Lysine-specific demethylase 2A gene expression in clear cell renal cell carcinoma and its clinical significance

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Research article

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Abstract

Background

Our study aimed to explore the expression of lysine-specific demethylase 2A (KDM2A) in clear cell renal cell carcinoma (ccRCC) and its relationship with clinical features of ccRCC.

Methods

A total of 50 patients with ccRCC were included. KDM2A expression was assessed by real-time PCR and immunohistochemistry (IHC). The correlations of KDM2A protein expression with clinicopathological parameters and survival rate were further verified.

Results

The KDM2A mRNA expression was significantly higher in ccRCC tissues than para cancer samples ($P < 0.05$). KDM2A protein was mainly expressed in the nucleus of tumor cells. Fifty (100%) of the ccRCC samples were immunopositive, 90% of which all showed high expression of KDM2A. Compared with para cancer tissues, ccRCC samples showed a larger proportion of high KDM2A expression in the overall and most stratified analysis ($P < 0.05$). KDM2A protein expression was positively correlated with TNM stage ($r = 0.307$, $P = 0.030$), proteinuria ($r = 0.385$, $P = 0.006$), and urine erythrocyte ($r = 0.307$, $P = 0.030$). Moreover, Kaplan-Meier survival analysis revealed that higher expression of KDM2A in ccRCC patients was associated with lower survival rate ($P = 0.004$).

Conclusions

Our findings provided the first evidence that KDM2A could be used as a promising diagnostic and prognostic biomarker in ccRCC patients.

Background

Renal cell carcinoma (RCC) is the main malignant tumor of the adult urinary system with an incidence rate second only to bladder cancer, and its biological characteristics and pathogenesis are very complex [1]. The RCC contains a cluster of heterogeneous tumors which originate from the renal tubular epithelial cells. At present, the accepted treatment of RCC is mainly surgical resection. Clear cell renal cell carcinoma (ccRCC) is the most frequent subtype of RCC, which nearly accounts for about 75% of RCC [2]. It derives from the proximal tubular epithelium and is characterized by the worst clinical process and prognosis among other classes of RCC. However, the genetic and epigenetic background of changes that occur during the initiation and development of ccRCC has thus far not been fully elucidated [3]. As to improving the early diagnosis rate of ccRCC, exploring new effective biomarkers and their correlation with clinical indicators is of great significance for the treatment of ccRCC.

Covalent histone modifications play a critical part in the regulation of chromatin dynamics and functions [4]. Histone methylation is an important form of epigenetic modification and occurs on both lysine and arginine residues. Through the methylation of different histone sites, gene expressions can be regulated by influencing the activation and inhibition of transcription [5]. The constant level of covalent histone methylation is under the control of histone methyltransferases and demethylases [6]. The lysine-specific demethylase 2A gene (KDM2A), found on chromosome 11q13.2, is a member of the KDM histone lysine demethylase family, which exhibits specificity for removal of methyl groups of histone H3K36 by binding directly to CpG islands in gene promoters [6]. The dysfunction of KDM2A has been reported in various cancers, and its loss-of-function mouse mutants are embryonically lethal [6]. However, KDM2A has a complex and tissue-specific role in tumorigenesis and tumor progression. Several studies have observed KDM2A functioning as an oncogene, and its expression was elevated in lung cancer, breast cancer, and cervical cancer to promote tumorigenesis and proliferation of cancer cells [7–10]. On the contrary, Frescas et al. found that KDM2A expression was often decreased in prostate cancer compared with normal prostate tissue [11]. However, its function in ccRCC remains poorly understood. The cellular localization and expression of KDM2A have not yet been elucidated, and the influence of their expression on the clinical characteristics of ccRCC patients still needs further elaboration.

Therefore, we decided to use RT-qPCR and immunohistochemistry (IHC) methods to assess the expression of KDM2A within cancer and para cancer tissue of ccRCC, and further analyze their correlation with the clinicopathological indicators. Then, the prognostic role of KDM2A in ccRCC was evaluated on the basis of survival data from TCGA. All together may provide novel insight into the development of therapeutic strategies for ccRCC treatment.

Materials And Methods

Patients and samples

Fifty patients with ccRCC who underwent radical nephrectomy at the First Hospital of China Medical University from January 2018 to October 2018 were enrolled in the study. ccRCC tissues and corresponding para cancer tissues were obtained from renal operation and immediately stored at -80°C. The para cancer tissues were taken from a location 5 cm away from the tumor. For some patients with a large tumor with the remaining para cancer tissues being less than 5 cm, the renal tissues more than 3 cm away from the tumor were taken and pathologically proven to be cancer-free. No patients had received any preoperative adjuvant therapy, such as radiotherapy, chemotherapy, immunotherapy, targeted therapy, interventional embolization, and so on, or had a history of other malignant tumors. Demographic and clinical data of ccRCC patients are summarized in Table 1. This study was approved by the Ethics Committee of the First Hospital of China Medical University (Shenyang, China). Written informed consent was obtained from each subject.

