Bioinformatics Analysis of the Expression of Key Long Intergenic Non-Protein Coding RNA Genes in Bladder Cancer

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Background: Evidence indicates that there is an important role for long non-coding RNAs (lncRNA) in numerous cellular processes and that lncRNAs dysregulation contributes to tumor progression. Improved insight into the molecular characteristics of bladder cancer is required to predict outcomes and to develop a new rationale for targeted therapeutic strategies. Bioinformatics methods, including functional enrichment and network analysis combined with survival analysis, are required to process a large volume of data to obtain further information about differentially expressed genes (DEGs) in bladder cancer. This study aimed to explore the role of lncRNAs and their regulation network in bladder cancer.

Material/Methods: We analyzed bladder cancer data by The Cancer Genome Atlas profiling to identify differentially expressed lncRNAs in bladder cancer. The genes involved in the circlncRNAnet database were evaluated using Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO), evolutionary relationship analysis, and protein-protein interaction (PPI) networks.

Results: Two new lncRNAs, ADAMTS9-AS1 and LINC00460, were shown to be differentially expressed in bladder cancer. Patients were divided into 2 groups (high expression and low expression) according to their median expression values. The overall survival and disease-free survival of patients with high ADAMTS9-AS1 bladder cancer were significantly shorter; the expression of LINC00460 had no significant correlation with survival. GO and KEGG analysis of the 2 lncRNA-related genes revealed that these lncRNAs played a vital role in tumorigenesis. Bioinformatics analysis showed that key genes related to LINC00460, including CXCL, CCL, and CSF2, may be related to the development of bladder cancer. The low expression of ADAMTS9-AS1 may influence the survival rate of bladder cancer with the hub gene as a target.

Conclusions: LncRNA, including LINC00460 and ADAMTS9-AS1, might play a crucial role in the biosynthesis network of bladder cancer. Differential expression results of ADAMTS9-AS1 suggests it may be correlated with a worse prognosis and a shorter survival time. We outlined the biosynthesis network that regulates lncRNAs in bladder cancer. Further experimental data is needed to validate our results.

MeSH Keywords: Computational Biology • Gene Regulatory Networks • RNA, Long Noncoding • Urinary Bladder Neoplasms

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Background

Bladder cancer is one of the most frequent malignant tumors among urological cancers [1]. Approximately 80% of patients suffer from non-muscle invasive bladder cancer [2]. However, approximately 20% of non-muscle invasive bladder cancer progress to muscle-invasive bladder cancer [2]. The prognosis of muscle-invasive bladder cancer remains poor due to aggressive metastasis and delayed diagnosis [1,3]. Although some routine treatments such as surgery and chemotherapy can be used, the recurrence rate is still as high as 70% within 2 years [4]. Thus, research on bladder cancer is essential, especially on its molecular mechanisms and the identification of biomarkers that are useful for early detection, risk stratification, determination of appropriate intervention, and prognostication.

Non-coding RNAs (ncRNAs) were a kind of RNA that lack protein-coding functionality [5]. With the development of high-throughput sequencing techniques, there are a growing number of ncRNAs being discovered. The ncRNAs are classified by 2 classes according to the transcript length [5]. The length up to 200 nucleotides is defined as short ncRNA, including microRNAs (miRNAs), small interfering RNAs (siRNAs), and small nuclear RNAs (snRNAs) [6,7]. The long ncRNA (lncRNA) class is composed of ncRNAs with nucleotides more than 200 [8]. LncRNAs were previously unknown entities within the genome. Coding and non-coding RNAs regulate the expression of each other through common miRNAs that target them. This mechanism is known as the competing endogenous RNA (ceRNA) [8]. There is a new approach used to reconstruct the ceRNA network for non-muscle invasive bladder cancer based on the expression data of coding and non-coding genes which shows that the lncRNAs CARMN, FENDRR, and ADAMTS9-AS2 may regulate MEG3 in non-muscle invasive bladder cancer through sponging some important miRNAs [9]. Recently, ncRNAs have attracted increasing attention in regard to their role in bladder cancer, and the possibility of using them to provide more specific treatment to patients. The effects of siRNA on tumor phenotypes and cancer behavior have been discussed widely [10,11]. For instance, it has been confirmed that tumor metastasis can be inhibited by metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) knockdown and that MALAT1 silencing can inhibit the epithelial-mesenchymal transition (EMT) [12,13]. The knockdown of snRNA host gene 16 (SNHG16) enhanced the effect of sorafenib in hepatocellular carcinoma patients [14].

Although it has been reported that some lncRNAs serve as tumor suppressor genes or oncogenes, the lncRNAs studied so far have been shown to be merely a small part of the lncRNAs defined in Gene Expression Profiling Interactive Analysis (GEPIA) database [15]. Therefore, it would be useful to further explore the role of lncRNAs in cancer, investigate the possibilities of siRNA mediated treatment in cancer management, and identify new lncRNAs that may help uncovering potential regulatory mechanisms and biomarkers in cancer.

In this study, we performed an investigation of differentially expressed lncRNAs in bladder cancer and their regulatory network with the aim of identifying potential biomarkers for bladder cancer.

Material and Methods

Analysis of The Cancer Genome Atlas (TCGA) datasets and differential expression of lncRNAs

RNA-Seq-based transcriptome data in The Cancer Genome Atlas (TCGA) database was obtained and analyzed using the DESeq R package in R software. The lncRNAs differentially expressed in bladder cancer were identified. In the TCGA program, 412 cases were available, including 304 men and 108 women, and 20 833 genes were associated with this group of bladder cancer cases. Finally, we selected the LINC00460 and ADAMTS9-AS1 genes as the hub lncRNAs in this study from the differentially expressed lncRNAs found in TCGA. The log2 transcripts per million (TPM+1) algorithm for TCGA data developed by the circlncRNAnet database and GEPIA (*http://gepia.cancer-pku.cn/*) were applied to explore lncRNAs expression in bladder cancer tissue and adjacent normal bladder tissue [15].

Prospective related genes in bladder cancer

Prospective related genes were identified using 2 online prediction databases: TANRIC and the circlncRNAnet database. The database is a web resource for the Department of Bioinformatics and Computational Biology that explores the interaction of lncRNAs in cancer, including experimentally supported genes related to mRNAs.

Gene-enriched pathway and functions

We used the Database for Annotation, Visualization and Integrated Discovery (DAVID) for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) approaches for the analysis of the differentially expressed genes. The DAVID is an online platform that offers functional annotation and analysis of genes derived from various genomic resources [16]. The species was restricted to *Homo sapiens,* and any *P*<0.05 was considered statistically significant.

Interaction and regulatory relationships

Protein-protein interaction (PPI) analysis was also used to develop network using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING), version 10.5, which can

Table 1. Top 10 upregulated and downregulated long noncoding RNAs in bladder cancer based on the Cancer Genome Atlas Database.

