RESEARCH ARTICLE

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High Expression of Actin Binding Proteins Predicts Hematogenous Metastases in Non-Small Cell Lung Cancer

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Abstract

Introduction: The high mortality rate in non-small cell lung cancer (NSCLC) patients is associated with tumor progression. Tumor cell motility, which is mediated by various actin-binding proteins (ABPs) plays a crucial role in tumor progression. Therefore, the study of the relationship between the expression of ABPs (*CAP1, cofilin-1, profilin-1, fascin-1* and *ezrin*) and NSCLC progression is of great significance. **Methods:** NSCLC tissue samples and tumor-adjacent unchanged lung tissue samples were collected from 46 NSCLC patients who were not previously treated. The mRNA expression of ABPs was determined by real-time PCR, and the ABP expression was analyzed using Western blotting assay. **Results:** All ABPs showed the correlation with 2-year metastasis-free survival. The increased mRNA levels of *CAP1, CFL1, EZR, FSCN1* and *PFN1* and their protein products in tumor tissue compared to tumor-adjacent unchanged tissue indicated the risk of hematogenous metastases. **Conclusion:** The data obtained demonstrated the feasibility of using these ABPs as additional predictors of NSCLC prognosis.

Keywords: Actin-binding proteins, non-small cell lung cancer, progression, metastasis

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Introduction

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer [1]. In addition to clinical and morphological parameters, the progression of NSCLC is influenced by individual molecular genetic characteristics of the tumor. Violation of adhesive properties and the ability to actively move within the extracellular matrix are crucial processes that influence the metastatic potential of tumor cells. The study of the expression levels of actin binding proteins (ABPs) is crucial because these proteins play a vital role in regulating the actin cytoskeleton [2, 3]. More than 100 proteins in the cytoplasm bind to actin, performing various functions: they regulate the volume of the G-actin pool, affect the polymerization rate, stabilize the ends of the threads, cross-link the filaments with each other, and destroy the double helix of F-actin [4, 5]. The group of actin-depolymerizing proteins include adenylate cyclase-associated protein 1 (*CAP1*) and cofilin. These proteins may work both in pairs and independently [6-8]. *CAP1* was shown to be involved in the pathogenesis of cancer of several localizations, including lung cancer [9-11]. Using real-time PCR methods, Western-blotting on cell lines of non-invasive (95-C) and invasive (95-D) lung cancer, it was shown that the *CAP1* protein level was higher in tumor tissues than in adjacent unchanged tissues and *CAP1* gene expression was significantly higher in metastatic tumors than in non-metastatic tumors. The relationship between *CAP1* gene expression and the stage of lung adenocarcinoma was found [12]. Using immunohistochemistry and Western blotting on NSCLC tissues taken from patients at different stages of the disease, a mathematical model was created with *CAP1* to predict brain metastases with sensitivity and specificity of 79.5% and 67.1%, respectively [13]. Cofilin and SAR1 are functional partners that are involved in the process of NSCLC metastasis. Cofilin-1 (*CFL1*) is expressed in the tissues of gastric cancer, squamous cell head and neck cancer and colorectal cancer [https://www.proteinatlas.org]. Inhibition of *CFL1* in the cell lines of the clinical and prognostic biomarkers of NSCLC stops the migration and invasion of tumor cells [14]. Preliminary analysis suggests that *CFL1* plays a role in the development of radioresistance and multiple drug resistance in lung adenocardinoma cells [15]. *CFL1* has been suggested can be used as a biomarker to prevent NSCLC [16, 17] and colorectal cancer [18]. We have previously indicated that an increase in the levels of *CAP1* and *CFL1* in tumor tissue increases the risk of distant metastases [19]. Profilin-1 (*PFN1*) belongs to the group of monomer-binding proteins, which provides G-actin recirculation during remodeling of the cytoskeleton [20].

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It has been shown that high levels of *PFN1* are associated with inhibition of growth and metastasis in pancreatic and liver cancers [21, 22]. However, in renal cell carcinoma and laryngeal squamous cell carcinoma, high *PFN1* levels are linked with tumor progression and aggressiveness [11, 23]. Fascin-1 (*FSCN1*) is a member of a group of cross-linking proteins that facilitate the formation of bundles and branching of actin filaments. *FSCN1* contributes to the disruption of intercellular contacts and promotes the release of tumor cells into the extracellular matrix [24]. Elevated *FSCN1* levels were reported in lung [25, 26], gastric [27, 28] and ovarian [29, 30] tumors. The increased level of *FSCN1* in breast cancer tissue is considered as a possible diagnostic marker of triple-negative cancer [31]. An experiment in mice showed that tumor cell migration and metastasis can be reduced by inhibiting the physiological function of fascin-1 [32]. Ezrin (*EZR*) refers to proteins that ensure the attachment of actin to the cell membrane [33]. However, the molecular mechanisms of *EZR* involvement in tumor metastasis promotion still not fully understood. The overexpression of this protein in the cancer tissue can be a therapeutic target for squamous cell carcinomas of the tongue [34]. The involvement of *EZR* in signaling cascades was shown on cell cultures of colorectal cancer [35, 36]. Thus, the study of the role of functionally different ABPs in pathological processes is important for understanding the processes associated with remodeling of the actin cytoskeleton, in particular, metastasis and invasion of tumor cells. The purpose of the study was to investigate the levels of ABPs: *CAP1*, *CFL1*, *PFN1*, *FSCN1*, and *EZR* and their gene expressions to predict hematogenous metastases in patients with NSCLC.

Materials and Methods

Patients and sample collection

The study included 46 patients with morphologically verified diagnosis of NSCLC: 35 (76%) men and 11 (24%) women aged 58.5±1.3years. The patients did not receive neoadjuvant chemotherapy. Specimens of adjacent (1 cm from the tumor) histologically unchanged lung tissue and primary tumor tissue were collected after informed consent of each patient after full explanation of the purpose and nature of all procedures used. The manipulations were carried out under conditions of voluntary participation and confidentiality in accordance with the Helsinki Declaration of the World Medical Association “Ethical Principles for Conducting Scientific Medical Research with Human Participation” as amended in 2000. The study was allowed by Ethic Committees of Tomsk National Research Medical Center and Tomsk Regional Oncology Center (protocol No.1 dated January 15, 2016).

