

The Analysis of lncRNA-miRNA-mRNA Competitive Endogenous RNA Network to Identify 4-lncRNA Signature Related to the Prognosis of Bladder Cancer

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Abstract

Background

Bladder cancer (BLCA) is one of the leading causes of cancer deaths in the world, and the molecular mechanism of its pathogenesis is very complicated. Long non-coding RNA (lncRNA) can interact with microRNA (miRNA) through the mechanism of competitive endogenous RNA (ceRNA), and affect the expression of Messenger RNA (mRNA), and affect the pathogenesis of bladder cancer. This study aims to construct the ceRNA-regulated bladder cancer network related to lncRNA and identify a novel lncRNA signature related to the survival prognosis of patients with bladder cancer. It was validated in GEPIA's online bioinformatics network server assists.

Methods

The RNA sequencing data of normal and adjacent bladder cancer tissues are from the Cancer Genome Atlas (TCGA). We identify differentially expressed (DE) genes by comparing gene expression between normal tissues and tumors in the TCGA dataset. Construct a ceRNA network and explore potential biological markers. Based on the ceRNA network, univariate regression analysis and multivariate regression analysis were used to screen out the lncRNA related to the overall survival (OS) of bladder cancer. It was validated in GEPIA's online bioinformatics network server assists. Receiver operating characteristic curve (ROC) analysis was used to evaluate the prognostic value of the risk score.

Results

We screened out 666 lncRNAs, 160 microRNAs (miRNAs), and 1,820 Messenger RNAs (mRNAs) by comparing normal bladder cancer tissues and adjacent tissues ($P < 0.05$). Then, we constructed a ceRNA regulatory network containing 44 DElncRNA, 22 DEmiRNA, and 52 DE mRNA. The survival analysis of differential genes in the ceRNA network identified 9 lncRNAs, 8 miRNAs, and 12 mRNAs that are associated with the prognosis of BLCA. Cox regression analysis of 9 lncRNAs related to the prognosis of bladder cancer showed that 4 lncRNAs (AC078778.1, ADAMTS9-AS1, ADAMTS9-AS2, and NAV2-AS2) can be independently used as prognostic markers of bladder cancer.

Conclusions

Based on the construction of the bladder cancer ceRNA network, a new prognostic signature of four lncRNA-based has been discovered. It will help to better understand the mechanism of bladder cancer occurrence, development and metastasis, and provide direction for future research.

Background

"Global Cancer Statistics" shown that the incidence of bladder cancer in 2018 ranked tenth among global cancers [1, 2]. Most bladder cancers originate in epithelial cells and can be cured if diagnosed before metastasis. The majority of bladder cancer patients are reported to be diagnosed with in-situ tumors and

a 5-year survival rate of 96%, which can be cured if diagnosed before metastasis[3]. However, as the tumor penetrates deeply into the muscle layer, the 5-year survival rate is greatly reduced[4]. Besides, The high recurrence rate of bladder cancer makes it difficult to remove and therefore difficult to treat[5]. Currently, the diagnosis method of bladder cancer is mainly based on cystoscopy and urine cytology[6]. But it cannot show the biological heterogeneity of BLCA. Therefore, understanding the molecular mechanism of the occurrence, development, and metastasis of BLCA is very important for early diagnosis. Deepening the research on molecular biology and genetic mechanism of bladder cancer can improve the efficiency of diagnosis and treatment of the development of bladder cancer, which will benefit bladder cancer patients and reduce the global burden of disease[7]. Therefore, early detection of bladder cancer can prevent disease progression and reduce mortality. Identifying potential molecular diagnostic markers is essential to combat bladder cancer.

Long non-coding RNAs (lncRNAs) account for a considerable proportion of the human transcriptome. Due to their evolutionary non-conservative characteristics, they have more responsibility for various regulatory functions than short-length microRNA (miRNA) and messenger RNA (mRNA). Initially, it was thought that lncRNA had no biological function and was just the "noise" of genome transcription [8]. But more and more literature research found lncRNA can be used as carcinogenic or tumor suppressor genes to participate in the occurrence and development of tumors [9]. In particular, a recent study found the vast majority of the expression of lncRNA in specific cancers is abnormal, which may be used as important biological markers for tumor diagnosis and treatment. Some of these cancer-related lncRNAs are associated with expression, including CCAT2 in colorectal cancer and PCAT-1 in prostate cancer [10]. Chun-Ni Fan et al provided new insights into the lncRNA-related ceRNA network in breast cancer, showing that four-lncRNA (LINC00491, AL391421.1, ADAMTS9-AS1, and LINC00536) may be an independent biological marker for predicting the survival of breast cancer patients [11]. Although non-coding RNA does not code for proteins, it participates in various levels of major life activities as regulatory molecules and other identities. Therefore, the identification of BLCA-specific lncRNA biomarkers has important clinical significance for the diagnosis and prognosis of BLCA. lncRNA mainly affects the stability of mRNA and translation regulation through the regulatory mechanism of competitive endogenous RNA (ceRNA) that adsorbs miRNA [12].

Salmena et al first presented the ceRNA (competing endogenous RNAs) hypothesis in 2011, which revealed a new mechanism of RNA interaction[13]. In the ceRNA gene interaction network including lncRNA, miRNA, and mRNA, lncRNA can act as an endogenous molecular sponge, through the shared microRNA response element and the reverse complementary binding seed region to competitively bind to the miRNA, thereby indirectly regulating the mRNA expression level. MiRNA is known to induce gene silencing by binding mRNA, while ceRNA can competitively bind miRNA and affect the gene silencing caused by miRNA through the binding of response elements to miRNA, which is of great biological significance. In recent years, a large number of researches confirmed the regulation mechanism of the ceRNA network is related to the occurrence, invasion, and development of tumor genesis. Such as, DANCR can sponge miR-149 through the ceRNA network mechanism, and positively regulate MSI2, thereby inhibiting the malignant phenotype (proliferation, migration, invasion, EMT, and tumorigenicity) of

bladder cancer cells [14]. Zhong et al. found that by comparing with normal tissues, MiR-20a in bladder cancer tissue is negatively correlated with PDCD4, and PTENP1/miR-20a/PTEN promotes the proliferation and migration of bladder cancer cells [15]. LINC01133 regulates APC expression and Wnt/ β -catenin pathway by acting as a miR-106a-3p ceRNA, thereby inhibiting the development and metastasis of gastric cancer [16]. Kong et al. found that the mechanism by which lncRNA-CDC6 can act as a competitive endogenous RNA in breast cancer is by sponging has-mir - 215 to regulate the expression level of CDC6 [17]. Nevertheless, very little information is available on BLCA ceRNAs. However, due to the lack of a comprehensive cancer genome database and mature research methods available, it is difficult to comprehensively analyze and construct a lncRNA-miRNA-mRNA ceRNA network that plays a regulatory role in the molecular mechanism of tumor pathogenesis. The TCGA database is an authoritative database containing comprehensive cancer genomic data and available clinical data, covering 36 cancer types. It's an important data source for cancer researchers. Broadening the path for research into malignancies and rare tumors. The database updates quickly and the data are ideal resources for biological information analysis and data mining. To a certain extent, it helps us to deepen and improve the research on cancer prevention, diagnosis and treatment. CeRNA network explains how transcripts construct gene expression regulatory networks from a new perspective, which will be helpful to explore gene functions and regulatory mechanisms at a deeper level.

The purpose of this study is to dig out the molecular markers related to the development, progression, and prognosis of bladder cancer and the potential biological molecular mechanism of its pathogenicity through bioinformatics analysis of BLCA data set in the TCGA database. The first is to identify differential genes by comparing cancer and paracancerous tissue. Then, the ceRNA network was constructed through a series of integrated analyses based on databases such as miRcode and miRDB. Subsequently, the DAVID database was used to enrich and analyze the differential genes in the ceRNA network, the possible cell components, biological processes, molecular functions, and signaling pathways involved were explored. Finally, the relationship between ceRNA and the overall survival time of bladder cancer patients was studied by survival analysis. Finally, the risk scoring system constructed by lncRNAs related to the prognosis of bladder cancer-derived from multivariate cox regression analysis can divide bladder cancer patients into high-risk groups and low-risk groups. This new research method of forecasting tumor ceRNA network-specific lncRNA markers may clarify the regulatory mechanism of the occurrence and development of BLCA. Through ceRNA analysis, we can explain how the transcriptome constructs the gene expression regulation network from a more macroscopic perspective, to further explore the regulation mechanism of genes in it.

Method

Data download and preparation

The RNA sequencing, miRNA sequencing and clinical data of bladder cancer were downloaded directly from the webpage of the TCGA database (<https://tcga-data.nci.nih.gov/tcga/>) on December 12, 2020 [18]. The inclusion criteria follow: (1) Both RNA sequencing and express data set clinical information available;

(2) The clinical survival time of each sample exceeds 1 day; (3) The survival status of each patient is complete and the status is two categories of mutually exclusive events. Then, the raw counts of the BLCA dataset including 414 BLCA samples and 19 adjacent normal bladder tissues for BLCA patients were obtained. And 402 BLCA patients with the corresponding clinical information were enrolled in this study. The approval of the ethics committee is not required, as all information needs to be obtained directly from the webpage of TCGA.

