

OPTICAL METHODS IN BIOLOGY AND MEDICINE

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Optical diagnostics of tumour cells at different stages of pathology development

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Abstract. **The differences in optical and biophysical properties between the cells of mammary gland tumour extracted from tumours of different diameter are described. It is shown that the spectral and spectrokinetic properties of fluorescent probes in the cells extracted from the tumours 1–3 cm in diameter are essentially different. Thus, the extinction coefficient of rhodamine 6G gradually increases with the pathology development. At the same time the rate of interaction of the triplet states of molecular probes with the oxygen, diluted in the tumour cells cytoplasm, decreases with the growth of the tumour capsule diameter. The observed regularities can be due to the changes in the cell structure, biochemical and biophysical properties. The reported data may be useful for developing optical methods of diagnostics of biotissue pathological conditions.**

Keywords: *fluorescence diagnostics, tumour, molecular probes, long-term luminescence.*

1. Introduction

Optical methods of diagnostics are widely used in biology and medicine for detecting pathological conditions of biotissues. The most widespread methods are the optical coherence tomography [1], the diffuse reflection spectroscopy [2] and the fluorescence spectroscopy [3]. However, since the existing methods do not allow tumour detection at the earliest stage, it is often possible to identify it only when the capsule formation has already occurred and the metastasis started. In this connection it is still urgent to develop optical methods of early diagnostics of malignant neoplasms.

It is known [4] that the normal and the tumour cells differ by the amount of proteins and carbohydrates dissolved in the cytoplasm, as well as by the steady-state oxygen concentration in them. The changes in the cells occur gradually, during the whole process of capsule formation. Earlier [5] we demonstrated the changes in the retarded fluorescence kinetics of molecular probes, localised in the cells of tumour and healthy mammary gland tissue. In the present paper the changes that occur in the tumour cells at different stages of the pathology development are studied using spectral methods with the aim of enhancing the sensitivity of optical biomedical diagnostics.

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2. The objects and methods of study

2.1. Spectrofluorimetry and spectrophotometry

We studied the cells of the mammary gland of female mice belonging to the line BYRB, for which the genesis of spontaneous cancer tumours of the mammary gland is specific. During the operation small tissue samples were extracted from the tumour capsules of different diameter (1–3 cm). Fragments of normal mammary gland tissue were taken from healthy female mice of the same line. The fragments of tumour and healthy tissue were placed in the solution of collagenase in the phosphate buffer (pH = 7.4) with the concentration 0.5 mg mL^{-1} and kept for 40 min at 37 °C to obtain a cell suspension, which then was freed from the enzyme molecules by centrifuging during 10 min with the rate of 3000 revolutions per minute. An aliquot of the rhodamine 6G solution was added to the resulting cell suspension. The final concentration of the dye in the cell suspension amounted to 2.9×10^{-5} M. The absorption spectra of cells and dye, as well as the fluorescence spectra of the dye were measured using the spectrofluorimeter SOLAR CM-2203 that operated alternately in the spectrophotometric and spectrofluorimetric mode. The absorption spectra of the cells were measured with respect to the phosphate buffer spectrum, and those of the dye with respect to the spectrum of uncoloured cell suspension (10 minutes after adding the dye solution). The exciting radiation wavelength in the fluorimetric measurements was equal to 515 nm. To obtain reliable results not less than 10 female mice were used for each type of samples.

2.2. Laser flash photolysis

For spectrokinetic measurements the obtained cell suspension was set on the surface of the solid nutrition medium (Giamatrix biomaterial [6]) and cultivated at 37 °C during 24 hours [7]. Fragments of the biomaterial with cells were stained with erythrosine with the initial concentration of 10^{-4} M. The choice of this type of dyes was determined by their spectral luminescence characteristics, namely, high quantum efficiency of the transition to the triplet state, intense delayed fluorescence (DF) and phosphorescence [8], high aqueous solubility, and ability to penetrate into cells and selectively get bound with proteins [9].

The basis of the setup for studying the kinetics of the DF decay and the phosphorescence of dye molecules was a repetitively pulsed solid-state Nd:YAG laser. To excite the molecules the second harmonic of the laser radiation (λ_{ex} = 532 nm) was used. The pulse duration was 10 ns, and the pulse energy

varied within the range 10–50 mJ. The retarded fluorescence of the probes was detected via the MDR-41 monochromator using the FEU-84 photomultiplier. The studied samples were placed in a special evacuated chamber. The air pressure above the surface of the stained biopolymer varied from 300 to 760 mm Hg. The measurements were implemented using the automated setup, including the PC and the CAMAC crate, with the transient response time constant 200 ns [6]. The recorded signal of the DF kinetics was the arithmetical mean curve of five accumulated pulses. In the experiments not less than 10 female mice were used for each type of cells.

