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GIANT FOAM-LIKE MACROPHAGES IN ADVANCED OVARIAN CANCER

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Abstract

Introduction. Ovarian cancer (OC) is the third most common gynecological cancer with the worst prognosis and highest mortality rate. The progression of OC can be accompanied by the detrimental functions of the components of the tumor microenvironment, including tumor-associated macrophages (TAMs). **The purpose of the study** to analyze distribution and morphological phenotype of TAMs in tumor tissue of patients with high-grade serous ovarian cancer (HGSOC). **Material and Methods.** Formalin fixed paraffin embedded tissue sections were obtained from ovarian cancer patients after tumor resection. The protein expression of general macrophage marker CD68 and M2-like markers CD206, CD163 and stabilin-1, belonging to scavenger receptors, was analysed by immunohistochemical staining in tumor tissue. Histological assessment of TAM distribution was performed by pathologist. Immunofluorescent analysis/confocal microscopy was applied to establish the co-expression of CD68 with the main macrophage scavenger receptors. **Results.** We were able to find giant CD68-positive macrophages with foamy cytoplasm in ovarian tumor tissue. The accumulation of these TAMs was specific only for patients with advanced stage (IIIC and IV stages). The presence of foam-like TAMs had a statistical tendency to be associated with ovarian cancer progression, including metastasis and recurrence. The distribution of stabilin-1-positive macrophages was matched to CD68 expression in almost all cases, as was shown by IHC. Confocal microscopy confirmed that stabilin-1 was expressed in at least 50 % of giant TAMs. IF analysis of tumor samples also demonstrated co-expression of other scavenger receptors, CD163 and CD36, in foam-like cells. Similar to IHC, in most samples the expression of CD206 in TAMs of foam-like morphology was limited. **Conclusion.** For the first time we demonstrated the accumulation of giant macrophages with fluffy foam cytoplasm in the tumor tissue of treated patients with advanced ovarian cancer. Such macrophages express diverse scavenger receptors (stabilin-1, CD163, CD36), thus indicating a high clearance activity of giant TAMs.

Key words: ovarian neoplasms, tumor-associated macrophages, foam-like cells, receptors, scavenger.

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ГИГАНТСКИЕ МАКРОФАГИ С ПЕНИСТОЙ ЦИТОПЛАЗМОЙ ПРИ ПРОГРЕССИРУЮЩЕМ РАКЕ ЯИЧНИКОВ

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Аннотация

Введение. Рак яичников (РЯ) занимает 3-е место среди гинекологических злокачественных новообразований и имеет наиболее неблагоприятный прогноз с самой высокой смертностью. Прогрессирование РЯ может сопровождаться активным вовлечением в опухолевую прогрессию компонентов опухолевого микроокружения, в том числе опухолеассоциированных макрофагов (ОАМ). **Цель исследования** – проанализировать распределение и морфологический фенотип ОАМ в опухолевой ткани больных серозным раком яичников высокой степени злокачественности (HGSOC). **Материал и методы.** Парафиновые срезы опухолевой ткани получены после операций по поводу рака яичников. С помощью иммуногистохимического окрашивания в опухолевой ткани анализировали белковую экспрессию общего маркера макрофагов CD68 и М2-подобных маркеров CD206, CD163 и стабилина-1, принадлежащих к скавенджер-рецепторам. Гистологическая оценка распределения ОАМ проводилась патологоанатомом. Иммунофлуоресцентный анализ/конфокальную микроскопию применяли для установления коэкспрессии CD68 с основными скавенджер-рецепторами макрофагов. **Результаты.** В ткани опухоли яичника мы обнаружили гигантские CD68-позитивные макрофаги с пенистой цитоплазмой. Накопление этих ОАМ было характерно для пациенток с распространенным опухолевым процессом (IIIC и IV стадии). Наличие ОАМ с пенистой цитоплазмой на уровне статистической значимости ассоциировалось с прогрессированием рака яичников, включая метастазирование и рецидивирование. Распределение стабилин-1-позитивных макрофагов практически во всех случаях соответствовало экспрессии CD68, что было показано методом ИГХ. Конфокальная микроскопия подтвердила, что стабилин-1 экспрессируется по крайней мере в 50 % гигантских ОАМ. Иммунофлуоресцентное окрашивание образцов опухоли также продемонстрировало ко-экспрессию других скавенджер-рецепторов, CD163 и CD36, в клетках с пенистой цитоплазмой. По данным ИГХ-исследования и конфокальной микроскопии экспрессия CD206 в ОАМ с пенистой цитоплазмой в большинстве образцов практически отсутствовала. **Выводы.** Впервые продемонстрировано накопление гигантских макрофагов с рыхлой пенистой цитоплазмой в опухолевой ткани больных раком яичников IIIC и IV стадии. Такие макрофаги экспрессируют разнообразные скавенджер-рецепторы (стабилин-1, CD163, CD36), что указывает на высокую клиренсную активность гигантских ОАМ.

