УДК 576.5:618.11-006.6-033.2
https://doi.org/10.20538/1682-0363-2021-2-44-53

Dissimilar populations of EpCam-positive cells in ascitic fluid of ovarian cancer patients: a relationship with the degree of carcinomatosis

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ABSTRACT

Background. Peritoneal carcinomatosis (PC) is one of the most unfavorable factors of ovarian cancer progression. It is often accompanied by accumulation of fluid in the abdominal cavity, which is called ascites. However, prognostic factors associated with malignant ascites are not well understood.

The aim of this study was to evaluate dissimilar populations of EpCAM-positive cells in ascitic fluid and their relationship with the presence of invasive peritoneal implants and the prevalence of carcinomatosis on the Predictive Index Value (PIV) scale in ovarian cancer patients.

Materials and methods. The prospective study included 22 patients aged 36–76 years with newly diagnosed FIGO stage Ic–IV ovarian cancer, who were admitted for treatment to Cancer Research Institute of Tomsk NRMC. The study material included EDTA-stabilized ascitic fluid sampled during laparoscopy.

Various populations of ascites tumor cells were identified by laser multicolor flow cytometry. The degree of carcinomatosis was determined according to the PIV scale.

Results. The study identified twelve populations of EpCAM-positive cells in the ascitic fluid of ovarian cancer patients. Epcam+CD45-CD44-CD24+CD133−Ncadherin+ cells (r = 0.58; p = 0.004) and atypical (hybrid) EpCam+CD45+CD44+CD24+/-CD133+/-Ncadherin+/- cells (r = = 0.51; p = 0.01) had a positive correlation with the PIV index.

Conclusion. The obtained results show a high degree of heterogeneity of tumor cells in the ascitic fluid of ovarian cancer patients. Identified atypical (hybrid) forms of EpCam-positive cells are of great interest for cell biology and require further investigation.

Key words: heterogeneity of tumor cells, carcinomatosis, Predictive Index Value (PIV), ascitic fluid, ovarian cancer, liquid biopsy.

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

Source of financing. The study was supported by the President of the Russian Federation grant (grant No. MD-2017.2020.7).

Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at Cancer Research Institute, Tomsk NRMC (Protocol No. 4 of 02.04.2018).

For citation: Kaigorodova E.V., Ochirov M.O., Molchanov S.V., Rogachev R.R., Dyakov D.A., Chernyshova A.L., Shpileva O.V., Kovalev O.I., Vtorushin S.V. Dissimilar populations of EpCam-positive cells in ascitic fluid of ovarian cancer patients: a relationship with the degree of carcinomatosis. *Bulletin of Siberian Medicine.* 2021; 20 (2): 44–53. https://doi.org/10.20538/1682-0363-2021-2-44-53.

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Различные популяции EpCam-положительных клеток в асцитической жидкости у больных раком яичников: связь со степенью канцероматоза

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РЕЗЮМЕ

Введение. Канцероматоз брюшины является одним из наиболее неблагоприятных факторов прогрессирования рака яичников. Он часто сопровождается накоплением жидкости в брюшной полости – асцитом. Прогностические факторы, связанные со злокачественным асцитом, изучены недостаточно.

Цель. Оценка различных популяций EpCam-положительных опухолевых клеток в асцитической жидкости, их связь с наличием перитонеальных «инвазивных» имплантатов и степенью распространенности канцероматоза по шкале Predictive Index Value (PIV) у больных раком яичников.

Материалы и методы. В проспективное исследование включены 22 больные с впервые диагностированным раком яичников, с Ic–IV стадиями по системе FIGO, в возрасте 36–76 лет, поступившие на лечение в НИИ онкологии Томского НИМЦ. Материалом для исследования служила асцитическая жидкость, стабилизированная ЭДТА, взятая во время лапароскопии.

Количество различных популяций опухолевых клеток в асцитической жидкости определяли методом многоцветной проточной цитометрии и проточной цитометрии с визуализацией. Степень канцероматоза определяли по шкале PIV.

Результаты. Показано, что клеточный состав асцитической жидкости больных раком яичников представляет собой гетерогенную популяцию. Положительная корреляционная связь с PIV, характеризующим степень распространенности канцероматоза, наблюдалась у асцитических клеток с фенотипом EpCam+CD45-CD44-CD24+CD133-Ncadherin+ ($r = 0{,}58$; $p = 0{,}004$) и у атипичных / гибридных форм клеток EpCam+CD45+CD44+CD24+/-CD133+/-Ncadherin+/- ($r = = 0{,}51$; $p = 0{,}01$).

