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ORIGINAL ARTICLE

Apoptosis-Inducing Factor (AIF) content in tumor cell mitochondria from colorectal cancer patients

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ABSTRACT

Purpose of the study. To investigate the level of protein AIF in the mitochondria of tumor cells and visually unchanged tissues of the colon in male and female patients with colorectal cancer.

Materials and methods. The study included results, obtained from 132 patients with stage T2-3N0M0 colon cancer, comprising 52 women and 80 men. Mitochondria were isolated from human colon and tumor tissue cells using differential centrifugation in a high-speed refrigerated centrifuge. The concentration of protein AIF (pg/mg protein) in mitochondria was determined using ELISA «Human AIF Elisa Kit» (Cloud-CloneCorp., China).

Results. It was established that in males, the AIF level in the mitochondria of rectal, sigmoid colon and ascending colon tumor cells was 2.4 times, 1.9 times ($p < 0.05$) and 3.1 times higher, respectively, than in the mitochondria of the corresponding tissues not affected by the tumor. In the mitochondria of the intestinal tissue not affected by the tumor, significant differences in the AIF content were observed, with levels varying depending on the anatomical location. In the sigmoid colon, the level of this factor was found to be 1.9 ($p < 0.05$) and 2.6 times higher than in the rectum and ascending colon, respectively. Concurrently, no notable discrepancies in the AIF concentration within the mitochondria of conditionally unimpaired tissues were observed in the female subjects. The AIF content was found to be higher in the mitochondria of tumor cells in women than in conditionally intact tissues. Specifically, it was observed to be 2.1 times higher in rectal tumors, 4.4 times higher in sigmoid colon tumors and 1.7 times ($p < 0.05$) higher in ascending colon tumors. Significant discrepancies in the AIF content between men and women, as well as between the mitochondria of tumor sample cells, were identified. In the rectal and ascending colon tumor, the AIF level in women was found to be markedly elevated in comparison to men, exhibiting a ratio of 1.3 ($p < 0.05$) and 2.4, respectively.

Conclusion. In patients with colorectal cancer, the content of AIF in tumor mitochondria is observed to increase. This can be considered to represent stimulation mechanism of tumor proliferative activity due to its NADH/NADPH oxidase function, which promotes the survival of malignant cells.

Keywords: mitochondria, colorectal cancer, males, females, AIF, tumor tissue, intestinal tissue

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Compliance with ethical standards: the study was carried out in compliance with the ethical principles set forth in the World Medical Association Declaration of Helsinki, 1964, ed. 2013. Signed informed consent was received from all patients for the removal and transfer of biological material for scientific research, government assignments for socially and socially useful purposes. Protocol No. 1 of the Ethics Committee of the National Medical Research Center for Oncology, the Russian Federation Ministry of Health, was approved on 01/30/2023

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3.1.6. Онкология, лучевая терапия
ОРИГИНАЛЬНАЯ СТАТЬЯ

Содержание апоптоз-индуцирующего фактора (AIF) в митохондриях клеток опухоли у больных колоректальным раком

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РЕЗЮМЕ

Цель исследования. Исследовать уровень белка AIF в митохондриях клеток опухоли и визуально неизмененных тканей отделов толстой кишки у больных колоректальным раком.

Пациенты и методы. В исследование включены результаты, полученные у 132 больных раком толстой кишки со стадией T2-3N0M0, из которых 52 составили женщины и 80 мужчин. Митохондрии из клеток тканей кишки и опухоли человека выделяли с применением дифференциального центрифугирования на высокоскоростной рефрижераторной центрифуге. В митохондриях методом ИФА определяли концентрацию белка AIF (пг/мг) с использованием тест-системы «Human AIF Elisa Kit» (Cloud-CloneCorp., China).

Результаты. Выявлено, что у мужчин, в митохондриях клеток опухоли прямой кишки, сигмовидной кишки и восходящего отдела ободочной кишки уровень AIF был выше, чем в митохондриях соответствующих тканей, не пораженных опухолью, в 2,4 раза, в 1,9 раз ($p < 0,05$) и в 3,1 раза соответственно. В митохондриях непораженной опухолью ткани кишки отмечали значимые различия в содержании AIF в зависимости от анатомического расположения: в сигмовидной кишке уровень данного фактора оказался в 1,9 ($p < 0,05$) и в 2,6 раза выше, чем в прямой и восходящем отделе ободочной кишки. При этом у женщин значимых различия в уровне AIF в митохондриях условно непораженных тканей не выявлено. В митохондриях клеток опухоли у женщин содержание AIF было выше, чем в условно интактных тканях: в опухоли прямой кишки в 2,1 раза, в опухоли сигмовидной кишки в 4,4 раза, в опухоли восходящего отдела ободочной кишки в 1,7 раза ($p < 0,05$). Установлены значимые различия в содержании AIF у мужчин и женщин и в митохондриях клеток опухолевых образцов: в опухоли прямой кишки и восходящего отдела ободочной кишки, у женщин уровень AIF был значимо выше, чем у мужчин от в 1,3 раза ($p < 0,05$) и в 2,4 раза.

