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Exploring vimentin expression and its protein interactors across diverse cancer types via the cancer genome atlas datasets: a comprehensive analysis

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ABSTRACT

Background: The global burden of cancer is escalating, with millions of individuals diagnosed and succumbing to the disease each year. Early detection is crucial for improving patient outcomes, yet many cancers are identified at advanced stages. Vimentin (VIM) has emerged as a promising biomarker with significant diagnostic and prognostic potential.

Materials and methods: This study investigates *VIM* expression and promoter methylation across various cancers using The Cancer Genome Atlas (TCGA) datasets. Additionally, we analyze protein-protein interactions and mutation frequencies using advanced bioinformatics tools.

Results: Our findings reveal that *VIM* is overexpressed in seven cancer types, including cholangiocarcinoma, glioblastoma multiforme, and breast invasive carcinoma. Notably, *VIM* expression is correlated with promoter methylation in specific cancers. Furthermore, we identify complex protein interactions involving *VIM*, highlighting its role in critical cellular processes such as proliferation and apoptosis.

Conclusion: These insights emphasize Vimentin's multifaceted role in cancer, suggesting its potential as both a therapeutic target and a diagnostic marker.

Keywords: vimentin; TCGA; STRING; expression; methylation; cancer; biomarker

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Introduction

The vimentin gene (*VIM*) is a single-copy gene located on the short arm of chromosome 10 (10p12) [1]. *VIM* encodes for a 57 kDa polypeptide, vimentin (VIM): one of the most widely expressed and highly conserved proteins of the type III intermediate filaments (IFs) protein family. Vimentin is expressed in many different tissues and its expression level varies between cell types. The data from the Human Protein Atlas show that human organs, such as ovaries, breasts, lungs, and bone marrow, are characterized by increased *VIM* expression, while limited gene expression is observed in the stomach,

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rectum, and liver [2]. Increased level of the *VIM* was also shown in activated macrophages, in contrast to limited expression of vimentin in T and B lymphocytes and a lack of its expression in Burkitt's lymphoma cell lines [3].

The primary biological function of vimentin is to maintain cellular integrity and provide resistance to cellular stress [4]. Moreover, vimentin may form a complex with cell signaling molecules and other adaptor proteins [5]. The protein forms networks around the cell nucleus and extends to the entire cytoplasm, creating a scaffold for cell organelles [6]. The cellular localization of vimentin is directly related to its function. The VIM is a multifunctional protein that interacts with many other proteins. Thus, it acts as a regulator of a number of physiological processes [7].

There has been growing evidence that during pathological conditions such as tissue injury, inflammation, or cancer progression, vimentin could also be localized outside a cell [8]. It has been proved that outside the cell vimentin might be identified in two different forms: as a protein attached to the outer cell surface as well as a protein secreted to the extracellular space [9]. The function of extracellular vimentin is mainly correlated with immune processes, such as weakened immune response activity or bacterial elimination. It is also linked to cancer progression-related processes such as cell migration, proliferation, adhesion, or cell apoptosis [10].

Overexpression of vimentin was reported in a variety of epithelial neoplasms, including prostate, gastrointestinal, central nervous system, breast, malignant melanoma, lung, and other cancer types. The *VIM* is also expressed in many hormone-independent mammary carcinoma cell lines [3, 11]. The gene transcriptional activity in cancer correlates well with increased tumor growth, invasion, and poor prognosis [12]. Moreover, vimentin has been shown to be an essential factor involved in the epithelial-mesenchymal transition (EMT). It controls the changes in cell shape occurring during EMT and, thus, it is strongly associated with cell invasion and poor tumor prognosis [12]. A growing body of evidence indicates that vimentin also controls cell proliferation [13]. Extensive research in vimentin-deficient animal models ($vim^{-/-}$ mice) showed that loss of vimentin caused a reduction in fibrosis and the mesenchymal phenotype of cells (e.g., in cholangiocytes), which could be reversed upon *VIM* re-expression [14, 15].

Expression of vimentin is regulated by the oncoproteins such as simian virus40 T-antigen (SV40T), proto-oncogene (c-Myc), and Cyclin E increase, which leads to promoting the collapse of the vimentin intermediate filament network [16].

Additional evidence suggests that vimentin may help to diagnose autoimmune disorders (e.g., Crohn's disease), viral infections [e.g., human immunodeficiency virus (HIV)], and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [17–20].

Since VIM, among other cancer-related processes, is linked with cancer cell invasion and thus poor tumor prognosis, the evaluation of the *VIM* expression in normal and cancer tissues can be of considerable value in tumor diagnosis and disease progression. Therefore, in the present study, we comprehensively analyzed the *VIM* expression and its promoter methylation in different cancer types using The Cancer Genome Atlas (TCGA) UALCAN database [21, 22].

