Competitive Endogenous RNA (ceRNA) Regulation Network of lncRNA–miRNA–mRNA in wilms tumor

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Abstract

Background

Competitive endogenous RNA (ceRNA) have revealed a new mechanism of interaction between RNAs. However, such comprehension of the ceRNA regulatory network in wilms tumor remains limited.

Methods

Raw RNA sequencing profiles regarding mRNAs, miRNAs and IncRNAs on wilms tumor samples and normal samples were obtained from Therapeutically Applicable Research to Generate Effective Treatment (TARGET). EdgeR package was applied to identify differentially expressed IncRNAs, miRNAs and mRNAs. Functional enrichment analysis were conducted via DAVID database and the ClusterProfile R package. The IncRNA–miRNA–mRNA interaction ceRNA network was established in Cytoscape according to the identified IncRNAs–miRNAs and miRNAs–mRNAs interactions. Subsequently, correlation between ceRNA network and overall survival prognosis were analyzed.

Results

A total of 2,037 IncRNAs, 154 miRNAs and 3,609 mRNAs were identified as differentially expressed RNAs in wilms tumor. 205 IncRNAs, 26 miRNAs and 143 mRNAs were included in ceRNA regulatory network. Analysis results showed that 14 out of the 205 IncRNAs, 1 out of 26 miRNAs and 8 out of 143 mRNAs were associated with overall survival in wilms tumor patients (P < 0.05).

Conclusions

CeRNA networks played an important role in wilms tumor. This might provide effective bioinformatics basis and novel insights for further understanding of the mechanisms underlying wilms tumor.

Background

Wilms tumor (WT) is the most common type of pediatric renal malignancy. WT have a poor prognosis although 5-year overall survival rate is constantly improved with the advancement of disease-associated therapies. Chemotherapy, surgery and radiation therapy are the main treatment strategies for WT. However, 50% of children who have a recurrence after these treatments go on to die from this tumor. Novel therapeutic treatment targeting specific mechanisms of WT is still lacking.
Previous studies demonstrated that numerous key Long non-coding RNA (lncRNA), microRNA (miRNA) and mRNAs are closely related with pathogenesis of WT, such as LINC004734, miR-483-5p5, miR-1954 and HACE16. However, there were little reports in prognosis biomarkers in WT. If WT patients who were more likely to have a poor prognosis according to these prognosis biomarker result could be identified, clinician might apply more aggressive treatment. If WT patients with low risk could be identified, treatment with less morbidity could be administered. Prognosis biomarkers and targeted cure in WT were required to be identified in order to improve the clinical outcomes.

In the last decade, complexity of the human genome could be revealed by advanced RNA sequencing analysis7. Under such circumstance, competing endogenous RNA (ceRNA) hypothesis was presented which demonstrated that lncRNAs could communicate with common miRNA response elements with miRNA to construct an intricate interplay network and ultimately crosstalk with mRNA. The involvement of ceRNA regulatory network tumor initiation and progression was validated in previous studies8,9. However, specific ceRNA regulatory network (lncRNA-miRNA-mRNA) in WT still remained unelucidated.

In present study, ceRNA regulatory network (205 mRNAs, 26 lncRNAs and 143 miRNAs) was constructed to promote the understanding how lncRNAs sponged miRNA to regulated gene expression in WT. Subsequently, survival analysis and functional analysis were used to promote new understanding of the role of ceRNA regulatory network in WT carcinogenesis. The present study might give insight into molecular mechanism which participate in the progression and tumorigenesis of WT.

Materials And Methods

Data collection and preprocessing

All the data were retrieved from Therapeutically Applicable Research to Generate Effective Treatment (TARGET: https://ocg.cancer.gov/programs/target) database. All the data (lncRNAs, mRNAs and miRNAs) in present study were publicly available. Ethics committee approval was not required because data in present study was obtained from TARGET database. Among them, miRNA expression data were acquired from 138 samples, including 6 normal samples and 132 WT samples. And mRNA
and lncRNA expression data were acquired from 132 samples, including 6 normal samples and 126 WT samples. The differentially expressed IncRNAs (DEls), differentially expressed miRNAs (DEMs) and differentially expressed genes (DEGs) between WT and normal samples were carried out via EdgeR package in R software (Version 3.2.2). \(|\text{logFC(fold change)}| > 1\) and false discovery rate (FDR) of <0.05 were defined as cut-off criteria.