Заключение. Полученные результаты показывают большую гетерогенность опухолевых клеток в асцитической жидкости больных раком яичников. Обнаруженный факт наличия атипичных (гибридных форм) EpCam-положительных клеток представляет интерес для клеточной биологии и требует дальнейших исследований.

Ключевые слова: гетерогенность опухолевых клеток, канцероматоз, Predictive Index Value, асцитическая жидкость, рак яичников, жидкостная биопсия.

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

Источник финансирования. Исследование выполнено при финансовой поддержке Президента РФ (грант № МД-2017.2020.7).

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом НИИ онкологии Томского НИМЦ (протокол № 4 от 02.04.2018).

Для цитирования: Кайгородова Е.В., Очиров М.О., Молчанов С.В., Рогачев Р.Р., Дьяков Д.А., Чернышова А.Л., Шпилёва О.В., Ковалев О.В., Вторушин С.В. Различные популяции EpCam-положительных клеток в асцитической жидкости у больных раком яичников: связь со степенью канцероматоза. *Бюллетень сибирской медицины.* 2021; 20 (2): 44–53. https://doi.org/10.20538/1682-0363-2021-2-44-53.

INTRODUCTION

Ovarian cancer is characterized by a unique metastatic process. The earliest and most common route of metastasis is implantation. It is often accompanied by accumulation of fluid in the abdominal cavity, which is called ascites. Ovarian cancer accounts for up to 38% of ascites cases associated with malignancies in females. Ascitic fluid is a promising biological mate-

rial to get information about the nature of the tumor. Unlike serum, ascitic fluid is more informative, especially at early stages of the malignant process [1].

In general, ascites is a multicomponent, dynamic system where all elements combined facilitate the formation of a proinflammatory and immunosuppressive environment. Ascites consists of a complex mixture of cell populations and a rich cytokine profile [2]. The variety of cells is related to several factors. The first factor is phenotypic plasticity arising from the influence of soluble factors and microenvironment signals from immune and stromal cells. Secondly, heterogeneity is associated with hydrodynamic forces that significantly change cell morphology [3]. Thirdly, the source of cancer cells in ascitic fluid is the tumor, and in ovarian cancer, in particular, it is a heterogeneous cell population [4].

Currently, the heterogeneity of tumor cells is evaluated based on their antigenic properties, spectrum of various cell surface markers, and activity of the key signaling pathways. If circulating tumor cells (CTCs) are detected, the epithelial cell adhesion molecule (EpCAM) is widely used as a specific biomarker, because it is overexpressed in more than 70% of ovarian cancer cases, and its level is closely associated with malignant ascites, chemoresistance, and decreased survival in patients. The role of EpCAM is not limited to cell adhesion; there is abundant evidence of its involvement in the epithelial–mesenchymal transition (EMT) [5]. The EMT is known to enable cells to separate, lose their apical–basal polarity typical of epithelial cells, demonstrate enhanced resistance to apoptosis, and return to a more mobile mesenchymal phenotype that promotes peritoneal dissemination. This molecule is also used as a marker of cancer stem cells (CSCs) [6].

Along with EpCAM, the CD44 receptor, widely present on the surface of tumor cells, is used to identify CTCs. It mediates attachment of ovarian cancer cells to the peritoneal mesothelium by binding to hyaluronic acid (HA). CD44, as a biomarker, has several advantages. Firstly, normal cells have a low level of CD44 expression and poor adhesion to hyaluronic acid. Secondly, HA is one of the main components of the tumor extracellular matrix that, along with binding to the CTCs, supports cell integrity [7].

Another CTC marker in ascitic fluid is CD24, which is expressed in 70.1% of ovarian cancer cases and is an independent predictor of survival. CD24 increases the metastatic potential of tumor cells, because it is a ligand of P-selectin, an adhesion receptor on activated endothelial cells. In addition, CD24 induces EMT, which leads to the formation of a highly proliferative phenotype and resistance to chemotherapy via activation of PI3K/Akt, NF-κB, and ERK signaling cascades [8].

