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The Competing Endogenous RNA Network of Hsa-mir-429/SEC23A as a Biomarker Pair of Rhabdoid Tumor

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***Abstract*—In this study, we aim at finding the biomarker and competing endogenous RNA (ceRNA) network analysis of rhabdoid tumor (RT) in kid's patients based on the RT subproject of the Therapeutically Applicable Research to Generate Effective Treatment (TARGET) kidney project. The differentially expressed genes (DEGs) were screened according to the RNA-seq, miRNA-seq and clinical files downloaded from TARGET website. 533 differentially expressed lncRNAs (DElncRNAs), 86 differentially expressed miRNAs (DEmiRNAs) and 2687 differentially expressed mRNAs (DEmRNAs) were found. In the ceRNA regulatory analysis, 57 DElncRNAs and 14 DEmiRNAs interacted in 223 pairs. And only one of the DEmiRNAs interacted with 4 DEmRNAs. In the clustering analysis of the DEGs, hsa-mir-429 was down-expressed while SEC23A was over-expressed. By using the kaplan-meier method in 5-year survival analysis, the tissues with dys-regulated hsa-mir-429 or SEC23A was in a 10% lower survival rate than the normal expression ones. hsa-mir-429 and SEC23A could be the regulatory biomarker pair of RT.**

Keywords*—target; rhabdoid tumor; ceRNA; survival analysis; clustering analysis

I. INTRODUCTION

Rhabdoid tumor (RT) is a highly malignant disease with poor prognosis among kids. It's one kind of aggressive kidney tumors [1, 2]. Our work was based on the RT subproject of the TARGET kidney project. Gene expression data of normal tissues and tumor tissues were downloaded from the TARGET database [1]. By using clustering Analytical limma R package, the DElncRNAs, DEmiRNAs and DEmRNAs between normal and tumor samples were screened [3-5]. Based on the regulations among the DElncRNAs, DEmiRNAs and DEmRNAs, the ceRNA network was built [4]. The dys-regulated mRNA in the ceRNA network was regarded as the RT biomarker and proved by kaplan-meier analytical method.

II. MATERIALS AND METHODS

Our research was based on the data that downloaded from TARGET online database (ocg.cancer.gov/programs/target) which focus on the project of childhood cancer research. 78 RNA-seq and 79 miRNA-seq txt files were obtained from the database which relied on illumine hi-seq 2000 platform [1]. 6 of the RNA-seq and 6 of the miRNA-seq files were from the same 6 normal samples. There were 1595 miRNAs expression data in each miRNA-seq file. As shown in Table I, 4534 lncRNAs, 17447 mRNAs and 1595 miRNAs were included in the RNA-seq files.

TABLE I. INFORMATION OF THE TARGET RT RNA-SEQ FILES

Data type	type	File number	Gene type	Gene number
RNA-seq	normal	6	lncRNA	4534
	tumor	72	mRNA	17447
miRNA-seq	normal	6	miRNA	1595
	tumor	73		

The downloaded files were merged into 3 matrixes: lncRNA-matrix, mRNA-matrix and miRNA-matrix. The clinical excel files were downloaded. 58 clinical data of the sample was extracted.

TABLE II. INFORMATION OF THE TARGET RT CLINICAL FILES

Gene type	Sample type	File number
RNA-seq	normal	6
	Tumor	52
miRNA-seq	normal	6
	Tumor	52

With the cutoff value of corrected P-value < 0.01 and |LogFoldChange| > 2 and the limma R package [5, 6], the DElncRNAs, DEmiRNAs and DEmRNAs between the normal samples and tumor samples would be screened.

Based on the DElncRNAs, DEmiRNAs and DEmRNAs, the regulation among them was found by the database, mircode (www.mircode.org) [7], starbase (starbase.sysu.edu.cn), miRDB (www.mirdb.org), miRTarBase (mirtarbase.mbc.nctu.edu.tw) and TargetScan (www.targetscan.org) step by step. The ceRNA network was constructed according to the regulatory of DElncRNA-DEmRNA-DEmiRNA. The up-regulated DEmRNAs in the ceRNA network would be selected as the biomarker of RT.

Survival analysis of the selected biomarker was by using survival R package and kaplan-meier method [8]. From the km-plot curve, we could find out the relationship between the biomarker and RT.

III. RESULTS

By using the limma R package with the cutoff value of corrected P-value < 0.01 and |LogFoldChange| > 2, 533 DElncRNAs, 2687 DEmRNAs and 86 DEmiRNAs were screened from the downloaded data. Among them, 182 DElncRNAs, 1010 DEmRNAs and 43 DEmiRNAs were up-regulated in RT tumor samples. And the others DEGs were down-regulated. It was shown in Table 3.