Prediction of lncRNA-miRNA and miRNA-mRNA interactions

DELs-DEM–DEGs interactions were divided into DELs-DEM and DEM-DEGs pairs. starBase database (http://starbase.sysu.edu.cn/) was applied to change the miRNA sequences. miRcode (http://www.mircode.org/) was effective online software which could provide the interactions between IncRNAs and miRNAs. MiRTarBase (http://mirtarbase.mbc.nctu.edu.tw), miRDB (http://www.mirdb.org/miRDB/), and TargetScan (http://www.targetscan.org/) online tools were applied to retrieve and predict target mRNAs of miRNA. Venny diagram was applied to acquire overlapping portion of target miRNAs and mRNAs. Matched DELs–DEM and DEM-DEGs interactions were screened for further bioinformatics analysis.

CeRNA network construction and functional enrichment analysis

Cytoscape software (Version 3.6.1) was applied to construct and visualize the DELs–DEM–DEGs ceRNA network. Cytoscape was an effective software to visualize the molecular interaction networks according to gene expression profiles and annotations. In order to better comprehend the tumorigenesis mechanisms in WT, Gene Ontology functional enrichment analysis (GO) and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis (KEGG) of DEGs in ceRNA network were performed via DAVID (the Database for Annotation, Visualization and Integrated Discovery). FDR <0.05 was defined as cut-off criteria.

Survival analysis

The log-rank test and Kaplan-Meier analysis were applied to identify prognostic DELs, DEMs and DEGs signatures. The survival curves were constructed using the ‘survival’ package. Survival analysis was
performed according to KaplanMeier univariate survival analysis. P<0.05 were selected as statistical threshold value. All the survival analysis was conducted in R software (version: 3.3.2).

Results

DELS, DEGs and DEMs in WT.

LncRNA, mRNA, and miRNA expression profiles between WT samples and normal samples acquired from TARGET were analyzed in present study. 2,037 DELs, 154 DEMs and 3,609 DEGs, were screened out in present study. 1,247 up-regulated and 790 down-regulated DELs were identified in WT with cut-off threshold of | logFC (fold change) | >1 and false discovery rate (FDR) of <0.05. DELs distribution between WT and normal controls were presented as heatmap plot in Figure 1A. According to the cut-off threshold of | logFC (fold change) | >1 and false discovery rate (FDR) of <0.05, 105 up-regulated and 49 down-regulated DEMs were identified in WT. Heatmap plot of the related DEMs between WT and normal controls is shown in Figure 1B. A total of 1,894 up-regulated and 1,715 down-regulated DEGs were identified in WT. DEGs distribution between WT and normal controls were presented as a heatmap plot in Figure 1C. The top 10 upregulated and downregulated DELs, DEMs and DEGs were showed in Table 1.

CeRNA network construction and Functional enrichment analysis

A dysregulated LncRNA-miRNA-mRNA ceRNA network in WT was constructed according to the interactions of 980 DEL–DEM pairs and 235 DEM–DEG pairs between 205 DELs, 26 DEMs, and 143 DEGs. Finally, LncRNA-miRNA-mRNA ceRNA regulatory network visualized in Cytoscape was presented in Figure 2.