Ключевые слова: рак яичников, опухолеассоциированные макрофаги, клетки с пенистой цитоплазмой, скавенджер-рецепторы.

Introduction

Ovarian cancer (OC) is the third among most common gynecological cancer after cervical and endometrial cancers and has the worst prognosis with the highest mortality rate [1, 2]. In 2020, 313,959 cases of ovarian cancer were detected, representing 3.4 % of all cancers in women and 207,252 deaths occurred [3]. The urgency of the OC research derives from the large number of issues associated with late-stage diagnosis, difficulties in choosing treatment tactics, low treatment efficacy and a high proportion of relapses [1, 4, 5]. There are no effective criteria for diagnosing OC in the early stages, and screening tests have limited sensitivity. Therefore, up to 70 % of OC cases are detected at late stages [6]. More than 80 % of patients with advanced OC recur and die within 5 years [7]. However, the life expectancy of patients with the early stages of OC (stages I–II) is very favorable, and the five-year survival rate of such women is 92 % [8, 9]. Tumors usually respond to the first-line standard platinum/taxane-based chemotherapy. Despite this, the development of relapses associated with multidrug resistance is detected within a short period of time in 70 % of patients. It can be accompanied by the detrimental effect of therapeutic agents on the components of the tumor microenvironment (TME), including tumor-associated macrophages (TAM) [10].

TAMs are key cells of the innate immunity in tumors. In most cancers, including breast cancer, ovarian cancer, prostate cancer, lung cancer, gastric

cancer, glioblastoma, and melanoma, TAM infiltration positively correlates with metastasis and short-term survival [11]. In ovarian cancer, the ratio of anti-tumor (M1)/pro-tumor (M2) TAMs has a prognostic value for predicting metastasis and recurrence, as shown in several patient cohorts [11–13]. However, plastic adaptation of macrophages to structural tumor development leads to TAM heterogeneity, which can significantly depend on tumor type, tumor microenvironment, and the location of TAMs in particular intratumoral compartments [11, 14, 15]. The major markers of TAMs belong to the scavenger receptors and include common marker of macrophages CD68, and M2 markers with pro-tumor polarization CD206, CD163, CD204, MARCO, stabilin-1, and others [16, 17].

In some cancers macrophages obtain the phenotype of foam cells, where the intracellular lipid content exceeds macrophage`s capacity to maintain lipid homeostasis, triggering lipid droplet formation and the foamy appearance [18–21]. Studies showed that foam cells tend to lose immune functions and induce tissue damage [18, 22, 23]. Foamy cells have been extensively studied in atherosclerosis and tuberculosis, however the role of these cells in cancer has only begun to be explored recently [19].

In the present study we analyzed M2-like TAM distribution and morphological phenotype in tumor tissue of patients with high-grade serous ovarian cancer (HGSOC). We were able to find foam-like CD68-positive macrophages with characteristics of incomplete endocytosis in advanced stage patients. We described the phenotypic profile of these cells.

The purpose of the study to analyze distribution and morphological phenotype of TAMs in tumor tissue of patients with high-grade serous ovarian cancer (HGSOC).

Material and Methods

Clinical samples

The study included 42 patients with histologically-verified high-grade serous ovarian carcinoma (HGSOC), treated at the Department of Gynecological Oncology, Cancer Research Institute of Tomsk National Research Medical Center (Tomsk, Russia) from 2015 to 2021. The study was carried out according to Declaration of Helsinki (from 1964, revised in 1975 and 1983) and was approved by the local committee of Medical Ethics of Tomsk Cancer Research Institute; all patients signed informed consent for the study. The clinical and pathological parameters of OC patients are presented in Table 1.