2.3. Optical microscopy

The cell size and shape were determined using the method of optical microscopy. Fragments of the biomaterial containing the cells were fixed on the microscope slide and stained with haematoxylin and eosin. The measurements were carried out with the Micros MSD 500 optical microscope, equipped with the JEM-7A digital camera, with the magnification 1600.

3. Results and discussion

Figure 1 presents the absorption spectra of the mammary gland cell suspension. The growth of the maximum at 420 nm with the increase in the tumour capsule size is clearly seen in the tumour cells. In healthy cells no absorption in this range is observed. This observation can be explained by the increase in the content of porphyrins in the tumour cells [10]. Their concentration grows with the development of the pathology and, as a consequence, an additional absorption maximum appears at $\lambda = 420$ nm. Progressive advance of absorption in the UV region indicates the growth of the number of protein molecules [4] in the tumour cells. The difference in the absorption spectra is observed even in the cells, extracted from the capsule with the size of 1 cm.

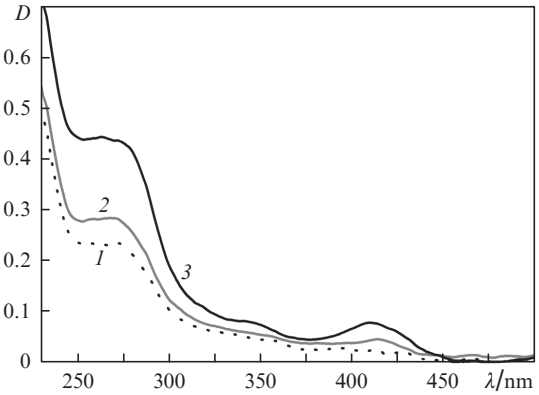

Figure 1. Absorption spectra of the suspension of cells of (*1*) a normal mammary gland tissue and tumour cells extracted from the tumour capsules with the size (*2*) 1 and (*3*) 3 cm.

The measurements of the DF kinetics demonstrated also its high sensitivity to the stage of the pathology development (Fig. 2). Since the curves illustrate the summary signal from the thermally retarded fluorescence and bimolecular processes (triplet–triplet and singlet–triplet annihilation [5]), the shape of the experimental curves in semi-logarithmic coordinates allows the calculation of the rate constant for the reaction of annihilation of the probe triplet states with the produced singlet oxygen $^1\Delta_g(O_2)$–T (the reaction of singlet-triplet annihilation). During the first 100 μs the character of the dependences is linear. Their slope in this temporal range determines the quasi-molecular rate constant of the annihilation reaction that is related to the true annihilation rate constant by the formula $k_{an} = k'_{an}N_\Delta$, where k'_{an} an is the true rate constant of the bimolecular reaction and N_Δ is the singlet oxygen concentration.

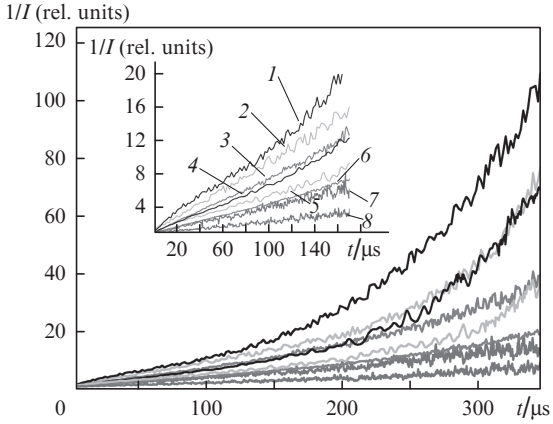

Figure 2. Kinetics of DF of erythrosine in (*1*, *4*) healthy cells and in tumour cells extracted from the tumour capsules with the size (*2*, *5*) 1, (*3*, *6*) 2 and (7, 8) 3 cm at the pressure (*4*, *5*, *6*, *7*) 300 and (*1*, *2*, *3*, *8*) 760 mm Hg. The initial probe concentration is 10^{-4} M.

With the increase in the tumour capsule size the rate constant of the annihilation reaction in cells decreases. Its values in healthy and tumour cells, extracted from a capsule with the size 1 cm, are comparable (Table 1). With the growth of the tumour size these distinctions become more pronounced. The sensitivity of the quantity k_{an} of the probe molecules to variations of the air pressure above the surface of the stained cells is also changed. When the pressure varies from 300 to 760 mm Hg, the quantity k_{an} for the cells from a 3-cm tumour changes, upon the average, by 27%, whereas in healthy cells this change is 38%.

Table 1. Rate constants of annihilation processes involving triplet erythrosine molecules in tumour and normal cells of mammary gland.

