Int J Clin Exp Med 2020;13(2):1129-1139
www.ijcem.com /ISSN:1940-5901/IJCEM0103397

Original Article

A signature of three miRNAs predicts survival in renal papillary cell carcinoma using bioinformatics methods

Yongheng Li[1*], Jie Zheng[2*], Xiaoru Xue[3]

[1]The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, China; [2]The Fifth Central Hospital of Tianjin, Tianjin, China; [3]Department of Pediatrics, The 940th Hospital of The Joint Logistic Support Force, PLA, Lanzhou, Gansu, China. [*]Equal contributors and co-first authors.

Received October 10, 2019; Accepted November 20, 2019; Epub February 15, 2020; Published February 28, 2020

Abstract: Objective: Growing evidence suggests that a large number of miRNAs are abnormally expressed in renal papillary cell carcinoma (pRCC) tissues and play crucial roles in tumorigenesis, progression, and metastasis. The aim of our present study was to identify and compare the differential miRNAs expressions between renal papillary cell carcinoma and normal renal tissues. Additionally, we evaluated the prognostic values of the differentially expressed miRNAs and constructed a three-miRNA signature that could effectively predict patient survival. Methods: We analyzed high-throughput miRNA data downloaded from the TCGA database. The differentially expressed miRNA profiles were normalized using log2 transformed. The prognostic value of each differentially expressed miRNA was evaluated using the Kaplan-Meier curve and log-rank methods. The target genes of the prognostic miRNAs were predicted using the Diana, miRWalk, TargetScan, and miRDB online analysis tools. Results: According to the cut-off criteria ($P<0.05$ and $|log2FC|>2.0$), a total of 50 differentially expressed miRNAs were identified between the renal papillary cell carcinoma tissues and the matched normal tissues, including 16 up-regulated miRNAs and 34 down-regulated miRNAs. The Kaplan-Meier survival method revealed the prognostic function of the three miRNAs (miRNA-599, miRNA-184, and miRNA-216b). Univariate and multivariate Cox regression analyses showed that the three-miRNA signature is an independent prognostic factor for renal papillary cell carcinoma. The functional enrichment analysis suggests that the target genes of the three miRNAs may be involved in various pathways related to cancer, including the ErbB receptor signaling network, the mTOR signaling pathway, the P53 signaling pathway, and the Wnt signaling pathway. Conclusion: Taken together, the present study finds that the three-miRNA signature can be used as a prognostic marker in renal papillary cell carcinoma.

Keywords: Renal, renal papillary cell carcinoma, renal cancer, bioinformatics, miRNA

Introduction

Renal cell carcinoma (RCC) is the most common type of kidney cancer, accounting for 2%-3% of all adult malignancies [1]. Renal papillary cell carcinoma (pRCC), a malignant tumor, accounts for 10% to 15% of all renal cell carcinomas [2]. According to its histological and cytogenetic features, this tumor can be classified into types 1 and 2 (carcinomas with MITF/TFE translocations, tubule mucinous carcinomas, etc.) [2-4]. However, there have been many difficulties in clearly distinguishing the two types of tumors. Ultrasound, CT, MRI, and MDCT are used most commonly in identifying renal cell carcinoma, including renal papillary cell carcinoma [5, 6]. However, these methods for pRCC clinical detection are not sensitive, and the early detection of the tumor is closely correlated with the 5-year survival rate [5]. Moreover, the traditional treatments, such as VEGFR-targeted therapies and mTOR inhibitors, have been shown to have a lower efficacy in pRCC [1]. Thus, understanding the molecular mechanisms of pRCC development and the identification of novel biomarkers are required for the early detection and treatment of renal papillary cell carcinoma.

microRNAs (miRNAs) are a large family of highly conserved, non-coding, small (22 nucleotides in length) RNA molecules that play important

roles in regulating post-transcriptional gene expression [7, 8]. miRNAs can function as oncogenes, affecting the development and progression of cancer and regulating the proliferation, differentiation, metastasis, autophagy [9], apoptosis [10] and invasion of tumor cells [11-13]. miRNA dysregulation is present in various types of tumors, including gastric, pancreatic, liver, and osteosarcoma [14-17]. Therefore, miRNAs have a large potential to serve as promising markers in cancer diagnosis and prognosis and in personalized targeted therapies.

Although a growing number of earlier studies have identified the value of miRNAs in predicting clinical outcomes in pRCC, they exist alone and are not in contact with each other. This may be due to the small sample sizes, the different histological types, the different detection approaches, and various data processing methods. The Cancer Genome Atlas Project (TCGA) is a National Cancer Institute initiative which makes an effort to profile at least 32 different tumor types using genomic platforms and to make the raw and processed data available to all researchers [18]. The TCGA released a large amount t of miRNA sequencing data for pRCC patients. The aim of the present study was to identify the differential miRNAs expressions between pRCC tissues and matched normal renal tissues by analyzing the high-throughput miRNA data downloaded from the TCGA database. Additionally, we evaluated the prognostic value of the differential expressed miRNAs and constructed a three-miRNA signature that could effectively predict patient survival. Meanwhile, the target genes of three-miRNAs were also determined through different databases to explore the biological processes, cellular components, and molecular functions. The KEGG pathways were also included, and they are the most important.

