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Comprehensive Analysis of a Long Noncoding RNA-Associated Competing Endogenous RNA Network in Wilms Tumor

Feng Zhang, PhD[1,2], Liping Zeng, MD[1,2], Qinming Cai, BS[1], Zihao Xu, BS[1], Ruida Liu, BS[1], Haicheng Zhong, BS[1], Robert Mukiibi, PhD[3], Libin Deng, PhD[1,2], Xiaoli Tang, PhD[1,4], and Hongbo Xin, PhD[1,2]

Abstract

Long noncoding RNA (lncRNA) plays crucial roles in various biological processes of different cancers, especially acting as a competing endogenous RNA (ceRNA). However, the role of lncRNA-mediated ceRNA in Wilms tumor (WT), which is the most common malignant kidney cancer in children, remains unknown. In present study, RNA sequence profiles and clinical data of 125 patients with WT consisting of 119 tumor and 6 normal tissues from Therapeutically Applicable Research To Generate Effective Treatments database were analyzed. A total of 1833 lncRNAs, 156 microRNAs (miRNAs), and 3443 messenger RNAs (mRNAs) were identified as differentially expressed (DE) using "DESeq2" package. The lncRNA-miRNA-mRNA ceRNA regulatory network involving 748 DElncRNAs, 33 DEmiRNAs, and 189 DEmRNAs was constructed based on miRcode, Targetscan, miRTarBase, and miRDB database. Gene Ontology term and Kyoto Encyclopedia of Genes and Genomes pathway analyses revealed that DEmRNAs were mainly enriched in cell proliferation-related processes and tumor-related pathways, respectively, and 13 hub genes were identified by a protein–protein interaction network. Survival analysis detected 48 lncRNAs, 7 miRNAs, and 16 mRNAs to have significant impact on the overall survival of patients with WT. Additionally, we found that 6 DElncRNAs with potential prognostic value were correlated with tumor stage (*DENND5B-AS1*) and histologic classification (*TMPO-AS1*, *RP3-523K23.2*, *RP11-598F7.3*, *LAMP5-AS1*, and *AC013275.2*) of patients with WT. Our research provides a great insight into understanding the molecular mechanism underlying occurrence and progression of WT, as well as the potential to develop targeted therapies and prognostic biomarkers.

Keywords

Wilms tumor, lncRNA, competing endogenous RNA, survival analysis, therapeutically applicable research to generate effective treatments

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[1] Nanchang University, Nanchang, China
[2] The National Engineering Research Center for Bioengineering Drugs and the Technologies, The Institute of Translational Medicine, Nanchang University, Nanchang, China
[3] Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada
[4] Department of Biochemistry, School of Medicine, Nanchang University, Nanchang, China

Corresponding Author:
Hongbo Xin, Nanchang University, Nanchang 330031, China.
Email: xinhb@ncu.edu.cn

Introduction

Wilms tumor (WT) or nephroblastoma is the most common pediatric kidney cancer in children of age below 5 years.[1] It accounts for nearly 95% of all pediatric renal malignancies, with an incidence of approximately 7 cases per million children.[2] The overall 5-year survival rate for WT in the United States is 92%, whereas the proportion plummets to just 78% in poor parts of the world with fewer medical resources.[3] Although a great improvement in survival rate has been achieved over past few decades through combination therapy including surgery, radiotherapy, and chemotherapy,[4] the prognoses of patients with WT with higher risks of treatment failure or a more advanced stage are still poor. In addition, the exposure to toxic therapies has potential side effects,[5] such as occurrence of renal dysfunction,[6] cardiotoxicity,[6] musculoskeletal problems,[7] and development of secondary malignant neoplasms.[8] It is thus urgent to improve outcomes for children with high risks of tumor characteristics and develop tailored therapy to reduce treatment burden for children with low risk of WT. Therefore, investigating the molecular mechanisms involved in WT for developing more effective targeted therapies and identifying biomarkers with highly sensitive and specific values for risk stratification are very essential.

Currently, the available information on the molecular background of WT pathogenesis largely implicates mutations in multiple genes, including *WT1*,[9,10] *CTNNB1*,[11] *AMER1* (*WTX*),[12] and *TP53*,[13] amplification of *MYCN* gene,[14] genomic imprinting of the *IGF2* and *H19*,[15] aberrations of 11p15 methylation,[16] loss of heterozygosity at 1p and 16q,[17] and dysregulation several microRNAs (miRNAs) including *miR-17*,[18] *miR-185*,[19] *miR-204*,[20] and *miR-483*.[21] However, the critical roles of long noncoding RNA (lncRNA), a class of noncoding RNAs > 200 nt in length, in WT are largely unknown. Numerous studies have demonstrated that lncRNA participates in several biological processes, especially as regulatory factors in various types of malignant tumors.[22,23] Notably, lncRNA could regulate messenger RNA (mRNA) expression by acting as a competing endogenous RNA (ceRNA) to affect tumorigenesis and progression. The ceRNA hypothesis was firstly proposed by Salmena et al[24] in 2011, which establishes a new regulatory mechanism among different RNA transcripts including lncRNAs, pseudogene transcripts, miRNAs, and mRNAs. According to this hypothesis, lncRNA can interact with mRNA by sharing miRNA response elements (MREs). For instance, lncRNA *FER1L4* can regulate *PTEN* expression by acting as a sponge for *miR-106a-5p* in gastric cancer and thus accelerate gastric cell proliferation.[25] *H19* can act as a ceRNA to regulate epithelial–mesenchymal transition and mesenchymal-to-epithelial transition in bladder cancer by targeting *miR-29b-3p*.[26] Recently, lncRNAs-associated ceRNA network have been constructed in colorectal cancer,[27] gastric cancer,[25] cholangiocarcinoma,[28] head and neck squamous cell carcinoma,[29] bladder cancer,[26] and others. Nevertheless, there is still a lack of comprehensively analysis of an lncRNA-mediated ceRNA network in WT, especially based on whole transcriptome profile and large-scale sample sizes.

Therefore, the aims of this study were to identify key differentially expressed RNAs and construct an lncRNA-related ceRNA network to reveal the underlying mechanisms in carcinogenesis and development of WT, and detect novel biomarkers with prognostic value through a large-scale high-throughput sequence database. The results are expected to help further develop more personalized treatment strategies to minimize toxicity exposure for patients with WT.

Materials and Methods

Data Collection and Processing

All data in this study were available and freely downloaded from Therapeutically Applicable Research To Generate Effective Treatments (TARGET) database (https://ocg.cancer.gov/programs/target) using the TCGA data portal (https://www.cancer.gov/tcga, accessed September 5, 2018). Our research was in accordance with the publication guidelines provided by TCGA (http://cancergenome.nih.gov/publications/publicationguidelines); therefore, further approval by an ethics committee was not required.

