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ADIPOSE TISSUE AND ITS ROLE IN MICROENVIRONMENT OF THE COLORECTAL ADENOCARCINOMA CANCER CELL

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Introduction. *The mechanisms of adipose-tissue's influence on tumor progression has been studied a lot, but the way of interaction of adipocytes with tumor cells have not been well defined until now.*

Objective. *The aim of this study was to evaluate the mechanisms of adipocytes and tumor cells interaction under the influence of radiation and chemo-radiation therapy in locally advanced rectal cancer (LARC) patients.*

Material and methods. *A prospective randomized single-center study was conducted. It involved 110 patients with LARC and pre-obesity. The patients were randomized into a main group A (radiation therapy and oxaliplatin-based chemotherapy) and a comparison group B (radiation therapy and fluoropyrimidine-based mono-chemotherapy). Superoxide free radicals and NO levels generated by mitochondria of adipocytes were evaluated In both groups'. Also, there was estimated the indices of MMP-2, MMP-9, 8-oxoG, and free fatty acids (FFA) level.*

Results and discussion. *Level of superoxide radicals in tumor-adjacent adipose tissue was 0.58±0.15 (main group) and 0.70±0.12 nmol/g·min (comparison group) (p<0.001). Blood levels of FFA increased in group A up to 2.05±0.15, and in group B up to 2.48±0.20 mmol/l (while in it was 0.57±0.11 mmol/L). 8-oxoG levels in tumor-adjacent adipose tissue had no statistically significant differences.*

Conclusions. *The tumor-adjacent adipose tissue is an energy depot that can act as a promoter of tumor progression supplying the locally advanced rectal cancer with an energy substrate FFA. It has been established that the level MMP-2 activity significantly reduces the degree of intercellular matrix remodeling by the XELOX chemotherapy.*

KEY WORDS: **locally advanced rectal cancer; adipose tissue; tumor progression; chemoradiotherapy.**

Introduction

A number of clinical studies have proved a direct effect of obesity on the epidemiology of colorectal cancer (CRC), disease progression, and a direct effect of adipose tissue (AT) on malignant neoplasms development, as well as the 'survival' of the latter. Adipocyte is the main structural unit of AT that provides a significant resource of lipids, cytokines and adipokines. It has been established that the lipids, cytokines and adipokines provide regulation of signaling metabolic cascades and spread of malignant cells, including CRC adenocarcinoma cells.

Adipocytes can modify a tumor microenvironment that in its turn stimulates/accelerates the tumor metabolism and leads to formation of an aggressive phenotype of CRC tumor due to paracrine secretion and the presence of significant levels of free fatty acids (FFA). The cells of malignant neoplasms can accelerate the rate of proliferation, which is impossible without the modification of energy metabolism and the initiation of *de novo*-lipogenesis [1]. FFA are used for the accumulation of adipose tissue and the membranes construction, which is the basis for the malignant cells' survival. Most studies have proved that lipids provide survival of adenocarcinoma cells while their own *de novo*-lipogenesis is in a state of inhibition [2]. Thus, it becomes clear that adenocarcinoma cells carry out proliferation without performing the synthesis of their own energy but using energy rich fatty acids of microenvironment [3]. FFA as a powerful source of energy becomes a key element in an aggressive rectal cancer (RC) phenotype formation. It worth remembering that lipids can enhance the Warburg effect in tumor cells [4].

Over the past decade, the awareness of the mechanisms of the AT influence on RC tumor progression has improved considerably. However, the mechanisms of adipocytes and tumor cells interaction under the influence of radiation and chemo-radiation therapy have not been revealed until now.

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Material and methods

A prospective randomized single-center study was conducted. The study involved 110 patients with locally advanced rectal cancer (LARC) (ymrT3-4aN0-2M0-1, CRM-positive) with overweight (pre-obesity), who were treated in National Cancer Institute in January 2016 – December 2018. The patients were randomized into a main group (A) n=57 (radiation therapy with a total focal dose of 50.4 Gy (1.8 Gy×28) and oxaliplatin-based polychemotherapy) and comparison group (B), n=53 (radiation therapy with a total focal dose of 50.4 Gy (1.8 Gy×28) and mono-chemotherapy based on fluoropyrimidine). The patients' division into groups was 1:1. The characteristic features of the groups of patients are provided in Table 1.

