RESEARCH ARTICLE

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Extracellular Vesicles as a Platform for Predicting Cancer Risk in Patients with Adenomas and Polyps of the Colon with Obesity

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Abstract

Abstract: The aim of the study was to investigate the level of heat shock proteins (HSPs) and matrix metalloproteinases (MMPs) on circulating extracellular vesicles (EVs) with the assessment to use EV markers to predict cancer risk in patients with colon adenomas and polyps. **Materials and Methods:** Multiparametric staining with flow cytometric visualization was performed to assess the expression of MMPs and HSPs on EVs isolated from blood plasma. Vesicles were characterized using transmission electron microscopy, nanoparticle tracking analysis, and flow cytometry. Groups of patients with colorectal cancer (CRC) n=40 (26 women and 14 men, T2-4N0-2M0, mean age 59.6±1.61 years) and patients with villous adenomas of the colon (CA) (n=20, 9 men and 11 women, mean age 57.5±10.55 years, with obesity, defined as a body mass index ≥30 kg/m² were formed. Logistic regression was used to calculate the cancer risk. **Results:** The logistic regression model for predicting the cancer risk in patients with CA with obesity included both CD9-positive EVs and the adipocyte-derived FABP4-positive EVs. Clinical parameters were not significant as indicators for predicting the cancer risk. The sensitivity and specificity of this model were 85.4% and 79.2%, respectively. Conclusion. The advantages of the calculated mathematical model are as follows: 1) the parameters included in the model are estimated in the blood plasma EVs, thereby not requiring invasive procedures (colonoscopy, biopsy sampling); 2) the model takes into account the presence of metabolic disorders, since the model includes subpopulations of CD9-positive vesicles and FABP4-positive (adipocyte-derived) EVs as predictors of cancer risk; 3) the calculation of cancer risk using this model can be repeated. This version of the application of the model can consider changes in cancer risk and allows optimization of management tactics for CA patients.

Keywords: Adipocyte-derived extracellular vesicles- colon adenomas- colon polyps- colorectal cancer

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Introduction

Cancer risk assessment can be used to determine the most suitable screening tests and appropriate diagnostic and treatment options for each patient. This may include lifestyle recommendations, preventive measures such as vaccination, or surgical removal of precancerous lesions. Identifying people at high cancer risk allows for the focus of health care resources on those most likely to develop cancer [1]. Assessment of the risk of malignant transformation of colon polyps can be challenging due to their diversity, including histological structure, size, shape, and morphology. Obesity or metabolic syndrome is the major factor for colorectal cancer (CRC) in more than 70% of patients. Similar metabolic changes are also observed in patients with colon polyps and adenomas (CA). Adenomatous polyps or villous adenomas of the colon are regarded as precursors of CRC [2]. Extracellular vesicles (EVs) are a heterogeneous population of membrane particles less than 1 μm in size, secreted by various cell types and bearing markers of the parent cell. Most EVs circulating in human blood are of platelet, leukocyte,

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erythrocyte, and endothelial origin. Adipocyte-derived EVs constitute a relatively small fraction of the total EV in circulating plasma, and their level is increased in obese patients [3-4].

Tumor-specific EVs carrying tumor-specific markers (EV-associated DNA, microRNA, proteins) may also present in circulation. These EVs can cross the blood-brain and histo-hematic barriers and can be detected in various biological fluids in humans [5-6]. However, the concentration of tumor-specific EVs in circulation is extremely low, and the diagnostic significance of markers in the composition of tumor-specific EVs has not been determined [7]. Typical adipocyte markers (peroxisome proliferator-activated receptor- ý (PPAR-ý), fatty acid binding protein 4 (FABP4), preadipocyte factor-1 (PREF1), and perilipin 1) are not strictly specific for adipose tissue cells and are also synthesized by macrophages and monocytes, which creates problems for the identification of adipocyte-derived EVs [3, 8-12].

Previously, our studies revealed that preliminary depletion of circulating vesicle samples with removal of monocyte-macrophage origin vesicles from patients with CRC and CA with metabolic disorders is not required to characterize adipocyte-derived EVs. In this work, it was also shown that in patients with and without obesity, circulating EVs of adipocyte origin overexpress FABP4 on their surface. Therefore, in the present study we used antibodies to the FABP4 protein to coat latex particles and subsequent sorption of adipocyte-derived EVs on these particles with flow cytometry detection [13]. Fractions of small adipocyte-derived EVs are specifically enriched in extracellular matrix proteins, including matrix metalloproteinases (MMPs) and their inhibitor (TIMP1), heat shock proteins (HSPs), enzymes involved in the synthesis of lipids and carbohydrates [14]. This was the reason for the choice of vesicular markers that are promising for predicting cancer risk in patients with precancerous lesions and obesity.

