Expression Profiles and Clinical Significance of MicroRNAs in Papillary Renal Cell Carcinoma

A STROBE-Compliant Observational Study

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Abstract: Papillary renal cell carcinoma (pRCC) is the second most prevalent subtype of kidney cancers. In the current study, we analyzed the global microRNA (miRNA) expression profiles in pRCC, with the aim to evaluate the relationship of miRNA expression with the progression and prognosis of pRCC.

A total of 163 treatment-naïve primary pRCC patients were identified from the Cancer Genome Atlas dataset and included in this retrospective observational study. The miRNA expression profiles were graded by tumor-node-metastasis information, and compared between histologic subtypes. Furthermore, the training-validation approach was applied to identify miRNAs of prognostic values, with the aid of Kaplan–Meier survival, and univariate and multivariate Cox regression analyses. Finally, the online DAVID (Database for Annotation, Visualization, and Integrated Discover) program was applied for the pathway enrichment analysis with the target genes of prognosis-associated miRNAs, which were predicted by 3 computational algorithms (PicTar, TargetScan, and Miranda).

In the progression-related miRNA profiles, 26 miRNAs were selected for pathologic stage, 28 for pathologic T, 16 for lymph node status, 3 for metastasis status, and 32 for histologic types, respectively.

In the training stage, the expression levels of 12 miRNAs (mir-134, mir-379, mir-127, mir-452, mir-199a, mir-200c, mir-141, mir-3074, mir-1468, mir-181c, mir-1180, and mir-34a) were significantly associated with patient survival, whereas mir-200c, mir-127, mir-34a, and mir-181c were identified by multivariate Cox regression analyses as potential independent prognostic factors in pRCC. Subsequently, mir-200c, mir-127, and mir-34a were confirmed to be significantly correlated with patient survival in the validation stage. Finally, target gene prediction analysis identified a total of 113 target genes for mir-200c, 37 for mir-127, and 180 for mir-34a, which further generated 15 molecular pathways.

Our results identified the specific miRNAs associated with the progression and aggressiveness of pRCC, and 3 miRNAs (mir-200c, mir-127, and mir-34a) as promising prognostic factors of pRCC.

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Abbreviations: AJCC = American Joint Committee on Cancer, ccRCC = clear cell renal cell carcinoma, CI = confidential interval, DAVID = Database for Annotation, Visualization, and Integrated Discover, HR = hazard ratio, KEGG = Kyoto Encyclopedia of Genes and Genomes, miRNA = microRNA, pRCC = papillary renal cell carcinoma, RCC = renal cell carcinoma, RPM = reads per million, STROBE = Strengthening the Reporting of Observational studies in Epidemiology, TCGA = The Cancer Genome Atlas, TNM = tumor-node-metastasis.

INTRODUCTION

Renal cell carcinoma (RCC) accounts for approximately 90% of all kidney tumors and 2% to 3% of all human malignancies, with an estimated 63,920 newly diagnosed cases and 13,860 RCC-related deaths for 2014 in the United States.[1] As the second most common histologic subtype after clear cell RCC (ccRCC), papillary RCC (pRCC) represents 10% to 15% of all RCC cases.[2] In the current clinical practice, the histopathologic parameters including tumor-node-metastasis (TNM) stage, histologic subtype (types 1 and 2), tumor necrosis and microvascular invasion, and preexisting health problems such as type 2 diabetes mellitus have been established and validated as potent prognostic factors; however, they are of limited values as the clinical behavior and long-term outcomes of pRCC are highly variable.[3–6] Hence, the identification of novel molecular biomarkers that are predictive of pRCC aggressiveness and patient outcome is of great importance, which could help identify the molecular mechanisms and improve the ability to manage pRCC patients.

MicroRNAs (miRNAs) are short (about 19–25 nucleotides in length), noncoding, and single-stranded RNAs that regulate gene expression post-transcriptionally through the epigenetic mechanism of RNA interference.[7] miRNAs are implied in a wide range of biological processes including cellular proliferation, differentiation, and apoptosis, via regulating the expression of hundreds of target genes.[8] Furthermore, miRNAs are aberrantly expressed or mutated in human cancers, suggesting that they may exert critical functions as a novel class of tumor suppressive or carcinogenic factors.[9–13] The diagnostic and prognostic capabilities of miRNAs have been thoroughly explored in ccRCC, and the cancer-specific miRNA expression profiles have been identified, which were significantly associated with patient survival.[14–16] However, the clinical utility of miRNAs in pRCC remains to be elucidated.

Hence, we stringently designed a stepwise study with the data from The Cancer Genome Atlas (TCGA) project, which provides a collection of clinicopathological data and the global miRNA expression profiles.[17,18] In the current study, we explored the miRNAs associated with the progression and prognosis of pRCC, with the hope to identify the specific miRNAs that could predict the clinical phenotypes and prognosis in pRCC.

