Original paper

Overexpression of KIF11 is a poor prognostic factor in clear cell renal cell carcinoma

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Unresectable renal cell carcinoma continues to be a great challenge due to our limited understanding of its underlying pathophysiology. We explored the relationship between KIF11 protein expression and the clinical courses of clear cell renal cell carcinoma (ccRCC) using a tissue microarray.
Material and methods: The tissue microarray contained specimens derived from 90 patients, cancer and matched adjacent non-cancerous tissue (2 cores per case), followed up for 7 years. Tumour samples were evaluated for KIF11 expression using the H-score, and their correlations with clinicopathological data and survival data were analysed.
72.7% of ccRCC tissues presented KIF11 cytoplasmic expression with a median value of 20 (interquartile range 0–200). The nuclear staining was positive in 36.36% of ccRCC tissues. Among controls, nuclear KIF11 expression was absent, but cytoplasmic expression was identified in all cases, with a median value of 230 (interquartile range 45–290). Cytoplasmic KIF11 expression in ccRCC tissues was lower than in the control tissues and was positively correlated with tumour grade and mortality ($p < 0.05$). KIF11 nuclear expression did not correlate with overall survival.
Elevated expression of KIF11 predicts poor clinical outcome in ccRCC patients. Downregulation of KIF11 may provide a new therapeutic strategy for ccRCC.

Key words: KIF11, ccRCC, kidney cancer, renal carcinoma, expression, prognosis, survival, OS.

Introduction

Renal cell carcinoma (RCC) is in the top 10 most common cancers, and its incidence is on the rise. Despite significant advances in medical management, the American Cancer Society estimates that in 2020 in the US, 14,830 people will die from this disease [1]. The most common subtype of RCC that accounts for 65–70% of cases, is the clear cell renal cell carcinoma (ccRCC). It originates from the proximal tubular epithelial cells of nephrons [1, 2]. The extraordinary heterogeneity of this tumour poses a great challenge

for its effective treatment [3]. Thus, the establishment of novel molecular targets is an attractive approach.

KIF11, as a motor protein encoded by the *KIF11* gene, assists in spindle dynamics. Among its main functions are chromosome positioning, centrosome separation, and establishing a bipolar spindle during mitosis [4]. Its overexpression reflects poor prognosis in various carcinomas including gastric, laryngeal, breast, prostate, and pancreatic [5–9]. Recent reports together with *in silico* analysis suggest that KIF11 may also contribute to ccRCC progression. We explored associations of KIF11 expression with the clinical course using a tissue microarray (TMA) and validated these findings in The Cancer Genome Atlas (TCGA).

Material and methods

Tissue microarray

The tissue microarray was purchased from a commercial supplier (US Biomax, Rockville, MD). The tissue microarray (HKid-CRC180Sur-01) contained specimens derived from 90 patients, cancer and matched adjacent non-cancerous tissue (2 cores per case), followed up for 7 years. Samples were consecutively collected from July 2006 to February 2008, following informed consent and under approval of the Ethics Committee. All of the specimens were obtained prior to any therapeutic manipulation. The diagnosis was made by at least 2 different evaluators in accordance with up-to-date World Health Organization guidelines. Two cores with ccRCC and 5 cores with normal adjacent tissue were missing and therefore were excluded from the analysis. Retrievable clinicopathological data included age, pathological diagnosis, TNM, stage, grade, and overall survival (OS). The quality of each specimen was additionally approved by our pathologists.

Immunohistochemistry

The tissue microarray slides were processed at the Department of Clinical Pathology. Primary rabbit polyclonal anti-KIF11 (HPA010568; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) antibody was used to estimate the expression of KIF11 protein. The standardization of the protocol was achieved using a series of control reactions: positive and negative. The positive control reaction was performed in accordance with reference sources (Human Protein Atlas: http://www.proteinatlas.org) and the antibody datasheet. KIF11-positive control reaction performed on pancreatic cancer tissue presented cytoplasmic and nuclear expression. Furthermore, all negative control reactions were performed on additionally analysed tissue sections by substituting the primary antibody with a solution of 1% bovine serum albumin diluted in phosphate-buffered saline. Immunohistochemical (IHC) staining was performed using primary rabbit polyclonal anti-KIF11 (1 : 200) antibody and visualization system EnVisionFlex+ Anti-Mouse/Rabbit HRP-Labelled Polymer (Dako, Agilent Technologies) on an Autostainer Link48 platform. Lastly, dehydration of tissue sections was performed in ethanol at increasing concentrations (80–98%), then cleared in a series of xylenes (I–IV) and cover-slipped in a medium (Dako, Agilent Technologies, USA).