Заключение. У больных колоректальным раком в митохондриях опухолей возрастает содержание AIF, которое можно рассматривать как механизм стимулирования пролиферативной активности опухоли за счет своей NADH/NADPH оксидазной функции, способствующей выживанию злокачественных клеток.

Ключевые слова: митохондрии, колоректальный рак, мужчины, женщины, AIF, ткань опухоли, ткань кишки

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Соблюдение этических стандартов: в работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Получено от всех пациентов подписанное информированное согласие на взятие и передачу биологического материала для проведения научных исследований, государственных заданий в общественно и социально-полезных целях. Протокол № 1 этического комитета ФГБУ «Национальный медицинский исследовательский центр онкологии» Министерства здравоохранения Российской Федерации утвержден 30.01.2023 г.

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BACKGROUND

Colorectal cancer (CRC) is a malignant tumor derived from the glandular epithelial cells of the colon; it is the third most frequently diagnosed cancer that ranks second in terms of mortality in the world [1–4]. CRC is a genetically heterogeneous disease that includes various molecular pathways of tumor formation and metastasis [5].

Mitochondria are vital for energy production, cell signaling, and metabolic homeostasis. Meanwhile, mitochondria influence processes such as cellular differentiation and proliferation. In malignant cells, mitochondrial functions undergo transformation, which promotes rapid proliferation, survival, and resistance to neoplasm death [6]. During the development of CRC, mitochondrial dysfunction and mutations in the tumor suppressor gene TP53 were revealed, which leads to impaired regulation of the cell cycle, mitochondrial respiration, cellular metabolism, and an imbalance between survival and cell death [7, 8]. Malignant cells have metabolic flexibility, using and regulating the tricarboxylic acid cycle not only for survival and proliferation, but also and to evade immune "surveillance" and suppress the cytotoxic function of immune cells [6].

The cellular protein AIF was initially identified as a 57 kDa soluble fragment that is released from mitochondria during apoptosis and translocated into the nucleus in a caspase-independent manner, causing caspase-independent chromatin condensation and DNA fragmentation [9].

It is currently recognized that AIF plays a vital role in mitochondrial bioenergetics under physiological conditions, as it supports normal oxidative phosphorylation of the cell, influencing multiple catabolic and anabolic pathways, as well as epigenetic processes that depend on mitochondrial metabolites [9]. It was found that the oxidoreductase activity of AIF gives malignant cells resistance to oxidative stress and supports their transformational status [10, 11]. Undoubtedly, the available data on the role of AIF in mitochondrial metabolism in the development of the malignant process, both in experiment and in the clinic, indicate the important role of this factor [12]. However, to date, there is not enough data on the functional features of AIF in human oncological diseases, in particular in the mitochondria of tissue cells in colorectal cancer.

Purpose of the study was to investigate the level of AIF protein in the mitochondria of tumor cells and visually unchanged colon tissues in patients with colorectal cancer.

PATIENTS AND METHODS

The study included the results obtained from 132 patients with T2-3N0M0 colon cancer, 52 of them women and 80 men. The average age was 66 (58–73) years, 68 (51.5 %) people were over the age of 65, and 64 (48.5) people were under the age of 65. 46 (34.8 %) patients suffered from sigmoid colon cancer, including 19 women, 44 (33.7 %) patients with rectal cancer, including 18 women, and 42 (31.5 %) patients with ascending colon cancer, including 15 women. The tumor differentiation grade in all patients corresponded to G2. None of the patients received neoadjuvant treatment before surgery. 98.5 % of patients had a good indicator status (ECOG 0 or 1). All patients were operated on. The work followed the ethical principles set forth in the World Medical Association Declaration of Helsinki, 1964, ed. 2013. Signed informed consent was received from all patients to take and transfer biological material for scientific research, government assignments for socially and socially useful purposes. Protocol No. 1 of the Ethics Committee of the National Medical Research Center for Oncology was approved on 01/30/2023.

