BPR MCS | Bulletin of Pioneering Researches of Medical and Clinical Science

Bulletin of Pioneering Researches of Medical and Clinical Science

Available online: https://bprmcs.com
2022 | Volume 2 | Issue 2 | Page: 143-152

Expression Patterns, Clinical Associations, and Prognostic Significance of ADAMTS Gene-Derived circRNAs in Non-Small-Cell Lung Cancer

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Abstract

This study investigates the association between circular RNA (circRNA) derived from three genes of the ADAMTS family—ADAMTS6, ADAMTS9, and ADAMTS12—and their corresponding host gene expression in non-small-cell lung cancer (NSCLC), considering various clinical parameters. A notable link was observed between ADAMTS12 expression and specific circRNAs, while distinct expression patterns of ADAMTS6 and its associated circRNAs were found to be subtype-specific. Survival analysis revealed that lower ADAMTS6 expression in squamous cell carcinoma correlated with improved survival, higher ADAMTS9 expression was associated with longer survival, and overexpression of ADAMTS12 predicted poorer outcomes. These findings indicate that circRNAs may serve as potential diagnostic or prognostic biomarkers in NSCLC, emphasizing the significance of molecular patterns across different cancer subtypes.

Keywords: ADAMTS, CircRNA, Non-small-cell lung carcinoma, Regulation of transcription

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How to Cite This Article: Peterson RL, Romano SL, Wilson JA. Expression Patterns, Clinical Associations, and Prognostic Significance of ADAMTS Gene-Derived circRNAs in Non-Small-Cell Lung Cancer. Bull Pioneer Res Med Clin Sci. 2022;2(2):143-52. https://doi.org/10.51847/PGbMpTrugy

Introduction

Non-small-cell lung cancer (NSCLC) remains the leading cause of cancer-related mortality, with 5-year survival rates under 15% despite therapeutic advances [1]. Approximately 80% of NSCLC cases harbor oncogenic driver mutations, including KRAS, EGFR, BRAF, MET, ERBB2, ALK, RET, ROS1, and NTRK1/2/3 [2], alongside dysregulation of genes controlling proliferation, apoptosis, and immune responses. Non-coding RNAs (ncRNAs), such as microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), are key genetic regulators [3]. CircRNAs are formed by covalently linking the 5′ and 3′ ends of RNA transcripts into a closed loop [4] and often act as miRNA sponges, bind regulatory proteins, or are translated, thereby influencing gene expression [5]. Their levels affect processes like proliferation, apoptosis, differentiation, aging, innate immunity, and stem cell maintenance [6]. Previously considered non-functional byproducts of pre-mRNA splicing, circRNAs are now recognized as important cellular regulators and mediators of intercellular signaling.

Cancer development involves changes not only in tumor cells but also in the surrounding microenvironment (TME), which can serve as biomarkers for staging, subtype classification, and treatment response prediction [7]. Tumor progression is associated with extracellular matrix (ECM) remodeling, where proteolytic enzymes such as metalloproteinases (MMPs), ADAMs, and ADAMTSs play crucial roles [8]. ADAMTS proteins influence tumor microenvironments by degrading ECM components, promoting invasion, regulating angiogenesis, and modulating growth factors and cytokines, with

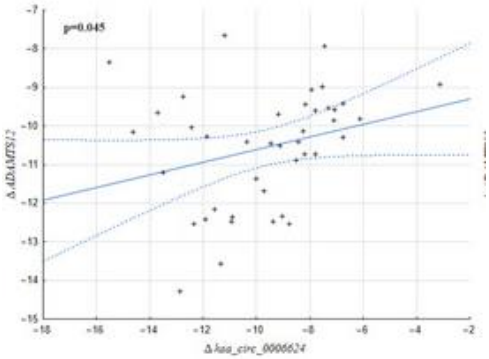
dysregulated activity observed across cancer types, making them potential therapeutic targets [9].

This study aimed to assess the expression of five circRNAs derived from ADAMTS6 (hsa_circ_0004418, hsa_circ_0072676), ADAMTS9 (hsa_circ_0066444), and ADAMTS12 (hsa_circ_0006624, hsa_circ_0072119) in NSCLC tissue, alongside the expression of their host genes. CircRNAs were selected based on the CircFunBase database for their reported involvement in cancer [10], with prior analyses limited to gastric cancer, where overexpression of these circRNAs was observed, and hsa_circ_0066444 was linked to poorer prognosis [11, 12]. This study thus provides one of the first evaluations of these circRNAs in NSCLC.

Results and Discussion

Correlation between circRNA and host gene expression

The analysis of correlations between ADAMTS6, ADAMTS9, and ADAMTS12 gene expression and their respective circRNAs revealed selective associations. No significant correlations were found for ADAMTS6 with hsa_circ_0004418 ($p = 0.589$) or hsa_circ_0072676 ($p = 0.338$), nor for ADAMTS9 with hsa_circ_0066444 ($p = 0.056$). However, significant correlations were detected for ADAMTS12 with hsa_circ_0006624 ($p = 0.045$) and hsa_circ_0072119 ($p < 0.001$) **(Figure 1)**.



