The microRNA prediction models as ancillary diagnosis biomarkers for urothelial carcinoma in patients with chronic kidney disease

An-Lun Li
National Central University
Che-Yi Chou
Asia University Hospital
Chien-Lung Chen
Taiwan Landseed Hospital
Kun-Lin Wu
National Central University
Shih-Chieh Lin
National Cheng Kung University
Chi-Ching Chen
Taiwan Landseed Hospital
Kuo-Hsiung Shu
Lin Shin Medical Corporation Lin Shin Hospital
Hung-Chun Chen
Kaohsiung Medical University
Ming-Chang Wang
National Cheng Kung University Hospital
Chia-Chu Chang
Buddhist Tzu Chi General Hospital Hualien: Hualien Tzu Chi Hospital
Bang-Gee Hsu
Hualien Tzu Chi Hospital
Mai-Szu Wu
Taipei Medical University Hospital
Chiu-Ching Huang
China Medical University Hospital
Nianhan Ma (nianhan.ma@gmail.com)
National Central University https://orcid.org/0000-0003-1800-2306

Research

Keywords: Urothelial carcinoma, chronic kidney disease, microRNA, biomarker, biofluid

Posted Date: March 26th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-353648/v1

Abstract

Background: Urothelial carcinoma is a common urological cancer in patients with chronic kidney disease in Taiwan. However, urinary cystoscopy is an invasive procedure and urine cytology showed low sensitivity for low-grade tumor. High accuracy with noninvasive diagnostic tools for UC is needed for patients with CKD.

Methods: A total of 272 urine and 138 plasma samples to detect the miRNA expression levels for establishing UC signatures from patients with CKD. 17 candidate miRNAs of urine and plasma were selected by the PCR array, and a single qRT-PCR assay was used to confirm the results. The website of a smRNA-seq analysis of the clinical specimens was compared to survival status. The miRNA prediction models were produced for predicting UC by multiple logistic regression.

Results: Urinary miR-1274a and miR-30a-5p expression levels were significantly lower but miR-19a-5p expression levels were higher in UC when compared with CKD. In plasma samples, miR-155-5p, miR-19b-1-5p, miR-378 and miR-636 showed significantly lower expression in UC compared to those with CKD. The Kaplan-Meier curve showed that lower expression of miR-19a, miR-19b, miR-636 and miR-378, and higher expression of miR-708-5p were associated with poor prognosis in patients with bladder cancer. In addition, we produced classifiers for predicting UC by multiple logistic regression. The urine signature was developed with four miRNAs, and the AUC was 0.8211. Eight miRNA expression levels from both urine and plasma samples were examined, and the AUC was 0.8595.

Conclusion: Two miRNA classifiers could improve the drawbacks of current UC biomarker screenings for patients with CKD.

Background

Urothelial carcinoma (UC) includes bladder cancer and urinary tract cancer. A worldwide report revealed approximately 549,393 newly diagnosed cases and 199,922 deaths from UC in 2018 (1). UC accounts for 90% of bladder cancer and is the most common malignancy involving the urinary tract (2). UC is responsible for 31% of urinary tract cancers in Taiwan (3). 51–58.6% of UC patients have chronic kidney disease (CKD) and patients with CKD are more at risk of UC (3–6).Advanced stages of CKD are associated with poor prognosis for UC treatment (7, 8). The association of CKD and UC makes it more difficult to diagnosis UC in patients with CKD. Painless hematuria is the most common presenting symptom in UC, but painless hematuria is also common in patients with CKD. The sensitivity and specificity of UC protein markers are decreased because the serum protein levels are increased in patients with CKD (9–13). Intravenous pyelography or urography cannot be performed in patients with CKD because of the exposure of contrast media (14). The high specificity of urinary cytology can be interfered by the presence of CKD (15). Invasive cystoscopy or ureteroscopy are usually needed to confirm the diagnosis of UC. In addition, the cost of cystoscopy or ureteroscopy is expensive, the procedure is invasive and uncomfortable, and patients need to experience the risk of anesthesia and surgery. Therefore, developing highly accurate noninvasive biomarkers for UC is urgently needed for patients with CKD.

The miRNA pattern in biofluids was thought to provide disease molecular markers to predict or differentiate different types of cancers because the development of cancer is associated with the expression levels of circulating miRNAs (16–18). In addition, miRNAs can be packed and released through exosomes or extracellular vesicles, enhancing their stability in biofluids such as urine and plasma. Some reports have discussed the difference in miRNA expression in biofluids for predicting urological tumors, but most of the studies compared healthy donors with patients with cancer (16, 19–23). Our previous study demonstrated that the miRNA prediction models of plasma predicted UC in patients with ESRD (24). In the present work, we investigated the expression levels of miRNAs in the urine and plasma of patients with CKD. We further used these miRNA signatures to develop prediction models of UC for patients with CKD.

Materials And Methods

Patients and samples

The Taiwan Urothelial Cancer Consortium (TUCC) organized a multicenter study of urothelial cancer (UC) from ten hospitals in Taiwan. The ten hospitals are distributed throughout the country (13). A total of 272 patients (50, 111 and 111 samples were healthy, CKD and CKD + UC, respectively) participated in this study. The urine and blood samples were collected from control patients after obtaining informed consents. The urine and blood samples were collected from CKD + UC patients within three days before the surgery. Samples were centrifuged at 1,700 and 2000 x g for 20 minutes. The supernatant was collected and stored at -80°C.

Ethics approval and informed consent

This study was approved by the internal review board (IRB) of China Medical University Hospital (CMUH 102-REC2- 043) and the IRB of each hospital. Written informed consent was obtained from all patients to use their urine and blood samples. All methods were followed in accordance with guidelines and regulations.

Total RNA Isolation from biofluids and miRNA Quantification by RT-PCR

Total RNA from urine and plasma was extracted using TRIzol® LS Reagent and a mirVana™ miRNA Isolation Kit according to the standard protocol. The spiked-in control of cel-miR-39-3p for technical variability followed the previously described (24). The RNA quality was detected by a spectrophotometer (BioTek Instruments). All RNA samples were stored at -80°C. The TaqMan™ MicroRNA Reverse Transcription Kit (Applied Biosystems) was used to produce the cDNAs from miRNAs, and the standard protocol or ingredients were followed as described previously (25). The microRNA profiling was generated using TaqMan® 2x Universal PCR master mix without UNG and TaqMan® Array Human MicroRNA Cards (4444913). TaqMan® miRNA assays quantified the specific miRNAs expression (4427975) (Thermo Fisher Scientific).