GO and KEGG analysis were also performed to reveal the functions of the 16 DEGs which were included in the ceRNA network. For “biological processes (BP)”, the top five terms were response to mechanical stimulus, G1/S transition of mitotic cell cycle, cell cycle G1/S phase transition, muscle cell proliferation, mesenchymal cell differentiation; for the “cellular component (CC)”, the top five terms were transcription factor complex, cyclin-dependent protein kinase holoenzyme complex, nuclear chromatin, nuclear transcription factor complex, Flemming body; The top five “molecular function (MF)” terms were transcription factor activity and RNA polymerase II proximal promoter sequence-
specific DNA binding, transcriptional activator activity and RNA polymerase II transcription regulatory region sequence-specific DNA binding, proximal promoter sequence-specific DNA binding, transcriptional activator activity and RNA polymerase II proximal promoter sequence-specific DNA binding, transcriptional repressor activity and RNA polymerase II transcription regulatory region sequence-specific DNA binding (Table 2). Additionally, KEGG pathway analysis showed that DEGs were enriched in 30 pathways, such as Cell cycle, Small cell lung cancer, p53 signaling pathway, MicroRNAs in cancer, Cellular senescence (Table 2 and Figure 3).

Survival analysis
Lapland-Meier univariate survival analysis was performed via combined information of clinical information and gene expression profiles of 205 IncRNAs, 26 miRNAs and 1 43 mRNAs in the ceRNA network in WT samples. According to analysis results, 14 IncRNAs (AC005609.1, AC135178.1, ADAMTS9-AS1, AL391832.1, AL445228.2, DENND5B-AS1, DLEU2, GRM7-AS3, LINC00303, LINC00473, MEG3, MYB-AS1, NRG1-IT1, RMST) among 205 DELs were closely related to overall survival of WT patients (Figure 4). For AC005609.1, AC135178.1, AL391832.1, AL445228.2, DENND5B-AS1, DLEU2, GRM7-AS3, LINC00303 and LINC00473, low expression was related to a high overall survival rate in WT patients. High expression of ADAMTS9-AS1, MEG3, MYB-AS1, NRG1-IT1, and RMST were related with high survival rates in WT patients. In addition, 1 miRNA (hsa-mir-200a) among 26 DEMs were closely related to prognosis of WT patients (Figure 5). High expression of hsa-mir-200a were related with high survival rates in WT patients. 8 mRNAs (CDCA4, CEP55, DEPDC1, KIAA0922, OSR1, PHF19, PLEKHA8, ZBTB4) among 143 DEGs were closely related to overall survival of WT patients (Figure 6). For CDCA4, CEP55, DEPDC1, OSR1, PHF19 and PLEKHA8, low expression was related to a high overall survival rate in WT patients. High expression of KIAA0922 and ZBTB4 were significantly related with high survival rates in WT patients.

Discussion
WT is a type of pediatric renal malignancy. Although overall survival in WT patients is constantly improved, disease recurrence and poor prognosis are still the main cause of cancer-related death in childhood. Dysregulated genes are considered as the major cause of tumorigenesis of WT.
Recently, more and more attentions are paid to the crucial roles of ceRNA network in gene expression regulation at three levels (transcription, post-transcription and translation). Previous study reported regulatory role of ceRNA networks in proliferation, metastasis and invasion of cancer\textsuperscript{14,15}. To better comprehend how ceRNA regulatory network affected WT, a large-scale WT data from TARGET database were analyzed and dysregulated ceRNA regulatory network in WT were constructed successfully. A dysregulated ceRNA network in WT was constructed according to the interactions of 980 DEL-DEM pairs and 235 DEM-DEG pairs between 205 DELs, 26 DEMs, and 143 DEGs. In addition, Kaplan-Meier curve analysis was also applied to identify prognosis biomarker in WT. And 14 differentially expressed lncRNAs (AC005609.1, AC135178.1, ADAMTS9-AS1, AL391832.1, AL445228.2, DENND5B-AS1, DLEU2, GRM7-AS3, LINC00303, LINC00473, MEG3, MYB-AS1, NRG1-IT1, RMST), 1 differentially expressed miRNA (hsa-mir-200a) and 8 differentially expressed mRNAs (CDCA4, CEP55, DEPDC1, KIAA0922, OSR1, PHF19, PLEKHA8, ZBTB4) were showed to be significantly associated with overall survival rate in WT.