RT-qPCR

A total of 29 fresh tissues were used to determine KDM2A mRNA levels. Total mRNAs of ccRCC and paracancer tissues were extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA.) following the manufacturer's protocols. Reverse-transcribed cDNA synthesis was performed with BioTeke super RT Kit (BioTeke, Beijing, China) following the manufacturer's protocols. PCR was conducted using 2×Power Taq PCR MasterMix (BioTeke, Beijing, China) and SYBR Green (BioTeke, Beijing, China). β -actin acted as an internal control. The primers were synthesized as follows: KDM2A Forward 5'-GGCAGTAGGAATCAAGGACC-3', Reverse 5'-ACCCGACAGCAGTGAGTAGA-3'; β -actin: Forward 5'-CACTGTGCCCATCTACGAGG-3', Reverse 5'-TAATGTCTACGACGATTTC-3'. PCR conditions were as follows: initial denaturation at 94°C for 5 min, followed by 40 cycles of 94°C for 15 sec, 60°C for 20 sec, and 72°C for 30 sec. Each reaction was set up 3 times and the expression level of KDM2A was quantified using the $2^{-\Delta\Delta C_t}$ method.

Immunohistochemistry

All tissues were fixed in 4% polyoxymethylene, embedded in paraffin, and sectioned at 4 μ m. Then, the slides were treated with xylene to remove the paraffin, followed by hydration with ethanol and the addition of EDTA for antigen retrieval. Endogenous peroxidase blocker solution was added to incubate for 10 min, and the sections were rinsed with PBS 3 times. To avoid nonspecific binding, normal goat serum was added to block tissue collagen for 10 min. Sections were incubated with KDM2A monoclonal antibody (1:250; Abcam, Cambridge, MA, USA) for 1 h at room temperature. After washing 3 times with PBS, the sections were incubated with biotinylated secondary goat anti-rabbit antibody, and then with streptavidin-biotin peroxidase for 10 min each. Finally, the diaminobenzidine (DAB) solution was used to stain the sections, which were then counterstained with hematoxylin.

The KDM2A IHC score was determined by both the intensity and percentage of cellular staining. The staining intensity was divided into scores of 0 (negative), 1 (mild), 2 (moderate), 3 (strong), and the percentage was classified as 1 (0-25%), 2 (26-50%), 3 (51-75%), and 4 (>75%). The intensity and percentage were multiplied to calculate the total IHC staining score, which was assigned as negative staining (-, 0), mild staining (+, 1-4), moderate staining (++, 5-8), severe staining (+++, 9-12). An IHC score ≥ 5 was defined as high expression, while a score of less than 5 was defined as low expression. Two independent observers were employed to assess and examine immunostaining.

Kaplan curve

Survival data from TCGA was obtained to clarify the prognostic value of KDM2A in ccRCC. They were submitted to OncoLnc (<http://www.oncolnc.org/>) and Kaplan-Meier curve was plotted for prognostic analysis.

Statistical analysis

Statistical analysis was performed using SPSS version 23. Quantitative data were expressed as the mean $\pm$ standard deviation (SD), and counting data were represented by the number and rate. The significance

of differences in expression levels of KDM2A between ccRCC tissues and para cancer tissues was calculated by independent sample t-test, χ^2 test, or Fisher's exact test, as appropriate. Spearman's rank correlation test was used to analyze the association between KDM2A expression and clinical parameters of ccRCC patients. All *P* values were two-sided, and values of less than 0.05 were considered statistically significant.

Results

Baseline characteristics of ccRCC patients

The patients' baseline characteristics are presented in Table 1. There were 29 (58%) male and 21 (42%) female cases with an average age of 58.78 years. TNM stage T1-T2 and T3-T4 accounted for 38 (76%) and 12 (24%) of all the patients, respectively. As for the International Society of Urological Pathology (ISUP) grade, 35 patients (70%) were classified as grade 1-2, while 15 patients (30%) were classified as grade 3-4. Moreover, most patients had no clinical symptoms.

KDM2A mRNA and protein expression in ccRCC

KDM2A mRNA expression was around 2.41-fold higher in ccRCC samples than in para cancer samples with a significant difference ($P < 0.001$; Figure 1).

