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Morphological Features of Mitochondria Isolated from Tumor and Apparently Healthy Tissue of the Rectum in Patients Depending on the Presence of Metastases

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ABSTRACT

Aim. To examine the ultrastructural characteristics of mitochondria isolated from rectal adenocarcinoma tissue and apparently healthy tissue along the tumor resection line in patients of both sexes, depending on the presence or absence of metastases.

Materials and methods. The study encompassed the analysis of 28 patients diagnosed with rectal cancer, classified as T2–3N0M0 and T2–3N1–3M1, with tumor differentiation grade G2. Mitochondria were isolated from tumor and intestinal tissue cells via differential centrifugation. The mitochondrial suspension was then placed in a fixative solution containing formaldehyde and glutaraldehyde, followed by immersion in pure Epon-812 resin (SPI Inc., USA). The resin was allowed to cure for a period of 72 hours at a temperature of 70° C. Standard methodologies were employed to prepare the sections, which were then examined using a Jeol JEM-1011 electron microscope (JEOL Inc., Japan). The mitochondrial perimeter on images obtained by electron microscopy was determined using the Fiji program.

Results. A comprehensive ultrastructural study and statistical analysis of the perimeter size of mitochondria isolated from rectal adenocarcinoma in patients of both sexes were conducted. The results indicated that there were no statistically significant intergroup differences in mitochondrial size, categorized as conditionally small (0–1 μm), medium (1–2 μm), and large (>2 μm), depending on the presence or absence of metastases. In contrast to the tumor tissue, in apparently healthy tissue along the rectal resection line, the proportion of mitochondria with a perimeter of > 2 μm, characterized by the most pronounced dysfunction, decreased by 3.6 times in women and by 4.8 times in men without metastases compared to the indicators in all patients with metastases ($p < 0.05$).

Conclusion. The significance of differences in the perimeter dimensions of mitochondria isolated from apparently healthy tissue at the resection line of rectal adenocarcinoma, regardless of gender differences in patients, indicated the possibility of a morphofunctional relationship between processes occurring at the ultrastructural mitochondrial level and metastasis of rectal cancer.

Keywords: rectal adenocarcinoma, mitochondria, metastasis

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Conformity with the principles of ethics. All patients signed an informed consent to collection and transfer of biological material and to carrying out of studies and state assignments for socially sensitive purposes. The study

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was approved by the Ethics Committee at N.N. Blokhin Russian Cancer Research Center (Minutes No. 1 dated 30.01.2023).

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Морфологические особенности митохондрий, изолированных из опухоли и условно здоровой ткани прямой кишки, у пациентов в зависимости от наличия метастазов

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РЕЗЮМЕ

Цель. Изучение ультраструктурных особенностей митохондрий, изолированных из ткани аденокарциномы прямой кишки и условно здоровой ткани по линии резекции опухоли, у пациентов обоего пола в зависимости от наличия или отсутствия метастазов.

Материалы и методы. В исследование включены результаты, полученные от 28 больных раком прямой кишки T2–3N0M0 и T2–3N1–3M1, степень дифференцировки опухоли – G2. Митохондрии из клеток тканей опухоли и кишки выделяли с применением дифференциального центрифугирования. Взвесь митохондрий помещали в фиксирующий раствор, содержащий формальдегид и глутаральдегид, далее в чистую смолу Epon-812 (SPI Inc., США) и отверждали в течение 72 ч при 70 °C. Применяли стандартные методы подготовки срезов, которые исследовали с помощью электронного микроскопа Jeol JEM-1011 (JEOL Inc., Япония). Определение периметра митохондрий на изображениях, полученных методом электронной микроскопии, проводилось с использованием программы FiJi.

Результаты. На основе ультраструктурного исследования и статистического анализа размерности периметра митохондрий, изолированных из аденокарциномы прямой кишки пациентов обоего пола, было установлено, что показатели условно малых (0–1 мкм), средних (1–2 мкм) и больших (> 2 мкм) размеров митохондрий не имели межгрупповых статистически значимых различий в зависимости от наличия или отсутствия метастазов. В отличие от опухоли, в условно здоровой ткани по линии резекции прямой кишки доля митохондрий с периметром более 2 мкм, характеризующихся наиболее выраженной дисфункциональностью, снижалась в 3,6 раза у женщин и в 4,8 раза у мужчин без метастазов по сравнению с показателями у всех пациентов с метастазами ($p < 0,05$).