Preparation of clarified homogenates

Frozen tissue (100 mg) was homogenized using a SONOPULS mini20 ultrasonic homogenizer (Bandelin, Germany) in 300 μL of 50 mM Tris-HCl (pH 7.5) containing 2 mM ATP, 5 mM magnesium chloride, 1 mM DTT, 1 mM EDTA, and 100 mM sodium chloride. The homogenate was centrifuged at 10 000 g at 4°C for 60 min. The total protein content in the supernatant (clarified homogenate) was measured by the Lowry assay [37].

ABPs level

The ABPs levels were estimated by Western-blotting. A sample (20 μg total protein) was separated via denaturing PAGE in vertical 10% gel in a Tris-glycine buffer at 200 V and 400 mA. Proteins were transferred onto a PVDF membrane (Millipore, United States) in a Tris-glycine buffer containing 10% ethanol at 100 V and 350 mA for 1 h. Protein levels were measured using primary antibodies against *CAP1*, cofilin, profilin, fascin, ezrin, and β-actin (Cell Signaling Technology, United States). Horseradish peroxidase conjugates of anti-mouse immunoglobulin goat antibodies and anti-rabbit immunoglobulin horse antibodies (Cell Signaling Technology) were used as secondary antibodies. Antibody binding and membrane washing were performed using a semi-automated iBind Western System (Thermo Fisher Scientific, USA). The membrane was treated with an ECL chemiluminescence detection system (GE Healthcare, United Kingdom). Immune detection was carried out using a ChemiDoc Touch Imaging System (BioRad, USA). Band intensities were evaluated using Image Lab software. Data were normalized to β-actin. The result was expressed as a tumor-to-unchanged tissue protein content ratio.

Isolation of mRNA and cDNA synthesis

RNA was isolated using a diaGene kit (Qiagen, USA) as recommended by the manufacturer. The RNA concentration and purity were assessed spectrophotometrically on NanoDrop-2000 (Thermo Fisher Scientific, USA). The RNA concentration varied from 80 to 250 ngμL; A260/A280 = 1.95–2.05; A260/A230 = 1.90–2.31. Synthesis of cDNA was carried out using a RealBest Master miks OT kit (Vector-Best, Russian Federation). The reaction was performed at 42°C for 30 min, and reverse transcriptase was inactivated by heating the mixture at 95°C for 2 min. The cDNA-containing reaction mixture (3μL) was immediately used as a template for the polymerase chain reaction (PCR).

Real-time PCR

Quantitative real-time PCR was carried out using a Rotor-Gene 6000 amplification system (Corbett Research, Australia). The reaction mixture (Biolambiks, Russian Federation) with a final volume of 25 μL contained 12.5 μL of BioMaster HS-qPCR (100 mM Tris-HCl, pH 8.5, 100 mM KCl, 0.4 mM dNTPs, 3 mM MgCl2, 0.06 units of Taq DNA polymerase, 0.025% Tween-20, HS-Taq DNA polymerase stabilizers, SYBR Green 1, and an inert dye), 1 μL of each of the forward and reverse primers, 3 μL of template cDNA (≈20 ng/μL), and 7.5 μL of deionized water. The glyceraldehyde 3-phosphate dehydrogenase gene (*GAPDH*) was used as a control. The expression level of each target gene was normalized to the *GAPDH* expression level according to the Pfaffl method [38]. Amplification included initial denaturation at 95^{o}C for 6 min and 40 cycles of denaturation at 95°C for 10 s and annealing and elongation at 62^{o}C for 35 s. The result was expressed in conventional units relative to the target gene expression in unchanged tissue. The following primers were designed using the program Vector NTI Advance 11.5 and the NCBI database: *CFL1* (F 5’-CTGCCGCTATGCCCTCTA-3’;R 5’-TTCTTCTTGATGGCGTCCTT-3’); *CAP1* (F 5’-CCAAACGAGCCACAAAGAA-3’;R 5’-ACCCATTACCTGAACTTTGACAT-3’); *PFN1* (F 5’-TGGAGCAAACCCTACCCTT-3’, R 5’-AGCCCAGACACCGAACTTT-3’); *FSCN1* (F 5’-TCAGAGCTCTTCCTCATGAAGCT-3’, R 5’-GTCCAGTATTTGCCTGTGGAGTC-3’); *EZR* (F 5’-CTTGATGTGATGTGGCAGGA-3’, R 5’-GGAATGAGTGGGCGGAA-3’); and *GAPDH* (F 5’-GGAAGTCAGGTGGAGCGA-3’, R 5’-GCAACAATATCCACTTTACCAGA-3’). The reaction specificity was checked for each sample and each gene after each PCR via automated electrophoresis, which was performed using a 2200 TapeStation instrument (Agilent Technologies, USA) and a R6K ScreenTape kit (Agilent Technologies, USA). The following negative controls were used additionally: no template + PCR mixture + primers, template + PCR mixture + no primer, and RNA + PCR mixture + primers. RNA quality was characterized using the RNA integrity number (RIN), which varied from 4 to 6 in the samples examined. The reaction efficiency varied from 1.8 to 1.9 with all primers.

Statistical analysis

Data were processed using IBM SPSS Statistics 20.0 software and presented as median and interquartile range Me (25-75%). Verification of the normality of the distribution of the studied samples was performed using the Kolmogorov-Smirnov criterion. The significance of differences between groups was determined using the nonparametric Mann-Whitney test for independent samples. Spearman’s rank correlation coefficient, linear and logistic regression methods were used to assess the relationship between the studied parameters. ROC analyses were carried out to evaluate the significance of parameters in developing hematogenous metastasis. The differences were assumed to be significant at $p<0.05$. The Kaplan-Meier method was used to construct cumulative survival curves. The survival of patients was determined using the dynamic (actuarial) method. Survival rates were calculated in the second year after the end of treatment, taking into account patients lost to follow-up and those who died from concomitant non-cancer diseases. Life expectancy was calculated from the end of treatment. The specificity and sensitivity of indicators that showed statistically significant results in terms of metastasis-free and overall survival were tested using ROC analysis.

