PROTEOME OF PLASMA EXTRACELLULAR VESICLES AS A SOURCE OF COLORECTAL CANCER BIOMARKERS

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The search for minimally invasive methods for diagnostics of colorectal cancer (CRC) is the most important task for early diagnostics of the disease and subsequent successful treatment. Human plasma represents the main type of biological material used in the clinical practice; however, the complex dynamic range of substances circulating in it complicates determination of CRC protein markers by the mass spectrometric (MS) method. Studying the proteome of extracellular vesicles (EVs) isolated from human plasma represents an attractive approach for the discovery of tissue-secreted CRC markers. We performed shotgun mass spectrometry analysis of EV samples obtained from plasma of CRC patients and healthy volunteers. This MS analysis resulted in identification of 370 proteins (which were registered by at least two peptides). Stable isotope-free relative quantitation identified 55 proteins with altered abundance in EV samples obtained from plasma samples of CRC patients as compared to healthy controls. Among the EV proteins isolated from blood plasma we found components involved in cell adhesion and the VEGFA–VEGFR2 signaling pathway (TLN1, HSPA8, VCL, MYH9, and others), as well as proteins expressed predominantly by gastrointestinal tissues (polymeric immunoglobulin receptor, PIGR). The data obtained using the shotgun proteomic profiling may be added to the panel for targeted MS analysis of EV-associated protein markers, previously developed using CRC cell models.

Key words: extracellular vesicles (EVs); human plasma; shotgun mass spectrometric analysis; colorectal cancer

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INTRODUCTION

Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality worldwide [1]. The 5-year survival rate for patients diagnosed with advanced CRC is only 12.5% [2]. At the same time, the introduction of CRC screening programs for detection of the early stages of the disease could result in remission of 85–90% of patients [2]. The screening methods include direct visualization of the tumor (colonoscopy, sigmoidoscopy) and stool tests to detect tumor components secreted into the intestinal lumen, such as abnormal DNA and/or hemoglobin as an indicator of occult blood [3].

However, stool screening tests are often inaccurate, especially for diagnostics of precancerous lesions that are less prone to bleeding [3], and colonoscopy accompanied by biopsy is associated with certain risks, such as bleeding and intestinal perforation [4]. In addition, the high degree of risk and discomfort associated with invasive techniques reduces motivation of patients for voluntarily participation in screening programs.

In this regard, the development of minimally invasive diagnostic methods, for example, the identification of CRC biomarkers in blood plasma represents an important scientific direction. To date, carcinoembryonic antigen (CEA) antibody testing is the most widely used approach for minimally invasive diagnostics of CRC in clinical practice. However, the method has limited sensitivity and specificity [2]. The use of mass spectrometry (MS) analysis of blood plasma has become a promising approach. For example, using multiplex MS analysis in whole blood plasma, it was possible to identify a signature including the proteins A1AT, APOA1, HP, LRG1, and PON3, which distinguished plasma samples obtained from CRC patients and healthy volunteers [5].

Blood plasma is an extremely complex biological matrix in which a dozen proteins, including albumin, immunoglobulins, and transferrin, represent up to 90% of the total protein [6]. Moreover, the dynamic range of plasma protein concentrations covers 10 orders of magnitude [7]. This complexity of the composition of human plasma determines the so-called “proteomic iceberg” effect, which complicates the MS analysis of low-copy proteins in the presence of highly abundant protein analytes. An increase in the sensitivity of plasma protein analysis may be achieved, for example, by immunoaffinity enrichment to remove highly abundant proteins [8]. However, the introduction of an additional sample preparation step leads to protein losses and distorted quantitative results. Another approach for detection of potential biomarkers consists in detection of tumor secreted elements (tumor cells, circulating tumor DNA, microRNAs, proteins, and extracellular vesicles) in the blood, which corresponds to the liquid biopsy concept.

Abbreviations used: EVs – extracellular vesicles; CRC – colorectal cancer; MS – mass spectrometry; TEAB – triethylammonium bicarbonate buffer.

Although all living cells in the body produce extracellular vesicles (EVs), cancer cells produce them especially intensively. The proteins, DNA, and RNA molecules included in the vesicles reflect the molecular landscape of tumor-producing cells, while the lipid membrane protects the EV contents from degradation by nucleases and proteases. Since proteins are direct performers of most cellular functions and direct drug targets in most modern cancer treatments, proteomic data will help to identify new CRC biomarkers.

