Research Article

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Metabolism and Microstructure of the Small Intestine Wall in Patients with Colorectal Cancer

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RELEVANCE In patients with colorectal cancer (CRC), the normal small intestine, located outside the pathological focus, undergoes changes that may be the cause of digestive dysfunction after radical surgery to remove the tumor.

The assessment of metabolic and microstructural changes in the ileum mucosa in patients with colorectal cancer is necessary to correct the algorithms of postoperative therapy and enteral nutrition. Modern means of optical bioimaging are potentially capable of solving this complex diagnostic problem.

AIM OF STUDY To study the features of metabolism and morphological structure of the wall of a conditionally normal small intestine in the mucosa in patients with stage 1–3 CRC using macro- FLIM and optical coherence tomography (OCT).

MATERIAL AND METHODS The object of the study was the wall of the ileum (66 samples) from the mucosal side of patients with histologically confirmed CRC stages 1–3 with tumor location in the right sections of the colon. Eight samples were obtained from patients with stage 1 CRC, 38 samples were obtained from patients with stage 2 and 20 samples were obtained from patients with stage 3 tumor. The volume of surgical intervention is right-sided hemicolectomy with total mesocolonectomy, CME (D2 lymph node dissection). Fresh tissue samples were examined using fluorescent lifetime macroimaging (macro-FLIM) and OCT, followed by histological analysis of the material.

RESULTS According to a histological study in the small intestine of patients with stage 1 CRC, the mucosa is covered with a normal single-layer prismatic border epithelium. In the intestine samples of patients with stage 2 CRC, mucus hypersecretion with areas of fibrosis and vascular congestion was observed. At the 3rd stage of CRC, the mucous membrane of the small intestine was loose, with local thickenings, areas of fibrosis with severe leukostasis, and foci of atrophy. None of the samples showed histological signs of a malignant tumor.

According to OCT data, in the mucous membrane of the small intestine in patients with the 1st and 2nd stages of CRC, the contours of the villi and, partially, the crypts were well visualized. The structure of the villi was smooth, not coarse, and the shape was regular. In patients with stage 3 CRC, the contours of the crypts and villi were indistinct. There were no differences in the OCT picture between histologic preparations in the 1st and 2nd stages of CRC: the structure of the villi of the small intestine was clear, the shape was unchanged. According to FLIM data, statistically significant differences were revealed in the mean fluorescence lifetime values of reduced nicotinamide dinucleotide (phosphate) NAD(P)H (τ_m) between 2nd and 3rd (p=0.031), 1st and 3rd (p=0.018) by CRC stages. At the 1st stage of CRC τ_m was 1.61 [1.30; 2.02] ns, at the 2nd stage 1.50 [1.36; 1.73] ns, at the 3rd stage 1.37 [1.22; 1.51] ns. The FLIM results suggest an increase in the role of glycolysis in enterocyte energy metabolism along with progression of the CRC stage.

CONCLUSION In patients with cancer of the right colon, lesions of the microstructure of the mucous membrane were revealed in the ileum not affected by the malignancy. At the same time, the severity of microstructural disorders in the wall of the small intestine is associated with the stage of tumor development in the colon. Bioimaging technologies, namely, methods of optical coherence tomography and fluorescence lifetime macroimaging, made it possible to objectively display microstructural and metabolic disorders in the ileum wall. The data of optical colorectal tomography demonstrated differences in the structural picture of the intestinal villi in patients with stages 1–2 and 3 of colorectal cancer. Results of fluorescence lifetime macroimaging of the metabolic cofactor nicotinamide dinucleotide (phosphate) confirmed an increase in the role of glycolysis in the energy metabolism of enterocytes along with an increase in the stage of colorectal cancer. The identified disorders in the state of the small intestine develop in patients with colorectal cancer before surgery and are highly likely to be an important pathogenetic link of malabsorption in the postoperative period. If the hypothesis is confirmed, the developed algorithm for the complex diagnosis of microstructural and metabolic disorders in tissues will expand the possibilities for the rehabilitation of patients with cancer of the right colon.

Keywords: ileum, fluorescent lifetime imaging FLIM, optical coherence tomography OCT, metabolism, colorectal cancer, malabsorption

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CRC – colorectal cancer
Macro FLIM – fluorescent lifetime macro imaging
MMD OCT – multimodal device for optical coherence tomography
NADH – nicotinamide dinucleotide
NAD(P)H – nicotinamide dinucleotide (phosphate)
OCT – optical coherence tomography
CME (D2 lymph node dissection) – right-sided hemicolectomy with complete mesocolic excision