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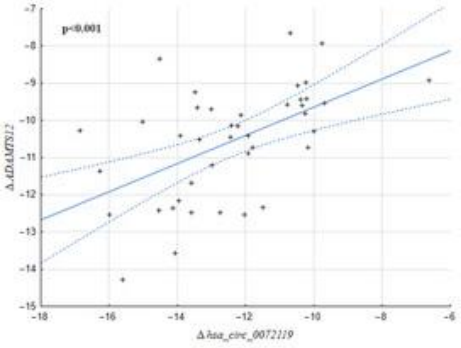

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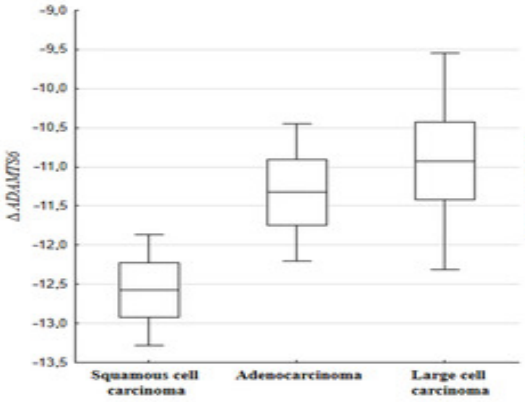
Figure 1. Relationship between ADAMTS12 expression and circRNAs hsa_circ_0006624 ($p = 0.045$) and hsa_circ_0072119 ($p < 0.001$), with the dashed regression band representing the 95% confidence interval.

Expression of circRNAs and host genes according to NSCLC histopathological subtype

The association between NSCLC histopathological subtype and the expression levels of the examined genes and circRNAs was analyzed. Significant differences were observed for ADAMTS6, with squamous cell carcinoma exhibiting lower ADAMTS6 mRNA levels compared to adenocarcinoma ($p = 0.0329$). Additionally, hsa_circ_0072676 expression was significantly higher in large-cell lung carcinoma than in squamous cell carcinoma ($p = 0.0243$) **(Figure 2)**.



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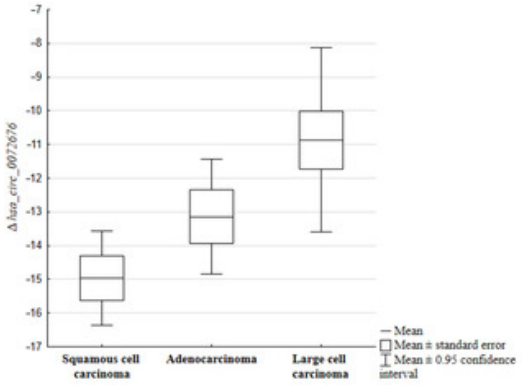

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Figure 2. Comparison of hsa_circ_0072676 ($p = 0.0243$) and ADAMTS6 ($p = 0.0329$) expression across NSCLC histopathological subtypes, highlighting significant differences among squamous cell carcinoma, adenocarcinoma, and large-cell carcinoma.

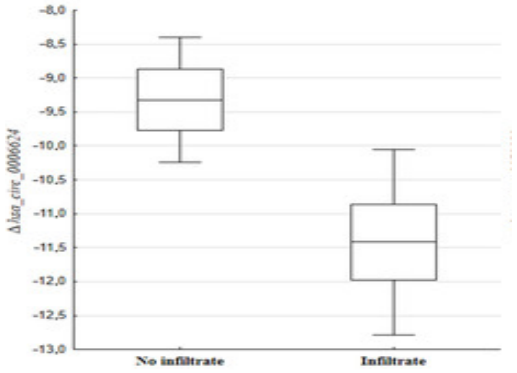
Relationship between circRNA/Host gene expression and TNM tumor stage

The study evaluated whether the expression levels of the examined circRNAs and their host genes were influenced by the size of the primary tumor [T] according to the TNM system, but no significant associations were identified. Likewise, the presence of metastases in regional lymph

nodes [N] did not show any correlation with gene or circRNA expression. Analysis of distant metastasis [M] also revealed no significant impact on the expression profiles of the genes or circRNAs studied.

circRNA and host gene expression in relation to local tissue invasion

A notable finding was that tumors invading adjacent tissues exhibited decreased expression of hsa_circ_0006624 (p = 0.0389) and hsa_circ_0072119 (p = 0.0021) **(Figure 3)**. In contrast, no significant changes were observed for hsa_circ_0004418 (p = 0.0519), hsa_circ_0072676 (p = 0.433), hsa_circ_0066444 (p = 0.5539), or their corresponding genes ADAMTS6 (p = 0.7795), ADAMTS9 (p = 0.4530), and ADAMTS12 (p = 0.7730).



