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Original Article

Relationship between LAPTM4B gene polymorphism and susceptibility of renal cell carcinoma and bladder cancer

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Abstract: Lysosomal-associated protein transmembrane-4 beta (LAPTM4B) gene has two alleles, LAPTM4B*1 and LAPTM4B*2. Previous studies have demonstrated that LAPTM4B*2 contributed to the risk of cancers. This study aimed to investigate the correlation between LAPTM4B polymorphism and the susceptibility to renal cell carcinoma (RCC) and bladder cancer (BCA). A case-control analysis was performed in 180 RCC and 91 BCA cases and 347 controls. LAPTM4B polymorphism was analyzed by polymerase chain reaction and electrophoresis. Chi-square test was used to calculate genotype frequency and other parametric distributions between cancer cases and controls. Unconditional logistic regression analysis models were used to calculate odds ratio (OR) with 95% confidence interval (CI). Results showed that allelic frequency of LAPTM4B*2 in the controls (24.06%) was significantly lower than in RCC (31.70%, P = 0.008) and BCA (35.71%, P = 0.009) cases. The risks for RCC and BCA increased 1.467 (95% CI 1.104-1.950) and 1.634 (95% CI 1.132-2.360) times in individuals carrying LAPTM4B*2 compared with those carrying LAPTM4B*1. RCC cases with LAPTM4B*1/2 and *2/2 have 1.476 (95% CI 1.008-2.161) and 2.231 (95% CI 1.089-4.569) higher risks than those carrying LAPTM4B*1/1. BCA risk in cases carrying LAPTM4B*1/2 and LAPTM4B*2/2 was 1.563-fold (95% CI, 0.932-2.622) and 2.900-fold (95% CI, 1.233-6.821) that of cases with LAPTM4B*1/1. No association between LAPTM4B genotypes and clinical parameters of RCC and BCA was found, except for smoking status in BCA. Conclusions: These findings suggested that LAPTM4B*2 was a risk factor for both RCC and BCA. LAPTM4B is a potential biomarker for susceptibility of RCC and BCA.

Keywords: LAPTM4B, polymorphism, primary renal cell carcinoma, bladder cancer, susceptibility

Introduction

Kidney cancer and urinary bladder cancer are among the most frequently diagnosed cancers and are the leading causes of cancer death, ranking ninth and sixth of estimated new cases worldwide [1]. There are two main types of kidney cancer, renal cell carcinoma (RCC) and renal pelvis carcinoma. RCC is the more prevalent, accounting for 90% of all kidney cancers with 33% of the cases being metastatic at diagnosis [2]. A gender discrepancy exists in the incidence of both bladder cancers (BCAs) and renal cell carcinomas, with more men presenting with these cancers than women. Smoking, tumor biology, occupational risk factors, and sex steroid hormones and their receptors could have a role in the observed gender disparities [3-5].

In RCC, there is a 1.5:1 predominance in men over women, with peak incidence found among those aged 60 to 70 years. From a clinical perspective, three main RCC subtypes are important: clear cell RCC (ccRCC, 80-90%), papillary RCC (pRCC types I and II, 10-15%, of which 60-70% are type I), and chromophobe RCC (chRCC, 4-5%) [6]. Risk factors for the development of RCC include cigarette smoking, obesity, continued misuse of analgesics, acquired cystic kidney disease, hypertension, and other genetic diseases [4, 5].

Men have a threefold greater risk of developing BCA than women, but female gender has been identified as an independent adverse prognostic factor for both recurrence and progression of this disease [5]. Smoking is the most well-established risk factor for BCA, with the risk

Table 1. Distribution of gender and age in renal cell carcinoma (RCC) cases and the controls

	Controls (n = 347)	RCC (n = 180)	Chi-square *value*	*P* value[a]
Gender, n (%)			0.647	0.441
Male	223 (64.27)	122 (67.78)		
Female	124 (35.73)	58 (32.22)		
Age (years), n (%)			42.968	< 0.001
≤ 40	55 (15.85)	16 (8.88)		
41-50	110 (31.70)	31 (17.22)		
51-60	71 (20.46)	53 (29.45)		
61-70	53 (15.27)	63 (35.00)		
> 70	58 (16.72)	17 (9.45)		

[a]Analyzed by Chi-square test.