Gene	log2 fold change	P-value
AF001548.6	−5.141418973	1.65E-23
CTC-296K1.4	−4.85531061	6.03E-17
CTC-296K1.3	−4.740860947	1.11E-18
ADAMTS9-AS1	−4.69361727	2.74E-18
PGM5-AS1	−4.628169171	1.64E-10
RP3-340N1.2	5.047029803	2.21E-16
RP11-54H7.4	5.136593686	2.67E-12
RP11-400N13.2	5.540092416	1.06E-10
AC011298.2	5.665766046	8.37E-14
LINC00460	5.901064657	1.13E-14

identify and predict the connection and interaction between differentially expressed proteins [17]. Data annotated with prior knowledge from high-throughput laboratory experiments and databases were applied to obtain more objective and reliable results, and the minimum interaction value was set to medium confidence (0.400).

Statistical analysis

SPSS version 18.0 was applied to analyze the data, and GraphPad Prism 7 software (GraphPad Software, San Diego, CA, USA) were used to plot the data. Data are presented as the mean±standard deviation (SD). Independent sample *t*-tests were used to analyze the expression of genes in bladder cancer and adjacent normal bladder tissues. Spearman's correlation test was used to analyze the relationships between lncRNAs, and the expression of LINC00665 and related genes were demonstrated by scatter plots and box plots. One-way analysis of variance (ANOVA) was used to process and demonstrate the differences in data from 3 or more groups. The total standardized mean difference (SMD) and 95% confidence interval (CI) were calculated for all included studies.

Results

### Differentially expressed lncRNAs in bladder cancer

Among the RNA expression data available that was downloaded from the TCGA portal, we identified 2796 lncRNAs. Comparison of bladder cancer tissues with normal bladder tissues revealed 2033 upregulated lncRNAs and 763 downregulated lncRNAs. The top 10 differentially expressed lncRNAs are shown in Table 1. Expression of the long intergenic non-protein coding RNA 460 (LINC00460) gene was increased in
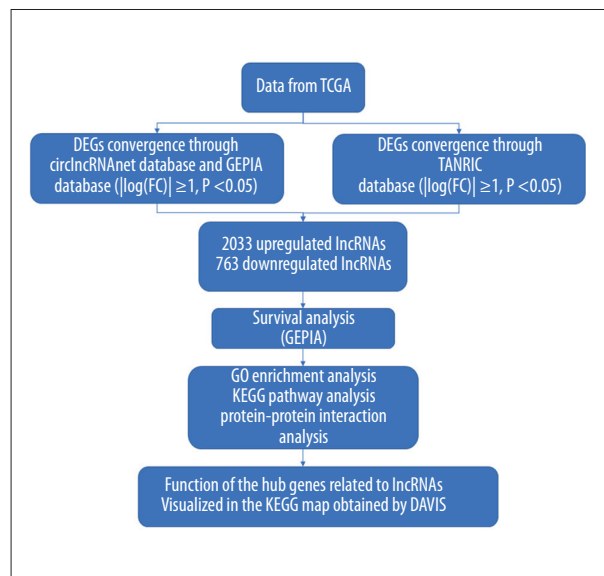
Figure 1. Flowchart of the research using bioinformatics data from The Cancer Genome Atlas.

bladder cancer tissues compared with paired normal bladder tissues, while the ADAMTS9-AS1 gene was expressed at low level in bladder cancer tissues compared with normal bladder tissues. Both of these lncRNAs had not been previously reported in the literature, and no studies have been published on these lncRNAs in bladder cancer; thus, we selected these lncRNAs for subsequent analyses (Figure 1).

Analysis of the TPM data from GEPIA showed that the expression of LINC00460 in 432 bladder cancer cases was increased in tumor tissues (n=404) compared with the paired normal tissues (n=28), with numerical values of 0.18 and 0, respectively (Figure 2A). In contrast, the expression of ADAMTS9-AS1 was decreased in the paired tumor tissues compared with normal
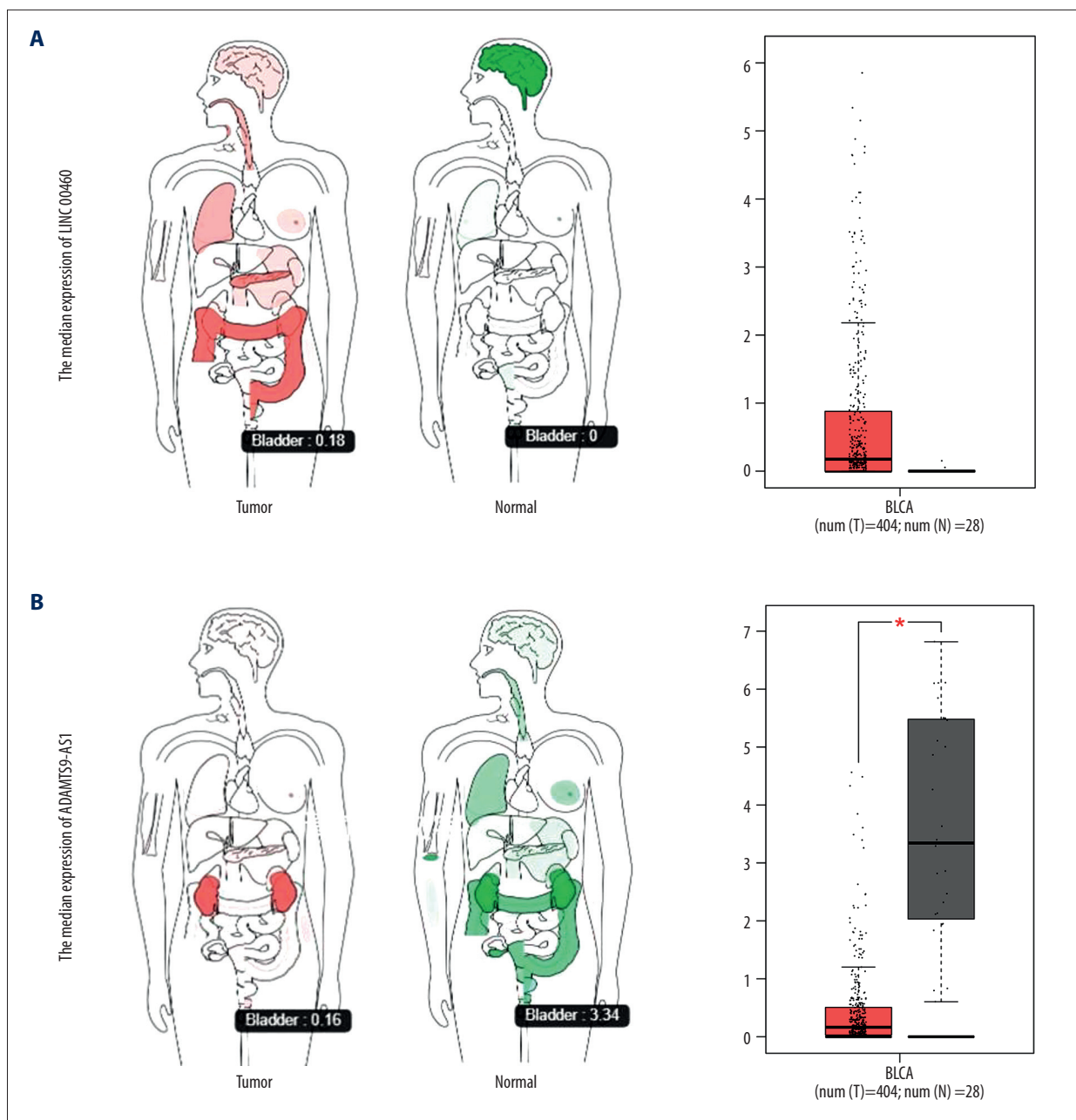
Figure 2. The median expression of LINC00460 (**A**) and ADAMTS9-AS1 (**B**) in normal and bladder carcinoma tissues in the body map by Gene Expression Profiling Interactive Analysis. Green represents healthy control tissues, and red represents bladder cancer tissues. Panels on the right show the expression of LINC00460 and ADAMTS9-AS1 in bladder cancer tissues and matched adjacent normal bladder tissues, based on The Cancer Genome Atlas database. BLCA – bladder cancer.