During the operation, after laparotomy, the colon affected by the tumor was mobilized with ligation and intersection of the feeding blood vessels, then lymphodissection was performed and the affected organ was resected (right-sided hemicolectomy, left-sided hemicolectomy, sigmoid colon resection, rectal resection) to remove the malignant tumor from the patient. A part of the tumor material and a fragment of intestinal tissue along the resection line were immediately placed in a cold sterile isolation medium containing 0.22 M mannitol, 0.3 M sucrose, 1 mM EDTA, 2 mM TRIS-HCL, 10 mM HEPES, pH 7.4. The further course of the operation was completed with a restorative stage with the application of an intestinal anastomosis, drainage of the abdominal cavity and suturing of the laparotomy wound.

Mitochondria from tumor and intestinal tissue cells were isolated using differential centrifuga-

tion on an Avanti J-E high-speed refrigerated centrifuge, BECMAN COULTER, USA using the method of Egorova MV, Afanasyev SA, 2011; Gureev AP, Kokina AV, 2015 [13, 14]. To destroy the intercellular connections, cell wall and plasma membranes, mechanical processing of tissues with crushing with scissors and homogenization in a glass homogenizer with a Teflon pestle (Potter-Elwegame homogenizer) was used. 10 ml of sterile isolation medium (0.22 M mannitol, 0.3 M sucrose, 1 mM EDTA, 2 mM TRIS-HCL, 10 mM HEPES, pH 7.4) was added to each gram of tissue. The tissues were homogenized and centrifuged for the first time for 10 min at a speed of 1000 g, a temperature of 0–2 °C. The second and third centrifugation was carried out at 20,000 g, 20 min, a temperature of 0–2 °C. Between centrifugation, the mitochondrial precipitate was resuspended in the isolation medium. Mitochondria were additionally purified from lysosomes, peroxisomes, melanosomes, etc., by centrifugation in a 23 % percolation gradient. The suspension of subcellular structures was layered on a Percall gradient, centrifuged for 15 min at 21000 g, after which separation into 3 phases was observed, the lower layer of mitochondria was left and resuspended with isolation medium. The next "washing" of mitochondria was carried out by centrifugation for 10 min at 15000 g, temperature 0–2 °C. The obtained mitochondrial samples were stored at –80 °C before analysis. Before the analysis, the samples were diluted to a protein concentration of 6 g/l [13]. ELISA was used to determine concentrations of: AIF (pg/mg protein) (Cloud-CloneCorp., China), protein by biuretic method (Olvex Diagnostics, Russia).

Statistical analysis

Statistical analysis of the results was performed using the Statistica 10.0 software package. The distribution of normality was evaluated using the Shapiro-Wilk criterion (for small samples). The comparison of quantitative data in groups (independent samples) was carried out using the Student and Mann-Whitney criteria. The value of $p < 0.05$ was retained as the limit of statistical significance. The table data is presented as M ± m, where M is the arithmetic mean and m is the standard error of the mean.

STUDY RESULTS

Statistically significant differences in the level of AIF factor were revealed, both in tumor tissue and in intact tissue, depending on gender. Thus, in women, the concentration of AIF in the mitochondria of cells of the rectal and ascending colon tissue exceeded the values in similar samples in men by 1.6 times ($p < 0.05$) and 2.4 times, respectively (Table 1). Only in the mitochondria of cells of the sigmoid colon tissue in men, the content of AIF was higher than in women 1.8 times ($p < 0.05$).

A comparative analysis of the AIF content in the mitochondria of tumor cells showed a statistically significantly higher level of the studied factor, compared with the values in the mitochondria of the corresponding tissue. Thus, in men, in mitochondrial samples of tumors of the rectum, sigmoid colon and ascending colon, the AIF level was statistically significantly higher than in the mitochondria of the corresponding tissues not affected by the tumor by 2.4 times, 1.9 times ($p < 0.05$) and 3.1 times, respectively (Table 1). The highest AIF index in men was found in the mitochondria of the sigmoid colon tumor – it exceeded the corresponding level in the mitochondria of the rectal and colon tumors by an average of 1.6 times. At the same time, significant differences in AIF content were also noted in the mitochondria of non-tumor-affected tissue, depending on the anatomical location: in the sigmoid colon, the level of this factor was 1.9 ($p < 0.05$) and 2.6 times higher than in the rectum and ascending colon.

In women with CRC, there were no significant differences in the level of AIF in the mitochondria of conditionally unaffected tissues, while in the mitochondria of tumor cells the content of this factor was statistically significantly higher than in conditionally intact tissues: in tumors of the rectum by 2.1 times, in tumors of the sigmoid colon by 4.4 times, in tumors of the ascending colon by 1.7 times ($p < 0.05$).