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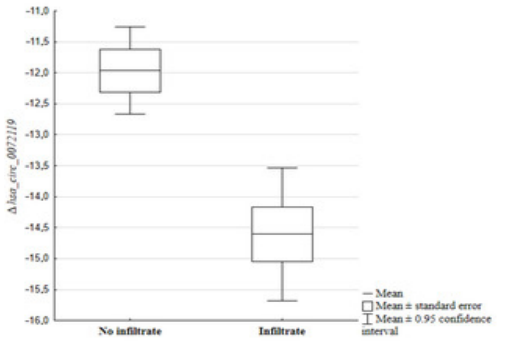
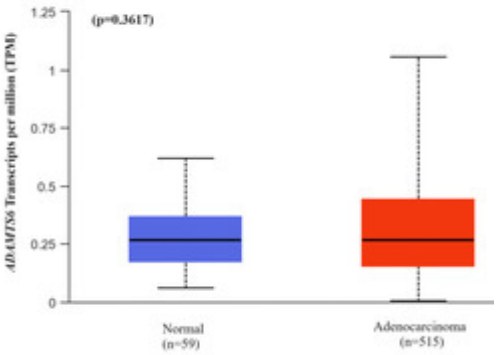


b)

Figure 3. Expression levels of hsa_circ_0006624 (p = 0.0389) and hsa_circ_0072119 (p = 0.0021) in tumors with versus without invasion of surrounding tissues.

Expression patterns of ADAMTS6, ADAMTS9, and ADAMTS12 in NSCLC tumors compared to normal lung tissue

Analysis using the UALCAN database evaluated differences in ADAMTS gene expression between NSCLC tumor samples, divided by histological subtype, and normal lung tissue. ADAMTS6 expression showed no significant variation between normal tissue and either adenocarcinoma (p = 0.3617) or squamous cell carcinoma (p = 0.4686). In contrast, ADAMTS9 expression was markedly reduced in both adenocarcinoma (p = 0.0154) and squamous cell carcinoma (p < 0.0001) relative to normal lung. ADAMTS12 displayed the opposite pattern, with significant upregulation in tumor tissue, observed in both adenocarcinoma (p < 0.0001) and squamous cell carcinoma (p < 0.0001). These differential expression patterns are summarized in **Figure 4**.



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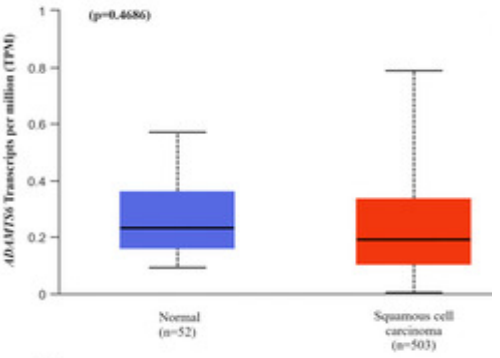

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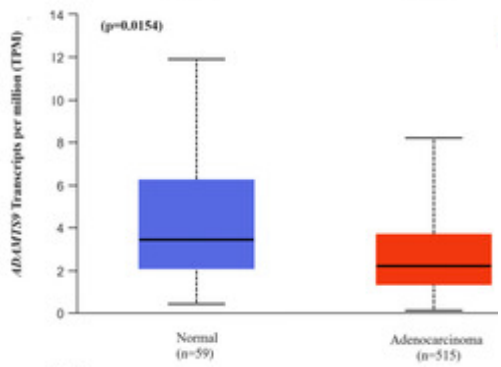

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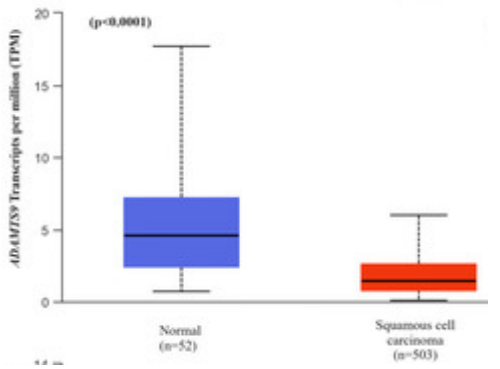

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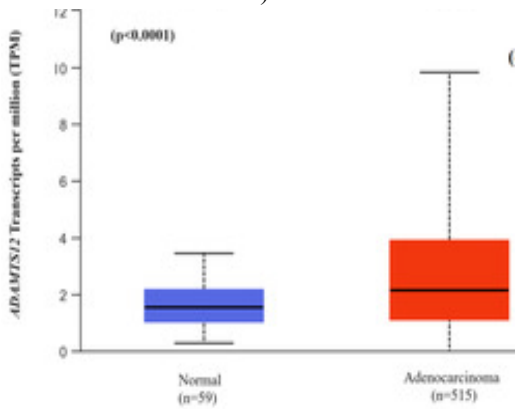