Differentially Expressed Gene Analysis

The lncRNA profile, mRNA profile, and miRNA profile were standardized by the R/Bioconductor package. In the R language (version number 3.6.1), use the "edgeR" package to compare whether there are significant changes in the expression of cancer tissues and adjacent tissues to screen for differentially expressed genes, and set the differential expression threshold were $FDR < 0.01$ and $|\log_2FC| \geq 2$ [19]. Use the ggplot2 program package in R software to draw the volcano map of the differential genes. At the same time, the heatmap package in the R software was used to draw heat maps to show the genes that were significantly differentially expressed.

Establishment of the ceRNA network

First, the name of DE miRNAs was converted into mature miRNA names by starBase V3.0. The relationships between lncRNA and miRNA were matched by using the miRcode database (<http://www.mircode.org>). Then, the potential target genes of predicting the target mRNAs of miRNAs were predicted via three online databases, including TargetScan (http://www.targetscan.org/vert_72/) [20], miRTarBase (<http://mirtarbase.cuhk.edu.cn/php/index.php>) [21] and miRDB (<http://mirdb.org/>) [22]. Through the number of miRNAs shared between lncRNA and mRNA and further screening, the ceRNA network of lncRNA-miRNA-mRNA was finally obtained. The visual image was constructed and the relationship diagram of the ceRNA regulatory network was made by Cytoscape software.

Construction of the bladder cancer-specific prognostic signatures

After the construction of the ceRNA network, to further explore the significance of differential lncRNA, miRNA, and mRNA in the ceRNA network for clinical prognosis, first of all, the expression data of differential lncRNA, miRNA, and mRNA in the ceRNA network were integrated with clinical data. Then, Univariate Cox regression analysis was used in the Survival package in R language to identify differential RNA in ceRNA network that affected overall survival (OS) in patients with bladder cancer. To further identify lncRNA associated with the prognosis of bladder patients, Prognostic lncRNA ($P < 0.05$) were significantly correlated with OS in multivariate CPHR analysis were used to establish risk score formula [23]. The formula = sum of coefficient \times the expression level of lncRNA. According to the expression of the screened genes and the over survival time of the patients, the best cutoff was found for each gene after screening. The risk score per bladder cancer patient was calculated based on the regression coefficient of each lncRNA and its expression status (high/low expression. When the risk score exceeds the best critical value, the expression level of the relevant gene is considered "high risk" and when

the risk score is less than or equal to the median value, the expression level is considered "low risk". Then the log-rank test was used to compare the differences in survival curves between the two groups. The difference in overall survival between the high expression group and the low expression group was considered statistically significant when $P < 0.05$. A 5-year time-dependent receiver operating characteristic (ROC) curve for bladder cancer patients was constructed using the "survival ROC" program package in R language software.

Functional enrichment analysis

The Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway analysis were analyzed by David database(<https://david-d.ncifcrf.gov/>) [24, 25] to analyze the mRNAs involved in the constructed lncRNA-related ceRNA network. $P < 0.05$, the difference was statistically significant.

Regression analysis of DElncRNA and DEmRNA

The correlation between differential lncRNA and differential mRNAs in the ceRNA network was analyzed by R software. When $P < 0.05$, $R > 0.3$, the correlation between variables is considered valid.

Results

Screening of differentially expressed RNAs in bladder cancer

RNAs (lncRNA, miRNA, and mRNA) sequencing data from TCGA were analyzed, 666 differentially expressed lncRNAs (420 up-regulated and 246 down-regulated), 160 differentially expressed miRNAs (139 up- and 21 down-regulated), and 1826 differentially expressed mRNAs (1031 up- and 789 down-regulated) were selected between 19 normal bladder tissues and 414 BLCA patients. The volcano plot of these differentially expressed RNAs was visualized in Fig. 1. The heatmap plot of these differentially expressed RNAs was visualized in Fig. 2.

Prediction of miRNA target genes and construction of ceRNA network

To illustrate the regulatory mechanisms of bladder cancer, according to the above results, the ceRNA network associated with lncRNA-miRNA-mRNA in bladder cancer was constructed. Firstly, we detected 666 potential DElncRNAs in the miRcode tool database, and then 292 pairs of interacting lncRNAs-miRNAs were got. Confirm that 22 DEmiRNA are interacting with 59 DElncRNA (Table S1).

Then, We used three target prediction databases to screen out 1219 mRNAs of 22 target genes targeted by DEmiRNAs. 1219 targeted mRNAs crossed further with 1819 DEmRNAs, excluding mRNAs that were not included in DEmRNAs. Finally, 52 mRNAs were included in the network (Table S2). Therefore, The final constructed ceRNA network in bladder cancer was composed of 59 lncRNAs, 22 miRNAs, and 52

mRNAs. The ceRNA network regulation pair was visualized by Cytoscape software (Fig. 3A). Besides, our study also suggested that ADAMTS9-AS2 in the bladder cancer ceRNA network may have the strong regulatory ability. ADAMTS9-AS2 interacts with 12 miRNAs (miR-301b, miR-372, miR-373, miR-96, miR-141, miR-200a, miR-143, miR-145, miR-182, miR-183, miR-205, and miR-31) and indirectly with 22 miRNA target mRNAs (TMEM100, HOXC13, SLC25A25, EPHA7, FBXL7, ELAVL2, FGF9, HOXB5, CADM2, CFL2, CCNE2, THBS1, AKAP12, TCEAL7, MEST, SHISA6, ZEB1 and TCEAL7) (Fig. 3B).

Survival analysis of ceRNA network-associated genes

We performed survival analysis on the differential RNA of the ceRNA network and found that 8 lncRNAs (AC078778.1, ADAMTS9-AS1, MYO16-AS1, AC110491.1, ADAMTS9-AS2, SACS-AS1, HCG22, and NAV2-AS2), 8 miRNAs (hsa-mir-96, hsa-mir-141, hsa-mir-143, hsa-mir-145, hsa-mir-192, hsa-mir-195, hsa-mir-200a, and hsa-mir-217) and 12 mRNAs (AIFM3, BTG2, CCNB1, CFL2, DUSP2, FAM129A, FGF2, HOXB5, MAP1B, RAB23, RUNX1T1, and TMEM100) were related to the survival and prognosis of bladder cancer ($P < 0.05$). Figure 4 shown the K–M survival curves of the first four lncRNAs, miRNAs, and mRNAs associated with the prognosis of BLCA patients.

Construction of the lncRNA-associated risk score system

Using Multivariate cox regression analysis to select lncRNAs associated with the prognosis of BLCA patients. Then, AC078778.1, ADAMTS9-AS1, ADAMTS9-AS2, and NAV2-AS2 have included in the final formula: $PI = (-0.32921 \times \text{the expression level of AC078778.1}) + (0.13558 \times \text{the expression level of ADAMTS9-AS1}) + (-0.17566 \times \text{the expression level of ADAMTS9-AS2}) + (0.18469 \times \text{the expression level of NAV2-AS2})$. Among these lncRNAs, AC078778.1 and ADAMTS9-AS2 have negative coefficients in multivariate Cox regression analysis (Table 1). The results indicate that t AC078778.1 and ADAMTS9-AS2 have a protective effect on the prognosis of BLCA patients, The higher expression of AC078778.1 and ADAMTS9-AS2 in bladder cancer, the longer OS about BLCA cancer patients. The maximum selective rank statistic was used to find the bound, the optimal cut-off value is 0.904 (Fig. 5),

According to the optimal cutoff value, patients with bladder cancer were divided into high and low-risk groups. Patients in the high-risk group had a risk score greater than 0.904 (211 cases), whereas patients in the low-risk group had a risk score less than or equal to 0.904 (191 cases). Patients in the high-risk group had a relatively poor prognosis compared to those in the low-risk group.

The ROC curve is used to reflect the accuracy and precision of the prediction model. The larger the area under the curve, the closer the turning point of the ROC curve is to the upper left corner, indicating the better the prediction ability (Fig. 6). The ability of the area under the time-dependent ROC curve labeled by 4-lncRNA to predict the 5-year prognosis is 0.666. Then, the expression of 4-lncRNA in bladder cancer tissue and normal tissue was analyzed, as well as the expression of the low-risk group and high-risk group of bladder cancer (Fig. 7). Compared with the high-risk group, the expression of AC078778.1 was higher in the low-risk group, whereas the expression of ADAMTS9-AS2 and NAV2-AS2 was higher in the high-risk group. Compared with normal tissues, AC078778.1 and NAV2-AS2 were highly expressed in bladder cancer tissues, whereas ADAMTS9-AS1 and ADAMTS9-AS2 were lowly expressed (Fig. 8).

