**INTRODUCTION**

Renal cell carcinoma (RCC) ranks the top among kidney cancers in terms of its morbidity, and it originates from the renal tubular epithelial cells. In the last decade, RCC shows an increasing morbidity, and more than 65,340 new RCC cases are reported annually, taking up about 4% of all adult malignancies in the United States. The diagnosis of RCC is usually made late because there is no early specific symptom, which has thus deprived the patients of the chance of being cured, leading to poor prognosis. However, in addition to tumor stage and grade, the influencing factors of survival for the diagnosed patients are largely unclear. Some studies have suggested

**miR-30b-5p up-regulation related to the dismal prognosis for patients with renal cell cancer**

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**Abstract**

The diagnosis of renal cell carcinoma (RCC) is often made late since there is no early symptom, which thus results in dismal patient prognosis. As a result, new biomarkers are urgently needed and efforts should be made to identify their functions in predicting RCC prognosis. microRNAs (miRNAs) are a class of small noncoding RNAs that are about 20-22 nucleotides in length, and they have been demonstrated to function as prognostic markers in numerous tumors. This study aimed to assess the role of miR-30b-5p in predicting the prognosis of RCC postoperatively. In this study, RNA was extracted from 284 formalin-fixed and paraffin-embedded kidney cancer tissue samples. After cDNA synthesis, real-time quantitative PCR (RT-qPCR) was adopted for detecting the relative miR-30b-5p level. Then, the Kaplan-Meier method, Cox regression analysis, and the receiver operating characteristic curve analysis were applied in analyzing the miR-30b-5p effect on the prognosis for patients. Our findings indicated that, following adjustment for age, gender, tumor stage, and tumor size, patients with low miR-30b-5p expression had remarkably longer overall survival. Thus, the miR-30b-5p level might be related to RCC prognosis.

**KEYWORDS**

miR-30b-5p, miRNAs, prognostic biomarkers, renal cell carcinoma
that obesity and the vitamin B6 content in circulation are the prognostic signatures in RCC, but the large-scale clinical application is impossible. Thus, it is of critical importance to identify the prognostic biomarkers for RCC.

microRNAs (miRNAs) are a class of small noncoding RNAs that are 20–22 nucleotides in length, and they can combine with 3-untranslated region (3-UTR) in target mRNAs, thereby resulting in the degradation or the blockade of translation. Besides, they show high stability following 24-hr incubation under ambient temperature or 8 freeze-thawing cycles. Specifically, the easily testable length and stability of miRNAs render them the appropriate candidates for biomarkers. According to the recent reports, miRNAs not only participate in a number of biological processes such as migration, proliferation, differentiation, and apoptosis, but are also associated with tumor pathological grade and stage. Various miRNAs, including miR-10b, miR-21, miR-23B/27B, miR-192, miR-183, miR-143, and miR-566, have been demonstrated to function as the accurate prognostic markers for RCC. Typically, miR-30b belongs to the miR-30 family, and its expression remarkably decreases within multiple malignancies, such as lung cancer, glioma, malignant peripheral nerve sheath tumor, and breast cancer. The above result suggests that miR-30 may play a vital regulatory part during human cancer development. As discovered in our prior work, the expression of miR-30 increases within RCC tissue samples, which may act as the oncogenic gene in RCC to regulate the proliferation of cells. However, it remains unclear about whether miR-30b-5p can be used to be the candidate biomarker for predicting RCC prognosis. It is extremely challenging to supplement to the widely accepted basis for making a clinical decision at present.

2 | PATIENTS AND SPECIMENS

Our study enrolled a total of 284 patients who underwent radical or partial nephrectomy and diagnosed as RCC at Peking University Shenzhen Hospital from April 2003 to May 2013. None of them had received any preoperative radiotherapy or chemotherapy. Their formalin-fixed paraffin-embedded (FFPE) tissue samples were obtained and classified according to the 2010 American Joint Committee on Cancer (AJCC) staging system. All the patients were followed up until May 2018 or death. The clinicopathological information of the RCC patients is present in Table 1. The Ethics Committees of Peking University had approved our study protocol. Each patient provided the informed consent before they participated in this study.

2.1 | RNA isolation, cDNA preparation, and RT-qPCR

RT-qPCR was conducted to detect relative miR-30b-5p levels. Then, the TRIzol (Invitrogen; Thermo Fisher Scientific, Inc) was used to extract total tissue RNA, and RNeasy Maxi Kit (Qiagen GmbH) was utilized for purification in accordance with protocols from manufacturer. Besides, RNA content was measured using the NanoDrop2000/2000c (Thermo Fisher Scientific). RNA samples with an optical density (OD) ratio at 260/280 of 1.8-2.1 were selected for further experiments. Then, reverse transcription was performed using the miScript Reverse Transcription Kit (Qiagen) to obtain cDNA, in accordance with the manufacturer’s protocol. miR-30b-5p expression was detected by miScript SYBR Green PCR kit (Qiagen) on the Roche lightcycler 480 Real-time PCR System (Roche Diagnostics GmbH), with U6 snRNA being used as the internal reference for data normalization. The primers utilized in this study were shown below: for miR-30b-5p: 5′-TGTAACATCCTACACTCACT-3′ (forward); universal primers (miScript SYBR Green PCR kit) (reverse); for U6: 5′-CTCGCTTCGGCAGCACA-3′ (forward), 5′-ACGCTTCAGAATTTGCGT-3′ (reverse). The following PCR running program was set, 2 min at 95°C, followed by 40 cycles of 10 s at 95°C, 30 s at 55°C, and 30 s at 72°C. Besides, relative miR-30b-5p expression in clinical specimens was determined according to the 2-ΔΔCT method.