Заключение. Значимость различий в показателях размерности периметра митохондрий, изолированных из условно здоровой ткани на линии резекции аденокарциномы прямой кишки, независимо от гендерных различий пациентов указывала на возможность морфофункциональной взаимосвязи процессов, протекающих на ультраструктурном митохондриальном уровне и метастазированием рака прямой кишки.

Ключевые слова: аденокарцинома прямой кишки, митохондрии, метастазирование

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии источника финансирования при проведении исследования.

Соответствие принципам этики. Все пациенты подписали информированное согласие на взятие и передачу биологического материала для проведения научных исследований, государственных заданий в общественно и социально полезных целях. Исследование одобрено этическим комитетом НМИЦ онкологии (протокол № 1 от 30.01.2023).

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INTRODUCTION

The principle of the unbreakable link between structure and function, that has long been developed in Russian science by the flagship of morphofunctional unity, Academician D.S. Sarkisov, has currently reached full understanding within the global community with the recognition of the equivalence of concepts previously considered as polar opposites at the fine ultrastructural level of mitochondria [1]. In major world publications, such as *Nature*, *Nat. Metabolism*, *Redox Biology*, and many others, hundreds of relevant articles and literature reviews have been published over the past 10 years, presenting a new paradigm regarding the versatility of mitochondria and declaring the expansion of mitochondrial science beyond the scope of function and dysfunction [2–5].

The tight link between mitochondrial structure and function led to the emergence of the concept of "morphofunctionality" of mitochondria [6], which included "morphofunctional analysis of mitochondria" as the "simultaneous quantitative determination of morphological and functional parameters of mitochondria." T.T. Renault et al. (2015) showed that both mitochondrial hyperfusion and hyperfragmentation protected cells from BAX-mediated apoptosis, suggesting that a specific mitochondrial shape is required for BAX to induce mitochondrial outer membrane permeabilization [7].

Furthermore, it was established that in response to low ADP concentrations, the structure of the inner mitochondrial membrane (IMM) changed: the mitochondrial matrix contracted and condensed ("condensed state"), and then became less dense and more stretched ("orthodox state"), which was associated with a more compact arrangement of the cristae [8]. A study by Y. Wang et al. (2017) demonstrated that mitochondrial fragmentation allowed for the release of Ca2+ from the endoplasmic reticulum into the cytosol, which promoted phagocytosis by stimulating Ca2+-mediated vesicle transport [9]. Mitochondrial structure has been shown to influence the cell cycle phase, and vice versa [10].

For example, cyclin-dependent kinase 5 (CDK5) inhibits dynamin-related protein 1 (DRP1) via phosphorylation [11], and mitochondria form a hyperfused network during the transition from the G1 to the S phase, associated with cyclin E accumulation [12]. The latter study also showed that $\Delta\psi$ depolarization at the onset of G1 prevented cell cycle progression into the S phase [12].

One of the most important aspects of mitochondrial research is associated with their unique and indispensable role in oncogenesis and cancer progression [5, 13, 14]. Although the mechanisms of mitochondrial reprogramming in cancer have recently received closer attention, the specifics of organelle adaptation during this process have not been widely considered [15–17]. Meanwhile, the microenvironment in which a tumor grows is extremely unfavorable for mitochondria, as unstable oxygen concentrations and oxidative radicals can disrupt organelle integrity, disintegrate the regulation of numerous mitochondrial functions, and activate cell death [18]. Questions regarding how mitochondria cope with the loss of their "functional shape" and what the impact of low-quality or damaged mitochondria is on tumor characteristics, including the transfer of malignant information, remain poorly understood and understudied [19].

In early 2025, Guidelines for the Nomenclature and Characterization of Mitochondrial Transfer developed by more than 30 scientists from different

countries around the world were published [20]. These guidelines significantly eased the complex task of describing all forms of mitochondria, whether structurally damaged, intact, or fragmented. They indicate the necessity for mitochondria to be characterized, at a minimum, in terms of size, as this is a physical characteristic that can be measured using various methods. Thus, studies at the level of mitochondria isolated from tissues can contribute not only to expanding the understanding of the link between functional and morphological aspects but also to determining the role of mitochondrial dimensions in tumor progression, particularly in the processes of metastasis.

The aim of this study was to investigate the ultrastructural features of isolated mitochondria derived from rectal adenocarcinoma tissue and apparently healthy tissue along the tumor resection line in patients of both sexes, depending on the presence or absence of metastases.