The high-throughput sequencing data (level 3) including miRNA count data for 137 WT samples and expression level of mRNA and lncRNA for 132 individuals, and the related clinical information of 652 patients with WT were downloaded. Data were filtered for samples with recurrent solid tumor and metastatic tumor, as well as samples without 3 different RNAs expression profiles simultaneously and complete clinical data information including tumor stage, survival status, and survival time. A total of 125 filtered samples were divided into 2 cohorts: 119 primary solid tumor and 6 normal tissues. The clinical characteristics of patients with WT are summarized in Table 1. Briefly, mean age of these people at initial pathologic diagnosis was 4.7 years. The number of patients with pathological WT stages I, II, III, and IV were 15 (12.60%), 48 (40.34%), 44 (36.97%), and 12 (10.08%), respectively. The majority of patients were FHWT (favorable histology WT; 67.22%) and the rest were DAWT (diffuse anaplastic WT; 32.77%). At the time of the last follow-up, 70 (58.82%) patients were still alive, while 49 (41.18%) individuals had died.

Identification of Differentially Expressed RNAs

A total of 14 852 lncRNAs, 20 271 mRNAs, and 1881 miRNAs were annotated based on Gencode version 22 (https://www.gencodegenes.org/) and miRBase version 21 (http://www.mirbase.org/). The RNAs with a mean expression less than 1 and a median read counts equal to 0 were removed. The differentially expression analysis then was performed between normal or adjacent tissues and tumor tissues using DESeq2,[30] a Bioconductor package in R, to detect potential RNAs involved in development of WT. The false discovery rate (FDR), as proposed by the Benjamini-Hochberg,[31] was utilized for each RNA to correct statistical significance of multiple testing. Therefore, lncRNAs, mRNAs, and miRNAs were deemed as

Table 1. Corresponding Clinical Features of 119 Patients With Wilms Tumor.

	Patients, N = 119	
Items	N	%
Tumor stage		
Stage I	15	12.6
Stage II	48	40.34
Stage III	44	36.97
Stage IV	12	10.08
Histologic classification		
FHWT	80	67.22
DAWT	39	32.77
Gender		
Female	67	56.3
Male	52	43.7
Race		
Black or African American	18	15.13
White	89	74.79
Other	5	4.2
Not reported	7	5.88
Ethnicity		
Hispanic or Latino	11	9.24
Not Hispanic or Latino	80	67.23
Not reported	28	23.53
Age (years)		
<5	74	62.18
≥5	45	37.82
Survival status		
Alive	70	58.82
Dead	49	41.18

Abbreviations: DAWT, diffuse anaplastic Wilms tumor; FHWT, favorable histology Wilms tumor.

differentially expressed (DE), that is, DElncRNAs, DEmiRNAs, and DEmRNAs, when they passed the threshold of $|\log_2$ fold-change (FC)$| \geq 2$ and FDR < 0.01. Finally, the cluster heat maps and volcano plots of above DERNAs were visualized using pheatmap (version 1.0.10, Raivo Kolde, 2019, pheatmap: Pretty Heatmaps) and ggplot2[32] packages in R.

Construction of a ceRNA Network

To construct a ceRNA regulation network in terms of ceRNA hypothesis, the DElncRNA–DEmiRNA intersections were firstly predicted according to the miRcode database.[33] These paired DEmiRNAs were used to identify targeted mRNAs based on miRTarBase,[34] miRDB,[35] and TargetScan[36] databases. Differentially expressed mRNAs only retrieved in all 3 databases were defined as candidate mRNAs. The co-expression network of DERNAs was built based on DElncRNA–DEmiRNA and DEmiRNA–DEmRNA intersections, which were visualized using Cytoscape version 3.7.0 software.[37]

Functional Enrichment Analysis and Protein–Protein Interaction Network Analysis of ceRNA

Functional enrichment analyses including Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis for targeted DEmRNAs in the ceRNA network were conducted by clusterProfiler R package.[38] The cutoff of q value < 0.05 for both GO and KEGG pathways was considered as statistically significant. Moreover, a protein–protein interaction (PPI) network was constructed using STRINGdb[39] R package to investigate the molecular and cellular mechanism underlying WT tumor. The protein intersection with over 700 scores was used to detect highly interacted hub proteins by cytoHhbba[28] plugin in the Cytoscape 3.7.0 software.

Survival Analysis

To identify potential prognostic signature in patients with WT, all RNAs in the ceRNA network were performed survival analysis using survival package[40] in R platform. The WT children were divided into a high expression group and a low expression group based on median expression level of the specific RNA. Log-rank test was used to compare differences of overall survival rates between subgroups, and Kaplan-Meier method was applied to plot survival curves. Generally, P value less than .05 was considered statistically significant.

Correlation Analysis Between Key lncRNAs and Clinical Features

After screening out key RNAs according to the above-described bioinformatics analyses, χ^2 test was performed to determine whether RNAs expression was correlated with different clinical features. These features included gender (female vs male), tumor stage (I+II vs III+IV), histologic classification (FHWT vs DAWT), race (black or African American vs white), ethnicity (Hispanic or Latino vs not Hispanic or Latino), and age (≥5 years vs <5 years). Notably, the histological classification followed protocol from the Children's Oncology Group (COG).[41] A P value <.05 was used as a significant cutoff value.

Results

Identification of DEmRNAs, DEmiRNAs, and DElncRNAs in WT

The expression level of RNAs between 119 primary solid tumor and 6 normal samples was compared in this study. A total of 1833 DElncRNAs (1115 upregulated and 718 downregulated), 156 DEmiRNAs (116 upregulated and 40 downregulated), and 3443 DEmRNAs (1777 upregulated and 1666 downregulated) were identified. The distribution of all DEmRNAs, DElncRNAs, and DEmiRNAs on $-\log_{10}$(FDR) and $\log_2$ FC are shown in volcano plot (Figure 1). Furthermore, heatmap in Figure S1 shows that the WT samples are clearly distinguishable from normal samples with respect to expression profiles of these DERNAs. The top 15 upregulated and 15 downregulated DElncRNAs, DEmiRNAs, and DEmRNAs in WT samples are presented in Tables S1 to S3.