A common EMT feature is reduced expression of epithelial cadherin (E-cadherin) and a concomitant increase in or *de novo* expression of neural cadherin (N-cadherin). This so-called "cadherin switch" is associated with increased migratory and invasive behavior of tumor cells. Increased expression of N-cadherin in solid tumors promotes collective cell migration, enhances transmission of fibroblast growth factor(FGF) signals, and modulates the canonical Wnt signaling pathway, which leads to the formation of an aggressive phenotype [9].

CD133 is the most commonly used cell surface antigen for detection and isolation of CSCs in various malignant diseases, including ovarian cancer. High expression of CD133 in tumors is considered a prognostic marker of disease progression. Despite the fact that the functional role of CD133 in malignancies is not fully understood, most studies suggest that CD133 has a significant prognostic value for assessing overall and progression-free survival in various cancer types [10].

Peritoneal dissemination caused by ascites is one of the most unfavorable factors for progression of malignancies. In 2006, Italian authors proposed a Predictive Index Value (PIV) scale for assessing the prevalence of carcinomatosis, taking into account the condition of the parietal peritoneum, diaphragmatic peritoneum, mesentery of the intestine, omentum, intestinal wall, stomach, and liver. The authors showed that with PIV ≥ 8, the probability of R0 resection is practically equal to 0, therefore, neoadjuvant chemotherapy (NACT) is recommended [11].

In connection with the foregoing, the aim of this study was to investigate various populations of tumor cells in the ascitic fluid of ovarian cancer patients and their relationship with the presence of invasive peritoneal implants and the prevalence of carcinomatosis on the PIV scale.

MATERIALS AND METHODS

The study was approved by the local Ethics Committee at Cancer Research Institute of Tomsk NRMC. The prospective study included 22 patients aged 36–76 years with newly diagnosed FIGO stage Ic–IV ovarian cancer, who were admitted for treatment to Cancer Research Institute of Tomsk NRMC. For the purpose of surgical staging, all patients included in

the study underwent laparoscopy using a Karl Storz laparoscopic unit (Germany), followed by morphological examination of biopsy specimens and ascitic fluid. PIV prevalence was also evaluated. The study material included 5 mL EDTA-stabilized ascitic fluid sampled during laparoscopy.

DETERMINING THE PIV INDEX

The PIV index in patients with ovarian cancer was calculated using video materials obtained during laparoscopy with the Karl Storz unit. The PIV scale (Fagotti score, range 0–14) allowed to assess the prevalence of carcinomatosis, taking into account the condition of the parietal peritoneum, diaphragmatic peritoneum, mesentery of the intestine, omentum, intestinal wall, stomach, and liver (Table 1) [11].

Assessing populations of tumor cells in ascitic fluid by multicolor flow cytometry. Different populations of ascites tumor cells (with stemness traits, with EMT traits, without stemness and EMT traits, with a combination of these traits, as well as atypical (hybrid) cell populations) were identified by laser multicolor flow cytometry on a BDFACSCanto apparatus (USA) using a molecular panel of EpCam, CD45, CD44, CD24, CD133, and N-cadherin markers and BD FACSDiva software.

Table 1

Determination of the PIV for assessing the prevalence of carcinomatosis

Parameter	Score = 2	Score = 0
Peritoneal carcinomatosis	Unresectable massive peritoneal damage	Carcinomatosis of isolated regions that can be surgically removed during peritoneoectomy
Diaphragmatic peritoneum	Widespread infiltrating carcinomatosis or confluent nodules in most of the diaphragmatic peritoneum	Focal damage to the peritoneum
Mesentery of the intestine	Big infiltrating nodules or involvement of the mesenteric root	Small nodules that are potentially resectable using argon plasma coagulation
Omentum	Tumor diffusion up to the large curvature of the stomach	Focal damage to the omentum
Infiltration of the bowel	Bowel resection is assumed to be required or miliary carcinomatosis	–
Infiltration of the stomach	Neoplastic damage to the stomach wall	–
Liver damage	Any surface lesions	–

For this purpose, ascitic fluid was incubated with fluorochrome-labeled monoclonal antibodies to CD45: clone HI30 (APC/Cy7) (Biolegend, USA), EpCAM clone 9C4 (PE) (Biolegend, USA), CD44 clone BJ18 (FITC) (Biolegend, USA), CD24 clone ML5 (PE/Cy7) (Biolegend, USA), N-cadherin clone 8C11 (PerCP/Cy5.5) (Biolegend, USA), and CD133 clone AC-133 (APC) (Miltenyi Biotec, USA). Then, erythrocytes in the sample were lysed with a BD Facs lysing solution and washed twice with CellWash buffer. Next, 1 ml of BD Flow was added to the cell pellet. All samples were stored in the dark at 4 °C and analyzed on a flow cytometer within an hour. The cell level was expressed as the number of cells per 1 ml of ascitic fluid.