Materials and methods

Data processing

The raw sequencing data and clinical information were downloaded from the TCGA database (https://cancergenome.nih.gov/). The inclusion criteria were: (1) the sample possessed miRNA sequencing data and clinical information; (2) the sample possessed prognosis information. (3) tumors with strict pathological diagnoses. (4) primary solid tumor. Finally, a total of 324 samples were enrolled in this study, including 290 KIRP tissues and 34 matched normal tissues. The detailed clinical characteristics and differentially expressed miRNAs are listed in **Table 2**. The miRNA sequencing data were processed using the R language package. The differentially expressed miRNAs between the KIRP and normal tissues were analyzed using the LIMMA package in R. The fold change (FC) in the expression of individual miRNAs were calculated and differentially expressed. miRNAs with log2|FC|>2.0 and P<0.05 were considered to be significant.

Association of differentially expressed miRNAs and patient prognosis

The differentially expressed miRNA profiles were normalized using a log2 transformation. The prognostic value of each differentially expressed miRNA was evaluated using the Kaplan-Meier curve and log-rank methods.

The target gene prediction of the prognostic miRNA signature

The target genes of the prognostic miRNAs were predicted using the Diana (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=site/index), miRWalk (http://mirwalk.umm.uni-heidelberg.de/), TargetScan (http://www.targetscan.org/), and miRDB (http://www.mirdb.org/miRDB/) online analysis tools. To further enhance the reliability of the bioinformatics analysis, the overlapping target genes were identified using a Venn diagram. Then, the overlapping genes were analyzed with FunRich. FunRich is a software program that aims to provide a comprehensive set of functional annotation tools for investigators to understand the biological mechanisms associated with large lists of genes [19]. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were then performed for the target genes. The *P*-value <0.05 and gene count ≥3 were set as the cut-off criteria.

Statistical analysis

The data are shown as the means ± standard deviations (SD). The expression levels of the miRNAs in pRCC and the matched normal tissues were analyzed using an unpaired *t* test. Chi-squared and *t* tests were performed to assess the relationship between miRNA expres-

Table 1. The clinical characteristics of renal cell carcinoma patients

Variables	Case, n (%)
Age at diagnosis	
≥60	166 (57.24)
<60	121 (41.72)
NA	3 (1.03)
Gender	
Male	213 (73.44)
Female	77 (26.55)
Race	
White	206 (71.03)
Other	69 (23.79)
NA+NE	15 (5.17)
Stage	
Stage1+2	193 (66.55)
Stage3+4	67 (23.10)
NA	28 (9.66)
DIS	2 (0.69)
T Stage	
T1+2	226 (77.93)
T3+4	62 (21.38)
TX	2 (0.69)
Lymph node status	
N0	50 (17.24)
N>0	239 (82.41)
DIS	1 (0.34)
Metastasis	
M0	94 (32.41)
M>0	181 (62.41)
NA	15 (5.17)
Histologic Type	
Type 1	76 (26.21)
Type 2	86 (29.66)
UN	72 (24.83)
NA	56 (19.31)
Smoking History Category	
<3	152 (52.41)
≥3	95 (32.76)
NA	10 (3.45)
UN	33 (11.38)

NA: not available; NE: not evaluated; DIS: discrepancy; UN: unknown.

sion and the clinical features. A Cox regression test and a univariate/multivariate Cox proportional hazard regression analysis were carried out to compare each miRNA (low level vs. high level). A *P* value less than 0.05 was considered statistically significant. The statistical analysis was performed using the IBM SPSS Statistics software program version 22.0 (IBM Corp, NY, USA).

Results

Identification of differentially expressed miRNAs in renal papillary cell carcinoma

In the present study, a total of 324 samples were involved, including 290 pRCC tissues and 34 matched normal tissues. The detailed clinical characteristics included age, gender, race, metastasis, lymph node status, pathological stage, T stage, histological type, and smoking history category at the time of the diagnosis (**Table 1**). According to the cut-off criteria (P<0.05 and |log2FC|>2.0), a total of 50 differentially expressed miRNAs were identified between the pRCC and matched normal tissues, including 16 up-regulated and 34 down-regulated miRNAs. In order to prove whether the *P* value and |log2FC| conformed to logic with different tests, we presented the result as Volcano plot (**Figure 1**).

The identification of three miRNAs associated with survival probability in renal papillary cell carcinoma

To identify the miRNAs which would be potentially associated with the Survival Probability (SP) of the pRCC patients, we evaluated the association between the miRNAs' expressions and the patients' survival using the Kaplan-Meier curve and log-rank methods. The results showed that one miRNA (miR-599) was negatively correlated with SP, and two miRNAs (miR-184 and miR-216b) were positively related to SP (**Figure 2**). The associations between the three miRNAs and the clinical features were evaluated in the pRCC patients (**Table 2**). The results showed that miR-184 was significantly associated with stage (P=0.007), T stage (P=0.008), Metastasis (P=0.022), Histological type (P=0.018), and smoking (P=0.016). miR-216b was associated with histological type (P=0.012). No significant difference was found between miR-599 and the clinical features (P>0.05).