Data Statistical Analysis

The expression of miRNAs was determined using the $2^{-\Delta CT}$ method relative to RNU6. The miRNA expression data were transformed to the $\log_{10}$ form to fit a normal distribution. The value of no detection of miRNA expression was replaced with the − 4.5 value in the $\log_{10}$ form. Clinical characteristics between healthy, CKD and CKD + UC patients were evaluated using Pearson's chi-squared test for each variable. Normality and Student's t-test were used for unpaired comparisons of two groups. All tests were two-tailed and were assessed by Levene's test. All statistical analyses were completed with GraphPad Prism software. Logistic regression of miRNA expression was combined with SigmaPlot software. All statistic methods or procedures were followed as described previously (24).

Survival Curve Analysis

A public website of a smRNA-seq analysis of the clinical specimens was compared to survival status with KM-plotter (http://kmplot.com/analysis/index.php?p=background) (26). Then, miRNA expression values from clinical specimens were used to perform Kaplan-Meier survival curve analysis according to the clinical parameters provided in the dataset. High and low expression groups were created using an automatic cutoff as described previously (25).

Results

Differentially Expressed Urine and Plasma miRNAs between CKD and CKD + UC

In order to discover an ancillary diagnostic tool for UC in patients with CKD, all samples were collected from ten hospitals throughout Taiwan from 2013 to 2018. We matched the patients with CKD and CKD + UC by sex, age, and CKD stage to select the difference in miRNA expression levels in this study (Table 1). For CKD + UC, blood and urine samples were collected within three days before surgery. For the control group, blood and urine samples were collected after tracking their renal functions as CKD. Next, we detected 754 miRNA expression levels from 22 (11 CKD and 11 CKD + UC) and 16 (8 CKD and 8 CKD + UC) samples of urine and plasma, respectively, by high-throughput and quantitative real-time PCR arrays. We not only calculated the relative expression levels by RNU6 but also calculated the miRNA expression of the ratio value of two different miRNAs from the same sample to remove the normalization problem in cell-free biofluids. To date, no literature has noted that any miRNA is a competent internal control in biofluids, and we found that the ratio value method could reduce individual sample differences. We compared CKD and CKD + UC samples in several ways, and 17 candidate miRNAs were selected (Table 2).

Next, we validated the expression levels of 17 candidate miRNAs from a screening set by the single qRT-PCR method and measured 200 urine samples (100 CKD and 100 CKD + UC) and 138 plasma samples (74 CKD and 64 CKD + UC) in training and testing set. Our results showed that the expression of seven miRNAs was significantly different between the CKD and CKD + UC samples (Fig. 1). In urine samples, miR-1274a and miR-30a-5p expression levels were significantly decreased ($p = 0.0243$ and 0.0356, respectively), but the miR-19a-5p expression level was significantly increased ($p < 0.001$) (Fig. 1A). In the plasma samples, miR-155-5p, miR-19b-1-5p, miR-378 and miR-636 expression levels were significantly decreased ($p = 0.0324$, 0.043, 0.287 and 0.0288, respectively) (Fig. 1B). Interestingly, previous study has shown that miR-30a-5p had significantly low expression levels in plasma samples of patients with BC (27). In addition, miR-155-5p expression was also reported to be significantly decreased in the urine sediment cells of patients with BC (28).

Many studies have compared the different miRNA expression levels between the healthy group and patients with UC (29–31). Unlike previous studies, we tried to compare miRNA expression differences to identify UC from patients with CKD. To determine whether these candidate miRNAs from this study also have the potential to distinguish from the healthy group, we further collected 50 healthy cases to analyze the differences within the healthy, CKD and CKD + UC groups. miR-1274a and miR-30a-5p had significant differences between healthy cases and CKD + UC ($p < 0.001$). Interestingly, we found that three miRNAs, namely, miR-30a-5p, miR-19a-5p and miR-708-5p, not only can provide a reliable ability to distinguish patients who were CKD or CKD + UC (AUC = 0.64, 0.61 and 0.63, respectively) but also had significantly different expression levels between healthy subjects and CKD ($p = 0.007$, 0.0326 and 0.009, respectively) (Table 3 and Fig. 2).

miRNA Expression Levels as a Prognostic Marker of Bladder Cancer and Kidney Cancer

It has been known that miRNA expression is associated with cancer prognosis. Therefore, we investigated these 17 candidate miRNAs in a public database (http://kmplot.com) to analyze the association between newly identified miRNA expression levels and the 5-year survival rate by the Kaplan-Meier method. Among these miRNAs, lower expression levels of miR-19a, miR-19b, miR-636, and miR-378 and higher expression levels of miR-708-5p were associated with poor prognosis in BC ($p = 0.0055$, 0.014, 0.041, 0.02 and 0.027, respectively) (Fig. 3A). In addition, lower expression of miR-30a and or higher miR-155 was associated with poor prognosis in urinary cancer, such as papillary cell carcinoma and clear cell renal cell carcinoma (Fig. 3B).

The Prediction Models to Predict UC for Patients with CKD

To develop a miRNA signature-based predicative model for UC of patients with all stages of CKD, receiver operating characteristic curve (ROC) analysis was performed. 17 candidate miRNA expression levels in urine or plasma from the training set samples were examined. The area under the receiver operating characteristic curve (AUC) is the most commonly used performance measure to indicate the discriminative ability of a prediction mode, and an AUC value higher than 0.6 could be a potential marker. Four miRNAs expressed in urine and four miRNAs expressed in plasma had AUC

values above 0.6. The AUC values of miR-1274a, miR-19a-5p, miR-30a-5p and miR-708-5p in urine were 0.71, 0.61, 0.64 and 0.628, respectively (95% confidence intervals: 0.6113 to 0.8198, 0.5016 to 0.7304, 0.5980 to 0.8073 and 0.5136 to 0.7424, respectively) (Table 3A). In plasma samples, miR-155-5p, miR-19b-1-5p, miR-210 and miR-636 could be potential markers, and their AUC values were 0.65, 0.66, 0.64 and 0.61, respectively (95% confidence intervals: 0.5168 to 0.7773, 0.5327 to 0.7875, 0.5107 to 0.7704 and 0.4758 to 0.7431, respectively) (Table 3B). Interestingly, these miRNAs have been reported in previous studies to play key functions not only in BC but also in clear cell renal cell carcinoma (32–35).