High-resolution imaging flow cytometry. Cells were incubated with fluorochrome-labeled monoclonal antibodies to CD45: clone HI30 (APC/Cy7) (Biolegend, USA), EpCAM clone 9C4 (PE) (Biolegend, USA), CD44 clone BJ18 (FITC) (Biolegend, USA), CD24 clone ML5 (PE/Cy7) (Biolegend, USA), N-cadherin clone 8C11 (PerCP/Cy5.5) (Biolegend, USA), CD133 clone AC-133 (APC) (Miltenyi Biotec, USA), and DAPI (ZytoVision, Germany). The aliquots were analyzed with an ImageStream Mk II Imaging Flow Cytometer (Luminex, Poland). All data were saved as raw image files for analysis in IDEAS software (version 6.2).

Statistical analysis. The obtained data were processed by variance statistical methods. All the statistical analyses were performed using Statistica 10.0 software package (StatSoft, Inc., USA). Assessment of the normal distribution of the results was performed using the Kolmogorov – Smirnov test. The significance of differences was assessed using the nonparametric Mann – Whitney test (for independent samples). Spearman's correlation analysis (*r)* was also used. The data were presented as the median and the interquartile range *Me* (*Q1*–*Q3*). The differences were considered statistically significant at $p < 0.05$.

RESULTS

Multicolor flow cytometry of ascitic fluid of ovarian cancer patients revealed 12 popu-

lations of Epcam-positive cells. These included ascites tumor cells without stemness and EMT traits – Epcam+CD45–CD44–CD24–CD133–Ncadherin– (AC-1); ascites tumor cells without stemness traits and with EMT traits – Epcam+CD45–CD44–CD24–CD133–Ncadherin+ (AC-2); ascites tumor cells with stemness traits, without EMT traits, and with phenotypes Epcam+CD45–CD44+CD24–CD133+/-Ncadherin– (AC-3); Epcam+CD45–CD44+CD24+CD133+/–Ncadherin– (AC-6); Epcam+CD45–CD44–CD24+CD133+/-Ncadherin– (AC-8); ascites tumor cells with stemness and EMT traits – Epcam+CD45–CD44+CD24–CD133+/-Ncadherin+ (AC-4); Epcam+CD45–CD44+CD24+CD133+Ncadherin+ (AC-5); Epcam+CD45–CD44–CD24+CD133+Ncadherin+ (AC-7); Epcam+CD45–CD44+CD24+CD133−Ncadherin+ (AC-9); Epcam+CD45–CD44–CD24+CD133–Ncadherin+ (AC-10); atypical (hybrid) cells without stemness traits – Epcam+CD45+CD44–CD24–CD133–Ncadh+/− (AC-11); and atypical (hybrid) cells with stemness traits: Epcam+CD45+CD44+CD24+/–CD133+/−Ncadh+/−(AC-12).

In patients with invasive peritoneal implants, the number of cancer stem cells with phenotypes Epcam+CD45-CD44+CD24+CD133−Ncadherin+ and Epcam+CD45-CD44-CD24+CD133−Ncadherin+ in ascitic fluid significantly exceeded the one in ascitic fluid of ovarian cancer patients with non-invasive peritoneal implants (Table 2).

