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Fluorescence lifetime optical biopsy of the hepatocellular carcinoma in murine model

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Abstract: In this paper we demonstrate results of combined measurements by diffuse reflectance and fluorescence lifetime measuring techniques for real time liver cancer differentiation using a needle optical probe.

1. Introduction

As cancer remains one of the most u rgent problems of modern medicine, the relevance of the development and application of new methods for its diagnostics and treatment cannot be underestimated. Speaking of liver cancer, physicians are in need of techniques for rapid and accurate determination of the type and histological structure of the tumor at the initiate stages of surgical treatment. In order to determine treatment tactics the histological analysis is performed after the tissue sample is obtained during puncture biopsy [1]. However, this procedure has a relatively high probability of getting a non-diagnostic sample and it takes 5-10 days to obtain the results of the following study [2].

A lot of biophotonics methods and approaches can be introduced into standard procedures of minimally invasive surgery and can be used to enhance the conventional diagnostics algorithms for liver cancer treatment. For several decades fluorescence methods have proven to be itself as a highly informative methods for differentiation of different tissue types. The values of fluorescence intensity and lifetime decay bear the information on endogenous fluorophores content and their biochemical interaction with surrounding substances which is very sensitive to the presence of pathological changes caused by cancer formation. Another spectroscopic method – diffuse reflectance spectroscopy (DRS) – provides the information on morphological changes in tissues as it reveals the changes in the amount of tissue chromophores. In particular, this method is sensitive to the hemoglobin content in tissues and can be used to evaluate the oxygen saturation, which changes are another sign of tumor growth [3].

Previously, we have demonstrated the possibilities and prospects of applying DRS [4], fluorescence intensity supplemented by DRS [5] or laser Doppler flowmetry [6] measurements to provide the additional diagnostic information that can be a valuable addition to assist a surgeon during minimally invasive intervention or a routine biopsy. The aim of this work is to demonstrate the multimodal approach based on fluorescence lifetime and diffuse reflectance data measurements to obtain the information on metabolic and morphological changes in liver cancer tissues in the real-time.