MATERIALS AND METHODS

The study included results obtained from 28 patients with rectal cancer classified as T2–3N0M0 and T2–3N1–3M1, with a median age of 66 (58–73) years. None of the patients included in the study received neoadjuvant treatment prior to surgery. The tumor differentiation grade in all patients corresponded to G2. Rectal resection of the affected organ was performed with the removal of the malignant tumor.

Mitochondria were isolated from tumor cells and apparently healthy rectal tissue using differential centrifugation on the Avanti J-E high-speed refrigerated centrifuge (BECKMAN COULTER, USA) according to generally accepted methods [21, 22]. To disrupt intercellular bonds, cell walls, and plasma membranes, mechanical tissue processing was used, including mincing with scissors and homogenization in a glass homogenizer with a Teflon pestle (Potter-Elvehjem homogenizer). For each gram of tissue, 10 ml of sterile isolation medium was added (0.22 M mannitol, 0.3 M sucrose, 1 mM EDTA, 2 mM TRIS-HCl, 10 mM HEPES, pH 7.4).

Tissues were homogenized and centrifuged for the first time for 10 min at 1,000 g at 0–2° C; the second and third centrifugations were performed at 20,000 g for 20 min at 0–2° C. Between centrifugations, the mitochondrial pellet was resuspended in the isolation medium. Mitochondria were further purified from lysosomes, peroxisomes, melanosomes, etc. by centrifuging in a 23% Percoll gradient. The suspension of subcellular structures was layered onto the Percoll gradient and centrifuged for 15 min at 21,000 g; after this, separation into 3 phases was observed, the bottom layer of mitochondria was retained and resuspended with the isolation medium. The next purification of the mitochondria was carried out by centrifugation for 10 min at 15,000 g at 0–2° C.

A portion of the intestinal tissues and the mitochondrial suspension isolated from the tumor and apparently healthy rectal tissue were immediately placed in a fixative solution containing formaldehyde and glutaraldehyde. After pre-treatment, the tissue sample was embedded in pure Epon-812 resin (SPI Inc., USA) and cured for 72 hours at 70° C. Ultrathin 90-nm sections were obtained using an ultramicrotome equipped with a diamond knife. The sections were mounted on copper slot grids and stained with a 2% aqueous solution of uranyl acetate for 40 min and lead citrate for 2 min. The sections were examined and photographed using the Jeol JEM-1011 electron microscope (JEOL Inc., Japan).

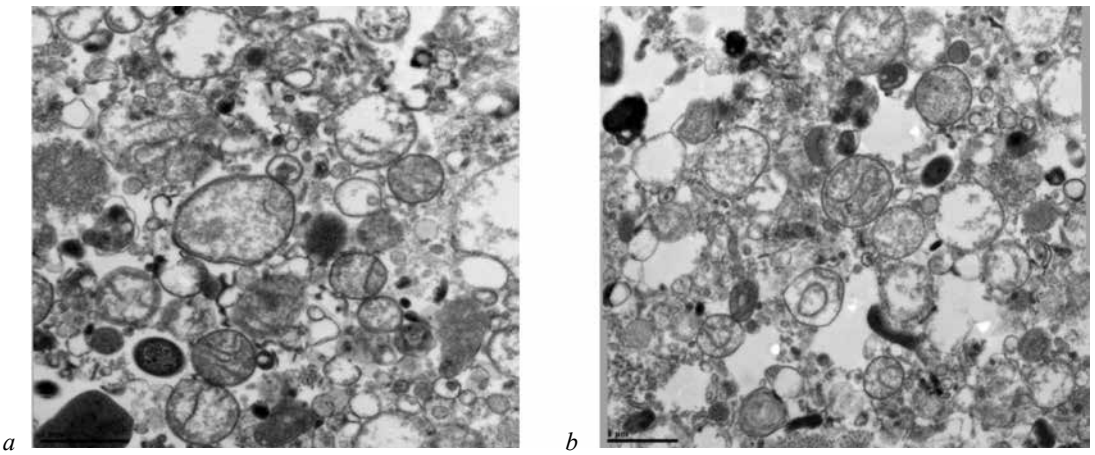
Mitochondrial perimeters were determined on electron microscopy images using Fiji software. Before starting the analysis, the image scale was manually set using the scale bar present on each micrograph. Mitochondria were selected using the Freehand Selection tool. When necessary, contrast correction and image smoothing were also performed to improve the visualization of mitochondrial boundaries. After tracing each mitochondrion, the result was saved as a region of interest (ROI), and the obtained contours were added to the ROI Manager. Based on these selections, the software automatically calculated the perimeter of each mitochondrion. To increase the reproducibility and transparency of the analysis, a markup map was created for each image: files with the applied mitochondrial contours were saved separately, which allowed for subsequent verification of the accuracy of object identification.