tissues, with numerical values of 0.16 and 3.34, respectively (Figure 2B).

To investigate the effect of the aberrant expression of LINC00460 and ADAMTS9-AS1 on the prognosis and survival of bladder cancer patients, the cases of bladder cancer from TCGA were split into high-expression and low-expression groups based on their median lncRNA expression level.

The Kaplan-Meier method was used for analysis. Survival curve analysis revealed no significant difference between the LINC00460 low-expression and high-expression groups in overall survival (OS) or disease-free survival (DFS) (log-rank *P*=0.063 and *P*=0.26 respectively) (Figure 3A). The hazard ratio (HR) of OS was 1.3 (95% CI: 0.726–0.152); the HR of DFS was 1.2 (95% CI: 0.845–0.134). In contrast, we found significant difference in OS and DFS in bladder cancer patients according
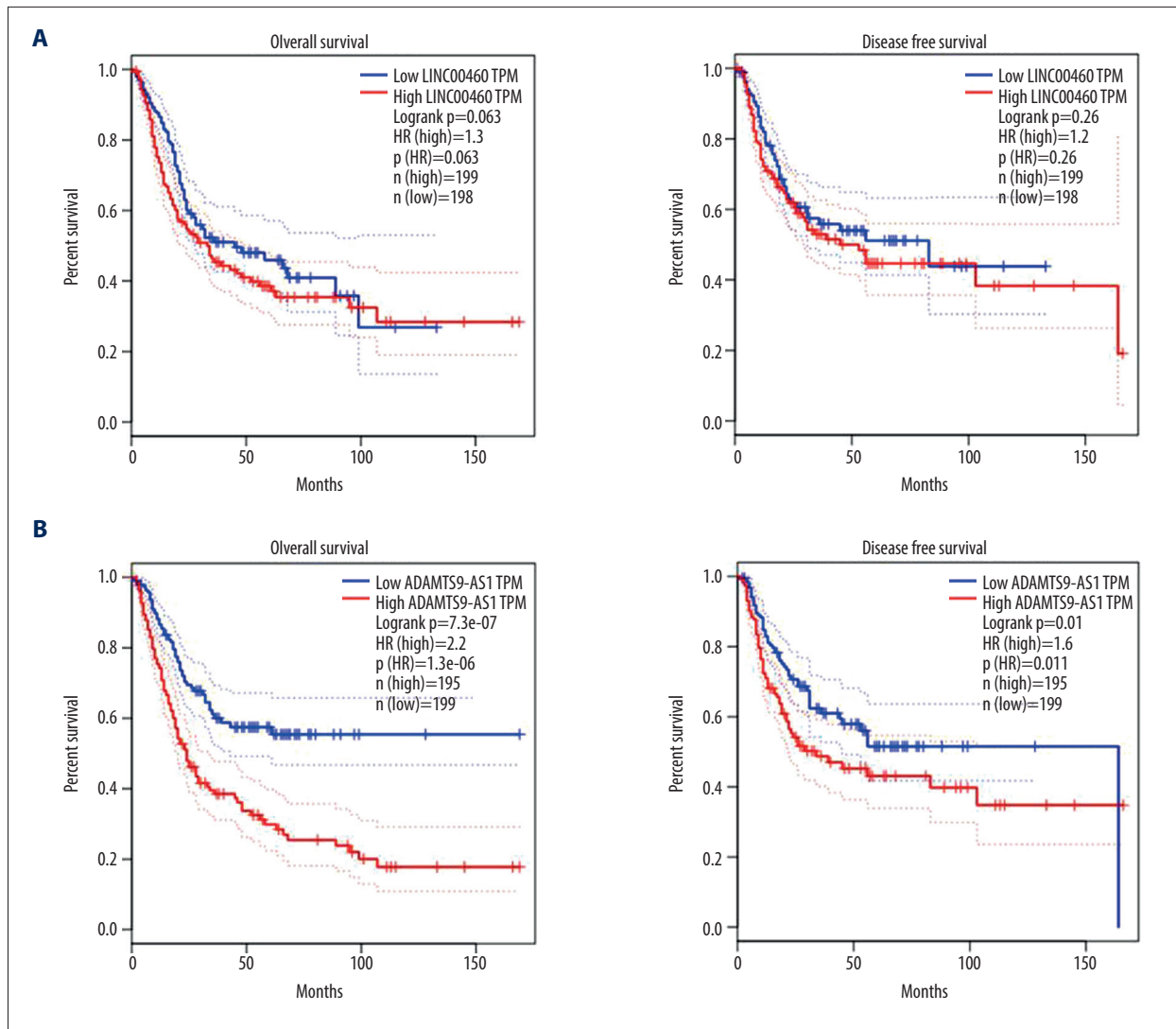
Figure 3. Kaplan-Meier plots of bladder cancer patients from The Cancer Genome Atlas dataset stratified by lncRNA expression.
(**A**) Overall survival and disease-free survival of the bladder cancer patients with high and low expression of LINC00460.
(**B**) Overall survival and disease-free survival of the bladder cancer patients with high and low expression of ADAMTS9-AS1.

to ADAMTS9-AS1 expression levels. Both the OS and DFS of bladder cancer patients with high ADAMTS9-AS1 expression were shorter than those of patients with ADAMTS9-AS1 expressed at low level (log-rank, P=7.3e-07 and P=0.01); the HR of OS was 2.2 (95% CI: 1.846–2.686) and that of DFS was 2.2 (95% CI: 1.846–2.686) (Figure 3B).

Related genes and gene annotation enrichment analysis

We obtained 2 sets of prospective related genes from the circlncRNAnet database and identified 615 overlapping genes for LINC00460 and 1158 overlapping genes for ADAMTS9-AS1. We used DAVID database to identify KEGG/PANTHER pathways and GO annotations.