Table 2. Distribution of gender and age in bladder cancer (BCA) cases and the controls

	Controls (n = 347)	BCA (n = 91)	*Chi-square value*	*P* value[a]
Gender, n (%)			10.924	0.001
Male	223 (64.27)	75 (82.42)		
Female	124 (35.73)	16 (17.58)		
Age (years), n (%)			34.533	< 0.001
≤ 50	55 (15.85)	14 (15.38)		
51-60	71 (20.46)	23 (25.27)		
61-70	53 (15.27)	30 (32.98)		
> 70	58 (16.72)	24 (26.37)		

[a]Analyzed by Chi-square test.

among smokers reported to be approximately 2-fold to 6-fold that among nonsmokers. In the developing world, particularly Africa and Western Asia, chronic infection with Schistosoma haematobium (a parasitic worm that causes urinary schistosomiasis) is associated with an increased risk of BCA [1].

Lysosomal-associated protein transmembrane-4 beta (LAPTM4B) was originally identified as a novel oncogene candidate in hepatocellular carcinoma (HCC), and resides at 8q22.1 (GenBank Accession Number: AY057051) containing seven exons and six introns [7]. It has two alleles, LAPTM4B*1 (GenBank Accession Number: AY219177) and LAPTM4B*2 (GenBank Accession Number: AY219176). LAPTM-4B*1 contains only one copy of a 19-bp sequence at the 5'UTR in the first exon, while LAPTM4B*2 has a tandem repeat of the 19-bp sequence [8]. The products of LAPTM4B are a 35-kDa protein and a 24-kDa protein. The full LAPTM4B mRNA predicts a putative protein of 317 amino acid with four transmembrane regions and a molecular mass of 35 kDa initiating from ATG at nt 157, with the 24-kDa protein initiating from ATG at nt 430. Both proteins locate in cell membrane. Previous studies have shown that LAPTM4B polymorphism is related to the susceptibility of HCC [9, 10], breast cancer [11-13], non-small cell lung cancer [14], gastric cancer [15], cervical cancer [16], endometrial carcinoma [17], colorectal cancer [18], lymphoma [19], gallbladder carcinoma [20], ovarian carcinoma [21], and malignant melanoma [8]; but not rectum carcinoma, esophageal carcinoma [18], and nasopharyngeal carcinoma [22]. However, the relationship between LAPTM4B polymorphism and the risk of RCC and BCA remains unclear.

Materials and methods

Patients

A total of 271 blood samples of 180 RCC and 91 BCA patients were collected from Peking University Cancer Hospital & Institute from April 2015 to November 2015. All patients underwent surgical resection and were diagnosed by at least two pathologists. Tumors were staged according to the tumor, node, metastasis (TNM) classification released by the American Joint Committee on Cancer (AJCC 7th edition, 2010). Each patient signed an informed consent. All study procedures were in accordance with the Helsinki Declaration and the study was approved by the Ethics Committee of Peking University Cancer Hospital & Institute.

347 controls were quoted from the healthy adult data published by Cheng et al [18]. And the controls were healthy individuals recruited from employees at the Peking University Cancer Hospital & Institute and the Health Science Center of Peking University, who never had a diagnosis for cancer. The controls from Peking University Cancer Hospital & Institute were checked for cancer history through their past medical records, while the others were directly asked for their cancer history. The nurse inter-

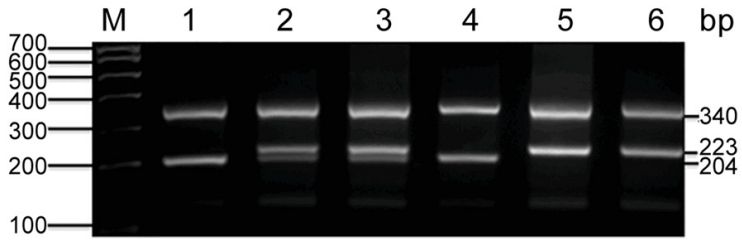

Figure 1. *LAPTM4B* polymorphism analysis. Samples were analysed after separation in a 3% agarose gel. The upper band in each lane was the PCR product of β-actin that served as a positive internal control. The lower bands were the amplified *LAPTM4B*: Lanes 1 and 4, genotype *LAPTM4B**1/1; Lanes 2 and 3, genotype *LAPTM4B**1/2; Lanes 5 and 6, genotype *LAPTM4B**2/2. Lane M: DNA marker (100, 200, 300, 400, 500, 600, and 700 bp).

viewers explained the aims of this study to the blood donors, who had all provided informed consent for participating.