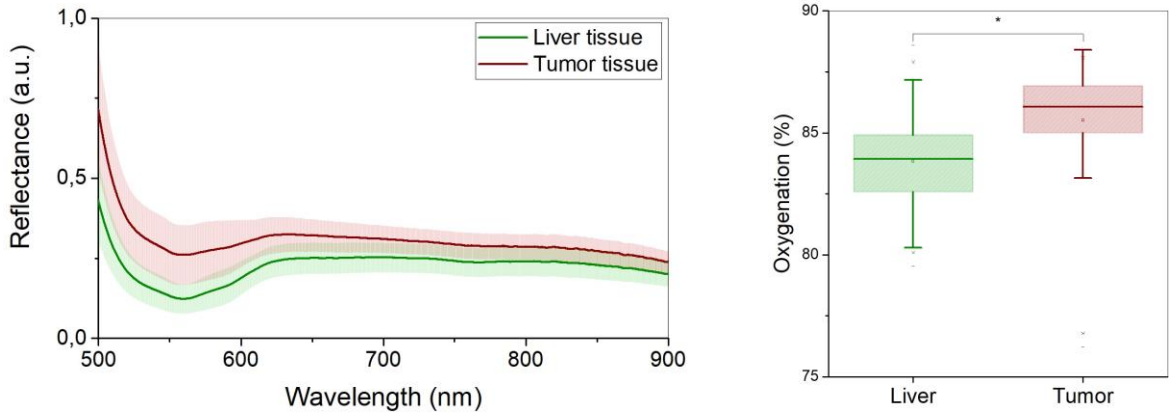
2. Materials and Methods

The study was performed using an experimental setup including diffuse reflectance and fluorescence lifetime measurement systems. To record fluorescence intensity values and lifetime decay curves the method of time-correlated single-photon counting (TCSPC) was used. The TCSPC system (Becker & Hickl, Germany) includes a 365 nm BDL-SMN ps laser as a source, a MonoScan2000 (Ocean Insight, USA) monochromator and two HPM-100-40 photomultiplier detectors equipped with MF445-45 and MF530-43 (Thorlabs, Inc., USA) fluorescence filters (hereinafter, the detector with the central wavelength of 445 nm is named Channel 1, the 530 nm one – Channel 2). The choice of excitation wavelengths was made according to the target fluorophore – nicotinamide adenine dinucleotide (NADH) coenzyme of mitochondria. The laser and detectors are connected to the specially developed fiber optic needle probe compatible with standard 17.5G Chiba type needles. The distal end of the probe has a 1 mm diameter and 20° bevel similar to the standard needles. The probe is also connected to the DRS measurement channel. It includes an HL-2000-FHSA (Ocean Insight, USA) halogen tungsten source (360-2400 nm) and FLAME-T-VIS-NIR-ES (Ocean Insight, USA) spectrometer to record spectra in the range of 350-100 nm.

To obtain the parameters of healthy and malignant liver tissues the murine model of hepatocellular carcinoma was used. The studies were approved by the ethics committee of Orel State University (minutes of the meeting No 12, September 6, 2018). The cells of H33 mouse hepatocellular carcinoma (50000 cells/μl, 100 μl per mouse) were inoculated by injecting a suspension of cells through a syringe into the middle lobe of the liver of male BDF mice (n=5) during laparotomy. The *in vivo* measurements were carried out after 2-3 month after the surgery as the weight increased by more than 15% of the normal weight and the health of the animal deteriorated. Each animal was anesthetized with Zoletil in the standard dosage and on a special platform in the back position and a laparotomy was performed. The measurements included recording of 5 fluorescence lifetime signals and 50 diffuse reflectance spectra at 5 different areas of intact liver and the tumor. The diffuse reflectance spectra were normalized by the spectra of the halogen lamp recorded from spectralon. The intensities of reflectance at 760 nm and 805 nm were used to calculate the values of oxygen saturation [7-9].

3. Results and Discussion

The results of diffuse reflectance measurements (Fig. 1) demonstrated the increase of reflectance in tumor tissues, especially in 500-600 nm range, which indicates the decrease of overall blood content in tumor tissues. The spectra of both tissue types expressed absorption peaks at 560 and 760 nm associated with deoxyhemoglobin. The average level of oxygen saturation in the liver was 83.8±1.7 %, which is less than in the tumor (85.5±2.2 %).



(a) (b)

Fig. 1. Average (mean ± standard deviation) diffuse reflectance spectra (a) and oxygen saturation values (b) of the areas of interest. Statistically significant difference was confirmed by One-Way ANOVA ($p < 0.001$).

The results of fluorescence lifetime measurements are shown in Table 1. It can be observed that cancer progression resulted in a decrease of both τ_1 and τ_2 lifetime decay components in the tumor, while the amplitude contribution of $\alpha 1$ component increased.

Table 1. Fluorescence lifetime parameters (mean ± standard deviation). * – Statistically significant differences confirmed by One-Way ANOVA ($p < 0.001$).

	Channel 1 (445±45 nm)		Channel 2 (530±43 nm)	
Parameter	Liver	Tumor	Liver	Tumor
α_1	63.6±3.1	75.3±5.4*	61.6±5.0	80.7±2.3*
α_2	36.4±3.1	24.7±5.4*	38.4±5.0	19.3±2.3*
τ_1	551.9±23.2	526.1±16.4	523.0±15.3	504.4±8.3
τ_2	2790.4±44.9	2548.9±23.9*	2798.9±64.0	2522.4±17.4*

The results obtained indicates that the influence of unbound NADH on the fluorescence is higher and it is usually indicates the preponderance of glycolysis in malignant tissues compared with oxidative phosphorylation in healthy ones.

4. Conclusion

The obtained data showed that atypical metabolic activity of malignant cells leads to changes in both morphological properties and metabolic state of tissues. Fluorescence lifetime parameters indicated the changes in the ratio of bound and unbound NADH. Diffuse reflectance spectra allowed us to observe the changes in tissue choromophores content and oxygen saturation. Our results provide evidence that the feature space obtained by the fluorescence lifetime measurements supplemented by the information about the blood oxygen saturation and blood volume fraction in the tumor area are of high predictive value to discover hepatocellular carcinoma. Thus, the proposed approach can be deemed to be promising for more detailed studies and the development of diagnostic criteria for analysis of different stages and types of tumors during minimally invasive diagnostics procedures.

5. Acknowledgements

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