Among the genes associated with LINC00460, GO: 0006955-immune response was the most significantly enriched GO term in Biological Process (P<0.001); the most enriched term in Cellular Component analysis was GO: 0005615-extracellular space; and the most enriched term in Molecular Functions was GO: 0004896-cytokine receptor activity (P<0.001, Table 2). KEGG enrichment analysis showed that the related genes of LINC00460 were involved in cytokine–cytokine receptor interaction (P<0.001, Figure 4A). PANTHER pathway analysis showed an enrichment of genes in the integrin signaling pathway (P=0.0018, Figure 4B).

Among the genes related to ADAMTS9-AS1, the most significantly enriched GO term in the Biological Process was GO: 0003012-muscle system process (P<0.001); the most enriched

term in Cellular Component analysis was contractile fiber part (*P*<0.001); and the most enriched term in Molecular Functions was glycosaminoglycan binding (*P*<0.001, Table 3). KEGG analysis showed that genes related to ADAMTS9-AS1 were related to the PI3K-Akt signaling pathway (*P*<0.001, Figure 5A). PANTHER pathway analysis showed an enrichment of genes in the heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway (*P*<0.001, Figure 5B).
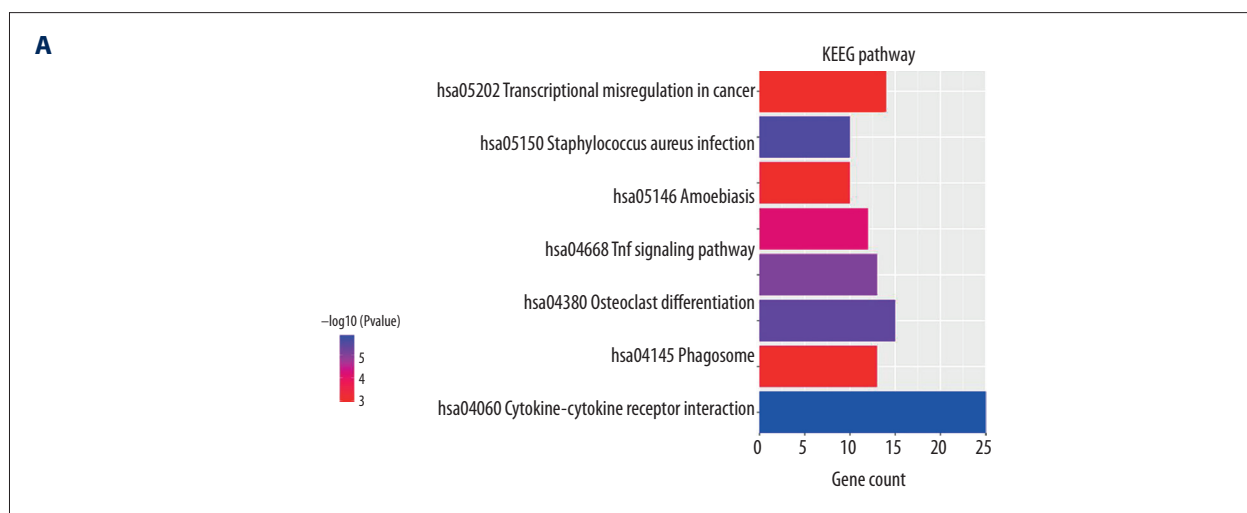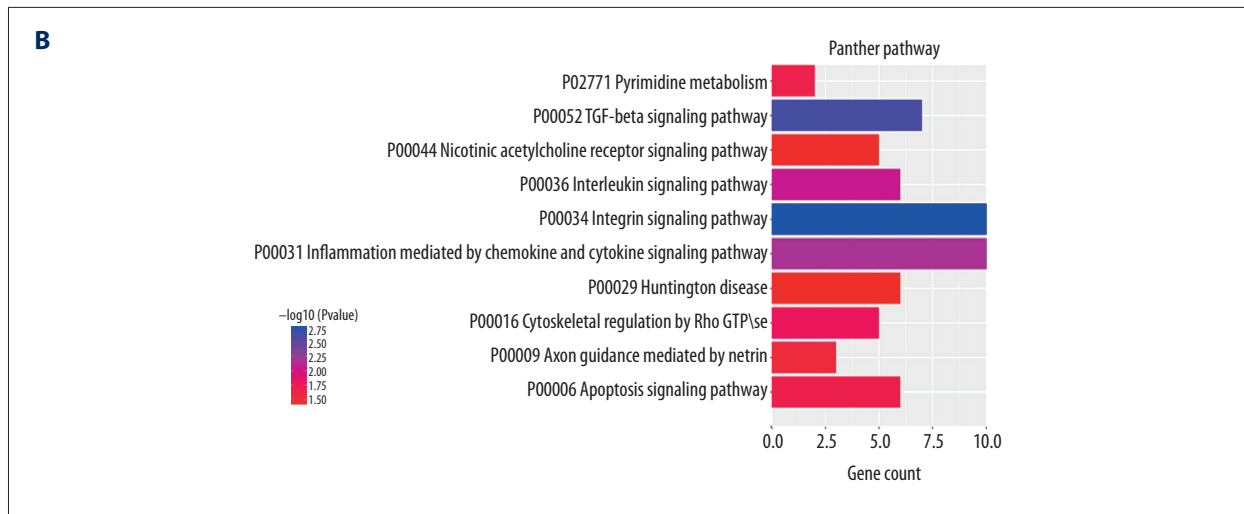
Construction of the PPI network and hub genes

We used the STRING database to analyze a functional protein network containing the aforementioned genes. A value greater than 2 degrees is defined as an indicator hub gene. Based on the constructed PPI network, the 30 hub genes with the most connections were obtained (Figures 6, 7). Among the genes related to LINC00460, the key hub genes are involved in cytokine–cytokine receptor interaction (Figure 8A). Among the genes related to ADAMTS9-AS1, the key hub genes are involved in the PI3K-Akt signaling pathway and may play critical function in the

Table 2. Gene ontology analysis of overlapping genes correlated with LINC00460.

Category	Term	Count	*P*-value
GOTERM_BP	GO: 0006955-immune response	96	1.52E-18
GOTERM_BP	GO: 0002682-regulation of immune system process	84	1.83E-16
GOTERM_BP	GO: 0002684-positive regulation of immune system process	65	5.95E-15
GOTERM_BP	GO: 0006952-defense response	85	3.93E-14
GOTERM_BP	GO: 0050776-regulation of immune response	60	2.29E-13
GOTERM_CC	GO: 0005615-extracellular space	67	5.91E-08
GOTERM_CC	GO: 0009986-cell surface	42	4.96E-07
GOTERM_CC	GO: 0009897-external side of plasma membrane	20	1.44025E-06
GOTERM_CC	GO: 0071437-invadopodium	4	0.000157469
GOTERM_CC	GO: 0070161-anchoring junction	34	0.000161924
GOTERM_MF	GO: 0004896-cytokine receptor activity	11	9.79E-06
GWOTEM	GO: 0004252-serine-type endopeptidase activity	16	7.39E-05
GOTERM_MF	GO: 0042379-chemokine receptor binding	8	9.37E-05
GOTERM_MF	GO: 0005125-cytokine activity	16	0.000128
GOTERM_MF	GO: 0005126-cytokine receptor binding	18	0.000139

Only the top 5 enriched pathways were listed for each category. BP – biological process; CC – cellular component; MF – molecular function.