In this work, we have performed shotgun MS analysis of EV samples obtained from the blood plasma of CRC patients and healthy volunteers. Based on the results of relative label-free quantitative analysis of proteomic data, proteins with altered abundance in EV samples obtained from the blood of CRC patients as compared to healthy controls were identified.

MATERIALS AND METHODS

Blood Plasma Sample Preparation

Plasma samples were provided by the biobank at the Institute of Biomedical Chemistry (IBMC) and included plasma samples from 11 CRC patients (4 women and 7 men, aged from 50 to 70 years) and 16 healthy volunteers (8 men and 8 women, aged from 45 to 74 years) (Table 1).

Nine patients had stage 3 CRC and two patients had stage 4 CRC. All the patients had metastases to regional lymph nodes. Two patients also had distant metastases: in the liver and abdominal cavity.

Isolation of Extracellular Vesicles from Plasma Samples

Extracellular vesicles were isolated from plasma samples using a commercial Total Exosome Isolation kit (Invitrogen, Thermo Fisher Scientific, Lithuania) in accordance with the manufacturer's recommendations. The initial volume of blood plasma was 30 μl. The sediment obtained after precipitation was dissolved in 100 μl 0.1 M Tris-HCl buffer, pH 8.5, and methanol-chloroform protein extraction was performed. After addition of 400 μl of 100% methanol (J.T. Baker, Avantor, Poland), 100 μl of chloroform (Sigma-Aldrich, USA), and 300 μl of deionized water the samples were thoroughly mixed and then centrifuged at 14,000 *g* for 2 min at room temperature. The supernatant was collected and after addition of 400 μl of 100% methanol it was then centrifuged again under the same conditions for 3 min. The resulting sediments were dissolved in 100 μl of 50 mM triethylammonium bicarbonate buffer (TEAB, Fluka Analytical, Switzerland) for subsequent proteomic analysis. The concentration of total protein in the samples was determined using a commercial Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, USA) in accordance with the manufacturer's recommendations.

Sample Preparation for Proteomic Analysis

For hydrolytic digestion of proteins, S-trap spin columns (Protifi, USA) were used in accordance with the manufacturer's recommendations.

10% SDS solution in 100 mM TEAB was added to each sample containing 100 μg protein so that the final concentrations were 5% SDS and 50 mM TEAB (pH 8.5). The resulting solutions were treated using a Bandelin Sonopuls ultrasonic homogenizer (BANDELIN electronic GmbH & Co., Germany) (power 30% for 30 s on ice) and then the samples were centrifuged at 14,000 *g* for 10 min at 4°C.

For alkylation and reduction of disulfide bonds, a solution containing 400 mM chloroacetamide (CAA, Sigma-Aldrich) and 500 mM tris(2-carboxyethyl) phosphine (Tris TCEP, Thermo Fisher Scientific, USA) was added to the samples to the final concentrations of these ingredients of 30 mM and 50 mM, respectively. Samples were incubated at 80°C for 40 min and then 12% phosphoric acid was added to the samples to a final concentration of 1.2%, mixed thoroughly and 6 parts of buffer (90% methanol in 100 mM TEAB (pH 8.5)) were added. Next, the samples were applied to S-trap micro-columns in 175 μl and centrifuged at 4000 *g* for 1 min at 20°C. The procedure was repeated until the sample was completely loaded, then the columns were washed four times with the same solution under the same conditions. Protein digestion was performed using 0.2 ng/μl trypsin solution (Promega, USA), which was added to the samples at the trypsin : total protein ratio of 1:50. Samples were incubated for 2 h at 47°C. Then the peptides were eluted: 40 μl of a solution containing 0.2% formic acid in 50 mM TEAB (pH 8.5) was added and samples were centrifuged at 4000 *g* for 1 min at 20°C. Next, 35 μl of a solution containing 0.2% formic acid in 50% acetonitrile was added and the samples were centrifuged again under the same conditions. The supernatant was dried in a vacuum concentrator (Concentrator 5301, Eppendorf, Germany).