The prognostic value of the three miRNAs in renal papillary cell carcinoma

We constructed a prognostic signature by integrating the expression profiles of the three miR-

Table 2. The association of the three miRNAs with the clinical features

Variables	Numbers	MiR-599	t value	*p* value	MiR-184	t value	*p* value	MiR-216b	t value	*p* value
Age at diagnosis										
≥60	166	5.62±14.86	-1.230	0.220	0.70±1.57	0.677	0.499	0.06±0.14	0.156	0.876
<60	121	8.39±21.31			0.58±1.40			0.05±0.19		
Gender										
Male	213	7.71±22.95	0.939	0.349	0.56±1.42	-1.958	0.051	0.48±0.13	-1.098	0.273
Female	77	5.11±13.25			0.96±1.80			0.72±0.22		
Race										
White	206	7.12±20.09	-0.120	0.905	0.63±1.46	-0.535	0.593	0.05±0.13	-0.712	0.477
Other	69	7.47±24.88			0.74±1.70			0.07±0.22		
Stage										
1+2	193	8.00±23.62	1.262	0.208	0.49±1.31	-2.768	0.007<0.01	0.05±0.16	-1.374	0.172
3+4	67	4.18±12.57			1.28±2.19			0.08±0.17		
T Stage										
T1+2	226	7.91±22.59	1.818	0.071	0.50±1.33	-2.747	0.008<0.01	0.05±0.16	-1.799	0.075
T3+4	62	3.92±12.59			1.26±2.06			0.09±0.18		
Lymph node status										
N0	50	5.25±12.42	-0.668	0.505	1.07±2.14	1.567	0.123	0.08±0.18	1.161	0.250
N>0	239	7.42±22.22			0.58±1.38			0.05±0.16		
Metastasis										
M0	94	6.34±20.68	-0.460	0.646	1.04±2.18	2.317	0.022<0.05	0.07±0.17	1.172	0.242
M>0	181	7.58±21.49			0.49±1.07			0.05±0.16		
Histologic Type										
Type 1	76	5.57±11.71	-0.793	0.429	0.33±1.00	-2.389	0.018<0.05	0.01±0.04	-2.572	0.012<0.05
Type 2	86	7.53±18.55			0.88±1.83			0.05±0.14		
Smoking History Category										
<3	152	9.01±26.29	1.275	0.203	0.47±1.01	-2.444	0.016<0.05	0.06±0.18	-0.111	0.912
≥3	95	5.73±14.00			1.05±2.16			0.06±0.15		

NAs and the corresponding estimated regression coefficient. Taking into account the following clinical features: age, gender, race, metastasis, lymph node status, stage, T stage, histological type, and smoking history category, univariate and multivariate Cox regression analyses were used to test the effect of the three-miRNA signature on SP. In the univariate analysis, stage (HR=0.454, P=0.023), T stage (HR=0.492, P=0.012), and the three-miRNA signature (HR=2.574, P<0.001) were associated with SP in the pRCC patients. In the multivariate analysis, the three-miRNA signature (HR=2.504, P=0.001) was shown to be an independent prognostic factor in the pRCC patients (**Table 3**).

Target prediction and function analysis

The target genes of the three miRNAs (miR-599, miR-184, and miR-216b) were predicted using the Diana, miRWalk, TargetScan, and miRDB online analysis tools. A total of 7 overlapping genes of miR-599, 8 overlapping genes of miR-184, and 160 overlapping genes of miR-216b were identified (**Figure 3**). Then, an enrichment analysis was performed to elucidate the biological process, cellular component, and molecular function. The KEGG pathways were also included with the consensus target genes. The KEGG pathways were significantly enriched in the ErbB receptor signaling network, the mTOR signaling pathway, the P53 signaling pathway, and the Wnt signaling pathway. In addition, the GO biological process (BP) terms were mainly enriched in CAMP-mediated signaling, bone remodeling and the regulation of the cellular process. The cellular component (CC) terms were mainly enriched nucleus, cell-cell junction, focal adhesion, and membrane junction. Molecular Function (MF) terms were mainly enriched RNA binding, auxiliary transport protein activity, cell adhesion molecule activity, and lipoprotein receptor activity (**Figure 4**).

Discussion

PRCC is the second most prevalent renal tumor in renal cell carcinoma, with two historical

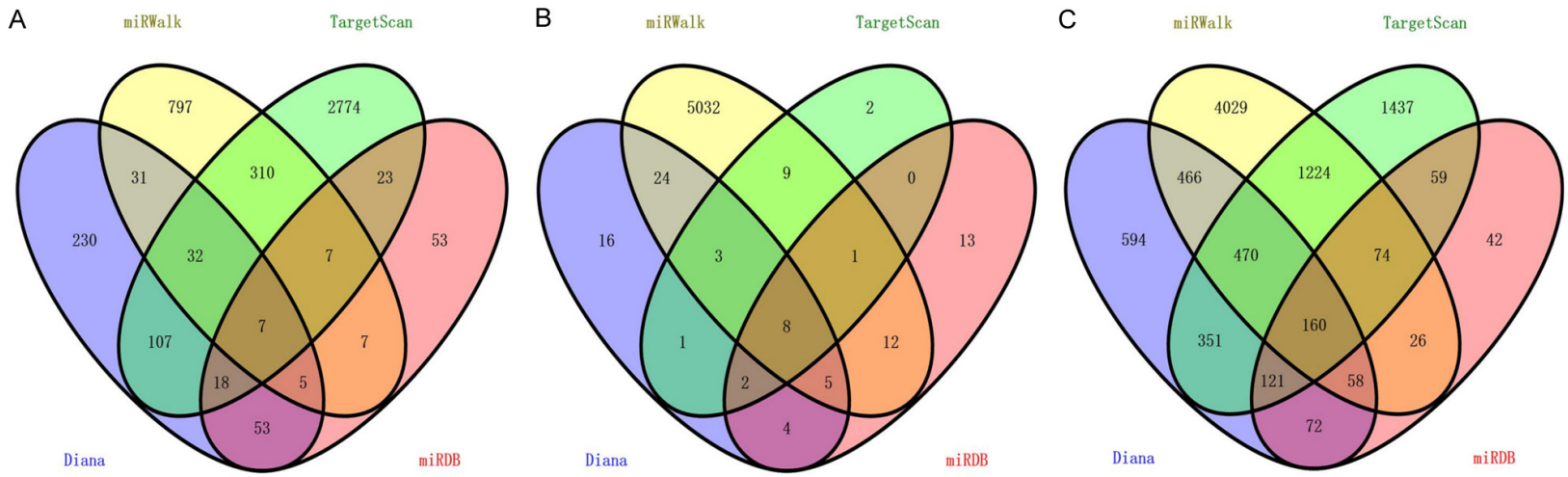

Figure 1. Volcano plot of differential miRNAs for renal papillary cell carcinoma.

