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A panel of three serum microRNAs as a potential diagnostic biomarker for renal cell carcinoma

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Hitherto there is no praiseworthy noninvasive methods in the early diagnosis of renal cell carcinoma (RCC). MicroRNAs (miRNAs) could be utilized as molecular markers for diverse malignancies. In this study, we aim to discern potential miRNAs as markers for screening RCC. We employed quantitative reverse transcription-polymerase chain reaction (RT-qPCR) to detect expression levels of candidate miRNAs in serum specimens of 108 RCC patients and 112 health volunteers. Diagnostic values of miRNAs were appraised, and panel was constructed by dint of receiver operating characteristic curves, the area under the ROC curve and backward stepwise logistic regression analysis. Moreover, we capitalized on bioinformatics analysis for exploration of miRNAs biological functions. The expression of five miRNAs (miR-30c-5p, miR-142-3p, miR-206, miR-223-3p, miR-200c-5p) were markedly alteration in serum specimens of RCC patients and health subjects. A three-miRNA panel combining miR-30c-5p, miR-142-3p and miR-206 was constructed and could discriminate RCC patients and healthy subjects satisfactorily with 0.872 (0.811–0.919, $P < 0.001$) AUC, 81.25% sensitivity and 86.90% specificity. ATF3 and MYC seem to be potential targets of the three-miRNA panel. The novel miRNA-based panel may perform as potential noninvasive markers to discriminate RCC patients and healthy subjects in advance.

Keywords MicroRNAs, Serum, Renal cell carcinoma, Biomarker, Bioinformatics

Renal cell carcinoma (RCC) is a lethal tumor with a global estimation of 431,000 new cases and 179,000 deaths in 2020[1], accounting for approximately 90% of renal cancer and 2–3% of malignant tumors in adult[2–4]. It is reported that the occurrence of RCC is associated with cigarettes, high blood pressure, obesity, chronic renal disease and occupational exposure to trichloroethylene[4,5]. Three quarters of RCC are diagnosed pathologically with clear-cell renal cell carcinoma (ccRCC)[3]. Generally, RCC is detected accidentally since there are no prominent clinical manifestations in early stage. The appearance of triad, namely, flank pain, visual blood in urine and abdominal mass often means that RCC has progressed to an advanced stage[4]. The five years survival rate is greater than 90% in early RCC with localize tumors, whereas it declines to 12% in metastatic tumors[3]. Thus, early detection is of great significance for RCC supervision.

A study in the UK elucidated that the impact of using symptoms to early diagnosed kidney cancer is of limitation and unreliable[6]. Pathological biopsy is the gold standard to diagnose kidney cancer but an invasive means, which is therefore not appropriate for screening. Ultrasound (US) is a reproduceable, economical and radiation-free imaging modality to diagnose renal lesions. However, renal lesions < 0.5 cm could not be detected by US and the diagnostic capability of US is dissatisfactory particularly in suspected renal malignancies[7,8]. Nowadays computed tomography (CT) is the primary choice for imaging diagnoses of kidney masses with high diagnostic accuracy, but radiation prevents CT from becoming a routine screening approach.

More attention should be paid to noninvasive and robust screening strategies to detect RCC early, such as molecular markers. Ideal markers should have high specificity to distinguish different pathologic conditions

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and could be isolated feasibly. Cumulative studies proved that microRNAs (miRNAs) conform to aforesaid characteristics. MicroRNAs are short (about 22 nucleotides) endogenous non-coding RNA that have been shown to be of fundamental importance in cancer, and miRNA molecules have entered the clinic as diagnostic and prognostic biomarkers for patient stratification, as well as as therapeutic targets and drugs[9]. Plenty of miRNAs are proven to be related with cancers, like lung carcinoma, breast carcinoma, prostate cancer and renal cell cancer[10–13]. With stability in blood and measurability through quantitative reverse transcription-polymerase chain reaction (RT-qPCR), miRNAs have great potential as molecular markers for screening RCC[14].

In this study, we searched the literatures in PubMed with the retrieval strategy as follow: "Carcinoma, Renal Cell"[Mesh]) AND "MicroRNAs"[Mesh]. Then we used the miRNA expression level of 517 patients with ccRCC and 71 cases of normal controls from ENCORI database for further screening. With filter condition for $P < 0.01$ and $|\log FC| > 1$, 10 abnormally expressed miRNAs greatly associated with renal cancer were selected as candidate miRNAs for succedent assay (Fig. 1). They were hsa-miR-24-3p, hsa-miR-30c-5p, hsa-miR-142-3p, hsa-miR-205-5p, hsa-miR-206, hsa-miR-223-3p, hsa-miR-200c-5p, hsa-miR-335-5p, hsa-miR-340-5p and hsa-miR-423-3p. We utilized serum specimens of RCC patients and specimens of health volunteers to determine expression profile of 10 candidate miRNAs by means of RT-qPCR and identify diagnostic capacities of serum miRNAs, and then constructed miRNA panel by dint of logistic regression analysis. Moreover, we capitalized on bioinformatics analysis for miRNAs target gene prediction and functional enrichment annotation.

Materials and methods

Serum specimens collection

Two hundred and twenty serum specimens were collected from 108 RCC patients and 112 HCs between December 2017 to April 2021 at Peking University Shenzhen Hospital. The diagnosis of RCC patients was based on the 2016 World Health Organization (WHO) standard of classification for RCC. Histological grading was based on Fuhrman grading system, while TNM staging and clinical staging were according to the 2017 American Joint Committee on Cancer (AJCC) TNM staging and clinical staging criteria for RCC. The patients had no other malignant tumors and did not receive any treatment before serological serum acquisition. We recruited 112 HCs who had no history of malignant tumors, infectious or chronic diseases. The trial procedure adhered to the World Medical Association Declaration of Helsinki and administration by Peking University Shenzhen Hospital ethics committee.

We drew 10 mL of venous blood and timely dealt with them within 2 h. We centrifuged them at 1,000×g for 10 min and subsequently at 15,000×g for 5 min at 4 °C. Ultimately, serum specimens were obtained and refrigerated at − 80 °C in fresh tubes for subsequent assay.