Results

Changes in the ABPs mRNA level of the NSCLC are correlated with lymph node metastasis

The mRNA expression and the protein levels of *CAP1*, *CFL1*, *PFN1*, *FSCN1*, and *EZR* in tumor tissue and adjacent unchanged tissue of patients with NSCLC were studied. The mRNA expression levels of *CAP1*, *CFL1*, *PFN1*, *FSCN1*, and *EZR* were shown to be significantly higher in tumor tissue than in adjacent unchanged lung tissue (Table 1). The mRNAs expression levels of other ABPs were elevated in about 60% of patients. The increased expression of *FSCN1* was observed in 80% of NSCLC patients. The Western-blotting technique identified higher levels of studied proteins in the tumor sample than in the non-tumor tissue (Figure 1).

Patients with NSCLC were divided into two groups: patients with lymphogenous metastases (T2-3N1-2M0) and without metastases (T2-3N0M0). The mRNA expression levels of genes encoding ABPs were significantly higher in tumors with regional lymph node metastases than in tumors without lymph node metastasis (Table 2). It should be noted that all mRNA levels of ABPs were significantly increased in NSCLC patients with lymph node metastases compared to those without metastasis. The most significant difference between stage T2-3N0M0 and stage T2-3N1-2M0 was shown for mRNA of *EZR*.

The increased mRNA expression of ABPs in tumor tissue compared to non-tumor tissue is an unfavorable predictor for hematogenous metastases in patients with NSCLC

To assess the 2-year metastasis-free survival, the prognostic significance of the studied ABPs was analyzed. At 2-years of follow-up, distant metastases occurred in 10 out of 46 patients. All ABPs studied showed an association with 2-year metastasis-free survival. A 2-3-fold increase in the mRNA expression levels of *CAP1*, *CFL1*, *EZR* and *FSCN1* and 6.6-fold increase in the *PFN1* level in the tumor tissue compared to the unaltered tissue was an unfavorable prognostic factor for the risk of developing hematogenous metastases (Figure 2).

We conducted a statistical analysis of the contribution

Table 1. The Relative mRNA Expression Levels of ABPs in Tumor Tissue Compared to Non-Tumor Adjacent Tissue.

mRNA	Tumor tissue (n=46)	p-value
CAP1	3.4 (0.9; 5.9)	0.004
CFL1	1.6 (0.4; 2.3)	0.023
PFN1	1.5 (0.3; 2.1)	0.054
FSCN1	2.0 (0.6; 3.9)	<0.001
EZR	1.6 (0.3; 2.2)	0.033

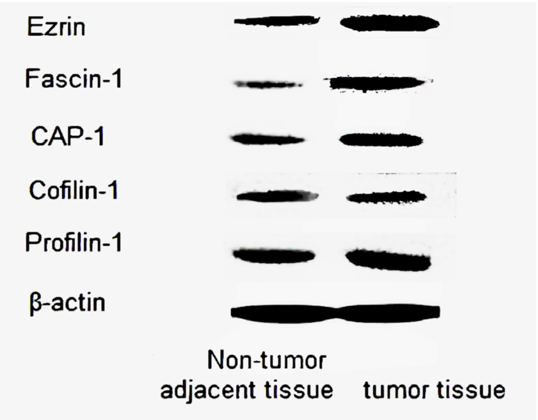

Figure 1. The Protein Level of ABPs in Tumor Tissue and in Non-Tumor Adjacent Tissue.

of ABPs to metastatic-free survival in NSCLC patients. Forty-six patients with NSCLC participated in the construction of the Cox regression model. Patients were divided into 6 groups depending on the increase in the mRNA expression of ABPs in tumor tissue compared to adjacent normal tissue (Table 3). In NSCLC patients of group 0, the mRNA expression level of ABPs in the tumor tissue was lower than or equal to that in the adjacent normal lung tissue. In this group, the mRNA expression score was equal to “0 points”. In patients of group 1, the mRNA expression level of 1 out of 5 ABPs was found to be increased; this group was given a score of “1 point”. In patients of group 2, the mRNA level of 2 ABPs was increased; this group received a score of “2 points”. In group 3, an increase in the expression of 3 ABPs was observed: “3 points”, etc. up to five points. In groups numbered 0, 1 and 2, 100% metastatic-free survival was observed. With an increase in the mRNA levels of three of the five ABPs, one-third of cases developed metastases, and with an increase in the mRNA levels of four and five proteins, metastases occurred in 100% of cases.

Table 2. The Relative mRNA Expression Levels of ABPs in the Primary Tumor Tissue with Respect to Lymph Node Metastasis (N)

mRNA	T2-3N0M0 (n=15)	T2-3N1-2M0 (n=31)	p-value
CAP1	1.7 (1.4; 2.0)	3.4 (2.7; 3.9)	0.021
CFL1	2.0 (1.0; 2.1)	5.4 (4.3; 6.0)	0.050
PFN1	1.6 (0.1; 2.8)	3.5 (3.3; 5.5)	0.020
FSCN1	1.6 (1.5; 2.5)	3.6 (2.8; 3.8)	0.026
EZR	1.0 (0.5; 2.1)	4.9 (4.3; 6.0)	<0.001

The effect of the increased mRNA levels of ABPs on the 2-year metastatic-free survival is shown in Figure 3. Figure 3 indicates 100% 2-year metastatic-free survival

Table 3. Distribution of NSCLC Patients According to the Relative mRNA Expression of ABPs in Tumor Tissue