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Figure 4. Eight enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways terms of differentially expressed genes in bladder cancer (**A**) and 10 significant PANTHER pathways (**B**) related to LINC00460 in bladder cancer. Count: number of genes related to the enriched KEGG or PANTHER pathway. The color of the bar denotes -$\log_{10}$ (*P*-value).

**Table 3.** Gene ontology of overlapping genes correlated with ADAMTS9-AS1.

Category	Term	Count	*P*-value
GOTERM_BP	GO: 0003012- muscle system process	97	6.95E-42
GOTERM_BP	GO: 0006936-muscle contraction	82	3.95E-36
GOTERM_BP	GO: 0003013-circulatory system process	89	4.26E-27
GOTERM_BP	GO: 0044057-regulation of system process	89	6.77E-27
GOTERM_BP	GO: 0072359-circulatory system development	127	1.08E-26
GOTERM_CC	GO: 0044449-contractile fiber part	56	1.39E-27
GOTERM_CC	GO: 0043292-contractile fiber	58	2.49E-27
GOTERM_CC	GO: 0031012-extracellular matrix	92	3.62E-27
GOTERM_CC	GO: 0030016-myofibril	56	7.51E-27
GOTERM_CC	GO: 0030017-sarcomere	51	5.06E-25
GOTERM_MF	GO: 0005539-glycosaminoglycan binding	46	1.72E-18
GOTERM_MF	GO: 0008201-heparin binding	40	3.24E-18
GOTERM_MF	GO: 1901681-sulfur compound binding	47	2.69E-17
GOTERM_MF	GO: 0005178-integrin binding	25	2.98E-11
GOTERM_MF	GO: 0008092-cytoskeletal protein binding	86	3.17E-11

Only the top 5 enriched pathways were listed for each category. BP – biological process; CC – cellular component; MF – molecular function.

growth, proliferation and survival of tumor cells. We identified an overlap among the top 30 genes connected within the PPI network and 14 genes in the PI3K-Akt signaling pathway, suggesting an involvement of ADAMTS9-AS1 and its core hub genes in tumor growth and development (Figure 8B). Among these genes, genes with a degree value greater than 35 were defined as hub genes. We have created a GitHub repository and uploaded all data of this study (link: *https://github.com/drlyz0218/R-*).

Discussion

Bladder cancer is frequently diagnosed late in the course of illness and late stage of bladder cancer are associated with much worse prognosis [18]. Besides clinical suspicion, it will be helpful if we can find suitable biomarkers with good diagnostic performance for identifying cases of early-stage bladder cancer. In this study, we identified 2 novel lncRNAs for bladder cancer:
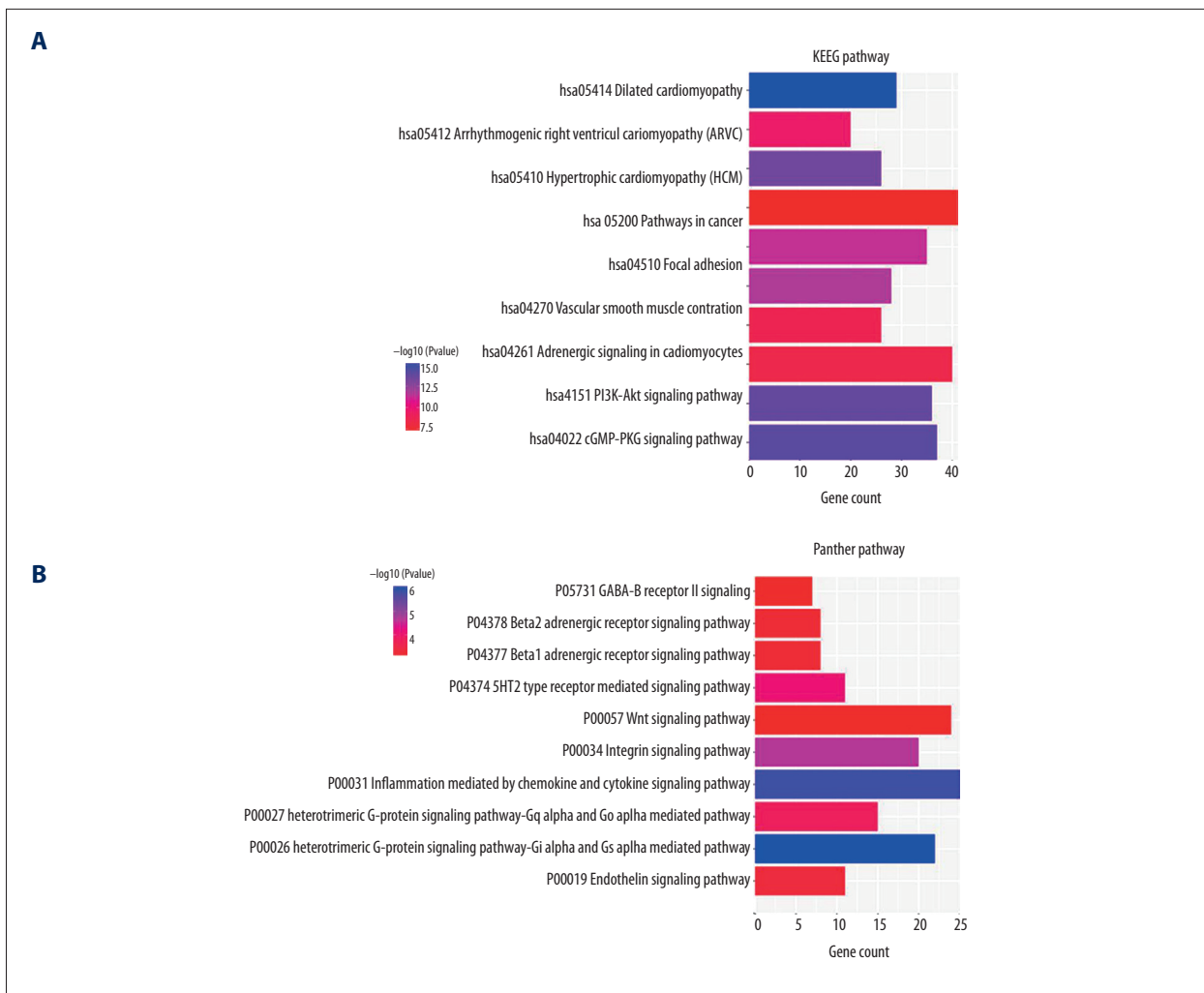
Figure 5. Ten significant KEGG (**A**) and PANTHER pathway (**B**) terms of differentially expressed genes related to ADAMTS9-AS1 in bladder cancer. Count: number of genes related to the enriched KEGG or PANTHER pathway. The color of the bar denotes -$\log_{10}$ (P-value).