Basic information from both cases and controls were shown in **Tables 1** and **2**.

Procedures

Anticoagulant blood (1 mL) was taken from the vein and kept in a freezer at -20°C. Genomic DNA was extracted by a DNA blood mini kit (Tiangen Biotech, China) following the manufacturer's protocol and then stored at -20°C for subsequent genotype analysis. The specific primers 5'-GCCGACTAGGG GACTGGCGGA-3' (forward) and 5'-CGAGAGCTCC GAGCTTCTGCC-3' (reverse) were used to determine the genotypes of LAPTM4B. Human β-actin was used as positive internal control, and the primers used were 5'-TCACCAACTGG GACGACAT-3' (forward), and 5'-AGGTAGTCAGT CAGGTCCCG-3' (reverse). The condition for the polymerase chain reaction (PCR) was 95°C denaturation for 5 min; 36 cycles of 30 sec at 94°C, 45 sec at 62°C, and 1 min at 72°C; and followed by autoextension at 72°C for 10 min. PCR products were identified by electrophoresis on 3% agarose gel containing GelStain (TransGen Biotech, China). The homozygous LAPTM4B*1 and LAPTM4B*2 genotypes were identified by a 204-bp band and a 223-bp band, respectively. The heterozygous LAPTM4B*1/2 genotype exhibited both the 204-bp and the 223-bp bands (**Figure 1**).

Statistical analysis

Statistical analyses were performed with SPSS (version16.0). The deviation of genotype frequencies from the Hardy-Weinberg equilibrium was made using the chi-square test. Chi-square test was also used to calculate genotype frequency and clinicopathological variables distributions between cancer cases and controls. All tests were two-sided and $P < 0.05$ was used as the significance level. Unconditional logistic regression analysis models were used to calculate the odds ratio (OR) and the corresponding 95% confidence interval (95% CI) to analyze relationship between LAPTM4B polymorphism and cancer risk adjusted by age and gender.

Results

PCR analysis of LAPTM4B

Among the 271 cancer cases (180 RCC and 91 BCA) and 347 healthy controls, three different genotypes were identified in the PCR products with the specific primers for LAPTM4B. The homozygous genotype LAPTM4B*1/1 and LAPTM4B*2/2 exhibited a 204-bp band and a 223-bp band, respectively. The heterozygous genotype LAPTM4B*1/2 had both the 204-bp and 223-bp bands. As a positive control, β-actin was amplified from each sample and was shown as a 340-bp PCR product (**Figure 1**).

LAPTM4B polymorphism and risk of RCC and BCA

LAPTM4B gene polymorphism in RCC cases, BCA cases, and the controls all was conformed to the Hardy-Weinberg equilibrium ($P = 0.786$, 0.997, and 0.915, respectively), indicating that the sampling was a good representative of the population.

Significant differences in age were observed between the RCC cases and the controls ($P < 0.001$), while not in gender ($P = 0.441$) (**Table 1**). Analysis of the BCA cases showed a significant difference compared with the controls in age ($P = 0.001$) and gender ($P < 0.001$) (**Table 2**).

The allelic frequency of the LAPTM4B*2 in the controls (24.06%) was significantly different

Table 3. Distribution of genotypes and alleles of LAPTM4B in renal cell carcinoma (RCC) cases and the controls

	Controls	RCC cases	OR (95% CI)[a]	Beta value[a]	*P* value[a]
Genotypes, n (%)					
LAPTM4B*1/1	198 (57.06)	83 (46.11)			
LAPTM4B*1/2	131 (37.75)	80 (44.44)	1.476 (1.008-2.161)	0.389	0.046
LAPTM4B*2/2	18 (5.19)	17 (9.45)	2.231 (1.089-4.569)	0.802	0.028
Total	347 (100.00)	180 (100.00)			
Alleles, n (%)					
LAPTM4B*1	527 (75.94)	246 (68.30)			
LAPTM4B*2	167 (24.06)	114 (31.70)	1.467 (1.104-1.950)	0.383	0.008
Total	694 (100.00)	360 (100.00)			

[a]Calculated by unconditional logistic regression adjusted by age and gender. LAPTM4B, lysosome-associated protein transmembrane 4 beta. OR, odds ratio. CI, confidence interval.