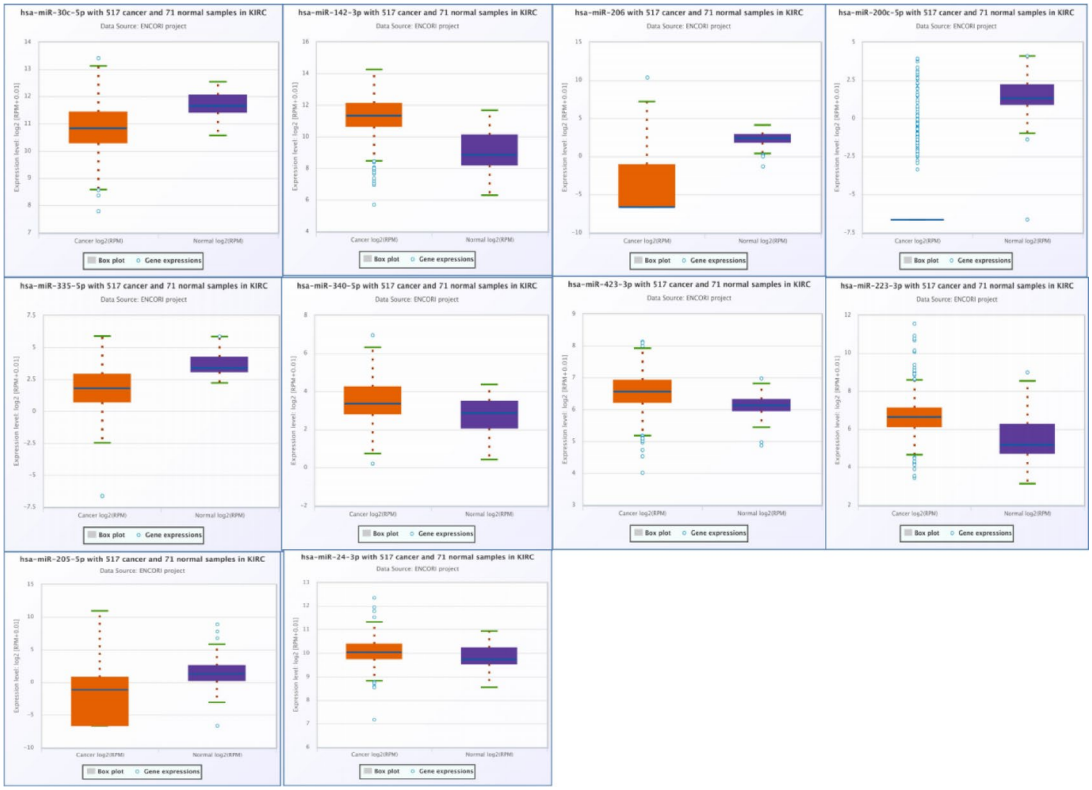

Fig. 1. In screening phase, through reviewing literatures and screened in ENCORI database with 517 RCC patients and 71HCs ($P < 0.01$ and $|\log FC| > 1$), 10 abnormally expressed miRNAs highly associated with RCC were found out. Filter criteria: p-value < 0.01 and $|\log FC| > 1$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Trial flow

The recapitulation of this study is demonstrated in Fig. 2. Firstly, in screening phase, we searched the literatures in PubMed with the retrieval strategy as follow: "Carcinoma, Renal Cell"[Mesh]) AND "MicroRNAs"[Mesh]. We then utilized the miRNA expression levels of 517 ccRCC patients and 71 normal control patients in the ENCORI database for further screening. Under the filtering conditions of $P < 0.01$ and $|\log FC| > 1$, we selected 10 aberrantly expressed miRNAs that were closely related to renal cancer as candidate miRNAs for successful detection. In training phase, we used 28 serum specimens of RCC patients and 28 specimens of health volunteers to test expression levels of 10 candidate miRNAs by means of RT-qPCR, and then selected critical miRNAs for subsequent phase with filter criteria: p-value < 0.05. Then, we utilized 80 samples of RCC patients and 84 samples of health volunteers in validation phase to profile expression of critical miRNAs. Finally, miRNA panel was constructed by dint of receiver operating characteristic (ROC) curves and backward stepwise logistic regression analysis. Moreover, we capitalized on bioinformatics techniques for miRNAs target gene prediction and functional enrichment annotation.

MiRNA isolation and qRT-PCR details

To normalize the variability in assay process, 2 μl of synthesized Caenorhabditis elegans miR-39 (cel-miR-39) (10 nM/L, RiboBio, Guangzhou, China) was added to serum specimens[15]. Then, we extracted total RNA from specimens pursuant to operational guideline of TRIzol LS isolation kit (Thermo Fisher Scientific, Waltham, USA). The RNA concentration and purity were gauged by virtue of NanoDrop 2000c spectrophotometer (Thermo Scientific, United States).

We implemented miRNAs amplification using the reverse transcription-specific primers from the Bulge-Loop miRNA RT-qPCR primer set (RiboBio, Guangzhou, China). The quantitative real-time polymerase chain reaction (qPCR) was achieved by dint of SYBR Green qPCR kit (SYBR Pre-mix Ex Taq II, TaKaRa) on LightCycler480 Real-Time PCR System (Roche Diagnostics, Mannheim, Germany). The processes were at 95 °C for 20 s, followed by 40 cycles of 95 °C for 10 s, 60 °C for 20 s and 70 °C for 10 s.

Statistical analysis

Relative miRNAs expressions were standardized to internal reference cel-miR-39 and computed through the 2-ΔΔCq method[16]. We utilized Student' t test or χ2 test to analyze differential expression of miRNAs in RCC and HCs specimens. Wilcoxon-Mann Whitney test was exerted to conduct statistical contrast among training and validation stages. Construction of miRNA panel was performed by virtue of multiple logistic regression analysis. To appraise diagnostic capacities of miRNAs and ascertain the optimal diagnostic panel, we utilized ROC curves, the area under the ROC curve (AUC) and Youden index (computed as J = Sensitivity + Specificity − 1). P-value < 0.05 was recognized as statistically significant. Confounding factors such as hypertension, obesity,

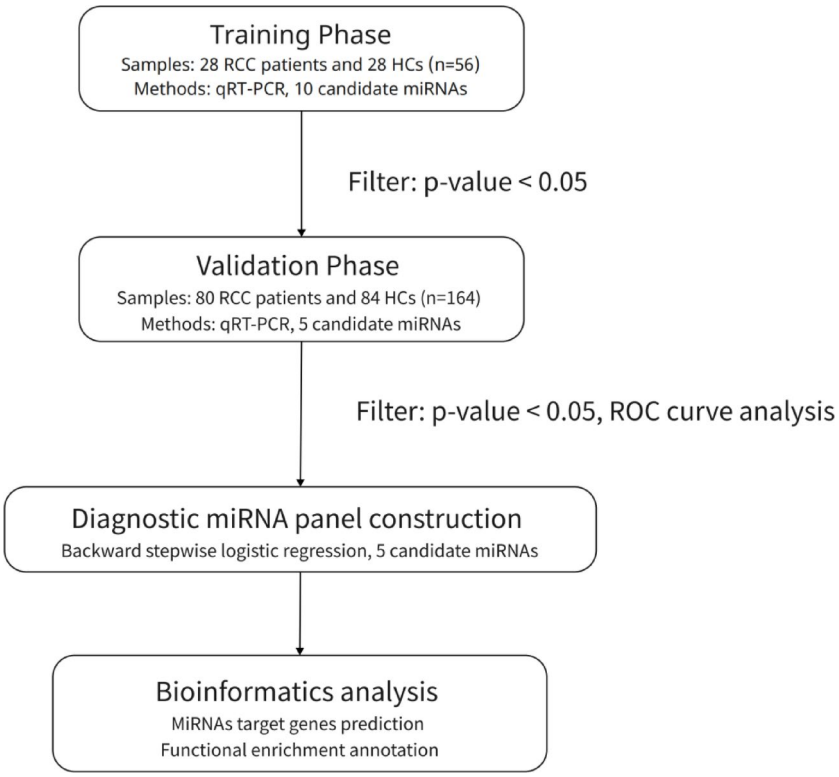

Fig. 2. The recapitulation of this study. RCC = Renal cell carcinoma; HC = Healthy control; ROC = receiver operating characteristic.