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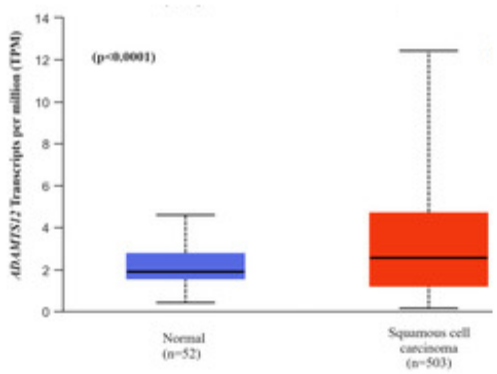
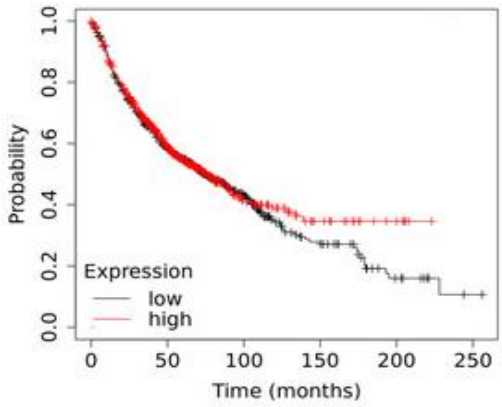


f)

Figure 4. Boxplots depicting ADAMTS6, ADAMTS9, and ADAMTS12 expression in NSCLC tumor tissue versus normal lung tissue: (a) ADAMTS6 in adenocarcinoma; (b) ADAMTS6 in squamous cell carcinoma; (c) ADAMTS9 in adenocarcinoma; (d) ADAMTS9 in squamous cell carcinoma; (e) ADAMTS12 in adenocarcinoma; (f) ADAMTS12 in squamous cell carcinoma. Data were obtained from the UALCAN database.

Survival analysis in relation to ADAMTS6, ADAMTS9, and ADAMTS12 expression in NSCLC tumors

Survival outcomes were assessed using Kaplan–Meier Plot data for NSCLC patients in relation to the expression of ADAMTS6, ADAMTS9, and ADAMTS12. Analyses included all NSCLC cases regardless of subtype, as well as the two predominant subtypes: adenocarcinoma and squamous cell carcinoma. The results indicated that ADAMTS6 expression did not significantly influence overall survival in the combined NSCLC cohort ($p = 0.4622$) or in adenocarcinoma patients specifically ($p = 0.2618$). Notably, in squamous cell carcinoma, lower ADAMTS6 expression was linked to improved survival compared to higher expression levels ($p = 0.0224$) **(Figure 5)**.



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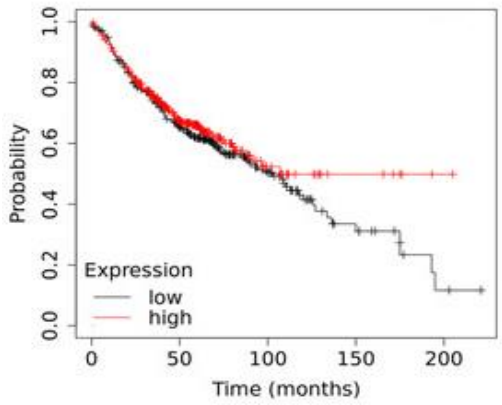

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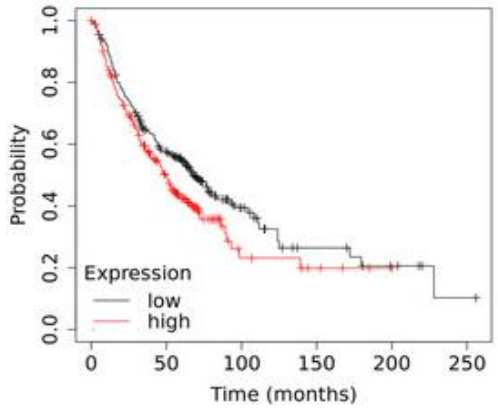

c)

Figure 5. Kaplan–Meier survival curves for NSCLC patients stratified by ADAMTS6 expression in tumor tissue: (a) all NSCLC cases without subtype separation ($p = 0.4622$); (b) adenocarcinoma ($p = 0.2618$); (c) squamous cell carcinoma ($p = 0.0224$). Data were obtained from the Kaplan–Meier Plotter database.

In contrast, ADAMTS9 expression showed a markedly different association with patient survival. Higher ADAMTS9 levels were significantly linked to improved survival in the overall NSCLC cohort without histological subdivision ($p = 0.0006$). This effect was especially pronounced in adenocarcinoma patients ($p < 0.0001$), where those with elevated ADAMTS9 expression survived an average of 127 months, nearly double the 76 months observed in patients with lower expression.

Conversely, ADAMTS9 expression did not significantly influence survival in squamous cell carcinoma patients (p = 0.1029) **(Figure 6)**.