In each clinical case, multidisciplinary approach was used, where surgeons, oncologists, chemotherapists and radiologists took part. In all cases, the diagnosis was confirmed cytologically/ histologically after the primary tumor biopsy taken during the colonoscopy in order to assess the resectability of the primary tumor, to detect metastatic lesions in regional lymph nodes and to identify possible distant metastases. A common method of magnetic resonance imaging (MRI) of the small pelvis and abdominal cavity with intravenous contrast and computer tomography (CT) of the chest cavity, abdominal cavity and small pelvis with intravenous contrast, according to international protocols was carried out [5]. Positron-emission tomography was conducted only for planned multivisceral resections and in clinically complicated cases as well as for differential diagnosis for the presence of metastatic lesions of other organs/sites.

Surgical techniques comprised implementation of standardized approaches for RC removing (Total mesorectal excision and D3 lymphatic dissection); most of the interventions were performed laparoscopically.

Sampling of AT was performed for 20 minutes since the removal of the tissue sample at a distance of 1-5 cm from the tumor; a surgeon and a morphologist took part in it. The samples were shaped by a special mold, frozen in liquid nitrogen, with subsequent electron paramagnetic resonance spectra (EPR), they were recorded in a paramagnetic pure quartz Devuar. The levels of superoxide free radicals (SR) generated by mitochondria of adipocytes, as well as the levels of NO content were evaluated by means of EPR method and Spin Traps technology.

The state of intracellular matrix was assessed by the activity of matrix metalloproteinases (MMP-2 and MMP-9) in polyacrylamide gel using zymography technique. After the washing the active forms of MMP-2 and MMP-9 in gel visualized as a discolored stripe on a blue background; their localization was determined according to the molecular weight standards (Sigma) and corresponded to the molecular weight of each of the enzymes (72 and 92 kDa, respectively). The proteolytic activity was evaluated by measuring the area of the lysis zone using a standard set of MMP-2 and MMP-9 (Sigma) for comparison. One a.u. of the enzyme activity assumed as 1 mcg of enzyme in 1 g of control sample. The results were pro-

Table 1. Characteristic features of the groups

Indices	Group A (n=57)	Group B (n=53)	P value
Age	61.4±2.3	64.2±1.9	0.16
Body mass index (BMI)	31.5±2.3	29.2±3.8	0.84
Sex (male/female), (n)	37/21	24/28	0.45
Presence of distant metastases, (n)	14	5	0.19
CRM positive	20	12	0.32
EMVI positive	9	3	0.44
TRG ≥3	10	4	0.25
pN+	28	14	0.21
CEA level	27.4±5.6	12.2±3.1	0.18
Patient's condition (ASA scale):			
I-II	47	39	0.65
III	10	14	0.67

Notes: CRM – 'circumferential resection margin'; EMVI – 'extramural venous invasion'; TRG – 'tumor regression grade'. ASA – American Society of Anesthesiologists scale.

cessed using the standard TotalLab 1.01 program. The levels of DNA guanine oxidation (marker 8-oxoG) in adipose tissue and blood FFA level were determined spectrophotometrically. The content of free (un-esterified) fatty acids (HF) in the blood was determined by spectrophotometric method at λ=546 nm (the intensity of the red dye formed by the interaction of hydrogen peroxide and Tronder compounds is directly proportional to the concentration of non-esterified fatty acids in the knowledge).

The attained results were statistically analyzed by means of SPSS 20.0 statistics (IBM, Armonk, New York, USA). The differences were statistically different at $p<0.05$.

Results

In AT superoxide radicals (SR) and their derivatives are produced by mitochondria and NOX immunocompetent cells. In physiological conditions there is a balance between the SR generation and its elimination by antioxidants. In cases of pathological conditions, in particular, in malignant tumors, there is an increase in SR levels both in tumor and in tissues surrounding it that affects signaling regulatory pathways of tumor cells.

The results of the AT studying are presented in Table. 2. The rate of SR generation in AT stripped at a distance of 1 cm from the macroscopically visible edge of the LARC tumors was determined. In the physiological conditions, the rate of SR generation was 0.18±0.03 nmol/g of tissue·min [6]. The rate of SR generation in tumor-adjacent AT was 0.58±0.15 (group A) and 0.70±0.12 nmol/g tissue·min (group B) ($p<0.001$). The increased blood level of FFA was evidenced in group A: 2.05±0.15 mmol/l, and in group B – 2.48±0.20 mmol/l, the control value was 0.57±0.11 mmol/L. Thus, the damaging effect on the surrounding tissues, in particular in cases of the radiation therapy and administration of platinum, is lower compare to the scheme comprising radiation therapy and fluoropyrimidines. The growth of SR and ROS, respectively, can cause lipids oxidation, 4-hydroxynonenal and malonic dialdehyde formation. The malonic dialdehyde can stimulate the activity of cyclooxygenase-2 (COX-2), which in turn catalyzes synthesis of prostaglandins and induces angiogenesis in RS tumors [7]. Fatty acids, the level of which increases in tumor during their own metabolism, produce electrons; their energy in mitochondrial ETC of tumor cells transform into ATP and SR due to fact that adipocytes mitochondria are dysfunctional (Table 2).