TABLE III. INFORMATION OF DEGS

Expression	Gene number		
	DElncRNA	***DEmiRNA***	***DEmRNA***
Up-regulated	182	43	1010
down-regulated	351	43	1677

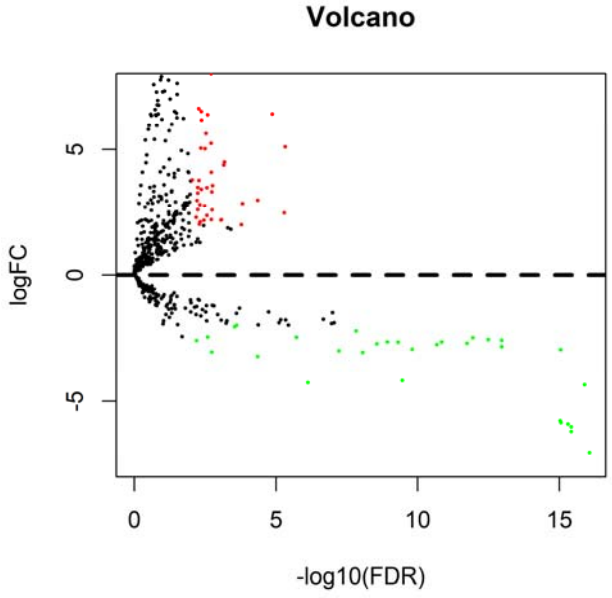

FIGURE I. volcano plot of DEmiRNA

In the ceRNA analysis, 57 DElncRNAs and 14 DEmiRNAs interacted in 223 pairs of regulatory by searching the mircode database. 33 mRNAs were found as the target mRNAs of the 14 DEmiRNAs by analyzing the miRDB, miRTarBase and TargetScan simultaneously. Among them, only 4 DEmRNAs, SEEC23A, PRRG4, ERMP1 and TPD52L1, were found as a target in regulatory with the screened DEmiRNA, hsa-mir-429. In the 223 regulation pairs of DElncRNAs-DEmiRNAs, hsa-mir-429 was interacted with DElncRNAs, GAS5, SNHG6, CECR7, LINC00501, MAGI2-AS3 and BOLA3-AS1.

The ceRNA of hsa-mir-429 and its interacted DElncRNAs and DEmRNAs in our research were shown in Figure III. And the expression of these DEGs were listed in Table 4.

SEC23A was the only up-regulated DEmRNA in Figure X. And it was selected as the potential biomarker of RT treatment.

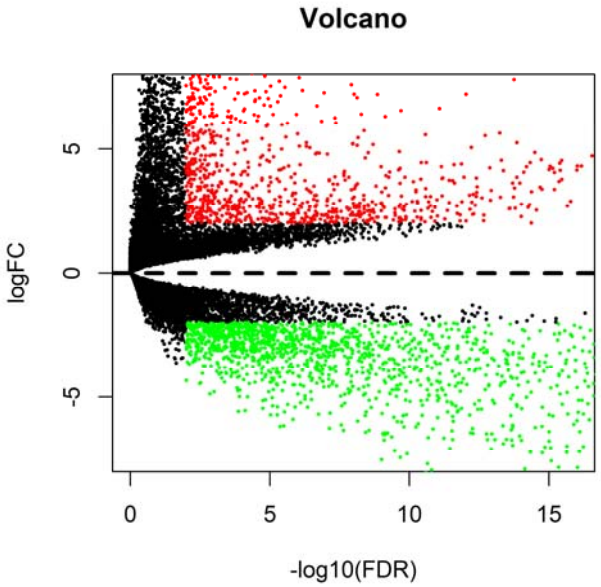

FIGURE II. VOLCANO PLOT OF DEmRNA

TABLE IV. DEGS IN CERNA NETWORK

Expression	Gene		
	DElncRNA	***DEmiRNA***	***DEmRNA***
Up-regulated	GAS5, SNHG6, CECR7, BOLA3-AS1	--	SEC23A
down-regulated	C00501, MAGI2-AS3	hsa-mir-429	PRRG4, ERMP1 TPD52L1