Statistically significant differences in the AIF content in men and women and in the mitochondria of tumor samples were revealed: in tumors of the rectum and ascending colon, the AIF level in women was significantly higher than in men by 1.3 times ($p < 0.05$) and 2.4 times. No significant sex differences were found in the mitochondria of sigmoid colon tumor cells.

DISCUSSION

There are scientific studies of several active substances that stimulate the death of malignant cells, both in cell lines and in tumors in patients by influencing apoptosis factors, including AIF [2, 3, 15]. These studies are relevant and promising, since limiting the uncontrolled growth of malignant cells and inducing apoptosis in them would be a promising direction in the fight against malignant tumors. However, there is a problem of reducing the expected percentage of positive effect of various targeted antitumor drugs, which can be explained by the multifunctionality of the selected targets. Thus, in addition to participating in the signaling pathway of cell apoptosis, AIF plays a key role in maintaining mitochondrial homeostasis, is an important factor in maintaining the functional integrity of the mitochondrial respiratory chain and participates in the regulation of the redox state of cells by regulating the activity of NADF-hydroxylase to influence the levels of reactive oxygen species (ROS) [9, 16].

Our study revealed a seemingly paradoxical (given its pro-apoptotic properties) increase in the level of AIF in all samples of the mitochondria of the CRC tumor, compared with the conditionally intact intestine in both men and women. There are several other works that also describe increased AIF levels in ovarian, prostate, and hemoblastosis cancers [17–19].

The revealed paradox of overexpression of AIF in mitochondrial tumor samples in colorectal cancer can be explained by the polyfunctionality of this factor, since AIF allows mitochondria to regulate cell survival due to the balance between pro-apoptotic and energetic pathways [16]. Malignant cells reprogram their metabolism to support the increased biosynthetic and energy requirements necessary for their growth and motility, and AIF is overexpressed to maintain cellular energy and metabolic homeostasis.

In order for AIF to perform its apoptotic function, it must be cleaved into a soluble apoptotic protein with a mass of 57 kDa in the mitochondria and translocated through the mitochondrial membrane to the nucleus. The release of apoptosis factors in mitochondria is caused by the loss of mitochondrial function, which is closely related to an increase in mitochondrial membrane permeability and depolarization of membrane potential [20]. At the same time, the release of AIF from mitochondria towards the nucleus, for the initiation of apoptosis, is regulated by many factors [21, 22]. In particular, studies on the example of breast cancer have shown that P21-activated kinase 5 (PAK5), which is an oncogenic pro-

Table 1. AIF content in the mitochondria of male and female colon tissue cells (pg/mg protein)

Samples	Males	Females
Rectal tissue		
Intact	237.7 ± 14.1	381.4 ± 30.9 p^2 = 0.0000
Tumor	559.3 ± 36.9 p^1 = 0.0000	817.7 ± 49.9 p^1 = 0.0000 p^2 = 0.0001
Sigmoid tissue		
Intact	451.5 ± 20.8	247.8 ± 16.6 p^2 = 0.0000
Tumor	859.3 ± 57.9 p^1 = 0.0000	1087.1 ± 69.9 p^1 = 0.0000
Ascending colon tissue		
Intact	176.8 ± 8.9	426.4 ± 39.1 p^2 = 0.0000
Tumor	541.4 ± 30.2 p^1 = 0.0000	716.4 ± 85.3 p^1 = 0.0044 p^2 = 0.0247

Note: statistically significant differences in relation to: p^1 is the indicator in the corresponding tissue along the resection line; p^2 is the indicator in men

tein and is overexpressed in multiple cancers and promotes tumor progression [23, 24], can prevent the release of AIF from mitochondria by reducing the permeability of mitochondrial membranes and increasing membrane potential and inhibit apoptosis by phosphorylation of AIF. This fact indicates that in malignant cells, the balance of AIF functional activities shifts from pro-apoptotic to a predominance of energetic, as a result of which AIF is localized on the inner membrane of mitochondria and performs the function of oxidoreductase [25]. It has been shown that depletion of AIF in tumor cells leads to metabolic reprogramming, inhibition of cell proliferation and tumor growth, elimination of cancer stem cells, changes in inflammation in the tumor microenvironment, and induction of differentiation of malignant cells into normal cells [26].

CONCLUSION

Thus, in patients with colorectal cancer, the content of AIF increases in the mitochondria of tumors, which can be considered as a mechanism for stimulating the proliferative activity of the tumor due to its NADH/NADPH oxidase function, which promotes the survival of malignant cells.

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