and smoking can affect miRNA levels. As a result, when we conducted our investigation, we took confounding bias control into account both throughout the study design and data analysis stages. Methods included: (1) reducing potential confounding factors; (2) matching confounding factors between experimental and control groups to maintain balance between the two groups; (3) grouping according to the randomization principle. We capitalized on Medcalc (Version 19), SPSS software (SPSS 26.0 Inc, Chicago, IL) and GraphPad Prism 8 (GraphPadSoftware Inc, La Jolla, CA) to implement statistical analyses.

Bioinformatic analysis

We predicted and tentatively verified information on miRNA-target interaction by means of miRWalk3.0 (http://mirwalk.umm.uni-heidelberg.de/)[17]. The screening criteria for putative target were that genes predicted in more than two miRNAs. Then, the predicted target genes were put into GEPIA to further verify their correlation with RCC[18]. We used the ENCORI database to conduct Kaplan-Meier survival analysis on the predicted target genes to explore the overall survival of RCC patients[19]. We took advantage of Enrichr database(http://amp.pharm.mssm.edu/Enrichr/) to accomplish enrichment analysis and functional annotation for putative target genes[20].

Results

Demographic manifestation of subjects

Overall 220 subjects containing 108 RCC patients and 112 healthy individuals were absorbed in this study. Demographic manifestation of subjects is displayed in Table 1. The RCC patients were diagnosed according to the 2016 World Health Organization (WHO) standard of classification for RCC. Histological grading was based on Fuhrman grading system, while TNM staging and clinical staging were according to the 2017 American Joint Committee on Cancer (AJCC) TNM staging and clinical staging criteria for RCC. The RCC patients accepted no therapy before blood drawing. The 112 HCs were subjects without tumors and diseases. Among training and validation stages, there was no significant difference between RCC patients and HCs in age and gender.

Selection of MiRNAs in screening phase and expression profile of MiRNAs in training phase

In screening phase, through reviewing literatures and screened in ENCORI database ($P < 0.01$ and $|\log FC| > 1$), 10 abnormally expressed miRNAs highly associated with RCC were found out (Fig. 1). They were miR-24-3p, miR-30c-5p, miR-142-3p, miR-205-5p, miR-206, miR-223-3p, miR-200c-5p, miR-335-5p, miR-340-5p and miR-423-3p and were selected for succedent assay.

In training phase, 10 candidate miRNAs were evaluated using specimens of 28 RCC patients and 28 HCs by dint of RT-qPCR approach. P-value < 0.05 was set as a filter criterion. As shown in Fig. 3, 5 miRNAs (miR-30c-5p, miR-142-3p, miR-206, miR-223-3p, miR-200c-5p) were eligible for further study.

Diagnostic capacities of critical MiRNAs in validation phase

As shown in Fig. 3, expressions of 5 critical miRNAs in serum were still significantly alteration with specimens of 80 RCC patients and 84 health subjects. To appraise diagnostic capacities of critical miRNAs, ROC curves

	Testing phase (n = 56)			Validation phase (n = 164)		
	RCC	HCs		RCC	HCs	
Total number	28	28		80	84	
Age at diagnosis	49.8 ± 11.3	51.1 ± 16.6	p = 0.729	50.3 ± 12.6	57.9 ± 14.1	p = 0.992
Gender			p = 0.403			p = 0.271
Male	20 (71.43%)	16 (57.14%)		50 (62.50%)	45 (53.57%)	
Female	8 (28.57%)	12 (42.86%)		30 (37.50%)	39 (46.43%)	
Location						
Left	16 (57.1%)			40 (50.0%)		
Right	12 (42.9%)			40 (50.0%)		
Fuhrman grade						
Grade I	2 (7.1%)			10 (12.5%)		
Grade II	15 (53.6%)			40 (50.0%)		
Grade III	8 (28.6%)			20 (25.0%)		
Grade IV	3 (10.7%)			10 (12.5%)		
AJCC clinical stage						
Stage I	23 (82.2%)			55 (68.8%)		
Stage II	2 (7.1%)			10 (12.5%)		
Stage III	2 (7.1%)			10 (12.5%)		
Stage IV	1 (3.6%)			5 (6.2%)		

Table 1. Demographic manifestation of 220 participants (RCC patients and HCs). Among two stages, there was no significant difference between RCC and HCs in age and gender. Parameters were shown as number(percentage). Statistical contrast was exerted through the Wilcoxon-Mann Whitney test.

