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Metalloproteinases at the surface of small extrcellular vesicles in advanced ovarian cancer: Relationships with ascites volume and peritoneal canceromatosis index

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ABSTRACT

Metalloproteinases and their extracellular matrix metalloproteinase inducer (EMMPRIN) play an essential role in the regulation of signaling from growth factors receptors and adhesion molecules, cell motility and extracellular matrix degradation. The aim of the study was to evaluate the relationship between the levels of small extracellular vesicles (sEVs) metalloproteinases, such as ADAM10, ADAM17, MMP2, MMP9 and EMMPRIN and ascites volume and peritoneal canceromatosis index in advanced ovarian cancer patients (OCPs).

The subpopulations of metalloproteinases at the surface of sEVs of borderline ovarian tumor patients (BOTPs) (n = 20, 36.5 ± 2.5 years) and previously untreated advanced OCPs (n = 35, 56.5 ± 2.5 years) were evaluated using flow cytometry. The metalloproteinase subpopulations of CD9-positive sEVs isolated from plasma of BOTPs and OCPs appeared to be quite similar. However, a significant difference in the expression of ADAM-metalloproteinases in ascites sEVs was found between BOTPs and OCPs. The level of sEVs metalloproteinases in OCPs significantly depended on the ascites volume. A statistically significant relationship between the level of ADAM10+/ADAM17- subpopulation in plasma sEVs and the peritoneal canceromatosis index was found (R = 0.66, p < .05). The levels of metalloproteinases and EMMPRIN in circulating sEVs, as well as the assessment of individual subpopulations may be promising approaches to OCPs managing.

1. Introduction

It's currently believed that, extracellular vesicles (EVs), such as exosomes (30–100 nm) and microvesicles (100–1000 nm), are great importance in the processes of tumor invasion, metastasis, the tumor chemo- and hormonoresistance [1,2]. They are detected in many biological fluids, such as plasma, blood serum, urine, saliva, breast milk, spinal fluid, as well as in pathological effusions (eg, ascites). Elevated levels of circulating EVs were detected in a number of solid tumors [3,4].

Proteases play an important role in the functional activity of the vesicles. Tetraspanin-associated proteases of EVs include ADAMs and matrix metalloproteinases (MMPs), as well as their inducer EMMPRIN [5,6]. ADAM10 and ADAM17 are transmembrane “molecular scissors”, which perform shedding - limited premembrane proteolysis of proteins, leading to the cleavage of the extracellular domain of transmembrane proteins. Substrates of sheddases are growth factor receptors (EGFR1, HER2, TGFβ-IIIR), adhesion receptors (CD44 and L1CAM), Fas-L receptor [7]. Both pro- and mature MMP9, MMP9, MMP14 forms with degrading activity exists in EVs from many tumor cells lines, including

Abbreviations: EVs, extracellular vesicles; MMPs, matrix metalloproteinases; MMP9, MMP2, MMP14, matrix metalloproteinases 9 and 2; ADAM10 and ADAM17, transmembrane metalloproteinase acting as sheddases; EMMPRIN (CD147), inducer of MMPs; PCI, peritoneal carcinomatosis index; BOTPs, Borderline ovarian tumor patients; OCPs, Ovarian cancer patients

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ovarian cancer cell lines as well as THP-1 monocytes cell line, human umbilical cord endothelial cells (HUVECs) [6,8]. Mature forms of ADAMs are predominant especially in exosomes [6]. Many MMPs, ADAM10 and ADAM17 were revealed in high concentration in exosomes and microvesicles from malignant ascites [6,9–12].

EMMPRIN (CD147) is often over-expressed in tumor cells, which can also be involved in the progression of malignant neoplasms by regulating the expression of MMPs in peritumoral stromal cells. CD147 has been shown to be secreted in microvesicles derived from cell cultures of epithelial ovarian cancer, and that these CD147-positive vesicles may contribute to the formation of an angiogenic phenotype in endothelial cells in vitro. The vesicles produced by human ovarian carcinoma cell lines OVCAR3, SKOV3 and A2780 expressed different levels of CD147 and stimulated the pro-angiogenic activity of HUVEC by CD147-dependent mechanisms (OVCAR3 > SKOV3 > A2780). The vesicles produced by the CABA I (ovarian carcinoma cell line with low CD147 expression) did not have any effect on the development of the angiogenic phenotype in HUVECs. Treatment of OVCAR3 cells with siRNA CD147 suppressed the angiogenic potential of vesicles derived from OVCAR3 [13].

One of the main prognostic factors for the effectiveness of advanced ovarian cancer treatment is the volume of residual tumors after cytoreductive surgery, and life expectancy is inversely proportional to the size of residual tumors [14]. At the same time, the currently used clinical classification of ovarian cancer (FIGO, 2013) does not always allow preoperative evaluation of the feasibility of performing optimal cytoreductive surgery. The difficulties are associated with the peritoneal carcinomatosis degree and assessment of the ascites volume. Currently, it has been shown that the volume of ascites itself has prognostic significance in patients with high-grade serous ovarian carcinomas [15]. In ovarian cancer, Fagotti A. et al. [16] proposed to use peritoneal carcinomatosis index (PCI) to evaluate peritoneal carcinomatosis. The authors showed that patients with PCI > 8 were unlikely benefit from undergoing complete cytoreductive surgery. When complete cytoreduction seemed unlikely, neoadjuvant chemotherapy was recommended [16]. Taking into account the important role of exosomal metalloproteinases and EMMPRIN in the regulation of signaling from receptors of growth factors, degradation of the extracellular matrix, cell motility, it seems relevant to study the exosomal metalloproteinase compositions in patients with advanced OC in relation with the ascites volume and PCI to clarify clinical significance of these exosomal markers.