The purpose of the study was to investigate the expression of HSPs, MMPs and TIMP1 on circulating EVs with an assessment of the feasibility of using EV markers to predict cancer risk in obese patients with precancerous colorectal lesions.

Materials and Methods

All patients were divided into two groups. Group I consisted of 40 patients (26 women and 14 men) with stage T2-4N0-2M0 colorectal cancer (CRC). The median age of the patients was 59.6±1.61 years. Group II comprised 20 patients (9 men and 11 women) with CA, the median age of the patients was 57.5±10.6 years. All patients had metabolic syndrome (according to IDF (2005) or metabolically healthy obesity. According to literature data, the prevalence of metabolically healthy obesity varies from 10 to 40%, which, in general, indicates the absence of standard definitions of this phenotype [15]. In our study, the criteria for metabolically healthy obesity were obesity (body mass index ≥30 kg/m²) in combination with another component of metabolic syndrome or obesity alone. For the analysis, the components of metabolic syndrome were as follows: abdominal obesity (waist circumference over 94 cm for men and >80 cm for women); hypertriglyceridemia – triglyceride level ≥1.7 mmol/l; decreased HDL cholesterol level – HDL cholesterol value <1.0 mmol/l in men and <1.3 mmol/l in women; arterial hypertension – blood pressure ≥130/85 mmHg; fasting hyperglycemia – blood plasma glucose value ≥6.1 mmol/l with HOMA-IR insulin resistance index value less than 2.7. Anthropometric parameters, such as waist circumference, hip circumference, and body mass index were measured and calculated. The levels of glucose, total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides in blood plasma were studied after 16 hours of fasting using a Torus biochemical analyzer 1220, Dixion (Russia).

MRI, endoscopic and histological examinations. All patients underwent magnetic resonance imaging on a magnetic resonance imaging scanner MAGNETOM ESSENZA 1.5 T (Siemens, Erlangen, Germany) with a surface-phased Body Matrix coil and endoscopic examination with sampling of material for histological examination. Endoscopic examination was performed on a video endoscopic complex EVIS EXERA III (Olympus-Evident, Japan). The use of this equipment allows for a standard endoscopic examination in white light and then an examination in a "narrow spectrum" (NBI technique). The NBI mode provides unique opportunities for visualizing the capillary pattern and surface structure. Video colonoscopy in white light made it possible to visualize the pathological process in various parts of the colon, determine its localization, size and prevalence in the intestine. An additional narrow-spectrum technique allowed differential diagnostics between non-neoplastic and neoplastic lesions. The Sano microvascular classification for colorectal lesions was used [16]. Classification of the capillary pattern allowed an endoscopic conclusion to be made about the nature of the detected colon structures, which was confirmed by subsequent histological examination. A total of 42 colon samples were removed. Each patient included in the CA group had at least 1 fragment classified as tubular, tubulovillous, or serrated adenoma (Table 1).

Isolation of EVs. Small EVs were isolated from patients' blood plasma by ultrafiltration with double ultracentrifugation (Optima XPN 80, Beckman Coulter, Brea, CA, USA) using filters with a pore diameter of 220 nm (PES, Wuxi NEST Biotechnology Co Ltd., Jiangsu, China) to remove large vesicles. The aliquots of small EVs were stored at -80°C.

Electron microscopy, NTA analysis and flow cytometry. Transmission electron microscopy was used to confirm the morphology of the isolated vesicles. The isolated vesicles were adsorbed for 1 minute on copper grids coated with a carbonized film. The grids were contrasted with a 2% aqueous solution of phosphotungstic acid. The preparations were examined on a Talos L120C electron microscope (Thermoscientific, Waltham, Massachusetts, USA). The distribution and concentration of isolated vesicles were studied using nanoparticle tracking analysis (NTA) using a NanoSight LM10 device (Malvern Instruments, Malvern, Worcestershire, UK). To confirm

Table 1. Clinical and Pathological Findings in Patients with Adenomatous Polyps of the Colon