Group No	Increased relative mRNA expression of ABPs, point	Number of patients with hematogenous metastasis, n (% of patients in the group)	Total number of patients, n (% of the total number of patients)
0	0	0 (0)	6 (13)
1	1	0 (0)	14 (30)
2	2	0 (0)	10 (22)
3	3	3 (33)	9 (20)
4	4	5 (100)	5 (11)
5	5	2 (100)	2 (4)
Total		10 (22)	46 (100)

Note: Increased relative mRNA expression of ABPs - mRNA expression in tumor tissue compared to adjacent normal tissue in NSCLC patients

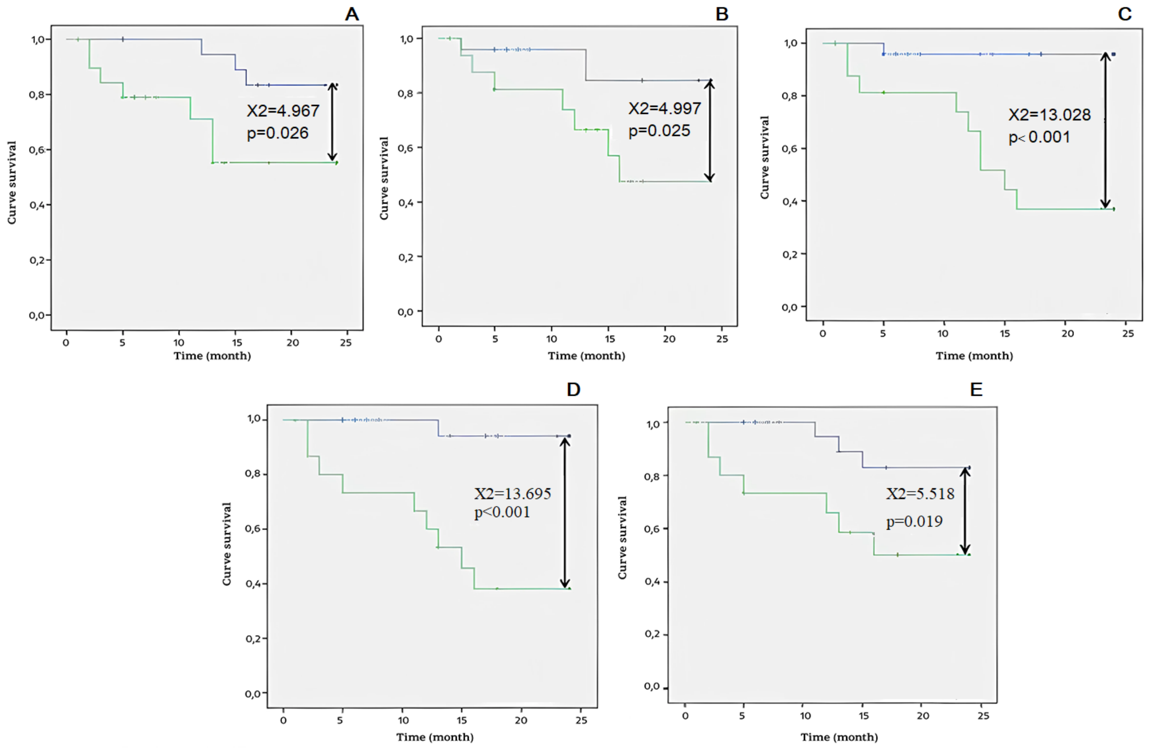

Figure 2. The 2-Year Metastasis-Free Survival of NSCLC Patients with Respect to RNA Expression Level of ABPs in Tumor Tissues. Note: the blue line depicts the survival for patients with tumor RNA level of *CAP1*>3.05 (A), *CFL1*>2.00 (B), *FSCN1*>3.00 (C), *EZR*>2.80 (D), and *PFN1*>6.60 (E); the green line depicts the survival for patients with tumor RNA level of *CAP1*≤3.05 (A), *CFL1*≤2.00 (B), *FSCN1*≤3.00 (C), *EZR*≤2.80 (E), and *PFN1*≤6.60 (D).

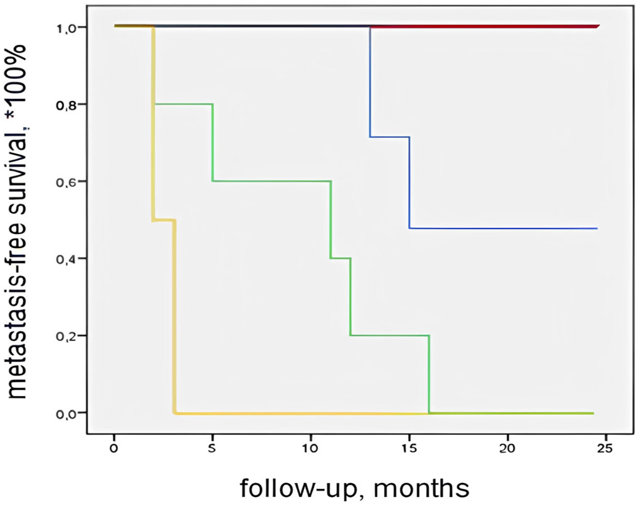

Figure 3. The 2-Year Metastasis-Free Survival Rates in Groups 0-5 Depending on the Increase in the Relative mRNA Expression of ABPs in Tumor Tissue. Note: the lines depicts survival curves for group 0 (yellow), group 1 (green), group 2 (blue), group 3 (red), group 4 (pink), and group 5 (black).

rate in the first three groups and 100% development of an unfavorable outcome in NSCLC patients with increased expression of four or five ABPs. In NSCLC patients of group 3, the expression of *CAP1*, *FSCN1*, and *EZR* mRNA had a greater impact on the risk of developing distant metastases. Moreover, an increase in at least one of these ABPs by one unit increased the risk of developing hematogenous metastases by almost 3 times (Table 4).

The significance of relative mRNA expression of ABPs in the prognosis of distant metastasis in patients with NSCLC was assessed using ROC analysis (Table 5, Figure 4).