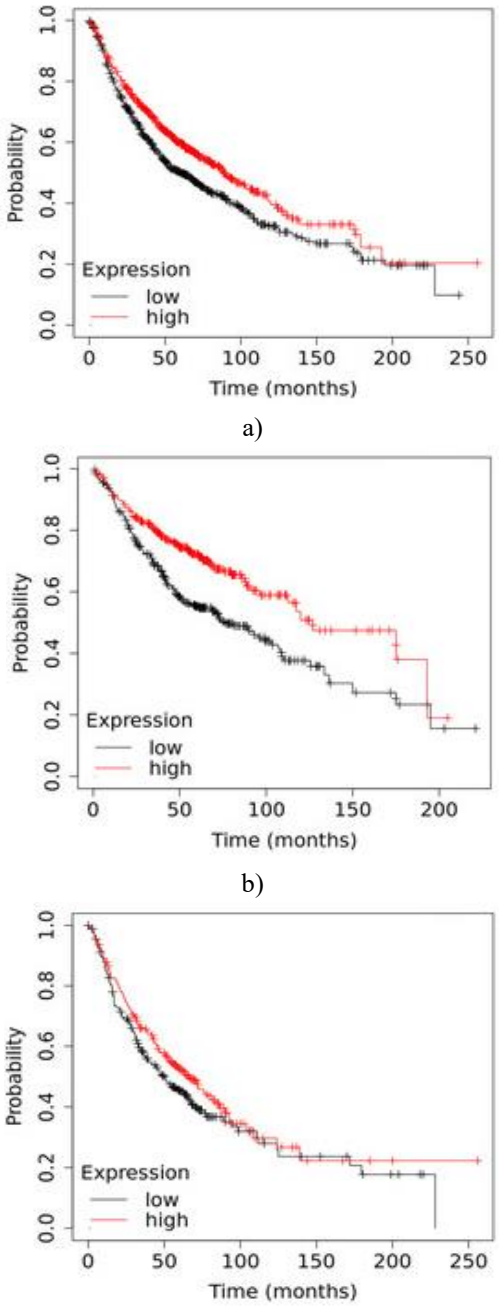

Figure 6. Kaplan–Meier survival plots showing the association between ADAMTS9 expression and NSCLC patient outcomes: (a) the entire cohort without separating histological subtypes (p = 0.0006); (b) adenocarcinoma cases (p < 0.0001); (c) squamous cell carcinoma cases (p = 0.1029), using data from the Kaplan–Meier Plotter database.

Investigation of ADAMTS12 expression revealed a clear negative impact on patient survival. NSCLC patients with higher ADAMTS12 levels had significantly shorter survival times (p < 0.0001). Among adenocarcinoma patients, elevated ADAMTS12 expression nearly halved survival, with median survival decreasing from 113 months in the low-expression group to 65 months in the high-expression group (p < 0.0001). Similar to ADAMTS9, ADAMTS12 expression did not significantly alter survival in squamous cell carcinoma patients (p = 0.3183) **(Figure 7)**.

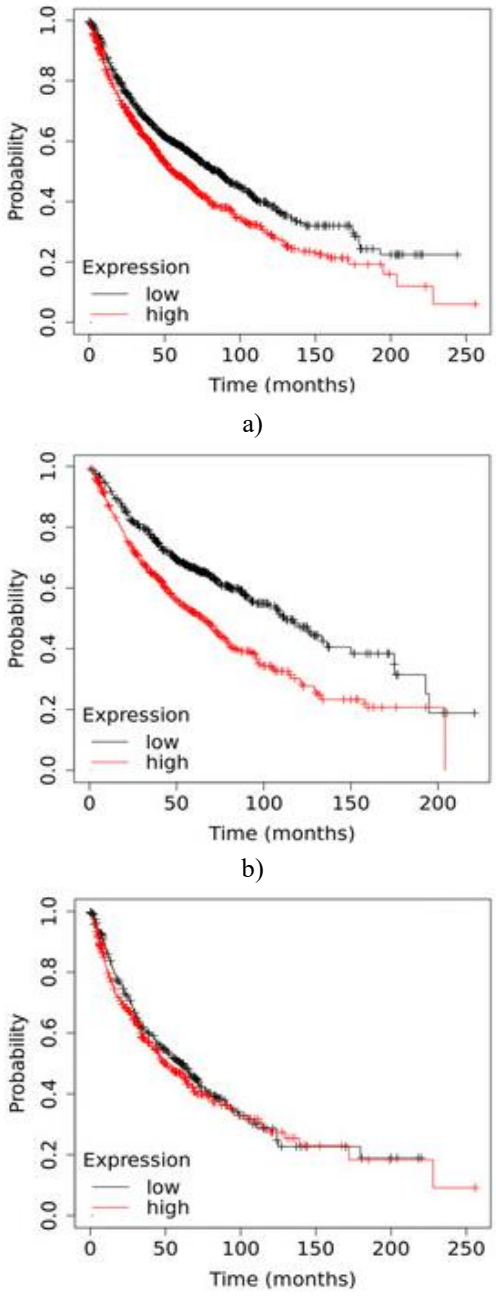

Figure 7. Kaplan–Meier survival plots showing NSCLC patient outcomes based on ADAMTS12 expression in tumor tissue: (a) all patients regardless of histopathological subtype (p < 0.0001); (b) adenocarcinoma (p < 0.0001); (c) squamous cell carcinoma (p = 0.3183). Data were obtained from the Kaplan–Meier Plotter database.

Predicted interactions between circRNAs, miRNAs, and ADAMTS family mRNAs

Potential regulatory relationships between the analyzed circRNAs and mRNAs of ADAMTS family genes, including their host genes, were investigated using external database resources. This analysis leveraged the role of circRNAs as miRNA sponges: by binding miRNAs, circRNAs reduce the pool of miRNAs available to interact with target mRNAs, thereby decreasing mRNA degradation, enhancing transcript stability, and potentially increasing protein expression.