LINC00460, which were elevated in bladder cancer tissues compared with paired normal bladder tissues, and ADAMTS9-AS1, which was expressed at low level in bladder cancer tissues compared with normal bladder tissues. We found that genes related to LINC00460 were associated with cytokines and mediators of inflammation. In the tumor microenvironment, inflammatory cells and molecules influence almost every aspect of cancer progression, including the ability of tumor cells to metastasize [19]. These clinicopathological characteristics that facilitate inflammation and metastasis could contribute to poor outcome [19]. We found that high expression of the ADAMTS9-AS1 gene was significantly associated with a shorter OS and DFS of patients with bladder cancer. Moreover, the hub genes related to ADAMTS9-AS1 were associated with tumorigenesis and progression of bladder cancer. Our findings suggested that LINC00460 and ADAMTS9-AS1 may play important roles in bladder cancer progression and could act as underlying biomarkers.

There have been increasing studies demonstrating that the abnormal expression of lncRNAs was of great importance in bladder cancer [11,20]. For instance, the lncRNA MALAT1 is overexpressed in urothelial carcinoma, inducing cell proliferation, migration and survival and promoting epithelial mesenchymal transformation by activating WNT signaling [11,12,21]. Furthermore, the upregulated lncRNA taurine gene 1 (TUG1) is also overexpressed in urothelial carcinoma, and high TUG1 expression is associated with higher stages [22]. Urothelial cancer associated 1 (UCA1) is another upregulated lncRNA in bladder cancer [23–26]. Overexpression of UCA1 enhances the activity of the erk1/2 MAPK and pi3-k/AKT kinases, increasing cell cycle progression, carcinogenesis and cancer invasion [24,26].

Our study may be a novel trial of the integration of data from the TCGA database with the published literature to investigate the possible differential expression of lncRNAs and their
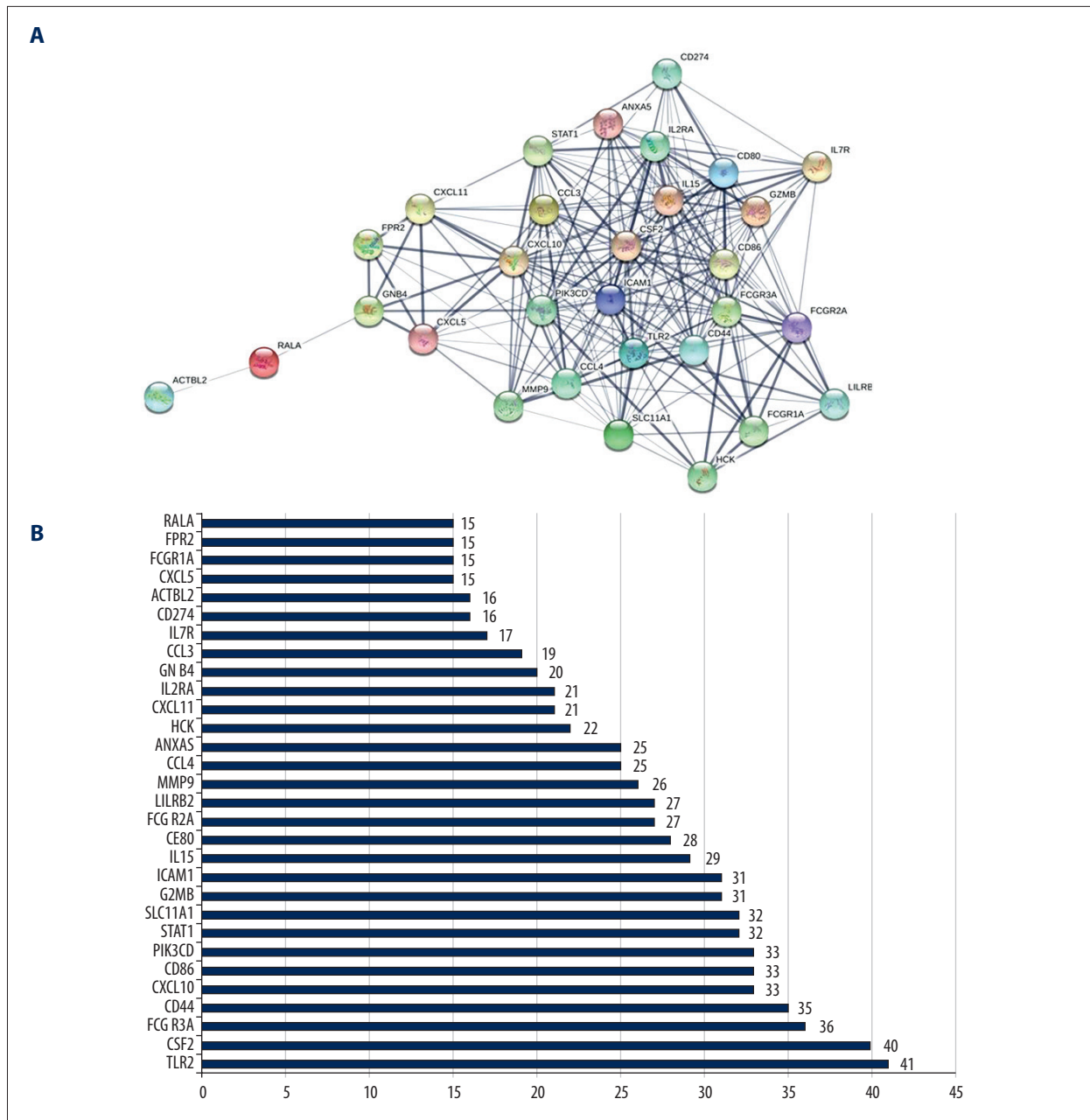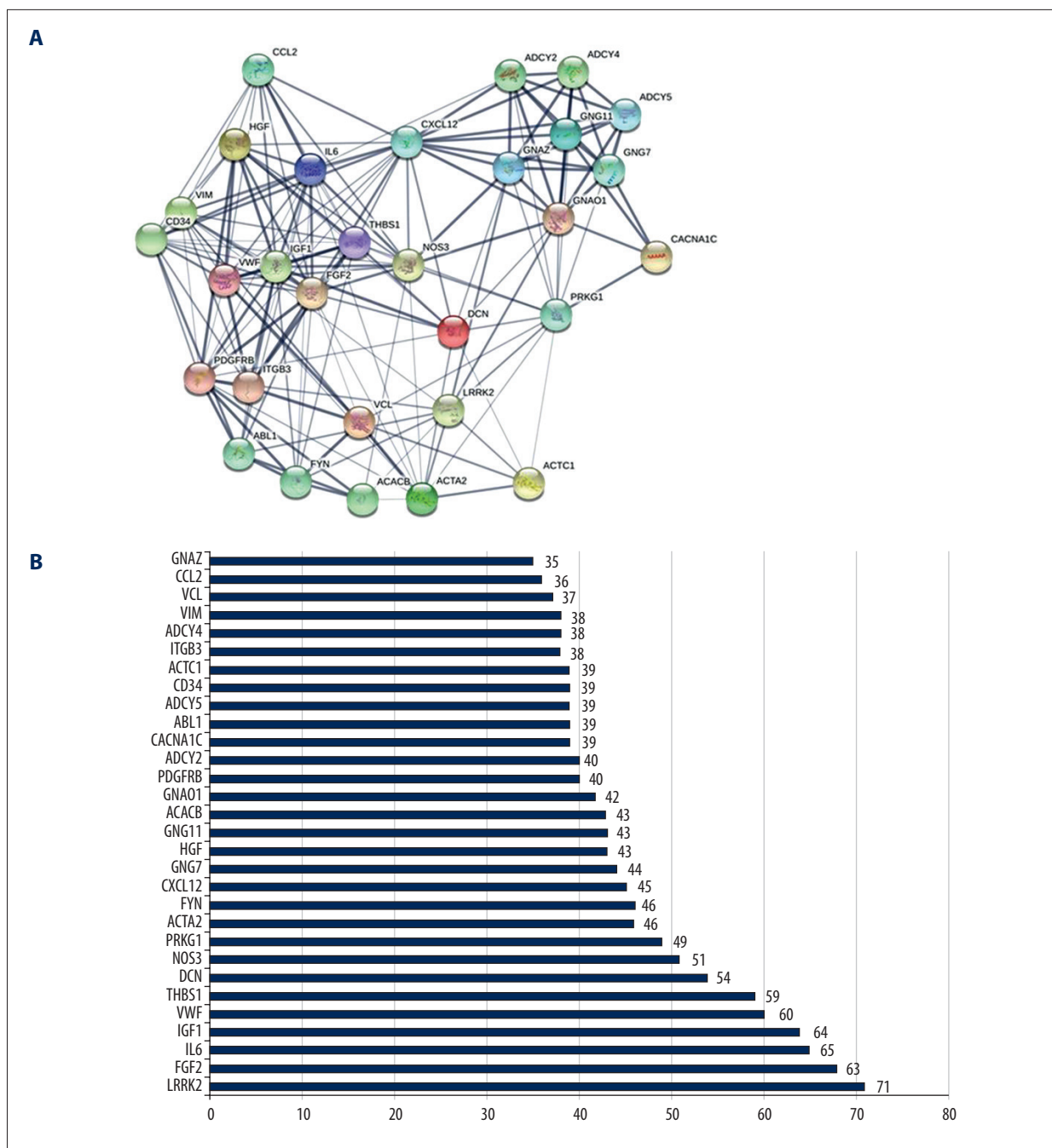
Figure 6. PPI network analysis. (**A**) PPI network of LINC00460 target genes in bladder cancer. (**B**) The top 30 most significant hub genes targeted by LINC00460 in bladder cancer.