Table 4. Distribution of genotypes and alleles of LAPTM4B in bladder cancer (BCA) cases and the controls

	Controls	BCA cases	OR (95% CI)[a]	Beta value	*P* value[a]
Genotypes, n (%)					
LAPTM4B*1/1	198 (57.06)	38 (41.76%)			
LAPTM4B*1/2	131 (37.75)	41 (45.05%)	1.563 (0.932-2.622)	0.447	0.091
LAPTM4B*2/2	18 (5.19)	12 (13.19%)	2.900 (1.233-6.821)	1.065	0.015
Total	347 (100.00)	91 (100.00)			
Alleles, n (%)					
LAPTM4B*1	527 (75.94)	117 (64.29%)			
LAPTM4B*2	167 (24.06)	65 (35.71%)	1.634 (1.132-2.360)	0.491	0.009
Total	694 (100.00)	182 (100.0)			

[a]Calculated by unconditional logistic regression adjusted by age and gender. LAPTM4B, lysosome-associated protein transmembrane 4 beta. OR, odds ratio. CI, confidence interval.

from that in the RCC (31.70%) and the BCA (35.71%) cases (P = 0.008 and 0.009, respectively). The risk of suffering from RCC and BCA increased to 1.467 (95% CI 1.104-1.950) and 1.634 (95% CI 1.132-2.360) times in the individuals carrying the allele LAPTM4B*2 compared with those carrying the allele LAPTM4B*1 (**Tables 3** and **4**).

The overall genotypic distribution between the cancer cases and the controls was significantly different. Adjusted by age, unconditional logistic regression analysis showed that RCC cases with genotypes of LAPTM4B*1/2 and LAPTM4B*2/2 had higher risk for RCC when compared with those carrying LAPTM4B*1/1, with the OR at 1.476 (95% CI 1.008-2.161) and 2.231 (95% CI 1.089-4.569), respectively. BCA risk of the cases with the LAPTM4B*1/2 and LAPTM4B*2/2 genotypes was 1.563-fold (95% CI, 0.932-2.622) and 2.900-fold (95% CI, 1.2336.821) that of the cases with LAPTM-4B*1/1, adjusted by age and gender (**Tables 3** and **4**).

LAPTM4B polymorphism and clinicopathological variables

The relationship between LAPTM4B genotype and clinical parameters of RCC (gender, age, stage, Fuhrman grade, histologic category, tumor size, tumor thrombus, smoking status, and hypertension) and BCA (gender, age, tumor size, recurrence, tumor grade, tumor thrombus, lymph node metastasis, tumor stage, smoking status, and hypertension) cases were analyzed by Chi-square test. No association between LAPTM4B genotype and these clinical parameters was found in this study, except that there was a significant association with smoking status in the BCA cases (**Tables 5** and **6**). Supplementary multivariate logistic regression

Table 5. Distribution of various genotypes of LAPTM4B in relation to clinicopathological and other variables in renal cell carcinoma (RCC) cases

Variable	LAPTM4B genotype		*P* value[a]	LAPTM4B genotype		*P* value[a]
	*1/1	*1/2		*1/1	*2/2	
Gender			1.000			0.573
Male	56	53		56	13	
Female	27	27		27	4	
Age (years)			0.716			0.136
≤ 50	19	21		19	7	
> 50	64	59		64	10	
Stage			0.868			0.289
I-II	40	39		40	12	
III-IV	32	34		32	5	
Unknown	11	7		11	0	
Fuhrman grade			1.000			1.000
I-II	36	40		36	11	
III-IV	23	25		23	6	
Unknown	24	15		24	0	
Histologic category			0.545			0.185
Clear cell	64	67		64	16	
Other	17	13		17	1	
Unknown	2	0		2	0	
Tumor size			0.733			0.573
≤ 50 mm	40	36		40	10	
> 50 mm	30	31		30	5	
Unknown	13	13		13	2	
Tumor thrombus			0.803			0.699
Visible	8	11		8	3	
Invisible	48	52		48	13	
Unknown	27	17		27	1	
Smoking status			0.711			1.000
Yes	19	22		19	4	
No	51	49		51	13	
Unknown	13	9		13	0	
Hypertension			0.731			0.400
Yes	24	27		24	8	
No	48	47		48	9	
Unknown	11	6		11	0	

[a]Analyzed by Chi-square test. LAPTM4B, lysosome-associated protein transmembrane 4 beta.

results also showed p > 0.05 in all clinicopathological and other variables of both RCC and BCA. Multivariate logistic regression results of RCC were shown in Supplementary Tables 1 and 2. And multivariate logistic regression results of BCA were not shown here.