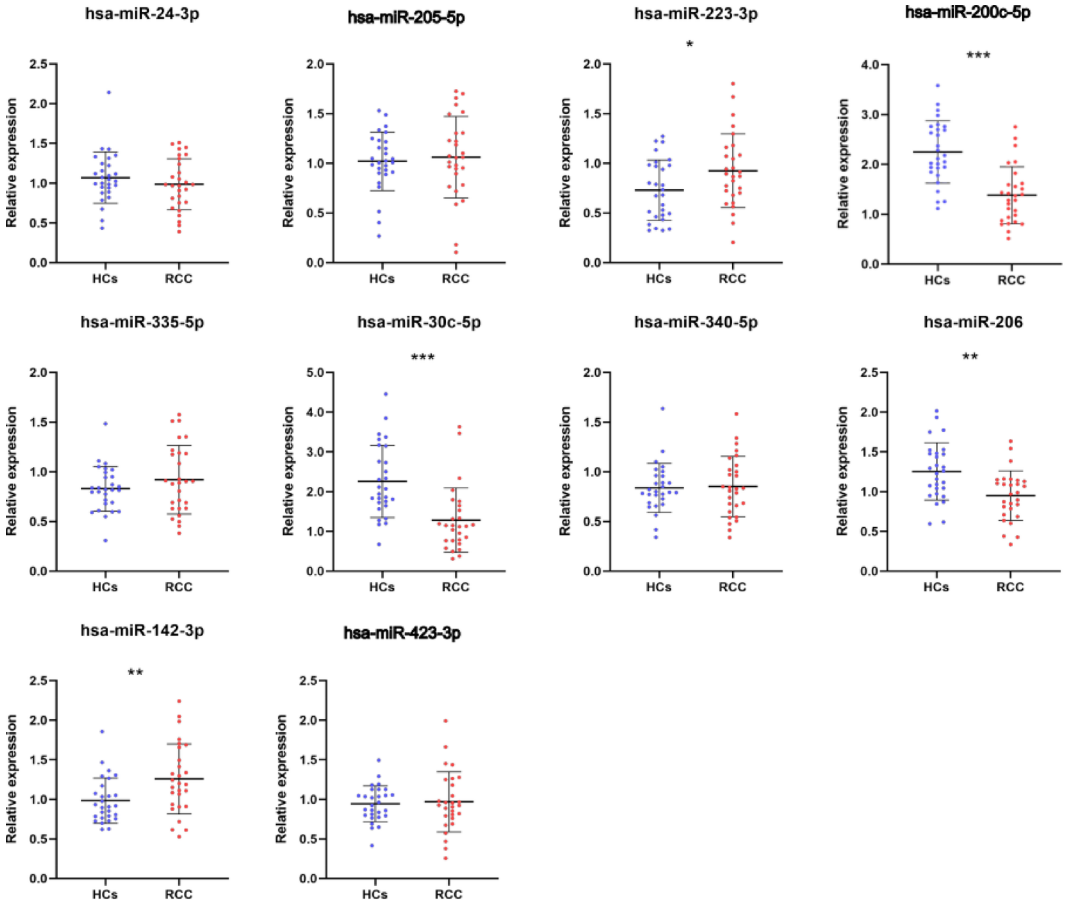

Fig. 3. The relative expression levels of ten candidate miRNAs in training phase. Filter criteria: p-value < 0.05. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

were plotted and whereafter AUCs and Youden index were figured out. The outcomes of five critical miRNAs were exhibited in Fig. 4; Table 2. Respectively, the AUCs for miR-30c-5p, miR-223-3p, miR-200c-5p, miR-142-3p and miR-206 were 0.769 (0.696–0.831, $P < 0.001$), 0.685 (0.608–0.756, $P < 0.001$), 0.639 (0.561–0.713, $P = 0.0013$), 0.716(0.641–0.784, $P < 0.001$) and 0.685 (0.608–0.755, $P < 0.001$). Calculation of Youden index revealed that miR-200c-5p and miR-206 had great sensitivity (78.75% and 75.00%) whereas miR-30c-5p had best specificity (83.33%).

Construction of diagnostic panel

For the purpose of having a better performance of miRNAs diagnosis, a three-miRNA panel combining miR-30c-5p, miR-142-3p and miR-206 was constructed by virtue of stepwise logistic regression analysis. This most remarkable panel was computed using the following formula: Logit(P) = 1.452 +(-1.594× miR-30c-5p) +(-1.871 × miR-206) + (3.204× miR-142-3p). In comparison with a single miRNA, the AUC of this panel had increased to 0.872 (0.811–0.919, $P < 0.001$; Fig. 5). Synchronously, sensitivity and specificity of this panel was respectively raised to 81.25% and 86.90% (Table 2).

Bioinformatics analysis

We detected putative target of the three-miRNA panel (miR-30c-5p, miR-142-3p and miR-206) using miRWalk3.0. As shown in Fig. 6(A), the number of genes predicted in more than two miRNAs were sum to 259 and that in all three miRNAs was 14. Through GEPIA with data from the TCGA and GTEx projects, expression analysis of 14 target genes was carried out. ATF3 and MYC were differentially expressed in kidney renal clear cell carcinoma contrasted to normal controls, thereof MYC upregulated and ATF3 downregulated (|log2Fold Change| cutoff > 1.5, $P < 0.01$, Fig. 6(B, C)). Therefore, they were identified as potential targeted genes of miR-30c-5p, miR-142-3p and miR-206. Kaplan-Meier survival analysis of 14 target genes was performed using ENCORI database. As shown in Fig. 6 (D, E, F), lower expression levels of GPATCH2L, SP1 and STRN were significantly associated with poor prognosis in kidney ccRCC patients (Log-Rank $p < 0.05$).

Enrichment analysis and function annotation for putative target genes were accomplished with Enrichr database. As illustrated in Fig. 7, the results compromised Biological Process, Cellular Component, Molecular Function and KEGG Pathways[21,22]. Biological Process implicated that target genes were prominently enriched in cell communication by electrical coupling involved in cardiac conduction, bundle of His cell to Purkinje