MATERIALS AND METHODS

Patients and Samples

This retrospective observational study was conducted and reported in accordance with the STROBE (Strengthening the Reporting of Observational studies in Epidemiology) guidelines.[19–22] All pRCC patients were identified from the multi-institutional TCGA project (http://cancergenome.nih.gov/) that underwent nephrectomy from 1996 to 2013 for sporadic pRCC.[23] The full clinical data (Level 1 and Level 2) were downloaded from the TCGA data portal (up to October 1, 2014) and double checked for the further assessment of the eligibility. The subjects with history of other malignancies or neoadjuvant therapy (chemotherapy or radiation therapy) were excluded. Furthermore, the pathological stage was reevaluated and confirmed by 2 experienced pathologists according to the 7th edition of TNM classification of the American Joint Committee on Cancer (AJCC). Overall, a total of 163 pRCC patients were enrolled with full annotation of the corresponding clinicopathological data including age, sex, race, histologic subtype, and AJCC TNM information (Table 1). In the current study, the 2-stage training-validation approach was adopted to identify miRNAs predictive of patient survival. Among the 163 pRCC patients routinely followed up at the corresponding centers, 129 subjects (79.1%) were followed up for >30 days and included in the training stage. In the subsequent validation stage, all subjects (n = 163) were included with limited follow-up time (2 year, 730 days) to confirm the prognostic power of the miRNAs selected in the training stage. All data collection and procession (including the consenting process) were conducted with approvals by the corresponding institutional review boards and in agreement with the human subject protection and data access policies of TCGA.

Microarray Data Procession

The miRNA expression profiling was performed with the Illumina HiSeq platform (Illumina Inc, San Diego, CA) and quantified by relative miRNA read counts to the total miRNAs read counts and presented as reads per million (RPM) counts.

TABLE 1. Clinicopathological Characteristics of Patients With Papillary Renal Cell Carcinoma

	pRCC Patients (n = 163)
Age, y	
Mean ± SD	59.9 ± 12.6
≥60, n (%)	87 (53.4%)
Sex, n (%)	
Female	50 (30.7%)
Male	113 (69.3%)
Race, n (%)	
Whites	120 (73.6%)
African	38 (23.3%)
Asian	5 (3.1%)
Histologic subtype, n (%)	
Type 1	40 (24.5%)
Type 2	51 (31.3%)
Unknown	72 (44.2%)
Pathologic stage, n (%)	
Stage I	97 (59.5%)
Stage II	19 (11.7%)
Stage III	34 (20.8%)
Stage IV	13 (8.0%)
Pathologic T, n (%)	
T1	100 (61.3%)
T2	21 (12.9%)
T3	40 (24.5%)
T4	2 (1.3%)
Pathologic N, n (%)	
N0	31 (19.0%)
N1–2	22 (13.5%)
NX	110 (67.5%)
Pathologic M, n (%)	
M0	89 (54.6%)
M1	9 (5.5%)
MX	65 (39.9%)
Follow-up time, n (%)	
>30 d	129 (79.1%)
<30 d	34 (20.9%)

MX = metastasis status unknown, NX = regional lymph node unknown, pRCC = papillary renal cell carcinoma, SD = standard deviation.

After downloading from TCGA data portal (up to October 15, 2014), the summary miRNA data was processed using BRB-Array tools (version 4.4.0; National Cancer Institute, Bethesda, MD) that were developed by Dr Richard Simon and BRB-Array Tools Development Team (http://brb.nci.nih.gov/BRB-Array-Tools.html).[24] Briefly, the miRNAs were retained when they were >1 RPM in at least 10% of all samples and had changes of >1.5 fold from the median value in at least 20% of samples. Subsequently, the expression level of each individual miRNA was log2 transformed for further analysis.

Target Gene Prediction and Pathway Analysis

Three computational algorithms, PicTar (http://pictar.mdc-berlin.de/),[25] TargetScan (http://www.targetscan.org/),[7] and Miranda (http://www.microrna.org),[26] were employed to predict miRNA targets, and the genes predicted by at least 2 independent tools were taken into consideration. The selected genes of each individual miRNA were uploaded to the online

Database for Annotation, Visualization, and Integrated Discovery (DAVID) program (http://david.abcc.ncifcrf.gov/)[27] for the analysis of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.[28] In the functional annotation analysis, the minimum number of genes for the corresponding term was set as 2 to identify appropriate functional categories. To examine the significance of gene-term enrichment, a modified Fisher test was conducted and the significant *P* value was set as 0.1.[27]

Statistical Analysis

The continuous variables were presented as mean ± standard deviation (SD), after the normality status was explored with Kolmogorov–Smirnov and Shapiro–Wilk tests. The miRNA expression levels between the 2 different groups (stages III + IV vs stages I + II, T3 + T4 vs T1 + T2, N1 + N2 vs N0, M1 vs M0, and type 2 vs type 1) were ascertained with Student *t* test (significance level was set as 0.001). To generate a tree cluster showing the separation of different classes, the hierarchical cluster analysis was performed by Euclidian distance and average linkage with BRB-Array tools.