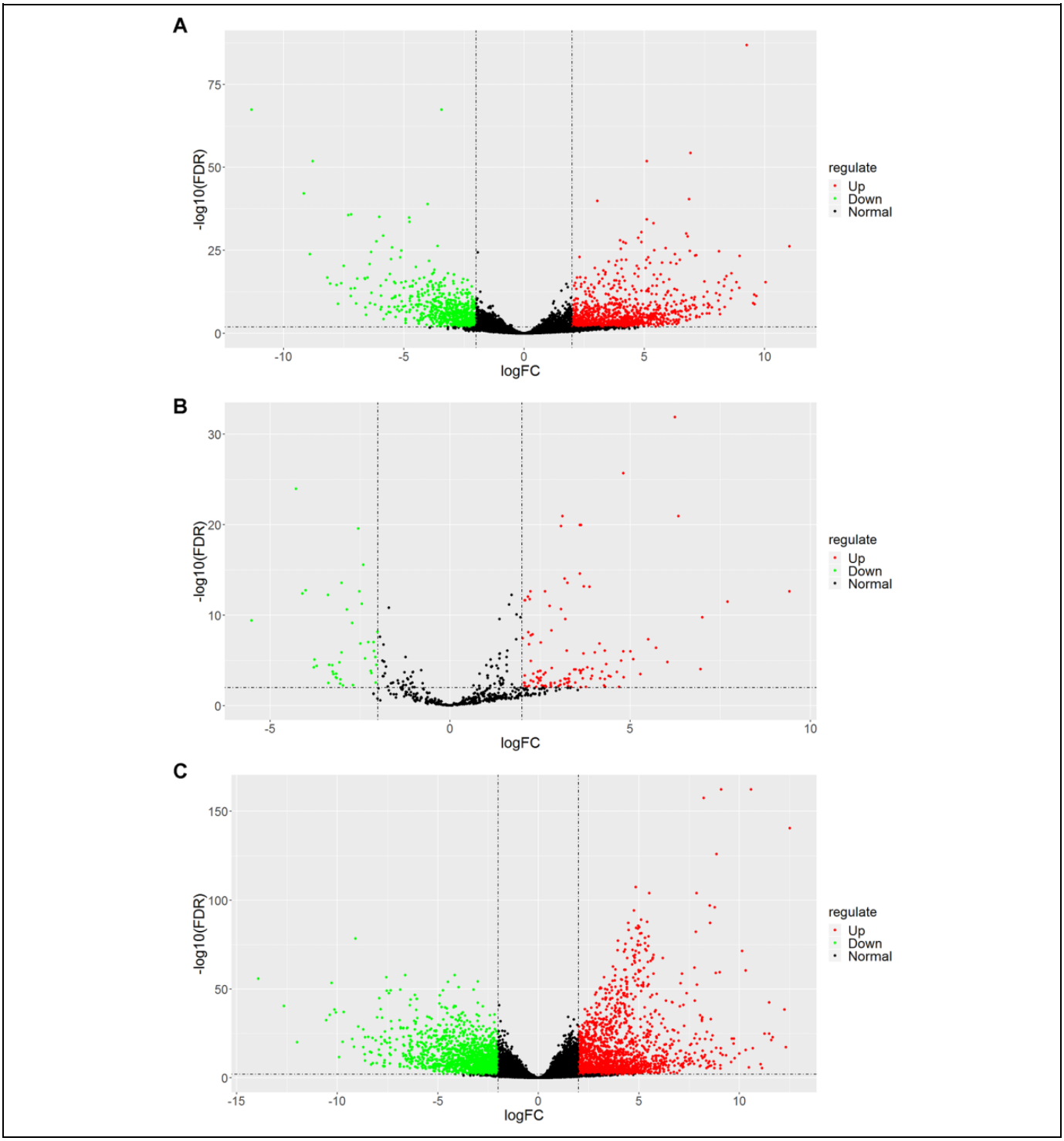

Figure 1. Volcano plots of differentially expressed lncRNAs (A), miRNAs (B), and mRNAs (C). The red spots represent upregulated RNAs and the green spots represent downregulated RNAs with statistical significance in Wilms tumor samples. lncRNAs indicates long noncoding RNAs; mRNAs, messenger RNA; miRNAs, microRNAs.

Construction of a ceRNA Network in WT

The ceRNA hypothesis suggests that lncRNAs could competitively bind with the MREs to communicate with mRNAs. To explore interactions among above DERNAs, a lncRNA-miRNA-mRNA-related ceRNA network of WT was built. First, the 1833 DElncRNAs were used to retrieve paired DEmiRNAs using miRcode database. As a result, 5032 pairs of DElncRNAs–DEmiRNAs involved in 759 DElncRNAs (eg, *SEMA3B-AS1*, *TMPO-AS1*, *RP11-576D8.4*, and *ATP1B3-AS1*)

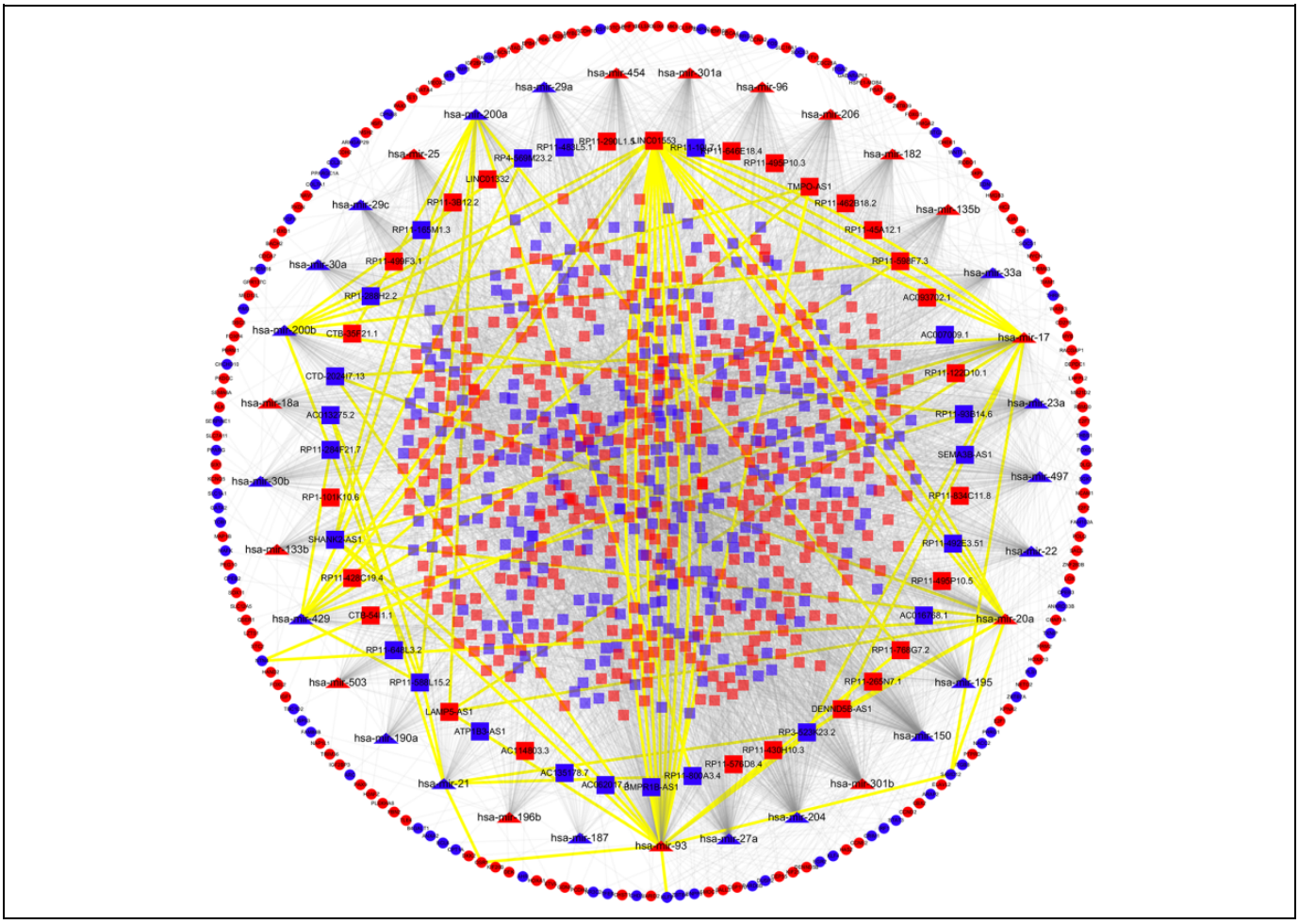

Figure 2. The lncRNA–miRNA–mRNA ceRNA regulatory network in Wilms tumor. LncRNAs, miRNAs, and mRNAs are denoted by rectangle, triangle, and ellipse, respectively. The nodes colored in red and blue, respectively, refer to upregulated and downregulated RNAs. The 62 lncRNAs–miRNAs interactions and 8 miRNAs–mRNAs interactions involved in RNAs with potential prognostic value were highlighted by yellow color. ceRNA indicates competing endogenous RNA; lncRNAs, long noncoding RNAs; mRNAs, messenger RNA; miRNAs, microRNAs.