Initial analysis focused on the binding potential between the selected circRNAs and various miRNAs. All examined circRNAs were capable of interacting with multiple miRNAs, including those targeting ADAMTS family gene transcripts. Notably, only circRNAs derived from the ADAMTS6 locus showed interactions with miRNAs that directly regulate ADAMTS6 expression. The detailed results of these interactions are summarized in **Figure 8**.

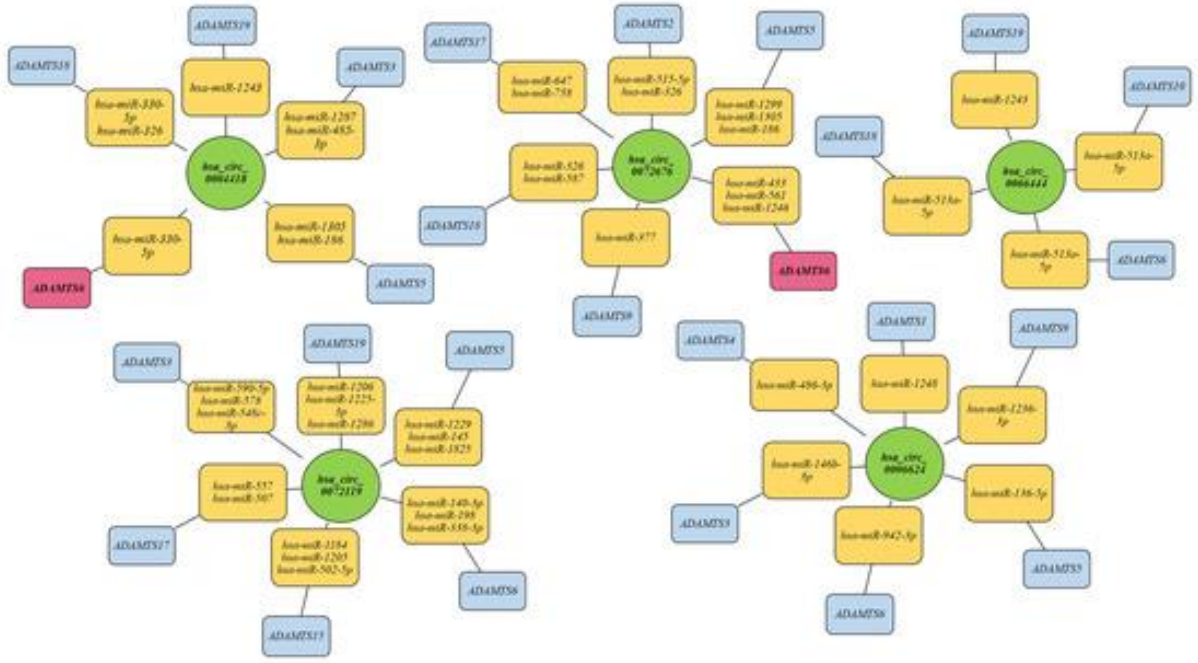

Figure 8. circRNA/miRNA/mRNA interaction network predicted using the CircInteractome database, miRDB, and miRTarBase. Host genes regulated by circRNAs in this network are highlighted with red rectangles.

The mammalian genome generates a vast array of non-coding transcripts [13], with estimates suggesting that more than 90% of the human transcriptome is not translated into proteins [14]. Nevertheless, these transcripts are biologically active, primarily modulating gene expression [15]. Clinically, their tissue-specific expression makes non-coding RNAs promising biomarkers for various diseases, including cancer.

Extensive research has shown that circRNAs play crucial roles in cellular function, including in cancer-transformed cells. These molecules act as transcriptional regulators, influencing genes involved in proliferation, apoptosis [7], and angiogenesis [16], all central to tumorigenesis. CircRNAs can modulate mRNA availability by acting as sponges for miRNAs [17], and some circRNAs may even serve as templates for protein synthesis, with the translated proteins exhibiting biological activity [18, 19]. CircRNAs are also highly stable and conserved; for instance, 15,000 circRNAs are shared among humans, mice, and rats [18]. Consequently, circRNAs hold potential as future diagnostic or prognostic cancer biomarkers.

In this study, the expression of five circRNAs originating from ADAMTS6 (hsa_circ_0004418, hsa_circ_0072676), ADAMTS9 (hsa_circ_0066444), and ADAMTS12 (hsa_circ_0006624, hsa_circ_0072119) genes was analyzed in lung tissue from NSCLC patients, alongside their respective host gene expression. The study also evaluated how various clinical and demographic factors influenced the expression of these gene structures. A significant correlation was observed only between ADAMTS12 expression and its associated circRNAs and host genes; no similar associations were found for ADAMTS6 or ADAMTS9. Bioinformatic analyses suggested a potential circRNA/miRNA/mRNA interaction for circRNAs derived from ADAMTS6, which might affect ADAMTS6 transcript stability; however, this was not corroborated by experimental data, as no correlation was observed between hsa_circ_0004418 or hsa_circ_0072676 and ADAMTS6 expression. Potential interactions for hsa_circ_0066444, hsa_circ_0006624, and hsa_circ_0072119 could also not be confirmed.