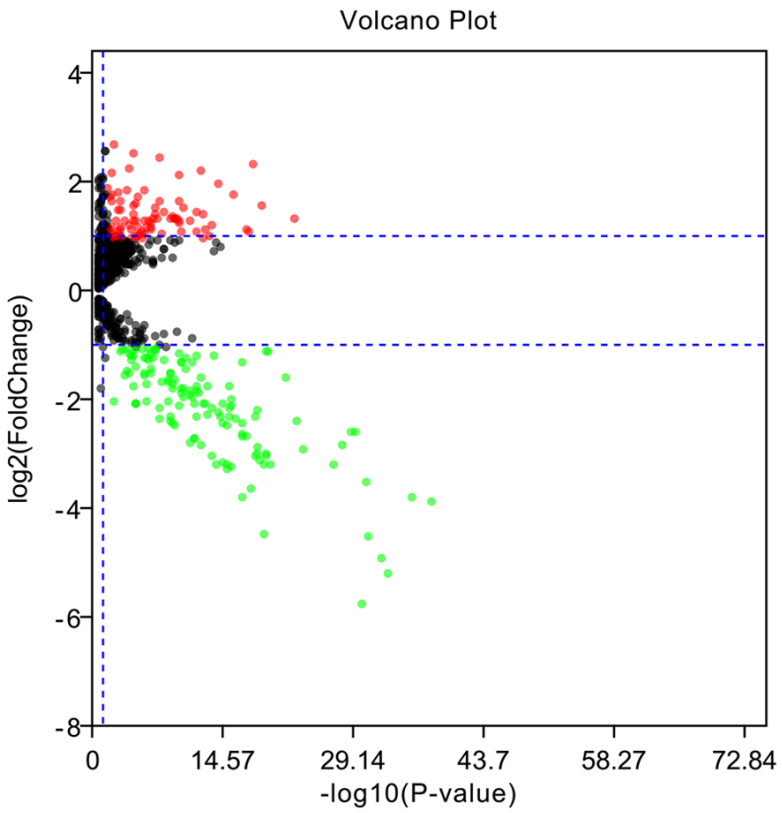

Figure 2. The survival probability in renal papillary cell carcinoma of three miRNAs.

types. Patients with type 1 tumors exhibit a pretty good, 92% 5-year survival rate. By contrast, the 5-year survival rate in type 2 tumors is only 65% [20, 21]. The pRCC patients' prognoses and 5-year survival rates would be improved considerably if tumor behavior could be predicted reliably at the time of early diagnosis. Thus, understanding the molecular mechanisms of pRCC development and the identification of novel biomarkers are needed. In the present study, a total of 50 differentially expressed miRNAs were identified, and three of them were associated with survival possibility in pRCC patients. The three-miRNA (miR-599, miR-184, and miR-216b) signature was established and found to be an independent prognostic factor for pRCC patients. Moreover, we screened the target genes of these three miRNAs and predicted the enrichment pathways and biological functions of the target genes using bioinformatics methods.

In the last decade, MiRNAs, as the master modulators of multiple biological and pathological processes, are a hot research topic in the field of cancer development. A growing body of evidence has suggested that miRNAs establish a complex combinatorial system of gene expression and pathway regulation, as well as prognostic indicators and therapeutic targets in different cancers, including pRCC [22, 23]. Previous studies have demonstrated that many miRNAs are crucial for the initiation, progression, and metastasis of pRCC by participating in various processes, including the Wnt signaling pathway, the TGF-β signaling pathway, and endocytosis [23]. To date, several studies have identified a number of miRNAs with prognostic values, such as hsa-miR-200c [23], hsa-miR-34a [23], hsa-miR-3199-2 [24], hsa-miR-1293 [24], and so on. However, the previous studies were based on small sample sizes, small sample types, different detection platforms, various assay methods, and a relatively limited number of miRNAs. In the present study, we analyzed high-throughput data and identified that two down-regulated miRNAs (miR-184 and miR-216b) and one up-regulated miRNA (miR-599) that are associated with the clinical outcomes of pRCC patients. It was reported in an earlier study that overexpression of miR-184 might play an oncogenic role in the antiapoptotic and proliferative processes of tongue squamous cell carcinoma (SCC) [25]. Studies by Foley revealed that miRNA-184 inhibits neuroblastoma cell survival by targeting the AKT2 [26], and so on [27, 28]. MiR-184 has also been reported in renal cell carcinoma [29, 30]. Moreover, miR-216b, as a tumor suppressor, is strongly downexpressed and related to proliferation and invasion in glioma [31]. A study by He Sy (2017) demonstrated that miR-216b, acting as a tumor suppressor in cervical cancer cells, inhibited cancer cell proliferation by targeting FOXM1 [32]. MiR-599 was confirmed to be down-regulated in human glioma [33] and hepatocellular

