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Research Paper

High expression of *F2RL3* correlates with aggressive features and poor survival in clear cell renal cell carcinoma

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Abstract

Background: Specific lifestyle factors including tobacco-exposure are vital etiologic factors for renal cell carcinoma (RCC), F2R Like Thrombin/Trypsin Receptor 3 (F2RL3) is associated with smoking but it is unknown whether its expression translate into poor survival of clear cell RCC (ccRCC). In the current study, the expression profiling and prognostic value of F2RL3 in Chinese patients with ccRCC were investigated.

Methods: Using Quantitative PCR analysis and immunohistochemistry, the relative expression levels of F2RL3 in 367 paired ccRCC and adjacent normal tissues were calculated. Cox regression analysis was used to identify independent prognostic factors and Kaplan-Meier analysis and a log-rank test were employed to evaluate the prognostic value of F2RL3.

Results: We observed that high expression of F2RL3 mRNA and protein were strongly correlated with shorten progression-free survival (PFS) of ccRCC with hazard ratios (HR; 95% confidence interval (CI)) of 2.060 (1.410-3.009) and 1.657 (1.193-2.300), respectively, as well as with poor overall survival (OS) with HRs (95%CI) of 2.826 (1.713-4.662) and 1.712 (1.140-2.569), respectively. After adjustment for confounding factors including smoking status, elevated HRs (95%CI) of 2.113 (1.445-3.089) and 1.692 (1.218-2.352) were presented for PFS, respectively, and 2.936 (1.777-4.851) and 1.811 (1.203-2.725) were present for OS, respectively. Meanwhile, increased F2RL3 mRNA and protein level were reported to significantly associate with smoking-exposure and well-known prognostic factors (higher TNM stage and ISUP grade).

Conclusion: These findings suggested that F2RL3 mRNA and protein level in ccRCC is a robust predictor of poorly prognostic phenotype. Exploring the causal relevance of F2RL3 in ccRCC development further warrants in the future study.

Key words: F2RL3 expression, aggressive features, poor survival, renal cell carcinoma

Introduction

Kidney cancer is one of the most commonly genitourinary carcinomas, and about 90% of all kidney cancers are renal cell carcinomas. Approximately 337,860 new cases of kidney cancer were diagnosed, and 143,406 deaths were occurred worldwide in 2012[1]. It is also statistically estimated that the incidence of kidney cancer in developed regions is higher than that of developing areas. This geographical variation is likely attributed to cancer-related risk factors such as obesity, smoking and hypertension [2]. Especially, tobacco exposure is an established risk factor for kidney cancer. A meta-analysis [3] including 24 studies confirmed that the relative risk (RR) for kidney cancer for frequent smokers compared to never smokers is 1.38. In addition, strong dose-dependent risk increases with heavier smoking and decreases with smoking-cessation >10 years [3]. There also exists evidence that higher exposure to environmental tobacco smoke among never-smokers increases higher risk for kidney cancer [4, 5]. Furthermore, tobacco exposure is associated with poor pathological and clinical stage, and unfavorable prognosis [6, 7]. The established mechanisms that explain how cigarette-smoking leads to carcinogenesis include as follows: (1) exposure to smoking-related carcinogens, (2) formation of DNA adducts between carcinogens and DNA, and (3) mutations induced in critical oncogenes or tumor suppressor genes [8]. For example, 4-(methylnitros-amino)-1-(3-pyridyl)-1-butanone is a carcinogenic chemical present in tobacco. It induces DNA damages-methylation and pyridyloxobutylation adducts that are related with higher risk of kidney cancer [9]. The insights on cancer-related risk factors provide promising avenues for preventing and treating kidney carcinoma.