Table 1. Characteristics of CRC patients and healthy volunteers

	CRC patients	Healthy volunteers
Total number	11	16
Age, years	50–70	45–74
Males	7	8
Females	4	8
Stage 3 (3, 3A, and 3B)	9	—
Stage 4	2	—

Shotgun Mass Spectrometry Analysis

For shotgun mass spectrometry analysis samples were dissolved in 0.1% formic acid to a final concentration of 2 μg/μl.

Each sample was analyzed in three technical replicates. Samples were applied onto a Zorbax 300SB-C18 enrichment column (particle diameter 5 μm, 5 mm × 0.3 mm) (Agilent Technologies, USA) and washed with mobile phase C (5% acetonitrile in 0.1% formic acid and 0.05% trifluoroacetic acid) at a flow rate of 3 μl/min for 5 min. Peptides were separated on a Zorbax 300SB-C18 analytical column (particle diameter 3.5 μm, 150 mm × 75 μm) (Agilent Technologies). The gradient was formed with mobile phase A (0.1% formic acid solution) and mobile phase B (80% acetonitrile solution in 0.1% formic acid) at a flow rate of 0.3 μl/min. The analytical column was washed with 2% mobile phase B for 3 min, then the concentration of mobile phase B was increased to 40% for 67 min. Then, within 2 min, the concentration of mobile phase B was increased to 100%, and the analytical column was washed for 9 min with 100% mobile phase B. Next, the concentration of mobile phase B was reduced to 2% over 2 min, and the analytical column was equilibrated with 2% mobile phase B for 7 minutes.

MS analysis was carried out using a Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ mass spectrometer (Thermo Fisher Scientific, USA), equipped with an Orbitrap mass analyzer in positive ionization mode with a resolution of 60000 (m/z = 400) for MS scans and 15000 (m/z = 400) for MS/MS scans. The maximum accumulation times for 3×10^6 and 2×10^5 ions were 25 ms and 150 ms for MS and MS/MS levels, respectively. For subsequent fragmentation, twenty most intense ions recorded in the MS scan were selected provided that their absolute intensity exceeded 1×10^4 relative units. The HCD fragmentation type was used with normalized collision energy (NCE) at 28%. Dynamic exclusion from tandem analysis was used: exclusion duration was 60 s.

Mass spectra processing and protein identification were performed using MaxQuant 1.5.5.0 software with the Andromeda search algorithm; identification was carried out using a FASTA file containing amino acid sequences of human proteins (25-10-2019) and its inverted analogue to calculate the false positive identification rate (FDR). Cysteine carbamidomethylation was taken into account as an obligatory modification of peptides, and methionine oxidation as a variable modification. The tolerance for parent and daughter ions was 20 ppm. For proteins and peptides, the FDR threshold value was set at 0.01. Quantitative analysis was performed based on the area under the peak of the parent ion with the calculation of the Label Free Quantification (LFQ) value by means of the built-in MaxQuant algorithm [9], using for quantification unique peptides without modifications.

Statistical analysis was performed using Perseus 1.6.0.7 software (Max Planck Institute of Biochemistry, Germany).

Biological annotation was performed using the DAVID Knowlegebase online resource (v2023q4) using the GeneOntology Cellular Component database categories. Proteome enrichment analysis of protein-protein interactions was performed using the online resource STRING (v. 12.0), considering the results for proteins with a minimum interaction confidence of 0.7, determined based on experimental data (“experiments”), as well as co-expression data (“co-expression”) and occurrence (“co-occurrence”).

Enrichment analysis was performed against the Reactome database (v. 2022), containing information on regulatory signaling pathways, as well as against the ARCHS4 repository (v. 8.0) [10], containing data on mRNA expression in various tissues and organs, and the TISSUES repository (Jensen) (v. 2.0) [11], containing transcriptomic and proteomic data on gene expression in various tissues and organs. The analysis was performed using the gsepy library (v. 1.0.4).

RESULTS AND DISCUSSION

The Proteome of EVs Isolated from Plasma is Enriched in Proteins of Tissue Origin

The shotgun MS analysis of EVs samples isolated from blood plasma resulted in identification of 370 proteins, which were registered by at least two peptides. A list of proteins with detailed information is given in Table 1 of the Supplementary Material (Table S1, the Proteins/2 Peptides sheet). These included 246 proteins classified as exosomal according to the annotation in the DAVID Knowledgebase (v2023q4) [12] (GeneOntology, Cellular Component) (Supplementary Materials, Table S1, the DAVID annotation sheet).