The combination of multiple factors compared to a single factor always presents more reliable prediction results for clinical classification. Therefore, we utilized multiple logistic regression calculation formulas to produce the prediction model combining different miRNA expression levels from the training group (Table 1). In the urine sample, the top four AUC values for miR-1274A, miR-30a-5p, miR-19b-3p and miR-708-5p were combined and calculated together, and the AUC was 0.8211 (95% confidence interval: 0.7359 to 0.9063). We also validated this panel in the testing group, and the data from 200 patients show that the accuracy of the 4-miRNA signature in urine was 70%, based on the cutoff value > 0.483 (Fig. 4A). Furthermore, we added another four miRNAs, namely, miR-155-5p, miR-19b-1-5p, miR-210 and miR-636, in plasma to increase the AUC value, and the AUC value increased up to 0.8507 (95% confidence interval: 0.7751 to 0.9439). The accuracy of the 8-miRNA signature was 72%, based on the cutoff value > -0.5940 (Fig. 4B)

Nomogram construction based on miRNAs expression signature

In order to validate the risk of UC, a nomogram integrated miRNAs expression signature was established. The miRNA expression level was transformed to the points based on the cutoff value from the training group. The cutoff of miR-1274a, miR-19a-5p, miR-30a-5p and miR-708-5p were < 34.41, > $2.24*10^{-4}$, < 3.798 and > $2.235*10^{-7}$, respectively. The AUC of the nomogram for urine samples were 0.7383 (n = 200, 95% confidence interval: to 0.6685 to 0.8080) (Fig. 5A). Furthermore, the cutoff of miR-155-5p, miR-19b-5p, miR-210-3p, miR-378 and miR-636 were < 1.21, < 0.5107, < 4.766 and < 0.5722, respectively. The AUC of the nomogram for urine and plasma samples were 0.8096 (n = 138, 95% confidence interval: 0.7365 to 0.8827) (Fig. 5B).

Discussion

We found that the expression levels of miR-1274a and miR-30a-5p were significantly lower in CKD + UC compared with patients with CKD in the urine samples, but conversely, miR-19a-5p was significantly higher in CKD + UC patients (Fig. 1A). High expression levels of miR-1274a have been demonstrated in clear cell renal cell carcinoma (ccRCC) compared with adjacent normal cells, which further induced cell apoptosis through the regulation of BMPR1B expression (32). The expression level of miR-30a-5p had significant decreased about 40% in the plasma sample of the BC patients when compared to the healthy, indicating lower expression of miR-30a-5p in urine due to the filtering on renal corpuscle (27). miR-30a-5p showed lower expression in UTUC compared with normal tissue, which was linked to decreased epithelial-to-mesenchymal transition (EMT) through regulation of the tight junction protein claudin-5 (34). Another study showed that miR-30a-5p expression was lower in muscle invasive BC and that overexpression of miR-30a inhibited the malignancy of UC through Notch-1 gene regulation (33). In addition, compared with the healthy group, miR-19a showed higher expression levels in the samples of BC such as cell lines, tissue and plasma (30).

Our results showed that the expression levels of miR-19b-1-5p, miR-378, miR-636 and miR-155-5p were significantly lower in CKD + UC plasma samples (Fig. 1B). The data of hazard ratio showed that the miR-19b expression level was highly correlated with the incidence of BC. Higher miR-19b expression levels were found in ccRCC tissue, and miR-19b promoted the malignancy of ccRCC through RhoB gene expression (36, 37). Lower miR-378 expression levels were significantly linked to the high-risk group suffering from prostate cancer (38). miR-155 showed a higher expression level in the urine and tissue of patients with BC (28, 39, 40). It has been demonstrated that miR-155 is a key regulator that promotes BC growth through DMTF1 regulation.

Interestingly, our results revealed that miR-155 had significantly lower expression in plasma samples of patients with UC compared to patients with CKD. However, other reports showed that miR-155 had higher expression in the urine, plasma and tissues of patients with BC compared to the healthy population (24, 28, 39, 40). In addition, three studies indicated different expression levels of miR-378 in RCC compared to the serum of healthy (41–43). Importantly, the statistical methods, including the calculation of expression levels and different internal controls, led to different results. The expression levels of miRNAs were inconsistent between cells and urine, possibly due to tissue specificity or the different functional effects between cellular and extracellular environments.

Our results showed that lower expression of miR-19a, miR-19b, miR-636 and miR-378 and higher expression of miR-708-5p were linked to the poor prognosis of patients with BC (Fig. 3A). On the other hand, the group with lower miR-30a expression and higher miR-155 expression was linked to the poor prognosis of ccRCC (Fig. 3B). Interestingly, a previous study showed that high miR-19a expression was associated with poor prognosis of prostate cancer (44). Low expression of miR-19b-1-5p in tissue was linked to poor prognosis of BC, and low miR-19b expression in patients suffering from prostate cancer also showed poor prognosis (24, 44). Poor prognosis was also found in the group with low miR-378 expression in the plasma of RCC (42). In a previous study, miR-708 was reported in non-small cell lung cancer, ovarian cancer and stomach cancer (45–47).

Conclusions

In this study, we aimed to establish predictive models of UC using miRNA expression levels in the urine and plasma. The prediction models could be an ancillary diagnostic marker for patients with CKD, who are at high risk of developing UC. As far as we know, this is the first study to investigate UC in CKD patients by miRNA expression levels in their biofluids.

In our study, we established miRNA prediction models with the combination of expression of four or eight miRNAs as a noninvasive screening or a diagnostic tool to predict the occurrence of UC in patients with CKD (Fig. 4). This study showed that the expression levels of specific miRNAs (miR-1274a, miR-30a-5p, miR-19a-5p, miR-155, miR-19b-1, miR-378 and miR-636) were significantly different between patients with CKD and those with UC. More importantly, the miRNA prediction models offered higher accuracy than the current serological test method such as HE-4 (13). This novel miRNA prediction models can improve the current problem of late diagnosis and be used to screen UC for patients with CKD in the future.