Oxidative DNA damage is also a result of unregulated increase of SR and ROS levels and their effects on human cells. The most common product of the pathological effect of SR on a DNA molecule is 8-oxoguanine (8-oxoG) formation; the level of it starts to exceed the normal values already in the early stages of the disease [8]. 8-oxoG can be accumulated both in nuclear and mitochondrial DNA. That is why they are considered to be a highly informative marker for the development of malignant neoplasms and their progression.

According to our previous studies, 8-oxoG levels in tumor-adjacent AT proved to have no statistically significant differences: 0.80±0.08 and 0.84±0.13, respectively for groups A and B, $p=0.052$ (Table 1 that may be explained by the fact that medicines used for hemotherapy lead to genotoxic effects). Platinum containing medicines can in addition initiate SR generation.

Nitric oxide (NO) is a pleiotropic, regulatory signaling molecule that is crucial for a variety of biological processes, including vasodilation, neurotransmission, and immune response [9]. NO is synthesized out of L-arginine by NO synthase (NOS) [10]. The increased expression of inducible NOS (iNOS) in tumor cells proves direct proportional correlation with survival of patients with RC and other malignant neoplasms of epithelial origin [11]. The activity of iNOS in tumor-adjacent adipose tissue is determined in accordance with the procedure described above. Normal value of the NO content is 1.16±0.20 nmol/g of tissue. Direct evaluation of NO level is 0.51±0.11 and 0.38±0.08 nmol/g of

Table 2. Comparison of the studied molecular markers between groups of patients.

Values	Norm values (Mean±SE)	Group A (Mean±SE)	Group B (Mean±SE)	t-value	P-value
Speed of SR generation, nmol/g tissue•min	0.18±0.03	0.58±0.15	0.70±0.12	4.43	<0.001
NO content level, nmol/g tissue	1.16±0.20	0.51±0.11	0.38±0.08	-7.24	<0.001
8-oxo-G level, nmol/g tissue	0.21±0.03	0.8±0.08	0.84±0.13	1.96	0.052
FFA levels, mmol/L	0.57±0.11	2.05±0.15	2.48±0.20	2.36	0.045

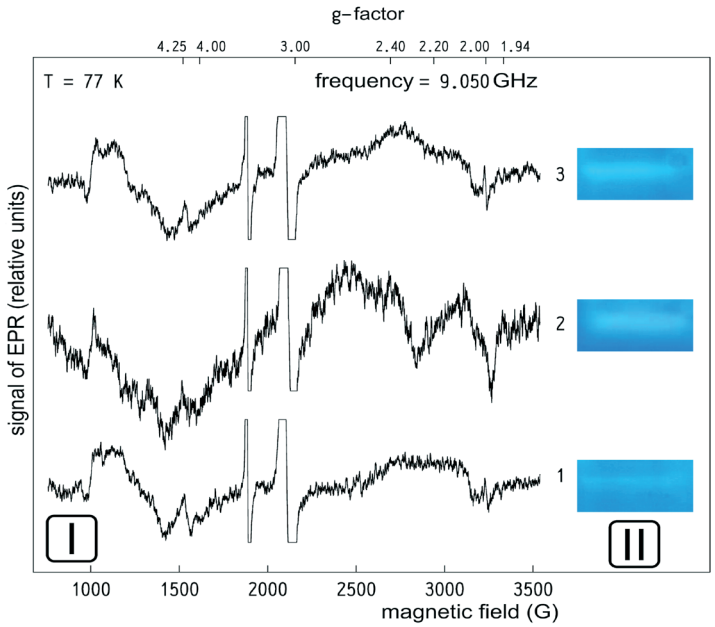

Fig.1. Graphic representation of EPR spectra in studied groups of patients.
I – EPR spectra of AT in patients with RC:
1 – AT of the patients with BMI 25.0–30.0.
2 – group B patients with BMI ≥25.
3 – group A patients with BMI ≥25.
II – zymograms showing MMP-2 activity in AT of the corresponding groups.

tissue in group A and B, respectively ($p<0.001$) (Table 2) that indicates a disturbance of NO bioavailability in tumor-adjacent AT.