Table 3. Univariate and multivariate Cox regression analysis in pRCC patients

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Age (≥60 vs. <60)	0.639 (0.256-1.595)	0.337		
Gender (male vs. female)	0.761 (0.304-1.906)	0.560		
Race (white vs. other)	1.152 (0.508-2.611)	0.735		
Stage (1, 2 vs. 3, 4)	0.454 (0.157-1.314)	0.023<0.05	1.826 (0.804-3.264)	0.031<0.05
T Stage (1, 2 vs. 3, 4)	0.492 (0.147-1.646)	0.012<0.05	1.765 (0.367-4.536)	0.026<0.05
Lymph node status (0 vs. ≥0)	3.040 (0.723-12.790)	0.129		
Metastasis (0 vs. ≥0)	1.182 (0.534-2.615)	0.680		
Histologic Type (type 1 vs. type 2)	0.903 (0.338-2.412)	0.838		
Smoking History Category (<3 vs. ≥3)	1.031 (0.446-2.385)	0.943		
Three miRNAs signature	2.504 (1.501-4.425)	0.001<0.05	2.183 (1.121-5.213)	0.001<0.05

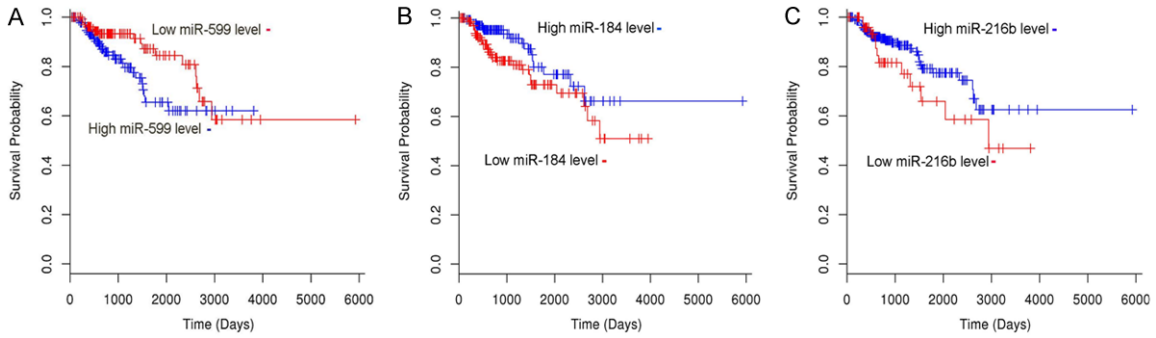

Figure 3. Overlapping target genes of the differential miRNAs.

carcinoma [34] and up-regulated in non-small cell lung cancer [35] and breast cancer [36], suggesting its complex role in cancer as it can act either as an oncogene or as a tumor suppressor depending on the origin of the cancer. Our results showed that miR-599 is up-regulated in pRCC and may function as an oncogene in the development of pRCC. Furthermore, miR-184 is significantly associated with pathological stage, T stage, histological type, metastasis, and smoking history category. miR-216b is associated with histological type, indicating that miR-184 and miR-216b are involved in the progression of pRCC. However, no significant differences were found between miR-599 and the clinical features. The TCGA database does not provide data on alcohol, hypertension, chromosome morphology, and so on. Maybe miRNA-599 is related to other factors. Future studies will focus on this point and further investigate the functions of miRNA-599 in pRCC.

In the present study, we found that miR-184, miR-216b, and miR-599 are differentially expressed and are significantly associated with overall survival possibility in pRCC patients, which is an independent prognostic factor in pRCC. To gain a deep insight into the molecular functions of the three miRNAs, we predicted the target genes and analyzed the related pathways and GO annotations. Abnormal signaling pathways play crucial roles in the pathogenesis and progression of pRCC. We found that the three miRNAs could regulate several key signaling pathways, including the ErbB receptor signaling network, the class I PI3k signaling events mediated by the AKT and mTOR signaling pathways, EGFR-dependent endothelin signaling events, the P53 signaling pathway and the Wnt signaling pathway. A growing body of evidence has demonstrated that the major signaling pathways are activated by the ErbB receptor which is crucial in cancer progression, invasion, and metastasis, such as the MAPK signaling pathway and the PI3K signaling pathway [37]. Moreover, it has been well established that the PI3K/Akt/mTOR signaling pathway plays a crucial role in cervical cancer development [38], and the inhibition of the mTOR kinase activity