Discussion

LAPTM4B is closely related to the biological behaviors of malignant tumors. The expression of LAPTM4B in HCC was inversely correlated with the differentiation of HCC, showing increased expression in the HCC tissue and low expression in the tissues adjacent to the carcinoma among most patients, which indicated that LAPTM4B played an important role in cell growth, proliferation, and differentiation. And high expression of LAPTM4B may be involved in or promote the occurrence and development of HCC [23]. Previous studies have confirmed that LAPTM4B could upregulate some proliferation-promoting transcription factors such as c-Myc, c-Jun and c-Fos, and cell cycle-promoting proteins such as cyclin D1 and E [24], which increased sharply in HLE cells stably transfected with LAPTM4B [25]. It also showed that LAPTM4B inhibited epidermal growth factor (EGF)-induced EGF receptor (EGFR) intraluminal sorting and lysosomal degradation, leading to enhanced and prolonged EGFR signaling, by which LAPTM4B can transform cells and promote tumor progression [26]. Through co-localization and interaction with MDR 1 (P-glycoprotein), LAPTM4B can motivate multidrug resistance (MDR) by activating the PI3K/AKT signaling pathway and enhancing the efflux of a variety of chemical drugs from cancer cells [27]. In regard to clinical treatment of cancers, LAPTM4B was a novel independent prognostic marker for hepatocellular carcinoma [28], breast cancer [29], colorectal carcinoma [30], and metastatic ovarian tumors [31]. It also indicated resistance to neoadjuvant chemotherapy in HER2-negative breast cancer [32]. Studies have demonstrated that cAMP responsive element binding protein-1 (CREB1) was an important factor regulating LAPTM4B gene transcription [33]. However, further investigations were needed to elucidate the role of LAPTM4B in cancers.

Table 6. Distribution of various genotypes of LAPTM4B in relation to clinicopathological and other variables in bladder cancer (BCA) cases

Variable	LAPTM4B genotype		*P* value[a]	LAPTM4B genotype		*P* value[a]
	*1/1	*1/2		*1/1	*2/2	
Gender			0.368			1.000
Male	30	36		30	9	
Female	8	5		8	3	
Age (years)			0.820			1.000
≤ 60	15	18		15	4	
> 60	23	23		23	8	
Tumor number			0.792			0.269
1	15	18		15	3	
≥ 2	12	12		12	7	
Unknown	11	11		11	2	
Tumor size			1.000			1.000
≤ 30 mm	19	22		19	7	
> 30 mm	10	12		10	3	
Unknown	9	7		9	2	
Recurrence			0.120			0.728
Yes	13	7		13	3	
No	25	34		25	9	
Tumor grade			0.792			0.664
G1-G2	8	10		8	1	
G3	29	30		29	8	
Unknown	1	1		1	3	
Tumor thrombus			0.491			0.659
Visible	8	8		8	3	
Invisible	6	11		6	5	
Unknown	24	22		24	4	
Lymphnode metastasis			0.132			0.131
Visible	7	6		7	1	
Invisible	4	13		4	5	
Unknown	27	22		27	6	
Tumor Stage			1.000			0.221
< pT2	9	11		9	5	
≥ pT2	18	19		18	3	
Unknown	11	11		11	4	
TNM stage			0.581			0.666
I-II	9	14		9	4	
III-IV	14	15		14	3	
Unknown	15	12		15	5	
Smoking status			0.003			0.435
Yes	7	20		7	4	
No	28	16		28	8	
Unknown	3	5		3	0	
Hypertension			1.000			0.731
Yes	12	11		12	5	
No	24	25		24	7	
Unknown	2	5		2	0	

[a]Analyzed by Chi-square test. LAPTM4B, lysosome-associated protein transmembrane 4 beta.

The results presented here demonstrated that LAPTM4B gene polymorphism was related to the risk of RCC and BCA. Significantly higher distribution of LAPTM4B*1/2 and LAPTM4B*2/2 among the cancer patients comparing with the controls indicated that LAPTM4B*2 was an important risk factor associated with susceptibility to cancer. In our study, the LAPTM4B*2 carriers had a 1.467-fold and 1.643-fold risk of suffering RCC and BCA than the LAPTM4B*1 carriers, respectively. Previous studies have reported that LAPTM4B*2 was associated with the susceptibility of various kinds of cancers [8-21], but was not for rectal cancer, esophageal carcinoma [18], and nasopharyngeal carcinoma [22]. It appeared that LAPTM4B protein is expressed in cancer tissues originated from single-layer cuboidal and columnar epithelia, such as hepatocellular carcinoma and colon cancer; whereas stratified epithelia tumor, such as esophageal cancer and nasopharyngeal cancer, showed a lacked of LAPTM4B expression [23]. However, a study from Iran showed that LAPTM4B genotype was not associated with the risk or the clinicopathological characteristics of breast cancer [34].