Immunohistochemical analysis and scoring

All immunostained samples were evaluated by 2 experienced pathologists blinded to the patients' clinical data. The level of KIF11 cytoplasmic and nuclear expression were assessed using the light microscope at 20× and 40× magnification. The extent of cytoplasmic immunoreactivity was assessed by H-Score. In this case, we distinguished 3 levels of expression intensity (1+ = 'low'/2+ = 'moderate'/3+ = 'high'). The percentage of those cells were applied to the following formula:

$1 \times (\%$ cells $1+) + 2 \times (\%$ cells $2+) + 3 \times (\%$ cells $3+) =$ H-score

The final score ranged 0–300. The nuclear expression of KIF11 was evaluated using a two-point scale (0 = 'negative IHC result'/1 = 'positive IHC result').

Statistical analysis

All the statistical analyses were performed using Statistica version 10 (StatSoft) and Microsoft Excel 2019. We used the log-rank test to compare the survival distributions of patients with different protein expression patterns. The Kaplan-Meier estimator was performed to estimate the survival functions from lifetime data. We used the Mann-Whitney *U* test to compare the protein expressions between cancerous and adjacent normal cells. Cox Proportional Hazards for analysing ccRCC survival data were considered. The data were divided into 4 groups according to patients' ages (age ≤ 65 and age > 65 years), grade (G1 and G2, G3), stage (T1 and T2, T3) and KIF11 expression level (KIF11 ≤ 42.5 – low and KIF11 > 42.5 – high). The p-value < 0.05 was considered statistically significant.

Ethical review and approval were waived for this study due to the lack of access to identifiable private information. Informed consent was obtained from all subjects involved in the study

Results

The study included 88 pairs of ccRCC and corresponding non-cancerous tissue. During the IHC staining procedure, 5 cores of corresponding tissue and 2 cores of ccRCC were lost. Summarized charac-

Table I. Baseline characteristics of the tissue microarray ($n = 88$) patient cohort

Clinical information	*n* (%)
Age [years]	
Mean	59.09
Range	29–83
Stage	
I	60 (68.20)
II	17 (19.30)
III	3 (3.40)
IV	2 (2.30)
Unknown	6 (6.81)
T stage	
T1	63 (71.59)
T2	17 (19.32)
T3	4 (4.55)
Unknown	4 (4.55)
Lymph nodes	
N1	1 (1.11)
N0/Nx	85 (96.59)
Unknown	2 (2.30)
Metastasis	
Yes	2 (2.28)
No	86 (97.72)
WHO/ISUP grade	
G1	33 (37.5)
G2	41 (46.59)
G3	13 (14.77)
G4	1 (1.14)
Median follow-up time [years]	7.0
Disease course	
Alive	60 (68.18)
Dead	28 (31.82)

ISUP – International Society of Urological Pathology, WHO – World Health Organization

teristics of the TMA cohort are presented in Table I. The median follow-up was 7.0 years.

64 of 88 ccRCC tissues (72.7%) presented KIF11 cytoplasmic expression with the median value of 20 (interquartile range 0–200). The nuclear staining was positive in 32 of 88 ccRCC tissues (36.36%). Among controls, nuclear KIF11 expression was absent, but cytoplasmic expression was identified in all cases, with a median value of 230 (interquartile range 45–290). Cytoplasmic KIF11 expression in ccRCC tissues was lower compared to control tissues ($p < 0.05$) (Fig. 1A).

Cytoplasmic KIF11 expression positively correlated with tumour grade ($p = 0.0013$) and mortality (HR 2.17; 95% Cl: 0.99–4.73; $p = 0.047$) (Figs. 1B, 2A). Cox Proportional Hazard was statistically significant only for T1 tumours. HR estimates of 0.19 (95% Cl: 0.04–0.96; $p = 0.045$) for low KIF cytoplasmic expression and 0.42 (95% Cl: 0.15–1.16; $p = 0.049$) for high KIF cytoplasmic expression were calculated. KIF11 nuclear expression did not correlate with OS ($p = 0.72$) (Fig. 2B). KIF11 cytoplasmic or nuclear expression did not correlate with tumour stage.