INTRODUCTION

Malabsorption is observed in 91% of patients after right hemicolectomy for cancer, and diarrhea persists in this group of patients for 3 months [1–3]. Recently, there has been an increase in survival rates after surgery for colorectal cancer (CRC); this positive trend has led to an increase in the clinical and social significance of late complications, which determine the duration and quality of life of successfully operated patients [4]. The incidence of chronic late gastrointestinal complications ranges from 6 to 66% and correlates with additional financial costs to the hospital [5]. In patients after right hemicolonectomy for colorectal cancer with resection of more than 10 cm of the ileum, frequent bowel movements are observed in addition to loose stools [6]. Malabsorption (intestinal malabsorption syndrome) is characterized by impaired digestion and absorption of nutrients due to changes in the normal structure and/or function of the intestine [7]. Protein-energy deficiency, which develops as a result of nutritional deficiency, aggravates the patient's condition in the early postoperative period and increases the risk of late complications [8]. In recent published works, the mechanism of development of malabsorption in patients with colon cancer is associated primarily with the consequences of surgical removal of functionally important parts of the intestine. According to D.J. Gracie et al. (2012), the pathogenesis of the development of malabsorption after right-sided hemicolectomy for cancer begins after resection of the terminal ileum with the ileocecal junction and includes: a decrease in the absorption of bile acids in the terminal small intestine; postoperative dyscoordination of intestinal motility; increased secretion of water with a dramatic decrease in its absorption; bacterial overgrowth syndrome [9, 10]. The role of resection of the ileocecal angle and right colon in the development of long-term malabsorption is described by J. Cosnes et al. (1978) [11]. The problem of the influence of rectal surgery on the function of the gastrointestinal tract after low anterior resection of the colon has been widely discussed [12–14]. Despite a large number of studies, the question still remains unclear: whether malabsorption develops in patients with colorectal cancer only after (and as a result of) resection of functionally significant areas of the intestine or whether the morphofunctional basis for malabsorption in the small intestine appears prior to surgery.

In the normal mucosa of the small intestine in patients with CRC, in contrast to patients without pathology of the gastrointestinal tract, there are changes in metabolism, in particular in the metabolism of carbohydrates, fats and amino acids, as well as microbiological, genetic and epigenetic changes. The research by Matthew L. Silviera et al. (2012) shows that the assimilation of food components in the normal small intestinal mucosa in patients with CRC occurs differently due to the high level of hormones that regulate glucose metabolism and low levels of glycolytic enzymes (key regulators of glucose metabolism) [15]. Identification of malabsorption in the colonic mucosa can be used in the early diagnosis of CRC.

In addition, the DNA of polyomavirus (JCV) and Fusobacterium nucleatum in the intact intestinal mucosa in CRC was found in more cases than in the colon mucosa in a healthy person [16, 17]. For example, Fusobacterium nucleatum DNA was found in the colonic mucosa in 16–48% of healthy patients, and in 63.9% of cases the colonic mucosa of patients with CRC was intact. Changes in the histologically intact intestine in patients with CRC at the genetic and epigenetic levels were characterized by increased expression of some microRNA genes: 21, 7, 31, 92, 181, 203, 106a, ERK1; proteins ITGB1, NLK, mtSSB; an increase in the number of estrogen receptors and its isoforms (ERβ) in comparison with the normal intestine of a healthy person.

To assess the structural and functional changes in the ileum, predict maldigestion and malabsorption in colon cancer, optical bioimaging methods may be promising. Modern methods of bioimaging such as optical coherence tomography (OCT) and fluorescence lifetime macro imaging (FLIM) open up the possibility of real-time visualization of microstructural and biochemical changes in tissues caused by the development of pathologies with high sensitivity and spatial resolution, respectively. Despite the fact that these bioimaging methods are objectively limited by the depth of tissue scanning, which is up to 100 μm in the case of FLIM and up to 1,500 μm in the case of OCT, due to the strong relationship of the anatomical sheaths of the intestine, data on metabolic disorders and

changes in the microstructure at the depth of the mucous layer are objectively reflect the state of the tissue of the entire intestinal wall. The possibilities of these methods in diagnosing disorders in the wall of the small intestine in the development of colorectal cancer have not been studied to date.

Aim of study: to study the features of metabolism and morphological structure of the wall of a conditionally normal small intestine from the mucosal side in patients with stage 1–3 CRC using macro-FLIM and OCT methods.

MATERIAL AND METHODS

The study was conducted on the basis of the Research Institute of Experimental Oncology and Biomedical Technologies of the Federal State Budgetary Educational Institution of Higher Education "PRMU" of the Ministry of Health of Russian Federation, the samples were obtained from the Coloproctology Center and the Department of Abdominal Oncology of the Semashko Nizhegorodsky Regional Clinical Hospital (Nizhny Novgorod). Object of study: the mucous membrane of the ileum in patients with cancer of the right colon of the 1st-3rd stages. Criteria for inclusion in the study: voluntary informed consent to participate in the study, uncomplicated cancer of the right colon, no organic pathology of the colon outside the tumor segment. Criteria for exclusion from the study: complicated colorectal cancer (intestinal obstruction, bleeding), stage 4 colorectal cancer, other pathology of the colon (colon polyps, ulcerative colitis, Crohn's disease), surgical complications of CRC.