2. Materials and methods

2.1. Patients

Blood and ascites samples of borderline ovarian tumor patients (BOTPs) ($n = 20$, 38.5 ± 2.7 years) and previously untreated advanced ovarian cancer patients (OCPs) ($n = 35$, 56.5 ± 2.5 years) were obtained from the Department of Gynecology of the Cancer Research Institute of Tomsk National Research Medical Center (Table 1). The volume of ascites was measured clinically and by ultrasound examination. Three clinical subgroups of OCPs were formed: patients having low-volume (< 200 mL), moderate-volume (200–1000 mL) and high-volume ascites (> 1000 mL). The peritoneal carcinomatosis index (PCI) was determined in all OCPs by thoracoabdominal computed tomography and/or preoperative laparoscopy [16]. The study was approved by the Local Ethics Committee of the Cancer Research Institute of Tomsk National Research Medical Center (Tomsk, Russia). All patients were fully informed of the purpose and nature of the treatment and provided an informed written consent.

Table 1
Clinical and histopathological characteristics of the BOTPs and the OCPs.

		BOTPs, No (%)	OCPs, No (%)
Histology	Serous	20 (100%)	35 (100%)
	Other		
FIGO (2013) staging	I-II	16 (80%)	
	IIIB	4 (20)	5 (14%)
	IIIC		30 (86%)
Tumor grade	High-grade		35(100%)
	Not specify	20 (100%)	
Total no. of patients		20 (100%)	35 (100%)

2.2. Small extracellular vesicles (sEVs) isolation

Blood plasma sEVs were isolated using ultrafiltration with ultracentrifugation [17]. In particular, venous blood (18 mL) from BOTs and OCPs was collected in K_3EDTA spray-coated vacutainers. The blood cells were pelleted by centrifugation for 20 min at 1200*g* (bucket rotor, Labofuge 400R, Thermo Fisher) and 4 °C. To remove the cell debris, plasma samples were centrifuged at 17000*g* (angular rotor, centrifuge 5415R, Eppendorf) and 4 °C for 20 min. To remove vesicles > 100 nm, the supernatant was diluted 5-fold with PBS (10 mM phosphate buffer, 0.15 M NaCl, pH 7.5) and filtered through 100 nm pore-size filter (Minisart high flow, 16553-K, Sartorius), than filtrate was centrifuged at 100000*g* (bucket rotor, Optima XPN 80, Beckman Coulter, USA) and 4 °C for 90 min, the pellet was resuspended in 10 mL PBS and twice centrifuged under the same conditions. Ascites fluid was centrifuged at 900*g* for 20 min at 4 °C, supernatant was transferred into a new tube, and centrifuged to pellet cell debris, than supernatants were diluted as 1:3 by PBS and were filtered and ultracentrifuged under the same condition listed above. Supernatant was removed and the pellet containing ascites fluid and plasma sEVs was re-suspended in 300 μL of PBS. sEVs samples from plasma and ascites fluid were aliquoted and stored either at −80 °C or in liquid nitrogen. The aliquots were thawed once before use [17].

2.3. Electron microscopy of sEVs

For negative staining, 10 μL of isolated sEVs samples were adsorbed for 1 min on copper grids covered with carbonized formvar film. Then the grids were exposed for 5–10 s on a drop a 2% phosphoric-tungstic acid. Grids were studied using Jem 1400 transmission electron microscope (Jeol, Japan), the images were obtained with a digital camera Veleta (Olympus Corporation, Japan).

2.4. Flow cytometry analysis

To evaluate the protein concentration of sEVs, a NanoOrange Protein Quantitation kit (Molecular Probes, USA) was used in accordance with the manufacturer's recommendations.

The 4 μm-diameter aldehyde/sulphate latex beads (Thermo Fisher Scientific, USA) were incubated with anti-CD9 (ab134375, Abcam) antibodies at room temperature for 14 h at gentle agitation. The aliquots of sEVs (about 30 μg vesicular protein) were incubated with antibody-coated latex beads in 150 μL of PBS at 4 °C for 14 h at gentle agitation. The reaction was blocked with 0.2 M glycine for 30 min at 4 °C. The exosomes-antibody-bead complexes were washed twice with washing buffer (2% EVs depleted bovine serum in PBS), were incubated with a blocking immunoglobulin G (BD Biosciences, USA) at room temperature for 10 min with washing. Then there was incubation with FITC-conjugated anti-tetraspanins (CD63, CD81, CD24) antibodies (BD Biosciences, USA) at 4 °C for 50 min. The complexes were washed twice with washing buffer. Single beads were gated, and acquired in a Cytoflex (Becman Coulter, USA). Data were analyzed with CytExpert 2.0 Software. The median fluorescence intensity (MFI) of the sEVs was analyzed in comparison with the isotypic control (BD bioscience, USA) [4].