2.2 | Statistical analysis

All statistical analyses were performed using the spss 19.0 software (IBM SPSS). The Cox proportional hazard regression analysis was applied in analyzing the relationships of miR-30b-5p level with survival or clinicopathological factors. Moreover, Fisher’s exact test or Pearson chi-square test was employed to analyze the correlations between clinicopathological factors (gender, age, tumor size, and tumor stage) and miR-106b expression. Further, the survival curves were plotted by the Kaplan-Meier curves, and differences among the survival curves were evaluated by log-rank test. The predictive ability of miR-30b-5p was determined by the receiver operating characteristic (ROC) curves. A difference of P < 0.05 indicated statistical significance.

| Variable          | Total | High (%) | Low (%) | P-value*
|-------------------|-------|----------|---------|-----------
| Gender            |       |          |         |           |
| Male              | 177   | 92       | 85      | 0.231     |
| Female            | 107   | 50       | 57      |           |
| Age (years)       |       |          |         |           |
| ≤60               | 206   | 96       | 110     | 0.042     |
| >60               | 78    | 46       | 32      |           |
| Tumor size (cm)   |       |          |         |           |
| ≤4.0              | 121   | 68       | 53      | 0.046     |
| >4.0              | 163   | 74       | 89      |           |
| Tumor stage       |       |          |         |           |
| I + II            | 175   | 85       | 90      | 0.313     |
| III + IV          | 109   | 57       | 52      |           |

*aCutoff point: median.

*bCalculated using Fisher’s exact test or Pearson chi-squared test.
2.3 | Validation from TCGA

We obtained data of 506 cases of RCC patients from The Cancer Genome Atlas (TCGA; www.cancergenome.nih.gov) as external validation. Similarly, we performed the Kaplan-Meier analysis with TCGA data to evaluate the relationship between miR-30b-5p expression and survival of RCC patients.

3 | RESULTS

3.1 | Patient clinical characteristics and miR-30b-5p expression

As observed from Table 1, a total of 284 patients, including 177 men and 107 women, were enrolled into this study. Among them, 175 cases were at stage I–II and 109 at stage III–IV. These patients had the median age of 50 years, and among them, 78 aged over 60 years, while the remaining 206 aged less than 60 years. After the follow-up period, 189 cases survived, with a median length of follow-up of 44 months (range, 4-163 months). The 284 patients were divided into high and low miR-30b-5p expression group with median cutoff for further analyses.

The first set of questions aimed to analyze the association of miR-30b-5p level with the clinical data within the renal cancer FFPE specimens. 284 cases were divided as low or high miR-30b-5p expression group according to the median miR-30b-5p level. As suggested by Fisher’s exact test or Pearson chi-square test, miR-30b-5p expression was not significantly related to age, gender, age, tumor stage, or tumor size.

3.2 | Patients’ survival and miR-30b-5p expression

Univariate Cox regression analysis was utilized to test the association of patient survival with the clinicopathological factors and the expression of miR-30b-5p. It was observed from Table 2 that the survival of patient was not related to age, gender, and tumor size, yet it was significantly related to tumor stage (HR = 0.583, 95% CI = 0.390-0.872, P = .009). Interestingly, the data in Table 2 also indicated that miR-30b-5p up-regulation was related to the shorter patient survival (HR = 0.550, 95% CI = 0.362-0.838, P = .005). Following adjustment for gender, age, tumor stage, and tumor size upon multivariate analysis, high miR-30b-5p expression still indicated the shorter overall for patients (HR = 0.572, 95% CI = 0.375-0.871, P = .009).

3.3 | Prognostic value of miR-30b-5p in RCC

As observed from those survival curves tested by Kaplan-Meier method in Figure 1, high miR-30b-5p expression predicted dismal survival of RCC patients (P = .005), which was similar with the data obtained from TCGA (P = .0388). The above findings suggested that miR-30b-5p might potentially serve as an independent factor to predict RCC prognosis. Additionally, we found that the prognostic value of miR-30b-5p in patients over 60 years old (P = .781) and those at advanced stage (P = .302) was not significant in the present study (Figure 2).

ROC curve analysis was used to evaluate the predictive ability of miR-30b-3p for the long-term survival of RCC patients. As shown in Figure 3, according to the ROC curve analysis, the AUC value of tumor stage was 0.591 (95% CI, 0.520-0.662). miR-30b-5p was a better prognostic marker, with the AUC value of 0.607 (95% CI, 0.537-0.776). Moreover, the combination of stage and miR-30b-5p achieved the AUC of 0.651 (95% CI, 0.584-0.717), which was superior to the individual factors.