Table 1
4 prognostic lncRNA significantly associated with OS in bladder cancer

Name	Coefficient	Type	Down/up-regulated	HR	95%CI	P value
AC078778.1	-0.3292	Protective	Up	0.7195	0.5990– 0.8642	0.0004
ADAMTS9-AS1	0.1356	Risky	Down	1.1452	1.0146– 1.2926	0.0282
ADAMTS9-AS2	-0.1757	Protective	Down	0.8389	0.7158– 0.9832	0.0301
NAV2-AS2	0.1847	Risky	Up	1.2029	1.0613– 1.3632	0.0038

Verification of lncRNA-associated prognostic genes

The results of GEPIA gene expression analysis shown the expression of ADAMTS9-AS1 and ADAMTS9-AS2 in bladder cancer was different from that in normal tissues, indicating that the prognosis of bladder cancer with low expression of ADAMTS9-AS1 and ADAMTS9-AS2 was markedly better than that of patients with high expression. This is consistent with TCGA cohort analysis (Fig. 9).

GO and KEGG enrichment analysis

The potential biological processes and pathways of 52 demRNAs in the ceRNA network were analyzed using the DAVID database. GO functional enrichment analysis revealed 34 significant enrichment biological processes ($P < 0.05$; Table S3). It mainly includes "positive regulation of cardiomyocyte proliferation", "positive endothelial cell migration regulation", "cell cycle regulation", "response to mechanical stimulation", and "positive regulation of smooth muscle cell proliferation", with decreasing P values. The relationships between the DEmRNAs and GO terms were visualized with R (Fig. 10A). The result of KEGG pathway analysis shown that different genes may be related to "romicroRNA in cancer", "proteoglycans in cancer", "PI3K-Akt signaling pathway" and "p53 signaling pathway". (Fig. 10B).

Interactions between lncRNA and mRNA in the ceRNA network

In the light of the principle of ceRNA, lncRNA can indirectly interact with mRNA to participate in the process of transcriptional regulation. lncRNA may interact with mRNA to participate in the process of transcriptional regulation. To verify whether the network conforms to the ceRNA principle, we analyzed the association between the expression level of lncRNA and the expression level of mRNA. The results have shown that there were 16 lncRNA-mRNA pairs with a strong positive correlation between the expression levels of ceRNA (Fig. 11). Besides, miR-373 and miR-372 are key genes involved in ceRNA pathways (Table S4). The results of GEPIA gene expression analysis shown the expression of ADAMTS9-AS2 positively interacted with TMEM100 and CFL2 in bladder cancer. This is consistent with TCGA cohort analysis (Fig. 12).

Discussion

Bladder cancer ranks 11th among all tumors in global incidence and 13th in mortality among all tumors [2]. The occurrence and development of bladder cancer is a complex, multi-factor, multi-step pathological process, and its specific pathogenesis has not been fully elucidated. Bladder cancer is difficult to treat and prone to recurrence after surgery, which may be due to the lack of corresponding biomarkers to diagnose the prognosis of bladder cancer. Therefore, to improve the clinical prognosis, it is a very important task to determine the prognostic markers that can predict the prognosis of bladder cancer by exploring the exact regulatory mechanism of the occurrence and evolution of bladder cancer. A growing number of experimental studies have shown that lncRNAs play an important part in many biological processes and are indispensable for gene regulation, especially in the ceRNA network, and the activity of the ceRNA network is bound up with the occurrence and evolution of cancer [26, 27].

To further understand the molecular mechanism of the early evolution of bladder cancer, as shown in the present study, we used the TCGA database to identify abnormal lncRNA, miRNA, and mRNA by comparing normal tissue and bladder cancer tissue. We predicted the interaction between DElncRNA and DEmiRNA or between DemRNA and DEmiRNA by using the STARBASE database. Then, a lncRNA-miRNA-mRNA regulatory network related to bladder cancer was constructed. The DEmRNA pathway enrichment in the network was analyzed by GO and KEGG. K-M survival analysis was used to investigate the notable genes related to bladder cancer survival prognosis in this network. Besides, the 9 differential lncRNAs of bladder cancers obtained by univariate cox regression analysis were included in the multivariate cox regression. Four DElncRNAs associated with bladder cancer survival were screened by multivariate Cox regression analysis to establish a predictive model for the diagnosis and prognosis of bladder cancer. Finally, the correlation analysis between lncRNA and mRNA was carried out.

The GO terms of different genes in the ceRNA network mainly include the following points: "positive regulation of cardiac muscle cell proliferation", "positive regulation of endothelial cell migration", "regulation of cell cycle response to mechanical stimulus", "positive regulation of transcription from RNA polymerase II promoter" and "protein binding", which suggests that bladder cancer may be a disease related to the urinary system. The six most abundant KEGG pathways include "PI3K-Akt signaling pathway," "pathways in cancer," "oocyte meiosis," "microRNAs in cancer," and "proteoglycan in cancer." Previous reports have also shown that these are common abnormal signaling pathways in cancer types. Such as phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling is one of the main cell signaling pathways and plays an important role in basic intracellular functions [28]. Notably, we performed survival analysis on the differential RNA of the ceRNA network and found that 8 lncRNAs, 8 miRNAs, and 12 mRNAs are closely related to the prognosis of BLCA patients ($p < 0.05$). Hence, many genes significantly affect the overall survival time of bladder cancer patients, indicating that the ceRNA regulatory network related to bladder cancer can identify potential candidate biomarkers for predicting the prognosis of bladder cancer patients. Besides, we also studied the relationship between lncRNA and OS in patients with bladder cancer, and multivariate analysis has shown that four of them (ADAMTS9-AS1, AC078778.1, ADAMTS9-AS2, and NAV2-AS2) had significant prognostic value in OS in

patients with bladder cancer. Cumulative risk scores of 4 lncRNAs were calculated, indicating that the 4 lncRNA signal independently predicted OS in BLCA patients. The risk score prediction model as an independent prognostic factor has a good predictive ability. Therefore, the ceRNA network was combined with TCGA data to establish a risk score system associated with lncRNA and to estimate reports of BLCA patients' OS. This research will help deepen the comprehension of the mechanism of lncRNA-mediated ceRNA regulation in BLCA and find new lncRNAs to assist in the diagnosis and determination of bladder cancer prognosis.

Among four lncRNAs, ADAMTS9-AS1 was one of the most studied oncogenic lncRNAs and ADAMTS9-AS1 an antisense lncRNA plays a key role in cancer [29, 30]. More and more researches shown ADAMTS9-AS1 is essential to the progression and prognosis of most tumors. It has been reported that long non-coding RNA antisense ADAMTS9-AS1 can predict the prognostic survival of a variety of tumors, including prostate cancer, colorectal cancer, and breast cancer. The latest study by Zhen Zhou et al. shown that ADAMTS9-AS1 inhibits the progression of prostate cancer by regulating the miR-142-5p/CCND1 axis [31]. Another study also showed the prognostic effect of ADAMTS9-AS1 in prostate cancer patients [32]. Besides, Wanjing Chen's study showed that the deletion of ADAMTS9-AS1 in colorectal cancer can significantly inhibit cell proliferation, G1/S transition, migration, and invasion, and inhibit CDK4/Cyclin D1 and epithelial-mesenchymal transition (EMT) [33]. ADAMTS9-AS2 is an antisense lncRNA, which plays a vital role in regulating gene expression and genome integrity. The same as ADAMTS9-AS1, it is vital for the progression and prognosis of most cancers. For example, Ming-Jian Huang et al. found that lncRNA ADAMTS9-AS2 controls the cartilage differentiation of human mesenchymal stem cells, and ADAMTS9-AS2 as ceRNA plays an important role in cartilage differentiation [34]. What's more noteworthy is that Zhan Zhang and other scholars found ADAMTS9-AS2 helps to inhibit the proliferation, migration and invasion of bladder tumor cells[35].

In this research, we noticed that the ADAMTS9-AS2 can contend with the hsa-mir-372 and the-mir-373, which affect regulating the expression of CFL2 and TMEM100 involved in the ceRNA network. The remaining 2 lncRNAs (AC078778.1/ NAV2-AS2) of the ceRNA network are involved in the progression of lung adenocarcinoma. So far, there have been very few reports on the regulatory mechanism of cancer. Dan Yang et al found that NAV2-AS2- miR-31- LATS2 was taken for the ceRNA network involved in the evolution of lung adenocarcinoma [36].In this study, the abnormal expressions of ADAMTS9-AS1, ADAMTS9-AS2, AC078778.1, and NAV2-AS2 were found in bladder cancer, suggesting that the 4-lncRNA signal has a potential prognostic role in bladder cancer. The results of the survival curve in GEPIA shown that ADAMTS9-AS2 and ADAMTS9-AS1 were associated with the survival prognosis of bladder cancer, and ADAMTS9-AS2 and ADAMTS9-AS1 were potential targets for accurate treatment of bladder cancer patients[37]. NAV2-AS2 is a novel biomarker for the prognosis of bladder cancer. This has laid the foundation and provided a new way of thinking for our future work. Furthermore, bioinformatics-based lncRNAs studies will be helpful for clinical experimental research in the future. Through regression correlation analysis, we found ADAMTS9-AS2 was highly positively correlated with CFL2 mRNA through miR-373 and TMEM100 mRNA through miR-372. Although our findings have important clinical significance, we must also pay attention to their limitations. First, after using bioinformatics to predict the

related miRNAs and mRNAs that lncRNA may regulate, a lot of detailed biological function studies are needed. Second, a longer tracking time is needed to verify our results. Besides, The biological role of NAV2-AS2 in bladder cancer remains to be further studied, and further functional studies are needed to clarify the molecular mechanism of lncRNA function in bladder cancer.