Additionally, to reveal the potential mechanism of the *VIM* action in cancers, we investigated the functional network of the vimentin partners using GeneMANIA, the protein-protein interaction using the Search Tool for the Retrieval of Interacting Genes (STRING), and mutation frequency by cBioPortal interactive online tool.

Materials and methods

Analysis of VIM mRNA expression levels in distinct types of human cancers using UALCAN web portal

UALCAN is an interactive online web portal of genomics data from The Cancer Genome Atlas (TCGA) (http://ualcan.path.uab.edu/index.html) [21–24]. This platform allows selected genes' expression to be analyzed and compared with clinical data. The UALCAN portal enables the comparison of the relative expression of selected genes in cancer and normal, non-tumorous samples. Moreover, it provides data on the patient's gender, age, body weight, race, and many clinicopathological features such as patient survival or individual cancer stages.

In this work, the expression of the gene encoding for VIM was analyzed in 19 out of 33 types of cancer accessible at the TCGA UALCAN portal.

Only 19 types of cancer possess complete and detailed information on VIM expression, including the gene promotor methylation and gene alteration status. Thus, only these 19 types of cancer were further analyzed. Thus, in our study, we analyzed the expression level of VIM in 19 cancer types, including bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head-and-neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), pancreatic adenocarcinoma (PAAD), pheochromocytoma and paraganglioma (PCPG), rectum adenocarcinoma (READ), sarcoma (SARC), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), thymoma (THYM). Moreover, we analyzed whether overexpression of VIM in those types of cancer is associated with gene promoter methylation.

All values of VIM expression are presented as transcripts per million (TPM) — a normalization method for RNA-seq representing the relative abundance of a gene or transcript in a sample. TCGA level 3 RNASeq V2 data corresponding to both normal tissue and primary tumor samples are presented as a box-and-whisker plot generated by the website tools.

The significant differences in the gene expression between primary tumor (according to clinical stages) and normal tissue were analyzed by StatView (SAS Institute, Cary, NC, USA). The student's t-test was used to calculate the level of statistical significance (p-value). The statistical significance of observed expression patterns is presented as p-values ($p \leq 0.05$). The data are presented using figures showing the interquartile range (IQR), and the median, minimum, and maximum values.

Analysis of VIM promoter methylation using UALCAN web portal

The UALCAN web portal was used to analyze and compare VIM promoter methylation patterns in normal tissue and primary tumor samples. The methylation level, ranging from 0 (unmethylated) to 1 (fully methylated) was estimated using the beta-value, which is the ratio of the methylated probe intensity to the sum of methylated and unmethylated probe intensity.

The boxplots were generated using the UALCAN web portal and represented the mean of beta-values from eight CpGs, including cg10790685, cg26306372, cg20198108, cg05151811, cg06460869, cg02236650, cg19111999, and cg23821329, located up to 1500 bp upstream of the VIM transcription start site (TSS200, TSS1500).

Differentially methylated promoters were identified based on statistical (Student's t-test ≤ 0.05) and biological (methylation level difference (Δβ-value) between the groups equal or higher than 0.05) thresholds.

Analysis of VIM networks using GeneMANIA and STRING web portal

The gene functional interactions of VIM were predicted using GeneMANIA analysis (http://www.genemania.org) [33, 67]. This online tool allows visualization of gene networks through bioinformatics methods, such as physical interaction, gene co-expression, gene co-localization, and gene enrichment analysis. Functional protein partners for VIM were identified using the Search Tool for the Retrieval of Interacting Genes (STRING) (version 11.0) analysis web portal (https://string-db.org/) [68]. The score of the minimum required interaction was medium confidence (0.4).

Analysis of VIM mutation in cancer using cBioPortal

Analysis of VIM nonsynonymous mutations in cancer genomes was performed using the cBioPortal for Cancer Genomics (https://www.cbioportal.org/) online platform [69]. cBioPortal collects NGS data and allows complex bioinformatics analyses to be performed, as well as generates graphical summaries showing the mutation status of selected genes. The graphical representation of VIM gene mutation analysis constitutes colored plots indicating mutations, fusions, amplifications, deep deletions, and multiple alterations.