Kaplan–Meier survival and univariate Cox proportional hazards regression analyses were conducted to explore the effects of age, sex, race, AJCC stage (stages III + IV vs stages I + II), tumor size (T3 + T4 vs T1 + T2), and miRNA expression levels (cutoff point: median value) on patient survival. Furthermore, the multivariate Cox proportional hazards regression analysis was performed by combining all potential prognostic factors, with the stepwise backward likelihood ratio method. In the Cox proportional hazards regression analyses, the results were expressed as hazard ratio (HR) with the corresponding 95% confidential interval (CI). Statistical significance was taken as a 2-sided *P* value <0.05 unless specifically indicated. The statistical analyses were performed with the use of BRB-Array Tools and SPSS (version 21.0; SPSS Institute Inc, Chicago, IL), as appropriate.

RESULTS

Patient Characteristics

All 163 patients enrolled in the current study were clinically and pathologically diagnosed with pRCC and underwent nephrectomy without history of other malignancies and neoadjuvant therapy (chemotherapy or radiation therapy). The mean age for all 163 patients was 59.9 years (SD: 12.6 years), and the detailed demographic and clinicopathologic information was summarized in Table 1.

MiRNAs in Relation to Tumor Progression of pRCC

To identify miRNAs related to progression for each clinical feature, the class comparison analyses were conducted. A summary of 26 miRNAs were selected for AJCC stage, 28 for pathologic T, 16 for lymph node involvement status, 3 for distant metastasis status, and 32 for histologic types (see Supplementary Table S1, http://links.lww.com/MD/A250, Supplementary Digital Content 1, which illustrates miRNAs associated with the histopathological characteristics of pRCC). Additionally, the unsupervised hierarchical clustering with the miRNAs expression data could clearly separate different classes according to the stage, pathologic T, lymph node involvement, and histologic type (Figure 1).

MiRNA Expression Profiles Associated With pRCC Prognosis

In the training stage, among the 129 pRCC patients followed up for >30 days, 18 (14.0%) participants had died
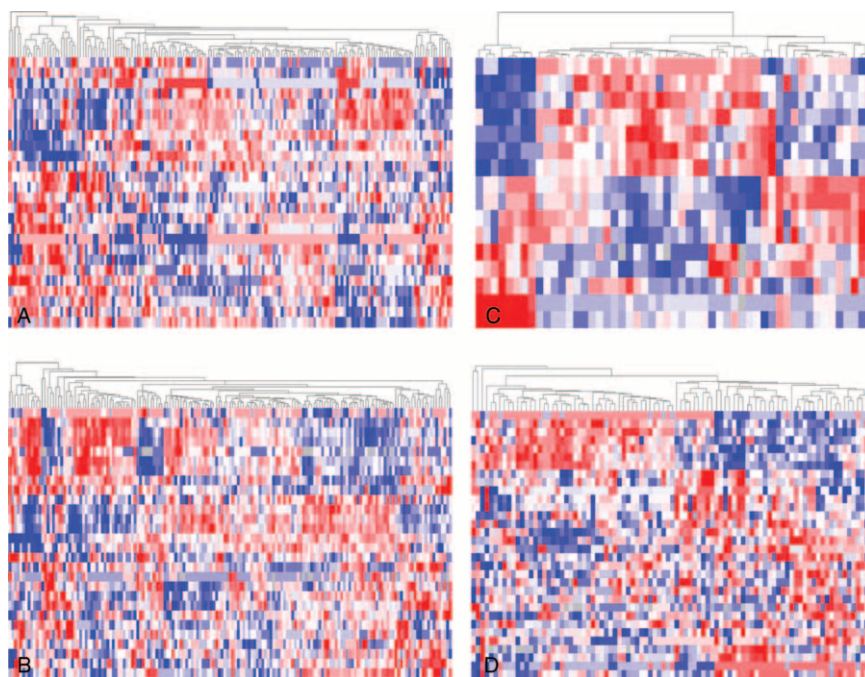

FIGURE 1. Unsupervised hierarchical cluster analysis of the differentially expressed microRNAs. The papillary renal cell carcinoma patients were compared between 2 groups according to (A) stage (stages III + IV vs stages I + II); (B) pathologic T (T3 + T4 vs T1 + T2); (C) pathologic N (N1–2 vs N0); and (D) histologic subtype (type 2 vs type 1).