Conclusion

In summary, we proposed a new idea for developing lncRNA-miRNA-mRNA ceRNA regulatory network based on the interaction between genes. The present research results offer new slants on the ceRNA network associated with lncRNA in BLCA. Four-lncRNA signatures can be used as potential prognostic biomarkers for BLCA patients. These target genes have great potential in the diagnosis, prognosis, and treatment of BLCA.

Abbreviations

BLCA: Bladder cancer

lncRNA: Long non-coding RNA

miRNAs: MicroRNAs

mRNA: Messenger RNA

ceRNA: Competing endogenous RNA

TCGA: The Cancer Genome Atlas

DEGs: Differentially expressed genes

OS: Overall survival

ROC: Receiver operating characteristic curve

KM: Kaplan–Meier

GO: Gene Ontology

KEGG: Kyoto Encyclopedia of Genes and Genomes

DAVID: The Database for Annotation, Visualization, and Integrated Discovery

Declarations

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Author information

Contributions

HLZ devised this research. HLZ, XZS, YLY, XCJ, and ZJS for the data download and analysis of the research. HLZ wrote this manuscript. HLZ, YLY, and YPW explained the data. YLY reviewed and revised the manuscript. All authors approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

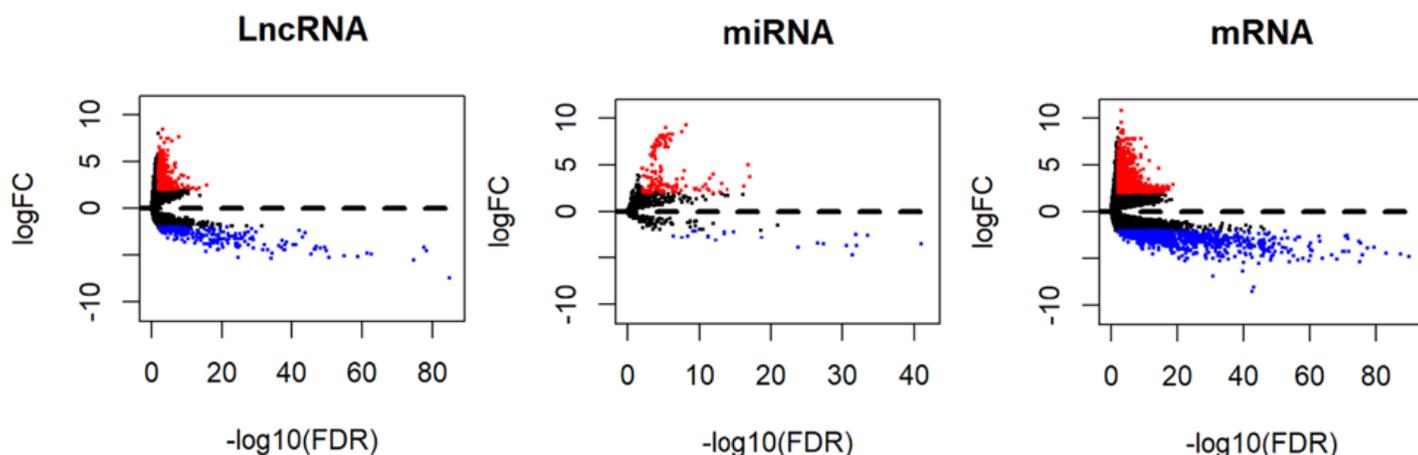


Figure 1

Volcanic map of differentially expressed RNAs (lncRNA, miRNA, and mRNA) in bladder

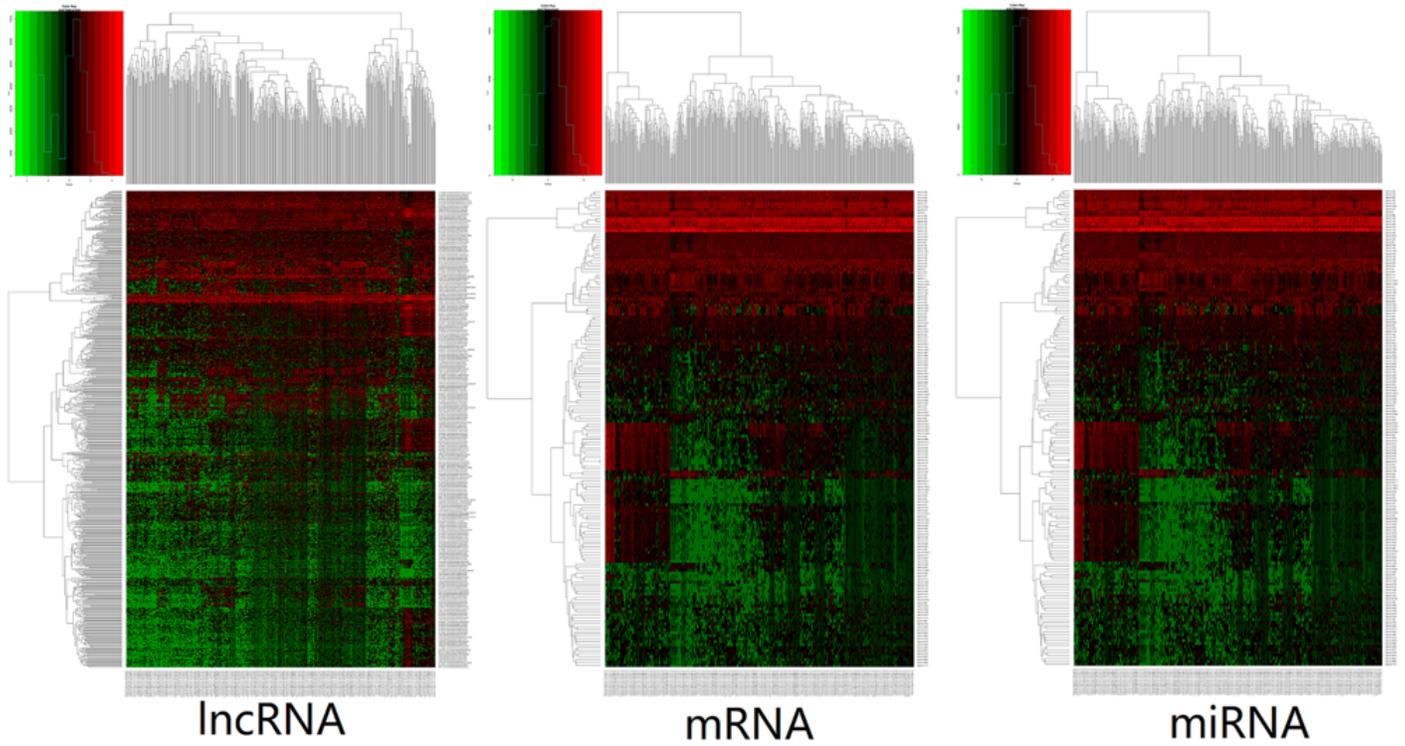


Figure 2

The heatmap of genome-wide differentially expressed RNAs (lncRNA, miRNA, and mRNA).