Difference of an extra 19-bp sequence between LAPTM4B*1 and LAPTM4B*2 may be the key point resulting the effect of LAPTM4B on cancers. With respect to clinical prognosis and treatment for patients with RCC and BCA, stage of cancer and tumor grade has been widely used as predictors [35, 36]. But in this study, no association between LAPTM4B genotype and the clinicopathological variables of RCC and BCA was found. It has been shown that smoking is the most well-established risk factor in BCA [1], and in this study significant association between LAPTM4B gene polymor-

phism and smoking status of the BCA cases was found.

Conclusions

To our best knowledge, this is the first case-control study to explore the association between LAPTM4B polymorphism and the risk of renal cell carcinoma and bladder cancer. LAPTM4B may be a potential biomarker for susceptibility of renal cell carcinoma and bladder cancer. However, the exact molecular mechanisms underlying the role of LAPTM4B polymorphism in the susceptibility of cancers remain unclear and further investigation of LAPTM4B*2 is interesting.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Distribution of various genotypes of LAPTM4B in relation to clinicopathological and other variables in renal cell carcinoma (RCC) cases

Variable	LAPTM4B genotype		OR (95% CI)[a]	Beta value	*P* value[a]
	*1/1	*1/2			
Gender					0.715
Male	56	53	1.196 (0.456-3.138)	0.179	
Female	27	27			
Age (years)					0.481
≤ 50	19	21	1.462 (0.508-4.206)	0.380	
> 50	64	59			
Stage					0.680
I-II	40	39	0.781 (0.241-2.528)	-0.247	
III-IV	32	34			
Unknown	11	7			
Fuhrman grade					0.968
I-II	36	40	1.022 (0356-2.934)	0.022	
III-IV	23	25			
Unknown	24	15			
Histologic category					0.782
Clear cell	64	67	1.225 (0.289-5.188)	0.203	
Other	17	13			
Unknown	2	0			
Tumor size					0.853
≤ 50 mm	40	36	0.915 (0.355-2.356)	-0.089	
> 50 mm	30	31			
Unknown	13	13			
Tumor thrombus					0.580
Visible	8	11	0.693 (0.189-2.540)	-0.367	
Invisible	48	52			
Unknown	27	17			
Smoking status					0.549
Yes	19	22	0.740 (0.276-1.982)	-0.301	
No	51	49			
Unknown	13	9			
Hypertension					0.916
Yes	24	27	0.954 (0.397-2.291)	-0.047	
No	48	47			
Unknown	11	6			

Supplementary Table 2. Distribution of various genotypes of LAPTM4B in relation to clinicopathological and other variables in renal cell carcinoma (RCC) cases

Variable	LAPTM4B genotype		OR (95% CI)[a]	Beta value	*P* value[a]
	*1/1	*2/2			
Gender					0.425
Male	56	13	0.553 (0.129-2.370)	-1.771	
Female	27	4			
Age (years)					0.050
≤ 50	19	7	4.083 (0.997-16.723)	1.407	
> 50	64	10			
Stage					0.493
I-II	40	12	1.926 (0.296-12.521)	0.655	
III-IV	32	5			
Unknown	11	0			
Fuhrman grade					0.888
I-II	36	11	1.126 (0.217-5.843)	0.119	
III-IV	23	6			
Unknown	24	0			
Histologic category					0.709
Clear cell	64	16	1.609 (0.133-19.537)	0.476	
Other	17	1			
Unknown	2	0			
Tumor size					0.920
≤ 50 mm	40	10	0.929 (0.221-3.908)	-0.074	
> 50 mm	30	5			
Unknown	13	2			
Tumor thrombus					0.671
Visible	8	3	0.647 (0.087-4.825)	-0.436	
Invisible	48	13			
Unknown	27	1			
Smoking status					0.742
Yes	19	4	1.283 (0.292-5.641)	0.249	
No	51	13			
Unknown	13	0			
Hypertension					0.313
Yes	24	8	0.510 (0.138-1.886)	-0.674	
No	48	9			
Unknown	11	0			