during the median follow-up time of 12.1 months. As indicated in the univariate Cox regression analyses (Table 2), a total of 12 miRNAs (mir-134, mir-379, mir-127, mir-452, mir-199a, mir-200c, mir-141, mir-3074, mir-1468, mir-181c, mir-1180, and mir-34a) were significantly associated with patient survival in pRCC, which were further confirmed by Kaplan–Meier survival analyses (Figures 2 and 3). Furthermore, the multivariate Cox regression analyses demonstrated that AJCC stage ($P = 0.019$), mir-200c ($P = 0.007$), mir-127 ($P = 0.001$), mir-34a ($P = 0.010$), and mir-181c ($P = 0.001$) were potential independent prognostic factors (Table 2 and Figure 3). Subsequently, the prognostic probabilities of mir-200c, mir-127, mir-34a, and mir-181c were explored in the validation stage with all subjects (n = 163) followed up for 2 years. As shown in Figure 4, mir-200c ($P = 0.010$), mir-127 ($P = 0.016$), and mir-34a ($P = 0.001$) except mir-181c ($P = 0.638$) were significantly associated with patient survival of pRCC patients, which were further confirmed by univariate Cox regression analyses: mir-200c (HR = 5.894, 95% CI: 1.271–27.33; $P = 0.023$), mir-127 (HR = 8.336, 95% CI: 1.066–65.21; $P = 0.043$), mir-34a (HR = 0.072, 95% CI: 0.009–0.561; $P = 0.012$), and mir-181c (HR = 1.329, 95% CI: 0.405–4.355; $P = 0.639$). Overall, mir-200c, mir-127, and mir-34a could serve as promising prognostic biomarkers in pRCC, even though only mir-34a (HR = 0.046, 95% CI: 0.004–0.485; $P = 0.010$) passed the multivariate Cox regression analysis in the validation stage.

Target Gene Prediction and Pathway Mapping Analysis

To explore the biological functions of the prognosis-related miRNAs, 3 computational algorithms (TargentScan, PicTar, and Miranda) were applied to predict the potential targets. Overall, a total of 113 target genes were selected for mir-200c, 37 for mir-127, and 180 for mir-34a by at least 2 of the 3 independent tools (see Supplementary Table S2, http://links.lww.com/MD/A250, Supplementary Digital Content 2, which illustrates the predicted target genes of mir-200c, mir-127, and mir-34a). Furthermore, the putative targets were assigned into KEGG pathway enrichment analysis via the online DAVID program. As summarized in Table 3, a total of 15 pathways were enriched, among which 9 were associated with mir-200c and 7 with mir-34a. Of note, the results provided evidence that mir-200c was involved in the pathway ''renal cell carcinoma'' through modulating 3 target genes (*CREBBP*, *EP300*, and *CRKL*), and 1 pathway could gather the putative targets for 2 miRNAs, such as Notch signaling pathway for mir-200c and mir-34a.

DISCUSSION

pRCC represents the second most common subtype of RCC, with highly variable clinical behaviors and long-term outcomes. However, the currently established prognostic models are based merely on clinicopathological and imaging data, which cannot compensate for the inherent limited information.[4,5,29] In the present study, we explored the global miRNA expression profiles in pRCC, with the aim to identify the specific miRNAs associated with the progression and prognosis of pRCC, which could fulfill the demands for improving risk stratification and prognosis prediction for pRCC patients.

As an endogenous class of small, noncoding RNAs, miRNAs have been documented to be involved in a wide range of

TABLE 2. Univariate and Multivariate Cox Regression Analysis of Overall Survival in Papillary Renal Cell Carcinoma Patients

Variables	HR	95% CI	P Value
Univariate analysis			
Age (≥60 vs <60)	1.712	0.603–4.859	0.312
Sex (female vs male)	1.293	0.476–3.513	0.615
Race (Caucasian vs non-Caucasian)	1.018	0.293–3.540	0.977
Pathologic T (T3 + T4 vs T1 + T2)	4.321	1.606–11.63	0.004[*]
Stage (stages III + IV vs stages I + II)	4.450	1.564–12.66	0.005[*]
Mir-134	6.795	1.559–29.62	0.011[*]
Mir-379	6.783	1.556–29.58	0.011[*]
Mir-127	6.759	1.552–29.43	0.011[*]
Mir-452	4.459	1.281–15.52	0.019[*]
Mir-199a	4.113	1.188–14.24	0.026[*]
Mir-200c	4.103	1.345–12.51	0.013[*]
Mir-141	3.428	1.123–10.46	0.030[*]
Mir-3074	0.357	0.135–0.943	0.038[*]
Mir-1468	0.302	0.112–0.816	0.018[*]
Mir-181c	0.283	0.100–0.800	0.017[*]
Mir-1180	0.264	0.086–0.806	0.019[*]
Mir-34a	0.254	0.089–0.721	0.010[*]
Multivariate analysis			
Stage (stages III + IV vs stages I + II)	4.174	1.267–13.75	0.019[*]
Mir-200c	31.93	2.525–403.9	0.007[*]
Mir-127	13.94	2.783–69.82	0.001[*]
Mir-34a	0.193	0.055–0.674	0.010[*]
Mir-181c	0.098	0.023–0.410	0.001[*]