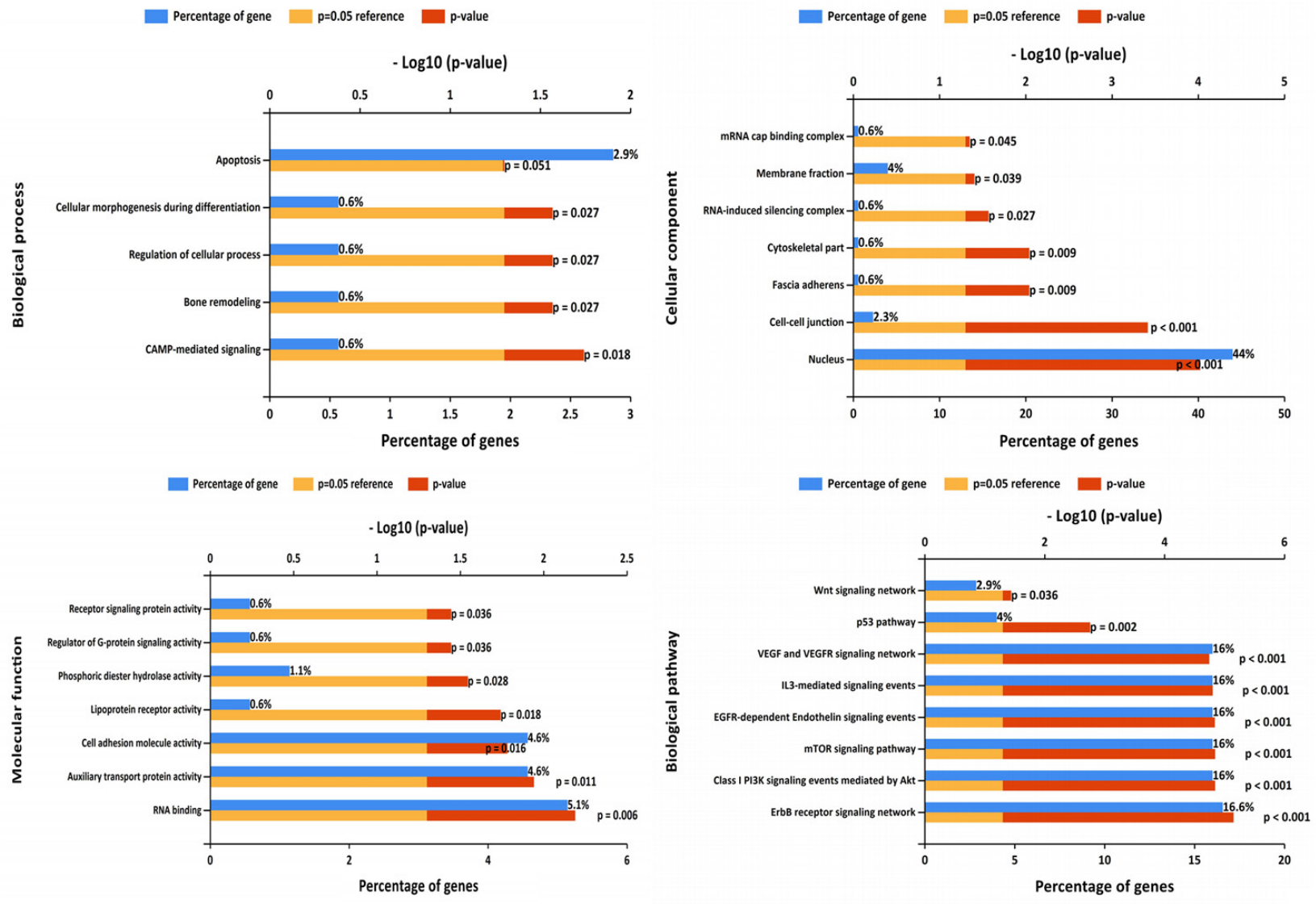

Figure 4. GO analysis of the overlapping target genes.

suppresses tumor growth [39]. EGFR overexpression has frequently been identified in renal cell carcinoma, and EGFR signaling is essential for malignant renal tubular cells [40, 41]. A study by Wang reported that the EGFR/MAPK signaling pathways participate in the immune escape in non-small cell lung cancer [42]. The tumor suppressor *p53* is the most frequently mutated gene in human cancers and mediates the cell-cycle arrest and apoptosis in renal cell carcinoma [43-45]. Previous studies have been indicated that the Wnt signaling pathways were related to cell proliferation [46], invasion [46], diagnosis [47] and progression [48] in renal cell carcinoma. Although we have devoted all our efforts to this research, further molecular investigations are needed to confirm these predictions, which can provide new therapeutic interventions for pRCC.

Taken together, we identified the three-miRNA signature as a potential prognostic predictor for pRCC patients. Further studies are needed to validate our findings with larger sample sizes, and further functional investigations are also needed to explore the molecular mechanisms of these miRNAs in renal papillary cell carcinoma progression.

Disclosure of conflict of interest

None.

Address correspondence to: Xiaoru Xue, Department of Pediatrics, The 940th Hospital of The Joint Logistic Support Force, PLA, No. 333 Henan Road, Qilihe, Lanzhou 730050, Gansu, China. Tel: +86-13919764830; E-mail: mjmy5b9@163.com

References

[1] Courthod G, Tucci M, Di Maio M and Scagliotti GV. Papillary renal cell carcinoma: a review of the current therapeutic landscape. Crit Rev Oncol Hematol 2015; 96: 100-112.

[2] Sibony M and Vieillefond A. Non clear cell renal cell carcinoma. 2008 update in renal tumor pathology. Ann Pathol 2008; 28: 381-401.

[3] Delahunt B and Eble JN. Papillary renal cell carcinoma: a clinicopathologic and immunohistochemical study of 105 tumors. Mod Pathol 1997; 10: 537-544.

[4] Delahunt B, Eble JN, McCredie MR, Bethwaite PB, Stewart JH and Bilous AM. Morphologic typing of papillary renal cell carcinoma: comparison of growth kinetics and patient survival in 66 cases. Hum Pathol 2001; 32: 590-595.

[5] Dunnick NR. Renal cell carcinoma: staging and surveillance. Abdom Radiol (NY) 2016; 41: 1079-1085.

[6] Ng CS, Wood CG, Silverman PM, Tannir NM, Tamboli P and Sandler CM. Renal cell carcinoma: diagnosis, staging, and surveillance. AJR Am J Roentgenol 2008; 191: 1220-1232.

[7] Zamore PD and Haley B. Ribo-gnome: the big world of small RNAs. Science 2005; 309: 1519-1524.

[8] Ambros V. The functions of animal microRNAs. Nature 2004; 431: 350-355.

[9] Shenoy A and Blelloch RH. Regulation of microRNA function in somatic stem cell proliferation and differentiation. Nat Rev Mol Cell Biol 2014; 15: 565-576.

[10] Otsuka K and Ochiya T. Genetic networks lead and follow tumor development: microRNA regulation of cell cycle and apoptosis in the p53 pathways. Biomed Res Int 2014; 2014: 749724.