The study was performed on 66 samples of the small intestine from patients with cancer of the right colon: 8 samples were obtained from patients with the 1st stage of CRC, 38 samples from the 2nd stage, 20 samples from patients with the 3rd stage of tumor development. The stages of CRC development were confirmed histologically. All patients underwent laparoscopic surgery — right-sided hemicolectomy with total mesocolonectomy — CME (D2 lymph node dissection) for cancer of the right colon. In accordance with the clinical guidelines "Malignant neoplasms of the colon and rectosigmoid" (2020), in addition to laboratory and instrumental methods of investigation (complete blood count, complete urinalysis, biochemical blood test, coagulogram, electrocardiogram, etc.), all patients underwent: fibrocolonoscopy with histological verification of the diagnosis of cancer of the right colon, fibrogastroscopy, computed tomography of the abdominal cavity and chest organs, cancer biomarkers - cancer-embryonic antigen and tumor marker CA19-9 [18]. The operation consisted in the removal of the right sections of the colon (cecum, ascending colon, hepatic flexure and the right sections of the transverse colon with a terminal ileum 10 ± 2 cm long) with the imposition of an extracorporeal antiperistaltic anastomosis using a hardware suture (side to side using a linear stapler Endo-Gia-60).

ALGORITHM FOR SAMPLING MATERIAL FOR RESEARCH

On the segment of the ileum included in the resection area, a 1.5 × 2.0 cm section of the wall was marked, located on the antimesenteric edge of the wall at a distance of 0.5–1 cm from the proximal edge (Fig. 1A, arrow). The marked area of the intestine in all cases was located at a distance of at least 10 cm and not more than 35 cm from the tumor, did not contain defects, fatty pendants (Fig. 1C). Immediately after cutting off the mesentery of the small intestine, the marked area was excised, immediately placed in a gauze napkin moistened with a sterile 10% solution of bovine serum albumin, and delivered to the site of macro-FLIM and OCT studies on ice, according to a previously developed protocol [19].

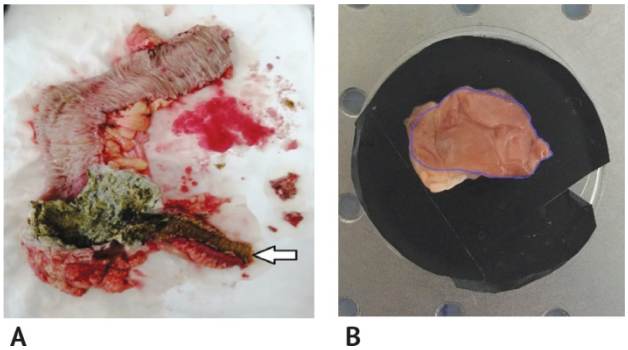

Fig. 1. Stages of sampling and preparation for the study of the ileum. A — macropreparation (on the example of patient V., 80 years old) after surgery — right-sided hemicolectomy (histologic — adenocarcinoma of the caecum), the arrow indicates the proximal border of tumor resection; C — the ileum section was prepared for examination using fluorescent-lifetime macroimaging and optical coherence tomography on the mucosal side

Macro-FLIM was performed on the original two-channel confocal FLIM/PLIM macroscanner (Becker&Hickl, Germany) with single-photon fluorescence excitation using picosecond lasers. The macroscanner makes it possible to obtain lifetime images from the field of view up to 18 × 18 mm in size with a spatial resolution of up to 15 μm [20]. Registration of the fluorescence lifetime was carried out according to the principle of time-correlated photon counting TCSPC (Becker&Hickl, Germany). Fluorescence was excited in the spectral channel of NAD(P)H using a picosecond laser at a wavelength of 375 nm and detected in the range of 435–485 nm. The excitation radiation power was 12 mW, and the photon collection time was 90 s. Autofluorescence decay curves were analyzed using the SPCImage 8.4 program (Becker&Hickl, Germany). The images were imported into the program, the attenuation was approximated by a biexponential model, areas with an acceptable chi-square (from 0.8 to 1.2) were selected, areas of interest were identified (2 areasin each image), excluding areas without an object and containing artifacts. The parameter τm was analyzed, which is the weighted average fluorescence lifetime ($\tau m = a1 \times \tau 1 + a2 \times \tau 2$, where $\tau 1$ and $\tau 2$ are the lifetimes of the short and long components, a1 and a2 are the relative contributions of the short and long attenuation components, a1+a2=100%).

After macro-FLIM, the microstructure of the samples was studied by OCT. A multimodal setup for optical coherence tomography (MMD OCT) was used, which operates on the spectral principle of receiving a signal backscattered from the tissue (Institute of Applied Physics, Russian Academy of Sciences, Nizhny Novgorod, Russia) [21, 22]. The central wavelength of the radiation source is 1,310 nm with a spectral width of 100 nm, the longitudinal resolution is 10 μm, and the transverse resolution is 15 μm. The data acquisition rate is 20,000 A-scans/s. A-scan is a signal intensity distribution profile with depth at the scanning point. Transverse tissue sections (B-scans) 2.4 mm long and 1.3 mm deep are obtained by 2D-lateral scanning. Sequential acquisition of 256 transverse B-scans makes it possible to reconstruct a 3D image in a tissue volume of 2.4×2.4×1.3 mm. OCT scanning was performed in contact mode.