Neoadjuvant chemotherapy regimens included standard platinum/taxane-based chemotherapy (cisplatin/carboplatin and paclitaxel/docetaxel). The chemotherapy response score (CRS) was used for the assessment of histological effect in ovarian cancer after neoadjuvant chemotherapy (NACT). All patients underwent surgical treatment. In adjuvant regime, patients received chemotherapy by the same schemes.

Immunohistochemical analysis

Formalin fixed paraffin embedded (FFPE) tissue sections were obtained from all ovarian cancer patients after tumor resection. Immunohistochemical analysis (IHC) was carried out by the standard method. Briefly, the slides with the samples were placed in xylene and alcohols for deparaffinization. Antigen unmasking was performed using EDTA buffer with pH 9.0 with heating. A protein block (Abcam, UK) and a hydrogen peroxide block (Spring BioScience, USA) were then used sequentially. Incubation with primary antibodies pre-diluted in 1 % BSA was carried out in a humid chamber. Antibodies to general marker of TAMs include monoclonal mouse anti-CD68 (1:100, clone KP1, NBP2-44539, Novus Biologicals, USA), and to specific M2-like subpopulations include rabbit anti-CD163 (1:250, ab182422, Abcam, USA), goat anti-CD206 (1:20, AF2534, R&D Systems, USA), and anti-stabilin-1 rabbit polyclonal antibody RS-1 (1:1000) [24]. To visualize the antigen-antibody reaction, rabbit anti-goat IgG (1:250, VB2932894, Invitrogen, USA) or poly-HRP anti-mouse/rabbit system (Bond oracle IHC system, TA9145, Leica Biosystems, Germany) were used. The nuclei were counterstained with hematoxylin.

Digital IHC analysis and quantification

Tumor tissue slides were scanned by using the Leica Aperio AT2 histoscanning station (Leica, Germany) and ScanScope software (Aperio ScanScope XT Leica). QuPath software (free from https://qupath.github.io) was used to analyze and quantify marker expression. Individual tumor regions were selected and analyzed using the cell detection and cell intensity classification. “Cell: DAB OD mean” was used for the analysis of both membranous and cytoplasmic staining of a selective antibody. Intensity thresholds were set to further subclassify cells as being negative, weak, moderate or strongly positive for CD68, CD163, CD206 and stabilin-1 staining based upon mean nuclear DAB optical densities. The results of analysis were presented with H-score parameter that was automatically calculated by Qupath software for each tissue section.

Immunofluorescent staining and confocal microscopy

Immunofluorescent staining was performed using monoclonal mouse anti-CD68 (1:100, clone KP1, NBP2-44539, Novus Biologicals, USA); monoclonal rabbit anti-CD163 (1:250, clone EPR19518, ab182422, Abcam, USA), monoclonal rat anti-CD68 (1:50, clone FA-11, GTX41864, GeneTex, USA), and monoclonal mouse anti-CD36 (1:50, clone 185-1G2, MA5-14112, ThermoFisher scientific, USA). The following combinations of secondary antibodies were used: Cy3-conjugated anti-rabbit, AlexaFluor488-conjugated anti-mouse, AlexaFluor647-conjugated anti-goat and AlexaFluor647-conjugated anti-rat

Table 1/Таблица 1

Clinical and pathological parameters of OC patients

Клинико-патологические параметры пациенток с раком яичника

Clinical and pathological parameters/ Клинико-патологические параметры	Number of patients/ Количество пациенток
Age, years/Возраст, лет	61.2 ± 13.2
Stage/Стадия	
I	2 (4.8 %)
II	4 (9.5 %)
III	22 (52.4 %)
IV	4 (33.3 %)
NACT/НАХТ	
Treated /Прошедших лечение	29 (69.0 %)
Untreated /Не прошедших лечение	13 (31.0 %)
Response to NACT/Эффект от НАХТ	
CRS1	5 (17.2 %)
CRS2	12 (41.4 %)
CRS3	12 (41.4 %)
Ascites/Асцит	
Yes /Да	20 (47.6 %)
No /Нет	15 (35.7 %)
Unknown /Неизвестно	7 (16.7 %)
Carcinomatosis/Канцероматоз	
Yes /Да	28 (66.7 %)
No /Нет	14 (33.3 %)
Recurrence/Рецидив	
Yes /Да	16 (38.1 %)
No /Нет	26 (61.9 %)
Progression/Прогрессирование	
Yes /Да	9 (21.4 %)
No /Нет	33 (78.6 %)
Foam-like macrophages/Пенообразные макрофаги	
Yes /Да	20 (47.6 %)
No /Нет	22 (52.4 %)