Abbreviations

AUC	Area under the curve
miRNA	microRNA
qRT-PCR	Quantitative Reverse Transcription Polymerase Chain Reaction
ROC	Receiver Operating Characteristic
UC	Urothelial carcinoma
CKD	Chronic Kidney Disease

Declarations

Acknowledgement

We would like to thank Yun-Ru Chiang and De-Xin Kong of National Central University and the assistants from ten hospitals for sample collection and processing. The authors thank the technical support provided by the Core Facilities for High Throughput Experimental Analysis of the Institute of Systems Biology and Bioinformatics, National Central University.

Author Contributions

Conceptualization, A.L., N.H. and C.C. Huang; methodology, A.L., N.H.; validation, A.L.; formal analysis, A.L., C.H., S.C., and C.C. Chen; resources, C.L., C.Y., K.H., H.C., M.C., C.C. Chang, B.G., M.S. and C.C. Huang; data curation, A.L. and C.H.; writing—original draft preparation, A.L.; writing—review and editing, N.H. and C.C. Huang; visualization, A.L.; supervision, N.H. and C.C. Huang; project administration, N.H.; funding acquisition, N.H. and C.C.H. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by the following programs: Academia Sinica, grant number BM10701010023, BM10601010037, BM104010113 and BM103010089, NCU-Landseed Interna-tional Chronic Disease Research Center, grant number NCU-LSH-108-A-005 and NCU-LSH-109-A-004, Ministry of Science and Technology, grant number MOST109-2628-B-008-001, National Health Research Institutes, grant number NHRI-109BCCO-MF-202018-01, Landseed International Hospital, grant number 2018-05.

Availability of data and materials

All data generated or analyzed in this study are included in this article.

Ethics approval and consent to participate

This study was approved by IRB ethics committee.

Consent for publication

No individual data were used in this study.

Competing interests

We declare no competing financial interest.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
2. Kaseb H, Aeddula NR. Cancer, Bladder. StatPearls. Treasure Island (FL)2019.
3. Yang MH, Chen KK, Yen CC, Wang WS, Chang YH, Huang WJ, et al. Unusually high incidence of upper urinary tract urothelial carcinoma in Taiwan. Urology. 2002;59(5):681–7.
4. Chen JS, Lu CL, Huang LC, Shen CH, Chen SC. Chronic Kidney Disease is Associated With Upper Tract Urothelial Carcinoma: A Nationwide Population-Based Cohort Study in Taiwan. Med (Baltim). 2016;95(14):e3255.
5. Hsieh JJ, Purdue MP, Signoretti S, Swanton C, Albiges L, Schmidinger M, et al. Renal cell carcinoma. Nat Rev Dis Primers. 2017;3:17009.
6. Lowrance WT, Ordonez J, Udaltsova N, Russo P, Go AS. CKD and the risk of incident cancer. J Am Soc Nephrol. 2014;25(10):2327–34.

7. Hatakeyama S, Koie T, Narita T, Hosogoe S, Yamamoto H, Tobisawa Y, et al. Renal Function Outcomes and Risk Factors for Stage 3B Chronic Kidney Disease after Urinary Diversion in Patients with Muscle Invasive Bladder Cancer [corrected]. PLoS One. 2016;11(2):e0149544.
8. Kodama H, Hatakeyama S, Fujita N, Iwamura H, Anan G, Fukushi K, et al. Preoperative chronic kidney disease predicts poor oncological outcomes after radical nephroureterectomy in patients with upper urinary tract urothelial carcinoma. Oncotarget. 2017;8(47):83183–94.
9. Lotan Y, Elias K, Svatek RS, Bagrodia A, Nuss G, Moran B, et al. Bladder cancer screening in a high risk asymptomatic population using a point of care urine based protein tumor marker. J Urol. 2009;182(1):52–7. discussion 8.
10. Ahmadi H, Djaladat H, Cai J, Miranda G, Daneshmand S. Precystectomy serum levels of carbohydrate antigen 19 – 9, carbohydrate antigen 125, and carcinoembryonic antigen: prognostic value in invasive urothelial carcinoma of the bladder. Urol Oncol. 2014;32(5):648–56.
11. Manvar AM, Wallen EM, Pruthi RS, Nielsen ME. Prognostic value of CA 125 in transitional cell carcinoma of the bladder. Expert Rev Anticancer Ther. 2010;10(12):1877–81.
12. Xi Z, LinLin M, Ye T. Human epididymis protein 4 is a biomarker for transitional cell carcinoma in the urinary system. J Clin Lab Anal. 2009;23(6):357–61.
13. Chou CY, Shu KH, Chen HC, Wang MC, Chang CC, Hsu BG, et al. Development and validation of a nomogram for urothelial cancer in patients with chronic kidney disease. Sci Rep. 2019;9(1):3473.
14. Sanli O, Dobruch J, Knowles MA, Burger M, Alemozaffar M, Nielsen ME, et al. Bladder cancer. Nat Rev Dis Primers. 2017;3:17022.
15. Gaggl M, Hofer M, Weidner S, Kleinert J, Fauler G, Wallner M, et al. Interfering parameters in the determination of urinary globotriaosylceramide (Gb3) in patients with chronic kidney disease. J Nephrol. 2015;28(6):679–89.
16. Fendler A, Stephan C, Yousef GM, Kristiansen G, Jung K. The translational potential of microRNAs as biofluid markers of urological tumours. Nat Rev Urol. 2016;13(12):734–52.
17. Andersen GB, Tost J. Circulating miRNAs as Biomarker in Cancer. Recent Results Cancer Res. 2020;215:277–98.
18. Mytsyk Y, Dosenko V, Skrzypczyk MA, Borys Y, Diychuk Y, Kucher A, et al. Potential clinical applications of microRNAs as biomarkers for renal cell carcinoma. Cent European J Urol. 2018;71(3):295–303.
19. Usuba W, Urabe F, Yamamoto Y, Matsuzaki J, Sasaki H, Ichikawa M, et al. Circulating miRNA panels for specific and early detection in bladder cancer. Cancer Sci. 2019;110(1):408–19.
20. Blanca A, Sanchez-Gonzalez A, Requena MJ, Carrasco-Valiente J, Gomez-Gomez E, Cheng L, et al. Expression of miR-100 and miR-138 as prognostic biomarkers in non-muscle-invasive bladder cancer. APMIS. 2019;127(8):545–53.
21. Gullu Amuran G, Tinay I, Filinte D, Ilgin C, Peker Eyuboglu I, Akkiprik M. Urinary micro-RNA expressions and protein concentrations may differentiate bladder cancer patients from healthy controls. Int Urol Nephrol. 2019.
22. Lin GB, Zhang CM, Chen XY, Wang JW, Chen S, Tang SY, et al. Identification of circulating miRNAs as novel prognostic biomarkers for bladder cancer. Math Biosci Eng. 2019;17(1):834–44.
23. Springer SU, Chen CH, Rodriguez Pena MDC, Li L, Douville C, Wang Y, et al. Non-invasive detection of urothelial cancer through the analysis of driver gene mutations and aneuploidy. Elife. 2018;7.
24. Chen CL, Lin CH, Li AL, Huang CC, Shen BY, Chiang YR, et al. Plasma miRNA profile is a biomarker associated with urothelial carcinoma in chronic hemodialysis patients. Am J Physiol Renal Physiol. 2019;316(6):F1094-F102.
25. Li AL, Chung TS, Chan YN, Chen CL, Lin SC, Chiang YR, et al. microRNA expression pattern as an ancillary prognostic signature for radiotherapy. J Transl Med. 2018;16(1):341.
26. Nagy A, Lanczky A, Menyhart O, Gyorffy B. Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets. Sci Rep. 2018;8(1):9227.