2.5. Analysis of metalloproteinases in sEVs

Aliquots of sEVs (about 30 μg vesicular protein) were incubated with 3×10^5 latex beads labeled anti-CD9 antibody in 150 μL of PBS at 4 °C overnight at gentle agitation, blocked in 0.2 M glycine for 30 min, then stained with anti-ADAM10 (CD156c)-PE (5 μL on test, N352704, Biolegend, USA), anti-ADAM17/TACE antibody (dilution 1:10, LS-C329200/109998, LifeSpan BioSciences, USA) for 20 min at room temperature. Then complexes were stained with anti-Rabbit IgG secondary antibody, Alexa Fluor 488 (dilution 1:3000, Thermo Fisher Scientific, USA). For analysis of vesucular MMPs and EMMPRIN latex beads-antiCD9 antibody-vesicles complexes stained with anti-EMMPRIN (CD147)-APC (5 μL on test, MAB5047, Abnova, USA), anti-MMP2-PE (0.3–0.5 μg on test, AAA444-575, Antibodies-online, Germany) and anti-MMP9-FITC (1 μg on test, AA1-708, Antibodies-online, Germany) for 20 min at room temperature. Single beads were gated, and acquired in a Cytoflex (Becman Coulter, USA). Data were analyzed with CytExpert 2.0 Software. We have submitted all relevant data of our experiments to the EV-TRACK knowledgebase (EV-TRACK ID: EV190021) (Van Deun J, et al. *EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research.* Nature methods. 2017;14(3):228–32).

2.6. Statistical analysis

Statistical analysis was performed using Statistica 10.0 software. Each data set was first tested for a Gaussian distribution by using a Shapiro-Wilk normality test. All data were expressed as medians with interquartile ranges or as means with standard errors. To evaluate the difference, Mann-Whitney *U* test and Kruskal-Wallis test were used. Correlation analysis on data was carried out with Spearman Rank Correlation test. *P*-values < .05 were considered to be statistically significant.

3. Results

3.1. Characterization of plasma and ascites sEVs

The morphology of sEVs was confirmed by the transmission electron microscopy. Cup-shaped low electron density particles with a clearly visible membrane were found in the preparations of EVs isolated from the blood plasma and ascites of OCPs and BOTPs (Fig. 1). In the all preparations, there were also particles without membrane. Some of them – compact roundish low electron density particles with clear-cut boundaries by morphological characteristics were determined as very low or low density lipoproteins and called “non-vesicles” [18,19]. Others with moderate or high electron density and irregular shape - as protein aggregates. The number of sEVs in OCPs ascites samples was significantly higher than in samples obtained from blood plasma. The number of “non-vesicle” did not differ in the sEVs samples from blood plasma and ascites in both OCPs and BOTs.

The isolated sEVs were also characterized for the presence of exosomal markers (CD9, CD81, CD63 and CD24) using flow cytometry. A combination of conjugated and unconjugated antibodies made it possible to identify different subpopulations of exosomes (Fig. 2). A subpopulation of CD9/CD24-positive sEVs predominated in both plasma and ascites of BOTPs and OCPs. In OCPs, the MFI of the CD9/CD24-positive sEVs population was significantly lower in plasma than in ascites (MFI = 2300 ± 250 and MFI = 4600 ± 380, respectively, $p < .05$) In BOTPs, no significant difference in the MFI of the CD9/CD24-positive sEVs population between ascites and plasma was found.

A NanoOrange Protein kit was used to determine the protein concentration in samples. It was found that median sEVs protein in OCPs ascites is significantly higher than in plasma (26.0 ± 3.2 μg/ml and 15.6 ± 3 μg/ml, correpondingly, $p < .05$. There was no significant difference in the level of vesicular protein in plasma and ascites samples in BOTPs and OCPs.