Coagulation factor II receptor-like 3 gene (*F2RL3*), coding for protease-activated receptor-4 (PAR4), has been proved to affect platelet activation and cardiovascular functions such as intimal hyperplasia and inflammation[10], and has been investigated as a drug target of cardiovascular importance features[10, 11]. A single locus in *F2RL3* out of 27578 loci tested in the 27K discovery and replication study was less methylated in smokers[12]. Importantly, methylation levels in *F2RL3* were consistently confirmed in several independent studies[12-14]. And dose-response associations with *F2RL3* methylation were demonstrated for both current and long-term tobacco exposure[15]. Furthermore, lower *F2RL3* methylation was a stronger predictor of all-cause, cardiovascular, cancer and other mortality[16]. In this study, the association of *F2RL3* with prognosis of clear cell renal cell carcinoma (ccRCC), which is the most commonly pathological type of kidney cancer, were evaluated to further understand *F2RL3* expression in related to smoking-related disease.

Materials and Methods

Patients and samples collection

Three hundred and sixty-seven patients with ccRCC, who underwent radical nephrectomy at Fudan University Shanghai Cancer Center (FUSCC) from 2008 to 2015, were enrolled into this study. Clinical and pathological information (e.g. age at surgery, gender, smoking status, clinical manifestation, laterality, tumor size, ISUP grade and TNM stage) for all participants included in this study were collected and then evaluated (Table 1). Tissue samples (ccRCC and adjacent normal tissues) collected at the time of surgery were obtained from FUSCC tissue bank. Paraffin-embedded specimens preserved at the department of pathology of FUSCC were also obtained for this study. The institutional review board of FUSCC approved this study. Written informed consent for each participant was also obtained.

Quantitative PCR analysis

Total RNA from 367 paired ccRCC and adjacent normal tissues stored in RNAlater™ Stabilization Solution (Invitrogen, Carlsbad, CA) were isolated using the TRIzol reagent (Invitrogen, Carlsbad, CA). Upon completion of total RNA collection, reverse-transcribed reaction was performed using SuperScript First Strand cDNA system (Invitrogen, Carlsbad, CA). Real-time PCR was performed using ABI Prism 7900 sequence detection system (Applied Biosystems) with β-actin as an internal reference. The forward and reverse primers for *F2RL3* were 5′-TTC CCTGGAGACTCACTGCAA-3′ and 5′-GGTTGCTCA AGAGACATCCCA-3′. For β-actin gene, the sense and antisense primers were 5′-GCCGACAGGATGCA GAAGGAGATCA-3′ and 5′-AAGCATTTGCGGTGG ACGATGGA-3′, respectively. For each assay, a total of 10uL reaction mixture was prepared using SYBR Green PCR master mix (Applied Biosystems) according to the manufacturer's instructions. Specific cycling conditions for β-action and *F2RL3* were carried out as follow: denaturation at 95℃ for 3 min, followed by 45 cycles of denaturation at 95℃ for 20 sec, annealing at 60℃ for 20 sec, extension at 68℃ for 20 sec, and measurement at 80℃ for 20 sec, followed by a final extension at 72℃ for 5 min. To confirm the specificity of amplification, melting curve analysis were performed and PCR products were sequenced and resolved in a 1% agarose gel. The *F2RL3* mRNA

expression was represented as $\Delta Ct = Ct_{(F2RL3)} - \Delta Ct_{(\beta\text{-actin})}$, and the relative expression of *F2RL3* in ccRCC was measured using the ratio of *F2RL3* expression in ccRCC/matched normal tissues. "Low *F2RL3* expression" denotes the ratio of *F2RL3* mRNA expression in ccRCC/matched normal tissues of less than 3. "Middle *F2RL3* expression" denotes the ratio of *F2RL3* mRNA expression in ccRCC/matched normal tissues of greater than 3 and less than 6. "High *F2RL3* expression" denotes the ratio of *F2RL3* mRNA expression in ccRCC/matched normal tissues of greater than 6. Paraffin slices were treated according to the immunohistochemical kit, and results were evaluated by two pathologists independently. Specimens were scored according to the intensity of the dye color. The intensity of the dye color was graded as 0 (no color), 1 (light yellow), 2 (light brown), or 3 (brown). Then specimens were assigned to one of two levels: 0-1 score (-) and 2-3 scores (+).