We have compared the proteome of plasma EVs, obtained in our experiment using high-resolution MS analysis, with data available in the public repository The Human Protein Atlas (v. 23.0) [13]. This database contains a list of 455 proteins potentially secreted into the blood and detected by quantitative immunoassay. The comparison results are shown in Figure 1.

For 266 proteins detected only in EVs isolated from human plasma (Fig. 1), we performed bioinformatics analysis using the online resource STRING (v. 12.0) [14]. The results have shown that the proteins in this pool are enriched in protein-protein interactions (PPI enrichment p-value: $<1.0\times10^{-16}$), and in the context of biological functions, they are involved in cell adhesion (GeneOntology, MF, 38 proteins, FDA = 3.77×10^{-17}) and VEGFA–VEGFR2 signaling pathway (WikiPathways, 27 proteins, FDA = 7.7×10^{-12}) (Fig. 2).

The VEGF family proteins (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PlGF) play a key role in the formation of blood vessels both in embryonic development and during pathological angio- and lymphangiogenesis. These processes promote the exponential tumor growth. Vascular endothelial growth factors influence the process of blood vessel formation in neoplasms, thus promoting tumor growth and the formation of metastases. Increased expression of these factors in CRC correlates with the progression of neoplastic disease [15].

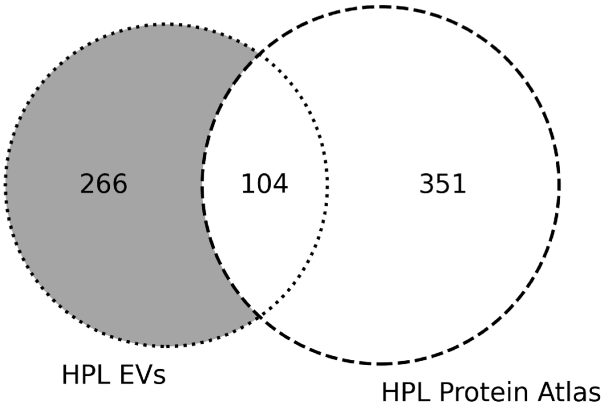

Figure 1. Venn diagram showing the intersection of proteins of EVs isolated from human plasma (HPL), which have been reliably identified by MS (n=370, registered by at least two peptides), and the list of proteins available in the Human Protein Atlas (v. 23.0) [13], which includes 455 proteins potentially secreted into blood and evaluated by quantitative immunoassay.

Targeting components of the vascular endothelial growth factor A (VEGFA)/VEGF receptor 2 (VEGFR2) signaling pathway inhibits tumor-induced regulatory T cell (Treg) proliferation in a mouse model of CRC and in patients with metastatic CRC [16].

Analysis of 266 proteins detected only in EV samples isolated from human plasma (Fig. 1) against the Reactome database, as well as the ARCHS4 (v. 8.0) and TISSUES (Jensen) (v. 2.0) repositories containing information on gene expression in various tissues and organs, showed enrichment of proteins expressed in the epithelium and immune cells of the gastrointestinal tract, including the colon (Supplementary Materials, Fig. 1S). Thus, MS analysis of EVs isolated from human plasma provided new insights in our understanding of the proteome associated with blood plasma, including the detection of protein markers in EVs of presumably tissue origin.