Patients	Sex	Age	Adenoma count	Pathological findings
1	F	61	3	Tubular ademoma/tubulovillous adenoma/ tubulovillous adenoma
2	M	46	3	Hyperplastic/hyperplastic/tubular ademoma
3	F	48	3	Tubular ademoma/tubular ademoma/tubular adenoma
4	F	43	1	Tubular adenoma with dysplasia
5	M	67	1	Tubular adenoma with dysplasia
6	F	44	1	Serrated adenoma with dysplasia
7	F	73	4	Hyperplastic/ hyperplastic/tubular ademoma/tubular ademoma
8	M	68	3	Hyperplastic/ hyperplastic/tubular ademoma
9	F	62	2	Tubular ademoma/tubular ademoma
10	F	66	3	Hyperplastic/ Tubular ademoma/tubular ademoma
11	M	52	2	Hyperplastic/ Tubular ademoma
12	F	41	2	Tubular ademoma/tubulovillous adenoma
13	M	60	4	Tubular ademoma/tubulovillous adenoma /tubular ademoma/tubulovillous adenoma
14	M	55	2	Tubular ademoma/tubular ademoma
15	F	45	1	Serrated adenoma with dysplasia
16	M	67	1	Tubular adenoma with dysplasia
17	M	65	2	Serrated adenoma/ hyperplastic
18	M	70	4	Hyperplastic/ hyperplastic/tubular ademoma/tubular ademoma
19	F	68	1	Tubular ademoma
20	F	49	2	Hyperplastic/tubular ademoma

the vesicular nature of the isolated particles, the level of tetraspanins CD9, CD81, CD63 and glycoprotein CD24 was studied by flow cytometry.

Five microliters of $3x10^5$ aldehyde-sulfate latex particles with a diameter of 4 μm (4%, A37304, Molecular Probes, Eugene, OR, USA) were washed twice with 100 μl of 0.1M MES buffer pH 5.5 (3000g, 15 min, room temperature) and resuspended in 25 μl of MES buffer. Then, 3 μg of monoclonal antibodies against CD9 (SAA0003, Antibody System, Schiltigheim, France) or FABP-4 (FAB693Hu01, Cloud-Clone Corp., China) were added to the particles and incubated at room temperature for 14 hours with stirring. To analyze the HSP60/HSP27/HSP90 subsets on the surface of CD9-positive and FABP4-positive EVs, EV aliquots (about 30 μg protein) were incubated with 3×10^5 anti-CD9 or anti-FABP4 latex particles in 150 μl PBS at 4°C for 14 hours with gentle stirring at 400 rpm and then blocked in 0.2 M glycine for 30 min and human BD Fc Block (Invitrogen, Elabscience, Houston, Texas, USA, 5 μl per test). Next, the particle-vesicle complexes were stained with antibodies (anti-HSP60-PE (2 μl per test, FAA822Hu41, Cloud-Clone Corp., China), anti-HSP27-FITC (2 μl per test, FAA693Hu81, Cloud-Clone Corp., China) and anti-HSP90-APC (2 μl per test, FAA863Hu51, Cloud-Clone Corp.) for 20 min at room temperature. The analysis of MMP9/MMP2/TIMP1 subpopulations on the EV surface was performed similarly. The antibodies used were anti-TIMP1-APC (2 μl per test, FAA522Hu51, Cloud-Clone Corp., China), anti-MMP2-PE (2 μl per test, FAA100Hu41, Cloud-Clone Corp., China) and anti-MMP9-FITC (2 μl per test, FAA553Hu81, Cloud-Clone Corp., China). Cytometry was performed on a Cytoflex device (Beckman Coulter, BioBay, Jiangsu, China), the obtained data were analyzed using CytExpert 2.4 Software. The study was performed in the Laboratory of Experimental Biochemistry and Biology of the Siberian State Medical University (headed by MD, L.V. Spirina).

Statistical analysis. For all types of analysis, differences were considered statistically significant at a significance level of $p < 0.05$. The normality of the distribution of the studied samples was checked using the Shapiro-Wilk test. In the table and figures, the data are presented as medians with interquartile range. To assess the significance of differences in the samples, the Mann-Whitney test and the one-sided Fisher test were used. Logistic regression was used to build a prognostic model. The sensitivity and specificity of candidate markers were determined using ROC (receiver operating characteristic) analysis, and it was also used to determine the cutoff thresholds for the indicators.

Results

A brief description of patients with CA and CRC is presented in Table 2. Patients with CRC were significantly more likely to have metabolic syndrome and type 2 diabetes mellitus compared to patients with CA, although body mass index did not differ in both groups.