Statistical analysis

The associations of *F2RL3* expression with clinical-pathological parameters were evaluated using Student's *t*-test for continuous variables and the χ^2-test for categorical variables. Progression-free survival (PFS) was measured as the time from the date of surgery to the occurrence of progression, relapse, or death from any cause. Overall survival (OS) was calculated as the time from the initiation of surgery to death from any cause or until the most recent follow-up. Kaplan-Meier method was used for PFS and OS analysis, and different Kaplan-Meier curves were compared by Log-rank test. Using the Cox regression model, univariate and multivariate survival analyses were performed. The level of significance was defined as p value < 0.05. SPSS software V13.0 (SPSS, Chicago, IL) was used for all statistical analyses.

Results

The mRNA and protein levels of *F2RL3* were examined in 367 couples of ccRCC tissues and cancer-adjacent counterparts. The associations of *F2RL3* mRNA and protein levels with clinical features were summarized in Table 1. We found that *F2RL3* mRNA and protein were significant higher in smokers than non-smokers, respectively (p=0.013 and 0.033). Importantly, upregulation of *F2RL3* mRNA and protein levels significantly correlated with higher T stage (p<0.001 and <0.001, respectively), N stage (p=0.004 and 0.002, respectively), M stage (p=0.015 and 0.001, respectively), and ISUP grade (p<0.001 and <0.001, respectively). However, no significant differences were observed with regard to the relationship of *F2RL3* expression and age at surgery, gender, clinical manifestation, laterality and tumor size (p>0.05). Moreover, the ratio of patients with high *F2RL3* expression presented in the group of stage III-IV was higher than in the group of stage I-II (66.7% and 57.5% versus 26.7% and 35.1%, respectively, all p<0.001), and in group with grade 3-4 was also more than in group with grade 1-2 (45.9% and 51.5% versus 12.5% and 23.9%, respectively, all p<0.001)), as shown in figure 1. We also found that the mean value of *F2RL3* mRNA expression was higher in patients with stage III-IV and grade 3-4, compared with participants with stage I-II and grade 1-2, respectively (all p<0.05, Fig. 2). Meanwhile, the ratio of patients with positive F2RL3 protein expression were found to be higher in stage III-IV and grade 3-4, compared with stage I-II and grade 1-2, respectively (all p<0.05, Fig.3).

Both in univariate and multivariate Cox regression analysis, well-known prognostic factors (e.g. T stage, N stage, and M stage) still significantly related to patients' PFS and OS in the cohort of our study, indicating the fine representativeness of population in the current research (Table 2-3). Importantly, high expression of *F2RL3* mRNA and protein were observed to be correlated with poor PFS (p<0.001 and =0.003, respectively) and OS (p<0.001 and =0.009, respectively) of ccRCC patients in univariate Cox regression analysis (Table 2-3). To eliminate the influence of covariates, *F2RL3* expression levels and clinical-pathological factors were further assessed in multivariate Cox regression analysis. Interestingly, high expression of *F2RL3* mRNA and protein remained an independently prognostic factor for PFS (p<0.001 and =0.002, respectively) and OS (p<0.001 and =0.004, respectively) in multivariate Cox regression model (Table 2-3). With regard to other factors, ISUP grade was detected to be prognostic parameters for PFS in univariate and multivariate Cox regression model, but significant association of ISUP grade with OS was observed in univariate analysis and only a trend was found in multivariate model. Age at surgery was detected to be a prognostic parameter for PFS and OS in univariate but not in multivariate Cox regression model. In addition, gender, smoking, clinical manifestation, laterality and tumor size were not found to be prognostic parameters for patients' PFS and OS in our study (Table 2-3).

As shown in Figure 4, patients with high *F2RL3* mRNA levels had a significantly worse PFS and OS (p<0.001 and <0.001, respectively), compared with patients with low *F2RL3* expression. Non-significant differences between population with low and middle *F2RL3* mRNA expression were found for PFS and OS. However, it was interestingly noted that a trend of significant difference in OS between the groups of low

and middle *F2RL3* expression was observed after follow-up of 70 months (Fig. 4). For the association of patients' prognosis with F2RL3 protein expression, we found that the PFS and OS in the patents with positive F2RL3 protein expression were significantly shorten than in negative F2RL3 protein expression, respectively (p=0.002 and =0.008, respectively, Fig. 5). In addition, the median PFS in the cohort of patients with low, middle and high *F2RL3* expression were 90, 74 and 37 months, respectively. The median OS in patients with low mRNA level of *F2RL3* has not yet reached, and in groups with middle and high *F2RL3* mRNA expression were 100 and 65 months, respectively (Supplementary Table S1).