B-scans and en-face images of the tissue were saved from the obtained volumetric OCT images. The images assessed the shape and structure of intestinal villi and the distance between villi. The data obtained were analyzed using the ImageJ program (NIH, USA). To compare OCT images depending on the stages of colorectal cancer, the "structure" criterion was used, characterized by the qualitative parameters of smoothness, roughness and regularity.

Histological examination of the resected section of the intestine with a tumor was performed in the department of pathomorphology of the State Budgetary Healthcare Institution NRCH of Semashko. Histological examination of tissue samples obtained from the proximal and distal ends of the resected section of the small intestinal wall was performed at the PRMU. Samples were fixed in 10% formalin for 48 hours. When preparing histological sections, the OCT scanning plane was taken into account: it coincided with the plane of the B-scans. Hematoxylin and eosin (H&E) staining was used. The pathologist performed a blind description of histological specimens and entered the results into a special table. The presence/absence of edema, inflammation and necrosis, as well as the condition of blood vessels, were assessed. Digital photographs were taken in transmitted light using a Leica DM 2500 DFC microscope (Leica Microsystems, Wetzlar, Germany) equipped with a Leica DFC 425 C digital camera. The results of the pathological examination were compared with the corresponding structural OCT images and macro-FLIM data.

STATISTICAL DATA PROCESSING

Statistical processing of the results was carried out using the statistical analysis package STATISTICA 10 (StatSoft, Inc., USA). In the work, the median values (Me) of the measured values and the upper (Q1) and lower (Q3) quartiles were calculated. The significance of differences between groups when comparing the quantitative parameters of the microstructure and blood circulation of the intestinal wall was performed using the nonparametric Mann–Whitney test. Differences were considered statistically significant at a significance level of $p < 0.05$.

RESULTS

It has been established that morphological and metabolic disorders in the ileum unaffected by the tumor process in patients with CRC appear before bowel resection, and their severity correlates with the stage of tumor development (table).

Table

The ratio of histological, metabolic (according to FLIM) and microstructural (according to OCT) changes in the tissues of the ileum with the stage of development of colorectal cancer

CRC stage	The result of histological examination	NAD(P)H fluorescence lifetime (ns)	OCT results
1 (n =8)	Conditional norm everywhere	1.61 [1.3; 2.02]	The surface of the villi of the small intestine is smooth and the shape is regular and unchanged
2 (n =38)	21% - hypersecretion of mucus, the presence of areas of fibrosis and vascular congestion; 2.6% - foci of atrophy of the mucous membrane, as well as leukostasis in single vessels; 2.6% - thickening of the mucous membrane, vascular plethora, moderate diffuse infiltration of the intestinal tissue with eosinophilic leukocytes	1.50 [1.36; 1.73]	
3 (n =20)	75% - conditional norm in the mucosa; 12.5% - villi are unevenly reduced in size or absent, inflammatory infiltration is minimal; 12.5% - severe diffuse chronic inflammation, the height of part of the villi is reduced	1.37 [1.22; 1.51]	Poor differentiation of the mucosa and submucosa, the contours of the villi are indistinct

Notes: CRC – colorectal cancer; NAD(F)H — nicotinamide dinucleotide (phosphate); OCT – optical coherence tomography

According to histological examination, in patients with stage 1 CRC, no signs of inflammation were found in the tissues of the ileum; all structures of the mucous membrane (single-layer prismatic bordered epithelium, lamina propria of the mucous membrane and muscular lamina), submucosa, muscular and serous membranes were normal (Fig. 2A).

In patients with stage 2 CRC, the structure of the small intestinal mucosa also looked conditionally normal, but the height of the villi was reduced (Fig. 2D) . Some patients had some morphological features: in 8 out of 38 samples there were signs of mucus hypersecretion, the presence of areas of fibrosis and vascular plethora: 1 patient had foci of atrophy of the villi, as well as leukostasis in single vessels; in 1 patient, the mucous membrane was thickened, the vessels looked full-blooded, moderate diffuse infiltration of the intestinal tissue with eosinophilic leukocytes was recorded.

In 12.5% of tissue samples from patients (1 out of 8) with stage 3 CRC, there was a pronounced diffuse chronic inflammation, the height of some of the villi was reduced. Also, in 12.5% (1 out of 8), villi were unevenly reduced in size or were absent, and inflammatory infiltration was minimal (Fig. 2G).

According to OCT data in the mucous membrane of the small intestine in the 1st (Fig. 2B, C) and 2nd (Fig. 2E, F) stages of CRC in all patients, the tissue structure is preserved: on cross-sectional images (Fig. 2B, E white arrows), the contours of the villi and, partially, the crypts are clearly visible, the border of the mucosa and submucosa is visible. There were no significant differences in the OCT picture between the 1st and 2nd stages of CRC, however, at the 2nd stage, the contours of the villi on the cross-sectional images are seen more clearly (Fig. 2E), on the en-face images, the villi have a larger diameter (Fig. 2F) compared with the 1st stage (Fig. 2C), which can be explained by a decrease in their height and a slight thickening. In preparations of the small intestine of patients with the 3rd stage of cancer development, according to the OCT study, the contours of the villi were fuzzy, in some cases indistinguishable; the mucosa and submucosa were poorly differentiated (Fig. 2H, I).