27. Jiang X, Du L, Wang L, Li J, Liu Y, Zheng G, et al. Serum microRNA expression signatures identified from genome-wide microRNA profiling serve as novel noninvasive biomarkers for diagnosis and recurrence of bladder cancer. Int J Cancer. 2015;136(4):854–62.
28. Wang G, Chan ES, Kwan BC, Li PK, Yip SK, Szeto CC, et al. Expression of microRNAs in the urine of patients with bladder cancer. Clin Genitourin Cancer. 2012;10(2):106–13.
29. Long JD, Sullivan TB, Humphrey J, Logvinenko T, Summerhayes KA, Kozinn S, et al. A non-invasive miRNA based assay to detect bladder cancer in cell-free urine. Am J Transl Res. 2015;7(11):2500–9.
30. Feng Y, Liu J, Kang Y, He Y, Liang B, Yang P, et al. miR-19a acts as an oncogenic microRNA and is up-regulated in bladder cancer. J Exp Clin Cancer Res. 2014;33:67.
31. Du M, Shi D, Yuan L, Li P, Chu H, Qin C, et al. Circulating miR-497 and miR-663b in plasma are potential novel biomarkers for bladder cancer. Sci Rep. 2015;5:10437.
32. Yoshino H, Yonezawa T, Yonemori M, Miyamoto K, Sakaguchi T, Sugita S, et al. Downregulation of microRNA-1274a induces cell apoptosis through regulation of BMPR1B in clear cell renal cell carcinoma. Oncol Rep. 2018;39(1):173–81.
33. Zhang C, Ma X, Du J, Yao Z, Shi T, Ai Q, et al. MicroRNA-30a as a prognostic factor in urothelial carcinoma of bladder inhibits cellular malignancy by antagonising Notch1. BJU Int. 2016;118(4):578–89.
34. Chung YH, Li SC, Kao YH, Luo HL, Cheng YT, Lin PR, et al. MiR-30a-5p Inhibits Epithelial-to-Mesenchymal Transition and Upregulates Expression of Tight Junction Protein Claudin-5 in Human Upper Tract Urothelial Carcinoma Cells. Int J Mol Sci. 2017;18(8).
35. Song T, Zhang X, Zhang L, Dong J, Cai W, Gao J, et al. miR-708 promotes the development of bladder carcinoma via direct repression of Caspase-2. J Cancer Res Clin Oncol. 2013;139(7):1189–98.
36. Niu S, Ma X, Zhang Y, Liu YN, Chen X, Gong H, et al. MicroRNA-19a and microRNA-19b promote the malignancy of clear cell renal cell carcinoma through targeting the tumor suppressor RhoB. PLoS One. 2018;13(2):e0192790.
37. Yin XH, Jin YH, Cao Y, Wong Y, Weng H, Sun C, et al. Development of a 21-miRNA Signature Associated With the Prognosis of Patients With Bladder Cancer. Front Oncol. 2019;9:729.
38. Nguyen HC, Xie W, Yang M, Hsieh CL, Drouin S, Lee GS, et al. Expression differences of circulating microRNAs in metastatic castration resistant prostate cancer and low-risk, localized prostate cancer. Prostate. 2013;73(4):346–54.
39. Zhang X, Zhang Y, Liu X, Fang A, Wang J, Yang Y, et al. Direct quantitative detection for cell-free miR-155 in urine: a potential role in diagnosis and prognosis for non-muscle invasive bladder cancer. Oncotarget. 2016;7(3):3255–66.
40. Peng Y, Dong W, Lin TX, Zhong GZ, Liao B, Wang B, et al. MicroRNA-155 promotes bladder cancer growth by repressing the tumor suppressor DMTF1. Oncotarget. 2015;6(18):16043–58.
41. Redova M, Poprach A, Nekvindova J, Iliev R, Radova L, Lakomy R, et al. Circulating miR-378 and miR-451 in serum are potential biomarkers for renal cell carcinoma. J Transl Med. 2012;10:55.
42. Fedorko M, Stanik M, Iliev R, Redova-Lojova M, Machackova T, Svoboda M, et al. Combination of MiR-378 and MiR-210 Serum Levels Enables Sensitive Detection of Renal Cell Carcinoma. Int J Mol Sci. 2015;16(10):23382–9.
43. Wang C, Hu J, Lu M, Gu H, Zhou X, Chen X, et al. A panel of five serum miRNAs as a potential diagnostic tool for early-stage renal cell carcinoma. Sci Rep. 2015;5:7610.
44. Stuopelyte K, Daniunaite K, Jankevicius F, Jarmalaite S. Detection of miRNAs in urine of prostate cancer patients. Medicina. 2016;52(2):116–24.
45. Jang JS, Jeon HS, Sun Z, Aubry MC, Tang H, Park CH, et al. Increased miR-708 expression in NSCLC and its association with poor survival in lung adenocarcinoma from never smokers. Clin Cancer Res. 2012;18(13):3658–67.
46. Lin KT, Yeh YM, Chuang CM, Yang SY, Chang JW, Sun SP, et al. Glucocorticoids mediate induction of microRNA-708 to suppress ovarian cancer metastasis through targeting Rap1B. Nat Commun. 2015;6:5917.