Table 1. Clinicopathological characteristics in relation to F2RL3 expression status.

Variable	Entire group (n=367)	F2RL3 mRNA expression				F2RL3 IHC expression		
		Low expression (n=91)	Middle expression (n=147)	High expression (n=129)	*P* value[a]	Negative (n=114)	Positive (n=253)	*P* value[b]
Age at surgery (y, mean±SD)	55.3±11.7	55.4±12.7	55.1±11.3	55.4±11.5	0.969	55.9±12.3	55.0±11.4	0.483
Sex (n, %)					0.980			0.464
Male	248 (67.6)	61 (67.0)	99 (67.3)	88 (68.2)		74 (64.9)	174 (68.8)	
Female	119 (32.4)	30 (33.0)	48 (32.7)	41 (31.8)		40 (35.1)	79 (31.2)	
Smoking (n, %)					**0.013**			0.033
No	163 (44.4)	35 (38.5)	79 (53.7)	49 (38.0)		60 (52.6)	103 (40.7)	
Yes	204 (55.6)	56 (61.5)	68 (46.3)	80 (62.0)		54 (47.4)	150 (59.3)	
Clinical manifestation (n, %)					0.729			0.562
Incidental	246 (67.0)	60 (65.9)	102 (69.4)	84 (65.1)		74 (64.9)	172 (68.0)	
Symptomatic	121 (33.0)	31 (34.1)	45 (30.6)	45 (34.9)		40 (35.1)	81 (32.0)	
Laterality (n, %)					0.902			0.425
Left	182 (49.6)	44 (48.4)	72 (49.0)	66 (51.2)		53 (46.5)	129 (51.0)	
Right	185 (50.4)	47 (51.6)	75 (51.0)	63 (48.8)		61 (53.5)	124 (49.0)	
Tumor size (cm, mean±SD)	5.2±2.4	4.9±2.6	5.1±2.5	5.5±2.2	0.197	4.8±2.6	5.3±2.3	0.064
T stage at presentation (n, %)					**<0.001**			**<0.001**
T1-T2	300 (81.7)	84 (92.3)	127 (86.4)	89 (69.0)		105 (92.1)	195 (77.1)	
T3-T4	67 (18.3)	7 (7.7)	20 (13.6)	40 (31.0)		9 (7.9)	58 (22.9)	
N stage at presentation (n, %)					**0.004**			**0.002**
N0	326 (88.8)	84 (92.3)	137 (93.2)	105 (81.4)		110 (96.5)	216 (85.4)	
N1	41 (11.2)	7 (7.7)	10 (6.8)	24 (18.6)		4 (3.5)	37 (14.6)	
M stage at presentation (n, %)					**0.015**			**0.001**
M0	330 (89.9)	85 (93.4)	137 (93.2)	108 (83.7)		111 (97.4)	219 (86.6)	
M1	37 (10.1)	6 (6.6)	10 (6.8)	21 (16.3)		3 (2.6)	34 (13.4)	
ISUP grade (n, %)					**<0.001**			**<0.001**
1-2	175 (47.7)	63 (69.2)	73 (49.7)	39 (30.2)		75 (65.8)	100 (39.5)	
3-4	192 (52.3)	28 (30.8)	74 (50.3)	90 (69.8)		39 (34.2)	153 (60.5)	