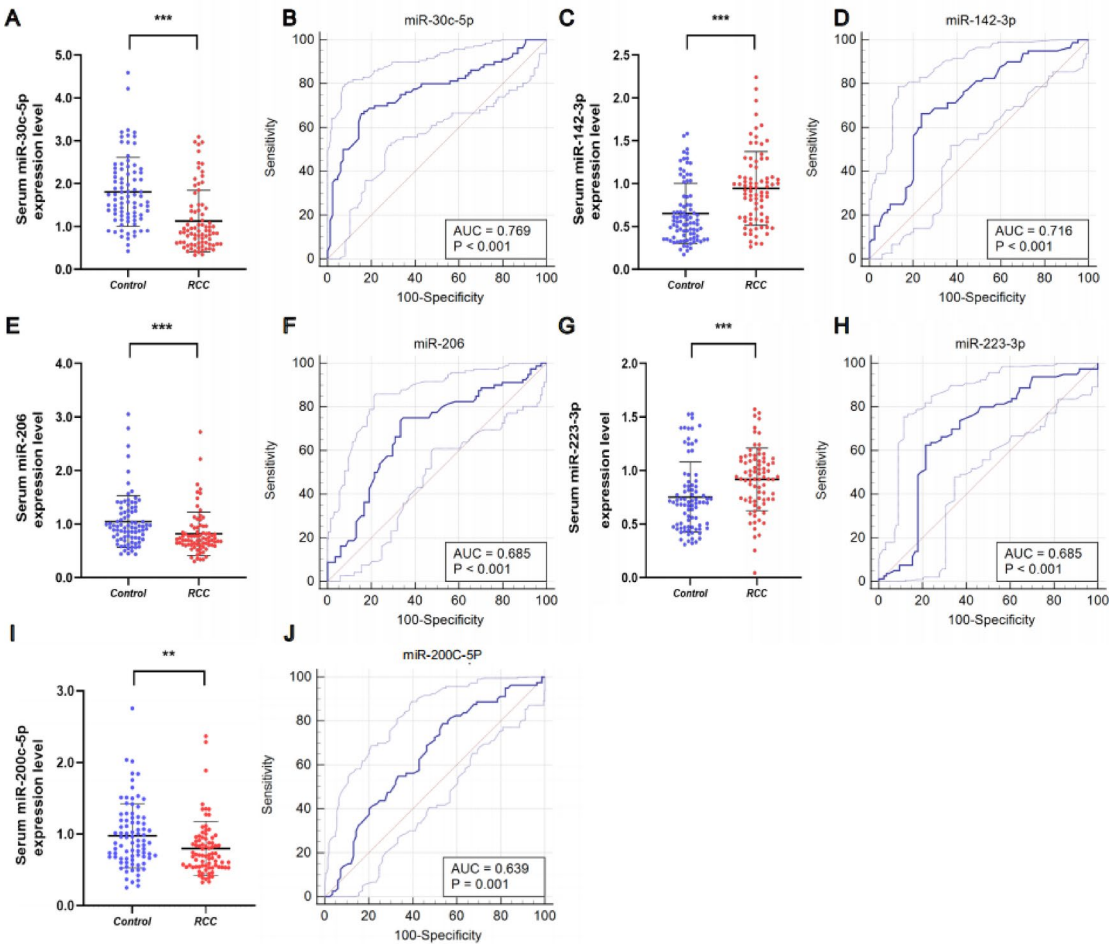

Fig. 4. Serum expression levels and receiver operating characteristic (ROC) curve of five chosen miRNAs in validation phase with 80 RCC patients and 84 HCs. (**C**) miR-142-3p and (**G**) miR-223-3p were significant upregulation in serum of RCC patients. (**A**) miR-30c-5p, (**E**) miR-206 and (**I**) miR-200c-5p were significant downregulation in serum of RCC patients. Respectively, AUCs of (**B**) miR-30c-5p, (**D**) miR-142-3p, (**F**) miR-206, (**H**) miR-223-3p, (**J**) miR-200c-5p were 0.769($P<0.001$), 0.716($P<0.001$), 0.685($P<0.001$), 0.685($P<0.001$), 0.639($P=0.001$). *$P<0.05$, **$P<0.01$, ***$P<0.001$.

	AUC	*P* value	95% CI	Associated criterion	Sensitivity (%)	Specificity (%)
miR-30c-5p	0.769	< 0.001	0.696–0.831	≤ 1.08	67.50	83.33
miR-223-3p	0.685	< 0.001	0.608–0.756	> 0.9	62.50	78.57
miR-200c-5p	0.639	= 0.0013	0.561–0.713	≤ 0.96	78.75	46.43
miR-142-3p	0.716	< 0.001	0.641–0.784	> 0.75	66.25	76.19
miR-206	0.685	< 0.001	0.608–0.755	≤ 0.85	75.00	65.48
three-miRNA panel	0.872	< 0.001	0.811–0.919	> 0.52135	81.25	86.90

Table 2. Outcomes of receiver operating characteristic curves and Youden index for 5 candidate MiRNAs and the three-miRNA panel. AUC: area under curve; CI: confidence interval.

myocyte communication, regulation of mitochondrial fission, regulation of cardiac muscle cell differentiation and positive regulation of cardiocyte differentiation. Cellular Component implicated that target genes were enriched in autophagosome membrane, intercalated disc, sarcoglycan complex, asymmetric synapse and postsynaptic density. Molecular Function elucidated that target genes were most enriched in transcription cis-regulatory region binding, MHC class I protein complex binding, cAMP-dependent protein kinase activity, leukemia inhibitory factor receptor activity and oncostatin-M receptor activity. KEGG pathways elucidated that target genes were enriched in Pathways in cancer, TGF-beta signaling pathway, Cocaine addiction Human T-cell leukemia virus 1 infection and Axon guidance.

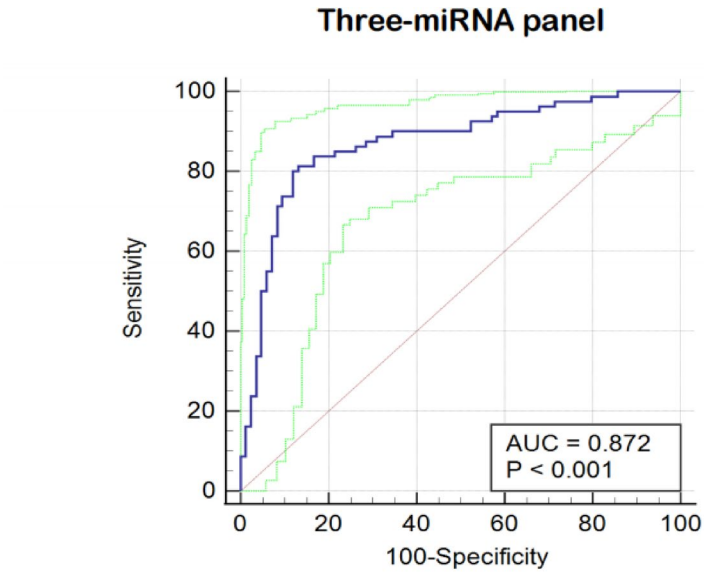

Fig. 5. The ROC curves analysis for the three-miRNA panel (miR-30c-5p, miR-142-3p and miR-206). The area under the ROC curves (AUC) of three-miRNA panel was 0.872 (95% CI:0.811–0.919, $P < 0.001$).