The largest area under the curve (AUC) was shown for the mRNA expression levels of *FSCN1*, *CAP1* and

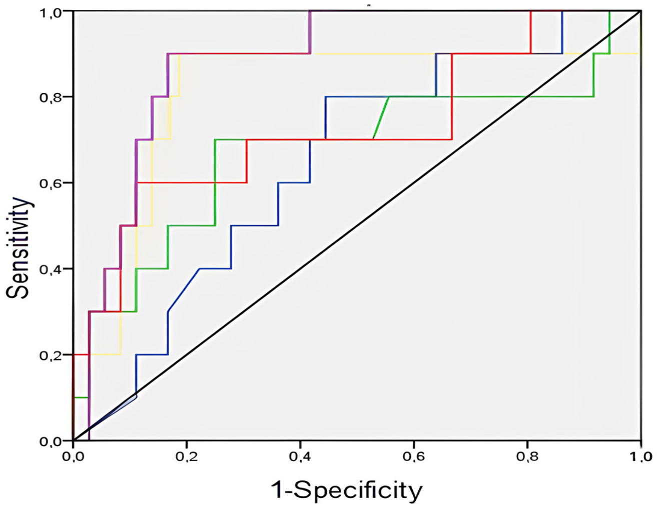

Figure 4. ROC Curves for Assessing the Quality of Binary Classification by Determining the mRNA Expression Level of ABPs in Tumor Tissue to Predict the Development of Distant Metastases. Note:the lines depicts ROC curves for RNA level of *CAP1* (blue), *CFL1* (green), *FSCN1* (purple), *EZR* (yellow), and *PFN1* (red).

Table 4. Significance of Relative mRNA Expression of ABPs in Tumor Tissue in the Cox Regression Model

mRNA	B	S.E.	Wald	p-value	Exp(B)	95% CI for exp(B)	
						Lower	Upper
CAP1	1.026	0.335	8.730	0.003	2.734	1.226	5.974
CFL1	0.500	0.306	11.876	0.001	2.011	1.000	3.004
PFN1	0.899	0.672	16.437	0.000	2.066	1.286	3.759
FSCN1	1.022	0.397	6.629	0.010	2.777	1.276	6.045
EZR	1.042	0.498	4.376	0.036	2.834	1.068	7.519

Note: S.E, standard error; 95% CI, 95% Confidence Interval

Table 5. The Significance of the relative mRNA Expression of ABPs in Tumor Tissue of NSCLC Patients in the ROC Analysis

mRNA	AUC	S.E.	p-value	95% CI	
				Lower	Upper
CAP1	0.775	0.102	0.031	0.526	0.924
CFL1	0.676	0.113	0.051	0.455	0.898
PFN1	0.725	0.102	0.031	0.526	0.924
FSCN1	0.883	0.052	0.000	0.781	0.986
EZR	0.803	0.095	0.004	0.617	0.989

Note: AUC, Area Under the ROC-Curve; S.E, standard error; 95% CI, 95% Confidence Interval

EZR. The sensitivity, specificity and overall significance of the model were 75%, 94.4%, and 84.5%, respectively.

Discussion

Lung cancer is the leading cause of cancer-related death and the second most common cancer worldwide. NSCLC is a highly aggressive tumor with early onset of metastases. Despite advances in diagnosis and treatment of NSCLC, its prognosis remains poor. The study of the mechanisms of NSCLC metastasis is necessary to understand the patterns of changes in cellular processes during the spread of tumors. The actin cytoskeleton and ABPs play a crucial role in cancer cell invasion and metastasis [39]. The participation of ABPs in the regulation of cell motility and the mechanisms involved in cell motility are extensively studied. Therefore, our results will complement the data on tumor growth and will be useful for developing methods for predicting metastasis and, possibly, selecting treatment tactics for patients with NSCLC.

We studied the mRNA expression level of *CAP1*, *CFL1*, *PFN1*, *FSCN1* and *EZR* and the levels of corresponding proteins in tumor tissue and tumor-adjacent unaltered tissue taken from patients with NSCLC. Our results showed a relationship between the changes in the mRNA expression levels of ABPs and lymphogenous and hematogenous metastasis in patients with NSCLC. In addition, the results obtained demonstrate the feasibility of using these ABPs as additional predictors of NSCLC progression.

Our data showed a significant increase in the mRNA expression of *CAP1*, *CFL1*, *PFN1*, *FSCN1* and *EZR*, and their protein products in the primary tumor tissue compared with tumor-adjacent unchanged lung tissue. The most pronounced changes in mRNA expression were observed for *FSCN1*. This finding is consistent with other studies. Currently, determination of the protein expression of *FSCN1* is proposed as a prognostic marker in kidney cancer, lung cancer and head and neck cancer (https://www.proteinatlas.org/ENSG00000075618-*FSCN1*).

In our study, the increased expression level of ABPs genes in tumors of patients with lymphogenous metastases compared to that in tumors of patients without lymphogenous metastases indicated the involvement of these ABPs in the pathogenesis of NSCLC. Using ROC analysis and Cox regression model, all ABPs were identified to be associated with 2-year metastasis-free survival in NSCLC patients. Thus, we can conclude that an increase in tumor aggressiveness is accompanied by an increase in the metastatic potential of tumor cells, which is reflected in the sequential activation of the ABP genes. Based on our results and data from https://string-db.org (a database of known and predicted protein-protein interactions), all of the ABP genes we studied are likely to be indirectly or directly functional partners. In addition, our data show that the increased mRNA expression levels of *CAP1*, *CFL1*, *PFN1*, *FSCN1* and *EZR* may be unfavorable prognostic factors for hematogenous metastases in NSCLC. Timely diagnosis and prediction of metastasis by assessing the mRNA expression levels of ABP functional partners in tumor tissue will help start appropriate treatment as soon as possible, thus increasing the metastatic-free survival of NSCLC patients.