[a]*P* velue for F2RL3 mRNA expression

[b]*P* velue for F2RL3 IHC expression

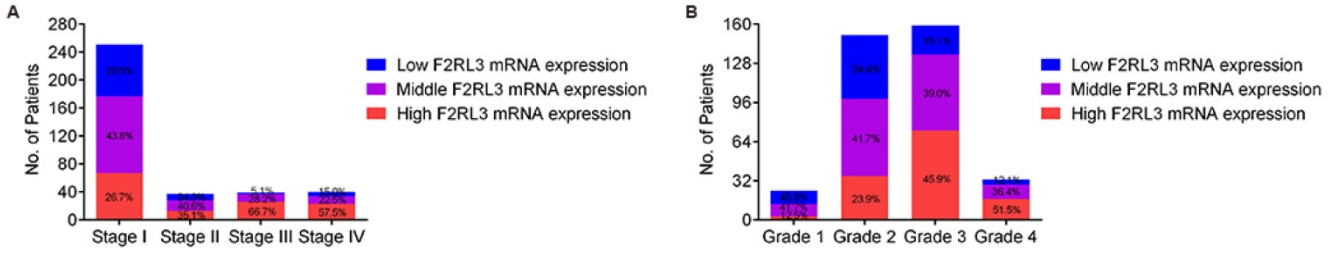

Figure 1. The percentage of high, middle and low *F2RL3* mRNA expression in patients with different TNM stage (A) and ISUP grade (B).

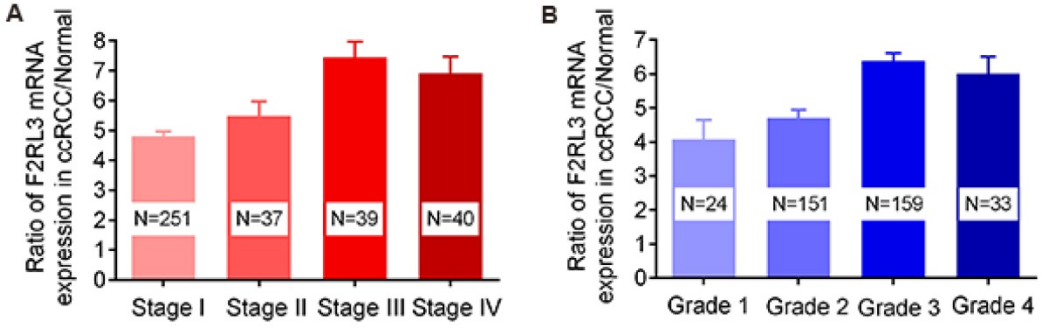

Figure 2. The mean value of *F2RL3* mRNA expression in enrolled participants with different TNM stage (A) and ISUP grade (B).

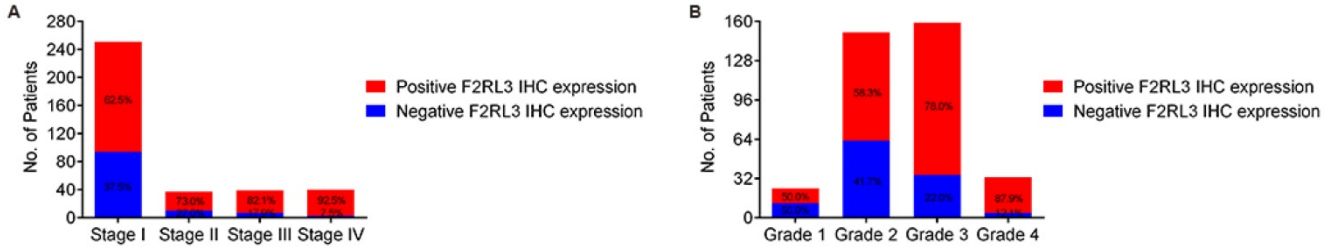

Figure 3. The percentage of positive and negative *F2RL3* protein expression in patients with different TNM stage (A) and ISUP grade (B).

Table 2. Univariate and multivariate Cox regression analyses of PFS in 367 enrolled ccRCC patients