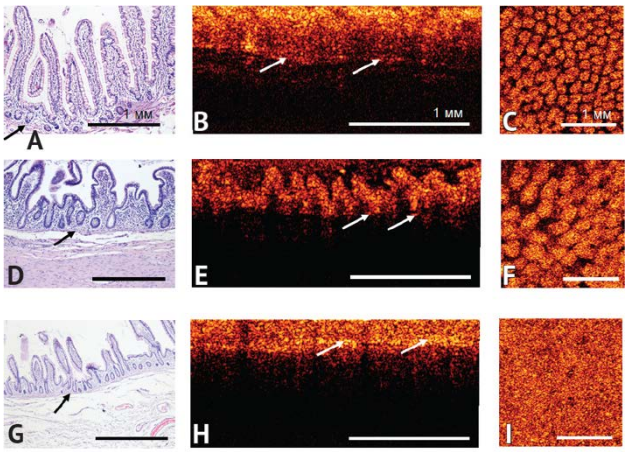

Fig. 2. Histologic picture (A, D, G); cross-sectional (B-scans) (B, E, H) (top view) and en-face (C, F, I) optical coherence tomography of the image in colorectal cancer of the 1st, 2nd and 3rd stages, respectively

The analysis of the mean lifetime of NAD(P)H fluorescence (τm) showed a statistically significant decrease in this parameter in patients with stage 3 compared with stages 1 and 2 of CRC. At the 1st stage of CRC Me [Q 1; Q 3] indicator tm was 1.61 [1.3; 2.02] ns. In patients with stage 2 CRC, the tm value was 1.5 [1.36; 1.73] ns. At the 3rd stage, the tm indicator was 1.37 [1.22; 1.51] ns. Statistically significant differences in the values of τm were also recorded between the 2nd and 3rd (p =0.031), 1st and 3rd (p =0.018) stages of CRC. There were no statistically significant differences between the 1st and 2nd stages. Figure 3 shows macro FLIM images typical for each group of the parameter tm (ns) of endogenous tissue fluorescence.

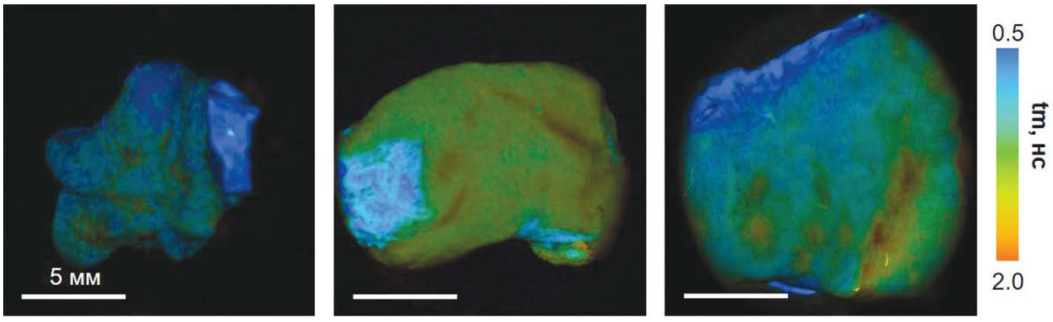

Fig. 3. Macro-FLIM images of tm (ns) parameter of endogenous fluorescence of the tissues of the mucous membrane of the small intestine of patients in 3 studied groups: with 1 (A), 2 (B) and 3 (C) stage of colorectal cancer. λ ex=375 nm, λ im=435–485 nm

Thus, in the study, for the first time, characteristic parameters of NAD(P)H autofluorescence in the small intestine in normal conditions and in colorectal cancer were obtained using macro-FLIM technology. The identified differences in the lifetime of NAD(P)H fluorescence in ileal enterocytes may indicate the predominance of the glycolysis process as the stage of colorectal cancer increases (from stage 1 to stage 3).

The study obtained the first characteristic parameters of the normal small intestine structure using FLIM technology. The metabolism in the mucosa was statistically significantly different in patients with stages 1 and 3, stages 2 and 3 of CRC. There were no statistically significant differences between stages 1 and 2.

DISCUSSION

In this work, a comprehensive study of the biochemical and microstructural features of the small intestine in patients with CRC of various stages was performed for the first time using modern methods of optical bioimaging — macro-FLIM and OCT.