Results

Complete and detailed information on *VIM* expression, including gene promoter methylation and gene alteration status, is available for nineteen out of thirty-three types of cancer accessible at

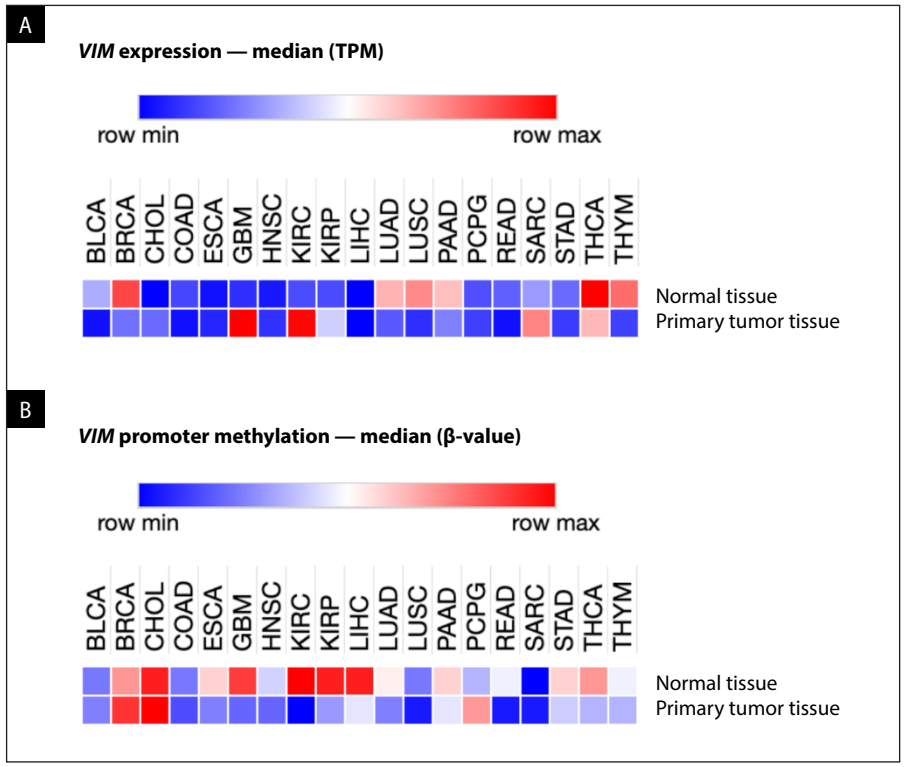

Figure 1. Heatmap for *VIM* expression (**A**) and *VIM* promoter methylation (**B**) across the studied The Cancer Genome Atlas (TCGA) samples. Low values of VIM expression/promoter methylation are represented by blue color, while high values of the studied parameters are indicated by red color

the TCGA UALCAN portal. Based on these data, a detailed analysis was conducted, and a heat map was plotted for *VIM* expression (Fig. 1A) and *VIM* promoter methylation (Fig. 1B). Overexpression of the VIM was found in 7 cancers: CHOL, GBM, HNSC, KIRC (Supplementary File — Fig. S1A, C, E, G), KIRP, liver hepatocellular carcinoma (LIHC), and PCPG (Supplementary File — Fig. S2A, C, E).

On the other hand, the expression of the VIM has significantly lowered in BLCA, BRCA, COAD, LUAD, LUSC, and READ (Supplementary File — Fig. S3A, C, E, G, I, K).

In ESCA, PAAD, SARC, STAD, THYM, and THCA no significant differences in the VIM expression between cancer tissues and normal tissues were observed.

From the TCGA database, we further retrieved a dataset containing complete information on the VIM expression, and its promoter methylation. These data are presented in Table 1.

It has to be noted that the analysis for BRCA (n = 1097) was performed on the highest number of primary tumor samples, in contrast to CHOL (n = 36), where the number of samples was the lowest. The highest median of the VIM transcript was found for GBM (3474.583), and the lowest for LIHC (142.187) (Tab. 1).

Further, we analyzed a correlation of the VIM expression with clinicopathological features of 19 cancers in the UALCAN database (Supplementary File — Figures S1–3).

Analysis of the VIM expression in cancer tissue as a function of gender showed significant differences between males and females in the case of BRCA, GBM, KIRC, LUAD, LUSC, PAAD, and THCA (p-values: $4.6 \times 10{-}3$, $4.0 \times 10{-}2$, $3.8 \times 10{-}2$, $4.3 \times 10{-}2$, $3.9 \times 10{-}2$, $4.7 \times 10{-}2$, and $3.6 \times 10{-}2$, respectively; Supplementary File — Figure S4). The TPM level of the VIM expression was higher for females in BRCA, GBM, LUAD, LUSC, and PAAD cancers. In KIRC and THCA, expression of the VIM was significantly elevated in males.