Discussion

Surgical resection is the best therapeutic strategy for localized RCC [10]. However, around 30% of patients experience tumour recurrence following complete resection [11, 12]. Immunotherapy and/or targeted therapy represent a standard of care for stage IV and recurrent RCC. Despite relatively high response rates to these agents, most patients eventually succumb to cancer progression. Versus KIF11 has been shown to promote the epithelial-mesenchymal transition and activate many molecular mechanisms involved in cancer progression, including Wnt/β-catenin, PI3K/AKT/mTOR, and MAPK/ERK pathway [9, 13, 14]. Targeting KIF11 inhibits invasion, proliferation, and self-renewal in glioblastoma cell lines [15]. A similar effect was observed in breast cancer and prostate cancer cells [9, 16–20]. Filanesib, a potent KIF11 inhibitor, has recently demonstrated clinical efficacy in patients with multiple myeloma [21].

The metastatic competence of ccRCC is afforded by chromosome complexity, in particular 9p and 14q loss [22]. We found that high KIF11 cytoplasmic (but not nuclear) expression correlates with poor survival in patients with ccRCC. KIF11 within the cytoplasm contributes to centrosome separation and bipolar spindle formation and can provoke vital chromosome-level alterations. Hence, it could play a significant role in driving ccRCC evolution and metastatic spread. Then, targeting KIF11 would be an attractive complement to evolution-targeted therapy. Evolution-targeted therapy in ccRCC is a novel concept that relies on patient stratification according to the deterministic evolutionary trajectory of the tumour [23]. Currently there are 7 well described evolutionary trajectories in ccRCC according to the tumour's genomic characteristics, evolution mode, and clinical course [24]. While the evolutionary trajectory could be used as a biomarker for guiding the intervention, inhibition of KIF11 could further curb cancer evolution, making this approach more effective.

According to the cBioportal for Cancer Genomics, a database with genome sequencing and comparative genome hybridization, KIF11 overexpression is driven by epigenetic alterations in 99.61% of cases. The remaining causes include genetic amplifications

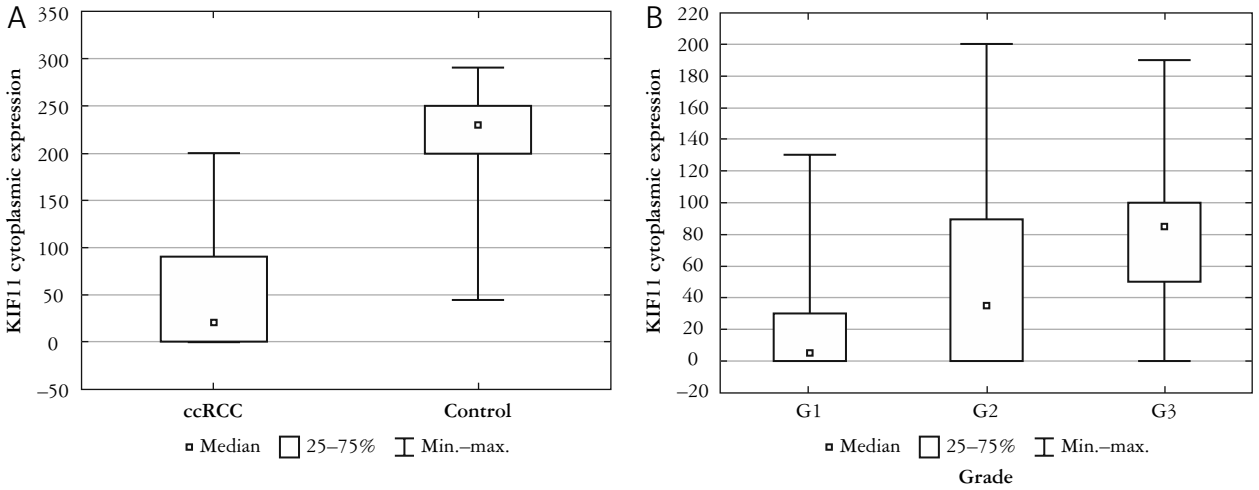

Fig. 1. A) Cytoplasmic KIF11 expression in clear cell renal cell carcinoma (ccRCC) and adjacent normal tissue (control). B) KIF11 expression according to ccRCC grade