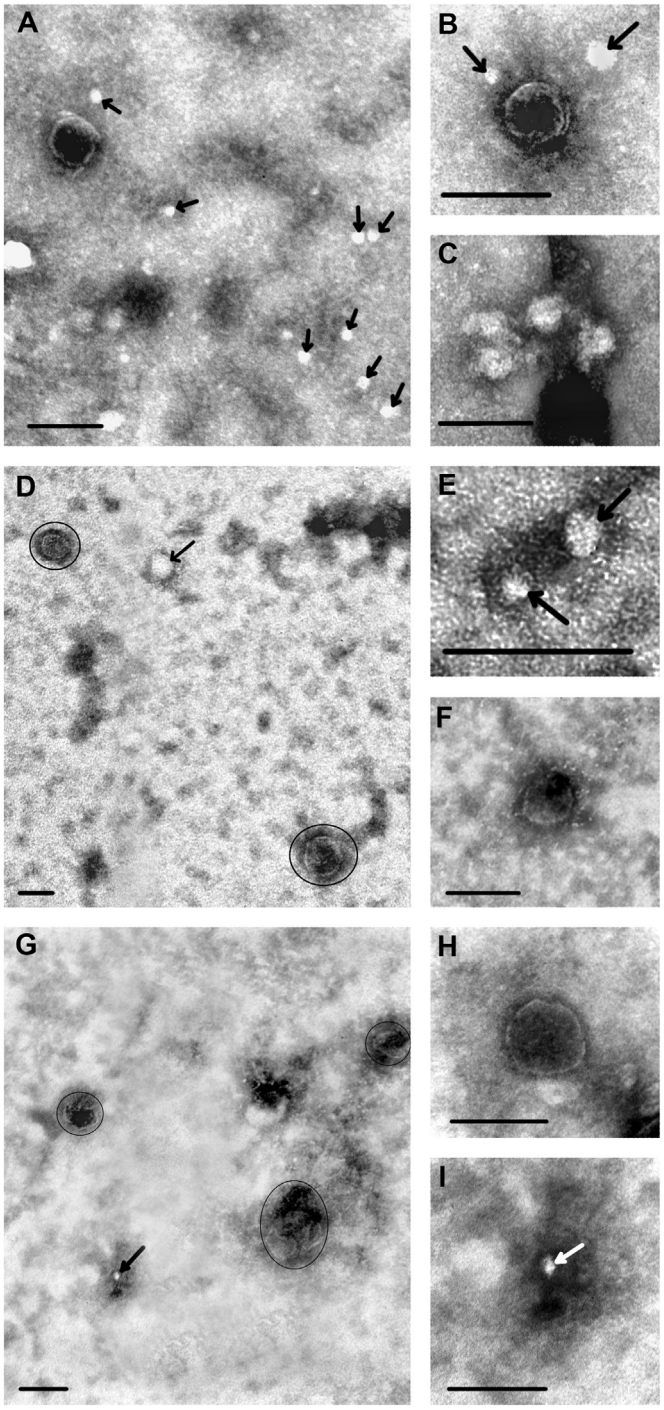

Fig. 1. Electron microscopic images of EVs and ‘non-vesicle’ structures isolated from: A-C – blood plasma BOTPs, D-F - blood plasma of OCPs, G-I – ascitic fluid of OCPs. A, D, G – total view. B, F, H – sEVs. C, E, I - ‘non-vesicles’. Ovals indicate sEVs, arrows - ‘non-vesicles’. Scale bars correspond to 100 nm. Electron microscopy, negative staining.

3.2. sEVs metalloproteinases distribution in of BOTs and OCs patients

We used anti-CD9 coated latex beads for the detection of ADAM10/ADAM17 and MMP9/MMP2/EMMPRIN subpopulations of sEVs in plasma and ascites BOTPs and OCPs (Fig. 3). Metalloproteinase compositions at the surface of the CD9-positive sEVs in BOTs and OCs patients are presented in Table 2. In the CD9-positive sEVs of blood