Long noncoding RNAs (lncRNAs) are defined as noncoding RNAs longer than 200 nucleotides\textsuperscript{16}. lncRNAs were showed to be involved in a variety of biological regulatory functions including metastasis and tumorigenesis of cancer\textsuperscript{17,18}. A total of 1,247 up-regulated and 790 down-regulated DELs were identified in present study. 205 DELs were included in construction of ceRNA network. And 14 out of the 205 DELs were associated with overall survival in WT patients (P < 0.05). Some differentially expressed lncRNAs in our analysis result have been investigated in WT: for example, LINC00473 was capable to decrease miR-195 expression level and inhibit miR-195’s function in WT\textsuperscript{19}. Dysregulated IncRNA signature LINC00473/miR-195/IKK\textalpha was showed to act as pro-tumour pathogenesis role in WT\textsuperscript{19}. The above-mentioned molecular experiments partially supported our analysis result in present study. Such identified IncRNAs might serve as prognosis biomarkers and therapeutic targets in WT. In previous studies, RMST might inhibit cell proliferation, invasion, migration, and enhance cell apoptosis, and regulate cell cycle to acted as a tumor suppressor in triple-negative breast cancer\textsuperscript{20}. MEG3, a myeloid-related IncRNA, played a tumor suppressor role in
various solid neoplasms\textsuperscript{21,22}. Dleu2 could control miR-16-1 to regulate proliferation, invasion and migration of laryngeal cancer\textsuperscript{23}. ADAMTS9-AS1 were related to overall survival of breast cancer patients\textsuperscript{24}, bladder cancer\textsuperscript{25} and colon adenocarcinoma\textsuperscript{26}. Few studies, however, have explored the relationship between above-mentioned DELs and tumorigenesis of WT. Additionally, little is known about the regulatory role of LINC00303, GRM7-AS3, DLEU2, DENND5B-AS1, AL445228.2, AL391832.1, AC135178.1 and AC005609.1 in cancer. Therefore, further studies are needed to illuminate the molecular and biological mechanism of these DELs in WT.

MicroRNAs are single-stranded and 18-25 nucleotide-long noncoding RNA which targets mRNAs to control gene expression\textsuperscript{27}. DEMs in WT, including 105 up-regulated and 49 down-regulated DEMs, were summarized in present study. In present study, ceRNA network contained 14 differentially expressed miRNAs. However, only one miRNA (miR-200a) were related to overall survival in WT patients. MiR-200a was an important member of miR - 200 family. It had been reported that miR-200a was involved in several biological processes to control progression of cancer\textsuperscript{28,29}. MiR-200a was showed to target FOXA1 and acts as a tumor suppressor on the survival, proliferation and invasion of glioma cells\textsuperscript{29}. And miR-200a might inactivate BRD4-mediated AR signaling to inhibit progression of prostate cancer\textsuperscript{28}. Moreover, previous studies reported that miR-200a is associated with the development and occurrence of esophageal cancer, breast cancer and endometrial cancer, breast cancer, and esophageal cancer by targeting specific genes, such as CRMP-1, EPHA2 and PTEN\textsuperscript{30-32}. However, there is no research to clearly elucidate the role of miR-200a in WT. The understanding of miR-200a in the progression of WT is limited which require more targeted molecular studies to confirm its role.

To further investigate related cellular mechanisms in WT, GO and KEGG analysis of 1 43 DEGs in the ceRNA network was performed. GO analysis result shows that that DEGs were mainly enriched in mechanical stimulus, G1/S transition of mitotic cell cycle, cell cycle G1/S phase transition, muscle cell
proliferation, mesenchymal cell differentiation. KEGG analysis result indicated that DEGs in ceRNA network were mainly enriched in Cell cycle, Small cell lung cancer, p53 signaling pathway, MicroRNAs in cancer, Cellular senescence. In recent years, many studies have found the same research findings. PI3K-AKT-p53 signaling pathway was involved in the tumorigenesis of WT which might represent a potential treatment in the future. MiRNAs, single-stranded and 18-25 nucleotide-long noncoding RNA, were involved in regulate the proliferation, cell cycle and apoptosis of WT. Cellular senescence was reported to responsible for restricted proliferation in WT, which had been linked to increased p21 expression and was independent of p53 expression. The above-mentioned related researches and experiments partially supported our GO and KEGG analysis result in present study.