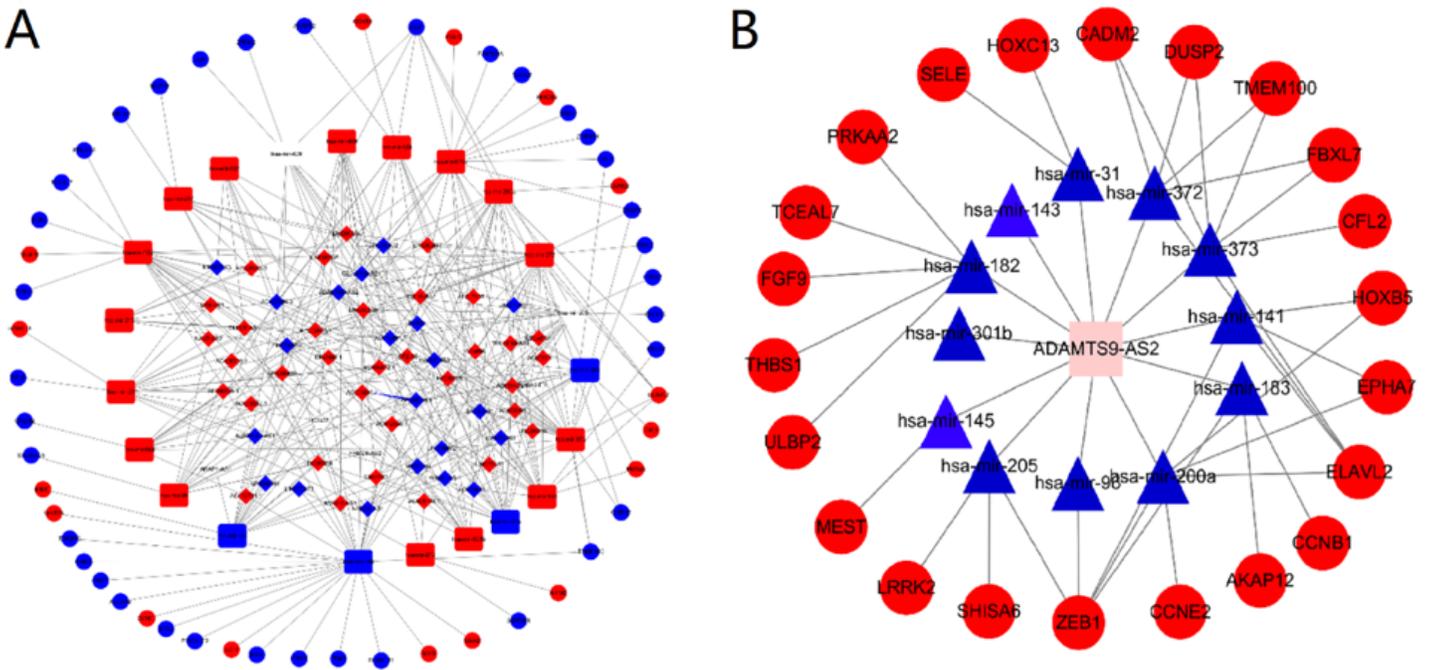


Figure 3

A: The lncRNA-miRNA-mRNA network in bladder cancer. B: The sub-network center on ADAMTS9-AS2.

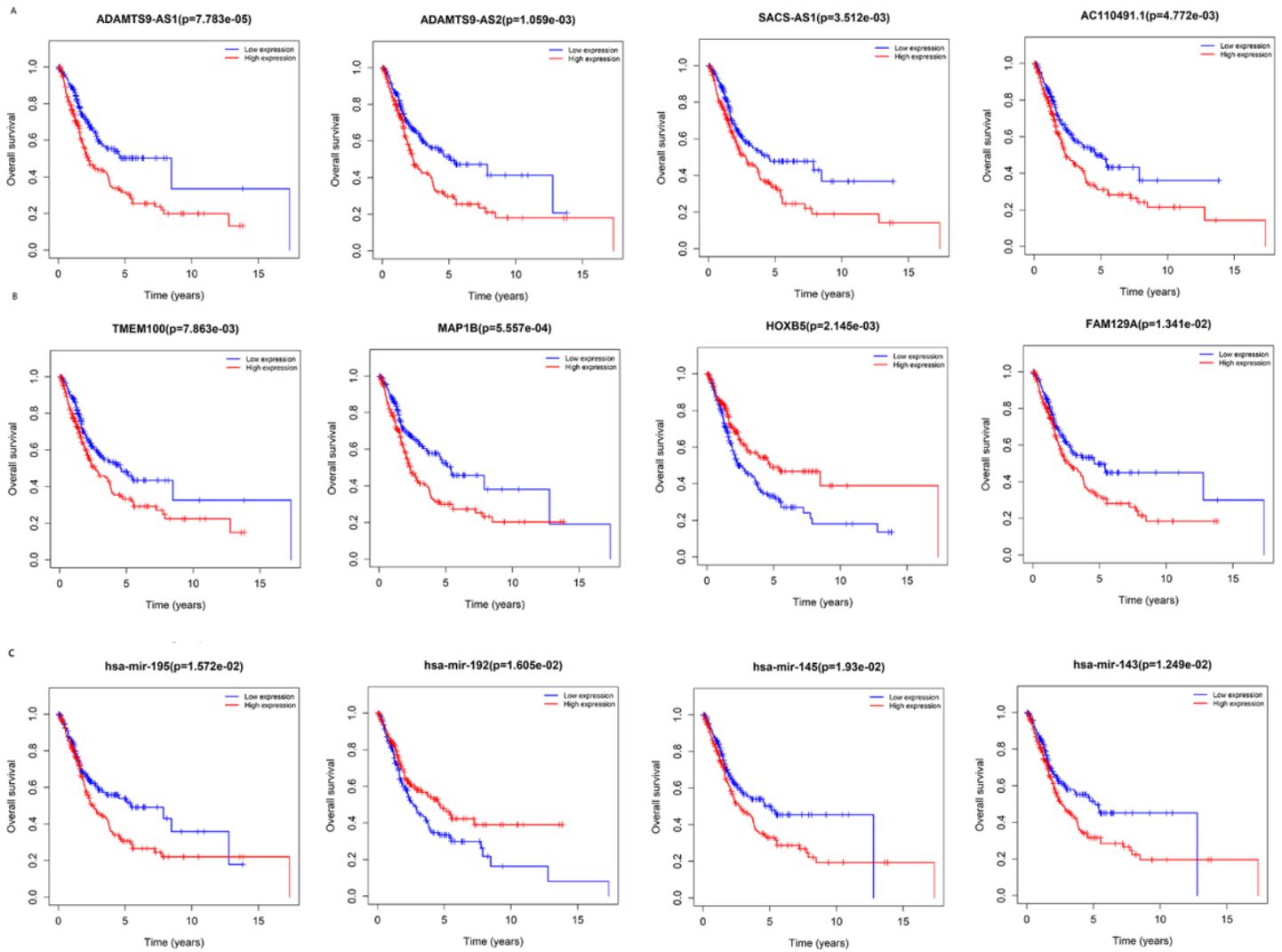


Figure 4

K-M survival analysis of DEGs in BLCA patients. The top 4 most relevant to survival lncRNA-miRNA and mRNA are shown based on their optimal cutoffs.

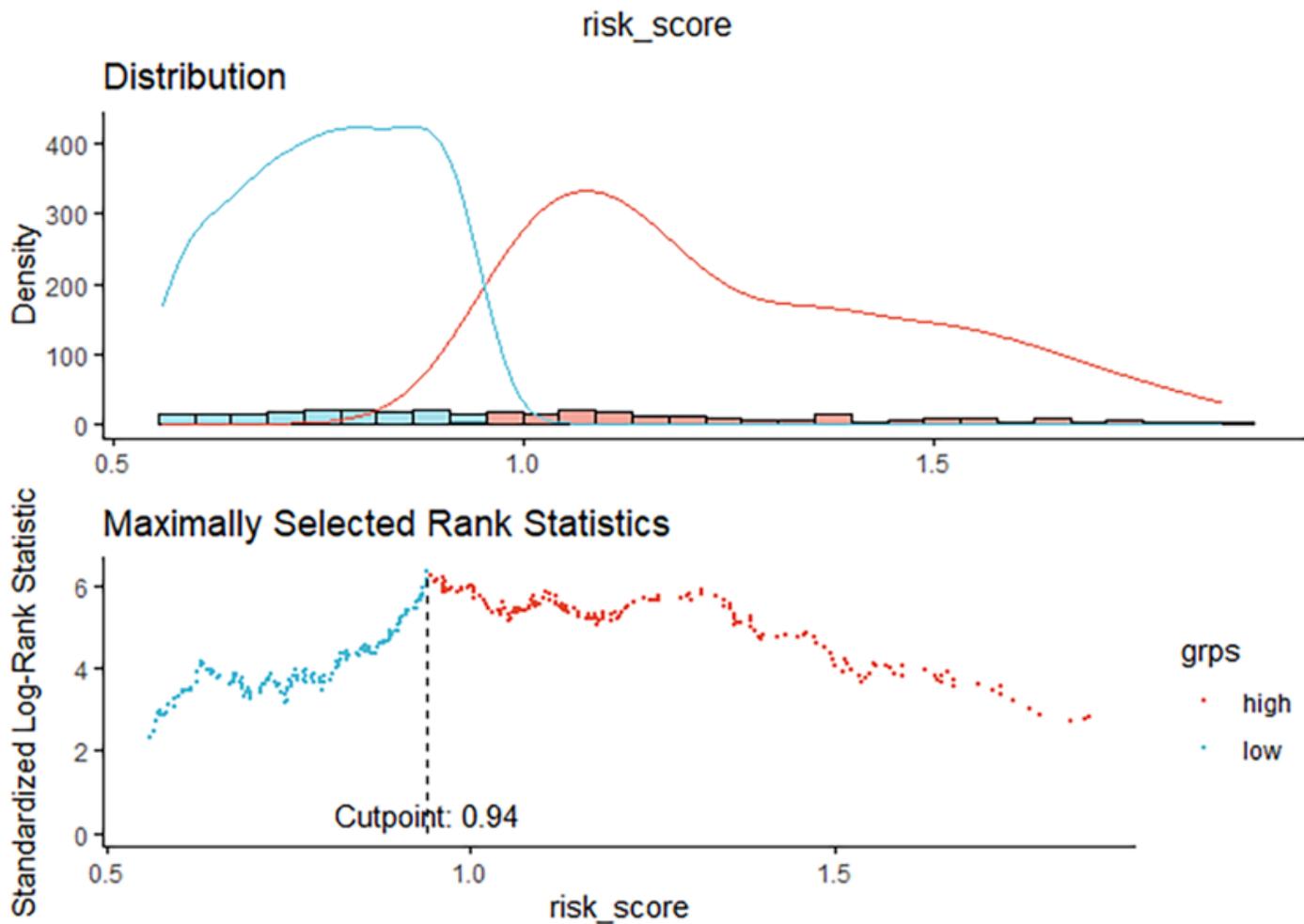


Figure 5

The standardized logarithmic scale statistics associated with risk scores are shown in the maximum lumped measurement are defined as the optimal truncation value.