[11] Chen B and Liu B. MiRNA-381 inhibits the invasion of renal carcinoma and the underlying mechanisms. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2015; 40: 1053-1059.

[12] Cho WC. OncomiRs: the discovery and progress of microRNAs in cancers. Mol Cancer 2007; 6: 60.

[13] Hayes J, Peruzzi PP and Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. Trends Mol Med 2014; 20: 460-469.

[14] Pan HW, Li SC and Tsai KW. MicroRNA dysregulation in gastric cancer. Curr Pharm Des 2013; 19: 1273-1284.

[15] Wei X, Wang W, Wang L, Zhang Y, Zhang X, Chen M, Wang F, Yu J, Ma Y and Sun G. MicroRNA-21 induces 5-fluorouracil resistance in human pancreatic cancer cells by regulating PTEN and PDCD4. Cancer Med 2016; 5: 693-702.

[16] Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST and Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology 2007; 133: 647-658.

[17] Mishra PJ. MicroRNAs as promising biomarkers in cancer diagnostics. Biomark Res 2014; 2: 19.

[18] Chandran UR, Medvedeva OP, Barmada MM, Blood PD, Chakka A, Luthra S, Ferreira A, Wong KF, Lee AV, Zhang Z, Budden R, Scott JR, Berndt A, Berg JM and Jacobson RS. TCGA expedition: a data acquisition and management system for TCGA data. PLoS One 2016; 11: e0165395.

[19] Pathan M, Keerthikumar S, Chisanga D, Alessandro R, Ang CS, Askenase P, Batagov AO, Benito-Martin A, Camussi G, Clayton A, Collino F, Di Vizio D, Falcon-Perez JM, Fonseca P, Fon-

seka P, Fontana S, Gho YS, Hendrix A, Hoen EN, Iraci N, Kastaniegaard K, Kislinger T, Kowal J, Kurochkin IV, Leonardi T, Liang Y, Llorente A, Lunavat TR, Maji S, Monteleone F, Overbye A, Panaretakis T, Patel T, Peinado H, Pluchino S, Principe S, Ronquist G, Royo F, Sahoo S, Spinelli C, Stensballe A, Thery C, van Herwijnen MJC, Wauben M, Welton JL, Zhao K and Mathivanan S. A novel community driven software for functional enrichment analysis of extracellular vesicles data. J Extracell Vesicles 2017; 6: 1321455.

[20] Kuthi L, Jenei A, Hajdu A, Nemeth I, Varga Z, Bajory Z, Pajor L and Ivanyi B. Prognostic factors for renal cell carcinoma subtypes diagnosed according to the 2016 WHO renal tumor classification: a study involving 928 patients. Pathol Oncol Res 2017; 23: 689-698.

[21] Kosaka T, Mikami S, Miyajima A, Kikuchi E, Nakagawa K, Ohigashi T, Nakashima J and Oya M. Papillary renal cell carcinoma: clinicopathological characteristics in 40 patients. Clin Exp Nephrol 2008; 12: 195-199.

[22] Iorio MV and Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. EMBO Mol Med 2012; 4: 143-159.

[23] Ge YZ, Xu LW, Xu Z, Wu R, Xin H, Zhu M, Lu TZ, Geng LG, Liu H, Zhou CC, Yu P, Zhao YC, Hu ZK, Zhao Y, Zhou LH, Wu JP, Li WC, Zhu JG and Jia RP. Expression profiles and clinical significance of microRNAs in papillary renal cell carcinoma: a STROBE-compliant observational study. Medicine (Baltimore) 2015; 94: e767.

[24] Luo W, Wang L, Luo MH, Huang YZ, Yang H, Zhou Y, Jia HT and Wang XX. hsa-mir-3199-2 and hsa-mir-1293 as novel prognostic biomarkers of papillary renal cell carcinoma by COX ratio risk regression model screening. J Cell Biochem 2017; 118: 3488-3494.

[25] Wong TS, Liu XB, Wong BY, Ng RW, Yuen AP and Wei WI. Mature miR-184 as potential oncogenic microRNA of squamous cell carcinoma of tongue. Clin Cancer Res 2008; 14: 2588-2592.

[26] Foley NH, Bray IM, Tivnan A, Bryan K, Murphy DM, Buckley PG, Ryan J, O'Meara A, O'Sullivan M and Stallings RL. MicroRNA-184 inhibits neuroblastoma cell survival through targeting the serine/threonine kinase AKT2. Mol Cancer 2010; 9: 83.

[27] Zhen Y, Liu Z, Yang H, Yu X, Wu Q, Hua S, Long X, Jiang Q, Song Y, Cheng C, Wang H, Zhao M, Fu Q, Lyu X, Chen Y, Fan Y, Liu Y, Li X and Fang W. Tumor suppressor PDCD4 modulates miR-184-mediated direct suppression of C-MYC and BCL2 blocking cell growth and survival in nasopharyngeal carcinoma. Cell Death Dis 2013; 4: e872.

[28] Wu GG, Li WH, He WG, Jiang N, Zhang GX, Chen W, Yang HF, Liu QL, Huang YN, Zhang L, Zhang T and Zeng XC. Mir-184 post-transcriptionally regulates SOX7 expression and promotes cell proliferation in human hepatocellular carcinoma. PLoS One 2014; 9: e88796.

[29] Leng HM, Qian WP, Zhou L, Zhai QN, Li XX, Guan ZC, Gui YT and Cai ZM. Abnormal expression and significance of MIR-184 in human renal carcinoma. Beijing Da Xue Xue Bao Yi Xue Ban 2011; 43: 509-513.