antibodies (all donkey, Dianova, Germany, dilution 1:400). Samples were mounted with Fluoroshield Mounting Medium with DAPI (#ab104135, Abcam, USA) and analyzed by confocal microscopy. Confocal laser scanning microscopy was performed with Carl Zeiss LSM 780 NLO laser scanning spectral confocal microscope (Carl Zeiss, Germany), equipped with 40x objective. Data were acquired and analyzed with Black Zen software. All three- and four-color images were acquired using a sequential scan mode.

Statistical analysis

Statistical analysis was performed using STATISTICA 8.0 for Windows. The Mann-Whitney test was implemented to compare two independent groups. Fisher Exact Probability Test was applied for the association of recurrence with the presence of foam-like cells. Data are presented as mean±standart deviation (M±SD) (Table 1). Results were considered to be significant with $p<0,05$. Data with marginal significance (p value <0.1) were also discussed.

Results

We characterized TAM morphology and amount in tumor tissue of HGSOC patients. Firstly, the expression of general macrophage marker CD68 and the main M2-like markers including CD163, CD206, and stabilin-1, was assessed by quantitative IHC. All analyzed markers belong to scavenger receptors, that are essential for TAM function [25]. Ligands for scavenger receptors include modified LDL, phospholipids, apoptotic cells, amyloid proteins, ferritin, hyaluronan (HA), heparin, and others [25]. TAMs actively display endocytosis altering extracellular landscape of TME and regulating tumor development [26].

Tissue slides were scanned and then the expression of TAM markers was analyzed. The level of protein expression of CD68, CD206, CD163 and stabilin-1 was quantified using the cell detection and cell intensity classification. Statistical analysis based on the comparison of two independent groups did not show significant differences in the expression of CD68, CD206, CD163 and stabilin-1 for the ovarian cancer stages, the present of recurrence, metastasis, and ascites, and for NACT response ($p>0.05$).

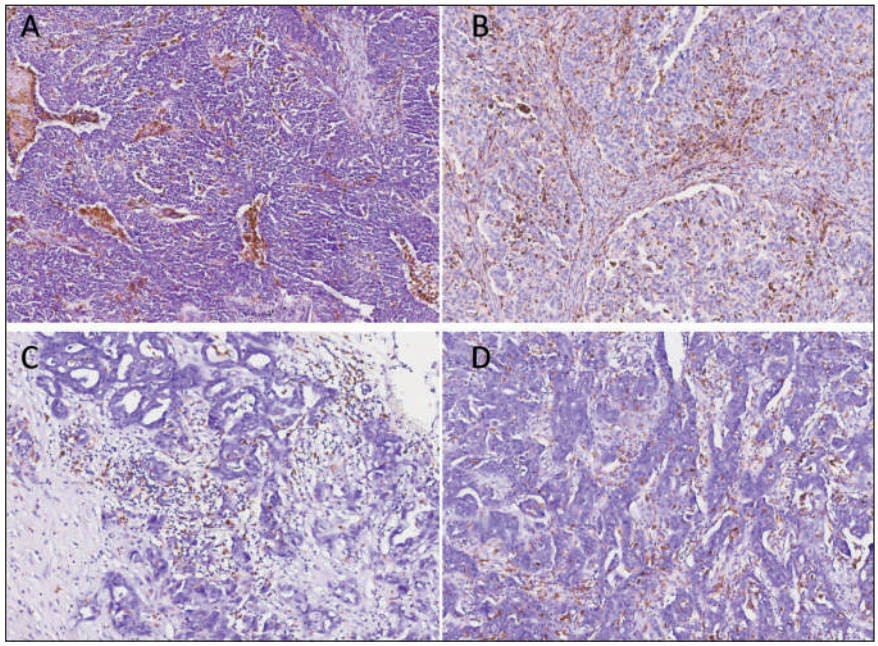

Fig. 1. Microphoto. Immunohistochemistry. The distribution of CD68-positive macrophages in tumor tissue. (A) intratumoral located macrophages with uniform and diffuse disposition, with increased amount around/inside necrosis focus and invasive front; (B) uniform disposition inside whole tumor area; (C) discrete macrophages or small groups of macrophages in stroma; (D) irregular intratumoral location of macrophages. ×40