Statistical analysis of the results was performed using the Statistica 10.0 software package. Normality distribution was assessed using the Shapiro – Wilk test (for small samples). Quantitative data in groups (independent samples) were compared using the Student's *t*-test and the Mann – Whitney *U*-test. A value of $p < 0.05$ was considered as the limit of statistical significance. Data in tables were presented

as $M \pm m$, where M is the arithmetic mean, and m is the standard error of the mean (SEM).

RESULTS

In all patients with rectal adenocarcinoma, morphological signs of dysfunction of mitochondria isolated from the tumor were noted on ultrathin serial sections, which was manifested by a significant variation in the size and shape of the organelles, ranging from ovoid and rounded to polygonal (Fig. 1). The electron micrograph shows that a significant part of the mitochondrial population (over 40%) with large and medium perimeters is in a swollen state. Total mitochondrial swelling could indicate greater coupling and increased ATP synthesis within them, i.e., the probability of a high metabolic rate. According to the literature, the intensity of mitochondrial respiration in pathological conditions can double, but without a significant change in the efficiency of oxidative phosphorylation [23, 24].



a *b*

Fig. 1. Electron micrograph of mitochondria isolated from rectal adenocarcinoma in men: *a* – with detected metastases; *b* – without metastases. Manifestation of local swelling (left arrow) and the granular osmiophilic component (right arrow); × 50,000

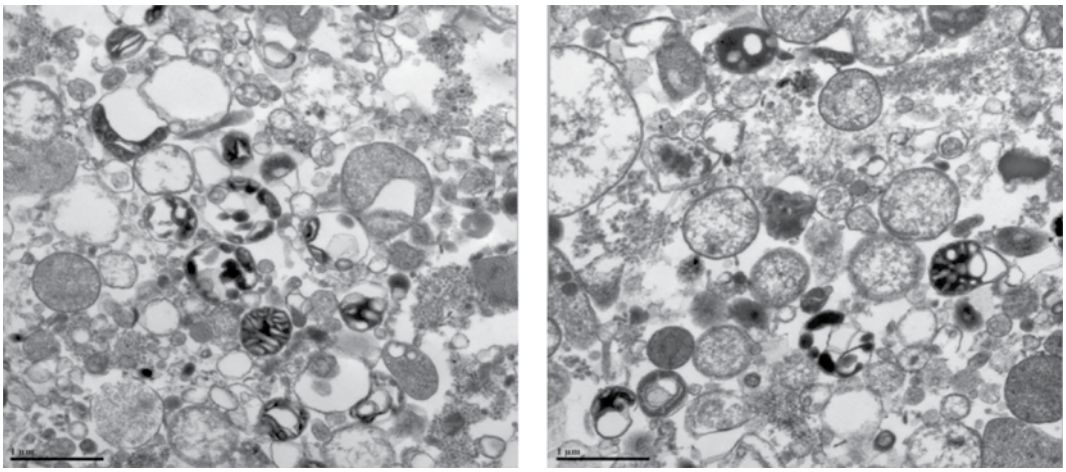
It was noted that in swollen, enlarged mitochondria, the structure of the cristae was substantially deformed and limited to single elongated or shortened invaginations of the inner membrane, oriented radially with an increase in the intermembrane space.

The ultrastructure of mitochondria in rectal adenocarcinoma of men without metastases and with metastases was similar in heterogeneity and was supplemented by data on the state of the mitochondrial matrix, which was completely or partially filled with inclusions of increased electron density. The matrix filling was heterogeneous, since the inclusions either filled the entire matrix or formed clumps in the marginal zones closer to the IMM (Fig. 1, *b*). On ultrathin sections, the outer membrane of the mitochondria was clearly contoured, in most cases retaining its integrity or having small ruptures. The pathological presentation of mitochondrial heterogeneity of the tumor in men without metastases was supplemented by the manifestation of local swelling and a slight deposition of the granular osmiophilic component inside individual organelles (indicated by arrows in Fig. 1, *b*).

In women with metastatic rectal adenocarcinoma, similar morphological signs of dysfunction of mitochondria isolated from the tumor were noted on ultrathin serial sections, as were observed in men (Fig. 2).