The EVs isolated from the blood plasma of patients were visualized by transmission electron microscopy as round membrane structures no more than 220 nm in size, corresponding to small EVs. The isolated vesicles expressed CD9, CD81, CD63, and CD24. According to NTA analysis, the average concentration of circulating EVs in patients with ATC and CRC did not differ

significantly and was 25.1±2.33×10^9 and 24.0±4.92×10^9 particles/ml of blood, respectively. The average vesicle size in patients with adenomas was 95.3±11.1 nm, and in patients with CRC - 96.2±9.70 nm (Figure 1). The gating strategy is shown in Figure 2. The whisker-box plots of the HSPs and MMPs levels on the surface of circulating CD9- and FABP4-positive EVs in patients with CRC and CA with metabolic disorders are shown in Figure 3.

Statistically significant differences in HSP60 expression on the surface of both CD9-positive and FABP4-positive EVs were found between patients with CRC and CA with metabolic syndrome or metabolically healthy obesity. In addition, populations of CD9-positive (MMP2+MMP9-TIMP1+ and MMP2+ vesicles) and populations of FABP4-positive (HSP60+HSP27+HSP90- and MMP2+MMP9+TIMP1-) EVs were identified, the level of which statistically significantly differed in patients with CA and CRC. The model included both CD9-positive EV populations (CD9+HSP60+, CD9+MMP2+, CD9+MMP2+MMP9-TIMP1+) and adipocyte-derived EVs (FABP4+HSP60+, FABP4+HSP60+HSP27+HSP90-, FABP4+MMP2+MMP9+TIMP1-) as predictors. The model also included such indicators as age, body mass index, and the presence/absence of metabolic syndrome as predictors of cancer risk. The contribution of each significant indicator was assessed using ROC analysis (Table 3).

The following characteristics of the variables are presented: area under the curve (AUC), cutoff point, asymptotic significance, 95% confidence interval limits (CI) boundaries, sensitivity, and specificity.

Based on the obtained results, the developed logistic regression model has the form:

F=0.34218*[CD9+HSP60+] - 0.1054*[CD9+MMP2+] - 0.3088*[CD9+MMP2+MMP9-TIMP1+] - 1.04413*[FABP4+HSP60+] - 0.8894*[FABP4+HSP60+HSP27+HSP90-] – 4.38529*[FABP4+MMP2+MMP9+TIMP1-] + 71.81, where the circulating EV populations indicated in square brackets are presented as percentages. The populations were calculated based on flow cytometry data. The probability of absence of cancer risk was calculated taking into account the value of the regression function F and the base of the natural logarithm (e):

P=1/(1+e^(-F))

Table 2. Characteristics of Patients with CA and CRC Included in the Study

Parameters	CRC	CA	P
Histology, n (%)	Adenocarcinoma G1- G3, 40 (100%)	Tubular, tubulovillous, or serrated adenoma; 20 (100%)	
Age, years, Mean ± SD	59.6±1.61	57.5±10.55	>0.05
BMI, Me (Q1-Q3)	30.7 (25.7; 32.6)	31.1 (28.4; 32.1)	>0.05
Metabolic syndrome, n (%)	12 (30%)	14 (70%)	<0.05
Diabetes type 2, n (%)	15 (37.5%)	1 (5%)	<0.05