Рис. 1. Микрофото. ИГХ-исследование. Распределение CD68-позитивных макрофагов в опухолевой ткани. (A) внутриопухолевые макрофаги с равномерным и диффузным расположением с увеличенным накоплением вокруг/внутри очага некроза и фронта инвазии; (B) равномерное расположение в пределах всей опухоли; (C) отдельные макрофаги или небольшие группы макрофагов в строме; (D) неравномерное внутриопухолевое расположение макрофагов. ×40

Then we analyzed the distribution and morphological features of TAMs. The distribution of CD68-positive TAMs varied greatly between tumors, but no correlation with clinical and pathological parameters was demonstrated. Tumors displayed diverse TAM content: a) intratumoral located macrophages with uniform and diffuse disposition, with increased amount around/inside necrosis focus and invasive front; b) irregular intratumoral location of macrophages; c) discrete macrophages or small groups of macrophages in stroma; d) uniform disposition inside whole tumor area; e) abundant accumulation of giant foam-like macrophages in stroma (Fig. 1). We were interested in the accumulation of giant CD68-positive macrophages that resemble foam-like cells. These TAMs have fluffy foamy granular cytoplasm, sometimes with inclusions. This morphology is the outcome of lipid over-intake and was found to be inherent to macrophages in atherosclerosis and tuberculosis, as well as in some cancers such as colorectal and renal cancers [21, 27, 28].

The presence of these macrophages in tumor was found in almost 50 % of patients (20/42 cases). Interestingly, all of them have advanced ovarian cancer: 9 patients with stage 3C and 11 patients with stage 4. Among 20 patients with giant fluffy macrophages, 18 patients underwent NACT, with different initial histological response (CRS1-CRS3). In most cases, foam-like macrophages form big aggregates in stroma surrounding tumor cells (Fig. 2A-C). In some patients, foam-like CD68+ cells are located in small aggregates or as discrete cells (Fig. 2F). Such cells can be merged and form 2 and 3-nuclei cells, resembling epithelioid cells (activated macrophages with pale foamy cytoplasm) [29]. The location of giant macrophages with cytoplasmic vacuolation around tumor cells after chemotherapy can indicate that they can have characteristics of incomplete endocytosis [30]. The presence of foam-like TAMs had a tendency to be associated with ovarian cancer progression, including metastasis and recurrence (Fisher's exact test, p=0.058). Further IHC analysis allowed us to reveal that foam-like macrophages express scavenger receptor stabilin-1 in almost 100 % of cases (Fig. 3A). Stabilin-1-positive cells had the same morphology and location in tumor tissue. The expression of other M2 marker CD163 was almost the same, but CD206 expression was absent in many samples (Fig. 3B and C).

These observations prompted us to perform immunofluorescent analysis to reveal the co-expression of CD68 and most common macrophage scavenger receptors (CD206, CD163, CD36, and stabilin-1). Using four-colour IF images we confirmed the co-expression of stabilin-1 in CD68+ macrophages (Fig. 3A). Stabilin-1 was expressed in at least 50 % of giant TAMs. IF analysis of several tumor samples also demonstrated co-expression of other SRs, CD163 and CD36, in foam-like cells (Fig. 3B). Similar to IHC, in most samples we did not find the expression of CD206 in TAMs of foam-like morphology (Fig. 3A).