Some circRNAs are known to regulate their parental gene expression, a key mechanism through which they impact cellular behavior. For instance, Li *et al*. demonstrated that knockdown of circITGA7 or its parent gene ITGA7 enhanced proliferation and metastasis in colorectal cancer [20]. Additionally, circ_MMP2 was identified in exosomes released by hepatocellular carcinoma cells into normal liver cells, promoting tumorigenesis and metastasis by sponging miR-136-5p and thereby increasing MMP2 expression [21]. Richardson *et al*. also reported that circPTEN regulates the tumor suppressor gene PTEN by acting as a molecular sponge for miR-155 and miR-330-3p, inhibiting the PI3K/AKT oncogenic pathway and suppressing cell growth in non-small-cell lung cancer [22].

Our results show that the expression of the ADAMTS6 gene and its derived circRNA, hsa_circ_0072676, differs significantly among the histological subtypes of non-small-cell lung cancer (NSCLC). Additionally, patient survival, which in some instances was associated with the expression levels of the analyzed genes, also varied according to the tumor's histological subtype. NSCLC is a heterogeneous disease, and both prognosis and therapeutic decisions are closely linked to the histopathological classification and specific genomic alterations in cancer cells [23]. Environmental factors further influence the development of particular subtypes; for example, tobacco use is strongly associated with squamous cell carcinoma. Distinct NSCLC subtypes also carry characteristic genetic alterations: adenocarcinoma frequently presents with EGFR and ALK mutations, squamous cell carcinoma commonly exhibits changes in PIK3CA and FGFR1, and large-cell carcinoma—which often includes cases not classified as adenocarcinoma or squamous cell carcinoma—shows typical NSCLC mutations such as KRAS and TP53 [24]. Because the molecular profiles of NSCLC subtypes are diverse, resulting in differential regulation of apoptosis, proliferation, and extracellular matrix remodeling genes, gene expression changes must be interpreted within the context of the specific NSCLC subtype.

Bioinformatic analyses indicated reduced ADAMTS9 expression and elevated ADAMTS12 expression in NSCLC tissue relative to normal lung tissue. Survival analyses revealed that this pattern—a decrease in ADAMTS9 combined with an increase in ADAMTS12—is associated with shorter patient survival. Notably, these findings are consistent with circRNA expression changes in tumors infiltrating surrounding tissues, where lower levels of hsa_circ_0006624 and hsa_circ_0072119 correlated with tissue invasion. ADAMTS family proteins possess both tumor-promoting and tumor-suppressing properties [25]. In cellular models, Koo *et al*. reported anti-angiogenic activity for ADAMTS9 [26], while El Hour *et al*. demonstrated similar properties for ADAMTS12 [27], although through different mechanisms. Du *et al*. found that ADAMTS12 overexpression reduces Akt and mTOR phosphorylation in gastric cancer, whereas ADAMTS9 knockdown enhances tumorigenic potential in colorectal and breast cancer [28]. Conversely, Fontanil *et al*. observed that ADAMTS12 overexpression increases metastatic potential in breast cancer [29]. Moreover, circRNAs can negatively regulate their host genes in cancer; for instance, elevated circSMARCA5 expression reduced SMARCA5 levels in breast cancer, thereby impairing DNA repair [30].

Materials and Methods

The study included 61 NSCLC tissue specimens collected intraoperatively. All procedures were approved by the Bioethics Committee (No. RNN/87/16/EC and KE/952/22). The clinicopathological features of the cohort, including smoking status, are summarized in **Table 1**.

Table 1. Docking result of selected ligands having excellent binding energy with higher number of amino acids interaction.

Clinical Parameter	Number of Patients			
Smoking tobacco products	Yes—40		Yes—40	
Pack–year	Mean—39.69 (SD—18.33)			
Histological subtype	Sqamous cell carcinoma 31	Adenocarcinoma 20	Large-cell carcinoma 5	Other 6
Primary tumor size (T in TNM classification)	T1—12	T2—23	T3—16	T4—10
Lymph node involvement (N in TNM classification)	N0—16	N1—27	N2—16	N3—2
Presence of distant metastasis (M in TNM classification)	Mx—25	M0—30		M1—6
Another cancer	Yes—12		No—59	

RNA extraction

Total RNA was obtained from tissue specimens using the miRNeasy Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer's guidelines. RNA purity and concentration were evaluated spectrophotometrically, and only samples with an A260/A280 ratio greater than 1.9 and concentrations above 250 ng/µL were included in subsequent experiments.

cDNA synthesis

Reverse transcription was carried out on 1 µg of extracted RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™, Waltham, MA, USA), following the provided protocol to generate complementary DNA for downstream analyses.