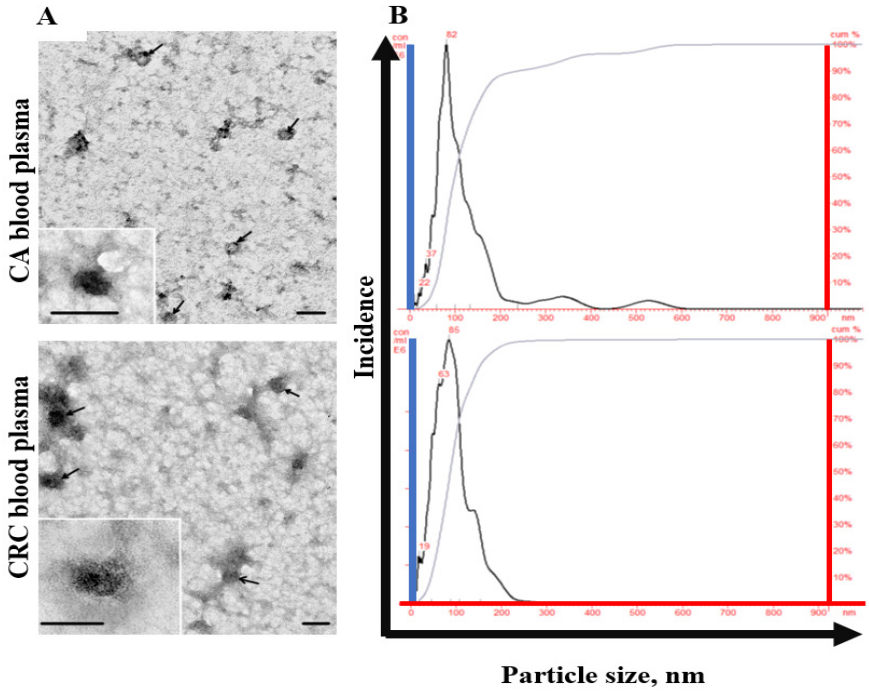

Figure 1. Identification of Isolated EVs. (A) Electron microscopy showed the presence of vesicles with typical morphology and the absence of vesicles larger than 220 nm. Scale bars correspond to 100 nm in the inset and 200 nm in the total view. Electron microscopy, 2% aqueous solution of phosphotungstic acid staining (X400); CA – colon adenomas, CRC – colorectal cancer. (B) NTA analysis data for EVs isolated from the blood plasma of patients with CA and CRC.

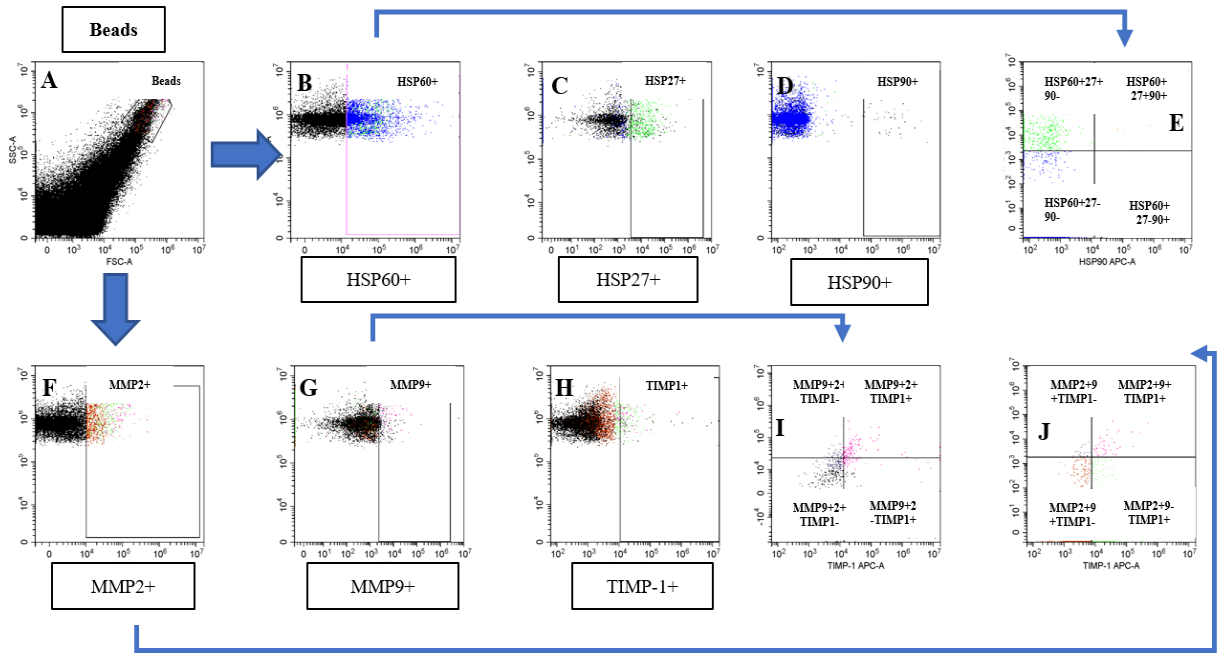

Figure 2. Gating Strategy of EV Staining. Initially, a gate of singlet particles with adsorbed EVs was isolated (2A), then gates of (2B, 2C, 2D) of HSPs and gate of MMP2-positive (2F), MMP9-positive (2G) and TIMP-1-positive EVs (2H) were isolated. Then the remaining dot plots were constructed from them (2E, 2I, 2J).