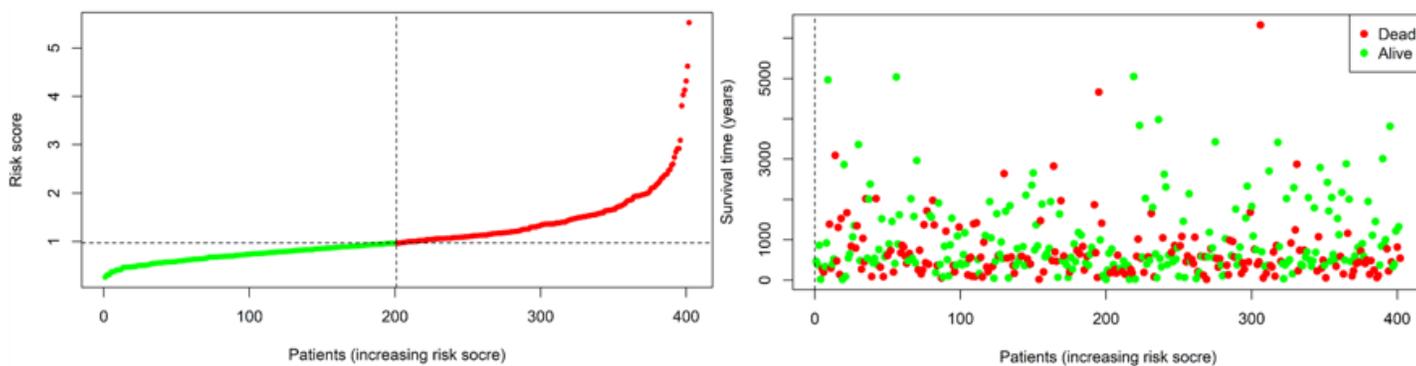


Figure 6

The distribution of bladder cancer risk score, OS, and OS status.

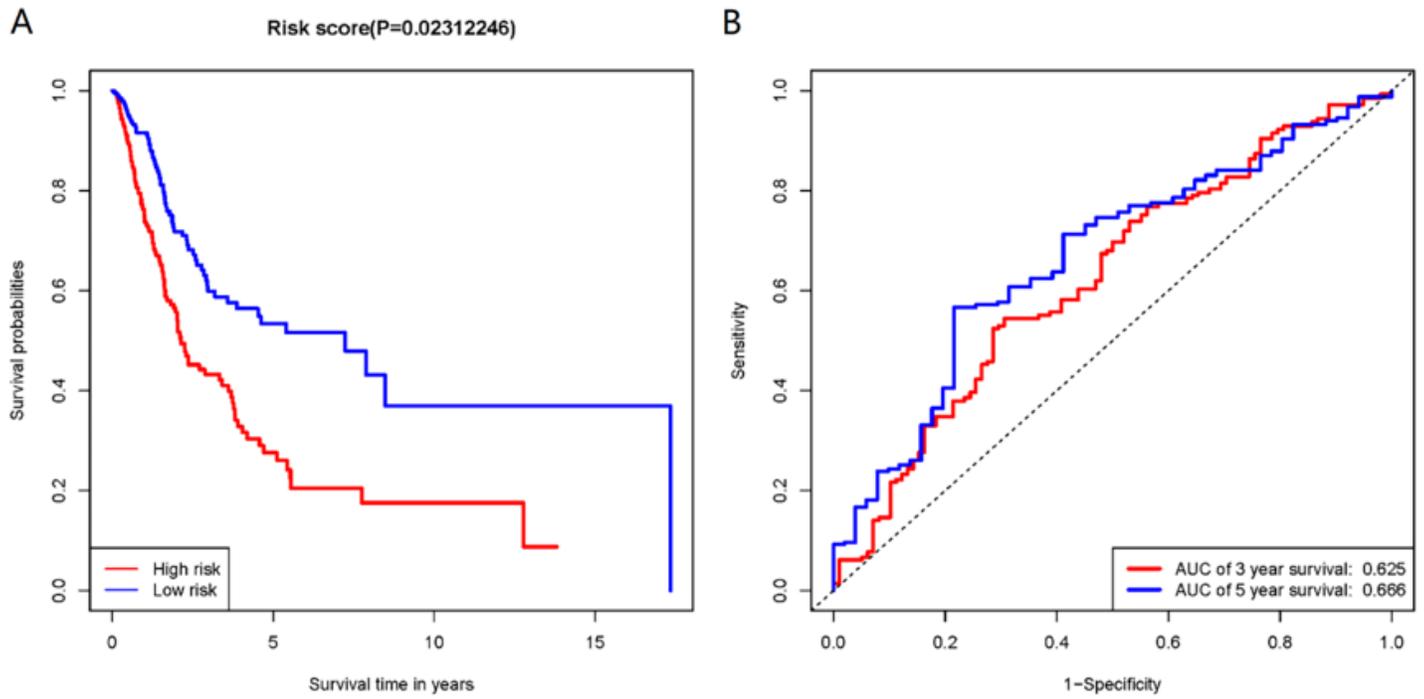
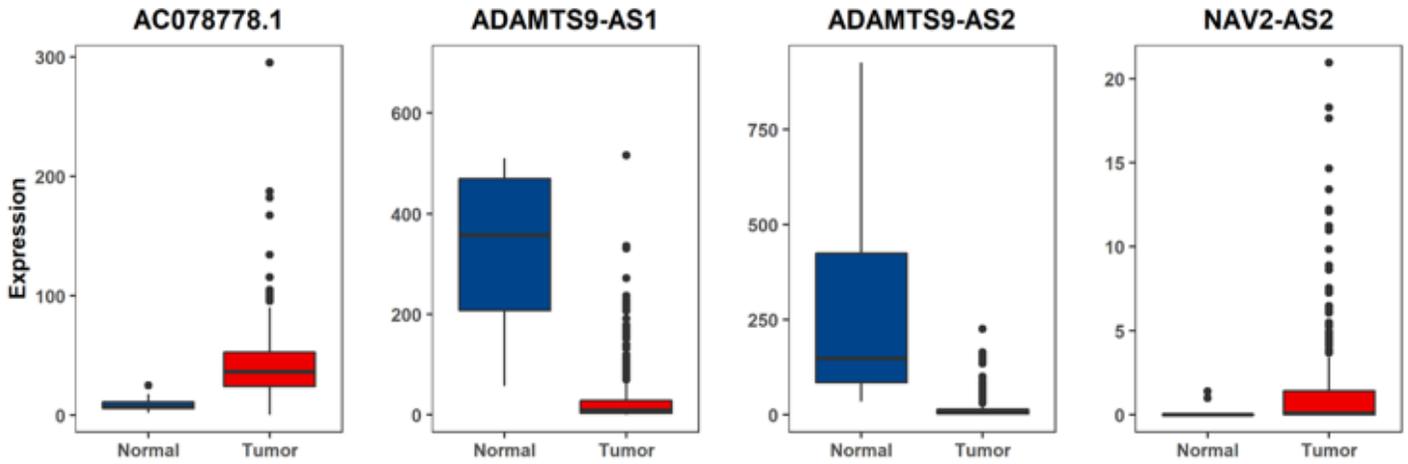


Figure 7

The 4 lncRNA signature of BLCA for the outcome. A. The survival differences between the high-risk and low-risk groups. B. ROC curves demonstrated that the area under the receiver operating characteristic (AUC) of the 4-lncRNA model.