potential molecular mechanism in bladder cancer using two different useful bioinformatic websites. In addition, there are many bioinformatics tools that can help us find and analyze existing bioinformatics data. DrugR+ (*http://www.drugr.ir*) is well-organized database that can be accessed in order to incorporate information about genes, drugs, and their different targets. It may be used for finding appropriate treatment for diseases induced by the aberrantly expressed lncRNAs detected in the other databases [27].

Our results in the current study suggest a potential relationship between the upregulated LINC00460 and the presence of inflammatory cells in the tumor microenvironment, which supports the probability of LINC00460 being a potential predictive biomarker for the occurrence and progression of bladder cancer. A previous study also reported that LINC00460 might be associated with a variety of biological behaviors in breast cancer [28], non-small cell lung cancer [29] and nasopharyngeal carcinoma [30]. Wen et al. [31] has shown that LINC00460 may play a crucial role in bladder cancer cell proliferation and migration and that

Figure 7. PPI network analysis. (**A**) PPI network of ADAMTS9-AS1 target genes in bladder cancer. (**B**) ADAMTS9-AS1 targets the 30 most significant potential hub genes in bladder cancer.

androgen receptors may be involved in this process. In addition, they also found that LINC00460 expression had a good negative correlation with the prognosis of bladder cancer patients, which was consistent with the results of this study.

The significant relationship between ADAMTS9-AS1 expression and the OS/DFS of bladder cancer patients was the most important finding in this study, indicating a prognostic function

for ADAMTS9-AS1 in bladder cancer. These findings also support the association of ADAMTS9-AS1 with the development of bladder cancer. ADAMTS9-AS1 expression may also be associated with worse OS and DFS in high-grade muscle-invasive bladder cancer. However, further confirmation with large sample size was required. Furthermore, the molecular mechanism of ADAMTS9-AS1 in bladder cancer remain unknown and should be examined in future studies.
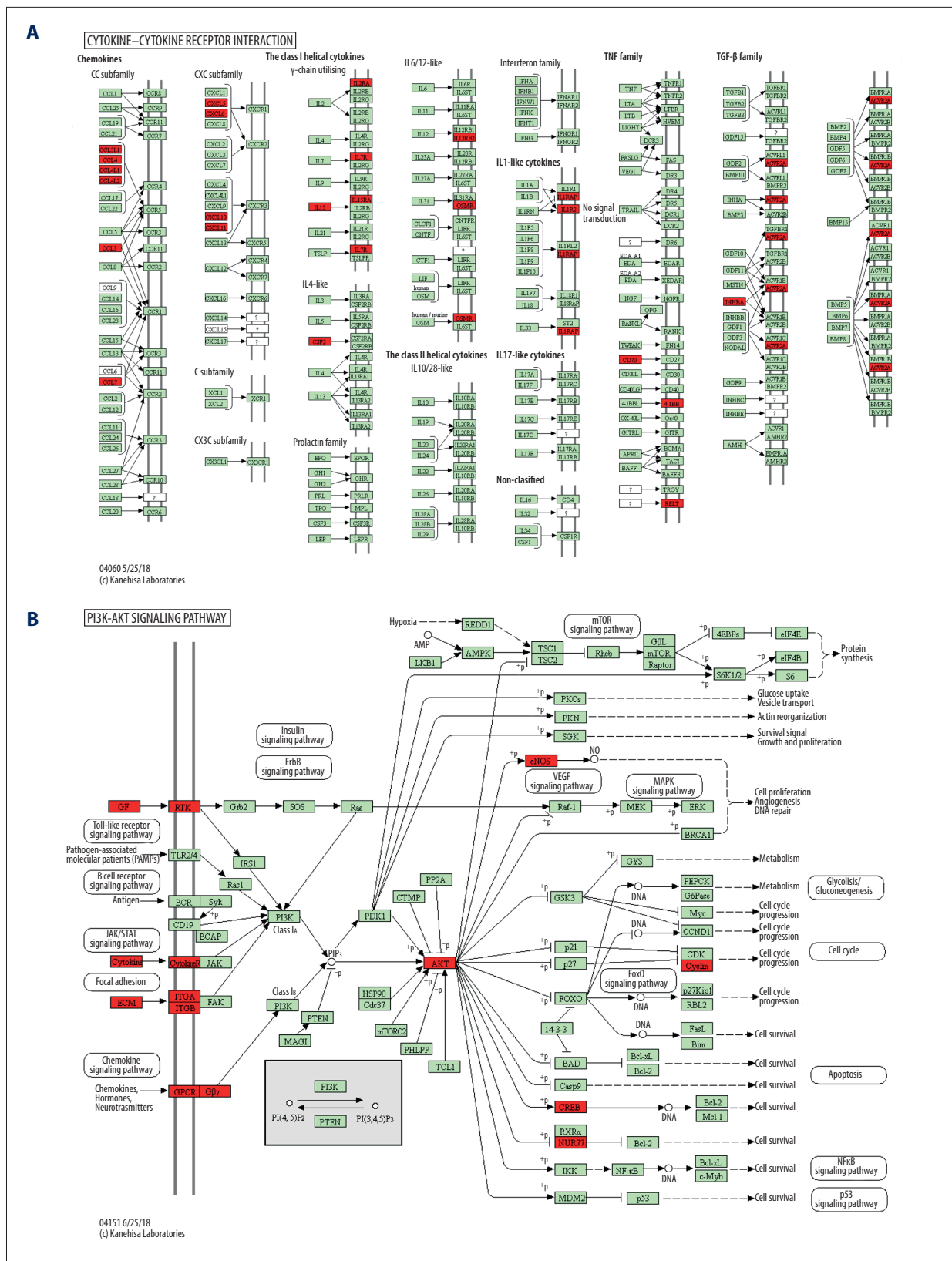
Figure 8. Function of the hub genes related to lncRNAs visualized in the KEGG map obtained from the DAVID. (**A**) LINC00460, (**B**) ADAMTS9-AS1.