Where P is the probability of cancer risk, e is (base of the natural logarithm) 2.718, F is the value of the regression function. If P>0.5, the high cancer risk is assumed, if P≤0.5, the low cancer risk is assumed. The sensitivity and specificity estimates for this model were 85.4% and 80.2%, respectively.

Discussion

Zambalova et al. [17] identified cancer risk predictors in patients with colon polyps, including tetraspanins (CD9, CD63, CD81, CD82, CD151 and Tspan8) and proteases (ADAM proteases, MMPs, EMMPRIN) in exosomes. Willms et al. [18] also showed that EMMPRIN-positive epithelial-cell-derived EVs can be used to form a group of increased cancer risk. Willms et al. [18] reported that the number of EpCAM+EMMPRIN-positive circulating vesicles was higher in patients with CRC than in patients with polyps and inflammatory bowel diseases. In the study by Uratani et al. [19], the authors observed that the tissue levels of four miRNAs (miR-21, miR-29a, miR-92a and miR-135b) increased according to tumor progression (normal tissue -adenoma-carcinoma). Moreover, the expression levels of three miRNAs (miR-21, miR-29a и miR-92a) were significantly higher in adenoma patients than in healthy volunteers; the level of microRNA correlated with the size of the adenoma and the total number of adenomas. The microRNA expression in exosomes isolated from the same blood serum samples was also assessed. Compared with exosomal miRNAs, serum levels of miR-21, miR-29a, and miR-92a were found to be better diagnostic biomarkers in patients with high-risk adenomatous polyps. However, the authors assessed the significance of free circulating and exosomal microRNAs for distinguishing patients with adenoma from healthy individuals, but not for assessing the risk of malignant transformation of adenomatous polyps/ adenomas of the colon [19].

In patients with precancerous colon lesions, the risk of developing CRC remains elevated even after surgical removal of precancerous lesions. Tubular-villous adenomas become malignant in 25-40% of cases, and the risk of malignancy of hyperplastic polyps of the colon is estimated at less than 10% [19-21]. The tactics of managing patients with hyperplastic polyps or villous adenomas of the colon with/without dysplasia

Table 3. Results of ROC Analysis of EV Subpopulations for Predicting Cancer Risk in Patients with CA

Variables	AUC	Cutoff point	Significance, p	95% Confidence interval limits		Sens. %	Spec. %
				Lower bound	Upper bound		
CD9+HSP60+	0.84	37.40	0.01	0.65	1.00	78.00	90.00
CD9+MMP2+	0.99	8.32	0.00	0.95	1.00	89.00	90.00
FABP4+HSP60+	0.92	48.20	0.00	0.77	1.00	89.00	90.00
FABP4+HSP60+HSP27+HSP90-	0.73	8.64	0.10	0.48	0.96	89.00	70.00
FABP4+MMP2+MMP9+TIMP1-	0.99	4.73	0.00	0.95	1.00	98.00	90.00
CD9+MMP2+MMMP9-TIMP1+	0.87	17.60	0.01	0.67	1.00	89.00	90.00