Table 2

The level of various populations of EpCAM-positive cells in the ascitic fluid of ovarian cancer patients with non-invasive or invasive implants, cells/ml, *Me* (Q_1–Q_3)

Phenotype of ascites tumor cells	Invasive peritoneal implants, *n* = 13	Non-invasive peritoneal implants, *n* = 9
AC-1 EPCam+CD45-CD44-CD24-CD133-Ncadherin-	290 (110–4,270)	55 (0–420) p_{1-2} = 0.082
AC-2 EpCam+CD45-CD44-CD24-CD133-Ncadherin+	40 (0–1,220)	170 (0–320) p_{1-2} = 0.84
AC-3 EpCam+CD45-CD44+CD24-CD133+/-Ncadherin-	10 (0–530)	0 (0–80) p_{1-2} = 0.35
AC-4 EpCam+CD45-CD44+CD24-CD133+/-Ncadherin+	300 (30–720)	40 (0–90) p_{1-2} = 0.126
AC-5 EpCam+CD45-CD44+CD24+CD133+Ncadherin+	360 (160–740)	110 (0–283) p_{1-2} = 0.160
AC-6 EpCam+CD45-CD44+CD24+CD133+/-Ncadherin-	50 (0,00–1,100)	0 (0–140) p_{1-2} = 0.640
AC-7 EpCam+CD45-CD44-CD24+CD133+Ncadherin+	312 (20–940)	0 (0–10) p_{1-2} = 0.064
AC-8 EpCam+CD45-CD44-CD24+CD133+/-Ncadherin-	170 (0–1,030)	0 (0–30) p_{1-2} = 0.194
AC-9 EpCam+CD45-CD44+CD24+CD133-Ncadherin+	2,030 (520–11,420)	150 (0–540) p_{1-2} = 0.025
AC-10 EpCam+CD45-CD44-CD24+CD133-Ncadherin+	1,640 (260–3,870)	10 (0–50) p_{1-2} = 0.003
AC-11 EpCam+CD45+CD44-CD24-CD133-Ncadherin+/-	185 (29–716)	219 (18–436) p_{1-2} = 0.940
AC-12 EpCam+CD45+CD44+CD24+/-CD133+/-Ncadherin+/-	2,476 (813–3,835)	907 (216–1,032) p_{1-2} = 0.016

Figures 1 and 2 present a part of the multicolor flow cytometry protocol in assessing different populations of Epcam+ cells in ascitic fluid of the ovarian cancer patient (Fig. 1) and photographs of the abdominal cavity of this patient obtained during laparoscopy (Fig. 2). The photos show diffuse infiltrating carcinomatosis, confluent nodules in different regions of the diaphragmatic peritoneum, and big infiltrating nodules.

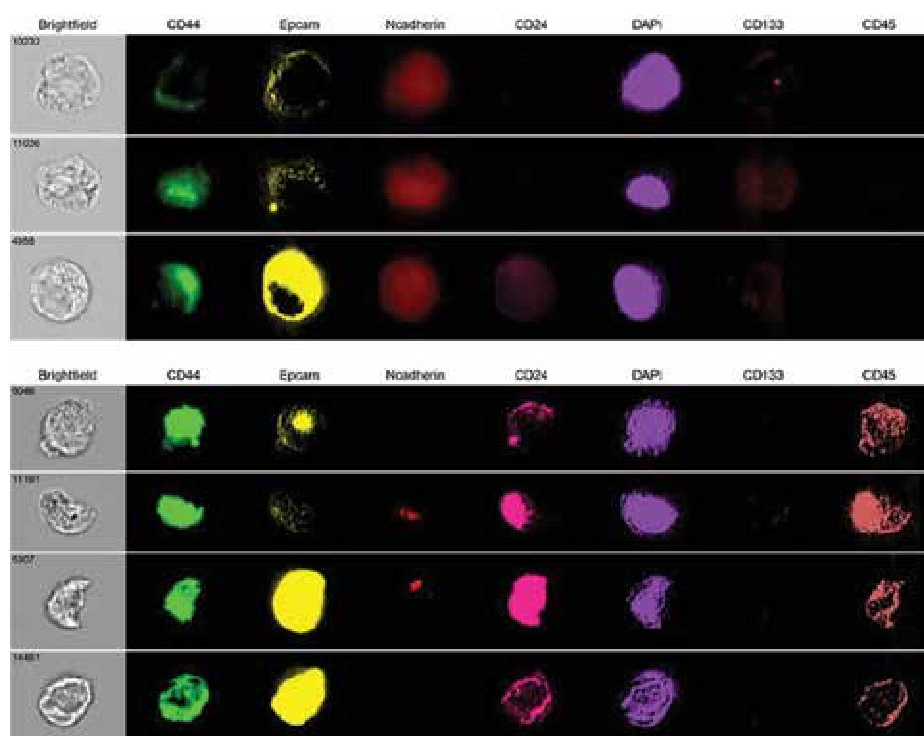

Fig. 1. Different populations of EpCam-positive cells in the ascitic fluid of the ovary cancer patient, high-resolution imaging flow cytometry (Amnis ImageStreamX)