A pronounced variety in the size and shape of the organelles was observed: ovoid, rounded, and less commonly, polygonal. In more than 50% of cases, mitochondrial swelling with deformation or absence of cristae was noted. Single mitochondria were identified with relatively uniform cristae striation, high osmiophilic activity of inclusions, and increased electron optical density of the matrix. Marginal deposits in the matrix of rounded fragments of the osmiophilic component, associated with deformed cristae, could be of a lipid nature.

When analyzing electron micrographs of mitochondria isolated from rectal adenocarcinoma cells in women without detected metastases (Fig. 2, *b*), no distinctive features of the ultrastructure were noted compared to previous studies.



a *b*

Fig. 2. Electron micrograph of mitochondria isolated from rectal adenocarcinoma in women: *a* – with detected metastases; *b* – without metastases; × 50,000

Thus, the microscopic presentation of mitochondria isolated from tumor cells in men and women, regardless of the presence of metastases, was characterized by comparatively similar features: heterogeneity of shape and enlarged size, total mitochondrial swelling with significant deformation of the cristae, altered electron density, and osmiophilic deposits in the matrix and cristae.

Upon studying the apparently healthy rectal tissue along the resection line in men and women with or without metastases, the infiltration of unaffected tissue by tumor mitochondria was revealed (Fig. 3).

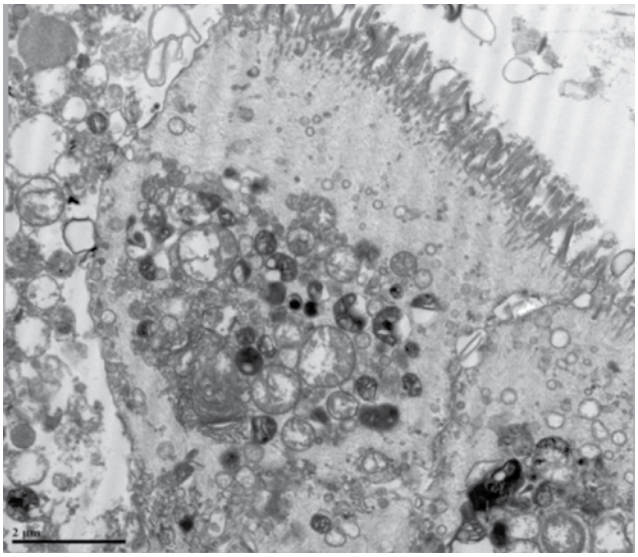


Fig. 3. Electron micrograph demonstrating the variation in the size and shape of mitochondria in apparently healthy rectal tissue along the tumor resection line; × 25,000

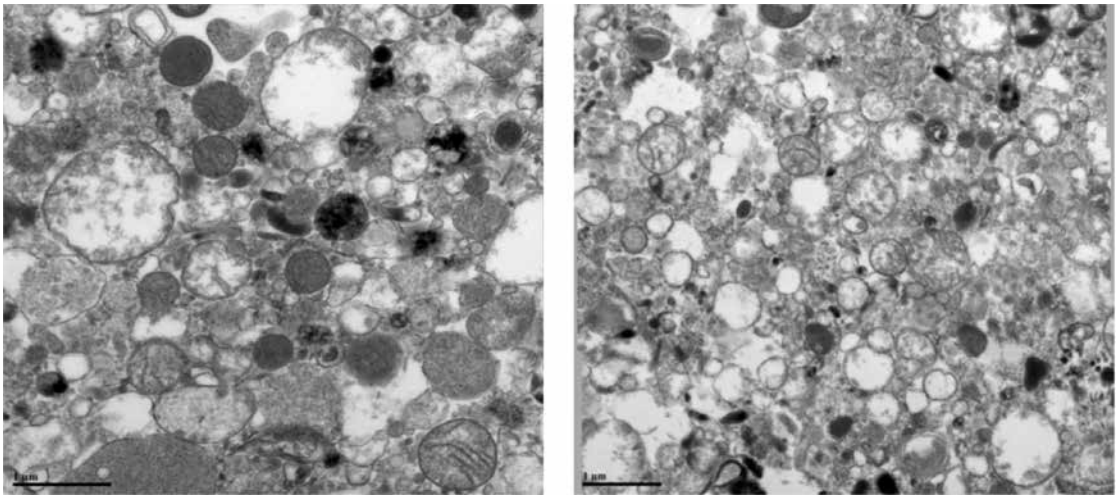
As an example, we present a micrograph that convincingly demonstrates the ability of tumor mitochondria to translocate into the areas adjacent to the resection line, which were visually (by light microscopy) unaffected by the tumor process.