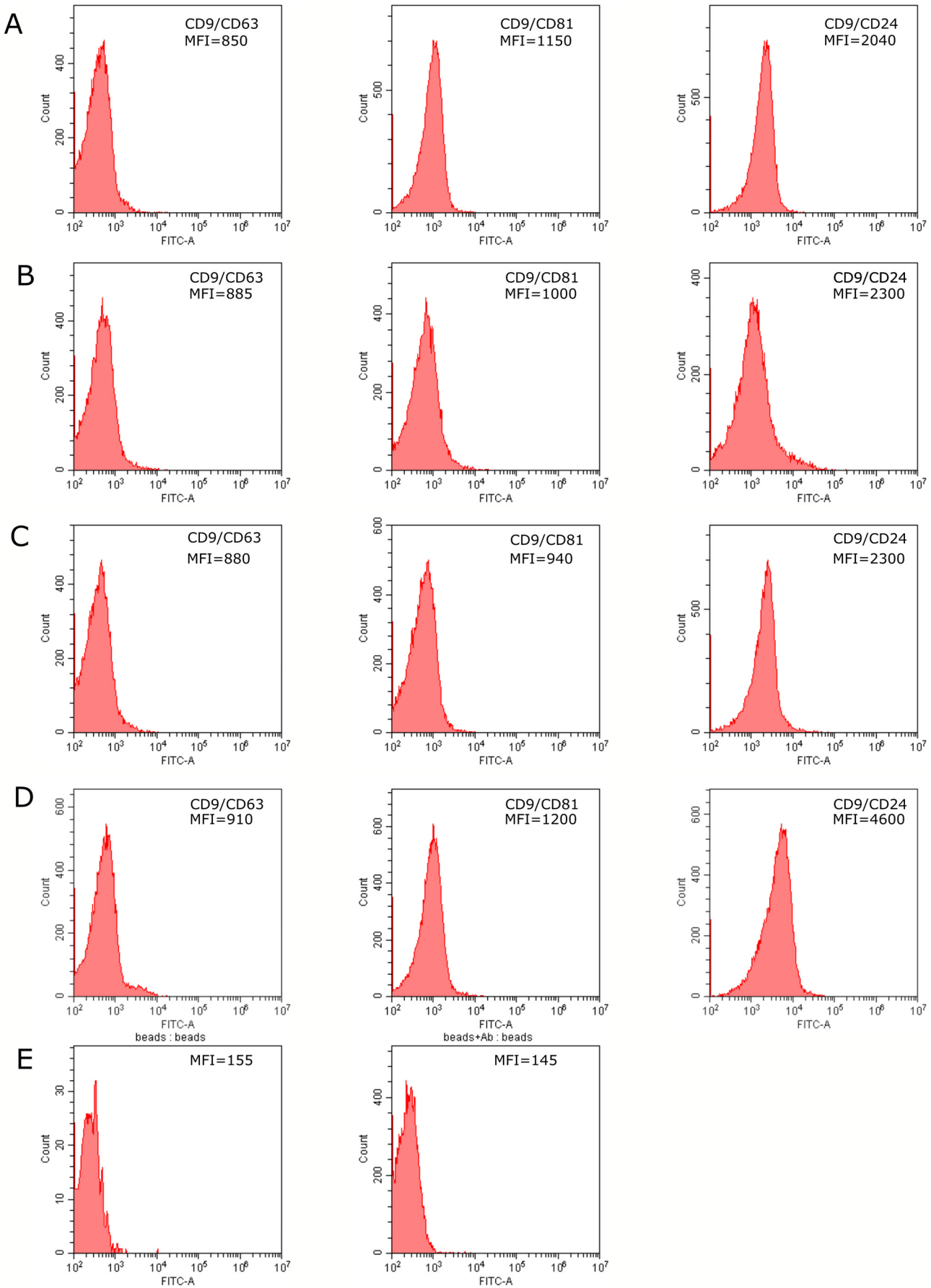

Fig. 2. Expression of CD63, CD81 and CD24 on CD9-positive sEVs of BOTs plasma (A), OCPs plasma (B), BOTs ascites (C), OCPs ascites (D). Isotype control and negative control (latex beads labeled anti-CD9 with anti CD81 FITC antibody) (E). Mean MFI are shown.