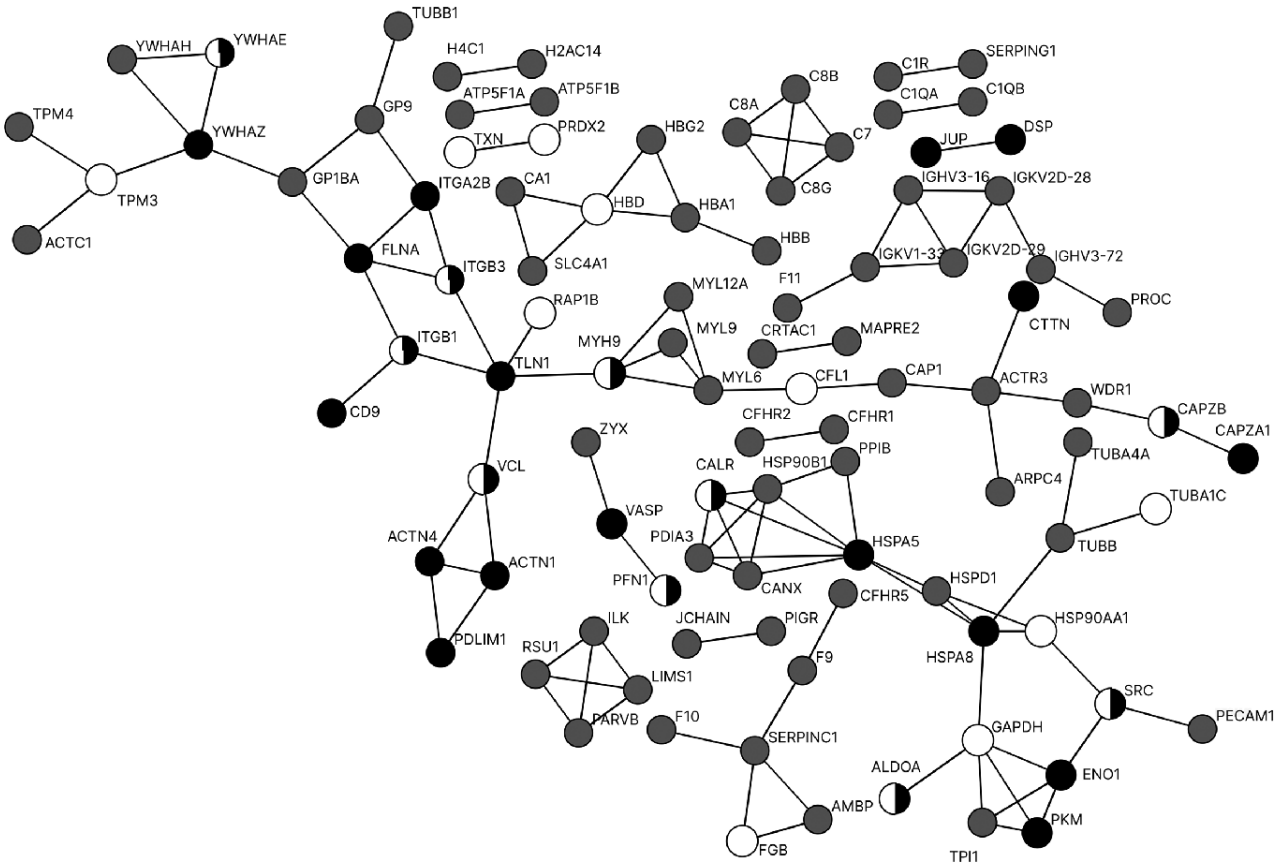

Figure 2. Scheme of protein-protein interactions for proteins (PPI enrichment *p*-value: $<1.0\times10^{-16}$) detected in EVs isolated from plasma (STRING, v. 12.0). Proteins that were annotated as involved in cell adhesion are highlighted in black. Proteins involved in the VEGFA-VEGFR2 signaling pathway are shown in white, proteins annotated to other groups are highlighted in gray.

Comparative Quantitative Analysis of the Proteome of EVs Isolated from Plasma Resulted in Determination of a Proteomic Signature Distinguishing Colorectal Cancer Patients and Healthy Volunteers

High-resolution MS data were quantified using a stable isotope label-free approach. The quantitative analysis was based on comparison of the normalized peptide precursor ion intensity (Label Free Quantification, LFQ) value. This resulted in identification of 55 proteins, which differed significantly (FC>1.5, FDR = 0.01) in EVs isolated from plasma of CRC patients and healthy volunteers (Fig. 3; Supplementary Materials, Fig. S2, Table S1, the DEB sheet).