	Univariate analysis		Multivariate analysis[a]		Multivariate analysis[b]	
Covariates	HR (95%CI)	P value	HR (95%CI)	P value	HR (95%CI)	P value
Age at surgery	**1.015(1.003-1.027)**	**0.014**	1.007 (0.994-1.020)	0.297	1.008 (0.995-1.021)	0.240
Sex (male vs. female)	0.774 (0.567-1.058)	0.108	0.757 (0.514-1.113)	0.157	0.765 (0.522-1.122)	0.171
Smoking (No vs. Yes)	1.079 (0.813-1.432)	0.599	0.856 (0.602-1.218)	0.388	0.871 (0.616-1.230)	0.433
Clinical manifestation (incidental vs. symptomatic)	1.010 (0.751-1.359)	0.945	0.965 (0.711-1.311)	0.822	0.956 (0.703-1.299)	0.772
Laterality (left vs. right)	1.053 (0.795-1.393)	0.720	1.018 (0.765-1.355)	0.902	1.031 (0.773-1.374)	0.838
Tumor size	1.033 (0.979-1.090)	0.233	1.035 (0.977-1.096)	0.245	1.037 (0.980-1.098)	0.211
T stage (T1-T2 vs. T3-T4)	**10.296 (7.287-14.549)**	**<0.001**	**2.001 (1.413-2.832)**	**<0.001**	**1.973 (1.404-2.772)**	**<0.001**
N stage (N0 vs. N1)	**12.415 (8.356-18.444)**	**<0.001**	**2.879 (1.677-4.941)**	**<0.001**	**2.864 (1.672-4.905)**	**<0.001**
M stage (M0 vs. M1)	**12.324 (8.227-18.461)**	**<0.001**	**5.047 (3.300-7.720)**	**<0.001**	**5.116 (3.360-7.791)**	**<0.001**
ISUP grade (1-2 vs. 3-4)	**3.019 (2.225-4.098)**	**<0.001**	**1.866 (1.062-3.280)**	**0.030**	**1.896 (1.083-3.319)**	**0.025**
F2RL3 mRNA expression (Low vs. middle)	1.149 (0.777-1.698)	0.487	1.159 (0.784-1.713)	0.460	-	-
F2RL3 mRNA expression (low vs. high)	**2.060 (1.410-3.009)**	**<0.001**	**2.113 (1.445-3.089)**	**<0.001**	-	-
F2RL3 IHC expression (negative vs. positive)	**1.657 (1.193-2.300)**	**0.003**	-	-	**1.692 (1.218-2.352)**	**0.002**

[a]Multivariate model include F2RL3 mRNA expression and covariates.
[b]Multivariate model include F2RL3 IHC expression and covariates.

Table 3. Univariate and multivariate Cox regression analyses of OS in 367 enrolled ccRCC patients

	Univariate analysis		Multivariate analysis[a]		Multivariate analysis[b]	
Covariates	HR (95%CI)	P value	HR (95%CI)	P value	HR (95%CI)	P value
Age at surgery	**1.018 (1.004-1.033)**	**0.012**	1.014 (0.999-1.029)	0.068	1.013 (0.998-1.029)	0.086
Sex (male vs. female)	0.922 (0.639-1.331)	0.665	1.015 (0.635-1.622)	0.952	1.028 (0.642-1.646)	0.908
Smoking (No vs. Yes)	1.046 (0.745-1.470)	0.794	0.968 (0.628-1.493)	0.884	0.985 (0.641-1.516)	0.947
Clinical manifestation (incidental vs. symptomatic)	0.968 (0.674-1.391)	0.861	0.951 (0.654-1.382)	0.791	0.951 (0.653-1.384)	0.793
Laterality (left vs. right)	1.030 (0.735-1.443)	0.866	0.914 (0.645-1.295)	0.613	0.938 (0.662-1.329)	0.719
Tumor size	0.995 (0.928-1.067)	0.890	1.004 (0.931-1.082)	0.923	1.010 (0.938-1.088)	0.792
T stage (T1-T2 vs. T3-T4)	**12.148 (8.359-17.654)**	**<0.001**	**1.651 (1.060-2.573)**	**0.027**	**1.737 (1.120-2.694)**	**0.014**
N stage (N0 vs. N1)	**12.684 (8.352-19.264)**	**<0.001**	**3.147 (1.777-5.571)**	**<0.001**	**2.972 (1.674-5.276)**	**<0.001**
M stage (M0 vs. M1)	**11.554 (7.618-17.525)**	**<0.001**	**5.456 (3.377-8.813)**	**<0.001**	**5.858 (3.640-9.428)**	**<0.001**
ISUP grade (1-2 vs. 3-4)	**3.466 (2.364-5.038)**	**<0.001**	1.658 (0.938-2.930)	0.082	**1.612 (0.909-2.858)**	**0.102**
F2RL3 mRNA expression (Low vs. middle)	1.473 (0.875-2.479)	0.145	1.497 (0.889-2.520)	0.129	-	-
F2RL3 mRNA expression (low vs. high)	**2.826 (1.713-4.662)**	**<0.001**	**2.936 (1.777-4.851)**	**<0.001**	-	-
F2RL3 IHC expression (negative vs. positive)	**1.712 (1.140-2.569)**	**0.009**	-	-	**1.811 (1.203-2.725)**	**0.004**