Normal vs Tumor



High risk vs Low risk

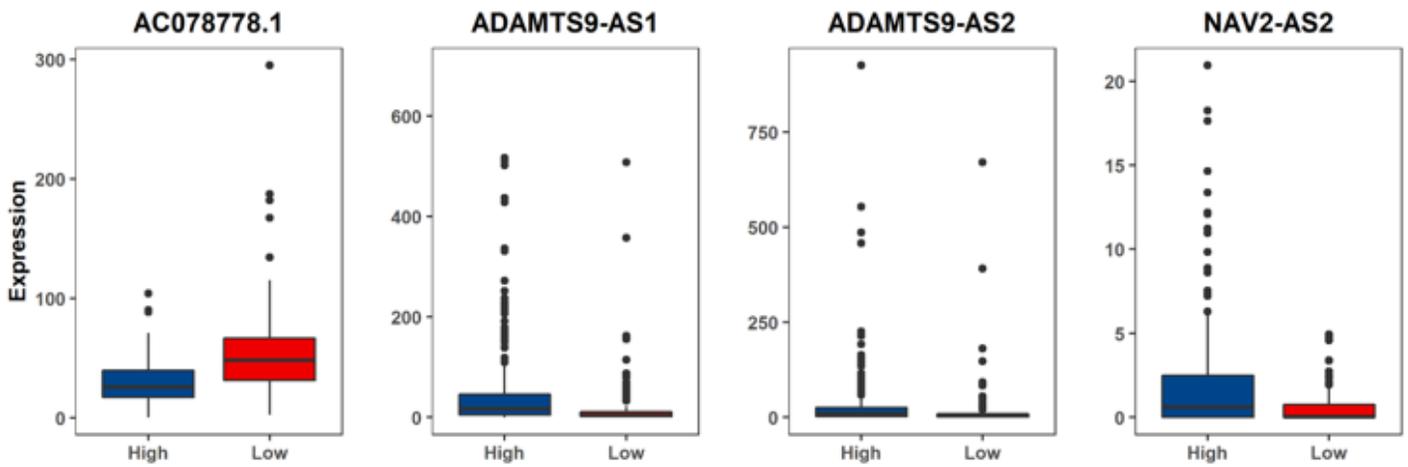


Figure 8

Expression pattern of the 4-lncRNA in BLCA and normal tissues, and in high-risk and low-risk groups.

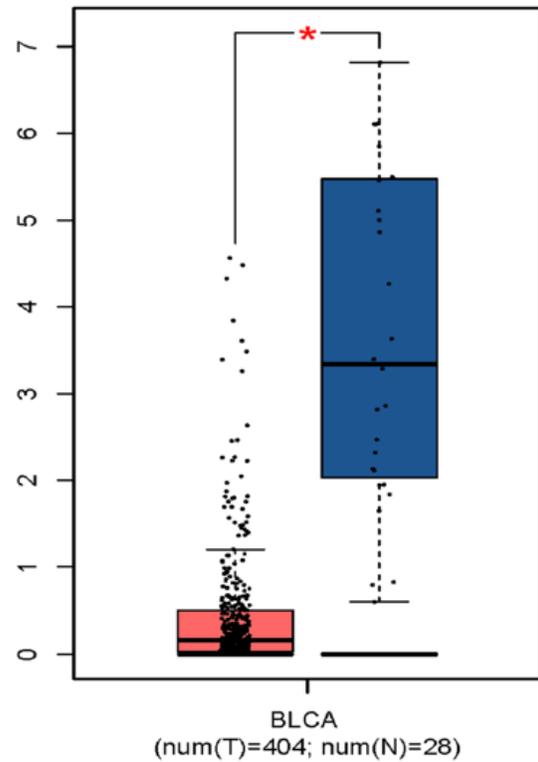
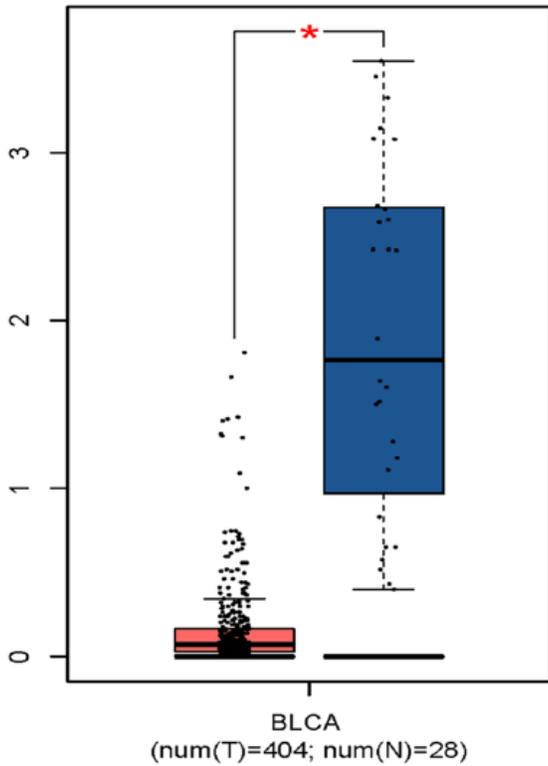
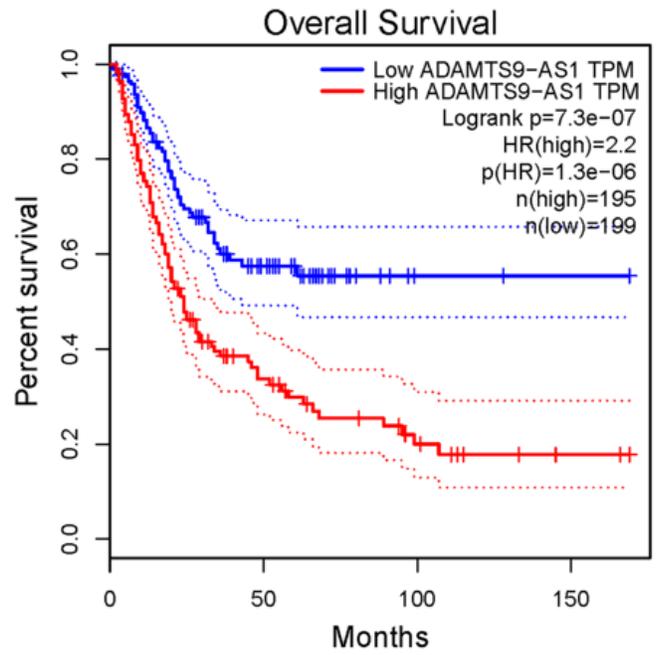
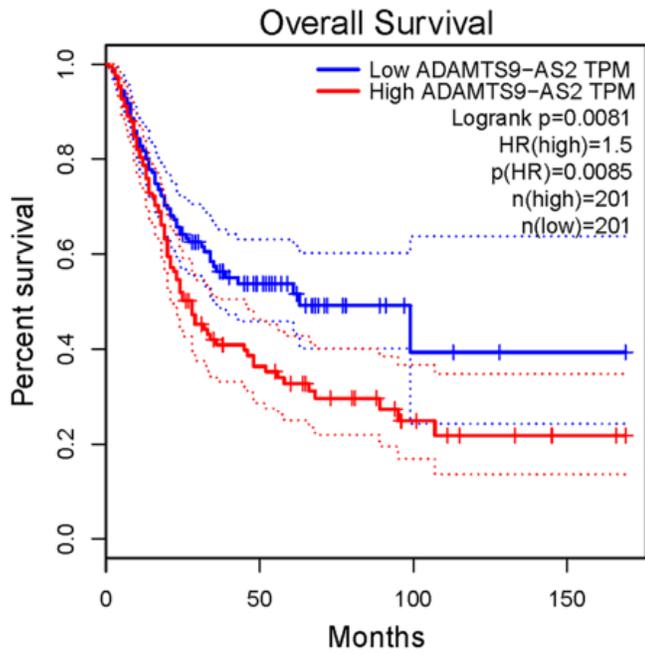


Figure 9

Expression pattern of the lncRNA in BLCA and normal tissues, and K-M survival curves of lncRNA