ccRCC – clear cell renal cell carcinoma

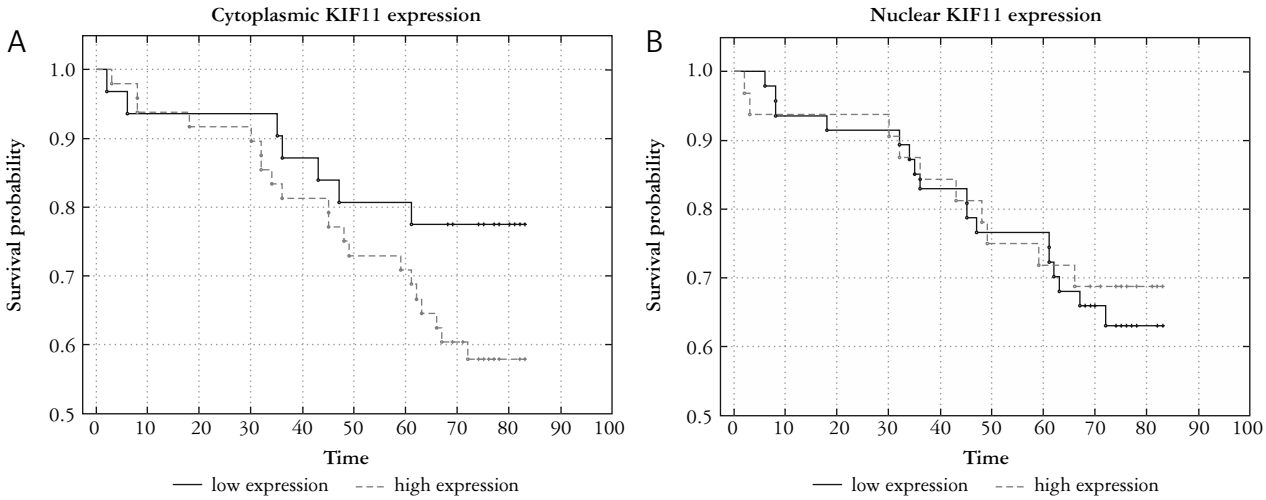

Fig. 2. A) The survival curve of clear cell renal cell carcinoma (ccRCC) patients according to cytoplasmic KIF11 expression. B) The survival curve of ccRCC patients according to nuclear KIF11 expression

and missense mutations. The epigenetic alterations are reversible and play a central role in renal carcinogenesis [25]. Hence, specifically targeting these alterations could restore a normal epigenetic pattern and potentially cure the disease. Currently, targeted epigenetic therapies are under investigation. Their combination with antiangiogenic or immune checkpoint treatments represents a particularly promising paradigm that could overcome frequent monotherapy resistance [26–29]. Epigenetic therapeutics are classified into agents that have a targeted effect, such as anti-miRNA oligonucleotides, and agents that have a more broad effect and lead to large-scale changes in gene expression, such as HDAC inhibitors (HDACi) [25]. The principal problem with the first group of agents is their difficult delivery to cancer cells [30]. The second group of agents, on the other hand, activate genes that are normally repressed, leading to adverse off-target effects that influence numerous processes in the body [31]. As a result, no epigenetic alteration can be both safely and precisely targeted, and therefore successful clinical translation of epigenetics in RCC remains to be seen.

To evaluate the association between KIF11 mRNA expression and the clinical course of ccRCC we accessed the TCGA database [32, 33]. In TCGA, patients were classified into 2 expression groups based on the KIF11 FPKM (number fragments per kilobase of exon per million reads) value. To choose the best FPKM cut-off for grouping the patients, significant differences in the OS of the groups were analysed, and the value yielding the lowest log-rank *p*-value (1.5e–8) was selected. KIF11 expression among 119 of 528 (22.54%) patients was higher than the established cut-off. The Kaplan-Meier survival estimators evaluated the prognosis of each group. The survival outcomes of the 2 groups were compared by log-rank tests. The five-year survival was reached

by 69% of patients with low KIF11 expression and 44% of patients with high KIF11 expression. According to data from the TCGA database, elevated KIF11 mRNA expression is associated with poor prognosis in ccRCC ($p < 0.05$). These results are in accordance with our findings.

Our study cohort comprised mainly low-grade and low-stage cases. Therefore, further research incorporating advanced, unresectable tumours is needed to translate our results toward a future potential clinical intervention.

Conclusions

Elevated expression of KIF11 predicts poor clinical outcome in ccRCC patients. Downregulation of KIF11 may provide a new therapeutic strategy for ccRCC.

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The authors declare no conflict of interest.

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