CI = confidential interval, HR = hazard ratio.
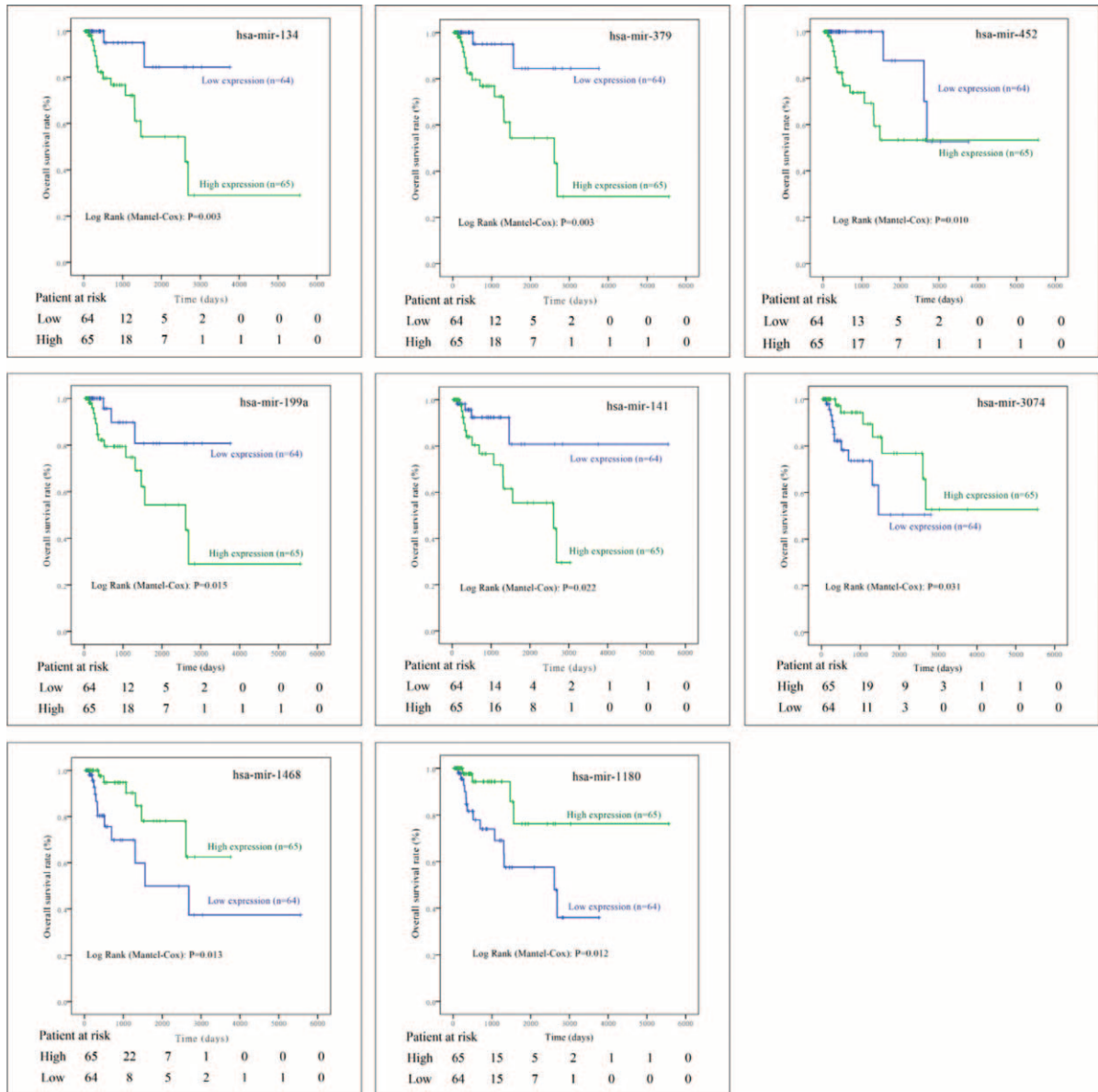[*] Statistically significant results.

FIGURE 2. Kaplan–Meier curve analysis of microRNAs for the survival in papillary renal cell carcinoma patients. A total of 8 microRNAs are presented, including mir-134, mir-379, mir-452, mir-199a, mir-141, mir-3074, mir-1468, and mir-1180.

pathophysiological processes including carcinogenesis and metastasis.[30,31] To gain more insight into the molecular mechanisms underlying carcinogenesis, the miRNA expression profiling has emerged as a potent technique approach and identified specific miRNA signatures associated with the aggressiveness and prognosis in human malignancies.[32,33] In the current study, we explored the genome-wide miRNA expression profiles in pRCC, after identifying 163 treatment-naïve primary pRCC patients from the multi-institutional TCGA project.