The changes of AT cells mitochondrial ETC in the patients with RC in cases of different treatment regimens were revealed. In EPR spectra of AT intensive EPR signals with g=2.03 were recorded that proved formation and increase of NO-FeS proteins complexes in Complex I (ETC) of adipocytes mitochondria in the group A (Fig. 1, EPR 3 spectrum, g=2.03). The level of NO-FeS proteins complexes was 0.27±0.14 a.u. This value in norm was 0.08±0.05 a.u. In the group B of patients, who received fluoropyrimidines instead of oxaliplatin, the level of NO-FeS proteins complexes in ETC were significantly higher (0.36±0.09 a.u.) that indicated that fluoropyrimidines-based AT formed hypoxic microenvironment, which was more favorable for the tumor. The level of NO-FeS proteins complexes in ETC of adipocytes mitochondria correlates with the degree of RC differentiation ($r=0.79$, $p<0.05$). It should also be noted that the level of MMP-2 activity in AT was significantly lower in the group A, indicating that oxaliplatin had less significant effect on redox state of AT and subsequently the intracellular matrix remodeling.

Tissue intracellular matrix remodeling, including degradation and reorganization of its structures developed with the participation of specific enzymes: matrix metalloproteinases (MMP). MMPs are a family of Zn^{2+} and Cu^{2+}-dependent endopeptidases with a common functional domain and activation mechanism.

At present, more than 20 types of MMPs different by their cellular localization, substrate specificity, functional activity, have been established: gelatinase, collagenase, stromelysines, membrane bound MMP as well as a group of insufficiently studied MMP [12].

The increased level of gelatinase subgroup members (MMP-2 and MMP-9) proves the presence of tissue matrix damage. The factors activating the inactive forms of MMP (pro-MMP) are intensification of SR generation in endothelial cells mitochondria and NOX immunocompetent cells of AT; SR reacts with NO forming $ONOO^-$ as a result that leads to disorder of NO bioavailability in AT [11, 13]. It was already noted that in the patients, involved into the research, independently from the treatment method a decrease in the NO content in AT adjusted to tumor was evidenced (Table 2).

In Fig.2 the results of the gelatinases activity (MMP-2 and MMP-9) in the patients with RC is presented.

It was proved that MMP-2 activity rates were (3.51±1.06) and (5.72±1.13) at $p<0.001$, respectively in groups A and B. MMP-9 activity

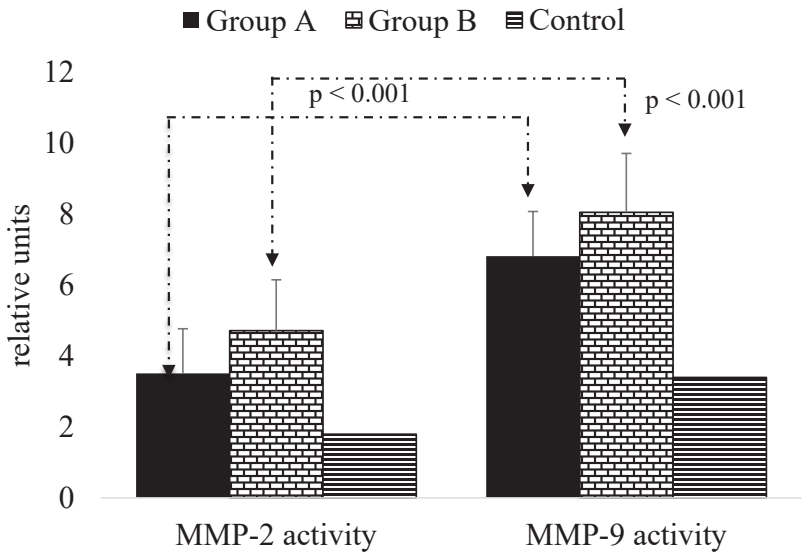

Fig. 2. MMP-2 AND MMP-9 activity in AT of the patients.

rate was (6.82±1.03) and (8.06±1.36) in the main and control groups, respectively (p<0.001). These results indicate that in the group A patients, who received oxaliplatin or capecitabine, the level of intercellular matrix degradation was lower compare to the group B patients administered with fluoropyrimidines.