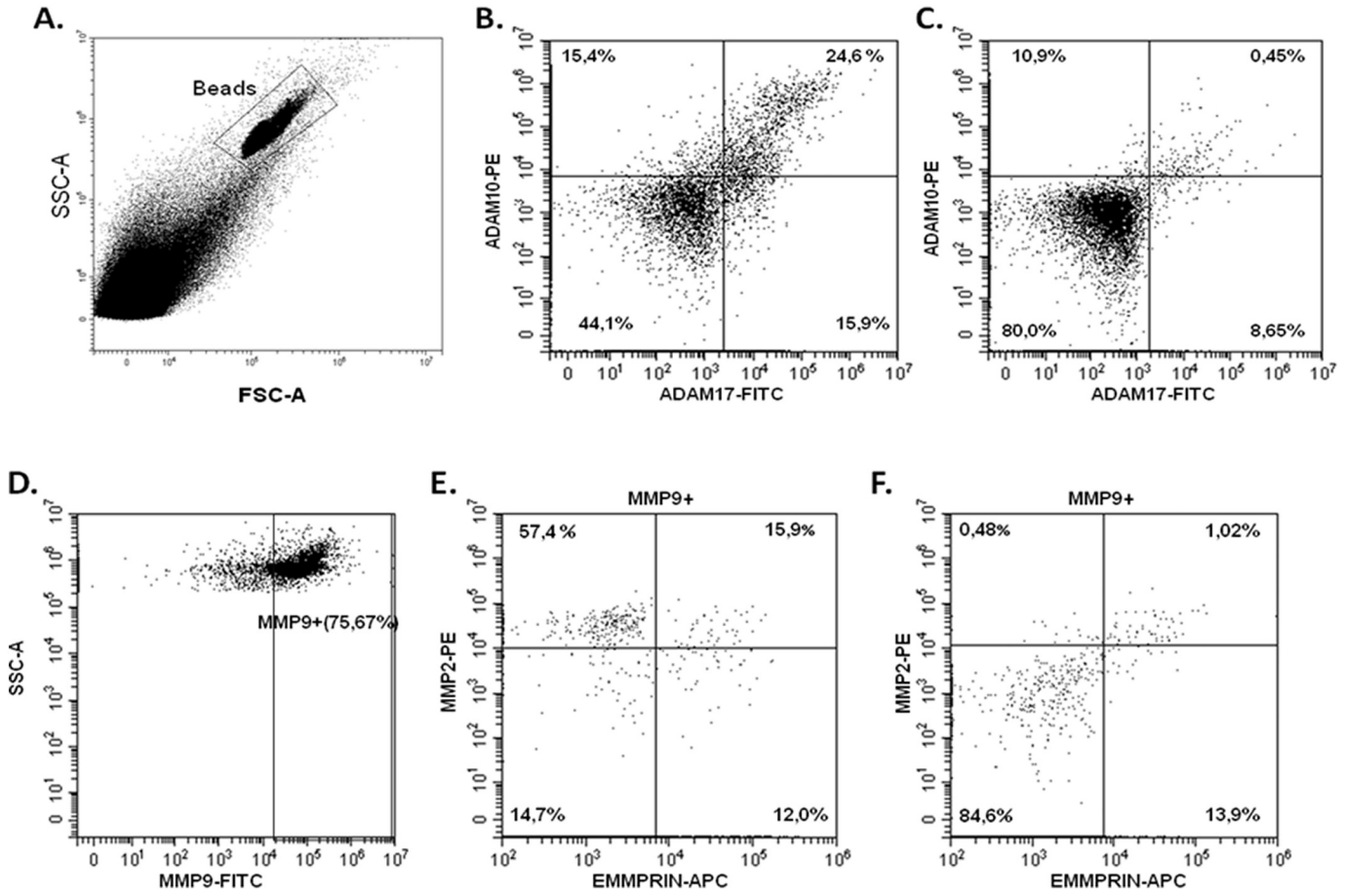

Fig. 3. Flow cytometry analysis of sEVs subpopulations. Forward scatter area (FSC-A) versus side scatter area (SSC-A) dot plot representing EVs samples adsorbed on aldehyde/sulphate latex beads labeled anti-CD9 antibody (A). Double labeling ADAM10 versus ADAM17 of ascites sEVs of BOTS (B) and OCPs (C). MMP9-positive plasma sEVs population in OCPs (D). Double labeling MMP2 versus EMMPRIN of plasma MMP9-positive (MMP9 +) sEVs of OCPs with low-volume ascites (E) and high-volume ascites OCPs (F).

Table 2
Metalloproteinase compositions (%) at the surface of sEVs in of BOTPs and OCPs samples, M ± m.

Subpopulations	BOTPs	OCPs	P-value
In plasma			
ADAM10 − /ADAM17 −	92.3 ± 2.6	89.9 ± 2.35	
ADAM10 + /ADAM17 −	2.63 ± 0.50	6.97 ± 2.16	
ADAM10 − /ADAM17 +	4.89 ± 0.63	2.81 ± 0.60	
ADAM10 + /ADAM17 +	0.21 ± 0.06	0.28 ± 0.05	
MMP9 +	78.0 ± 2.90	73.3 ± 5.98	
MMP9 −	22.0 ± 3.1	26.6 ± 6.10	
MMP9 + /MMP2 + /EMMPRIN +	2.20 ± 0.87	4.77 ± 1.60	
MMP9 + /MMP2 − /EMMPRIN −	90.0 ± 11.0	77.6 ± 6.00	
MMP9 + /MMP2 − /EMMPRIN +	7.77 ± 1.66	12.8 ± 2.62	
MMP9 + /MMP2 + /EMMPRIN −	0.12 ± 0.05	5.54 ± 2.54	< 0.05
In ascites			
ADAM10 − /ADAM17 −	38.1 ± 2.90	80.0 ± 5.39	< 0.05
ADAM10 + /ADAM17 −	15.8 ± 3.30	10.9 ± 2.02	
ADAM10 − /ADAM17 +	17.9 ± 3.90	8.65 ± 2.50	< 0.05
ADAM10 + /ADAM17 +	28.2 ± 4.04	0.41 ± 0.09	< 0.05
MMP9 +	73.0 ± 10.3	69.4 ± 6.75	
MMP9 −	27.0 ± 4.19	30.7 ± 6.03	
MMP9 + /MMP2 + /EMMPRIN +	8.23 ± 1.5	6.70 ± 4.23	
MMP9 + /MMP2 − /EMMPRIN −	78.9 ± 9.7	80.3 ± 3.60	
MMP9 + /MMP2 − /EMMPRIN +	12.5 ± 1.9	14.3 ± 2.25	
MMP9 + /MMP2 + /EMMPRIN −	0.16 ± 0.10	2.46 ± 1.75	

plasma of BOTPs and OCPs, ADAM10 − /ADAM17- subpopulation predominated (up to 90%). In the CD9-positive sEVs from ascites in BOTPs, subpopulations of ADAM10 + /ADAM17- and ADAM10 − /ADAM17 + (up to 18%) occurred equally. The level of ADAM10 + /ADAM17 + the CD9-positive sEVs in ascites was higher in BOTPs than OCPs, while the level of ADAM10 − /ADAM17- sEVs was significantly lower in BOTPs than in OCPs (Fig. 3B, C). In plasma and ascites, MMP9-positive population predominated in both BOTPs and OCPs. Statistical significant differences in the level of MMP9 + /MMP2 + /EMMPRIN- subpopulation were revealed ($p < .05$).

3.3. CD9-positive sEVs metalloproteinase compositions depending on ascites volume and peritoneal canceromatosis index in OCPs

Metalloproteinase compositions in CD9-possitive sEVs in OCPs depending on ascites volume are presented in Table 3. In low-volume ascites OCPs (up to 200 mL), the level of ADAM10 + /ADAM17- plasma CD9-positive sEVs was increased, whereas in OCPs with moderate- and high-volume ascites, the level of ADAM10 + /ADAM17- plasma CD9-positive sEVs was decreased. The increased ADAM10 − /ADAM17 + level in circulating CD9-positive sEVs was observed in OCPs with a high-volume ascites. The redistribution of subpopulations of plasma-derived CD9-positive sEVs expressing MMPs and EMMPRIN in OCPs was shown to be dependent on the ascites volume. The proportions of MMP9 + /MMP2 + /EMMPRIN + and MMP9 + /MMP2 + /EMMPRIN- plasma CD9-positive sEVs were higher in low-volume ascites OCPs than in OCPs with moderate- and high-volume ascites (Fig. 3D–F). The same

Table 3
Metalloproteinase compositions (%) in at the surface of sEVs in OCPs samples depending on ascites volume, M ± m.