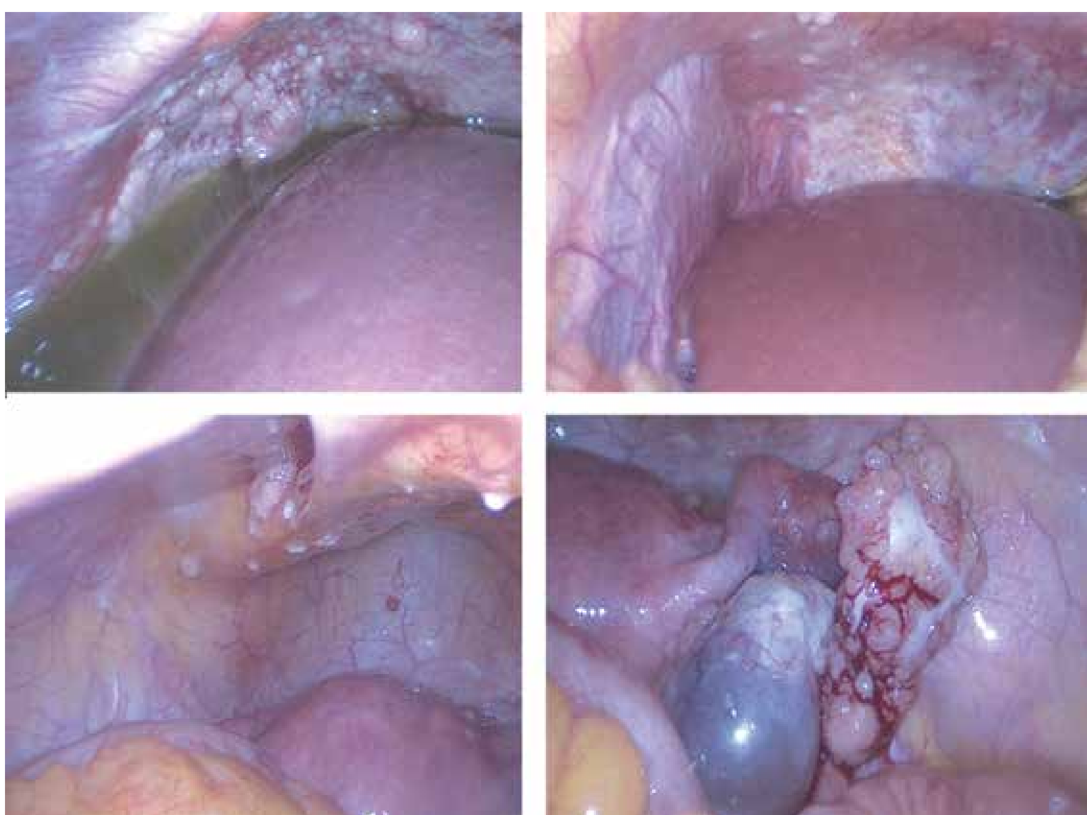

Fig. 2. Photographs of the abdominal cavity of the ovary cancer patient V, 50 years old, T3c Nx M0, obtained during laparoscopy using a Karl Storz laparoscopic unit. PIV index = 8

We assessed the extent of carcinomatosis using the PIV scale in our study. The range of the PIV index in patients included in the study ranged from 0 to 11. The correlation analysis showed that a statistically significant, positive relationship with the PIV index was observed in AC-9 cells with the Epcam+CD45-CD44-CD24+CD133− Ncadherin+ phenotype (r = –0.58; *p* = 0.004) and in atypical (hybrid) cell forms with stemness features Epcam+CD45+CD44+CD24+/−CD133+/− Ncadherin+/− (r = –0.51; *p* = 0.01) (Table 3).