[a]Multivariate model include F2RL3 mRNA expression and covariates.
[b]Multivariate model include F2RL3 IHC expression and covariates.

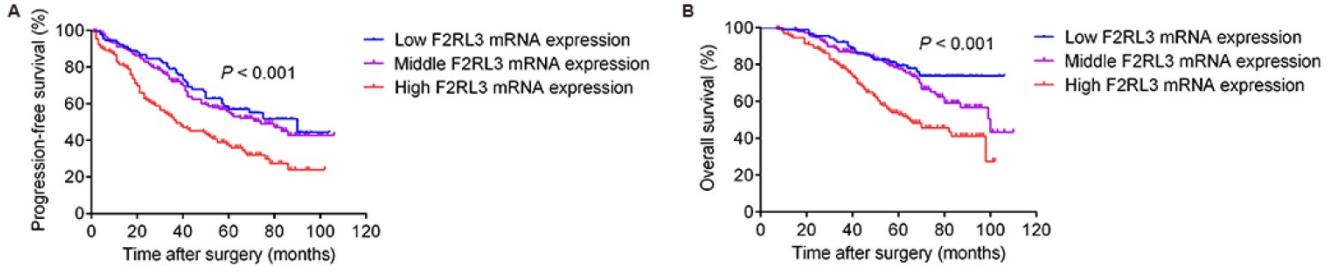

Figure 4. Correlated of *F2RL3* mRNA expression with PFS (A) and OS (B) in the enrolled 367 ccRCC patients. High *F2RL3* mRNA expression is significantly associated with poor PFS and OS, compared with middle and low *F2RL3* expression.

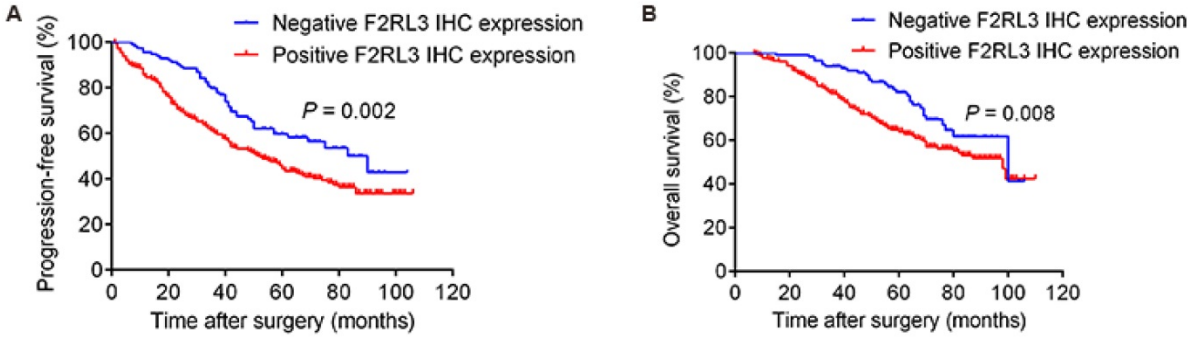

Figure 5. Correlated of *F2RL3* protein expression with PFS (A) and OS (B) in the enrolled 367 ccRCC patients. The PFS and OS in the patents with positive *F2RL3* protein expression were significantly shorten than in negative *F2RL3* protein expression

Discussion

The current study indicated that upregulated expression of *F2RL3* contributes to progressive characteristics and poor prognosis of ccRCC. More importantly, the association of elevated expression of *F2RL3* with PFS and OS of ccRCC patients persisted after adjusting for a variety of confounding factors (e.g. smoking-exposure).