Overall, 26 miRNAs were selected for AJCC stage, 28 for pathologic T, 16 for lymph node involvement status, and 3 for metastasis status. Of note, as pRCC is relatively indolent with low incidences of metastasis,[4] and the patients with distant metastasis are rarely recommended to undergo surgery, the

number of M1 patients was only 9, which could explain the result of 3 metastasis-related miRNAs to some extent. Furthermore, as pRCC can be divided into 2 distinct subtypes (type 1 and type 2), and type 2 pRCC appears to have a worse prognosis than type 1,[4,34] we compared the miRNA expression profiles according to the histologic subtypes and identified 32 differentially expressed miRNAs. In the training stage of survival analysis, a summary of 12 miRNAs (mir-134, mir-379, mir-127, mir-452, mir-199a, mir-200c, mir-141, mir-3074, mir-1468, mir-181c, mir-1180, and mir-34a) were demonstrated to be significantly associated with prognosis by univariate Cox regression and Kaplan–Meier survival analyses. After final stepwise multivariate Cox regression analysis, 4 miRNAs (mir-200c, mir-127, mir-34a, and mir-181c) were identified as potential independent prognostic parameters,
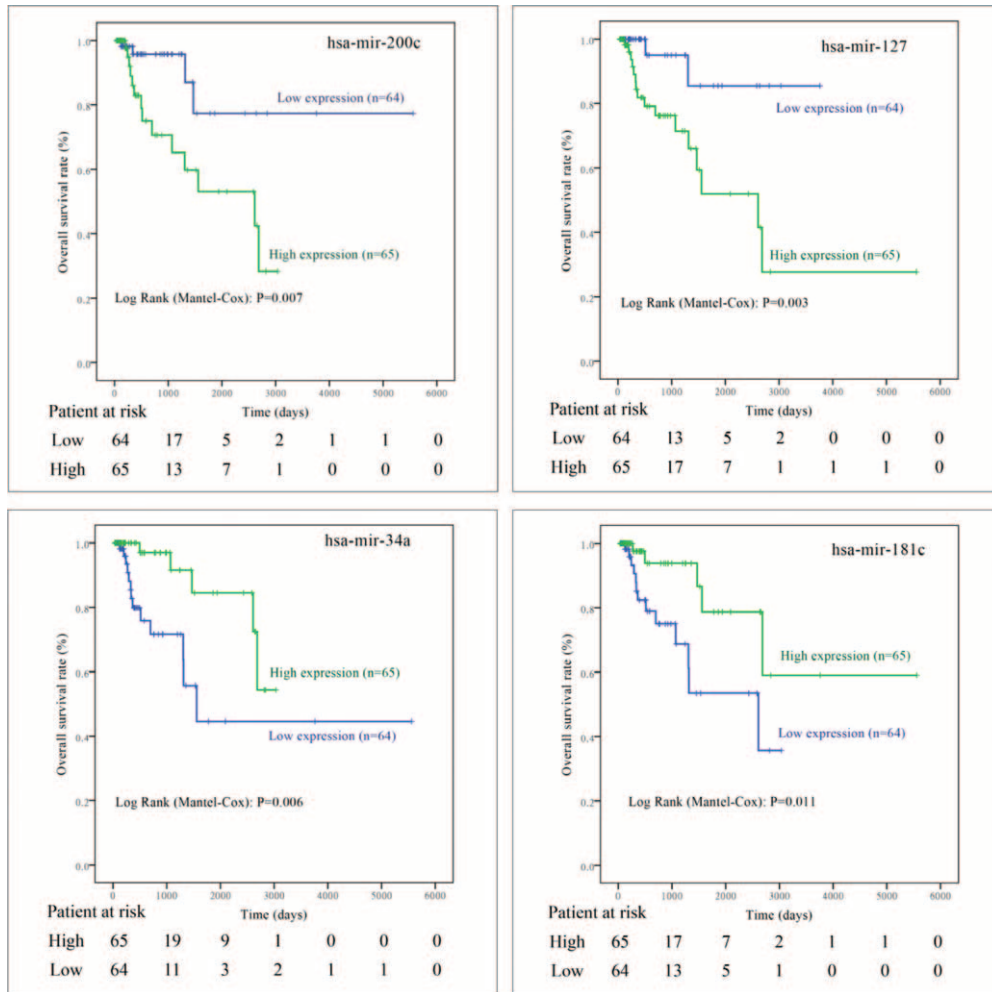
FIGURE 3. Kaplan–Meier curve analysis of microRNAs for the survival in papillary renal cell carcinoma patients. The 4 microRNAs (mir-200c, mir-127, mir-34a, and mir-181c) are potent prognostic factors confirmed by multivariate Cox regression analyses in the training stage.

and the high expression of mir-200c and mir-127 predicts poor outcomes in pRCC. In the subsequent validation stage, mir-200c, mir-127, and mir-34a were confirmed to be significantly correlated with overall survival of pRCC patients.