The content of 37 proteins (ACTN1, AFM, APOC4, CAP1, CAVIN2, F13A1, F13B, FCN2, FCN3, FERMT3, FGA, FGB, FGG, FLNA, HSPA5, HSPA8, ITGA2B, ITGB3, KLKB1, MMRN1, MYH9, MYL12A, MYL6, PARVB, PDLIM1, PKM, PLEK, PON1, PPBP, PZP, RAP1B, TLN1, TPM4, TUBA1C, TUBA4A, TUBB1, and VCL) was lower in EVs isolated from plasma of CRC patients as compared to controls. The most pronounced decrease was observed for myosin-9 (MYH9, FC = 6.0, p-value = 6.16×10^{-11}), talin-1 (TLN1, FC = 4.3, p-value = 2.27×10^{-7}), and filamin-A (FLNA, FC = 5.7, p-value = 3.35×10^{-7}).

The MYH9 protein was initially identified as a tumor suppressor in mice with squamous cell skin cancer; however, subsequent experiments showed that reduced levels of MYH9 were associated with reduced tumor cell proliferation, metastasis formation, and resistance to the antitumor kinase inhibitor drug sorafenib [17]. Another study found a correlation between increased expression of this marker in CRC tissues and poor prognosis in patients [18]. At the same time, there is insufficient information on the plasma levels of MYH9 in CRC patients. It is possible that simultaneous study of MYH9 levels in tumor tissues and blood of patients will clarify the role of MYH9 in the context of CRC.

The results of the analysis in the STRING database (v. 12.0) have shown that proteins with reduced abundance in CRC samples are enriched in protein-protein interactions (PPI enrichment p-value: $<1.0\times10^{-16}$), and in the context of biological functions, are involved in cell adhesion (Gene Ontology, molecular function is shown in black, biological process is shown in white) (Fig. 4).

Potentially interacting proteins with decreased abundance in CRC patients included TLN1, ITGB3, and HSPA8, which were also shown to be decreased in CRC in our previous targeted MS studies [19]. Studies from other research groups have shown that low *TLN1* expression at both the RNA and protein levels was found in CRC patients as compared with healthy donors, and was associated with poor survival [20].

The importance of heat shock protein HSPA8 in the prognosis of the course of CRC is controversial. For example, in one study increased expression