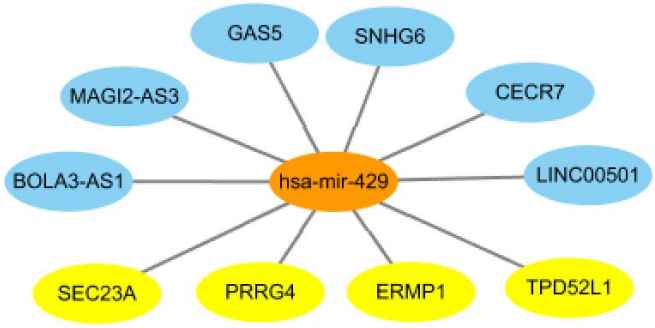

FIGURE III. THE ceRNA NETWORK

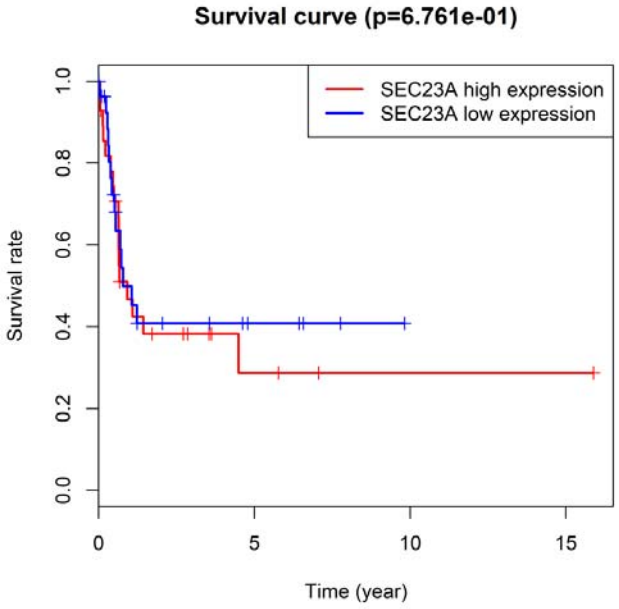

FIGURE IV. SURVIVIAL CURVE OF SEC23A

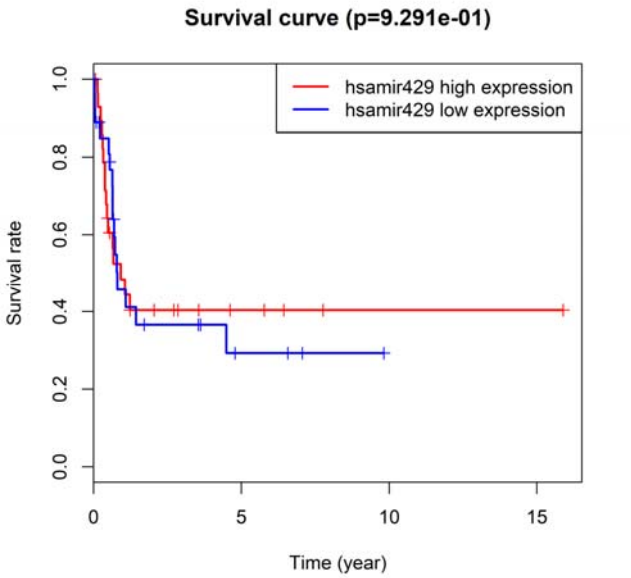

FIGURE V. SURVIVIAL CURVE OF HSA-MIR-429

By using the kaplan-meier analytical method, the relationship of SEC23A expression and its clinical data was shown in Figure IV. The 5 years' survival rate was about 10% lower when the gene was high expression in the clinical samples (p=6.761e-01). On the opposite, the down-regulated DEmiRNA, has-mir-429, was about 10% lower when it as low expression in a 5 years' survival analysis (p=9.291e-01) and shown in Figure V.

IV. DISCUSSION

As one kind of kidney tumors, rhabdoid tumor was still in the high lethal rate. It was found that RT was poor prognosis in young patents. In this paper, we aimed to research the dys-regulated genes and their relationship between the RT disease and normal tissues based on the RT subproject of the TARGET kidney project.

533 DElncRNAs, 2687 DEmRNAs and 86 DEmiRNAs were extracted from 4534 lncRNAs, 17447 mRNAs and 1595 miRNAs. Among them, 57 DElncRNAs interacted with 14 DemiRNAs in 223 regulatory pairs. And 4 regulatory pairs were found between 4 DEmRNAs, SEC23A, PRRG4, ERMP1 and TPD52L1 and DEmiRNA, has-mir-429. At the same time, the 6 DElncRNAs, GAS5, SNHG6, CECR7, LINC00501, MAGI2-AS3 and BOLA3-AS1, also interacted with hsa-mir-429. SEC23A was the only up-regulated DEmRNA interacted with hsa-mir-429.

Hsa-mir-429 has already known in some cancer treatment including bladder cancer, osteosarcoma, lung cancer and renal cell carcinoma [9-12]. The meaning of SEC23A has found in colorectal cancer and prostate cancer diagnosis [13, 14].

Compared to the normal tissues, SEC23A was up-expressed while hsa-mir-429 was down-expressed in RT tumor tissues.

And from the 5 years' kaplan-meier analysis of clinical data [15], it could be known that survival rate of the patients with a higher-expressed SEC23A or a lower-expressed hsa-mir-429 were only 30%. It was 10% lower than the low-risk ones. It could be known that SEC23A and hsa-mir-429 were in a negative regulation in RT. SEC23A and hsa-mir-429 might be the regulatory biomarker pair of RT.

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