Electron microscopy of mitochondria isolated from apparently healthy rectal tissue in men with metastatic adenocarcinoma revealed the entire spectrum of morphological disorders. The micrograph (Fig. 4, *a*) shows swollen organelles of various size clusters, predominantly with increased electron density of the matrix and single elongated as well as multiple shortened cristae.

The outer mitochondrial membrane largely retained its integrity; however, fragmented areas of damage were also detected, including splitting of the outer and inner membranes with filling of the intermembrane space. The presence of process-like inclusions with high osmiophilic activity, associated with the cristae, within the mitochondria was notable. Pathological forms of mitochondria isolated from apparently healthy rectal tissue cells were also noted in men without metastases. However, the microscopic presentation changed somewhat, highlighting the predominance of small forms. With reduced mitochondrial dimensions, the mitochondrial matrix was characterized by moderate and low optical density, the membrane retained its integrity, and a cristae structure of varying visualization intensity and configuration was revealed.

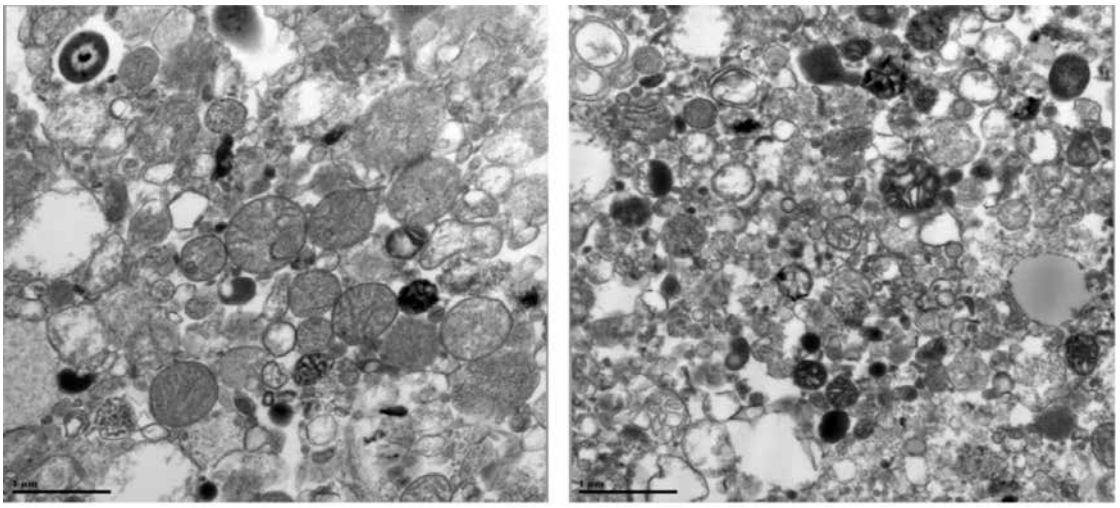
A similar discrepancy in mitochondrial size was visualized in apparently healthy rectal tissue among

women, depending on the presence or absence of adenocarcinoma metastases. It is important to emphasize that a fundamental condition was met during all studies of mitochondrial ultrastructure for the adequate comparison of their sizes: an identical electron microscope magnification of ×50,000, which allowed for the standardization of their dimensions.



a *b*

Fig. 4. Electron micrograph of mitochondria isolated from apparently healthy tissue along the resection line in men: *a* – with detected metastases; *b* – without metastases; × 50,000



a *b*

Fig. 5. Electron micrograph of mitochondria isolated from apparently healthy rectal tissue along the tumor resection line in women: *a* – with detected adenocarcinoma metastases; *b* – without metastases; × 50,000

Mitochondria isolated from apparently healthy tissue along the tumor resection line in women with metastases appeared larger compared to mitochondria without metastases (Fig. 5*, a*, *b*). Nevertheless, in both cases, swelling and a pronounced cristae structure were noted, associated to a greater or lesser extent with inclusions of high osmiophilic activity. The outer mitochondrial membranes generally maintained their integrity; however, thinning or even blurring of mitochondrial boundaries could be observed, as well as membrane splitting with the gap filled by the active medium resulting from mitochondrial metabolism.

Given the significant variation in mitochondrial sizes, we formed clusters of conditionally small (0–1 μm), medium (1–2 μm), and large (>2 μm)

mitochondria. Their perimeters were measured, followed by statistical processing of the results and calculation of the occurrence frequency of these mitochondrial dimensions in women and men with the presence and absence of metastases (Table).