and 39 DEmiRNAs (eg, *hsa-mir-20a*, *hsa-mir-17*, and *hsa-mir-21*) were identified for further analysis. The top 10 DElncRNAs targeted by most DEmiRNAs are presented in Table S4. Subsequently, the DEmiRNAs–DEmRNAs intersection was constructed based on the 39 DEmiRNAs through Targetscan, miRTarBase, and miRDB databases. We detected 33 DEmiRNAs interacting with 189 DEmRNAs in all 3 databases (Table S5). The 6 remaining DEmiRNAs and their 11 paired DElncRNAs were discarded. In total, 748 DElncRNAs, 33 DEmiRNAs, and 189 DEmRNAs were incorporated into the ceRNA regulatory network of WT, as shown in Figure 2. Noticeably, the lncRNA *RP11-205M3.3* with most connections could interact with 9 DEmiRNAs and 28 DEmRNAs.

Functional Enrichment Analysis and PPI Network Analysis

We subsequently explored the biological functions of 189 DEmRNAs in the ceRNA network by GO and KEGG analyses. The GO analysis revealed that these DEmRNAs significantly enriched in 440 GO terms where the top 20 GO biological processes are depicted in Figure 3A. The main biological processes implicated in WT contained in response to mechanical stimulus, muscle cell proliferation, mesenchyme development, and appendage development. The 16 significantly enriched KEGG pathways mainly included tumor-related pathways (Figure 3B), such as transcriptional misregulation in cancer, p53 signaling pathway, cell cycle, cellular senescence, and miRNAs in cancer. Finally, PPI network analysis involved 96 DEmRNAs with combined score >700 was conducted to understand crucial proteins involved in WT. Then, we used cytoHhbba app to screen out top 13 hub proteins (*E2F1*, *CCNE1*, *E2F2*, *CCNA2*, *FOS*, *GATA4*, *STAT3*, *MYBL2*, *EDN1*, *CCNE2*, *GATA2*, *CDC25A*, and *SKP2*) ranked by Matthew's correlation coefficient method from the constructed PPI network (Figure 3C).

Important RNAs Relevant to the Prognosis of Patients With WT

In order to identify potentially significant RNAs associated with the survival of patients with WT, Kaplan-Meier survival curve

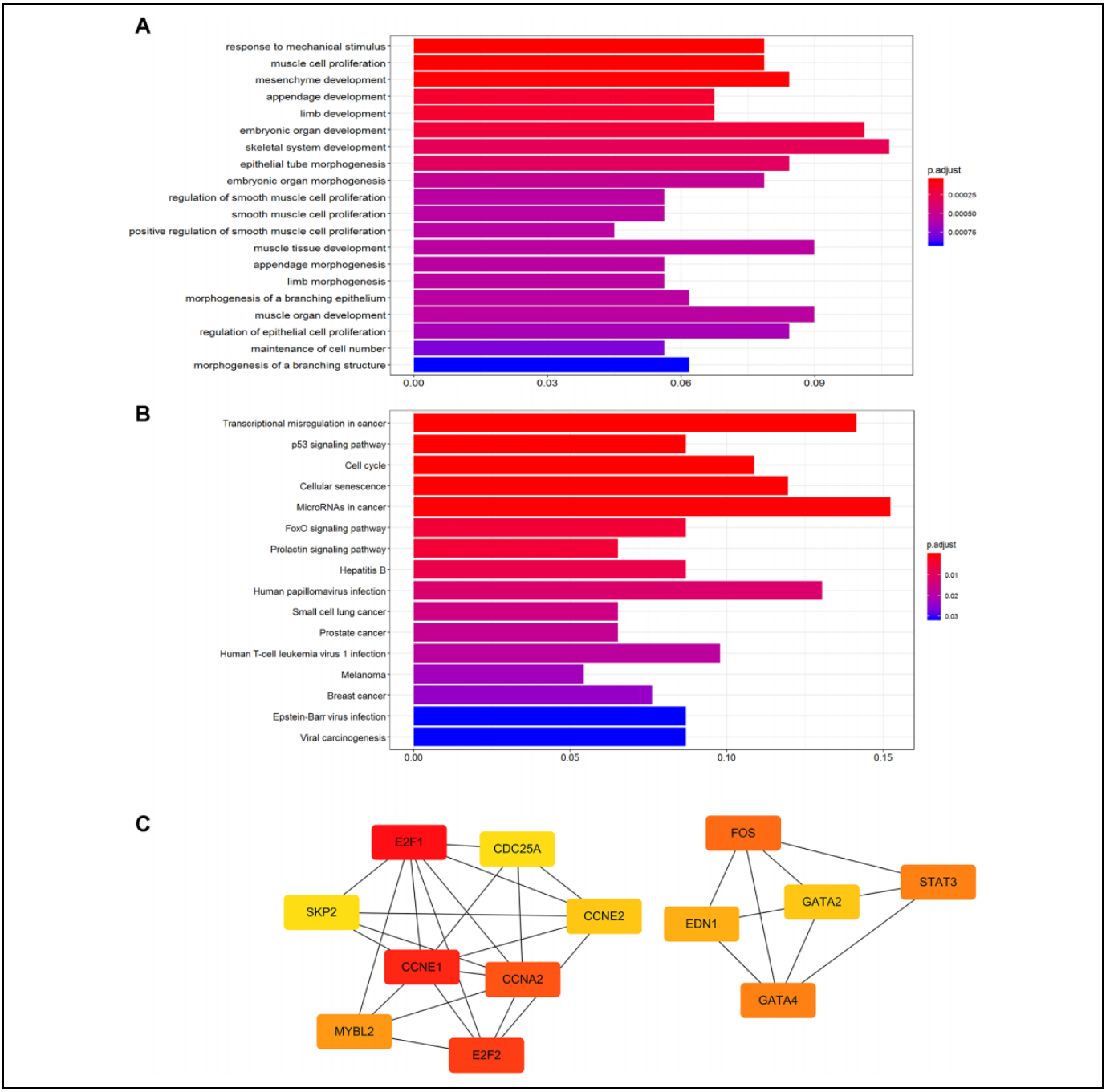

Figure 3. The functional enrichment analysis of 189 DEmRNAs in the ceRNA network and PPI network of top 13 hub DEmRNAs identified by MCC method. A, Top 20 significantly enriched GO terms (q value <0.05). B, Sixteen significantly KEGG pathways (q value <0.05). C, The node color in PPI network changes gradually from red to yellow in decreasing order according to the intersection scores. DEmRNAs indicates differentially expressed messenger RNAs; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MCC, Matthew's correlation coefficient; PPI, protein–protein interaction.