Of all the 3 prognosis-related miRNAs (mir-200c, mir-127, and mir-34a), none has been explored in pRCC. As 1 member of the tumor suppressive mir-200 family, mir-200c has been involved in the process of mesenchymal-epithelial transition.[35] However, the high expression of mir-200c was proved to be associated with a poor prognosis in pRCC, which were in consistent with the reports in other malignancies such as breast cancer,[36] colorectal cancer,[37] and ovarian cancer.[38] The computational studies demonstrated that mir-200c was targeting 113 genes and implied in 9 pathways including ''renal cell carcinoma'' by KEGG enrichment analysis. Mir-127 is located on chromosome region 14q32.2 with 37 putative target genes. Even though no pathway was identified because of the small number of target genes, mir-127 has been proven to promote glioblastoma cell migration and invasion via targeting the tumor

suppressor genes.[39] In pRCC, mir-127 was upregulated in pathologic T3+T4 tumors and associated with a poor prognosis, which totally differed from ccRCC, as the high expression of mir-127 was significantly associated with longer relapse-free survival time in ccRCC.[40] The remaining 1 miRNA, mir-34a, was a protective miRNA, as the higher expression levels correlated with a better prognosis in pRCC. In agreement with the current study, mir-34a acts as a tumor suppressor in ccRCC via inhibiting cell proliferation and metastasis.[41,42] Furthermore, the bioinformatics analysis identified a total 180 target genes and 7 pathways including ''endocytosis,'' ''Notch signaling pathway,'' ''heparan sulfate biosynthesis,'' and others for mir-34a, which could deepen the understanding of mir-34a in RCC (including ccRCC and pRCC). Overall, all these 3 miRNAs exert important functions in the carcinogenesis of pRCC through targeting a wide range of genes, regulating various pathways and complicated cross-talk with each other, which warrant further functional analysis.

Some limitations should be acknowledged in interpreting the results. First, as the status of lymph node involvement,
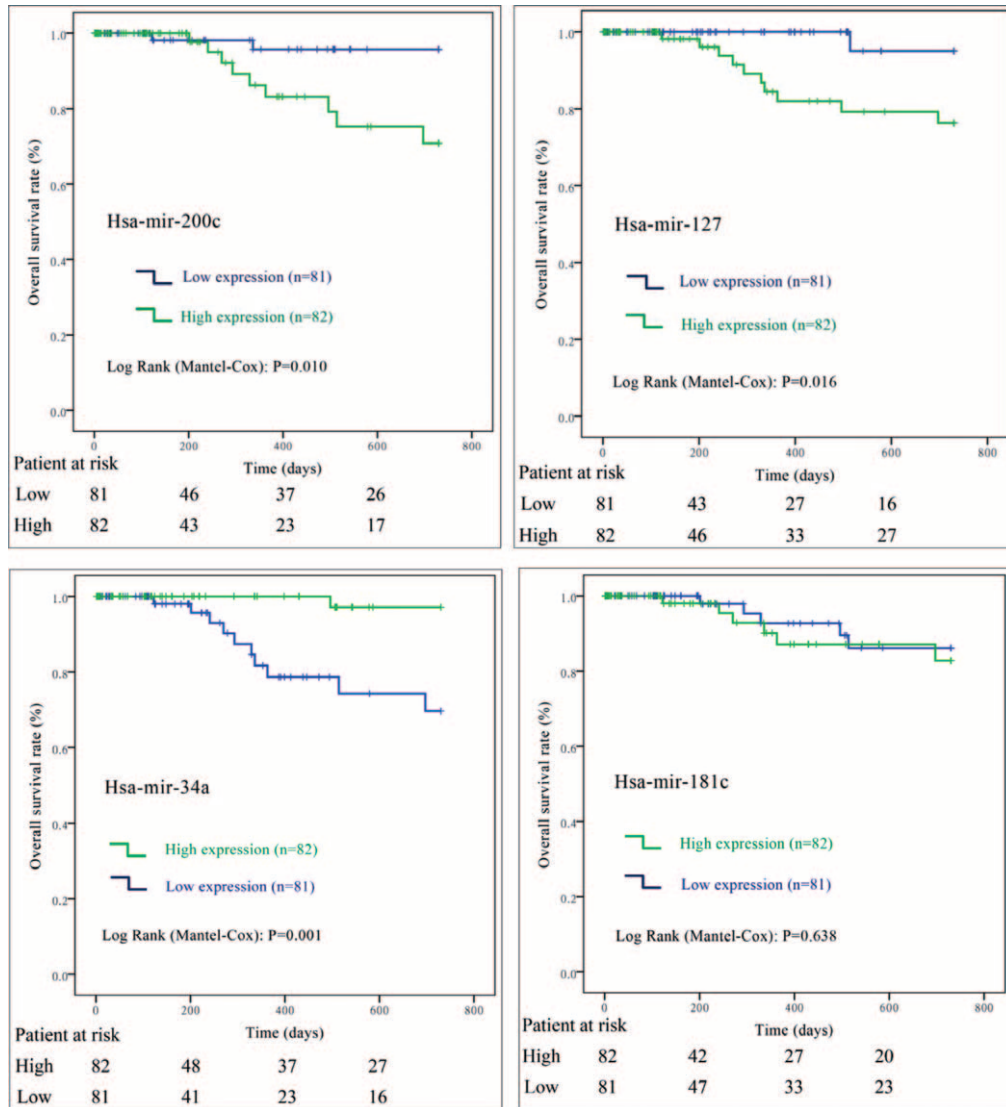
FIGURE 4. Kaplan–Meier curve analysis of microRNAs for the survival in papillary renal cell carcinoma patients (the validation stage). The 3 microRNAs (mir-200c, mir-127, and mir-34a) except mir-181c are significantly associated with patient survival.