Elevated VIM expression was correlated with individual cancer stages (Supplementary File — Fig. S5). In the case of CHOL, HNSC, KIRC, and LIHC statistically increased levels of gene expression were observed in all cancer stages (stage from 1 to

Table 1. The expression level and promoter methylation of the VIM in normal and primary tumor tissues according to data deposited in the The Cancer Genome Atlas (TCGA) database. A p-value ≤ 0.05 was considered statistically significant (bolded in table). The Δβ-value indicates differences in the promoter methylation level of the VIM between normal and primary tumor tissue

Tumor type	*VIM* expression								*VIM* promoter methylation							
	Normal tissue			Primary tumor tissue				p-value	Normal tissue			Primary tumor tissue				p-value
	Samples no.	Median TPM	IQR	Samples no.	Sex (M/F)	Median TPM	IQR		Samples no.	Median β-value	IQR	Samples no.	Median β-value	IQR	Δβ	
BLCA	19	946.84	806.90	402	297/105	243.59	333.02	1.92×10^{-3}	21	0.13	0.02	418.00	0.17	0.14	-0.04	1.63×10^{-12}
BRCA	114	2266.87	1679.57	1087	12/1075	877.85	687.01	1.62×10^{-12}	97	0.21	0.07	793.00	0.30	0.21	-0.09	$< 1 \times 10^{-12}$
CHOL	9	91.38	101.33	36	16/20	826.36	728.04	7.63×10^{-4}	9	0.25	0.04	36.00	0.32	0.31	-0.07	1.22×10^{-2}
COAD	41	434.87	222.92	286	156/127	245.46	289.32	3.62×10^{-4}	37	0.13	0.07	313.00	0.15	0.06	-0.02	2.45×10^{-10}
ESCA	11	173.87	291.37	184	157/26	382.02	398.88	6.91×10^{-1}	16	0.19	0.08	185.00	0.17	0.12	0.02	5.93×10^{-1}
GBM	5	315.13	42.89	156	101/54	3474.58	2141.85	1.63×10^{-12}	2	0.24	0.01	140.00	0.16	0.07	0.08	6.44×10^{-1}
HNSC	44	205.48	222.61	520	383/136	453.82	517.89	9.78×10^{-5}	50	0.16	0.03	528.00	0.16	0.11	0.00	1.57×10^{-1}
KIRC	72	466.96	201.55	533	345/188	3433.38	1744.88	1.62×10^{-12}	160	0.26	0.04	324.00	0.12	0.04	0.14	1.62×10^{-12}
KIRP	32	452.62	205.15	290	214/76	1486.24	723.44	$<1 \times 10^{-12}$	45	0.25	0.02	275.00	0.18	0.14	0.07	1.02×10^{-7}
LIHC	50	88.59	70.18	371	245/117	142.19	135.34	1.16×10^{-11}	50	0.25	0.05	377.00	0.21	0.02	0.04	5.22×10^{-1}
LUAD	59	1729.38	451.63	515	238/276	725.15	573.35	$<1 \times 10^{-12}$	32	0.18	0.03	473.00	0.17	0.06	0.01	6.60×10^{-2}
LUSC	52	1929.41	691.97	503	366/128	440.28	487.73	1.63×10^{-12}	42	0.13	0.02	370.00	0.13	0.07	0.00	2.17×10^{-8}
PAAD	4	1669.09	434.14	178	97/80	984.38	692.16	1.70×10^{-1}	10	0.19	0.03	184.00	0.21	0.07	-0.02	1.73×10^{-7}
PCPG	3	477.67	4.42	179	78/101	553.12	471.06	2.13×10^{-7}	3	0.15	0.01	179.00	0.26	0.17	-0.11	1.63×10^{-12}
READ	10	561.82	218.48	166	90/76	247.13	206.22	9.60×10^{-4}	7	0.17	0.01	98.00	0.13	0.05	0.04	4.55×10^{-2}
SARC	2	850.43	374.77	260	141/119	2603.49	2132.70	1.24×10^{-1}	4	0.09	0.01	261.00	0.13	0.07	-0.04	5.99×10^{-4}
STAD	34	613.8	536.76	415	268/147	529.51	418.89	7.88×10^{-1}	2	0.19	0.03	395.00	0.20	0.12	-0.01	5.49×10^{-1}
THCA	59	2614.33	710.91	505	136/369	2278.46	1107.04	4.95×10^{-1}	56	0.21	0.07	507.00	0.19	0.11	0.02	3.08×10^{-1}
THYM	2	2080.5	1310.76	120	63/57	558.07	369.14	5.22×10^{-2}	2	0.17	0.05	124.00	0.19	0.08	-0.02	4.23×10^{-1}