and log-rank test analysis were conducted for each RNA in the ceRNA network. Consequently, 48 lncRNAs, 7 miRNAs, and 16 mRNAs were considered as potential prognosis-related RNAs (P value <.05, Table 2). Among these, 17 of the 48 DElncRNAs were positively correlated with overall survival of the patients with WT, while the remaining 31 DElncRNAs were negatively associated with overall survival (Table 2). As shown in Figure 4A, survival curves for *RP11-576D8.4* appeared to be protective, as patients with high expression levels of *RP11-576D8.4* tended to have longer survival time, whereas *TMPO-AS1* and *SEMA3B-AS1* were considered as risk factors, as their high expression were associated with shorter overall survival time. The high expression of 7 DEmiRNAs (*hsa-mir-93*, *hsa-mir-20a*, *hsa-mir-17*, *hsa-mir-429*, *hsa-mir-21*, *hsa-mir-*

Table 2. The 48 DElncRNAs, 7 DEmiRNAs, and 16 DEmRNAs in the ceRNA Network Significantly Associated With Overall Survival of Patients With Wilms Tumor

Node	RNA[a]	Ensembl ID	Regulate	*P* value	Function[b]
DElncRNA	LINC01332*	ENSG00000250446	Up	1.01E-03	Risky
	RP11-430H10.3*	ENSG00000254651	Up	1.98E-03	Risky
	RP11-93B14.6*	ENSG00000167046	Down	2.79E-03	Risky
	AC062017.1	ENSG00000222020	Down	3.15E-03	Risky
	TMPO-AS1*	ENSG00000257167	Up	4.02E-03	Risky
	BMPR1B-AS1*	ENSG00000249599	Down	4.64E-03	Protective
	RP11-495P10.5*	ENSG00000238107	Up	5.47E-03	Risky
	CTB-54I1.1	ENSG00000254299	Up	6.74E-03	Risky
	RP11-834C11.8	ENSG00000248576	Up	7.12E-03	Risky
	RP11-45A12.1*	ENSG00000255079	Up	7.24E-03	Protective
	RP1-101K10.6*	ENSG00000227627	Up	8.42E-03	Risky
	AC135178.7	ENSG00000226871	Down	8.57E-03	Risky
	RP11-165M1.3*	ENSG00000261394	Down	9.33E-03	Protective
	AC013275.2*	ENSG00000231013	Down	1.20E-02	Protective
	RP11-483L5.1	ENSG00000255462	Down	1.23E-02	Risky
	LAMP5-AS1*	ENSG00000225988	Up	1.37E-02	Protective
	RP11-462B18.2*	ENSG00000231193	Up	1.47E-02	Risky
	RP11-800A3.4*	ENSG00000260401	Down	1.62E-02	Protective
	DENND5B-AS1*	ENSG00000255867	Up	1.77E-02	Risky
	RP11-10L7.1*	ENSG00000246375	Down	1.81E-02	Risky
	AC093702.1	ENSG00000231156	Up	2.01E-02	Risky
	CTB-35F21.1*	ENSG00000249526	Up	2.02E-02	Risky
	RP11-576D8.4*	ENSG00000224717	Up	2.08E-02	Protective
	RP11-428C19.4	ENSG00000255308	Up	2.12E-02	Risky
	SEMA3B-AS1	ENSG00000232352	Down	2.21E-02	Risky
	RP11-265N7.1*	ENSG00000259450	Up	2.34E-02	Risky
	RP11-646E18.4*	ENSG00000260377	Up	2.47E-02	Risky
	RP11-598F7.3*	ENSG00000256948	Up	2.49E-02	Risky
	RP11-122D10.1*	ENSG00000259280	Up	2.73E-02	Risky
	RP11-768G7.2*	ENSG00000241213	Up	2.88E-02	Risky
	LINC01553*	ENSG00000235931	Up	3.15E-02	Risky
	RP11-499F3.1	ENSG00000259610	Up	3.20E-02	Risky
	RP1-288H2.2	ENSG00000257989	Down	3.57E-02	Protective
	CTD-2024I7.13*	ENSG00000246422	Down	3.58E-02	Protective
	AC016768.1*	ENSG00000232451	Down	3.68E-02	Protective
	RP11-588L15.2*	ENSG00000250893	Down	3.70E-02	Protective
	ATP1B3-AS1*	ENSG00000244124	Down	3.74E-02	Protective
	AC007009.1	ENSG00000244239	Down	3.76E-02	Risky
	RP11-290L1.5	ENSG00000257329	Up	4.08E-02	Risky
	RP11-284F21.7	ENSG00000229953	Down	4.15E-02	Protective
	SHANK2-AS1*	ENSG00000226627	Down	4.21E-02	Protective
	RP11-492E3.51	ENSG00000239353	Down	4.57E-02	Protective
	RP11-3B12.2	ENSG00000237764	Up	4.60E-02	Risky
	AC114803.3	ENSG00000230432	Up	4.67E-02	Risky
	RP11-648L3.2	ENSG00000253116	Down	4.74E-02	Protective
	RP3-523K23.2*	ENSG00000261116	Down	4.75E-02	Protective
	RP11-495P10.3*	ENSG00000224481	Up	4.84E-02	Risky
	RP4-569M23.2	ENSG00000231119	Down	4.91E-02	Risky
DEmRNA	CDCA4	ENSG00000170779	Up	4.14E-03	Risky
	CCNE1	ENSG00000105173	Up	6.67E-03	Risky
	OSR1	ENSG00000143867	Up	7.81E-03	Risky
	BCL6	ENSG00000113916	Down	1.67E-02	Protective
	NTN4	ENSG00000074527	Down	2.12E-02	Protective
	KLF9	ENSG00000119138	Down	2.46E-02	Protective
	SAMD12	ENSG00000177570	Down	2.74E-02	Risky
	DEPDC1	ENSG00000024526	Up	2.88E-02	Risky
	ARC	ENSG00000198576	Down	3.47E-02	Protective

(continued)

Table 2. (continued)

Node	RNA[a]	Ensembl ID	Regulate	*P* value	Function[b]
	FOXP4	ENSG00000137166	Up	3.90E-02	Risky
	QSER1	ENSG00000060749	Up	4.14E-02	Risky
	DBF4	ENSG00000006634	Up	4.37E-02	Risky
	MYCN	ENSG00000134323	Up	4.38E-02	Risky
	IGF2BP2	ENSG00000073792	Up	4.66E-02	Risky
	CHEK1	ENSG00000149554	Up	4.68E-02	Risky
	FGF9	ENSG00000102678	Down	4.97E-02	Protective
DEmiRNA	hsa-mir-93		Up	1.71E-03	Protective
	hsa-mir-20a		Up	8.77E-03	Protective
	hsa-mir-17		Up	1.71E-02	Protective
	hsa-mir-429		Down	2.89E-02	Protective
	hsa-mir-21		Down	3.12E-02	Protective
	hsa-mir-200a		Down	3.63E-02	Protective
	hsa-mir-200b		Down	4.46E-02	Protective

Abbreviations: DE, differentially expressed; lncRNAs, long noncoding RNAs; miRNAs, microRNAs; mRNAs, messenger RNA.
[a]Star highlights DElncRNA that has a positive association with DEmRNAs through linear regression analysis.
[b]The relationship between expression level of RNAs and survival of patients with Wilms tumor. "Protective" refers to patient with high expression levels of RNA tended to have longer survival time, whereas "risky" represents their high expression were associated with shorter overall survival time.