The pathogenetic role of ABPs in the development and progression of cancer, including NSCLC, have mainly been studied using a limited set of targets from a large family of proteins involved in cytoskeletal remodeling. A comprehensive assessment of the expression level of genes and their protein products in the relation with metastases in NSCLC patients has not yet been carried out. A model was built on NSCLC tissues using *CAP1* to predict brain metastases with sensitivity and specificity of 79.5 and 67.1%, respectively. Based on this data the authors suggested that *CAP1* was involved in the pathogenesis of brain metastases. The relationship between *CAP1* expression in tissue and blood serum and the presence of lymphogenous metastases was also observed in other types of cancer: pancreatic, breast, esophageal and head and neck cancers [10-13]. There are many publications on the functional partnership with *CAP1* and *CFL1* [40]. Currently, protein interaction network map and protein complexes identified in breast cancer cells has been registered and entered into the public database https://www.bu.edu/dbin/cnsb/BrCa3CL/, where the physical interaction of *CAP1* and *CFL1* is indicated [41]. Thus, the functional activity of these proteins can influence various cellular processes that participate in the cell motility. The increase in the level of ABPs and their mRNA in tumor tissue compared to adjacent unaltered tissue may likely indicate active invasion of tumor cells, as well as their preparation for metastatic processes [19].

CFL1 is the most studied ABPs and is one of the key factors in the regulation of the cell cycle, participating in the pathogenesis of various tumors [14 –17, 42]. Overexpression of *CFL1* has been reported in several cancers, and, nevertheless, its role in carcinogenesis continues to be discussed [43]. The contribution of *PFN1* and *EZR* to tumor growth and progression is still not fully understood. The role of *PFN1* in tumor progression is not clear and likely depends on the histological type and location of the tumor [22]. Thus, a high level of *PFN1* is associated with both inhibition of the development of metastases in pancreatic and liver cancer and tumor progression in renal cell carcinoma and squamous cell carcinoma of the larynx [21, 23, 44]. According to the database (https://www.proteinatlas.org/), high levels of mRNA *EZR* are a favorable prognostic marker for kidney cancer and unfavorable for pancreatic cancer. An increase in the expression of ezrin in the tissue of squamous cell carcinomas of the tongue was found [34]. The participation of *EZR* in signaling cascades was shown in colorectal cancer cell cultures [36].

Increased levels of *FSCN1* have been reported in tumors of the lung [25, 26], stomach [27, 28], and bladder [24]. Assessment of the level of *FSCN1* in breast cancer tissue is considered as a potential marker of triple-negative cancer [31]. An experiment on mice showed that the migration of tumor cells and metastasis can be reduced by inhibiting the function of fascin. The participation of *FSCN1* in the regulation of autophagy processes has been demonstrated in endometrial cells. In the lungs, during tumor growth, *FSCN1* is translocated into mitochondria to stabilize oxidative phosphorylation under metabolic stress, which promotes metastasis [12]. *FSCN1* is considered as a promising target for therapy to prevent invasion and metastasis [29, 30, 32].

Thus, there are few data available on the participation of the ABPs complex in tumor growth, in particular, in NSCLC. The informative value of assessing the described ABPs complex as diagnostic markers is confirmed by the presence of significant differences in the expression of their mRNA between the tissues of head and neck squamous cell carcinoma and laryngeal dysplasia as well as between the tissues of the primary tumor and lymphogenous metastasis [11].

In conclusion, changes in the expression level of selected ABPs indicate the actin-cytoskeleton reorganization processes, which are closely related to the preparation of the tumor for proliferation and growth. The dynamics of changes in membrane structures determines the metastatic and invasive potential of a tumor cell. The assessment of changes in the ABP composition and/or transcriptional activity of genes encoding them can be a good diagnostic tool. In addition, regulation of the cytoskeleton activity can influence tumor progression.

Author Contribution Statement

E.K. conceived the study, designed the experiments and wrote the manuscript. E.K., G.K., and E.E.S.. performed the studies and acquired data. D.K. and A.D. participated in patient recruitment and supervised the clinical aspects of the research. E.A.S. and I.K. were involved in the creation of figs, tables and technical support. All the authors gave the approval to the final version of the manuscript.

Acknowledgements

Not applicable

Ethics approval

The manipulations were carried out under conditions of voluntary participation and confidentiality in accordance with the Helsinki Declaration of the World Medical Association "Ethical Principles for Conducting Scientific Medical Research with Human Participation" as amended in 2000. The study was allowed by Ethic Committees of Tomsk National Research Medical Center and Tomsk Regional Oncology Center: protocol No.1 dated January 15, 2016.

Data Availability

The datasets used and/or analyzed during the current study will be made available from the corresponding author on reasonable request.

Conflict of interest

All authors have read the journal's policy on disclosure of potential conflicts of interest and have none to declare.

References

1. Alduais Y, Zhang H, Fan F, Chen J, Chen B. Non-small cell lung cancer (NSCLC): A review of risk factors, diagnosis, and treatment. Medicine [Internet]. 2023 February 22; 102(8): e32899. Available from: https://doi.org/10.1097/MD.0000000000032899
2. Kondakova I V, Yunusova N V, Spirina LV, Kolomiets LA, Villert A B Association between intracellular proteinase activities and the content of locomotor proteins in tissues of primary tumors and metastases of ovarian cancer. Russian Journal of Bioorganic Chemistry. 2024; 40: 681-7. https://doi.org/10.1134/S1068162014060089
3. Lai WF, Wong W T Roles of the actin cytoskeleton in aging and age-associated diseases. Ageing research reviews [Internet]. 2020 March; 58: 101021. Available from: https://doi.org/10.1016/j.arr.2020.101021
4. Zhao X, Jiang M, Wang Z. TPM4 promotes cell migration by modulating F-actin formation in lung cancer. OncoTargets and therapy. 2019 April;12:4055-63. https://doi.org/10.2147/OTT.S198542
5. Banerjee S, Gardel ML, SchwarzU S. The actin cytoskeleton as an active adaptive material. Annual review of condensed matter physics.2020; 11: 421-39. https://doi.org/10.1146/annurev-conmatphys-031218-013231
6. Purde V, Busch F, Kudryashova E, Wysocki V H, Kudryashov D S. Oligomerization affects the ability of human cyclase-associated proteins 1 and 2 to promote actin severing by cofilins. International Journal of Molecular Sciences. 2019 November; 20(22): 5647. https://doi.org/10.3390/ijms20225647
7. Rust M B, Khudayberdiev S, Pelucchi S, Marcello E. CAPt'n of actin dynamics: Recent advances in the molecular, developmental and physiological functions of cyclase-associated protein (CAP). Frontiers in cell and developmental biology [Internet]. 2020; 8: 586631. Available from: https://doi.org/10.3389/fcell.2020.586631
8. Kolegova E S, Kakurina GV, Shashova E E, Yunusova N V, Spirina L V, Sidenko E A, et al. Relationship of intracellular proteolysis with *CAP1* and cofilin1 in non-small-cell lung