Table 3

Correlation between the number of dissimilar populations of Epcam-positive cells in the ascitic fluid of ovarian cancer patients and the PIV index

A pair of parameters	*r*	*p*
AC1 – PIV	0.32	0.137
AC2 – PIV	−0.02	0.92
AC3 – PIV	0.20	0.35
AC4 – PIV	0.19	0.38
AC5 – PIV	0.29	0.27
AC6 – PIV	0.12	0.64
AC7 – PIV	0.46	0.07
AC8 – PIV	0.21	0.42
AC9 – PIV	0.34	0.11
AC10 – PIV	0.58	0.004
AC11 – PIV	0.15	0.49
AC12 – PIV	0.51	0.01

DISCUSSION

Metastasis of ovarian cancer occurs mainly due to detachment of cells from the primary tumor and their invasion of the abdominal cavity filled with malignant ascites. The cells spread widely with fluid flow and cause secondary tumor growth. At all stages of this unique process, tumor cells change their phenotype and co-evolve together with surrounding fibroblasts, macrophages, adipocytes, endothelial, and other cells.

This study revealed different tumor cell populations in ascitic fluid: atypical forms (hybrid cells) both with and without stemness traits, with EMT traits, and with a combination of these traits and stromal and immune cell populations, identification and characterization of which may be a useful tool in predicting the disease course and response to chemotherapy.

According to the literature data, out of 150 different marker combinations, the most common panel includes three markers: CD44, CD24, and Epcam. Expression of these molecules in OVCAR-5, SKOV-3, and IGROV-1 lines corresponded to cells with greater colony-forming ability. These cells demonstrated a short *in vivo* relapse-free interval after xenotransplantation and a greater migratory capacity in an *in vitro* invasion study compared to CD44-CD24-Epcam cells. In addition, doxorubicin, cisplatin, and paclitaxel promoted an increase in this population, which indicates drug resistance, but Müllerian inhibiting substance (MIS) effectively suppressed its growth [12].

In our study, we showed that the concentration of tumor cells with phenotypes Epcam+CD45-CD44+CD24+CD133−Ncadherin+ and Epcam+CD45-CD44-CD24+CD133−Ncadherin+ was higher in the ascitic fluid of ovarian cancer patients with invasive peritoneal implants compared to the level of these cells in the ascitic fluid of ovarian cancer patients with non-invasive peritoneal implants. We also showed the presence of atypical (hybrid) cells in the ascitic fluid of ovarian cancer patients. The number of atypical (hybrid) cells with stemness features (Epcam+CD45+CD44+CD24+/− CD133+/− Ncadherin+/−) was also significantly higher in the ascitic fluid of patients with invasive peritoneal implants compared to the level of these cells in the ascitic fluid of ovarian cancer patients with non-invasive peritoneal implants. It should be noted that these cell populations are positive for the CD24 marker. In a study aimed at investigating the mechanisms of acquired drug resistance, it was shown that the CD24+ fraction obtained from samples of tumor tissue from the ovarian cancer patient was relatively resistant to cisplatin *in vitro* compared to its CD24− cells. In addition, the tumorigenicity of CD24+ also surpasses that of CD24− cells, as evidenced by the shorter period before the appearance of tumors in mice (Nude mice) injected with an equal number of CD24+ and CD24− cells. It was also found that CD24+ cells express higher mRNA levels of several stemness-related genes (including Nestin, β-catenin, Bmi-1, Oct4, Oct3/4, Notch1, and Notch4, which are involved in modulating many stem cell functions) and a lower E-cadherin mRNA level than CD24− cells [13].

Our clinical trial results are consistent with the literature. Numerous studies have shown that the Epcam+CD44+CD24+CD133+CD117+ population has an increased ability to initiate cancer and/or stimulate metastasis *in vivo* [13, 14].

In a mouse model (NOD/Shi-scid/IL-2Rγ null mice), CD24+ and CD133+ cells were demonstrated to be more capable of forming spheres, spreading, and initiating tumors *in vivo*. In addition, CD24+ cells showed a more mesenchymal phenotype with higher expression of Twist1, Snail, and Vimentin, which relates the CD24 marker to the EMT phenotype. Interestingly, CD24- cells are also capable of initiating tumor growth, albeit to a lesser extent than CD24+.

This is probably determined by the fact that a subset of CD24- cells with stemness traits has a lower proliferation rate than CD24+ cells [15].