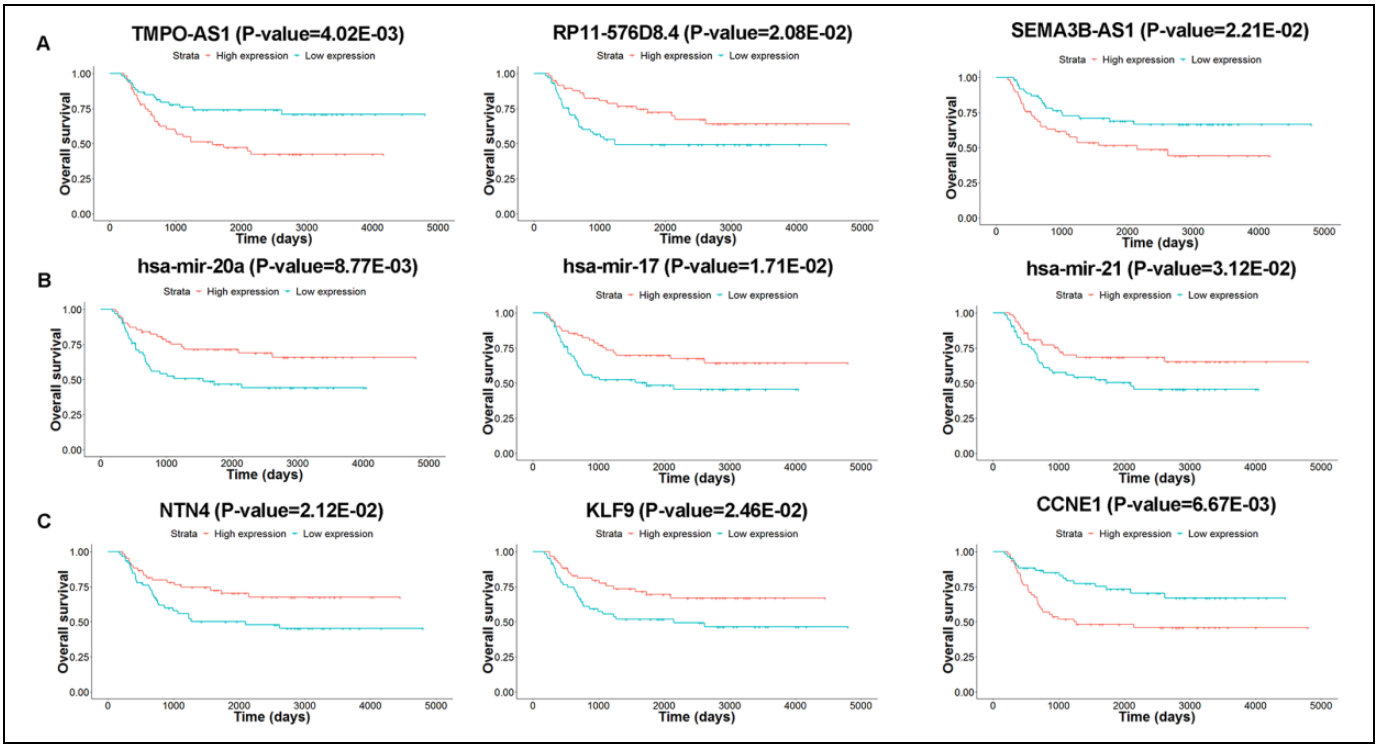

Figure 4. The Kaplan-Meier curves for RNAs associated with overall survival in Wilms tumor. A, lncRNAs for *TMPO-AS1*, *RP11-576D8.4*, and *SEMA3B-AS1*. B, miRNAs for *hsa-mir-20a*, *hsa-mir-17*, and *hsa-mir-21*. C, mRNAs for *NTN4*, *KLF9*, and *CCNE1*. Horizontal axis represents overall survival time (days) and vertical axis shows survival probability. lncRNAs indicates long noncoding RNAs; mRNAs, messenger RNA; miRNAs, microRNAs.

200a, and *hsa-mir-200b*) and 5 DEmRNAs (*BCL6*, *NTN4*, *KLF9*, *ARC*, and *FGF9*) were associated with prolonged survival time, on the contrary, high expression level of the rest of 11 DEmRNAs were related to poor prognosis (Figure 4B and C).

To confirm the speculation that lncRNAs may indirectly interacted with mRNAs in the constructed ceRNA regulatory network of WT, a linear regression analysis was conducted to analyze the relationships between expression levels of 48 prognostic DElncRNAs and 189 DEmRNAs. Consequently, 30 of 48 lncRNAs were positively correlated with 76 DEmRNAs mediated by 28 DEmiRNAs. The *P* value and correlation coefficient of 4 DElncRNAs against DEmRNAs expression level

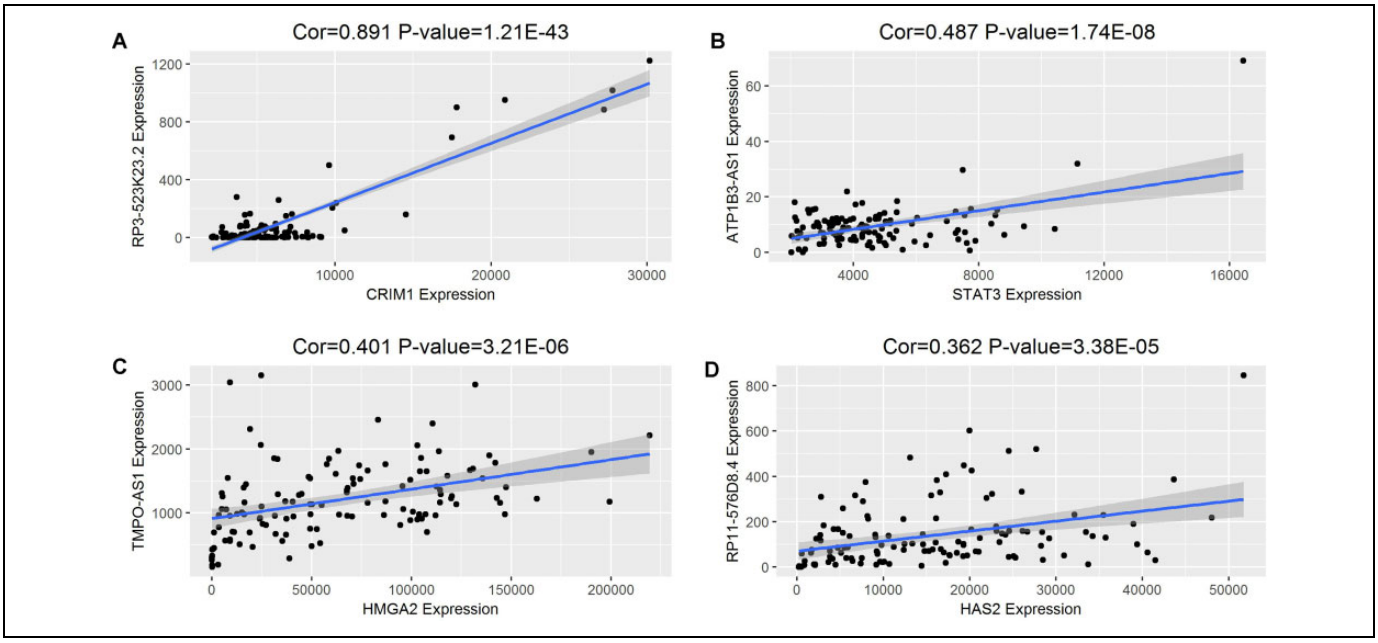

Figure 5. The linear regression of 4 representative DElncRNAs against DEmRNAs expression level including RP3-523K23.2 versus CRIM1 (A), ATP1B3-AS1 versus STAT3 (B), TMPO-AS1 versus HMGA2 (C), and RP11-576D8.4 versus HAS2 (D). The blue line represents the linear model fitted by the dots in each figure, while gray region refers to the 95% CI. "Cor" is the correlation coefficient between the expressions of lncRNA and mRNA. DE indicates differentially expressed; lncRNAs, long noncoding RNAs; mRNAs, messenger RNA; miRNAs, microRNAs.