BLCA — bladder urothelial carcinoma, BRCA — breast invasive carcinoma, CHOL — cholangiocarcinoma, COAD — colon adenocarcinoma, ESCA — esophageal carcinoma, GBM — glioblastoma multiforme, HNSC — head-and-neck squamous cell carcinoma, KIRC — kidney renal clear cell carcinoma, KIRP — kidney renal papillary cell carcinoma, LIHC — liver hepatocellular carcinoma, LUAD — lung adenocarcinoma, LUSC — lung squamous cell carcinoma, PAAD — pancreatic adenocarcinoma, PCPG — pheochromocytoma and paraganglioma, READ — rectum adenocarcinoma, SARC — sarcoma, STAD — stomach adenocarcinoma, THCA — thyroid carcinoma, THYM — thymoma; TPM — transcripts per million; IQR — interquartile range

4) in comparison to normal tissues (with one exception noted for stage 3 of one sample of CHOL). The VIM expression in individual cancer stages of GBM and PCPG was not analyzed because of the lack of adequate data in the UALCAN database.

We further investigated the differences in methylation of the VIM gene promoter between cancer and normal samples in all analyzed types of cancers (Table 1; Supplementary File — Fig. S1–3). We identified significant methylation differences (see Methods for details) for six cancers: BLCA, BRCA, CHOL, KIRC, KIRP, and PCPG (Supplementary File — Fig. S3B, S3D, S1B, S1H, S2B, S2F). The expression level of the VIM in those cancers was significantly different between the studied groups, suggesting a biological function of methylation in gene expression regulation. However, considering that the expression level of a gene is at least to some instances inversely correlated with promoter methylation, only in the case of KIRC, KIRP, BLCA, and BRCA cancers methylation and expression of the VIM were associated [29–32]. Thus, although in some cancer types methylation may regulate the expression of VIM, the involvement of regulation by mechanisms other than methylation must be considered.

We also investigated the potential effect of VIM gene expression level on patients' survival (data not shown). No potential effect of vimentin on patient's survival was indicated.

To explore the interactive functional association among VIM and genes related to its biological functions in cancer, the GeneMANIA analysis was performed [33]. As vividly shown in the Figure 2A, the central node representing VIM was surrounded by 20 nodes representing the genes that were found to be related with VIM in many biological processes such as physical interaction, co-expression, predictions, co-localization, and genetic interactions. The results of the analysis suggested that the VIM gene interacts with the SERPINH1, which plays a crucial role in collagen biosynthesis as a collagen-specific molecular chaperone. The VIM was also predicted to have a significant association with desmin (DES), titi-Cap (TCAP), titin (TTN), nebulin (NEB), and tropomodulin-1 (TMOD1).

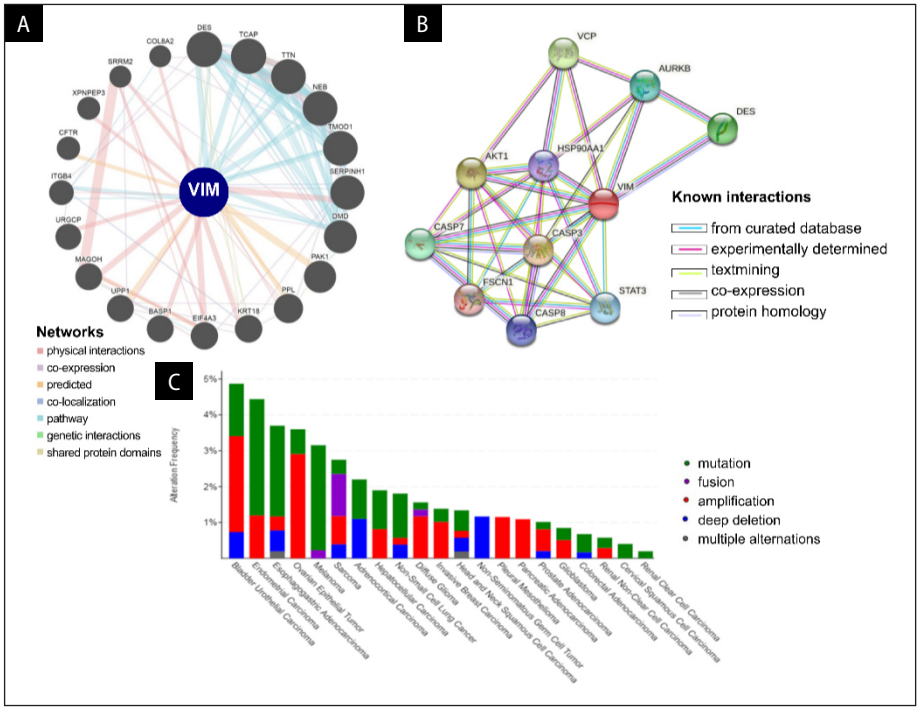

Figure 2. Protein-protein interaction (PPI) network of vimentin. **A.** GeneMANIA analysis with the *VIM* placed at the center of the diagram. The different colors of network edges indicate the bioinformatics methods applied: physical interaction, co-expression, prediction, co-localization, pathway, genetic interaction, and shared protein domains; **B.** Search Tool for the Retrieval of Interacting Genes (STRING) analysis of the VIM. Interacting nodes are displayed in colored circles; **C.** Copy number alterations of the *VIM* genes and cancer subtypes according to cBioPortal. All abbreviations are listed in the dedicated subsection at the end of the document

Moreover, the VIM is co-expressed with caspase-6 (CASP6), serpin H1 precursor (SERPINH1), and TTN.