Subpopulations	Ascites volume, mL			P-value
	Up to 200	200–1000	More 1000	
In plasma				
ADAM10−/ADAM17−	85.5 ± 9.00	94.1 ± 1.70	89.1 ± 1.62	
ADAM10+/ADAM17−	12.5 ± 3.50	4.15 ± 1.15	6.44 ± 0.95	< 0.05
ADAM10−/ADAM17+	1.43 ± 0.36	1.42 ± 0.41	4.21 ± 0.66	< 0.05
ADAM10+/ADAM17+	0.23 ± 0.09	0.27 ± 0.08	0.30 ± 0.07	
MMP9+	60.0 ± 10.0	76.7 ± 10.0	72.9 ± 5.02	
MMP9−	40.0 ± 15.1	20.3 ± 5.10	25.0 ± 5.07	
MMP9+/MMP2+/EMMPRIN+	13.9 ± 2.88	2.37 ± 0.80	0.92 ± 0.12	< 0.05
MMP9+/MMP2-/EMMPRIN-	30.5 ± 7.09	83.6 ± 6.07	84.5 ± 5.85	< 0.05
MMP9+/MMP2-/EMMPRIN+	10.2 ± 2.00	13.7 ± 1.66	13.9 ± 3.60	
MMP9+/MMP2+/EMMPRIN−	35.4 ± 8.05	0.29 ± 0.08	0.11 ± 0.02	< 0.05
In ascites				
ADAM10−/ADAM17−	83.7 ± 9.00	71.5 ± 10.0	83.2 ± 10.1	
ADAM10+/ADAM17−	8.73 ± 3.01	16.5 ± 3.42	8.69 ± 3.40	< 0.05
ADAM10−/ADAM17+	7.16 ± 3.01	11.4 ± 3.80	7.68 ± 3.00	
ADAM10+/ADAM17+	0.35 ± 0.06	0.52 ± 0.12	0.38 ± 0.09	
MMP9+	66.6 ± 7.72	70.6 ± 11.7	65.1 ± 11.0	
MMP9−	33.4 ± 5.12	29.4 ± 6.03	34.9 ± 5.23	
MMP9+/MMP2+/EMMPRIN+	3.80 ± 0.80	12.1 ± 5.08	1.36 ± 0.22	
MMP9+/MMP2−/EMMPRIN−	72.3 ± 8.70	81.1 ± 5.00	82.14.20	
MMP9+/MMP2−/EMMPRIN+	12.4 ± 0.35	14.4 ± 3.08	16.3 ± 3.12	
MMP9+/MMP2+/EMMPRIN−	12.0 ± 5.10	0.59 ± 0.33	0.04 ± 0.01	< 0.05

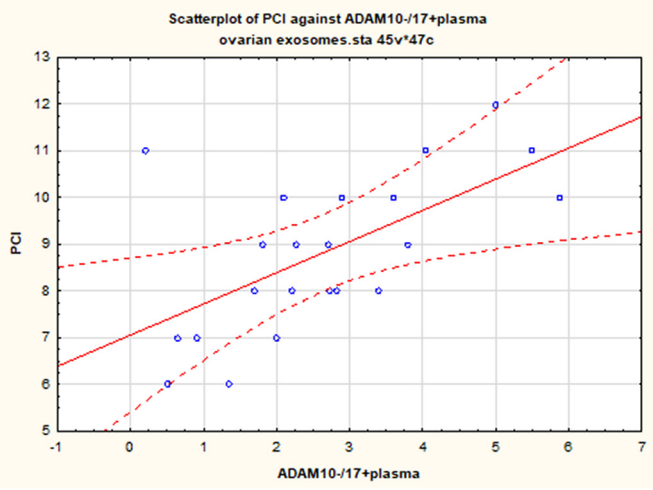

Fig. 4. Scateplot of PCI against ADAM10−/ADAM17+ subpopulation of plasma sEVs. Note: The solid line is the trend line, the dotted line is the 95% confidence interval.

changes in the level of MMP9+/MMP2+/EMMPRIN- population in ascites-derived CD9-positive sEVs were observed. The relationship between the metalloproteinases and EMMPRIN expressions in plasma and ascites CD9-positive sEVs and PCI (ranged from 5 to 12 units) in OCPs was revealed. A statistically significant correlation between the level of the ADAM10+/ADAM17- subpopulation in plasma-derived CD9-positive sEVs and PCI was found, $R = 0.66$, $p < .05$ (Fig. 4).