47. Liang L, Zhang L, Cui D, Yang D. Identification of the key miRNAs associated with survival time in stomach adenocarcinoma. Oncol Lett. 2017;14(4):4563–72.

Tables

Tab 1. Distribution of the clinical status of patients in this study.

Each group was well matched for age, sex and CKD stage. n, number in each group. Mean, average of each group. SD, standard deviation. CKD, chronic kidney disease. CKD+UC, the urothelial carcinoma patients with CKD. [a]Independent samples test. [b]Pearson chi-square test.

		Screening (n= 22)						Training (n= 100)						Testing (n= 100)						Testing (n= 50)		
		CKD			UC			CKD			UC			CKD			UC			Normal		
		n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
Age		11	66.82	11.25	11	64.45	9.50	50	65.4	10.6	50	65.78	11.49	50	61.12	11.22	50	67.44	10.95	50	61.88	14.52
Sex	F	2			2			15			13			20			18			18		
	M	9			9			35			37			30			32			32		
Grade	-	11						50			5			50			14			50		
	low							0			13						9					
	high				11			0			32						27					
CKD stage	0																			50		
	I	1			1			9			11			10			7					
	II	1			1			10			11			10			10					
	III	3			3			15			14			11			18					
	IV	2			2			10			11			10			7					
	V	4			4			6			3			9			8					
							Pvalue[a]						Pvalue[a]						Pvalue[b]			
							0.600						0.849						0.005			
							Pvalue[b]						Pvalue[b]						Pvalue[b]			
							1						0.656						0.591			
							Pvalue[b]						Pvalue[b]						Pvalue[b]			
							1						0.856						0.680			

Tab 2. miRNA names and sequences.

miRNA name	Mature miRNA Sequence
hsa-miR-586	UAUGCAUUGUAUUUUUAGGUCC
hsa-miR-129-5p	CUUUUUGCGGUCUGGGCUUGC
hsa-miR-33b-5p	GUGCAUUGCUGUUGCAUUGC
hsa-miR-30a-5p	UGUAAACAUCCUCGACUGGAAG
hsa-miR-1274A	GUCCCUGUUCAGGCGCCA
hsa-miR-126-3p	UCGUACCGUGAGUAAUAAUGCG
hsa-miR-210-3p	CUGUGCGUGUGACAGCGGCUGA
hsa-miR-202-3p	AGAGGUAUAGGGCAUGGGAA
hsa-miR-19a-5p	AGUUUUGCAUAGUUGCACUACA
hsa-miR-708-5p	AAGGAGCUUACAAUCUAGCUGGG
hsa-miR-19b-1-5p	AGUUUUGCAGGUUUGCAUCCAGC
hsa-miR-183-3p	GUGAAUUACCGAAGGGCCAUAA
hsa-miR-636	UGUGCUUGCUCGUCCCGCCCGCA
hsa-miR-155-5p	UUAAUGCUAAUCGUGAUAGGGGU
hsa-miR-378	ACUGGACUUGGAGUCAGAAGG
hsa-miR-487a-3p	AAUCAUACAGGGACAUCCAGUU
hsa-miR-150-5p	UCUCCCAACCCUUGUACCAGUG

Tab 3. The area under the curve of candidate miRNAs in the training group.
The receiver operating characteristic curve analysis for the candidate miRNAs is shown to distinguish patients with UC from those with CKD through urine samples (A) and plasma samples (B) (n=100 and 70, respectively).

A

Urine sample	AUC	95% CI
miR-1274a	0.71	0.6090 - 0.8110
miR-19a-5p	0.61	0.4943 - 0.7169
miR-30a-5p	0.64	0.5342 - 0.7514
miR-708	0.63	0.6717 - 0.8611

B

Plasma sample	AUC	95% CI
miR-155	0.65	0.5168 - 0.7773
miR-19b-1-5p	0.66	0.5327 - 0.7875
miR-210-3p	0.64	0.5107 - 0.7704
miR-636	0.61	0.4758 - 0.7431