Further examination with the Search Tool for the Retrieval of Interacting Genes (STRING) database allowed the identification of the direct protein-protein interaction (PPI) network between vimentin and 10 different proteins (Fig. 2B). The VIM as the center node was associated with: proteins responsible for apoptosis execution (CASP3, CASP7, and CASP8), protein involved in intermediate filaments organization — fascin (FSCN1), regulator of mitosis aurora kinase B (AURKB), signal transducer and activator of transcription 3 (STAT3), DES as well as RAC-alpha serine/threonine protein kinase (AKT1), heat shock protein HSP90-alpha (HSP90AA1), and transitional endoplasmic reticulum ATPase (VCP). The line thickness (Fig. 2B) indicates the strength of data support from the sources of text mining and experiments with a cut-off value of medium confidence (0.4) The PPI enrichment p-value was 0.004.

To establish the frequency of the VIM mutations in different cancer types, the cBioPortal database was utilized (Fig. 2C). This analysis included 1084 samples of different tumor types deposited in this database. The highest occurrence of the gene alterations was found in BLCA (4.87%) and endometrial carcinoma (4.44%), while the lowest frequency (0.57%) was found in renal carcinoma. Endometrial carcinoma presented the most heightened number of mutations (3.24%), in contrast to diffuse glioma, which had the lowest number of mutations (0.19%). Ovarian epithelial tumor showed the highest amplification of the VIM (2.91%), whereas the lowest value was revealed in non-small-cell lung cancer and HNSC (0.19% for both). In nonseminomatous germ cell tumor, the VIM alterations appeared only in the form of a deep deletion (1.16%). Interestingly, in adrenocortical carcinoma, an equal frequency of mutations and deep deletions was observed (1.1% for both alterations).

Discussion

Decades of research attempting to impinge on the so-called hallmarks of cancer led to the development of adequate strategies for cancer diagnostic and targeted therapies. The differences observed in every type of cancer highlight the importance of individualized treatment approaches.

In the tumor metastasis process, the most pivotal steps are based on degrading cell-cell junctions and the cell matrix, activating pathways that control the cytoskeletal reorganization of cancer cells' microenvironment. The proteins involved in the development of tissue microenvironments, such as e-cadherin, beta-catenin, and vimentin, seem to be sufficient for tumor cells to metastasize. The presence of these proteins, specific tumor microenvironments, can change and maintain the cells' plasticity and motility and their related function (survival, migration, and proliferation).

There has been growing interest in the biological function of vimentin lately [13, 17–19]. As a multifunctional protein, vimentin is differentially expressed in diverse cell types. Thus, it may play a tissue-specific function.

A study on knockout mice (*vim* $^{-/-}$) proved that a lack of vimentin or destabilization of the vimentin network enhances lamellipodia formation in all directions without net cell displacement [25]. Therefore, vimentin as the protein involved in a cytoskeleton rearrangement, may play a role in the acquisition of invasive phenotype of cancer cells.

To verify the hypothesis that vimentin is involved in cancerogenesis, we analyzed the expression of the *VIM* gene in different cancer types using such databases as TCGA UALCAN, GeneMANIA, STRING, and cBioPortal.

Only 19 out of 33 types of cancer accessible at the TCGA UALCAN portal possess complete and detailed information on the *VIM* expression, including the gene promoter methylation and gene alternation status. Thus, the study, for the first time, comprehensively analyzes and demonstrates overexpression of the *VIM* in those 19 types of cancer. The analysis of these records and comparison of healthy and cancerous tissues revealed overexpression of the *VIM* in seven out of 19 cancers. Cancers characterized by the increased level of the *VIM* include CHOL, GBM, HNSC, KIRC, KIRP, LIHC, and PCPG.

This high transcriptional activity of the *VIM* was observed in every stage of the disease, in the case of CHOL, HNSC, KIRC, KIRP, and LIHC cancers. On the other hand, decreased expression of the *VIM* was reported for six types of cancers, namely BLCA, BRCA, COAD, LUAD, LUSC, and READ.

For the remaining six cancers, ESCA, PAAD, SARC, STAD, THYM, and THCA, no statistical significance of *VIM* expression was observed.