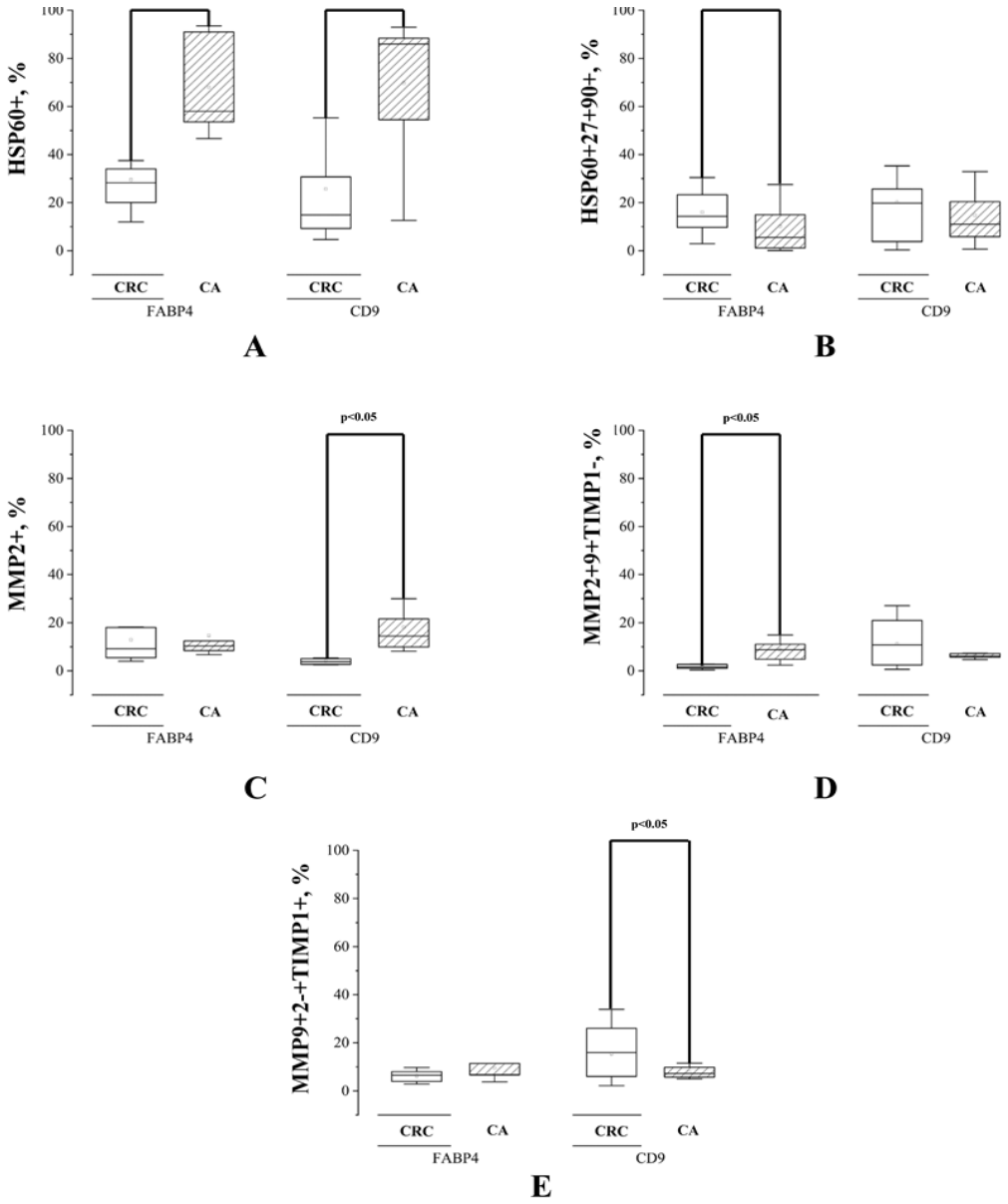

Figure 3. Whisker-Box Plots of HSPs and MMPs Levels on the Surface of CD9- and FABP4-positive EVs in Patients with CRC and CA. In each figure: left – FABP4-positive EVs, right – CD9-positive EVs; CRC – colorectal cancer, CA – colon adenomas. Y-axis – EV populations as a percentage, p – significance level according to the Mann-Whitney criterion (A) – HSP60+ subpopulations, (B) – HSP60+HSP27+HSP90- subpopulations, (C) – MMP2+ subpopulations, (D) – MMP2+MMP9+TIMP1- subpopulations, (E) – MMP2+MMP9-TIMP1+ subpopulations of EVs.

in patients without metabolic disorders or with obesity/metabolic syndrome are not different. Currently, there is an opinion that only adenomas of 5 mm in diameter require endoscopic resection, since smaller lesions rarely become malignant. Patients with adenomas less than 5 mm are offered surveillance. However, a more common strategy is to remove all adenomas, regardless of the size of the neoplasm, since histological studies show that polyps even less than 5 mm in diameter have areas of a tubular structure and can undergo malignant transformation in 60-70% of cases.

There is currently no effective conservative treatment for tubular adenomas. Patients are recommended to undergo surgical treatment. Unfortunately, tubular adenomas tend to recur. Therefore, after removal of adenomas, it is necessary to carry out a set of measures aimed at preventing the occurrence of new lesions. Since nutritional factors such as high fat and low-fiber diet are directly related to the occurrence of colon adenomas, dietary modifications after adenoma removal may not only prevent the occurrence of new tubular adenomas, but also have a positive effect on the growth of existing adenomas. Finally, since a sedentary lifestyle and excess body weight contribute to the development of colon adenomas, physical activity and weight normalization may also be a reasonable recommendation for the prevention of tubular adenomas. The second important and necessary component of monitoring of this cohort of patients is endoscopic examination. The frequency of follow-up colonoscopies is determined individually:

• every 6 months in the first year, and then annually after removal of large pedunculated adenomas