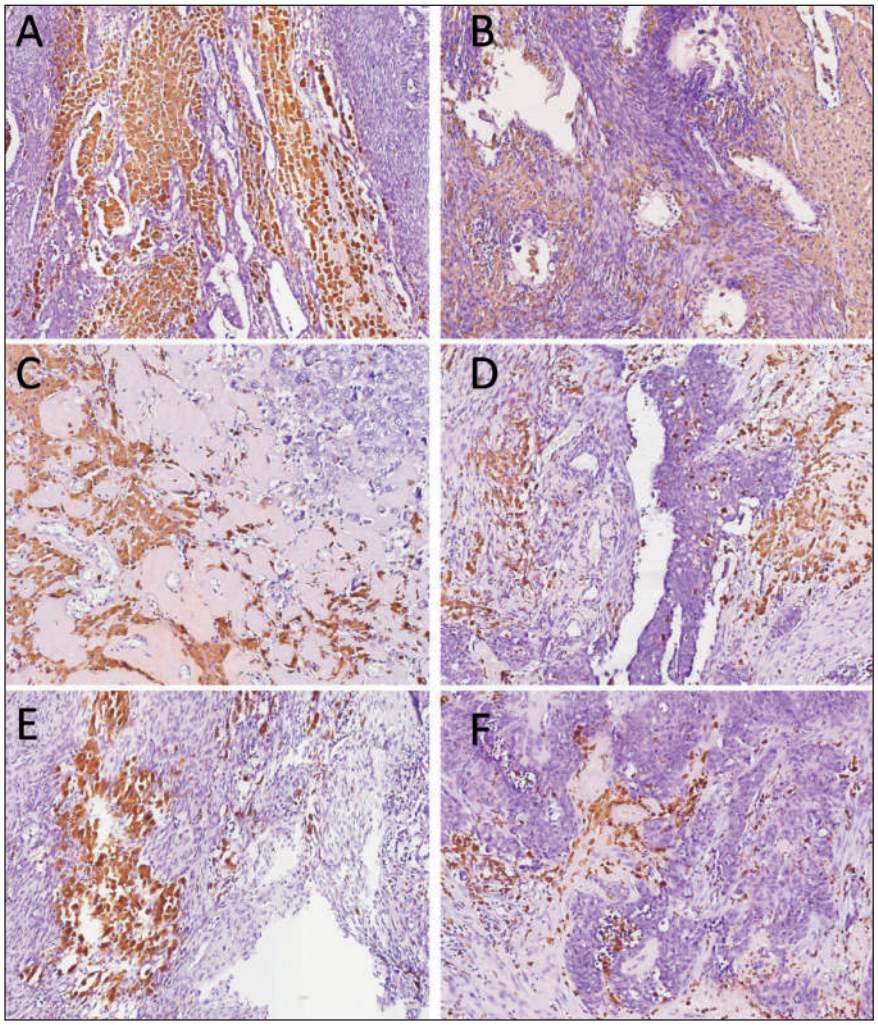

Fig. 2. Microphoto. Immunohistochemistry. The distribution of large foam-like CD68+ macrophages in tumor tissue of HGSOC. (A) and (B), big aggregates of foam-like macrophages surrounded tumor cells. (C) and (D), fluffy macrophages in fibrosis around tumor cells. (E) – medium aggregates of foam-like macrophages. (F) – small aggregates or discrete cells with foam-like morphology. ×40

Рис. 2. Микрофото. ИГХ-исследование. Распределение крупных CD68+ макрофагов с пенистой цитоплазмой в опухолевой ткани HGSOC. (A) и (B) — большие скопления макрофагов с пенистой цитоплазмой, окружающих опухолевые клетки; (C) и (D), рыхлые макрофаги в фиброзе вокруг опухолевых клеток; (E) – средние агрегаты макрофагов с пенистой цитоплазмой; (F) – мелкие агрегаты или дискретные клетки с пенообразной морфологией. ×40

Discussion

Foam macrophages are cells with extensive amount of fluffy looking cytoplasm. This morphology is due to aberrant lipoprotein metabolism and accumulation of LDL or cholesterol either via macropinocytosis or scavenger receptor-mediated pathways (mostly by SRA and CD36) [31]. In our study for the first time we found the aggregates of giant macrophages with foamy cytoplasm in patients with advanced ovarian cancer. Cancer cells were able to activate adipocytes and other stromal cells to lipolyze their triglyceride storage resulting in release of fatty acids (FA) outside of cells, that was linked to an increased risk of developing lung cancer, gastric cancer, thyroid cancer, rectal cancer, colon cancer, and ovarian cancer [27, 32]. Excessive amount of FA in intratumoral milieu prompts TAMs to uptake FA through SR-mediated phagocytosis, which in turn leads to formation of lipid-laden TAMs [28].