Table

Occurrence Frequency (%) and Perimeter (μm) of Mitochondria Isolated from Tumor Cells and Apparently Healthy Rectal Tissue of Male and Female Patients with or without Metastases, $M \pm m$

Patient groups	Tumor			Apparently healthy tissue		
	0–1	1–2	> 2	0–1	1–2	> 2
Women with metastases, $n = 7$	65.5 ± 5.1% 0.63 ± 0.009 $n = 272$	22.9 ± 2.0% 1.46 ± 0.03 $n = 95$	11.6 ± 1.4% 2.43 ± 0.05 $n = 48$	50.6 ± 4.9% 0.69 ± 0.01 $n = 167$	35.2 ± 2.9% 1.45 ± 0.03 $n = 116$	14.2 ± 1.7% 2.41 ± 0.06 $n = 47$
Women without metastases, $n = 7$	49.6 ± 4.4% 0.69 ± 0.01 $n = 130$	36.3 ± 3.1% 1.45 ± 0.03 $n = 95$	14.1 ± 1.6% 2.43 ± 0.06 $n = 37$	61.5 ± 5.4% 0.68 ± 0.01 $n = 220$	34.6 ± 2.7% 1.32 ± 0.02 $n = 124$	3.9 ± 0.5% * 2.32 ± 0.06 $n = 14$
Men with metastases, $n = 7$	58.1 ± 5.3% 0.68 ± 0.01 $n = 122$	27.1 ± 2.2% 1.41 ± 0.04 $n = 57$	14.8 ± 1.9% 2.65 ± 0.12 $n = 31$	42.5 ± 3.9% 0.69 ± 0.02 $n = 119$	42.5 ± 3.7% 1.41 ± 0.03 $n = 119$	15.0 ± 1.6% 2.63 ± 0.09 $n = 42$
Men without metastases, $n = 7$	55.9 ± 4.9% 0.7 ± 0,02 $n = 85$	32.3 ± 2.7% 1.4 ± 0.04 $n = 49$	11.8 ± 1.5% 2.5 ± 0.08 $n = 18$	64.5 ± 5.1% 0.76 ± 0.01 $n = 82$	32.4 ± 2.5% 1.37 ± 0.03 $n = 71$	3.1 ± 0.2% * 2.48 ± 0.16 $n = 19$

* differences between the frequency of detection of the maximum mitochondrial dimension in the apparently healthy tissue of patients with and without metastases are statistically significant ($p < 0.05$).

The calculations revealed that in all patient groups across the three mitochondrial perimeter dimension clusters isolated from the tumor, the conventional ratio of perimeter values was maintained, with a predominance of small mitochondria (0–1 μm) (Table). Perimeter values of mitochondria > 2 μm in women and men with and without rectal adenocarcinoma metastases showed no significant intergroup differences and varied with an insignificant difference of 3%. Meanwhile, the relative content of small organoid forms (perimeter of 0–1 μm) exceeded the proportion of large forms by 5.6–3.1 times. The relative number of mitochondria belonging to the medium perimeter dimension of 1–2 μm occupied an intermediate position between the large and small mitochondria.

Upon the analysis of the perimeter values of mitochondria isolated from apparently healthy tissue cells, statistically significant differences were found in both women and men, depending on the presence or absence of metastases. These differences were characteristic only of the conditionally large mitochondrial cluster.

Thus, while the proportion of isolated mitochondria with a perimeter > 2 μm from apparently healthy tissue cells in women and men with metastases showed no statistically significant differences either between one another or compared to the mitochondria isolated from the tumor, a completely different situation was revealed in patients without metastases. In women without rectal adenocarcinoma metastases, the frequency of occurrence of mitochondria with a perimeter > 2 μm isolated from apparently healthy tissue decreased by 3.6 times compared to the mitochondria of the same dimension in women with detected metastases ($p < 0.05$). Simultaneously, a reciprocal increase in the proportion of small-dimension mitochondria (0–1 μm) was observed together with a decrease in the proportion of large organoids; however, the differences in the values were not significant in the context of detected metastases.

A similar pattern of changes was observed in men without metastases: against the background of an increase in the frequency of detecting organoids with a small perimeter (0–1 μm), the content of mitochondria with a perimeter > 2 μm isolated from apparently healthy tissue was 4.8 times smaller compared to the values when metastases were present ($p < 0.05$). In other words, the statistical significance of differences in the indicators of the large-perimeter mitochondrial cluster pointed to the possibility of a morphofunctional interrelation between processes occurring at the ultrastructural level and rectal cancer metastasis. A question arose as to what might cause this kind of determination of

mitochondrial morphogenesis in apparently healthy rectal tissue with metastasis?