Gene expression analysis by qPCR

The expression levels of ADAMTS6 (Hs01552731_m1), ADAMTS9 (Hs00172025_m1), and ADAMTS12(Hs00917098_m1) were determined via quantitative real-time PCR using TaqMan probes (Applied Biosystems™, USA) and TaqMan Fast Advanced Master Mix on a QuantStudio™ 5 Real-Time PCR System (Applied Biosystems™, USA). GAPDH (Hs02758991_g1) and ACTB (Hs01060665_g1) were employed as internal controls. All reactions were performed in duplicate, and relative gene expression was calculated as the difference between the Ct values of the target genes and the average Ct values of the reference genes.

Removal of Linear RNA

To selectively enrich circRNAs, linear RNA molecules were digested with RNase R (ab286929, Abcam, UK). For this reaction, 10 µg of total RNA was incubated with 20 U of RNase R and 40 U of RiboLock RNase Inhibitor (Thermo Scientific™, Waltham, MA, USA) in the supplied reaction buffer, bringing the final volume to 20 µL with RNase-free water, and incubated at 37 °C for 150 minutes. Following digestion, the RNA was purified using the miRNeasy Micro Kit (Qiagen, Germany), and reverse transcription was performed with the Thermo Scientific Maxima First Strand cDNA Synthesis Kit (Thermo Scientific™, USA).

circRNA quantification by qPCR

Expression of the selected circRNAs was assessed using Power SYBR™ Green PCR Master Mix (Applied Biosystems™, USA) according to the manufacturer's instructions. All assays were carried out in triplicate. Primers for each circRNA were designed using NCBI Primer-BLAST [31] and are listed in **Table 2**.

Table 2. Primer sequences used for assessing the expression of the investigated circRNA.

Primer Name	Sequence
hsa-ADAMTS6 0002 forward	5′-CTGTGACAGTCCAGCGTAAGT-3′
hsa-ADAMTS6 0002 reverse	5′-TGACACAGCGGTTGCTTTTG-3′
hsa-ADAMTS6 0003 forward	5′-GTGACAGTCCAGCACCTTCAG-3′
hsa-ADAMTS6 0003 reverse	5′-ACGCTTGGGAGGCTCATTAT-3′
hsa-ADAMTS9 0002 forward	5′-GACGCTGCATGGAGTACTGG-3′
hsa-ADAMTS9 0002 reverse	5′-GACATACAACAACACGCCGC-3′
hsa-ADAMTS12 0001 forward	5′-CTCAGTGGCACGGTTCTACA-3′
hsa-ADAMTS12 0001 reverse	5′-TCTACTCGGACTGGACCCAC-3′
hsa-ADAMTS12 0004 forward	5′-CCTCCTTTCCTGCAACAGAGA-3′
hsa-ADAMTS12 0004 reverse	5′-GCGGCCCTTCTTTATGCAATG-3′

Analysis using public databases

To compare gene expression between tumor and normal lung tissues, data from the UALCAN portal [32, 33] were analyzed, with a focus on samples from The Cancer Genome Atlas (TCGA). Survival outcomes were investigated in relation to ADAMTS6, ADAMTS9, and ADAMTS12 expression using the Kaplan–Meier plotter database [34]. Analyses were conducted both for the overall NSCLC population and separately for adenocarcinoma and squamous cell carcinoma subtypes. The CircInteractome database [35] was employed to explore potential regulatory roles of the studied circRNAs, predicting miRNAs that could bind and sequester these circRNAs, thus modulating mRNA availability. Complementary analyses of miRNA-mRNA interactions were carried out using miRDB [36, 37] and miRTarBase [38], allowing the construction of a network depicting the interactions between circRNAs and genes of the ADAMTS family, including their corresponding host genes.

Statistical methods

All analyses were performed using Statistica 13.1 software (TIBCO, Palo Alto, CA, USA). The Shapiro–Wilk test

evaluated whether continuous variables followed a normal distribution. Group comparisons were conducted using Student's t-test or one-way ANOVA, depending on the number of groups. Survival differences were assessed via the log-rank test, and relationships between quantitative variables were examined using Pearson correlation. A p-value of less than 0.05 was considered statistically significant.

Conclusion

Circular RNAs are emerging as important regulators of cellular behavior in both normal and cancerous tissues. In this study, five circRNAs derived from ADAMTS family genes, which play roles in extracellular matrix remodeling, were investigated. The results suggest that alterations in circRNA abundance are associated with changes in host gene expression and with clinical features of NSCLC. Future studies should examine whether these circRNAs can be translated into functional proteins and evaluate the miRNAs capable of interacting with them, as these interactions could further influence tumor biology. Given their stability and regulatory potential, circRNAs may serve as valuable biomarkers for diagnosis or prognosis in non-small-cell lung cancer.

Acknowledgments: None

Conflict of interest: None

Financial support: This study was supported by funds from of National Science Centre, Poland (No. 2022/06/X/NZ5/00743) and the Department of Pharmaceutical Biochemistry and Molecular Diagnostics, the Medical University of Lodz (No. 503/3-015-02/503-31-001).

Ethics statement: The investigation was in accordance with the principles of the Declaration of Helsinki and was approved by the Ethical Committee of the Medical University of Lodz (No KE/952/22 and RNN/87/16/KE). Written informed consent has been obtained from the patients.

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