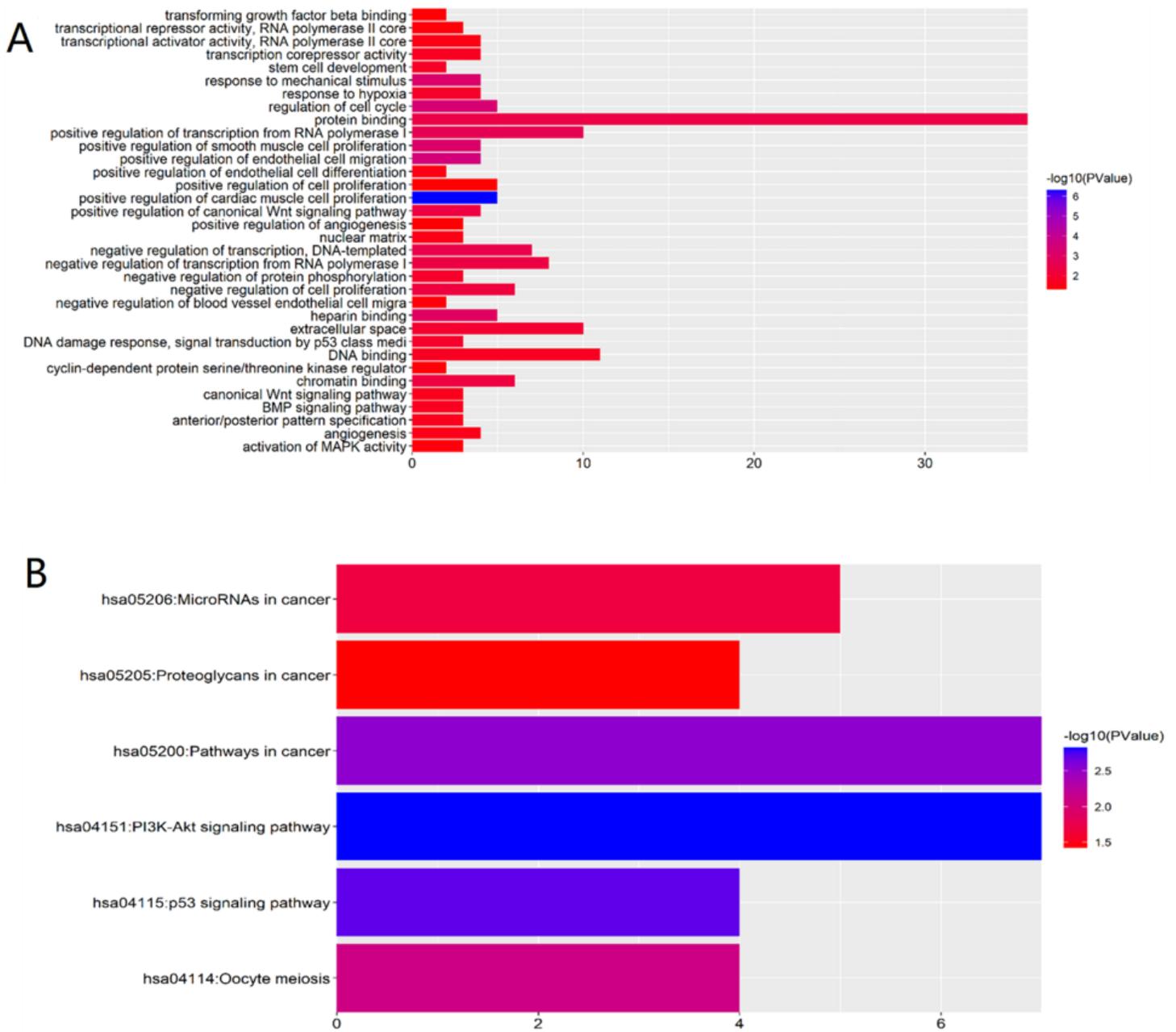


Figure 10

Biological function and pathway analysis of differentially expressed mRNAs. A. The significant functional annotations in the GO biological process. B. The significant functional annotations in the pathway.

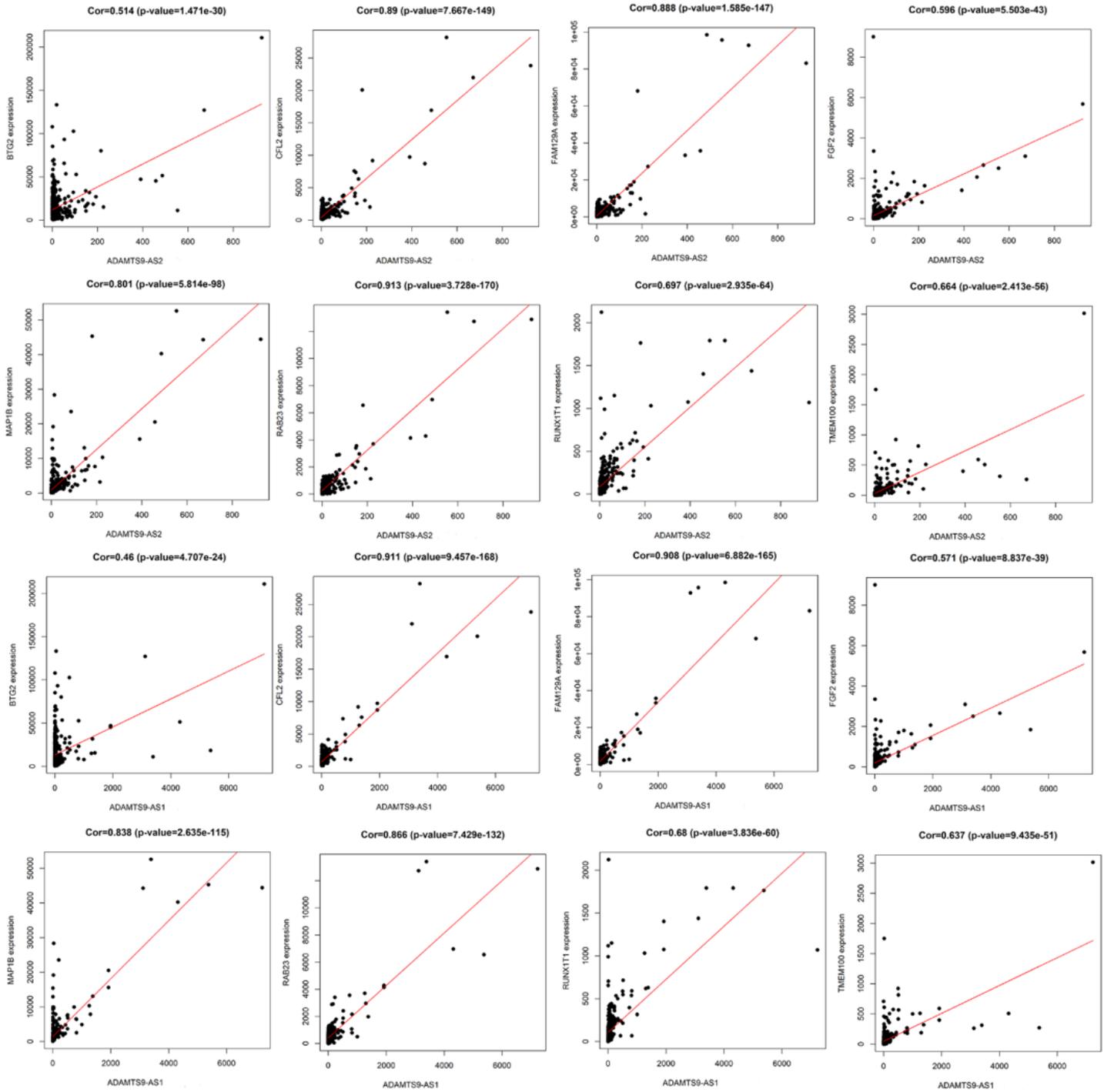


Figure 11

Correlation analysis and linear regression analysis between lncRNA and mRNA.

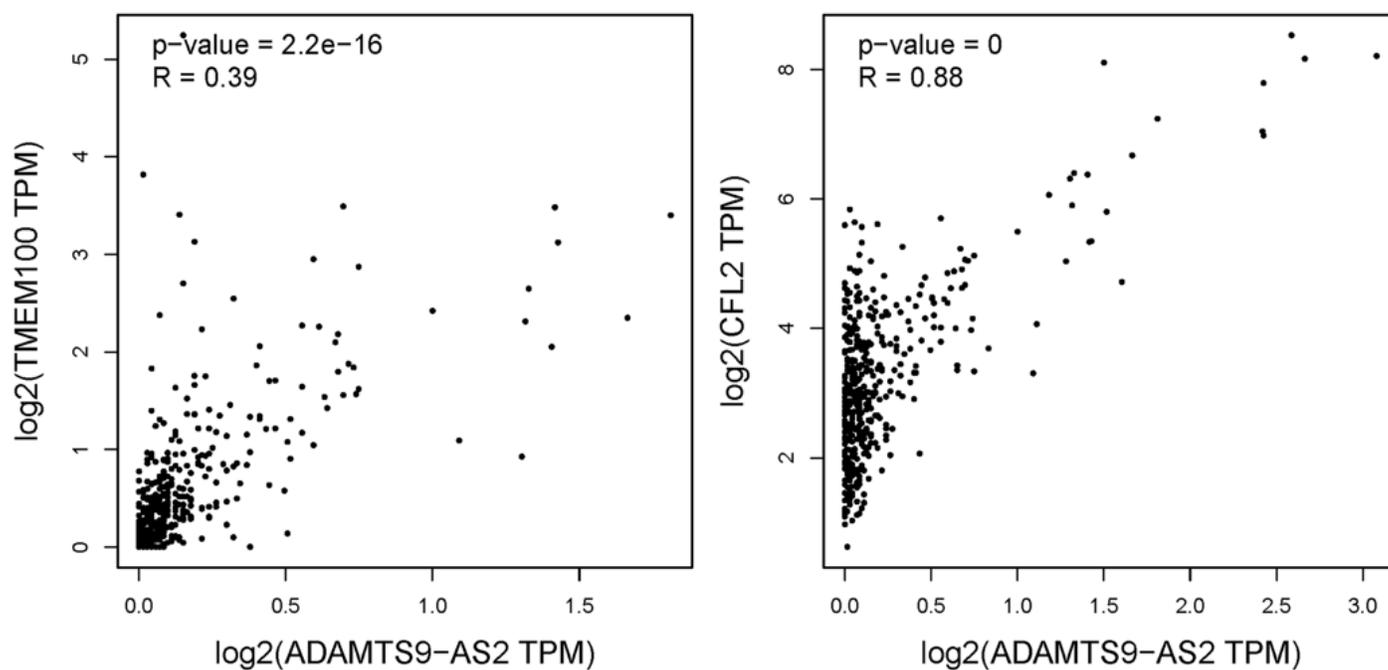


Figure 12

Correlation analysis and linear regression analysis between lncRNA and mRNA.

Supplementary Files

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