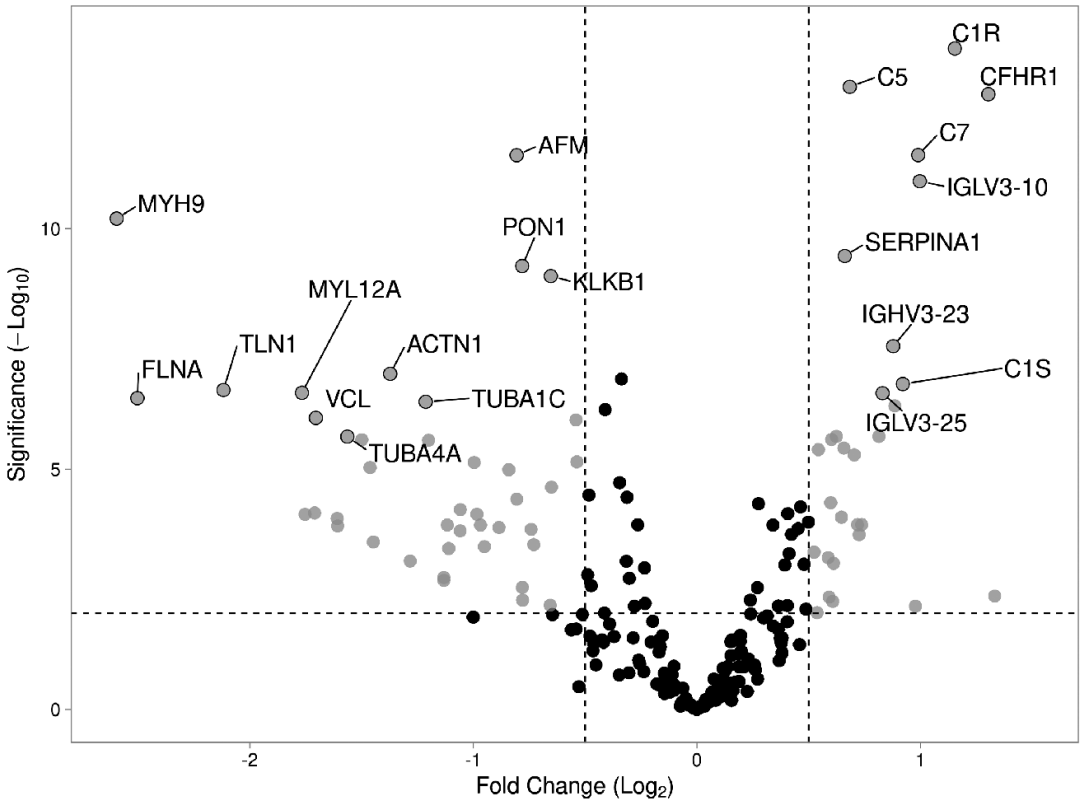

Figure 3. Scatterplot for proteins with significantly different levels (FC>1.5, FDR=0.01) in EV samples obtained from the plasma of CRC patients (left) and healthy volunteers (right).

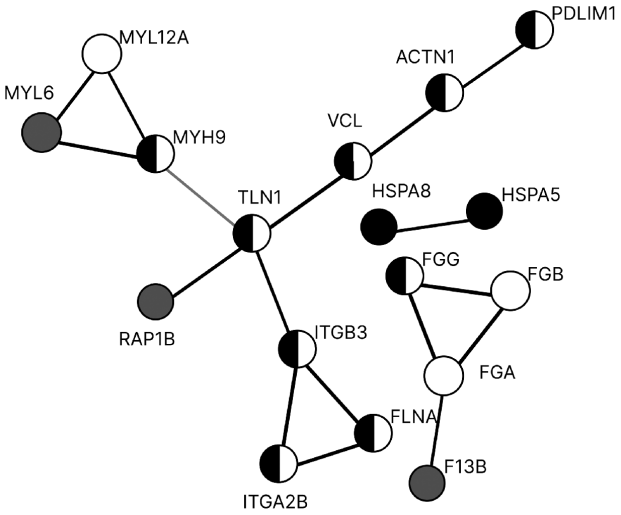

Figure 4. Scheme of protein-protein interactions for proteins (PPI enrichment *p*-value: $<1.0\times10^{-16}$) with reduced levels in EV samples obtained from plasma of CRC patients as compared to healthy controls (STRING, v. 12.0). Proteins annotated as related to cell adhesion, Gene Ontology, molecular function are highlighted in black, biological process are highlighted in white. Proteins annotated to other groups are highlighted in gray.

of the HSPA8 protein was associated with a favorable outcome in CRC patients with colorectal cancer [21], while another study showed that increased expression of the HSPA8 protein was associated with a poor prognosis in CRC patients with a mutation in the *BRAF* gene (V600E). At the same time, the HSPA8 inhibitor, the substance VER155008, increased sensitivity to targeted drugs in such patients, thus inspiring certain optimism in therapeutic strategies [22].

Vinculin (VNC) is also implicated in the CRC biology; this cytoskeletal protein plays a role in the structure and function of focal adhesion. In a study performed on rats with induced colon adenomas, a decrease in the expression of VNC [23] and profilin-1 (Pfn1), a protein involved in the regulation of actin polymerization in response to extracellular signals, was found. The role of Pfn1 in the development of CRC remains poorly understood; however, the expression of its analog profilin-2 (Pfn2) is associated with the regulation of microelements in the intestine, and the loss or decrease in Pfn2 activity correlates with the metastatic and migration ability of CRC cells [24].

The content of 18 proteins (C1R, C1S, C4A, C5, C7, CFHR1, IGHV3-23, IGHV4-59, IGKV1D-39, IGLV3-10, IGLV3-25, ITIH3, LPA, LRG1, PIGR, SERPINA1, SERPING1, and STRADB) was increased in EV samples isolated from the plasma of CRC patients as compared to controls. The most pronounced changes in abundance were observed for the polymeric immunoglobulin receptor (PIGR, FC = 2.5, *p*-value = 4.4×10^{-4}), complement factor H-related protein 1 (CFHR1, FC = 2.5, *p*-value = 1.6×10^{-13}), and complement component C1r (C1R, FC = 2.2, *p*-value = 1.8×10^{-14}).