Currently, one of the promising methods for assessing the energy metabolism of living cells and tissues is fluorescent time-resolved imaging (FLIM) with the determination of the fluorescence decay parameters of the metabolic cofactor NAD(P)H [23]. The non-phosphorylated form of the cofactor in the form of the NAD/NADH redox pair is one of the main cofactors involved in cellular respiration, which is carried out through the processes of oxidative phosphorylation in mitochondria and anaerobic glycolysis in the cytosol. The phosphorylated analog of NADH, NAD(P)H, regulates lipid, amino acid, and nucleotide biosynthetic pathways and protection against reactive oxygen species by glutathione (GSH) 2. The fluorescence spectra of NADH and NAD(P)H are almost identical, but the concentration of NAD(P)H in the cell is usually extremely low and is not recorded by FLIM, so the results of the study are often interpreted as NADH.

The NADH molecule exists in free and protein-bound forms, which differ in fluorescence lifetime [24]. The free form of NADH is found in the cytoplasm of the cell and is associated with glycolysis, in contrast to the bound form, which is located in the mitochondria and is associated with oxidative phosphorylation. It has been shown that an increase in the intensity of glycolysis or inhibition of cellular respiration leads to an increase in the contribution of the free form of NAD(P)H to fluorescence decay and, as a consequence, to a decrease in the mean lifetime [25].

FLIM method can be implemented in the form of laser scanning microscopy and macroimaging [26]. On the one hand, FLIM microscopy, having a subcellular resolution, makes it possible to analyze the metabolic status at the level of individual cells, but, on the other hand, the small size of the field of view (no more than 1 mm) and complex technical design limit its use in the clinic. The advantage of FLIM macroimaging over two-photon lifetime microscopy is the ability to quickly examine sufficiently large tissue samples, which is of interest for further clinical use of this method. FLIM is mainly used in cell culture experiments and animal disease models. There are few works using FLIM to study material from patients. For example, articles have been published describing the first results of the clinical application of FLIM to assess cell metabolism in bladder cancer [27], CRC [28], glioblastomas [29], and skin neoplasms [30]. Studies on the use of the FLIM method in assessing the morphofunctional state of the small intestine in patients have not yet been conducted.

The transformation of the intestinal structure leads to disturbance of its function in the form of malassimilation syndrome, which includes malabsorption and maldigestion. Extended surgical interventions on the colon are also very often accompanied by the above syndromes due to disturbances in the processes of humoral and nervous regulation of the alimentary canal and changes in the intestinal biocenosis [31]. Malabsorption refers to impaired absorption of nutrients in the intestine, and maldigestion refers to impaired digestion of nutrients in the intestinal lumen or intestinal wall.

For a comprehensive solution of urgent clinical problems in patients with CRC in the postoperative period, as the first stage of the study, we proposed the study of the morphofunctional state of the small intestine using FLIM and OCT technologies. However, OCT in this work acts as a method for detecting gross structural disorders, when histologically, the villi are unevenly reduced in size or absent. At the same time, local changes in the villi, such as atrophy of individual sections of the villi, fibrosis zones, etc., cannot be traced due to the deformation of the villi from the probe during the study and their uneven compression, as well as the inability to straighten all the folds, install the probe and see the true height and shape of the villi.

The problem of finding the optimal method for assessing the metabolism of the small intestine epithelium in patients with various pathologies of the gastrointestinal tract is relevant. Due to the peculiarities of the anatomical location of the small intestine, there is a problem of limited use of the endoscopic method; Of the existing methods of endoscopic examination, only capsule endoscopy can be used, but the above method does not allow studying the structure of the intestine over its entire thickness. Magnetic resonance imaging and positron emission tomography do not allow assessing the microstructure of the intestine due to their low resolution. The use of the SDFM (sidestream dark field imaging method) method is limited due to insufficient depth of examination of the intestinal wall. OCT and fluorescein angiography methods are used to assess the structure and function of the small intestine, but they only allow visualization of tissue morphology, but not its functional state. Thus, the advantages of metabolic macro-FLIM technology in assessing the structure and function of the small intestine in CRC are beyond doubt. It should be noted that this method can be used to assess metabolic changes in the intestine under various pathological conditions.

CONCLUSIONS

FLIM method, based on recording endogenous fluorescence of the cofactor nicotinamide dinucleotide (phosphate), was used for the first time in assessing the metabolic status of the small intestinal mucosa in patients with stage 1–3 colorectal cancer. The identified changes presumably indicate the predominance of the glycolysis process with increasing stage of colorectal cancer. Concomitant structural changes in the small intestine were verified by optical colorectal tomography and standard histological examination. Thus, it has been demonstrated that FLIM allows ex vivo assessment of the structure and metabolism of the intestinum in general, and in the ileal mucosa in particular in patients with colorectal cancer, which will further expand the scope of its use in patients with urgent conditions after extensive intestinal surgery with malassimilation syndrome.