IHC analysis revealed that KDM2A protein was predominantly located in the cell nucleus and that there were significantly higher KDM2A expression levels in ccRCC samples than in para cancer samples (Figure 2). In addition, all the ccRCC samples were immunopositive, 90% of which showed a high expression of KDM2A protein, which was significantly higher than in para cancer tissues (Table 2).

We also compared KDM2A protein expression between ccRCC and para cancer tissues stratified by selected characteristics (Table 3) and found that the rate of high KDM2A expression was significantly elevated in ccRCC tissues in each stratified analysis when compared with para cancer tissues (all $P \leq 0.05$), except for the comparison in the smoking and renal venous thrombosis subgroups.

Correlation of KDM2A protein expression with clinicopathologic features and common biochemical indicators

As shown in Table 4, KDM2A protein expression level was positively related to TNM stage ($r = 0.307$, $P = 0.030$) in ccRCC patients. Furthermore, there was a positive correlation of KDM2A expression with the levels of proteinuria ($r = 0.385$, $P = 0.006$) and urine erythrocyte ($r = 0.307$, $P = 0.030$).

The prognostic value of KDM2A in ccRCC

Kaplan-Meier survival analysis suggested that higher expression of KDM2A in ccRCC patients was related to lower survival rate ($P = 0.004$, Figure 3).

Discussion

In the current study, we first, to our knowledge, made a comprehensive evaluation of the correlation between KDM2A expression and ccRCC disease. We found that KDM2A level was often upregulated in ccRCC tissues, and high KDM2A expression was positively associated with TNM stage and the levels of proteinuria and urine erythrocyte. Moreover, ccRCC patients with high KDM2A expression showed a poor survival prognosis. Our research provides new information about the diagnostic and prognostic importance of KDM2A in ccRCC, which enable adequate risk stratification and predict the tumor's reaction to individual therapy.

The occurrence and development of ccRCC is a complicated multi-step biological process, which may involve the instability of the genome, the gradual accumulation of gene mutations, epigenetic mechanism alterations, and abnormal gene expression [12, 13]. Histone lysine methylation was regarded as a central modification for the post-transcriptional regulation of chromosome structure and DNA replication, repair, and transcription procedure [14], and was relevant to the activation or silencing of gene expression [15]. KDM2A can specifically catalyze the demethylation of histone H3K36, which is a conserved epigenetic marker influencing gene transcription, alternative splicing, and DNA repair [16, 17]. During cell mitosis, KDM2A plays a role in maintaining genomic stability and centromeric integrity [11]. Meanwhile, the overexpression of KDM2A might antagonize the senescence of embryonic fibroblasts and promote somatic reprogramming [16]. The deletion of KDM2A may also inhibit the proliferation of stem cells from apical papilla by inhibiting p15 (INK4B) and p27 (Kip1) [14].

Emerging studies have shown that the level of KDM2A expression is up-regulated in a variety of tumor cells and affects the biological behavior of tumor cells. In the present study, KDM2A expression in ccRCC was evaluated using RT-qPCR and IHC methods, which have never been reported before. Our results found that the mRNA and protein expression levels of KDM2A in ccRCC tissues were significantly increased compared to para cancer tissues. Moreover, KDM2A protein was found in the cell nucleus. Furthermore, an elevated percentage of high KDM2A protein expression was also markedly demonstrated in ccRCC tissues in most stratified analysis when compared with para cancer tissues. The above findings suggest that ccRCC may play a key role in the initiation of ccRCC and could serve as a diagnostic biomarker for this disease.

Similarly, gastric cancer tissues have been found to have an increased level of KDM2A expression and forced expression of KDM2A promoted cell growth and migration via a downregulation of the expression of programmed cell death 4 (PDCD4), a known tumor suppressor in the progression of gastric cancer [18]. In breast cancer, KDM2A was found to be highly expressed and significantly correlated with shortened survival of breast cancer patients [9, 19]. In non-small cell lung cancer (NSCLC), Wagner et al. demonstrated that KDM2A was overexpressed and could promote the proliferation and metastasis of NSCLC cells by regulating the activity of ERK1/2 pathway, which is correlated with a poor prognosis of NSCLC patients [8]. An up-regulated expression of KDM2A was also shown to play a critical role in the onset and progression of cervical cancer and promote the proliferation and invasion of cervical cancer

cells [10, 20]. In addition, Xu et al. found that KDM2A might be an important regulator of cell proliferation and cell cycle via impacting TGF- β signaling pathway [21]. However, Frescas et al. revealed a lower level of KDM2A expression in prostate carcinomas compared to normal prostate tissue [11]. These diverse observations may be attributed to the heterogeneity of the disease and a variety of sample sizes.