Moreover, analysis of individual cancer stages among the groups showed that in CHOL, HNSC, KIRP, and LIHC, the *VIM* expression was extremely high in the early stage of the disease. While in KIRC the expression of *VIM* was increased and stayed at the same level in all stages of the disease. These findings imply that the analysis of the *VIM* expression can be used as a diagnostic biomarker of a selected cancer. The VIM expression in early breast and its role in the early diagnosis of cancer has been shown before [26, 27]. Our study does not confirm those results. Still, we agree that *VIM* might be a valuable marker to diagnose tumor cells and our findings may be useful for scientists working on personal medicine development.

Numerous studies propose the *VIM* promoter methylation status as a diagnostic biomarker in multiple cancers, including bladder cancer [28], breast cancer [29], cervical cancer [30], colorectal cancer [31, 32], and epithelial ovarian cancer [33], as well as a prognostic biomarker in e.g. pancreatic cancer [34]. Thus, in this study, we also investigated the *VIM* promotor methylation. The performed analysis showed that overexpression of the *VIM* may be the result of the loss of gene promoter methylation, as was revealed in the case of KIRC and KIRP, and opposite, the lower *VIM* expression may be associated with a gain of the gene promoter methylation, as was observed in case of BLCA and BRCA. We confirmed hypermethylation of the *VIM* promoter compared to normal samples in the case of BLCA and BRCA. The promoter was also hypermethylated in cholangiocarcinoma, pheochromocytoma, and paraganglioma. We also observed significant hypomethylation of the gene promoter in both kidney carcinomas. For BLCA, BRCA, KIRC, and KIRP cancers change in methylation corresponded with changes in the expression level of the gene. The differences in *VIM* methylation levels between studied cancers suggest the importance of these mechanisms in the different cancer types of pathogenesis. However, due to the fact that not for all types of cancers methylation status corresponded to changes in the *VIM* expression levels, there are probably other mechanisms involved in the gene regulation.

The functional analysis of the *VIM* expression in cancer using GeneMANIA and STRING indicates the potential direct and/or indirect relations between other genes associated with *VIM*-mediated cancerogenesis. The GeneMANIA analysis revealed interactions between the *VIM* and genes involved in the cytoskeletal matrix [*NEB,* dystrophin (*DMD*), periplakin (*PPL*)] and genes associated with muscle functioning (*TTN*, *TCAP*, *DES*) [35–37]. The *VIM* was co-expressed with *NEB*, *TMO1*, *CASP6*, and *SERPINH1*, which plays a crucial role in collagen biosynthesis as a collagen-specific molecular chaperone. These genes which are strongly related to the *VIM* may be used as a diagnostic biomarker for cancer (independently or as a panel). Operating more than one marker for a given tumor may be more costly but provides more confidence in diagnosing. In fact, overexpression of *SERPINH1* or P21(RAC1) activated kinase 1 (*PAK1*) has previously been proposed to be useful in some types of cancer diagnosis [38, 39].

The protein-protein interactions play diverse roles in cancer biology. The analysis of protein-protein interactions by the STRING database confirmed that vimentin is directly involved in a range of interactions, and thus biological processes. Proteins that interacted with VIM can be categorized into three distinct clusters. The first contains CASP3, CASP7, and CASP8 proteins related to creating death-inducing complex. The second consists of filament proteins: DES, VCP, and AURKB, and the function of this cluster is correlated with its members' functions. These proteins are engaged in cell cycle control, intermediate filament network structure, and function. The last cluster collects proteins mainly involved in cytokine-induced signaling pathways and signal transduction (AKT1, FSCN1, HSP90AA1, STAT3).

The results of both GeneMANIA and STRING analysis indicate that the VIM together with its protein partners is related to many cellular functions including cell organization, proliferation, migration, and cell death processes, regulating oncogenesis. Thus, the identified protein partners predicted to directly interact with the VIM might be involved in regulating cancer progression and prognosis.

The overexpression of the *VIM* documented in numerous cancer-related studies indicates that the gene expression may also be directly linked to cancer aggressiveness, however, depending on

the cancer type, different mechanisms underlying vimentin's contribution action may be observed [40, 41]. Overexpression of the *VIM* in LIHC cells has been shown to suppress their proliferative and invasive capabilities [42]. In stomach cancer, *VIM* overexpression is correlated with a significantly higher incidence of lymph node metastasis [43]. Moreover, the enhanced expression of the *VIM* in colorectal cancer is positively correlated with expanded migration and invasive potential, which seems to allow cancer cells to infiltrate into surrounding tissues and create a specific microenvironment [44].