[30] Su Z, Chen D, Li Y, Zhang E, Yu Z, Chen T, Jiang Z, Ni L, Yang S, Gui Y, Ye J and Lai Y. microRNA-184 functions as tumor suppressor in renal cell carcinoma. Exp Ther Med 2015; 9: 961-966.

[31] Chen Z, Wu Y, Song S, Zhu X and Zhu J. MicroRNA216b inhibits cell proliferation and invasion in glioma by directly targeting metadherin. Mol Med Rep 2017; 16: 9749-9757.

[32] He S, Liao B, Deng Y, Su C, Tuo J, Liu J, Yao S and Xu L. MiR-216b inhibits cell proliferation by targeting FOXM1 in cervical cancer cells and is associated with better prognosis. BMC Cancer 2017; 17: 673.

[33] Zhang T, Ma G, Zhang Y, Huo H and Zhao Y. miR-599 inhibits proliferation and invasion of glioma by targeting periostin. Biotechnol Lett 2017; 39: 1325-1333.

[34] Tian J, Hu X, Gao W, Zhang J, Chen M, Zhang X, Ma J and Yuan H. Identification a novel tumor-suppressive hsa-miR-599 regulates cells proliferation, migration and invasion by targeting oncogenic MYC in hepatocellular carcinoma. Am J Transl Res 2016; 8: 2575-2584.

[35] Tian W, Wang G, Liu Y, Huang Z, Zhang C, Ning K, Yu C, Shen Y, Wang M, Li Y, Wang Y, Zhang B and Zhao Y. The miR-599 promotes non-small cell lung cancer cell invasion via SATB2. Biochem Biophys Res Commun 2017; 485: 35-40.

[36] Wang Y, Sui Y, Zhu Q and Sui X. Hsa-miR-599 suppresses the migration and invasion by targeting BRD4 in breast cancer. Oncol Lett 2017; 14: 3455-3462.

[37] Olayioye MA, Neve RM, Lane HA and Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. EMBO J 2000; 19: 3159-3167.

[38] Feng T, Zheng L, Liu F, Xu X, Mao S, Wang X, Liu J, Lu Y, Zhao W, Yu X and Tang W. Growth factor progranulin promotes tumorigenesis of cervical cancer via PI3K/Akt/mTOR signaling pathway. Oncotarget 2016; 7: 58381-58395.

[39] Li S, Li Y, Hu R, Li W, Qiu H, Cai H and Wang S. The mTOR inhibitor AZD8055 inhibits proliferation and glycolysis in cervical cancer cells. Oncol Lett 2013; 5: 717-721.

[40] Dordevic G, Matusan Ilijas K, Hadzisejdic I, Maricic A, Grahovac B and Jonjic N. EGFR protein overexpression correlates with chromosome 7 polysomy and poor prognostic parameters in clear cell renal cell carcinoma. J Biomed Sci 2012; 19: 40.

[41] Szymanska K, Moore LE, Rothman N, Chow WH, Waldman F, Jaeger E, Waterboer T, Foretova L, Navratilova M, Janout V, Kollarova H, Zaridze D, Matveev V, Mates D, Szeszenia-Dabrowska N, Holcatova I, Bencko V, Le Calvez-Kelm F, Villar S, Pawlita M, Boffetta P, Hainaut P and Brennan P. TP53, EGFR, and KRAS mutations in relation to VHL inactivation and lifestyle risk factors in renal-cell carcinoma from central and eastern Europe. Cancer Lett 2010; 293: 92-98.

[42] Wang J, Jia Y, Zhao S, Zhang X, Wang X, Han X, Wang Y, Ma M, Shi J and Liu L. BIN1 reverses PD-L1-mediated immune escape by inactivating the c-MYC and EGFR/MAPK signaling pathways in non-small cell lung cancer. Oncogene 2017; 36: 6235-6243.

[43] Ku BM, Kim DS, Kim KH, Yoo BC, Kim SH, Gong YD and Kim SY. Transglutaminase 2 inhibition found to induce p53 mediated apoptosis in renal cell carcinoma. FASEB J 2013; 27: 3487-3495.

[44] Zhu Z, Xing S, Cheng P, Li G, Yang Y, Zeng F and Lu G. The relationship of expression of bcl-2, p53, and proliferating cell nuclear antigen (PCNA) to cell proliferation and apoptosis in renal cell carcinoma. J Huazhong Univ Sci Technolog Med Sci 2004; 24: 354-357.

[45] Miyake H, Hara I, Gohji K, Arakawa S and Kamidono S. p53 modulation of Fas/Apo-1 mediated apoptosis in a human renal cell carcinoma cell line. Int J Oncol 1998; 12: 469.

[46] Huang JL, Liao Y, Qiu MX, Li J and An Y. Long non-coding RNA CCAT2 promotes cell proliferation and invasion through regulating Wnt/beta-catenin signaling pathway in clear cell renal cell carcinoma. Tumour Biol 2017; 39: 1010428317711314.

[47] Liu Z, Liu XW, Liu SA, Lv JJ and Fu Q. Clinical significance of changes of expression of the Wnt/beta-catenin signaling pathway in renal clear cell carcinoma. Eur Rev Med Pharmacol Sci 2016; 20: 4840-4845.

[48] Li M, Pei X, Wang G, Zhan J, Du J, Jiang H, Tang Y, Zhang H and He H. Kindlin2 promotes clear cell renal cell carcinoma progression through the Wnt signaling pathway. Oncol Rep 2017; 38: 1551-1560.