Currently, factors such as tumor stage, size, and grade, along with other preoperative laboratory variables, are considered to have the prognostic significance of ccRCC [22, 23]. Given this, we analyzed the relationship of KDM2A expression with clinicopathological parameters and laboratory values. Interestingly, KDM2A expression was indicated to have a positive and significant association with TNM stage and the levels of proteinuria and urine erythrocyte. Indeed, using TNM as a prognostic factor is recommended by the European Association of Urology (EAU) RCC guideline panel. In all RCC subtypes, the prognosis was found to worsen with increasing TNM stage [24]. Proteinuria was regarded as an independent risk factor for adverse renal outcomes in ccRCC patients, and assessment of proteinuria should be performed to inform prognosis and select optimal treatment strategy [25]. Severe preoperative proteinuria was also reported to be associated with worse overall survival following radical or partial nephrectomy for ccRCC [26], while preoperative urine erythrocyte could have prognostic importance for ccRCC patients in the univariate analysis [22]. Our correlation analysis results suggested that increased KDM2A expression might help to promote the malignant biological behavior of the tumor and imply a poor prognosis for ccRCC. Furtherly, based on the survival data from TCGA, it was demonstrated that ccRCC individuals with high KDM2A level would have a relatively worse survival prognosis. Therefore, our results indicated that KDM2A also has potential as a prognostic marker of ccRCC.

Some limitations exist in our study. Firstly, there was a lack of comparison with normal kidneys. Secondly, due to incomplete prognostic information of our ccRCC patients, the prognostic results relied on the data from TCGA. In addition, the sample size was relatively small. Further studies with a larger number of subjects are necessary to confirm our findings, while the underlying mechanisms deserve further exploration.

Conclusions

In summary, our study tested the expression of KDM2A in ccRCC samples for the first time, with the findings suggesting that KDM2A might be a potential marker for the diagnosis and prognosis of ccRCC patients. High KDM2A expression was observed in ccRCC tissues and had an adverse effect on the overall survival of ccRCC patients. KDM2A protein expression was closely related to worse TNM stage and renal function. Our results may be useful in understanding the pathogenesis and progression of ccRCC.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Hospital of China Medical University. Written informed consent was obtained from each patient, including consent for their samples to be taken and used for research purposes, before surgery.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

XW conceived and supervised the study. XW and JW designed the experiments. JW and XL performed the experiments. JW and YZ analyzed the data. JW and TL wrote the manuscript. XW revised the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Baseline characteristics of ccRCC patients (n=50).

Parameters	ccRCC
Gender, n (%)	
Male	29 (58%)
Female	21 (42%)
Age, years	58.78±8.85
<60, n (%)	28 (56%)
≥60, n (%)	22 (44%)
Location, n (%)	
Left	22 (44%)
Right	28 (56%)
BMI (kg/m ²)	24.79±3.22
<24, n (%)	20 (40%)
≥24, n (%)	30 (60%)
Tumor size(cm)	6.20±2.38
≤7, n (%)	35 (70%)
>7, n (%)	15 (30%)
Smoking, n (%)	
No	39 (78%)
Yes	11 (22%)
Thrombus of renal vein, n (%)	
No	46 (92%)
Yes	4 (8%)
TNM stage, n (%)	
T ₁	27 (54%)
T ₂	11 (22%)
T ₃	10 (20%)
T ₄	2 (4%)
T ₁ -T ₂	38 (76%)
T ₃ -T ₄	12 (24%)
ISUP grade, n (%)	
1	4 (8%)
2	31 (62%)
3	8 (16%)
4	7 (14%)
1-2	35 (70%)
3-4	15 (30%)
Symptoms, n (%)	
No	35 (70%)
Yes	15 (30%)
Hypertension, n (%)	
No	29 (58%)
Yes	21 (42%)
Proteinuria, n (%)	
Negative	18 (36%)
Trace	20 (40%)
1+	10 (20%)
2+	1 (2%)
3+	1 (2%)
Urinary occult blood, n (%)	
Negative	30 (60%)
Trace	5 (10%)
1+	5 (10%)
2+	6 (12%)

3+	4 (8%)
Urine erythrocyte (/μl)	307.64±825.37
Urine specific gravity	1.02±0.01
White blood cell (×10 ⁹ /l)	6.79±2.86
Hemoglobin (g/l)	138.62±23.47
Creatinine (μmol/l)	60.82±11.61
D-Dimer (μg/ml)	1.25±2.93

ccRCC, clear cell renal cell carcinoma; BMI, body mass index; TNM, tumor, node, metastasis; ISUP, international society of urological pathology.