Figures

Figure 1

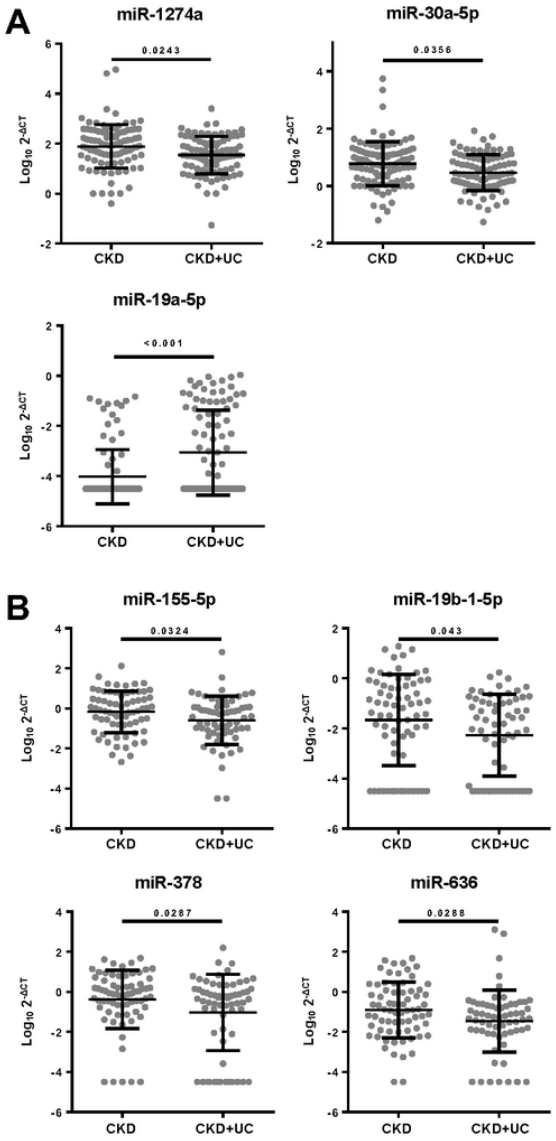

Figure 1

Significant different miRNA expressions in the urine or plasma between CKD and CKD+UC. (A) miRNA levels from the urine of patients detected by qRT-PCR using RNU6 as a control (n=200). (B) miRNA levels from the plasma were detected by qRT-PCR using RNU6 as a control (n=134). The Y axis presents the expression level (Log10 2-ΔCT). CKD, patients with chronic kidney disease. CKD+UC, the urothelial carcinoma patients with CKD. The p-value was analyzed by Student's t-test for each miRNA.

Figure 2

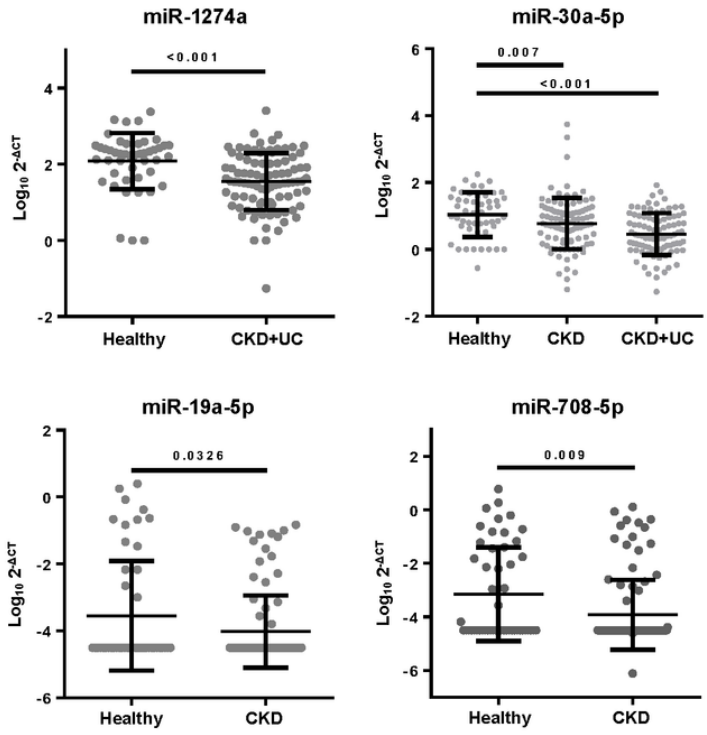

Figure 2

Significant changes in the urine miRNA expression levels between the healthy, CKD and CKD+UC groups. The Y axis presents the expression level (Log102-ΔCT). Healthy, healthy donors. CKD, patients with chronic kidney disease. UC, the urothelial carcinoma patients with CKD. The p-value was analyzed by Student's t-test for each miRNA.