cancer. Journal of Biosciences [Internet]. 2021; 46(3): 55. Available from: https://doi.org/10.1007/s12038-021-00177-z

9. Hasan R, Zhou G L. The cytoskeletal protein cyclase-associated protein 1 (*CAP1*) in breast cancer: context-dependent roles in both the invasiveness and proliferation of cancer cells and underlying cell signals. International journal of molecular sciences [Internet]. 2019; 20(11): 2653. Available from: https://doi.org/10.3390/ijms20112653
10. Zeng J, Li X, Liang L, Duan H, Xie S, Wang C. Phosphorylation of *CAP1* regulates lung cancer proliferation, migration, and invasion. Journal of Cancer Research and Clinical Oncology. 2022; 148:137-53. https://doi.org/10.1007/s00432-021-03819-9
11. Kakurina G V, Cheremisina O V, Sereda E E, Kolegova E S, Kondakova I V, Choinzonov E L. Serum levels of cytoskeleton remodeling proteins and their mRNA expression in tumor tissue of metastatic laryngeal and hypopharyngeal cancers. Molecular Biology Reports. 2021; 48(6): 5135-42. https://doi.org/10.1007/s11033-021-06510-x
12. Lin S, Huang C, Gunda V, Sun J, Chellappan S P, Li Z, Izumi V, et al. Fascin controls metastatic colonization and mitochondrial oxidative phosphorylation by remodeling mitochondrial actin filaments. Cell reports. 2019; 28(11): 2824-36. https://doi.org/10.1016/j.celrep.2019.08.011
13. Xie S S, Tan M, Lin H Y, Xu L, Shen C X, Yuan Q, et al. Overexpression of adenylate cyclase-associated protein 1 may predict brain metastasis in non-small cell lung cancer. Oncology reports. 2015; 33(1): 363-71. https://doi.org/10.3892/or.2014.3577
14. Xu J, Huang Y, Zhao J, Wu L, Qi Q, Liu Y, et al. Cofilin: a promising protein implicated in cancer metastasis and apoptosis. Frontiers in cell and developmental biology [Internet]. 2021; 9: 599065. Available from: https://doi.org/10.3389/fcell.2021.599065
15. Zhou T, Zhang L Y, He J Z, Miao Z M, Li Y Y, Zhang Y M, et al. Mechanisms and perspective treatment of radioresistance in non-small cell lung cancer. Frontiers in Immunology [Internet]. 2023; 14: 1133899. Available from: https://doi.org/10.3389/fimmu.2023.1133899
16. Müller C B, de Barros R L, Castro M A, Lopes F M, Meurer R T, Roehe A, et al. Validation of cofilin-1 as a biomarker in non-small cell lung cancer: application of quantitative method in a retrospective cohort. Journal of cancer research and clinical oncology. 2011; 137: 1309-16. https://doi.org/10.1007/s00432-011-1001-5
17. Zhang Y G, Niu J T, Wu H W, Si X L, Zhang S J, Li D H, et al. Actin-binding proteins as potential biomarkers for chronic inflammation-induced cancer diagnosis and therapy. Analytical Cellular Pathology [Internet]. 2021; 2021:15. Available from: https://doi.org/10.1155/2021/6692811
18. Mousavi S, Safaralizadeh R, Hosseinpour-Feizi M, Azimzadeh-Isfanjani A, Hashemzadeh S. Study of cofilin 1 gene expression in colorectal cancer. Journal of Gastrointestinal Oncology. 2018; 9(5): 791-6. https://doi.org/10.21037/jgo.2018 .05.17
19. Kolegova E S, Kakurina G V, Kondakova I V, Dobrodeev A Y, Kostromitskii D N, Zhuikova L D. Adenylate cyclase-associated protein 1 and cofilin in progression of non-small cell lung cancer. Bulletin of Experimental Biology and Medicine. 2019; 167: 393-5. https://doi.org/10.1007/s10517-019-04534-9
20. Pinto-Costa R, Sousa M M. Profilin as a dual regulator of actin and microtubule dynamics. Cytoskeleton. 2020; 77(3-4): 76-83. https://doi.org/10.1002/cm.21586
21. Davey R J, Moens P D. Profilin: many facets of a small protein. Biophysical Reviews. 2020; 12(4): 827-49. https://doi.org/10.1007/s12551-020-00723-3
22. Wang Z, Shi Z, Zhang L, Zhang H, Zhang Y. Profilin 1, negatively regulated by microRNA-19a-3p, serves as a tumor suppressor in human hepatocellular carcinoma. Pathology-Research and Practice. 2019; 215(3): 499-505. https://doi.org/10.1016/j.prp.2018.12.012
23. Wang Y, Wang Y, Wan R, Hu C, Lu Y. Profilin 1 Protein and Its Implications for Cancers. Oncology [Internet]. 2021 Jule; 35(7): 420. Available from: https://doi.org/10.46883/onc.2021.3507.0402
24. Zhang X, Cho I H, Park J H, Lee M K, Hwang Y S. Fascin is involved in cancer cell invasion and is regulated by stromal factors. Oncology Reports. 2019; 41(1): 465-74. https://doi.org/10.3892/or.2018.6847
25. Lin S, Li Y, Wang D, Huang C, Marino D, Bollt O, et al. Fascin promotes lung cancer growth and metastasis by enhancing glycolysis and PFKFB3 expression. Cancer letters. 2021; 518: 230-42. https://doi.org/10.1016/j.canlet.2021.07.025
26. Sarantelli E, Mourkakis A, Zacharia L C, Stylianou, A., Gkretsi V. Fascin-1 in cancer cell metastasis: Old target-new insights. International Journal of Molecular Sciences [Internet]. 2023;24(14): 11253. Available from: https://doi.org/10.3390/ijms241411253
27. Qiu H, Zhao R, Shi Z, Li L, Guo Y, Zhao, W. Effects of fascin1 on epithelial-mesenchymal transition and metastasis of human gastric cancer cells. Int J Clin Exp Med. 2019; 12(4): 4043-9.
28. Ristic B, Kopel J, Sherazi S A, Gupta S, Sachdeva S, Bansal P. et al. Emerging role of fascin-1 in the pathogenesis, diagnosis, and treatment of the gastrointestinal cancers. Cancers [Internet]. 2021; 13(11): 2536. Available from: https://doi.org/10.3390/cancers13112536
29. McGuire S, Kara B, Hart P C, Montag A, Wroblewski K, Fazal S, et al. Inhibition of fascin in cancer and stromal cells blocks ovarian cancer metastasis. Gynecologic Oncology. 2019; 153(2): 405-15. https://doi.org/10.1016/j.ygyno.2019.01.020
30. Gupta I, Vranic S, Al-Thawadi H, Al Moustafa A E Fascin in gynecological cancers: an update of the literature. Cancers [Internet]. 2021; 13(22): 5760. Available from: https://doi.org/10.3390/cancers13225760
31. Barnawi R, Al-Khaldi S, Majid S, Qattan A, Bakheet T, Fallatah M, et al. Comprehensive transcriptome and pathway analyses revealed central role for fascin in promoting triple-negative breast cancer progression. Pharmaceuticals [Internet]. 2021; 14(12):1228. Available from: https://doi.org/10.3390/ph14121228
32. Francis S, Croft D, Schüttelkopf A W, Parry C, Pugliese A, Cameron K, et al. Structure-based design, synthesis and biological evaluation of a novel series of isoquinolone and pyrazolo [4, 3-c] pyridine inhibitors of fascin 1 as potential anti-metastatic agents. Bioorganic & Medicinal Chemistry Letters. 2019; 29(8): 1023-1029. https://doi.org/10.1016/j.bmcl.2019.01.035
33. Kawaguchi K, Asano S. Pathophysiological roles of actin-binding scaffold protein, ezrin. International Journal of Molecular Sciences [Internet]. 2022; 23(6): 3246. Available from: https://doi.org/10.3390/ijms23063246
34. Noi M, Mukaisho K I, Murakami S, Koshinuma S, Machida Y, Yamori M, et al. Expressions of ezrin, ERK, STAT3, and AKT in tongue cancer and association with tumor characteristics and patient survival. Clinical and experimental dental research. 2020; 6(4): 420-7. https://doi.org/10.1002/cre2.293
35. Haase G, Gavert N, Brabletz T, aBen-Ze'ev A. The Wnt target gene L1 in colon cancer invasion and metastasis.