REFERENCES

1. Palmisano S, Silvestri M, Troian M, Germani P, Giudici F, de Manzini N. Ileocaecal valve syndrome after surgery in adult patients: myth or reality? ColorDis . 2017;19(8):288–295. PMID: 28622448 https://doi.org/10.1111/codi.13778
2. Hope C, Reilly J, Lund J, Andreyev H. Systematic review: the effect of right hemicolectomy for cancer on postoperative bowel function. Support Care Cancer . 2020; 28(10):4549–4559. PMID: 32430603 https://doi.org/10.1007/s00520-020-05519-5
3. Massironi S, Cavalcoli F, Rausa E, Invernizzi P, Braga M, Vecchi M. Understanding short bowel syndrome: current status and future perspectives. Dig LiverDis . 2020;52(3):253–261. PMID: 31892505. https://doi.org/10.1016/j.dld.2019.11.013
4. Larsen HM, Borre M, Christensen P, Mohr Drewes A, Laurberg S, Krogh K, Fassov J. Clinical evaluation and treatment of chronic bowel symptoms following cancer in the colon and pelvic organs. Acta Oncol. 2019;58(5):776-781. PMID: 30698052 https://doi.org/10.1080/0284186X.2018.1562211
5. Louis M, Johnston SA, Churilov L, Ma R, Christophi C, Weinberg L. Financial burden of postoperative complications following colonic resection: A systematic review. Medicine (Baltimore) . 2021;100(27):e26546. PMID: 34232193 https://doi.org/10.1097/MD.0000000000026546
6. Yde J, Larsen HM, Laurberg S, Krogh K, Moeller HB. Chronic diarrhoea following surgery for colon cancer-frequency, causes and treatment options. Int J Colorectal Dis . 2018;33(6):683–694. PMID: 29589108 https://doi.org/10.1007/s00384-018-2993-y
7. Zuvarox T, Belletieri C. Malabsorption Syndromes. 2021 Jul 30. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–. PMID: 31971746
8. Vasil'ev SV, Popov DE, Semenov AV. Rannyaya posleoperatsionnaya enteral'naya podderzhka bol'nykh rakom tolstoy kishki. Koloproktologia . 2015;S1(51):63–63a.
9. Gracie DJ, Kane JS, Mumtaz S, Scarsbrook AF, Chowdhury FU, Ford AC. Prevalence of, and predictors of, bile acid malabsorption in outpatients with chronic diarrhea. Neurogastroenterol Motil . 2012;24(11):983–538. PMID: 22765392 https://doi.org/10.1111/j.1365-2982.2012.01953.x
10. Rao SSC, Tan G, Abdulla H, Yu S, Larion S, Leelasinjaroen P. Does colectomy predispose to small intestinal bacterial (SIBO) and fungal overgrowth (SIFO)? Clin Transl Gastroenterol. 2018;9(4):146. PMID: 29691369 https://doi.org/10.1038/s41424-018-0011-x
11. Cosnes J, Gendre JP, Le Quintrec Y. Role of the Ileocecal valve and site of intestinal resection in malabsorption after extensive small bowel resection. Digestion. 1978;18(5–6):329–336. PMID: 750260 https://doi.org/10.1159/000198220
12. Keane C, Wells C, O'Grady G, Bissett IP. Defining low anterior resection syndrome: a systematic review of the literature. Color Dis. 2017;19(8):713–722. PMID: 28612460 https://doi.org/10.1111/codi.13767
13. Koda K, Yamazaki M, Shuto K, Kosugi C, Mori M, Narushima K, et al. Etiology and management of low anterior resection syndrome based on the normal defecation mechanism. Surg Today. 2019;49(10):803–808. PMID: 30937634 https://doi.org/10.1007/s00595-019-01795-9
14. Ketelaers SHJ, van Heinsbergen M, Orsini RG, Vogelaar FJ, Konsten JLM, Nieuwenhuijzen GAP, et al. Functional Bowel Complaints and the Impact on Quality of Life After Colorectal Cancer Surgery in the Elderly. Front Oncol. 2022;12:832377. PMID: 35242714 https//doi.org/10.3389/fonc.2022.832377
15. Silviera ML, Smith BP, Powell J, Sapienza C. Epigenetic differences in normal colon mucosa of cancer patients suggest altered dietary metabolic pathways. Cancer Prev Res (Phila) . 2012;5(3):374–384. PMID: 22300984 https://doi.org/10.1158/1940-6207.CAPR-11-0336
16. Toumi W, Ripalti A, Ricciardiello L, Cherif A, Gargouri D, Bouhafa A, et al. Detection of a new JCV strain of genotype A in a subpopulation of colorectal adenocarcinomas in Tunisia. New Microbiol. 2017;40(2):99–106. PMID: 28255604 PMID: 28255604
17. Coelho TR, Gaspar R, Figueiredo P, Mendonça C, Lazo PA, Almeida L. Human JC polyomavirus in normal colorectal mucosa, hyperplastic polyps, sporadic adenomas, and adenocarcinomas in Portugal. J Med Virol . 2013;85(12):2119–2127. PMID: 24009184 https://doi.org/10.1002/jmv.23705
18. Zlokachestvennye novoobrazovaniya obodochnoy kishki i rektosigmoidnogo otdela. Klinicheskie rekomendatsii. 2020. Available at: https://legalacts.ru/doc/klinicheskie-rekomendatsii-zlokachestvennye-novoobrazovanija-obodochnoi-kishki-i-rektosigmoidnogo-otdela/ [Accessed Apr 27, 2023]
19. Lukina MM, Shimolina LE, Kiselev NM, Zagainov VE, Komarov DV, Zagaynova EV, et al. Interrogation of tumor metabolism in tissue samples ex vivo using fluorescence lifetime imaging of NAD(P)H. Methods Appl Fluoresc . 2019;8(1):014002. PMID: 31622964 https://doi.org/10.1088/2050-6120/ab4ed8
20. Shcheslavskiy VI, Shirmanova MV, Dudenkova VV, Lukyanov KA, Gavrina AI, Shumilova AV, et al. Fluorescence time-resolved macroimaging. Opt Lett. 2018;43(13):3152–3155. PMID: 29957804 https://doi.org/10.1364/OL.43.003152
21. Gelikonov VM, Romashov VN, Shabanov DV, Ksenofontov SYu, Terpelov DA, Shilyagin PA, et al. Cross-Polarization Optical Coherence Tomography with Active Maintenance of the Circular Polarization of a Sounding Wave in a Common Path System. Radiophys Quantum Electronics. 2018;60:897–911. https://doi.org/10.1007/s11141-018-9856-9
22. Moiseev A, Ksenofontov S, Sirotkina M, Kiseleva E, Gorozhantseva M, Shakhova N, et al. Optical coherence tomography-based angiography device with real-time angiography B-scans visualization and hand-held probe for everyday clinical use. J Biophotonics . 2018;11(10):e201700292. PMID: 29737042 https://doi.org/10.1002/jbio.201700292