distant metastasis, and histologic subtype remains unclear in a substantial group of pRCC patients (65.9%, 42.6%, and 44.2%, respectively), these histopathological parameters were excluded from the survival analysis, which could reduce the statistical power of the survival analysis. Second, the participants were majorly recruited from Caucasians, which limited the application of the miRNA profiles in the whole population, even though ethnicity has been disproved as an independent prognostic factor.[43,44] Third, the limited number of pRCC patients enrolled in the current study and relatively short follow-up time, which could reduce the statistical power to identify more significant miRNAs. Fourth, even though we strictly selected the subjects to control the potential heterogeneity, the false-positive results do potentially exist, which need an external validation cohort to validate the results with different methods such as quantificational real-time polymerase chain reaction.

CONCLUSION

In summary, by analyzing the global miRNA expression profiles in an independent pRCC patient cohort, our study identified specific miRNAs in relation with the progression and aggressiveness of pRCC and 3 miRNAs (mir-200c, mir-127, and mir-34a) as the promising prognostic factors of pRCC. Additionally, further well-designed and unbiased studies with larger sample size and longer follow-up time should be conducted to verify our findings. Furthermore, the functional studies of miRNAs in pRCC are warranted that will help to understand the underlying molecular mechanisms.

ACKNOWLEDGMENTS

TABLE 3. Kyoto Encyclopedia of Genes and Genomes Terms of the Predicted Target Genes for Mir-200c and Mir-34a

Pathway Terms	No. (%)[*]	*P* Value[†]	Genes
Hsa-mir-200c			
Wnt signaling pathway	5 (0.4)	0.025	*CTBP2, CREBBP, EP300, PPP2R5E, SIAH1*
TGF-β signaling pathway	4 (0.3)	0.027	*CREBBP, EP300, SMURF2, NOG*
Notch signaling pathway	3 (0.2)	0.048	*CTBP2, CREBBP, EP300*
Neurotrophin signaling pathway	4 (0.3)	0.064	*NTF3, YWHAB, YWHAG, CRKL*
Cell cycle	4 (0.3)	0.066	*CREBBP, EP300, YWHAB, YWHAG*
Axon guidance	4 (0.3)	0.071	*EFNA1, GNAI3, NGEF, SEMA6D*
Ubiquitin-mediated proteolysis	4 (0.3)	0.081	*SMURF2, SIAH1, SYVN1, UBE2I*
Pathways in cancer	6 (0.5)	0.095	*CTBP2, CREBBP, EP300, GLI3, FN1, CRKL*
Renal cell carcinoma	3 (0.2)	0.096	*CREBBP, EP300, CRKL*
Hsa-mir-34a			
Endocytosis	8 (0.4)	0.010	*EHD4, GRK6, AP2S1, CSF1R, MET, PIP5K1A, PDGFRA, VPS37B*
Notch signaling pathway	4 (0.2)	0.023	*APH1A, DLL1, JAG1, NUMBL*
Heparan sulfate biosynthesis	3 (0.2)	0.045	*NDST1, B3GAT3, GLCE*
Galactose metabolism	3 (0.2)	0.045	*B4GALT2, HK1, PGM1*
Fructose and mannose metabolism	3 (0.2)	0.073	*ALDOA, FUK, HK1*
Adherens junction	4 (0.2)	0.080	*WASF1, MET, PTPRM, VCL*
Colorectal cancer	4 (0.2)	0.098	*MET, MAP2K1, PDGFRA, RALGDS*

[*] Number of target genes involved in the corresponding pathway and the percentage of all genes.
[†] *P* value derived from the modified Fisher exact test to determine the significance of gene-term enrichment.

providing high-quality biological and clinical data about papillary renal cell carcinoma.

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