In colorectal cancer, the formation of lipid droplet-bearing $CD68^{+}CD206^{+}$ TAMs was specifically found in tumor tissue, but not in adjacent benign tissue, indicating that specific quality of TAMs could serve as a parameter to predict the prognosis of cancer [28]. Moreover, the lipid droplet-dependent FA metabolism was shown to induce the immunosuppressive phenotype of TAMs, and targeting lipid droplets with chemical inhibitors impaired tumor growth in vivo [28]. A correlation between the presence of large tumor-associated macrophages (L-TAMs) in colorectal cancer metastasis and poor outcomes was observed. The presence of L-TAMs correlated to low 5-year disease-free survival rates, while in case of small TAMs (S-TAM) the survival rate was higher [20]. Transcriptomic analysis demonstrated that the gene profile of L-TAMs was associated with lipid metabolism; however, S-TAMs had a distinct profile characterized by

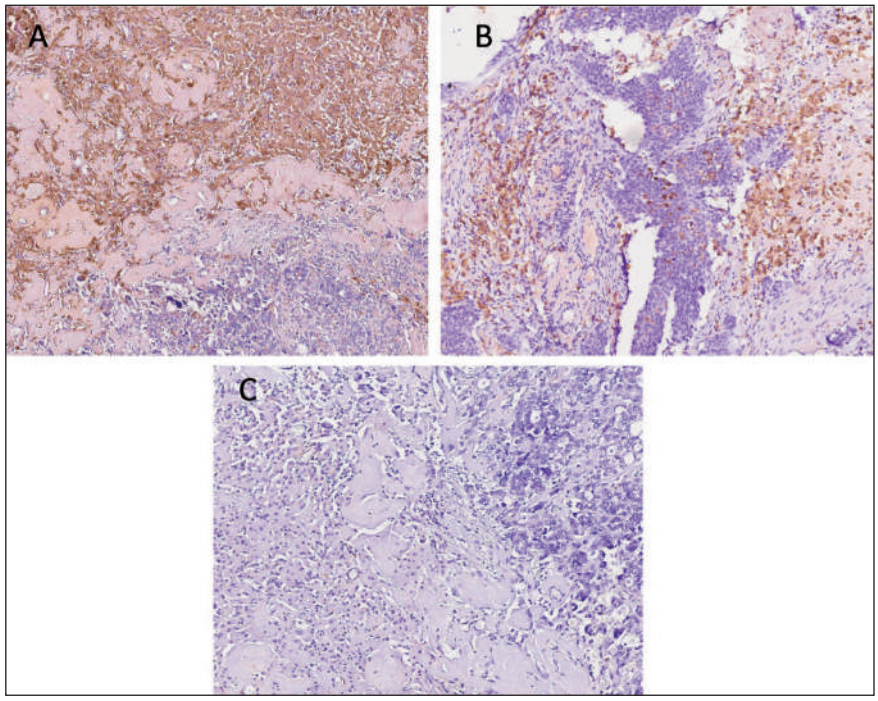

Fig. 3. Microphoto. Immunohistochemistry. Representative images of M2 foam-like macrophages in tumor tissue of HGSOC. (A) Stabilin-1-positive TAMs located in fibrosis tissue surrounding tumor cells. (B) CD163-positive TAMs located in fibrosis tissue surrounding tumor cells. (C) The absence of CD206 expression in foam-like macrophages. ×40

Рис 3. Микрофото. ИГХ-исследование. Примеры пенообразных М2 макрофагов в опухолевой ткани HGSOC. (A) Стабилин-1-положительные ОАМ, расположенные в фиброзной ткани, окружающей опухолевые клетки. (B) CD163-положительные ОАМ, расположенные в фиброзной ткани, окружающей опухолевые клетки; (C) Отсутствие экспрессии CD206 в пенообразных макрофагах. ×40