are depicted in Figure 5. The relatively high positive correlations suggested a possibility of indirect interactions between lncRNA and mRNA expression. For example, *RP3-523K23.2* and *ATP1B3-AS1* may regulate the expression of *CRIM1* (cor = 0.891 and *P* value = 1.21E-43) and *STAT3* (cor = 0.487, *P* value = 1.74E-08), respectively.

Correlation Analysis Between Key lncRNAs and Clinical Features

To further investigate relationship between expression level of 748 lncRNAs involved in the ceRNA network and clinical features of the patients, a comprehensive analysis was performed by comparing lncRNAs expression profiles with gender, tumor stage, histologic classification, race, ethnicity, and age. A total of 133 lncRNAs were found to be associated with clinical features as summarized in Table 3. Notably, *DENND5B-AS1* and 5 DElncRNAs (*TMPO-AS1*, *RP3-523K23.2*, *RP11-598F7.3*, *LAMP5-AS1*, and *AC013275.2*) were correlated with tumor stage and histologic classification, respectively, which suggested their great value in diagnosis, therapy, and prognosis for patients with WT.

Discussion

Although numerous studies have endeavored to elucidate on the molecular mechanisms underlying WT, the exact pathogenesis remains elusive, particularly the roles of related lncRNAs. Recently, the ceRNA hypotheses have been proposed that different RNAs including lncRNAs and mRNAs could interact with each other through shared MREs. To comprehensively illustrate how lncRNA-associated ceRNA network involved in pathogenesis and progression of WT, we analyzed the large cohort of patients with WT with high-throughput sequencing data from TARGET database in this study. The aberrantly expressed RNAs (DElncRNAs, DEmiRNAs, and DEmRNAs) were identified and incorporated into construction of ceRNA network. To evaluate biological roles of dysregulated RNAs in the ceRNA network, we investigated their associations with the overall survival and clinical features of patients with WT. In addition, the functional enrichment analysis and PPI network of DEmRNAs were performed to detect hub genes.

Recently, accumulative studies have been reported that lncRNA acting as a ceRNA plays critical roles in different cancers, for instance, *UCA1*[42] in renal cell carcinoma, *FER1L4* in gastric cancer,[25] and *H19* in bladder cancer.[26] Despite considerable literature revealing key lncRNA roles in various cancers, a few studies have reported crucial association of dysregulated lncRNA as a ceRNA with progression and development of WT. To our knowledge, only 1 lncRNA (*LINC00473*) has been reported as a potential oncogene for WT through sponging *miR-195* and activating IKKα to promote tumor proliferation.[43] In our current study, 1833 DElncRNAs including *LINC00473* were detected. Additionally, 48 DElncRNAs in the ceRNA network were significantly correlated with overall survival, indicating their potential prognostic value for WT. Guo et al[44] detected that overexpression level of *SEMA3B-AS1* inhibited gastric cancer cell proliferation, migration, and invasion in vitro and had a prognostic value in gastric cardia adenocarcinoma. *TMPO-AS1* has been found to be a

Table 3. Correlations Between DElncRNAs and Clinical Features of Patients With Wilms Tumor.

Clinical parameters	Upregulate	Downregulate
Tumor stage (I+II vs III+IV)	CASC6, DENND5B-AS1, AC012462.1, AC011288.2, AC114808.2, AC007285.7, RP11-650L12.2, LINC00574, RP11-524C21.2, AC010729.2, AC010974.3, RP11-119F7.5, RP11-626G11.4, RP11-573J24.1	RP11-438D8.2, SHANK2-AS3, GRM5-AS1, CTC-321K16.1
Histologic classification (FHWT vs DAWT)	RP11-472M19.2, CTA-254O6.1, BARX1-AS1, AC079135.1, CTD-3007L5.1, RP11-429By14.4, RP11-881M11.4, TMPO-AS1, RP11-626H12.2, RP1-182D15.2, CTD-2320G14.2, LINC01280, RP11-626G11.4, RP11-847H18.3, LINC01284, LINC00337, RP11-348F1.2, AC068057.1, RP11-443B7.2, RP11-553A10.1, AP000462.1, CTD-2298J14.2, HCG23, RP1-170O19.14, RP11-343B18.2, RP11-384P7.7, LINC00595, RP1-45N11.1, CTD-2591A6.2, GPR50-AS1, RP11-3B12.5, RP11-434D9.2, AC005592.1, RP11-1084I9.1, RP11-152L7.2, AC092159.3, RP11-219B4.3, RP11-314D7.2, AC011288.2, RP11-343J3.2, RP11-379B8.1, LINC01231, RP11-560A15.3, RP11-567G11.1, AP000432.2, RP11-608O21.1, RP11-67H24.2, LAMP5-AS1, RP3-522D1.1, RP11-598F7.3, RP3-510O8.4, AC133633.2, LINC01059, DEPDC1-AS1, CTD-2587M23.1, RP11-524C21.2	LINC01484, RP4-781K5.4, RP11-386B13.4, RP11-22C11.2, AC104794.4, RP3-523K23.2, RP11-327J17.2, WWTR1-AS1, RP11-311H10.4, LINC00505, RP11-106M7.1, RP5-884G6.2, RP1-18D14.7,RP1-187B23.1, KCNC4-AS1, RP11-710F7.2, GSN-AS1, AC013275.2, RP11-67L3.5,
Gender (female vs male)	AC012442.5, AC079150.3, RP11-907D1.1, RP11-54O7.1, TMPO-AS1, AC003092.2, RP11-9L18.3, RP11-430H10.3	CTC-498J12.1, DDX39B-AS1, RP11-165M1.3, RP11-403P17.2
Race (white vs black or African American)	RP11-1042B17.3, CTD-2147F2.1	AP001627.1, RP1-187B23.1, AC012485.2, LINC01123
Ethnicity (not Hispanic or Latino vs Hispanic or Latino)	RP11-626G11.4, RP11-13E5.2, RP11-847H18.3, CTD-2130F23.2, RP11-897M7.1, RP11-495P10.6, CTD-2544H17.1, LINC01096	KCNC4-AS1, GLIS3-AS1, RP11-21A7A.4, RP5-1021I20.2
Age (≥5 vs <5 years)	FAM225A, CTD-2383M3.1, CTD-2116N20.1, RP11-67H24.2, RP11-474D1.4, AC005592.1, RP11-266N13.2, LINC01310, AC068057.1, RP11-575H3.1, RP11-316I3.1, STARD4-AS1, RP11-64P14.7, RP11-893F2.5, RP11-307C19.1, RP11-292D4.1, RP11-58A18.1, LINC00390 RP11-16O9.2	RP11-800A3.7, WWTR1-AS1, GATA2-AS1, RP11-386B13.4