According to the Proteome Atlas database, PIGR, found among proteins with increased content in EVs samples isolated from plasma of CRC patients (identified by 15 proteotypic peptides), is included in the list of proteins secreted by the gastrointestinal tract into the blood [13]. This protein has previously been shown to have prognostic value: its increased expression in CRC patients correlated with a more favorable prognosis [25, 26].

Complement components (C1R, C1S, C4A, C5, C7, and CFHR1) may have immunosuppressive functions in the tumor microenvironment; however, the activation mechanism in this process is not completely clear. Complement protein expression has been found to be increased in malignant tumors [27]. Elevated levels of certain complement components are associated with an increased risk of CRC development, and serum levels of C1 and C3 were significantly increased in CRC patients as compared with healthy controls [28].

In the context of CRC, the biological role of leucine-rich glycoprotein alpha 2 (LRG1) is especially interesting. Increased expression of this protein by tumor-associated fibroblasts in CRC promotes the migration and invasion of cancer cells and induces epithelial-mesenchymal transition [29].

CONCLUSIONS

MS profiling of EVs isolated from human plasma revealed protein markers, presumably of tissue origin; this is consistent with the concept of liquid biopsy. This approach facilitates solution of the problem of detecting diagnostically significant proteins in such a complex biological matrix as human plasma.

The results obtained using shotgun proteomic profiling of EVs isolated from human plasma may be added to the panel for targeted MS analysis of EV-associated protein markers, previously developed using the CRC cell models [19]. It is expected that such expanded panel of EV-associated protein markers will increase the specificity and sensitivity of CRC recognition using a minimally invasive method.

However, proteins with increased levels in EVs isolated from plasma of CRC patients belong to components of the innate immune system, including the complement system, which may reflect a nonspecific inflammatory reaction accompanying the tumor process. In addition, the present study was carried out on plasma samples from patients with advanced CRC. For future work aimed at identifying more specific protein markers of EVs, suitable for early diagnostics, it is planned to involve patients with early stages of CRC, as well as patients with inflammatory non-cancerous bowel diseases as additional control.

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COMPLIANCE WITH ETHICAL STANDARDS

Blood plasma samples stored in the IBMC biobank used in this study were obtained in compliance with all necessary ethical standards.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Supplementary materials are available in the electronic version at the journal site (pbmc.ibmc.msk.ru).

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ПРОТЕОМ ВНЕКЛЕТОЧНЫХ ВЕЗИКУЛ ПЛАЗМЫ КРОВИ КАК ИСТОЧНИК БИОМАРКЕРОВ КОЛОРЕКТАЛЬНОГО РАКА

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Поиск малоинвазивных способов диагностики колоректального рака (КРР) является важнейшей задачей для выявления заболевания на ранней стадии и последующего успешного лечения. Плазма крови человека представляет основной тип биологического материала, используемого в клинике, однако сложный динамический диапазон циркулирующих в ней веществ затрудняет определение белков-маркеров КРР масс-спектрометрическим (МС) методом. Исследование протеома внеклеточных везикул (ВнВ), выделенных из плазмы крови человека, представляет собой привлекательный подход для обнаружения секретируемых тканями маркеров КРР. Мы провели панорамный масс-спектрометрический анализ образцов ВнВ, полученных из плазмы крови пациентов с КРР и здоровых добровольцев. В результате было идентифицировано 370 белков, зарегистрированных, как минимум, по двум пептидам. Относительный количественный анализ без использования стабильных изотопных меток позволил определить 55 белков, содержание которых различалось в образцах ВнВ, полученных из крови больных КРР, по сравнению со здоровым контролем. Среди белков ВнВ, выделенных из плазмы крови, оказались компоненты, вовлечённые в клеточную адгезию и сигнальный путь VEGFA–VEGFR2 (TLN1, HSPA8, VCL, MYH9 и другие), а также белки, экспрессирующиеся преимущественно тканями ЖКТ (полимерный рецептор иммуноглобулина, PIGR). Полученные с помощью панорамного протеомного профилирования данные позволят дополнить панель для направленного МС анализа ассоциированных с ВнВ белковых маркеров, разработанную ранее с использованием клеточных моделей КРР.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: внеклеточные везикулы (ВнВ); плазма крови человека; панорамный масс-спектрометрический анализ; колоректальный рак

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