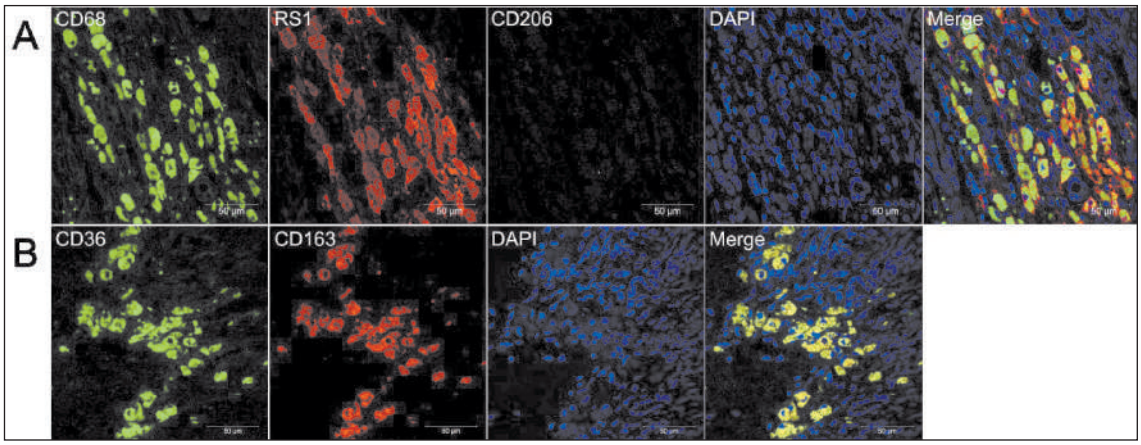

Fig. 4. Multicolor immunofluorescent analysis of scavenger receptor co-expression in CD68-positive macrophages. (A) Co-expression of CD68, CD206 and stabilin-1 in tumor samples from a patient with ovarian cancer. (B) Co-expression of scavenger receptors CD163 and CD36 in tumor samples from a patient with ovarian cancer. Scale bars correspond to 50μM

Рис. 4. Многоцветный иммунофлюоресцентный анализ ко-экспрессии скавенджер-рецепторов в CD68-позитивных макрофагах (A) Ко-экспрессия CD68, CD206 и стабилина-1 в образцах опухоли пациентки с раком яичника; (B) Ко-экспрессия скавенджер-рецепторов CD163 и CD36 в образцах опухоли пациентки с раком яичника. Шкала соответствует 50μM

inflammatory gene expression. In single-cell analysis S-TAM and L-TAM signatures were also differentially enriched in individual clusters [20].

An increase of lipids, which co-localized with CD68 staining in tissue sections of patients with breast, colon, and prostate cancers, was observed [33]. $CD11b^{+}CD68^{+}$ macrophages isolated from colon cancer tissues displayed significantly more lipid accumulation compared with macrophages from normal tissues. In addition, an increased lipid accumulation in TAMs from multiple myeloma patients' bone marrow was positively and significantly associated with the progression of monoclonal gammopathy of undetermined significance to multiple myeloma [33].

Conditioned medium from papillary renal cell carcinoma (pRCC) cultures skewed human monocytes toward the M2 macrophage phenotype. These macrophages became enlarged and loaded with lipids, adopting the foam cell morphology found in pRCC tissue. The formation of these cells was linked to the

expression of IL-8, CXCL16, and chemerin by pRCC primary tumor cells [21].

In a study of 30 non–small cell lung carcinomas after neoadjuvant therapy the formation of foam cells/giant cells was stated to be a histologic feature of tumor regression along with coagulative necrosis, fibrosis, and mixed inflammatory infiltrate [34]. Pretreatment non-small cell lung cancer tissue samples from all patients with hyperprogression showed tumor infiltration by M2-like $CD163^{+}CD33^{+}PD\text{-}L1^{+}$ clustered epithelioid macrophages [35].

In the present study giant foam-like TAMs displayed high scavenging activity, that was confirmed by high expression of scavenger receptors, including stabilin-1, CD36 and CD163. The expression of CD206 was limited almost in all foam-like CD68+ macrophages. Although CD206 participates in endogenous and exogenous molecule clearance as scavenger receptor, there is no data about the involvement of CD206 in lipid metabolism, as was shown for other scavenger receptors, including CD68, CD163, CD206 and stabilin-1 [25, 36, 37]. Statistical analysis showed the tendency to the association between the presence of foam-like TAMs and ovarian cancer progression, including metastasis and recurrence. Further analysis of foam-like macrophages will help to clarify their function in lipid metabolism and to relate their biological activity to ovarian cancer progression and NACT response.

Conclusion

For the first time we demonstrated the accumulation of giant macrophages with fluffy foam cytoplasm in tumor tissues of NACT-treated advanced ovarian cancer patients. Such macrophages express diverse scavenger receptors (stabilin-1, CD163, CD36), playing role in processing of long-chain free fatty acids, ox-LDL, collagens I and IV, hemoglobin-haptoglobin complex, thus indicating high scavenging activity of giant TAMs.

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Irina V. Larionova: study conception and design, data collection and analysis, writing of the manuscript, study supervision.

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Conflict of interests

The authors declare that they have no conflict of interest.

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