Furthermore, elevated *VIM* expression has been reported in lung cancer [25], especially in non-small-cell lung cancer, in which the VIM has been shown to be a prognostic factor of poor survival [45]. Moreover, the *VIM* overexpression in breast cancers is correlated with increased invasion and promotion of epithelial cell migration [46, 47]. Finally, silencing of the *VIM* in cisplatin-resistant ovarian cancer cell lines A2780-DR and HO-8910 increased the expression levels of exocytotic proteins, which have been proposed as a new therapeutic target for treating drug-resistant ovarian cancer [48].

Recent studies showed that vimentin could play a crucial role in cancer development and subsequent reaction of the immune system. It is suggested that vimentin is involved in the apoptosis of neutrophils and lymphocytes [49, 50]. Thus, the possible role played by vimentin may be more complicated than we assume. Future studies investigating the VIM expression and action are needed to reveal the protein's importance and its use as a target in anticancer therapy.

We are aware that our research has some limitations, typical for bioinformatic analysis. The most important of them is linked with the limited number of data, especially in the case of noncancerous samples providing controls for CHOL, ESCA, GBM, PAAD, PCPG, and THYM. This problem might affect the statistical significance calculator, especially if more normal tissues' samples were available for the VIM expression analysis. The second limitation is the lack of wet lab experiments, which could confirm the conclusions of bioinformatic analyses. The third one is the fact that the data obtained from the TCGA UALCAN did not allow for extracellular *VIM* expression evaluation, which may have some diagnostic value. Also, due to differences in the databases, we cannot compare cBioPortal analysis with the TCGA database. It must also be mentioned that this analysis was completed using current TCGA bioinformatics tools rather than performing a new analysis of the raw TCGA data. Still, we believe that these analyses bring important information that may be useful in the evaluation of the *VIM* expression in specific types of cancer.

Further study is required to confirm the clinical importance of the VIM, especially as a potential diagnostic biomarker. Several experimental approaches can be used to validate the bioinformatic findings derived from the TCGA dataset. Quantitative PCR (qPCR) can be used to confirm differential gene expression patterns observed in the analysis, providing accurate quantification at the mRNA level. Western blotting offers the potential to validate changes in protein expression corresponding to the identified genes, ensuring that transcriptional differences are translated to the protein level. In addition, CRISPR/Cas9 gene editing could be used to investigate the functional role of specific genes in the context of the observed abnormalities, further corroborating the bioinformatic predictions. Taken together, these methods provide a robust validation of the computational findings and strengthen the overall conclusions of the study.

A promising avenue for future research is to explore the therapeutic targeting of VIM (vimentin) in cancer treatment. The development of small molecule inhibitors or RNA-based therapies that specifically target VIM may provide new avenues for intervention. In addition, investigating the role of VIM in cancer cell migration, invasion and metastasis may provide critical insights into its function in tumor progression. Preclinical studies evaluating the efficacy of VIM-targeted therapies, particularly in combination with existing treatments, may improve therapeutic outcomes and offer novel strategies to combat cancer.

Conclusions

Personalized medicine is an emerging practice that allows treatment strategies. Therapies based on a translational medicine approach are dedicated to cancer patients, even those who undergo radi-

cal resection surgery. While milestones have been achieved during the last decades for several cancer types, some cancers still comprise features limiting the effectiveness of treatment. Vimentin expression dynamics, intricately linked with gene promoter methylation, unveil a compelling narrative in cancer diagnostics. Particularly noteworthy is its pronounced overexpression in select malignancies such as cholangiocarcinoma, head-and-neck squamous cell carcinoma, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, and liver hepatocellular carcinoma. Our investigation unravels a rich tapestry of interactions between vimentin and a cadre of protein partners, including DES, NEB, TCAP, SERPINH1, and FASCN1, underscoring its pivotal role in orchestrating diverse cellular processes. Yet, the clinical significance of vimentin's expression and functionality in cancer demands further scrutiny, paving the way for future research endeavors to illuminate its therapeutic potential.

Author contributions

Conceptualization, M.B. and B.H.-Sz.; methodology, M.B., P.B., O.T.-L., and B.H.-Sz.; software, M.B.; formal analysis, M.B., P.B., O.T.-L., S.M., and B.H.-Sz.; investigation, M.B.; resources, M.B.; data curation, M.B.; writing—original draft preparation, M.B., P.B., O.T.-L., B.H.-Sz, and A.J.; writing—review and editing, M.B., B.H.-Sz., and A.J.; visualization, M.B.; supervision, M.B., P.B., O.T.-L., B.H.-Sz., and A.J.; project administration, M.B.; funding acquisition, M.B., and B.H.-Sz.

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Conflicts of interest

The authors declare no conflicts of interest.

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