23. Shirmanova MV, Shcheslavskiy VI, Lukina MM, Becker W, Zagaynova EV. Exploring tumor metabolism with time-resolved fluorescence methods: from single cells to a whole tumor. In: Tuchin V, Popp J, Zakharov V. (eds). Multimodal optical diagnostics of cancer. Chapter 3. Springer; 2020. p. 133–155. https://doi.org/10.1007/978-3-030-44594-2_3
24. Rück A, Hauser C, Mosch S, Kalinina S. Spectrally resolved fluorescence lifetime imaging to investigate cell metabolism in malignant and nonmalignant oral mucosa cells. J Biomed Opt . 2014;19(9):96005. PMID: 25202900 https://doi.org/10.1117/1.JBO.19.9.096005
25. Liu Z, Pouli D, Alonzo CA, Varone A, Karaliota S, Quinn KP, et al. Mapping metabolic changes by noninvasive, multiparametric, high-resolution imaging using endogenous contrast. Sci Adv . 2018;4(3):eaap9302. PMID: 29536043 https://doi.org/10.1126/sciadv.aap9302
26. Shcheslavskiy VI, Shirmanova MV, El'tsov A, Bekker V. Lyuminestsentnaya mikroskopiya na osnove mnogoparametricheskogo vremya-korrelirovannogo scheta fotonov. Uspekhi biologicheskoy khimii. 2019;59:103–138. (In Russ.)
27. Suarez-Ibarrola R, Braun L, Pohlmann PF, Becker W, Bergmann A, Gratzke C, et al. Metabolic Imaging of Urothelial Carcinoma by Simultaneous Autofluorescence Lifetime Imaging (FLIM) of NAD(P)H and FAD. Clin Genitourin Cancer. 2021;19(1):e31–e36. PMID: 32771335 https://doi.org/10.1016/j.clgc.2020.07.005
28. Lukina MM, Shimolina LE, Kiselev NM, Zagainov VE, Komarov DV, Zagaynova EV, et al. Interrogation of tumor metabolism in tissue samples ex vivo using fluorescence lifetime imaging of NAD(P)H. Methods Appl Fluoresc . 2019;8(1):014002. PMID: 31622964 https://doi.org/10.1088/2050-6120/ab4ed8
29. Lukina M, Yashin K, Kiseleva E, Alekseeva A, Dudenkova V, Zagaynova EV, et al. Label-Free Macroscopic Fluorescence Lifetime Imaging of Brain Tumors. Front Oncol. 2021;11:666059. PMID: 34109119 https://doi.org/10.3389/fonc.2021.666059
30. Luo T, Lu Y, Liu S, Lin D, Qu J. Phasor-FLIM as a Screening Tool for the Differential Diagnosis of Actinic Keratosis, Bowen's Disease, and Basal Cell Carcinoma. Anal Chem. 2017;89(15):8104–8111. PMID: 28661125 https://doi.org/10.1021/acs.analchem.7b01681
31. Sivokhina TA, Zolotareva TG, Kozhina MV. Opyt primeneniya preparata Rezolor u patsientok, perenesshikh khirurgicheskoe lechenie po povodu raka sigmovidnoy kishki. In: Novye tekhnologii v onkologii: sbornik materialov nauchno-prakticheskoy konferentsii . Samara; 2014: 188–189. Available at: https://www.elibrary.ru/download/elibrary_25358234_95890786.pdf [Accessed Apr 27, 2023]

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