DISCUSSION

We believe that the causal chain of events may be primarily linked to the specific characteristics of mitochondrial dysfunction during carcinogenesis. Large mitochondria represent the most unfavorable, yet effective, form of dysfunctional mitochondria, the excessive presence of which in the tumor poses a high risk of its death. What is this associated with? The increase in mitochondrial size, including the perimeter, is associated with swelling processes, which intensify when the production of Bcl-2, Bax, and Bak increases within the cell. These very apoptotic signals cause mitochondrial swelling and the formation of a special pore for the exit of biologically active molecules into the medium: cytochrome C, flavoproteins, reactive oxygen species (ROS), and mtDNA, which, upon entering the cytosol, trigger the program of cell death [25].

Based on this fundamental process, the following can be hypothesized. The statistically significant increase in the frequency of detection of mitochondria with perimeter > 2 μm in the apparently healthy rectal tissue of patients with metastases, compared to the same perimeter dimension in cases without metastases, may be related to various metabolic changes in the surrounding cellular environment. First and foremost, this may be due to the release of factors involved in the pathological reprogramming of genetic and metabolic processes, including ROS, acting as mediators of targeted mitochondrial transfer [26, 27].

Additionally, cancer-associated fibroblasts (CAFs), which participate in the metabolic reprogramming of cancer cells and promote tumor progression, may also contribute to this [28]. Consequently, the morphological differences in mitochondria observed by us in the apparently healthy rectal tissue of patients with metastases may indirectly indicate involvement in the processes of metastasis. These prerequisites may be associated with the accumulation of unfavorable factors in the form of enlarged, deformed mitochondria, whose dysfunction is combined with the ability to actively translocate in media and tissues and which possess potent signaling regulation of structural and metabolic processes during tumor growth.

As noted previously, the present study was aimed at identifying the ultrastructural features of mitochondria isolated from rectal adenocarcinoma tissue and apparently healthy tissue along the tumor resection line in patients of both sexes, depending on the presence or absence of metastases.

However, this problem is much broader than just the morphological aspects and may encompass many more unaccounted factors. These include violations of mitophagy, an increase in extracellular vesicles (EVs) which can engulf large mitochondria and facilitate their transfer to the extracellular space [29, 30]. In this regard, it should be noted that a population of mitochondria-derived extracellular vesicles (EV-Mitos) plays an important role in the realization of mitochondrial dysfunction toward metastasis. These vesicles contain numerous mitochondrial components (the mitochondrial cargo), the composition of which reflects changes in the parent cell.

It is fundamentally important to consider that metastasis is a complex, multi-stage process dependent not only on intracellular changes but also on angio- and lymphangiogenesis, expression of adhesion molecules, EMT, and features of the immune microenvironment. For more detailed investigation, control of variables, such as tumor stage, level of invasion, tumor mutational profile, tumor microenvironment, Ki-67 index, etc., is required. The present study represents only a limited portion of changes at the subcellular level within the multifaceted processes of rectal cancer metastasis.

Nevertheless, considering the strategic role of mitochondria in the processes of carcinogenesis, one cannot but to note that even the observed morphological changes in these organoids already at the tumor resection line may manifest events related to the implementation of the tumor progression program. An increase in the number of dysfunctional, enlarged mitochondria in the zone relatively close to the tumor may serve as one of the significant signals on the path to metastasis.

CONCLUSION

Morphological differences were revealed when comparing the spatial dimension of mitochondria isolated from adenocarcinoma and apparently healthy rectal tissue in patients of both sexes with or without metastases. These differences were associated with a statistically significant increase in the population of large-dimension mitochondria

(perimeter > 2 μm) only in the apparently healthy tissue along the tumor resection line in cases where metastasis was detected, compared to their content in the absence of metastases ($p < 0.05$). The increase in mitochondrial dimension, coupled with morphological signs of dysfunction, characterizes an increase in the malignant potential of apparently healthy rectal tissue, which may contribute to the progression of the tumor process.

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Kit O.I., Kolesnikov E.N. – final approval of the manuscript for publication. Shikhlyarova A.I., Ilchenko S.A. – substantiation of the manuscript and critical revision of the manuscript for important intellectual content. Neskubina I.V., Cheryarina N.D., Kozhushko M.A., Anisimov A.E. – critical revision of the manuscript for important intellectual content. Kirichenko E.Yu., Logvinov A.K., Legenko N.N. – analysis and interpretation of the data. Rodkin S.V., Frantsiyants E.M. – conception and design.

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