Cells	$k_{an}/10^{-4}$ s^{-1}	
	0.3 atm	1 atm
Tumour	$6.5 \pm 0.9^{*}$	$10.1 \pm 2.2^{*}$
	$5.5 \pm 0.8^{**}$	$7.8 \pm 1.7^{**}$
	$2.6 \pm 0.6^{***}$	$3.6 \pm 0.7^{***}$
Normal	6.8 ± 1.5	11.0 ± 2.4

Note: * – tumour capsule diameter 1 cm, ** – 2 cm, *** – 3 cm.

The decrease in k_{an} with the pathology development may be caused by the hypoxia of the tumour cells [10]. At the same time, in Ref. [11] it was shown that in the tumour cells the viscosity of cytoplasm is significantly higher. It leads to the reduction of the diffusion coefficient of the dye molecules in the cytoplasm and, as a consequence, of the rate constant of annihilation processes.

In addition to the changes in the biochemical composition of the tumour cells, the metamorphoses in organelles are also

probable. It is known [12] that rhodamine dyes penetrate into cells and get bound with mitochondria, the cell organelles responsible for the ATP synthesis and the cell energy balance. One can suppose that with the increase in the tumour size the number of mitochondria in its cells also increases due to the necessity of a greater amount of energy for enhanced cell division. Figure 3 presents the absorption and luminescence spectra of rhodamine 6G in healthy and tumour cells of the mammary gland. In the latter the increase in the dye optical density can be clearly seen, the growth of the optical density becoming more expressed with the increase in the tumour capsule size. At the same time, the dye luminescence spectra in healthy cells and in tumour cells, extracted from the capsule with the size 1 cm, demonstrate insignificant difference. With the increase in the tumour size the shift of the fluorescence maximum towards the long-wavelength region occurs.

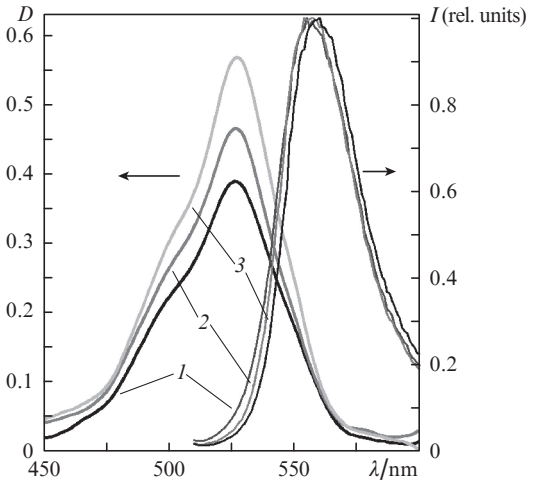

Figure 3. Absorption and luminescence spectra of rhodamine 6G in (*1*) healthy cells and tumour cells extracted from the capsules with the size (*2*) 1 and (*3*) 3 cm.

In the process of capsule formation the composition and structure of the tumour cells experience significant changes. This leads to the nucleus polymorphism and the increase in the cell size. Figure 4 presents the photographs obtained by means of optical microscopy. The changes in the morphometric cell indicators are clearly seen. Thus, the cell of healthy mammary gland tissue has the dimensions 20×20 μm and the regular shape. The development of the pathological process leads to the increase in the cell size (40×60 μm) and disproportions in its shape.

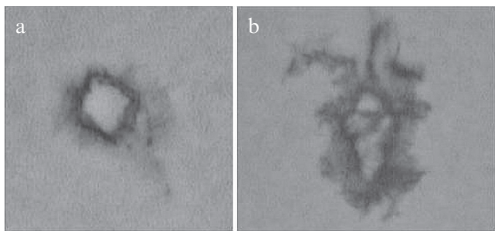

Figure 4. Microphotographs of (a) the normal mammary gland tissue cell and (b) the cell extracted from the tumour capsule with the size 3 cm.

4. Conclusions

Despite a large number of experimental and theoretical results obtained in the study of tumour cell properties, many questions are still poorly studied and open to discussion. One of them is the concentration of oxygen in the tumour cells. In Refs [4, 10, 13] the data are presented on the lower concentration of oxygen in the tumour cells as compared with the healthy ones. Our results agree with the results of these research teams.

In the present paper we studied the changes that occur in the cancer cells at different stages of the pathology development. It is worth noting that by using optical methods one can detect the differences between the normal and the tumour cells even if the size of the tumour is small. However, the spectral and spectrokinetic properties of the tumour cells are essentially different at all stages of the pathological process development.

Since the elaboration of carcinogenesis theories is in progress [14–18], the data reported in the present paper may be useful for the analysis and correction of the existing theories, as well as for further development of the optical methods in biomedical diagnostics.

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