4 | DISCUSSION

RCC shows an increasing morbidity in the past several decades, which has surpassed lung cancer, and the diagnosed cases increase by 6% annually. Around 25% RCC cases are diagnosed at the metastatic stage, and 20%-40% of them suffer from metastasis or

| Variable                          | Univariate analysis | Multivariate analysis |
|-----------------------------------|---------------------|-----------------------|
|                                   | HR (95% CI)         | P-value               |
|                                   |                     |                       |
| Gender (Female vs Male)           | 0.803 (0.518-1.243) | 0.324                 |
| Age (≤60 y vs > 60 y)             | 0.912 (0.573-1.451) | 0.697                 |
| Tumor size (≤4.0 cm vs > 4.0 cm)  | 1.131 (0.755-1.693) | 0.550                 |
| Tumor stage (I + II vs III + IV)  | 0.583 (0.390-0.872) | 0.009                 |
| miR-30b-5p (low vs high)          | 0.550 (0.362-0.838) | 0.005                 |

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.
Thanks to the integrative efforts made on RCC research and the available targeted therapies, several targeted treatments are replacing IFN-α and IL-2 immunotherapy to be the major treatments for metastatic RCC over the last decade. Targeted therapeutic agents, including sunitinib and sorafenib, can greatly improve patient survival; however, it is estimated that there are still 14,970 deaths associated with renal pelvic cancer and kidney cancer in the United States in 2018. Therefore, novel biomarkers are required and more efforts should be made to discover their functions in predicting RCC prognosis.

miRNAs, which belong to the noncoding RNAs family, exert vital parts during tumor development. More and more studies have demonstrated that miRNAs may be potentially used as the biomarkers to detect cancer early and as the accurate prognostic markers and therapeutic targets for cancer. Recent evidence shows that miRNAs expression is associated with disease severity as well as cancer survival. For instance, the increased hsa-mir-101 expression and decreased hsa-let-7c expression are related to the poor prognosis for lung cancer. Meanwhile, miR-10b, miR-21, miR-126, miR-223, miR-30a-5p, miR-338, and let-7a are related to the OS as well as relapse-free survival for gastric cancer (GC) cases.

In RCC, the expression of miR-566 increases within RCC tissues, and patients with low miR-566 expression have remarkably longer survival. Another study have identified that the overexpression of miR21/10b is markedly related to the shortened survival for RCC cases.

The present study detected the association of miR-30b-5p with RCC survival. In our study, several methods were used to analyze the data collected from 284 patients. And a similar result was obtained, which was that miR-30b-5p independently predicted OS. RCC patients with high miR-30b-5p expression had worse survival, according to both our own data and TCGA data. However, for patients in old group (>60y) and advanced stage group (III+IV), miR-30b-5p expression did not show significant prognostic value in our study. A larger sample of external validation is required in the future. Additionally, as observed from ROC curve analysis, stage combined with miR-30b-5p showed excellent predictive ability for RCC survival after surgery. Our results indicated that miR-30b-5p had the potential to be a novel prognostic biomarker for RCC.

As far as we know, few experiments have been performed to investigate the role of miR-30b within human cancers. According to the results obtained by Zhong et al and Chen et al, miR-30b might
target Rab18 and collagen triple helix repeat containing 1 and play a role of the tumor suppressor gene in the pathogenesis of non-small cell lung cancer. A recent study reports that the expression of miR-30b decreases within GC tissues and cells, thus inhibiting GC cell invasion, migration, and proliferation, while enhancing their apoptosis through targeting EIF5A2. These studies suggest that miR-30 is a potential tumor suppressor gene. However, as suggested by our prior work, miR-30b increased within RCC tissues and act as an oncogene, which prompted us to investigate its relationship with RCC prognosis.

Some limitations of our study should be mentioned. The sample size of the present study was relatively small. It is necessary to conduct multicenter studies to further validate the clinical value of miR-30b-5p. Moreover, FFPE samples are not applicable for tumor postoperative surveillance while body fluids are more ideal biomarkers. As suggested in many recent studies, the abnormal expression of miRNAs in circulation is discovered during early tumor growth, which suggests that miRNA expression in circulation is related to cancer development and prognosis. For example, miR-17-5p/20a, miR-21, miR-148a, and miR-146a are demonstrated to be the candidate non-invasive biomarkers for predicting the presence of lymph node metastasis. However, it remains unclear about whether miR-30b-5p could work as a non-invasive prognostic biomarker in RCC, which needs further studies in the future.

5 | CONCLUSIONS

To sum up, the present work has first detected the relationship between the miR-30b-5p expression level and the prognosis for RCC.

Our results suggest that patients with low miR-30b-5p expression are associated with remarkably prolonged OS, revealing that miR-30b may serve as a candidate biomarker for RCC prognosis. This may help us to recognize the patients with high risk and gain more benefits for RCC treatment.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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