Table 2. The overall expression of KDM2A protein in ccRCC and para cancer tissues.

Group	(-)	(+)	(++)	(+++)	<i>P</i>	Low, n (%)	High, n (%)	<i>P</i>
Para cancer tissues	8 (16%)	17 (34%)	14 (28%)	11 (22%)	Reference	25 (50%)	25 (50%)	Reference
ccRCC tissues	0 (0%)	5 (10%)	19 (38%)	26 (52%)	≪0.001	5 (10%)	45 (90%)	≪0.001

KDM2A, lysine-specific demethylase 2A; ccRCC, clear cell renal cell carcinoma.

Table 3. Stratified analysis of KDM2A protein expression in ccRCC patients.

Parameters	Para cancer tissues		Cancer tissues		<i>P</i>
	Low, n (%)	High, n (%)	Low, n (%)	High, n (%)	
Gender					
Male	13 (26%)	16 (32%)	1 (2%)	28 (56%)	0.001
Female	12 (24%)	9 (18%)	4 (8%)	17 (34%)	0.011
Age, years					
<60	12 (24%)	16 (32%)	3 (6%)	25 (50%)	0.007
≥60	13 (26%)	9 (18%)	2 (4%)	20 (40%)	0.001
Location					
Left	10 (20%)	12 (24%)	2 (4%)	20 (40%)	0.007
Right	15 (30%)	13 (26%)	3 (6%)	25 (50%)	0.001
BMI (kg/m ²)					
<24	10 (20%)	10 (20%)	0 (0%)	20 (40%)	0.001
≥24	15 (30%)	15 (30%)	5 (10%)	25 (50%)	0.006
Tumor size (cm)					
≤7	18 (36%)	17 (34%)	5 (10%)	30 (60%)	0.001
>7	7 (14%)	8 (16%)	0 (0%)	15 (30%)	0.006
Smoking					
No	22 (44%)	17 (34%)	5 (10%)	34 (68%)	0.001
Yes	3 (6%)	8 (16%)	0 (0%)	11 (22%)	0.214
Thrombus of renal vein					
No	23 (46%)	23 (46%)	5 (10%)	41 (82%)	0.001
Yes	2 (4%)	2 (4%)	0 (0%)	4 (8%)	0.429
TNM stage					
T ₁ -T ₂	20 (40%)	18 (36%)	5 (10%)	33 (66%)	0.001
T ₃ -T ₄	5 (10%)	7 (14%)	0 (0%)	12 (24%)	0.037
ISUP grade					
1-2	15 (30%)	20 (40%)	4 (8%)	31 (62%)	0.003
3-4	10 (20%)	5 (10%)	1 (2%)	14 (28%)	0.002
Symptoms					
No	14 (28%)	18 (36%)	4 (8%)	28 (56%)	0.005
Yes	11 (22%)	7 (14%)	1 (2%)	17 (34%)	0.001
Hypertension					
No	15 (30%)	14 (28%)	4 (8%)	25 (50%)	0.002
Yes	10 (20%)	11 (22%)	1 (2%)	20 (40%)	0.002

KDM2A, lysine-specific demethylase 2A; ccRCC, clear cell renal cell carcinoma; BMI, body mass index; TNM, tumor, node, metastasis; ISUP, international society of urological pathology.

Table 4. Correlations of KDM2A protein expression with clinicopathologic features and common biochemical indicators.

Parameters	r	P
Tumor size (cm)	0.235	0.100
TNM stage	0.307	0.030
ISUP grade	0.210	0.144
Proteinuria	0.385	0.006
Urinary occult blood	0.239	0.094
Urine erythrocyte (/μl)	0.332	0.019
Urine specific gravity	0.155	0.284
White blood cell (×10 ⁹ /l)	0.042	0.770
Hemoglobin (g/l)	-0.031	0.833
Creatinine (μmol/l)	0.215	0.133
D-Dimer (μg/ml)	0.030	0.840

KDM2A, lysine-specific demethylase 2A; TNM, tumor, node, metastasis; ISUP, international society of urological pathology.

Figures

Figure 1

KDM2A mRNA expression was detected by RT-qPCR and shown to be about 2.41-fold higher in ccRCC samples than in para cancer samples.

Figure 2

Immunohistochemical staining for KDM2A protein expression in ccRCC and para cancer tissues. A: High expression of KDM2A in ccRCC tissues; B: Low expression of KDM2A in para cancer tissues. (Magnification, ×400)

Figure 3

Kaplan-Meier survival curve was performed to assess the prognostic value of KDM2A in ccRCC.