diagnostic and prognostic marker for prostate cancer[45] and could be a prognostic signature for stages I and II lung adenocarcinoma.[46] The linear regression of expression of *TMPO-AS1* against mRNAs in the present study indicated that *TMPO-AS1* may interact with 5 genes (*FSCN1*, *HMGA2*, *MYB*, *DENND5B*, and *ETV5*) mediated by *hsa-mir-200b*, *hsa-mir-429*, *hsa-mir-33a*, and *hsa-mir-150* (Figure 5). In addition, we observed that *RP11-576D8.4* had a potential to indirectly regulate 3 genes (*HAS2*, *JARID2*, and *CDH2*) through *hsa-mir-204* (Figure 5) in this study. Yuan et al[47] reported that *RP11-576D8.4* was upregulated in triple negative breast cancer. Moreover, *ATP1B3-AS1* has been recently reported to be aberrantly expressed in uninfected infants of HIV-positive mothers and HIV-negative mothers.[48] The linear regression results showed that *ATP1B3-AS1* interacted with 24 genes mediated by *hsa-mir-96*, *hsa-mir-93*, and *hsa-mir-182*. To our best knowledge, the other 44 lncRNAs identified in our study are novel potential biomarkers in survival prediction, which provide new clues to further investigate the molecular mechanism underling initiation and progression of WT.

It is widely acknowledged that miRNA, a bridge of the ceRNA network, binds with lncRNA and mRNA in different cancers, including WT. In our study, 7 miRNAs in the ceRNA were associated with overall survival rate, 3 (*hsa-mir-20a*, *hsa-mir-17*, and *hsa-mir-21*) of which agreed with reports by other literature. He et al[49] identified that the *hsa-mir-20a* and *hsa-mir-17* were involved in the DE regulatory network of WT, which were targeted by transcription factor *E2F3.* In addition, *hsa-mir-20a* and *hsa-mir-17-5p* were upregulated in WT and acted as an oncogene.[18,49] Upregulated *miR-21* indicated a poor prognosis of WT, and the *miR-21* expression level was associated with clinicopathological parameters.[50] However, the roles

of *hsa-mir-93*, *hsa-mir-429*, *hsa-mir-200a*, and *hsa-mir-200b* in WT have not been reported.

In our constructed ceRNA network, 16 of 189 DEmRNAs were detected to be significantly correlated with survival time of patients with WT. Five DEmRNAs (*BCL6*, *NTN4*, *KLF9*, *ARC*, and *FGF9*) were protective and the other 11 DEmRNAs (*CDCA4*, *CCNE1*, *OSR1*, *SAMD12*, *DEPDC1*, *FOXP4*, *QSER1*, *DBF4*, *MYCN*, *IGF2BP2*, and *CHEK1*) were unfavorable. These results suggested that these 16 genes might play important roles in progression of WT and could serve as potential prognostic factors for patients with WT. Some of the 16 mRNAs have been reported to have a substantial influence on several diseases. Paschou et al[51] revealed that *NTN4* directed axon outgrowth and guidance as an extracellular protein and could be a candidate gene for Tourette syndrome susceptibility. The *KLF9* was a valuable prognostic factor and its high expression level meant longer survival time in human pancreatic ductal adenocarcinoma.[52] Huang et al[53] showed that *DEPDC1* promoted cell proliferation and might be a potential therapeutic target of prostate cancer. Furthermore, GO and KEGG analyses revealed that DEmRNAs in the ceRNA network might be involved in cell proliferation and tumor-related biological processes, such as p53 signaling pathway, a well-known cancer-related pathway. Lahoti et al[54] detected that *p53* expression associated with the development of WT, and *p53* immunopositivity indicated negative outcome. Besides, most of the hub DEmRNAs identified by PPI network have been well documented as closely correlated with carcinogenesis and progression of WT. The *CCNE1* gene, which is an essential gene for controlling cell cycle, was detected to be negatively associated with *WWOX* (a tumor suppressor gene) in WT using real-time reverse transcript polymerase chain reaction experiment.[55] *STAT3* activation can promote WT proliferation and indicated unfavorable prognosis in WT.[56] In addition, Cao et al[57] found that *STAT3* could inhibit *WTX* expression by regulating *microRNA-370* in WT cells.

Due to the high correlation of 133 RNAs expression level with clinical features (Table 3), we performed multivariable Cox regression analyses for each RNA with potential prognostic value and 4 vital clinical variables including tumor stage (I+II vs III+IV), histologic classification (FAWT vs DAWT), gender (female vs male), and age ($\geq$5 vs <5) to investigate the independence of RNAs in survival prediction. The regression coefficients of RNAs and clinical features are listed in Table S6, where red color represents significance of variables due to its P value less than .05. We found that tumor stage, gender, and age had large coefficients and were significant for most RNAs, whereas histologic classification derived from COG had slightly impact on survival prediction. Despite of the effect of clinical features, few RNAs still had significant prognostic value, which were 4 DElncRNAs (*AC135178.7*, *AC093702.1*, *RP11-483L5.1*, and *RP3-523K23.2*) and 3 DEmiRNA (*hsa-mir-200a*, *hsa-mir-200b*, and *hsa-mir-429*). These results suggested single RNA as a prognostic biomarker was largely dependent from clinical factors, and integrating multiple RNAs as a risk index would be promising as previously proposed by Golub et al.[58]

Conclusion

In the current study, we identified WT-specific DElncRNAs, DEmiRNAs, and DEmRNAs through multiple bioinformatics analyses of genome-wide transcriptome profiling. We successfully constructed an lncRNA-associated ceRNA network and found some key RNAs significantly associated with overall survival and clinical features. Some of these identified key RNAs were agreement with the previous reports; however, majority of them were identified to be associated with WT for the first time by our study. These results further provide a novel insight into better understanding of the molecular genetic mechanisms underlying pathogenesis of WT and enrich the database to develop targeted therapies and biomarkers that would minimize toxicity for patients. In addition, the mismatched data on WT and normal samples from TARGET database may lead to systematic bias in identification of DERNAs, and therefore in vitro and in vivo experiments for above candidate RNAs are needed for further validation.

Authors' Note

XH and ZF conceived and designed the study. ZF, ZL, DL, and TX downloaded clinical and RNA sequence data and performed data analyses. CQ, XZ, LR, and ZH prepared figures and tables. ZF, ZL, and MR wrote the manuscript. All authors read, commented, and approved the final manuscript.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics

All data in this study were freely downloaded from Therapeutically Applicable Research To Generate Effective Treatments (TARGET) database (https://ocg.cancer.gov/programs/target) using the TCGA data portal (https://www.cancer.gov/tcga, accessed September 5, 2018). Our research was in accordance with the publication guidelines provided by TCGA (http://cancergenome.nih.gov/publications/publicationguidelines); therefore, further approval by an ethics committee was not required.

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ORCID iD

Feng Zhang https://orcid.org/0000-0003-2723-4037

Supplemental Material

Supplemental material for this article is available online.

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