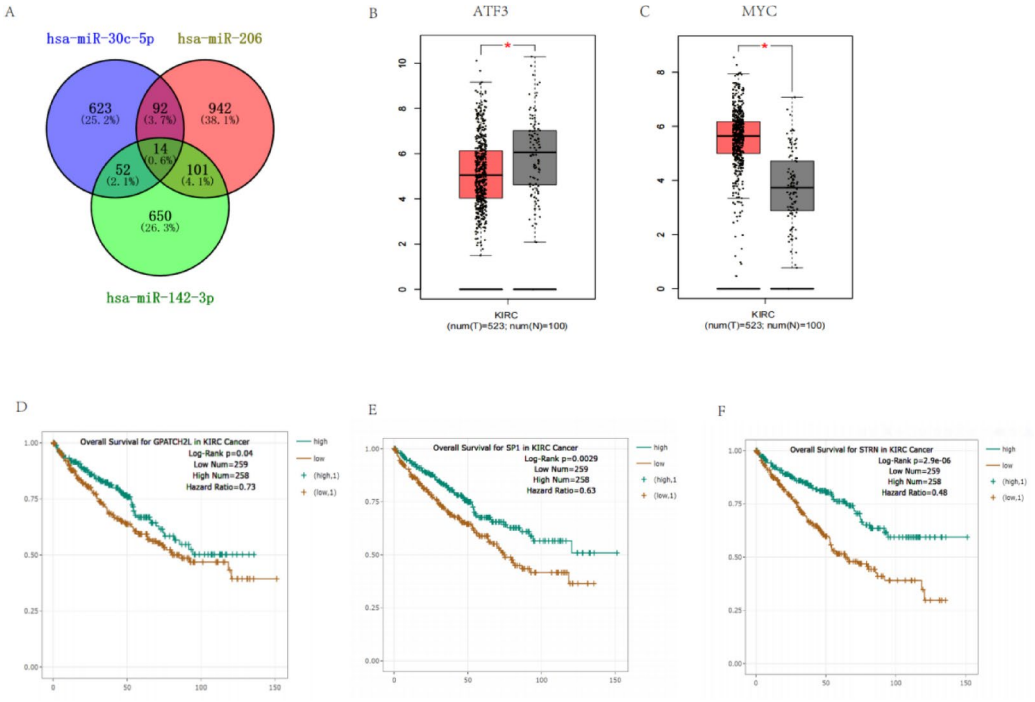

Fig. 6. Putative target genes of the three-miRNA panel were detected. (**A**) 259 genes targeted by two or three miRNAs were predicted by miRWalk 3.0. The expression analysis of 14 target genes was carried out through GEPIA. (**B**,**C**) ATF3 and MYC were differentially expressed in 523 kidney renal clear cell carcinoma (KIRC) contrasted to 100 normal controls with |log2Fold Change| cutoff > 1.5, $P < 0.01$, thereof MYC upregulated and ATF3 downregulated. Kaplan-Meier survival analysis of 14 target genes was conducted using ENCORI database. (**D**–**F**) Lower expression levels of GPATCH2L, SP1 and STRN were significantly associated with poor prognosis in kidney ccRCC patients (Log-Rank $p < 0.05$). $^*P < 0.01$.

Discussion

In spite of the endeavors over the past few decades, there is no praiseworthy noninvasive methods in the diagnosis of renal cell cancer. Disparity can be observed in 5 years survival with 90% in early stage and 12%

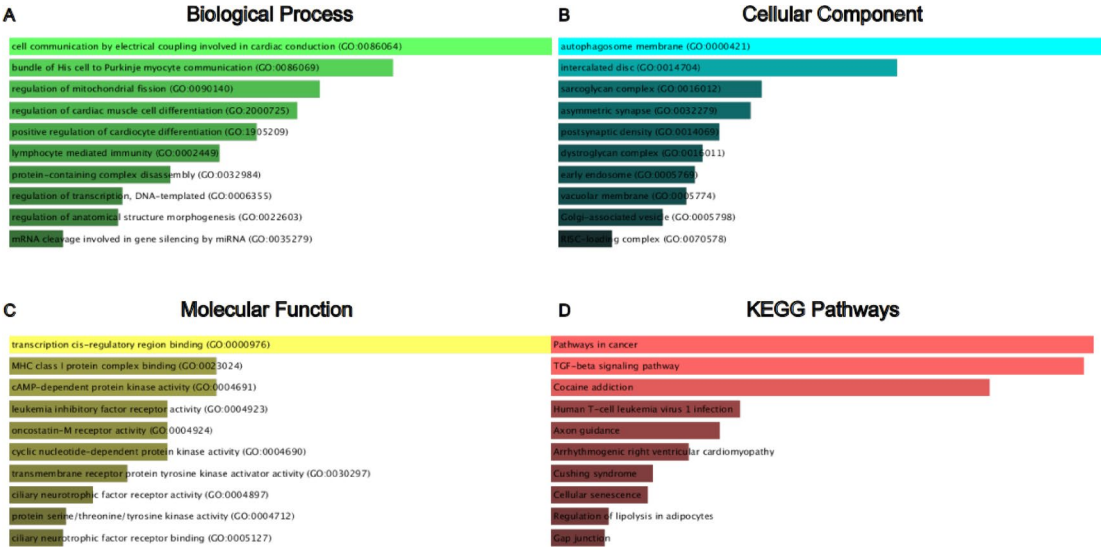

Fig. 7. GO functional annotation and KEGG pathway enrichment analysis of the target genes of miR-30c-5p, miR-142-3p, miR-206. The top ten (**A**) Biological process analysis, (**B**) cellular component analysis, (**C**) molecular function analysis, (**D**) KEGG pathway enrichment analysis are shown.

in advanced RCC[3]. Therefore, it is of great significance for early surveillance. Unfortunately, no manifested biomarkers could be applied to early diagnose RCC hitherto. Numerous studies elucidated that miRNAs are related with diverse malignant tumors and stably existed in circulating blood, which prompted miRNAs to serve as molecular markers for screening RCC[14].

Firstly, literatures associated with RCC were retrieved on PubMed and miRNA expression level was screened in ENCORI database, then ten miRNAs highly correlated with renal cancer were chosen as candidate miRNAs. Following with selection of miRNAs, we employed RT-qPCR to detect expression of ten miRNAs in serum samples of RCC patients and health volunteers, whereafter 5 miRNAs (miR-30c-5p, miR-142-3p, miR-206, miR-223-3p, miR-200c-5p) with statistic difference were selected for further assay. With more serum samples, expression levels of above 5 miRNAs were further identified and diagnostic value of them were appraised. Through regression analysis, we constructed a miRNA panel with miR-30c-5p, miR-142-3p, miR-206 which could discriminate RCC patients and healthy subjects satisfactorily.

In this study, we unraveled miR-30c-5p downregulation in serum of RCC patients in comparison with healthy individuals, which is in keeping with previous findings[23–25]. An experiment proved that downregulation of miR-30c-5p induced by TGF-β results in TGFBR3 down-regulated expression in RCC to promote cancer progression[26]. A study of ccRCC revealed that miR-30c-5p hampers ccRCC development via targeting heat-shock protein 5[25]. Another study suggested that miR-30c-5p was involved in restraint of ccRCC cell aggressiveness via directly targeting TWF1 and suppressing epithelial-mesenchymal transition (EMT)[23]. Taken together with our results, miR-30c-5p may function as RCC suppressor and seems to be a potential target in treating malignancies.

Numerous studies had proven that miR-142-3p showed markedly overexpression in RCC tissues[27–29]. In the present study we further validated that miR-142-3p represented upregulation in serum of RCC patients contrasted to healthy subjects. Zhang Y et al. unraveled that RhoBTB3 is targeted by miR-142-3p to modulate HIF-1 pathway and GGT/GSH signaling to facilitate the development of RCC[30]. An experiment conducted by Wu R et al. elucidated that miR-142-3p carried by small extracellular vesicles which were derived from kidney cancer stem cells modulated ERp44 to trigger endoplasmic reticulum stress and eventually result in apoptosis of normal kidney cells and impairment of kidney function[31]. We previously revealed that miR-142-3p was associated with terrible overall survival and could be utilized as prognostic marker for RCC patients[32]. Synthesizing the above evidence, we speculated that miR-142-3p may exert an oncogene function and serve as poor prognosis marker in RCC.