Cancers [Internet]. 2016; 8(5): 48. Available from: https://doi.org/10.3390/cancers8050048

36. Wang Y, Yang Y, Wang X, Jin T, Zhu G, Lin Z. Ezrin as a prognostic indicator regulates colon adenocarinoma progression through glycolysis. Journal of Gastroenterology and Hepatology. 2021; 36(3): 710-20. https://doi.org/10.1111/jgh.15195
37. Lowry O H. Measurement with the folin phenol reagent. J Biol Chem. 1951; 193: 265-75.
38. Pfaffl M W. A new mathematical model for relative quantification in real-time RT–PCR. Nucleic acids research. 2001; 29(9): e45-e45. https://doi.org/10.1093/nar/29.9.e45
39. Alexandrova A Y, Chikina A S, Svitkina T M. Actin cytoskeleton in mesenchymal-to-amoeboid transition of cancer cells. International review of cell and molecular biology. 2020; 356: 197-256. https://doi.org/10.1016/bs.ircmb.2020.06.002
40. Heinze A, Schuldt C, Khudayberdiev S, van Bommel B, Hacker D, Schulz T G, et al. Functional interdependence of the actin regulators *CAP1* and cofilin1 in control of dendritic spine morphology. Cellular and Molecular Life Sciences [Internet]. 2022; 79(11): 558. Available from: https://doi.org/10.1007/s00018-022-04593-8
41. Havugimana P C, Goel R K, Phanse S, Youssef A, Padhorny D, Kotelnikov S, et al. Scalable multiplex co-fractionation/mass spectrometry platform for accelerated protein interactome discovery. Nature communications [Internet]. 2022; 13(1): 4043. Available from: https://doi.org/10.1038/s41467-022-31809-z
42. Kang C G, Han H J, Lee H J, Kim S H, Lee E O. Rho-associated kinase signaling is required for osteopontin-induced cell invasion through inactivating cofilin in human non-small cell lung cancer cell lines. Bioorganic & medicinal chemistry letters. 2015; 25(9): 1956-1960. https://doi.org/10.1016/j.bmcl.2015.03.024
43. Leonov S, Inyang O, Achkasov K, Bogdan E, Kontareva E, Chen Y, et al. Proteomic Markers for Mechanobiological Properties of Metastatic Cancer Cells. International Journal of Molecular Sciences [Internet]. 2023; 24(5): 4773. Available from: https://doi.org/10.3390/ijms24054773
44. Wang Y, Lu Y, Wan R, Wang Y, Zhang C, Li M, et al. Profilin 1 induces tumor metastasis by promoting microvesicle secretion through the ROCK 1/p-MLC pathway in non-small cell lung cancer. Frontiers in Pharmacology [Internet]. 2022; 13: 890891. Available from: https://doi.org/10.3389/fphar.2022.890891