We also examined the possible functional mechanism of LINC00460 and ADAMTS9-AS1 in bladder cancer using bioinformatics. We first obtained genes that were correlated with the lncRNAs through TANRIC and the circlncRNAnet database and performed GO, KEGG, PANTHER, and PPI analysis on an overlapping set of the correlated genes. The results indicated that these genes participate in numerous molecular functions and biological processes. KEGG analysis demonstrated that the cytokine–cytokine receptor interaction for LINC00460 and the PI3K-Akt signaling pathway for ADAMTS9-AS1 were the most significant enriched pathway. We also detected enriched genes in other important biological processes, such as the TNF signaling pathway and toll-like receptor signaling pathway, which may correlate with bladder cancer tumor progression and development.

The PI3K-Akt signaling pathway regulates several fundamental cellular functions, such as transcription, translation, proliferation, growth, and survival [32]. This pathway is overactive in many cancers, resulting in a reduction of apoptosis and increased in proliferation [33]. PI3K is vital for cancer emergence and development, underscoring the significance of understanding the regulatory mechanism of PI3K-Akt signaling pathway. PIK3CA frequently shows gain-of-function mutations in urothelial cancer [34]. Liu et al. [35] reported that PI3Kb also plays a vital role in tumorigenesis and tumor cell proliferation of PTEN negative cancers. In addition, they found that interfering with PI3Kb may be a treatment for high-risk bladder cancer with e-cadherin loss and mutated PTEN. Singh et al. [36] has reported that loss of PTEN was one of the major factors leading to the initiation of prostate cancer through the PI3K/Akt signaling pathway. Furthermore, they used a multifunctional antioxidant nanoliposome containing PTEN plasmid and cerium oxide nanoparticles (CeNPs) to deliver the PTEN protein efficiently and found that the viability of prostate cancer cells was weakened after inhibiting the PI3K/AKT signaling pathway. In addition, it has been reported that an interaction between hypoxia and the PI3K/Akt pathway was implicated in bladder cancer progression [37]. The expression of hypoxia-inducible factor-1 (HIF-1α) contributes to tumor angiogenesis in bladder cancer cells and can be downregulated significantly by inhibiting the PI3K/Akt pathway [38]. Lv et al. [39] also reported that PI3K/Akt pathway was involved in hypoxia-induced epithelial-mesenchymal transition (EMT) in bladder cancer cells.

Our results together with these studies suggest that ADAMTS9-AS1 could promote the development of bladder cancer by influencing the PI3K-Akt signaling pathway. Further studies are required to confirm the target molecule of ADAMTS9-AS1 in bladder cancer.

Among the 30 significant hub genes identified from PPI analysis, 24 hub genes associated with LINC00460 are involved in tumor immune surveillance. Ten genes were enriched in cytokine–cytokine receptor interactions for LINC00460: CSF2, CXCL10, ICAM1, IL15, CCL4, CXCL11, IL2RA, CCL3, IL7R, and CXCL5. These 10 genes showed significant overexpression in bladder cancer and have correlation with LINC00665. Therefore, LINC00665 likely regulates the cytokine–cytokine receptor interaction through these ten genes. Some reports have been published on the effects of the tumor microenvironment on tumor progression, and IL, the CCL and CXCL families, inflammatory cells, costimulatory molecules in tumor cells, and serum cytokines all have prognostic significance.

A previous review analyzed studies evaluating the impact of the inflammatory response on bladder cancer progression [40]. Inflammatory cytokines are involved in the STAT and T cell proliferation pathways, which are associated with cellular growth, proliferation, cancer, inflammatory response, cell death, and survival in bladder cancer [40]. Previous studies showed that high CSF2 expression has a significant association with poor outcome in urothelial carcinoma patients [41]. Increased CSF2, CCL3, and IL6, as well as increased CD8a expression, can recruit immune cells [42]. MB49 and MBT-2 cells and human bladder tumors express the chemokine CXCL10 (IP10), which has angiostatic effects and might promote inflammatory responses [42]. Local production of CCL2 might be crucial in the progression of bladder cancer [42]. Therefore, the expression and upregulation of LINC00460 and the 10 related hub genes likely lead to changes that may affect the development and progression of bladder cancer, especially the tumor microenvironment.

This study had several limitations. First, the sample size was as there were only 413 cases in the TCGA database. Our results indicated that the clinical prognosis of bladder cancer patients with upregulated LINC00460 or downregulated ADAMTS9-AS1 was poorer. However, to provide statistically significant power for these potential prognostic biomarkers requires confirmation in larger, multicenter studies. Moreover, as the potential mechanism of these lncRNAs in bladder cancer was still unelucidated, further research is required.

Conclusions

In this study, we demonstrated high LINC00460 expression and low ADAMTS9-AS1 expression in bladder cancer as compared with normal bladder tissues. Bioinformatics analysis found that key pivotal genes related to LINC00460, including the CXCL, CCL, and CSF2 genes, might play an important role in the progression of bladder cancer. The low expression of ADAMTS9-AS1 can also target the hub genes to affect the survival of bladder cancer patients. As far as we known, this is the first study identifying these lncRNAs as useful biomarkers for bladder cancer. Our findings provide further insight into the underlying mechanisms of lncRNA in bladder cancer.

CLINICAL RESEARCH

Availability of data and materials

The authors thank the TCGA and the GEPIA databases for providing the data.

Conflicts of interest

None.

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