station xCELLigence system, simultaneously measuring the electrical resistance at the bottom of the wells.

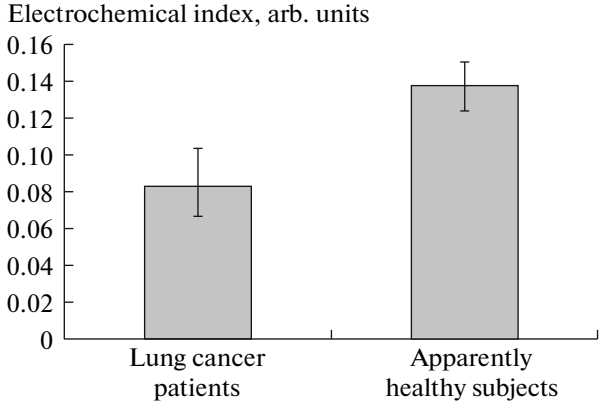

Fig. 3. Specific resistance on the aptasensor-coated electrodes in the analysis of blood plasma of lung cancer patients (0.083 ± 0.019 arb. units, $n = 7$) and apparently healthy subjects (0.137 ± 0.014 arb. units, $n = 7$); $p < 0.002$.

Before incubation, electrode readings were stabilized in the wells containing PBS supplemented with Ca^{2+} and Mg^{2+}, after which the buffer was removed and the blood plasma samples of patients and healthy subjects were added. After incubation, the wells were washed 5 times with PBS at equal time intervals, performing repeated measurements after each washing. The electrochemical index (Cell index) was measured every 5 min. Figure 2 presents the plots showing the changes in the resistance on aptasensor-coated electrodes for the blood plasma of healthy subjects and cancer patients. Figure 3 shows the mean resistance on the electrodes after the incubation with the blood plasma of patients and healthy subjects. As can be seen from the diagrams, the electrochemical index values of healthy subjects significantly exceeded those of lung cancer patients. The individual values of the Cell index and the histological types of lung cancer corresponding to them are summarized in the table.

Thus, using the developed biosensor, significant differences in the Cell index we detected in the study of blood plasma samples of lung cancer patients and apparently healthy subjects. Further comparative studies of clinical blood plasma samples of patients with other types of malignant and benign tumors at different stages of tumor progression will determine the clinical significance of the new test.

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