For kidney cancer, about 20-30% patients are presented with metastatic disease at the time of diagnosis[17]. Meanwhile, another 20% of all patients received nephrectomy will have a relapse and develop metastatic RCC during follow-up[18]. The fact that the prognosis of metastatic RCC is poor[17] makes the occurrence of metastatic RCC a serious burden for oncologic healthcare worldwide. Specific lifestyle factors including tobacco-exposure are vital etiologic factors for RCC[3]. Investigating the association of tobacco-exposure related gene with RCC incidence and prognosis extend our comprehension of mechanisms by which smoking-related gene increase risk of RCC and we have potential to explore novel therapeutic interventions.

The *F2RL3* gene, which codes for the thrombin PAR-4, was strong associated with smoking in previous studies[12-14]. It should be importantly noted that PAR4 has been suggested to be involved in the development of several carcinoma[19, 20] and metastasis[21]. For example, Baglietto *et al*[20] found that smoking related *F2RL3* methylation changes measurable in peripheral blood may improve prediction of lung cancer risk. In addition, a study conducted by Zhang *et al*[16] indicated that *F2RL3* methylation is a strong predictor of cancer mortality and pathways associated with *F2RL3* methylation may regulate harmful effects of smoking. Moreover, PAR-4 is a thrombin receptor and regulate blood coagulation processes[22]. Hyper-coagulation is a common feature in patients with cancer, which from other side provides plausible explanation for the relationship of *F2RL3* gene and carcinogenesis.

The main question proposed in this study was whether mRNA changes of smoking-related *F2RL3* gene are involved in ccRCC prognosis. Although our data is impossible to deeply answer the question such as its underlying mechanism, the results of our study indicate the close association of increased *F2RL3* mRNA level with progressive feature and poor prognosis of ccRCC. In the current study, we observed that high mRNA expression of *F2RL3* present in patients with T3-4, N1, M1, or ISUP grade 3-4. Meanwhile, well-known prognostic factors such as TNM stage and ISUP grade still significantly correlated with PFS and OS in the cohort of this study, which are consistent with a variety of guidelines and previous studies[23]. This supports that high *F2RL3* expression associated with advanced TNM stage or ISUP grade is an adverse prognostic factor for ccRCC. Furthermore, we confirmed that elevated *F2RL3* mRNA expression are associated with ccRCC poor survival after adjustment for smoking status and so on. It may partly reflect that smoking-exposure can result in various changes in cells, of which only some vital variations will trigger carcinogenesis. We speculated that smoking-exposure leads to higher expression of *F2RL3* in cells and clonal reproduction, this anomaly in upregulated *F2RL3* expression persists and maintains the stability of mitosis through subsequent cell divisions. Analyzing the association of *F2RL3* gene methylation status and protein expression with patients' prognosis and building a prediction model based on *F2RL3* mRNA/protein measured in blood samples are required for fully understand the value of this gene in ccRCC. Additional investigations such as a mechanism analysis are necessary for the full assessment of the causal relevance of F2RL3 in ccRCC development (work in preparation).

In conclusion, our study indicates that high expression of smoking-related *F2RL3* gene is correlated with progressive characteristics and worse prognosis of ccRCC. It provides a basic message for the notion that elevated expression of *F2RL3* in ccRCC

may be crucial to gain the aggressive features and poorly prognostic phenotype. These findings encourage further work to explore the potential function of *F2RL3* in ccRCC development.

Supplementary Material

Supplementary table.
http://www.jcancer.org/v09p3400s1.pdf

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Authors' contributions

DLC, YYQ, and XZ performed the experiments, wrote and revised the paper, collected clinical data and analyzed the data. FJX, SXZ, GMZ, BD, YZ, GHS, YJS and YPZ revised the paper and collected the clinical data. HLZ, DWY and JYZ guided the experimental design and revised the manuscript. All authors read and approved the final the manuscript.

Competing Interests

The authors have declared that no competing interest exists.

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