The major limitation of present study is that tumor tissue and blood verification of these differentially expressed IncRNAs, miRNAs, mRNA and relative pathways is lacking. Further targeted studies related to this ceRNA network still need to be designed to verify and investigate these valuable ncRNAs in the progression of WT.

Conclusion
In summary, differentially expressed IncRNAs, mRNAs, and miRNAs were identified and functional IncRNA-miRNA-mRNA ceRNA regulatory network for WT tumorigenesis was successfully constructed. Significantly altered IncRNAs, mRNAs and miRNAs might serve as prognosis biomarkers and therapeutic targets for tumorigenesis of WT. The ceRNA regulatory network might illuminate inner molecular mechanism which was involved in progression and tumorigenesis of WT.

Abbreviations
WT – Wilms tumor
ceRNA – Competitive endogenous RNA
TARGET – Therapeutically Applicable Research to Generate Effective Treatment
IncRNA – Long non-coding RNA
miRNA – microRNA
DELS – differentially expressed IncRNAs
DEMsdifferentially expressed miRNAs
DEGs
differentially expressed genes
FDRfalse discovery rate
GOGene Ontology
KEGGKyoto Encyclopedia of Genes and Genomes pathway
DAVIDThe Database for Annotation, Visualization and Integrated Discovery

Declarations

Funding
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Availability of data and materials
The datasets used and analyzed during the current study download from Therapeutically Applicable Research to Generate Effective Treatment database(https://ocg.cancer.gov/programs/target).

Authors’ contributions
Zhaohui He, Fucai Tang and Zechao Lu conceived the study; Zhaohui He and Fucai Tang conducted the work; Zhibiao Li, weijia Wu and Haifeng Duan obtaining and analyzed the data; Fucai Tang, Zechao Lu and Jiaming Wang wrote the paper. All the authors listed have read and approved the manuscript.

Ethics approval and consent to participate
The present study, the data download from Therapeutically Applicable Research to Generate Effective Treatment database, therefore, this article does not contain any studies with human participants or animals performed by any of the authors. thus no ethical approval and patient consent are required.

Consent for publication
Not applicable.

Conflicts of Interests
All authors declare that they have no conflict of interest to state.

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Tables
Due to technical limitations, Tables 1 and 2 are only available as downloads from the supplemental file section.

Table 1. The top 10 up-regulated and down-regulated differentially expressed genes

Table 2. The enriched Gene Ontology terms and Kyoto Encyclopedia of Genes and Genomes of differentially expressed genes.

Figures
Figure 1

Heatmap analysis of differentially expressed IncRNAs (A), miRNAs (B), and mRNA (C) (top 30). Each row represents a sample, and each column represents an IncRNA, miRNA, or mRNA. High- or low-relative expression is displayed as a red or green strip, respectively.
Competitive endogenous RNA (ceRNA) regulation network of 205 lncRNAs, 26 miRNAs and 143 mRNAs in wilms tumor. The diamond indicates LncRNAs; The triangle indicates miRNAs; The circle indicates mRNAs; Blue indicates down-regulated RNAs; red indicates up-regulated RNAs.
Figure 3

The top 20 Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis of genes involved in the ceRNA regulation network of Wilms tumor.
Figure 4

The top 20 Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis of genes involved in the ceRNA regulation network of Wilms tumor.
Kaplan-Meier curve of hsa-mir-200a that are significantly associated with overall survival in Wilms tumor patients.

$P = 0.0225$
Figure 6

Kaplan-Meier curve of mRNAs that are significantly associated with overall survival in Wilms tumor patients.

Supplementary Files
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- table 2.pdf
- table 1.pdf