The former studies showed a marked reduction in miR-206 expression of RCC[33–36], which justifies our results. It has been revealed that miR-206 has tumor suppressor properties in RCC. A study by Xiao H et al. expounded that through targeting CDK4, CDK9 and Cyclin D1, which are cell cycle correlative gene, cell cycle arrest is induced by miR-206 and thereby ccRCC cell proliferation is repressed[34]. Similarly, Guo Z et al. found that proliferation and EMT of RCC was hampered by miR-206 via targeting CDK6[35]. Studies revealed that miR-206 inhibits the proliferation of renal clear cell carcinoma by targeting ZEB2[37]. Collectively, miR-206 may exert a repressive effect on RCC and therapeutic targets of it need to be further exploration.

Several previous studies have shown that when combining miRNAs with ultrasound/CT examination, the overall diagnostic accuracy can be improved[38–40]. We therefore hypothesized that the combination of serum microRNA and ultrasound/CT examination may also provide added clinical value to the existing clinical pathway for RCC. The potential of this combined application for RCC diagnosis is well worth exploring in subsequent studies.

ATF3 and MYC seem to be potential targets of miR-30c-5p, miR-142-3p and miR-206. Downregulation of ATF3 (activating transcription factor 3) in malignancies was substantiated by previous research, like esophageal squamous cell carcinoma[41], hepatocellular cancer[42], prostate cancer[43]. An experiment demonstrated that ATF3 could hamper cell proliferation, colony formation, and distant spread of ccRCC via β-Catenin Pathway[44]. Thus, ATF3 may exert a tumor repressive gene function on RCC. MYC is defined as oncogene and is often upregulated in RCC[45,46]. Accumulating evidence proven that MYC plays pivotal role in tumorigenesis, cell proliferation, tumor growth of RCC[45–48]. Therapy on RCC by targeting MYC may be a hot spot in the future, like MYC inhibitor.

This study also has some limitations. Firstly, the study's inclusion of 108 patients with RCC and 112 healthy controls is reasonable, but comprehensive validation of biomarkers in diverse populations may still be relatively small for direct consideration of clinical applications. A larger cohort from diverse population backgrounds would strengthen the generalizability of the findings. Secondly, only ten miRNAs are involved in our research, but there are large quantities of miRNAs interrelated with kidney cancer. Thirdly, although we predicted potential target gene of miRNA panel, the underlying mechanism was not investigated. In addition, the study lacks functional experiments. Future functional investigations are required to establish that these miRNAs influence kidney cancer cell proliferation, migration, or resistance to treatment. Lastly, whether treatment like nephrectomy can influence regulation of the expression of miRNAs is obscure. Thus, more experiments are needed.

Conclusions

In this study, we discovered three aberrantly expressed miRNAs in serum specimens of RCC patients and healthy volunteers and developed a novel miRNA-based panel with miR-30c-5p, miR-142-3p and miR-206. This three-miRNA panel has remarkable diagnostic capacity and could be utilized as potential noninvasive markers to discriminate RCC patients and healthy volunteers in advance.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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References

1. Sung, H. et al. Global Cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Cancer J. Clin.* **71**(3), 209–249 (2021).
2. Perazella, M. A., Dreicer, R. & Rosner, M. H. Renal cell carcinoma for the nephrologist. *Kidney Int.* **94**(3), 471–483 (2018).
3. Padala, S. A. et al. Epidemiology of renal cell carcinoma. *World J. Oncol.* **11**(3), 79–87 (2020).
4. Gray, R. E. & Harris, G. T. Renal cell carcinoma: diagnosis and management. *Am. Family Phys.* **99**(3), 179–184 (2019).
5. Capitanio, U. et al. Epidemiology of renal cell carcinoma. *Eur. Urol.* **75**(1), 74–84 (2019).
6. Vasudev, N. S. et al. Challenges of early renal cancer detection: symptom patterns and incidental diagnosis rate in a multicentre prospective UK cohort of patients presenting with suspected renal cancer. *BMJ Open.* **10**(5), e035938 (2020).
7. Sankineni, S., Brown, A., Cieciera, M., Choyke, P. L. & Turkbey, B. Imaging of renal cell carcinoma. *Urol. Oncol.* **34**(3), 147–155 (2016).
8. Jamis-Dow, C. A. et al. Small (< or = 3-cm) renal masses: detection with CT versus US and pathologic correlation. *Radiology* **198**(3), 785–788 (1996).
9. Jansson, M. D. & Lund, A. H. MicroRNA and cancer. *Mol. Oncol.* **6**(6), 590–610 (2012).
10. Yang, Y. et al. MicroRNA-218 functions as a tumor suppressor in lung cancer by targeting IL-6/STAT3 and negatively correlates with poor prognosis. *Mol. Cancer*. **16**(1), 141 (2017).
11. Fabris, L. et al. The potential of microRNAs as prostate cancer biomarkers. *Eur. Urol.* **70**(2), 312–322 (2016).
12. Wang, J. et al. Breast cancer cell-derived microRNA-155 suppresses tumor progression via enhancing immune cell recruitment and antitumor function. *J. Clin. Investig.* **132**(19). (2022).
13. Bao, N. et al. miR-378a-3p promotes renal cell carcinoma proliferation, migration, and invasion by targeting TOB2. *Clin. Translational Oncology: Official Publication Federation Span. Oncol. Soc. Natl. Cancer Inst. Mexico*. **25**(3), 748–757 (2023).
14. Wang, J., Chen, J. & Sen, S. MicroRNA as biomarkers and diagnostics. *J. Cell. Physiol.* **231**(1), 25–30 (2016).
15. Yu, Z., Lu, C. & Lai, Y. A serum MiRNAs signature for early diagnosis of bladder cancer. *Ann. Med.* **55**(1), 736–745 (2023).
16. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods (San Diego Calif)*. **25**(4), 402–408 (2001).
17. Sticht, C., De La Torre, C., Parveen, A. & Gretz, N. MiRWalk: an online resource for prediction of MicroRNA binding sites. *PloS One*. **13**(10), e0206239 (2018).
18. Tang, Z. et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res.* **45**(W1), W98–w102 (2017).
19. Li, J. H., Liu, S., Zhou, H., Qu, L. H. & Yang, J. H. StarBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res.* **42**(Database issue), D92–97 (2014).
20. Kuleshov, M. V. et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res.* **44**(W1), W90–97 (2016).
21. Kanehisa, M., Furumichi, M., Sato, Y., Matsuura, Y. & Ishiguro-Watanabe, M. KEGG: biological systems database as a model of the real world. *Nucleic Acids Res.* **53**(D1), D672–d677 (2025).
22. Kanehisa, M. Toward understanding the origin and evolution of cellular organisms. *Protein Science: Publication Protein Soc.* **28**(11), 1947–1951 (2019).
23. Outeiro-Pinho, G. et al. Epigenetically-regulated miR-30a/c-5p directly target TWF1 and hamper CcRCC cell aggressiveness. *Translational Research: J. Lab. Clin. Med.* **249**, 110–127 (2022).
24. Onyshchenko, K. V. et al. Expression of micro-RNA hsa-miR-30c-5p and hsa-miR-138-1 in renal cell carcinoma. *Exp. Oncol.* **42**(2), 115–119 (2020).
25. Song, S. et al. Urinary exosome miR-30c-5p as a biomarker of clear cell renal cell carcinoma that inhibits progression by targeting HSPA5. *J. Cell. Mol. Med.* **23**(10), 6755–6765 (2019).
26. Hanusek, K. et al. TGF–β1 affects the renal cancer mirnome and regulates tumor cells proliferation. *Int. J. Mol. Med.* **49**(4). (2022).