4. Discussion

OCPs were found to release sEVs not only into the blood circulation but also into ascites. There are contradictory data on the number of circulating and ascites-derived sEVs in OCPs. Apparently, this is due to various techniques employed in the isolation of exosomes and different methods available for determining the protein content in vesicles [20]. Most authors report that both the total number of sEVs and the expression of exosomal markers in OCPs can significantly vary, especially in ascites [9,10,21]. CD24 is often expressed in both the ascites exosomes and plasma exosomes in OCPs [10].

The role of metalloproteinases (ADAMs, MMPs) and their inducer (EMMPRIN) in the progression of cancer has been well studied, but the role of vesicular metalloproteinases in the formation and progression of malignant tumors has been poorly studied. Data on the level of exosomal metalloproteases in circulating and ascites exosomes in OCPs is extremely scarce. Meanwhile it is known that ADAM10 exhibits proteolytic activity in multivesicular bodies, hence, mature ADAM10 is already included in exosomes [7]. Annexin V-binding EVs have higher level of MMP9 in malignant compared to portal-hypertensive ascites [21]. There is no evidence of the presence of pro- or mature forms of MMPs in multivesicular bodies, but proteomics have shown the presence of both membrane-type (MMP14, MMP24, MMP25) and secreted-type MMPs (MMP-1, -13, -2,-9, -3, -10, -7, -19) in EVs derived from various cell type, some of which possess proteolytic activity. Gelatinases MMP2 and MMP9 in EVs are most studied [6,22]. Currently, there are several theories explaining the presence of many components of the extracellular matrix (fibronectin, glycosaminoglycans, secreted-types MMPs) on EVs. It is assumed that EV membrane deposition of matrix molecules results from binding of these molecules onto EV surfaces extracellularly. It is assumed that the binding of pro- and mature forms of MMPs is mediated through the binding of EVs integrins with fibronectin. While on the surface of the EVs the pro-MMP2 shown can be activated by MMP14. Thus, vesicles become the centers of MMP-2 activation [22,23]. It has been proposed recently that cells endocytose of matrix molecules and re-secrete them on the exofacial surface of EVs [22].

The pool of EVs isolated from blood plasma and ascites of OCPs is a complex mixture of vesicles of different origin, where, in addition to tumor vesicles, there are a large number of vesicles of hematopoietic and endothelial origins. Mesothelial cell-derived vesicles can be also found in ascites. The MMP2, MMP9, ADAM10, ADAM17 and EMMPRIN were shown to be expressed not only by tumor cells, but also by platelets [6], endotheliocytes [24], monocytes/macrophages [25], stem and progenitor cells [26], fibroblasts [27], mesothelial cells [28], and all these cells can be sources of circulating and ascites EVs.

The simultaneous determination of a large number of surface molecules on EVs using flow cytometry is often problematic. Taking into account a similar surface density of surface receptors and antigens, EVs

will carry ~10,000-times less surface molecules than the parent cells [29]. Therefore, we separately analyzed the ADAM10/ADAM17 subpopulations and MMP9/MMP2/EMMPRIN subpopulations of plasma and ascites CD9-positive sEVs in BOTPs and OCPs.

Our previously published data indicated a high expression of ADAM10 in ascites in the CD24-positive exosomes population in OCPs, while in CD9-positive exosomes from ovarian tumor patients (BOTPs and OCPS) both in plasma and ascites was revealed low level of ADAM10 [20]. The metalloproteinase subpopulations of CD9-positive sEVs isolated from plasma of BOTPs and OCPs appeared to be quite similar. However, a significant difference in the expression of ADAM-metalloproteinases between CD9-positive sEVs isolated from ascites of BOTPs and OCPs was found. Similar data were obtained for MMP9 by Reiner et al. [21]. We showed that the level of metalloproteinases and EMMPRIN at the surface of the CD9-positive sEVs in OCPs significantly depended on the volume of ascites and correlated with PCI. The most changes were revealed in the plasma-derived CD9-positive sEVs. PCI for all histological types of ovarian cancer is the most powerful tool that determines the feasibility of achieving complete or optimal cytoreduction, as well as the tactics of patient management. The ascites volume for serous high-grade carcinomas correlated both with the outcome of primary surgery and overall survival [15]. Our preliminary results suggest that the level of metalloproteinases (ADAM10, ADAM17, MMP2, MMP9,) and EMMPRIN in circulating sEVs, as well as the assessment of individual sEVs subpopulations may be promising approaches to the management of patients with advanced OC.

5. Conclusion

A subpopulation of CD9/CD24-possitive sEVs predominated both in plasma and ascites in BOTPs and OCPs. The metalloproteinase subpopulations of sEVs isolated from plasma of BOTPs and OCPs appeared to be quite similar. However, a significant difference in the expression of ADAM-metalloproteinases in ascites sEVs was found between BOTPs and OCPs. The CD9-positive sEVs metalloproteinases level in OCPs significantly depended on the ascites volume. A statistically significant relationship between the level of the ADAM10+/ADAM17- subpopulation in plasma CD9-positive sEVs and peritoneal canceromatosis index was found. The levels of metalloproteinases (ADAM10, ADAM17, MMP2, MMP9) and EMMPRIN at the surface of the circulating CD9-positive sEVs, as well as the assessment of individual subpopulations may be promising approaches to the management of advanced OCPs.

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