Discussion

Redox molecules are capable of inducing lipolysis in adenocarcinoma cells that results in glycerol production, which can be incorporated into the glycolytic metabolic pathway [14]. Such pathological changes in normal metabolism can activate more rapid growth and subsequently much significant malignant metastatic tumor phenotype, including RC [15]. Such changes are accompanied by changes in the microenvironment of healthy cells, infiltration of tumor-associated immune cells that trigger the process of chronic inflammation and enhance oxidative stress. The analysis proves that activation of *de novo*-lipogenesis in most of the patients with CRC, is caused by high levels of CR and, consequently leads to MMP activation. The MMP activation, in its turn, proves that the adipocytes of tumor-adjacent AT in the patients with RC are an 'additional' source of energy involved in metabolic processes of the RC adenocarcinoma that contributes to their faster growth and formation of metastases.

In addition, a significant difference in the studied molecular markers proved a promising use of a modified scheme of preoperative course of chemotherapy-based oxaliplatin and capecitabine (XELOX). We believe that such approach can help reduce the percentage of distant metastasis and local relapses.

Conclusions

Tumor-adjacent AT is an energy depot that can act as a promoter of tumor progression, supplying the RC tumor with the energy substrate – FFA. In addition, the oxaliplatin based chemotherapy reduces the level of DNA oxidative-induced point mutations in adipocytes mitochondria. It has been established that the level MMP-2 activity significantly reduces the degree of intercellular matrix remodeling by the XELOX chemotherapy.

Conflict of interest

The author declares no conflict of interest.

ЖИРОВА ТКАНИНА ТА ЇЇ РОЛЬ В МІКРООТОЧЕННІ КЛІТИН АДЕНОКАРЦИНОМИ КОЛОРЕКТАЛЬНОГО РАКУ

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Вступ. *За останні десять років наше розуміння механізмів впливу жирової тканини (ЖТ) на пухлинне прогресування значно покращилось, однак до цього часу не розкрито механізми взаємодії адипоцитів з клітинами пухлин раку прямої кишки (РПК) за умов проведення променевої та хіміотерапії*

Мета. *Дослідити механізми взаємодії адипоцитів та клітин аденокарциноми за умов променевого та хіміопроменевого лікування у хворих на місцево поширені форми раку прямої кишки.*

Методи. *Було проведено проспективне рандомізоване одноцентрове дослідження. У дослідженні брали участь 110 хворих на місцево-поширений рак прямої кишки (мпРПК), (ymrT3-4aN0-2M0-1, CRM – positive) з надлишковою вагою в період з січня 2016 р. до грудня 2018 р. Пацієнтів рандомізували у співвідношенні 1:1 на основну групу (А) n=57 (променева терапія сумарною осередковою дозою 50,4 Гр (1,8 Гр х 28) та поліхімотерапія на основі оксаліплатини), та на групу порівняння (Б), n=53 (променева терапія сумарною осередковою дозою 50,4 Гр (1,8 Гр х 28) та монохіміотерапія на основі фторпіримідинів). У обох групах вивчали рівні мітохондріальних супероксидиних радикалів та NO в адипоцитах прилеглої до пухлини жирової тканини. Також досліджували рівень матриксних металопротеїназ (ММП-2 та ММП-9), маркери окисного пошкодження ДНК (8-oxoG) та рівень жирних кислот.*

Результати. *Зареєстровані рівні швидкості генерування супероксидних радикалів (СР) в ЖТ, яка контактувала з пухлиною складали 0,58±0,15 (група А) та 0,70±0,12 нмоль/г тканини•хв (група Б) (p<0,001). В крові цих хворих виявлено зростання рівня вільних жирних кислот (ВЖК): у групі А до значень 2,05±0,15 ммоль/л, а в групі Б цей показник склав 2,48±0,20 ммоль/л при значеннях норми 0,57±0,11 ммоль/л. Рівні 8-oxoG в прилеглій до пухлини ЖТ хворих досліджуваних груп не мали статистично значущої відмінності – 0,80±0,08 та 0,84±0,13 відповідно для груп А та Б, при p = 0,052.*

Висновки. *Прилегла до пухлини ЖТ представляє собою енергетичне депо, здатне виступати в ролі промотора пухлинного прогресування, забезпечуючи пухлину мпРПК енергетичним субстратом – ВЖК. Було показано, що хіміпроменева терапія (XELOX) знижує рівень ремоделювання міжклітинного матриксу прилеглої жирової тканини.*

КЛЮЧОВІ СЛОВА: **місцево-поширений рак прямої кишки; жирова тканина; пухлинне прогресування; хіміопроменева терапія.**

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