The hybrid cells and multicellular aggregates which role in cancer has long been examined [16–18], were also discovered in our study. Other researchers revealed fusion of blood cells and epithelial cells in Lewis lung carcinoma and metastatic epithelial ovarian carcinoma [19]. Similar results were obtained by A.E. Powell et al. for colorectal cancer [20]. Another study claimed that their formation and form depend on the cadherin expression profile. For instance, Ncad+ cells formed stable and dense spherical structures, and Ecad+ cells – clusters with lower adhesion (compared to Ncad+) [21].

In our study, we showed that the number of Epcam+CD45-CD44-CD24+CD133− Ncadherin+ cells and atypical (hybrid) Epcam+CD45+CD44+CD24+/− CD133+/-Ncadherin+/− cells has a direct correlation with the PIV index, characterizing the prevalence of carcinomatosis. This index takes into account the condition of the parietal peritoneum, diaphragmatic peritoneum, mesentery of the intestine, omentum, intestinal wall, stomach, and liver [11, 22–24]. A. Fagotti et al. (2006) showed that with PIV ≥ 8, the probability of R0 resection is practically equal to 0, and NACT is recommended [11]. The effectiveness of this approach was subsequently confirmed by a series of randomized trials.

In 2017, data from a Dutch multicenter randomized trial were published, including treatment analysis for 201 patients with advanced ovarian cancer [24]. A total of 102 patients underwent laparoscopy with PIV determination to assess the possibility of performing primary optimal cytoreductive surgery (treatment group), and 99 patients underwent primary cytoreductive surgery without laparoscopic assessment (control group). The authors considered a decrease in the number of unjustified laparotomies (diagnostic operations, suboptimal cytoreductions), that reduce the effectiveness of treatment in this category of patients, as one of the main advantages of the proposed technique [24].

The results obtained in our study showed a direct correlation between the PIV index and the level of ascites tumor cells with EMT traits (AC-10) and atypical (hybrid) cells with stemness features (AC-12). It can be assumed that determining Epcam+CD45-CD44-CD24+CD133−Ncadherin+ and Epcam+CD45+CD44+CD24+/−CD133+/−Ncadherin+/− cells in ascitic fluid will be useful for specifying the treatment strategy for ovarian cancer patients.

CONCLUSION

The results of the study show high heterogeneity of tumor cells in the ascitic fluid of patients with ovarian cancer. The presence of atypical (hybrid) forms of Epcam-positive cells is of interest for cell biology and requires further research. The cell populations identified in our study (Epcam+CD45-CD44-CD24+CD133− Ncadherin+ and Epcam+CD45+CD44+CD24+/− CD133+/−Ncadherin+/−) and their detection in ascitic fluid can be useful for determining the treatment strategy for patients with ovarian cancer.

Further study of various populations of tumor cells in ascitic fluid and their relationship with the clinical course of the disease and effectiveness of chemotherapy for patients with ovarian cancer is reasonable and opens up great prospects for practical developments in the field of targeted therapy and liquid biopsy.

ACKNOWLEDGMENTS

The authors are grateful to S.V.Vtorushin, head of the Pathological Anatomy and Cytology Department of Cancer Research Institute, Tomsk NRMC, Prof. V.M.Perelmuter and Prof. L.A.Kolomiets (head of the Gynecology Department of Cancer Research Institute, Tomsk NRMC) for providing consultations on selection of patients for the study. The authors would also like to thank Grigoriy Marchenko from Luminex Corporation in Russia and the CIS for assistance in organization of the experiment using the ImageStreamX imaging flow cytometer (Luminex, Poland).

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Acknowledgments

The authors are grateful to The Core Facility «Medical genomics», Tomsk NRMC for the opportunity to use scientific equipment. Also authors thank Grigory Marchenko, Flow Cytometry Supervisor, Russia&CIS Luminex Corporation for help in experiment organization using flow cytometer with ImageStreamX visualization (Amnis).

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Kaigorodova E.V., Ochirov M.O., Molchanov S.V., Dyakov D.A., Rogachev R.R., Kovalev O.V., Vtorushin S.V. – carrying out of research, analysis and interpretation of data. Ochirov M.O., Molchanov S.V, Shpileva O.V., Chernyshova A.L. – diagnosis and treatment of patients with ovarian cancer. Kaigorodova E.V. – conception and design, drafting of the manuscript.

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Received 06.07.2020
Accepted 28.12. 2020