Figure 3

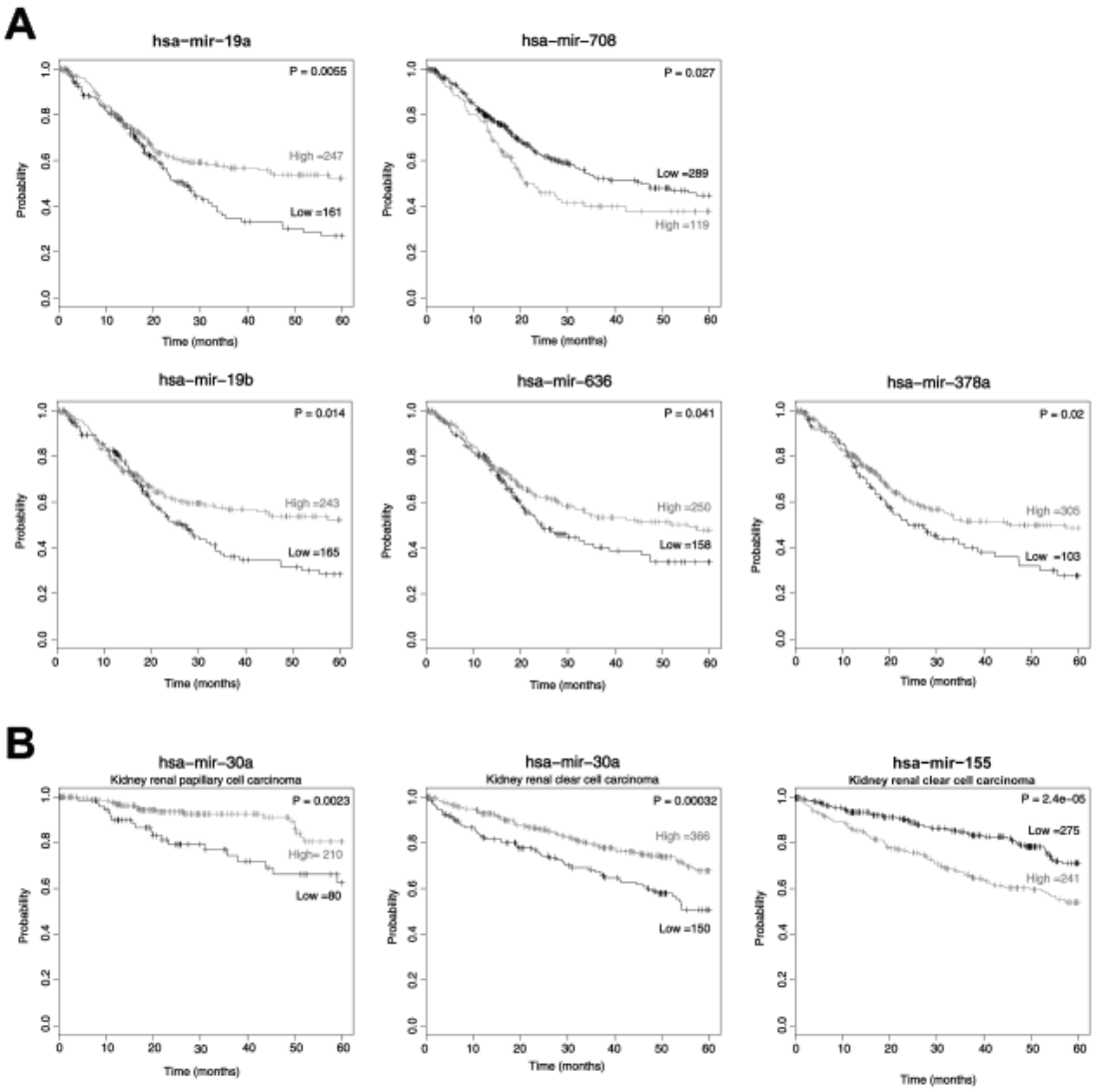

Figure 3

The expression of miRNAs in cancer tissue is associated with survival. The Kaplan-Meier survival curve of patients: low miRNA expression versus high miRNA expression according to the automatic best cutoff from the database. The statistical significance of the difference in bladder cancer (A) and kidney renal papillary cell carcinoma and kidney renal clear cell carcinoma (B) are shown.

Figure 4

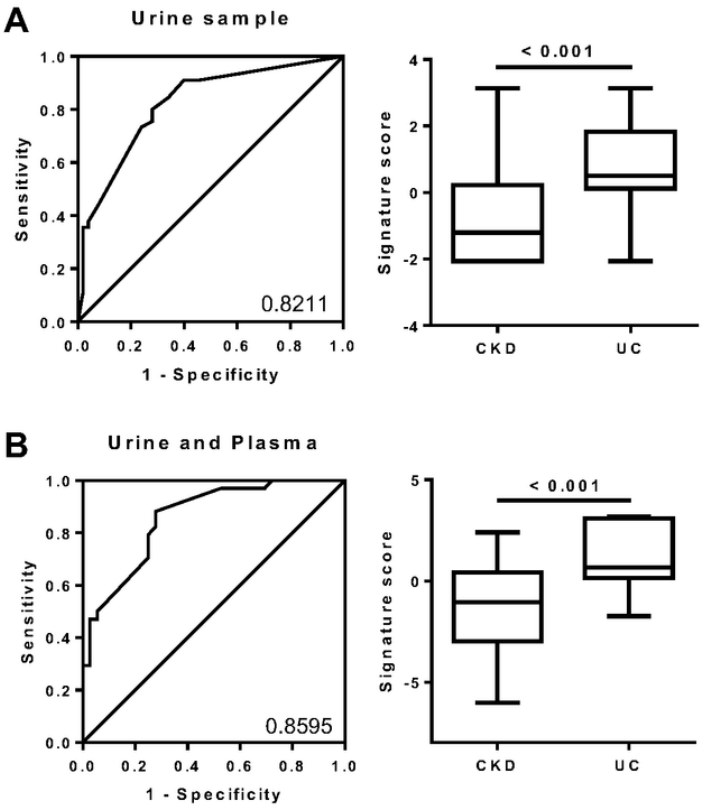

Figure 4

ROC curve analysis of miRNA combinations. (A) ROC curve analysis for miR-1274a, miR-19a-5p, miR-30a-5p and miR-708-5p in urine was shown to distinguish patients with CKD+UC from those with CKD. (B) The ROC analysis for eight miRNAs (4 in urine：miR-1274a, miR-19a-5p, miR-30a-5p and miR-708-5p, and 4 in plasma：miR-155-5p, miR-19b-1-5p, miR-210 and miR-636) was shown to distinguish patients with CKD+UC from those with CKD. The box plots show the two prediction models distribution that combine the miRNA expression levels from the training group.

Figure 5

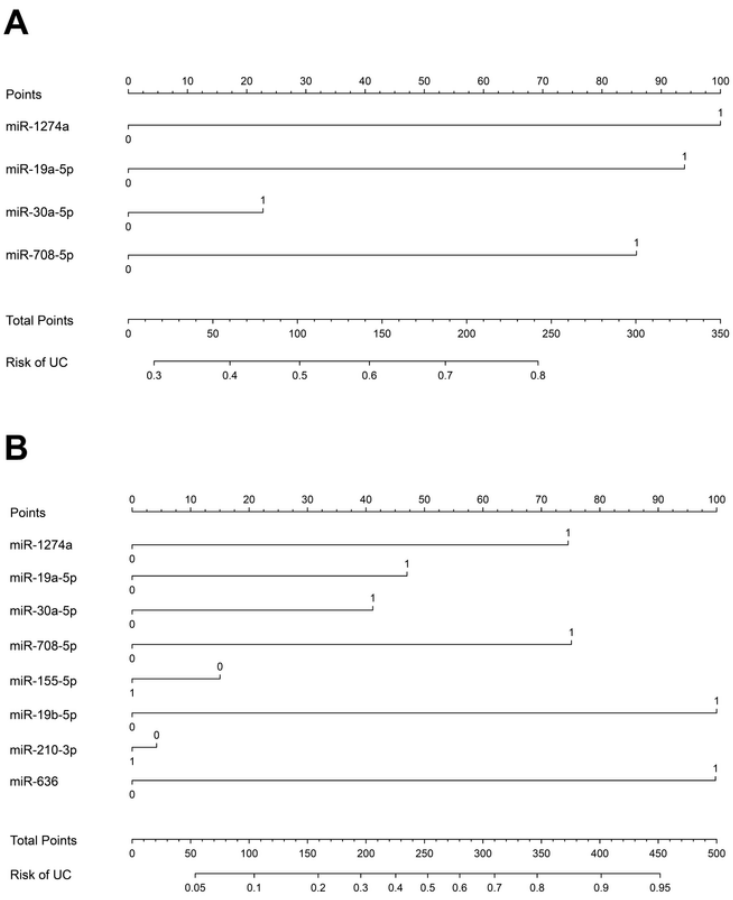

Figure 5

Nomogram for the diagnosis of urothelial cancer. (A) Nomogram plot from the points of four miRNA expressions in urine sample. (B) Nomogram plot from the points of eight miRNA expressions in urine and plasma sample.