27. Lokeshwar, S. D. et al. Molecular characterization of renal cell carcinoma: A potential three-microRNA prognostic signature. *Cancer Epidemiol. Biomarkers Prevention: Publication Am. Association Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* **27**(4), 464–472 (2018).
28. Li, Y. et al. Oncogenic microRNA-142-3p is associated with cellular migration, proliferation and apoptosis in renal cell carcinoma. *Oncol. Lett.* **11**(2), 1235–1241 (2016).
29. Nientiedt, M. et al. Identification of aberrant tRNA-halves expression patterns in clear cell renal cell carcinoma. *Sci. Rep.* **6**, 37158 (2016).
30. Zhang, Y. et al. MicroRNA-142-3p promotes renal cell carcinoma progression by targeting RhoBTB3 to regulate HIF-1 signaling and GGT/GSH pathways. *Sci. Rep.* **13**(1), 5935 (2023).
31. Wu, R. et al. Renal cancer stem cell-derived sEVs impair renal function by inducing renal cell ERS and apoptosis in mice. *Translational Androl. Urol.* **11**(5), 578–594 (2022).
32. Peng, X. et al. miR-142-3p as a novel biomarker for predicting poor prognosis in renal cell carcinoma patients after surgery. *Int. J. Biol. Mark.* **34**(3), 302–308 (2019).
33. Heinemann, F. G. et al. Serum miR-122-5p and miR-206 expression: non-invasive prognostic biomarkers for renal cell carcinoma. *Clin. Epigenetics*. **10**, 11 (2018).
34. Xiao, H. et al. miR-206 functions as a novel cell cycle regulator and tumor suppressor in clear-cell renal cell carcinoma. *Cancer Lett.* **374**(1), 107–116 (2016).
35. Guo, Z., Jia, H. & Ge, J. MiR-206 suppresses proliferation and epithelial-mesenchymal transition of renal cell carcinoma by inhibiting CDK6 expression. *Hum. Cell.* **33**(3), 750–758 (2020).
36. Cai, Y., Li, H. & Zhang, Y. Downregulation of microRNA-206 suppresses clear cell renal carcinoma proliferation and invasion by targeting vascular endothelial growth factor A. *Oncol. Rep.* **35**(3), 1778–1786 (2016).
37. Chen, X. F., Guo, J. F., Xu, J. F., Yin, S. H. & Cao, W. L. MiRNA-206 inhibits proliferation of renal clear cell carcinoma by targeting ZEB2. *Eur. Rev. Med. Pharmacol. Sci.* **23**(18), 7826–7834 (2019).
38. Wang, C. et al. Integrating plasma exosomal MiRNAs, ultrasound radiomics and tPSA for the diagnosis and prediction of early prostate cancer: a multi-center study. *Clin. Translational Oncology: Official Publication Federation Span. Oncol. Soc. Natl. Cancer Inst. Mexico*. **27**(3), 1248–1262 (2025).
39. Wang, Y., Yang, J. & Wang, Y. Serum MiRNA combined with transvaginal color doppler ultrasound diagnosis clinical research on cervical cancer. *J. Ultrasound Medicine: Official J. Am. Inst. Ultrasound Med.* **43**(4), 791–798 (2024).
40. Zhang, Y. et al. Combination of serum microRNAs and ultrasound profile as predictive biomarkers of diagnosis and prognosis for papillary thyroid microcarcinoma. *Oncol. Rep.* **40**(6), 3611–3624 (2018).
41. Li, J. et al. ATF3 suppresses ESCC via downregulation of ID1. *Oncol. Lett.* **12**(3), 1642–1648 (2016).
42. Xiaoyan, L. et al. Low expression of activating transcription factor 3 in human hepatocellular carcinoma and its clinicopathological significance. *Pathol. Res. Pract.* **210**(8), 477–481 (2014).
43. Wang, Z. & Yan, C. Emerging roles of ATF3 in the suppression of prostate cancer. *Mol. Cell. Oncol.* **3**(1), e1010948 (2016).
44. Gao, S. et al. ATF3 suppresses growth and metastasis of clear cell renal cell carcinoma by deactivating EGFR/AKT/GSK3β/β-catenin signaling pathway. *Front. Cell. Dev. Biology*. **9**, 618987 (2021).
45. Shroff, E. H. et al. MYC oncogene overexpression drives renal cell carcinoma in a mouse model through glutamine metabolism. *Proc. Natl. Acad. Sci. U.S.A.* **112**(21), 6539–6544 (2015).
46. Tang, S. W. et al. MYC pathway is activated in clear cell renal cell carcinoma and essential for proliferation of clear cell renal cell carcinoma cells. *Cancer Lett.* **273**(1), 35–43 (2009).
47. Gordan, J. D., Bertout, J. A., Hu, C. J., Diehl, J. A. & Simon, M. C. HIF-2alpha promotes hypoxic cell proliferation by enhancing c-myc transcriptional activity. *Cancer Cell.* **11**(4), 335–347 (2007).
48. Bailey, S. T. et al. MYC activation cooperates with Vhl and Ink4a/Arf loss to induce clear cell renal cell carcinoma. *Nat. Commun.* **8**, 15770 (2017).

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Author contributions

SL. and XL. wrote the main manuscript text and ZG. WC. YL. PZ. YW. WW.SC. HZ. prepared Figs. 1, 2, 3, 4, 5, 6 and 7 and all tables. LT and YL. provided Conceptualization. All authors reviewed the manuscript.

Declarations

Ethical approval and consent to participate

All participants recruited in our study provided written informed consent. The protocol of this study was approved by the Ethics Committee of Peking University Shenzhen Hospital (2017-007).

Competing interests

The authors declare no competing interests.

Additional information

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