• every 3 months in the first year, every 6 months in the second year, and then annually after removal of large broad-based adenomas and tubular adenomas with dysplasia [21-23]. In the study by Kim et al. [24], a survey on the frequency and necessity of colonoscopic surveillance after removal of colonic lesions was conducted among Korean endoscopists. Participants were asked about their preferred surveillance intervals and the age of the patients at which surveillance was stopped. Compliance with the latest recommendations of the US Multisociety Task Force on Colorectal Cancer (USMSTF) was also analyzed. The average compliance rate with the USMSTF guidelines was 30.7%. The highest proportion of respondents (40.8–55.1%) stopped follow-up when patients were 80–84 years old. The authors noted a significant discrepancy between the preferred follow-up intervals after polypectomy and the latest international recommendations, leading to the conclusion that individualized approaches to surveillance are needed to improve compliance with guidelines [24].

Given the research evidence on the timing of surveillance colonoscopy, we believe that a combination of standard clinical guidelines and individual cancer risk data is most appropriate. Data on the level of MMPs and HSPs in circulating EVs can be used as additional data. Available literature data suggest that the impact of obesity or metabolic syndrome on the colonic epithelium is mediated through activation/inactivation of expression of growth and transcription factors. This causes activation or inhibition of multiple signaling pathways responsible for proliferation, apoptosis, neoangiogenesis, chronic inflammation, cell motility and adhesion. It is believed that obesity and individual components of metabolic syndrome increase the risk of developing CRC in the population as a whole [1, 2], but it is quite difficult to assess to what extent the presence of metabolic disorders increases the risk of developing cancer in the presence of precancerous changes in the colon in each specific case. Since predicting the cancer risk in obese patients with polyps and adenomas of the colon is quite difficult, the vesicular markers could potentially help to clarify the individual risk and optimize surveillance.

Our study has certain limitations. These limitations may be attributed to the relatively small sample size. The proposed predictive model incorporated six predictor variables and was developed based on data from 60 patients. However, it is important to note that all patients with colorectal adenomas/polyps included in our study presented with precancerous lesions classified as tubular, tubulovillous, or serrated adenomas. Additional limitations of the regression model are associated with the parameter estimation method, which employed a jackknife resampling algorithm.

In conclusion, our study showed that clinical parameters (sex, age, duration of anamnesis, presence of obesity or metabolic syndrome, body mass index) in patients with CA were not indicators of cancer risk. However, individual subpopulations of circulating EVs turned out to be significant predictors and can be used in the future to clarify the cancer risk. The advantages of the mathematical model are as follows: 1) the parameters included in the model are estimated in EVs isolated from patients' blood plasma, not requiring invasive manipulations (colonoscopy, obtaining biopsy material); 2) the model takes into account metabolic disorders. In addition to subpopulations of CD9-positive vesicles, the model also includes subpopulations of FABP4-positive (adipocytic EVs); 3) the calculation of the relative cancer risk using the model can be repeated. These calculations can reflect changes in the cancer risk during follow-up visits and allow optimization of the management of patients with adenomas and polyps of the colon.

Author Contribution Statement

Conceptualization, N.V.Y., O.V.Ch.; investigation, D.A.S., O.V.Ch., S.G.A., D.N.K. and G.V.K.; resources, L.V.S.; statistical analysis, E.E.S. and E.E.K.; writing -original draft preparation, N.V.Y., D.A.S., O.V. Ch.; review and editing – N.V.Y., O.V.Ch and A.L. Ch. All authors have read and agreed to the published version of the manuscript. The authors thank Tatyana Shtam, Senior Researcher of the Cellular and Biomedical Technologies Group, P.N. Konstantinov Petersburg Institute of Nuclear Physics (St. Petersburg) for her consultation during the nanoparticle tracking analysis.

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The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Cancer Research Institute of the Tomsk National Research Medical Center (protocol No. 21 dated November 9, 2023). Informed consent was obtained from all patients participating in the study.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Conflicts of interest

The authors declare no potential related to the publication of this article.

List of abbreviations

EVs – extracellular vesicles
CRC – colorectal cancer
CA – colon adenomas
NTA – nanoparticle tracking analysis
FABP4 – fatty acid binding protein 4
HSPs – heat shock proteins
MMPs – matrix metalloproteinases
TIMP1 – tissue inhibitor of metalloproteinases 1
CD9 – tetraspanin, transmembrane protein, typical EVs marker.

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