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Immunohistochemical expression of renin is a prognostic factor for recurrence in nonmetastatic renal cell carcinoma

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Abstract

Purpose: To analyze the intratumoral immunohistochemical expression of renin and its value as a prognostic factor for recurrence in nonmetastatic clear cell renal cell carcinoma (ccRCC).

Methods: A total of 498 patients with nonmetastatic ccRCC from the *Latin American Renal Cancer Group* database who underwent partial or radical nephrectomy between 1990 and 2016 were selected. All cases were revised, and 2 distinct samples were obtained for tissue microarray construction. Ten years of follow-up was assessed, and disease-free survival rates (DFS) were analyzed. Renin expression was classified qualitatively as negative or positive. For the quantitative analysis, a cutoff was estimated using the maximum of the standardized log-rank statistic.

Results: Nuclear renin was qualitatively positive in 360 cases (72%) and negative in 138 (28%), whereas quantitatively, an equal number of cases had ≤35% or >35% renin-positive nuclei. The absence of renin expression was associated with high-grade tumors (by ISUP and Fuhrman classification, both $P < 0.001$), greater microscopic venous invasion ($P = 0.046$), and renal vein invasion ($P = 0.026$). In the multivariate analyses, qualitatively negative renin expression was an unfavorable prognostic factor for DFS (RR = 2.923, $P < 0.001$). With regard to quantitative renin expression, a cutoff of ≤35 was associated with worse DFS (RR = 4.085, $P < 0.001$).

Conclusions: The intratumoral immunohistochemical expression of renin in patients with ccRCC provides valuable prognostic data regarding the likelihood of recurrence.

Keywords: Kidney Neoplasms; Renin; Biomarkers; Disease-free survival

Abbreviations: AJCC, American Joint Committee on Cancer; ccRCC, clear cell Renal Cell Carcinoma; DFS, Disease-Free Survival; ECOG, Eastern Cooperative Oncology Group; EPO, erythropoietin; ISUP, International Society of Urological Pathology; RR, Relative Risk; TMA, Tissue Microarray; VHL, von Hippel-Lindau

1. Introduction

Over the past several decades, there has been a progressive increase in the incidence of renal cell carcinoma (RCC) in the United States, Europe, and South America, reaching approximately 3% per year [1–3]. This increase is related in particular to early-stage incident tumors, the rates of which reach 4% per year, which has traditionally been attributed to greater use of imaging methods [4,5].

Recent publications have reported a decline in the use of imaging tests, during which the incidence of RCC climbed

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[6]. It has been hypothesized that the increased incidence is attributed exclusively to improved detection of clinically localized disease and, consequently, that these rates should stabilize and the proportion of patients with locally advanced or metastatic disease should decrease over time. Yet, current findings indicate that there is no such concomitant decline or long-term trend [7].

Mortality rates have also risen due to localized disease, supporting that although the prognosis of these patients appears to be favorable, certain cases that are detected early are not clinically insignificant as believed [8,9]. In this context, although surgical intervention is the preferred modality, overtreatment can occur [10]. The criteria that are based on tumor characteristics and nomograms models are not accurate [11,12]. Thus, improving the selection of candidates who will benefit from active treatment has become imperative, for which the study of molecular biomarkers has shown promise.

Most studies have aimed to identify markers that correlate with the adaptive advantages of tumor cells, such as self-sufficient production of growth factors, insensitivity to signals that inhibit cell growth, avoidance of programmed death (apoptosis), unlimited replicative potential, sustained angiogenesis and tissue invasion, and metastasis. Little attention had been to the prognostic value of hormones that are secreted by the kidney, which is also an endocrine organ. Because the kidney is the major source of renin and because, like most RCCs, this enzyme is found in the renal cortical region, an examination of its expression and impact on the nature of RCC is warranted.

Our group analyzed the intratumoral immunohistochemical expression of renin in a retrospective series of 498 patients with initially nonmetastatic clear cell RCC (ccRCC). To determine the prognostic potential of this biomarker, we analyzed its relationship with disease-free survival over a follow-up of 10 years.

2. Material and methods

2.1. Patients

A total of 498 consecutive cases, involving radical or partial nephrectomy for nonmetastatic ccRCC between 1990 and 2016, were selected from the medical records of a 2-center cohort (members of the LARCG-Latin American Renal Cancer Group: http://www.larcg.org). Abdominal CT or MRI was used as the standard imaging method for diagnostic confirmation. Patients were evaluated quarterly during the first year, every 6 months for 2 years, and yearly thereafter. Loss of follow-up was defined when more than twice the time that the patient was expected to return had elapsed.

Two uropathologists reviewed all of the cases for uniform reclassification, based on the new renal tumor classification system [13,14]. The most representative tumor areas, with higher Fuhrman grade and concentration of nuclear expression, were selected for construction of the tissue microarray (TMA). Two areas at different positions and depths in the donor paraffin block were harvested to represent various regions of the same tumor. Our internal review board approved this study. Samples were provided by the biobanks of both institutions with the patients' informed consent.

Epidemiological, anatomopathological, and staging variables were included in the database, as were the immunohistochemical expression patterns of renin (Table 1) [15,16].

During radical nephrectomy, retroperitoneal lymphadenectomy was restricted to the renal hilum and was performed for staging purposes only, except in cases with intraoperative detection of suspicious lymph nodes. In nephron-sparing procedures, lymph node dissection was not conducted. Patients with metastatic disease at presentation were excluded.

2.2. Tissue microarray construction and renin immunohistochemistry

Seven TMAs were prepared. Each TMA was constructed by transferring a representative part of approximately 100 samples of ccRCC to a paraffin block. Two cylinders, measuring 1 mm in diameter, taken from the original paraffin blocks of various parts of the tumor, were used to build the TMA. Sequential 4-μm sections were obtained for the immunohistochemical study. H&E staining was also performed to assess the quality of the TMA and the presence of the tumor in the microscopic field. Benign peritumoral tissue samples from 80 patients, 0.5 cm from the edge of the tumoral lesion, were also included.

Sections were mounted on positively charged glass slides, dried for 30 minutes at 37°C, deparaffinized in xylene, and rehydrated in a series of graded alcohols. The sections were then incubated with primary rabbit polyclonal antibody against renin (Abcam, San Francisco, CA) at 1:50 for 60 minutes. All immunohistochemical procedures were performed in the Benchmark ULTRA autostainer (VENTANA) using the Flex Plus visualization system per the supplier's specifications.

The same pathologists who were blinded to the outcome of the cases semiquantitatively scored the nuclear staining intensity of renin in all specimens, based on the number of positive cells per 1000 counted cells (ranging from 0% to 100%). To assess the immunohistochemical scores, all spots were evaluated in duplicate, and the mean of the 2 spots for each case was used for analysis. To improve the interpretation, we used 2 evaluation methods: the categories "negative expression" and "positive expression" were used, based on the absence (0%) or presence (1% to 100%) of nuclear renin, respectively, and cases were divided through statistical tests, based on being above or below a cutoff of expression level.

2.3. Statistical analysis

Statistical analyses were performed using Windows Statistical Package for Social Science - SPSS, version 24.0

Table 1
Quantitative and qualitative expression of renin according to clinical and anatomopathological variables of 498 patients with nonmetastatic CCRcc.

		Renin Expression (Quantitative)			Renin Expression (Qualitative)		
		Negative	Positive	*P* value[a]	≤35	>35	*P* value[a]
		n (%)	*n (%)*		*n (%)*	*n (%)*	
Age (y)	≤40	17 (12,3)	44 (12,2)		27 (10,9)	34 (13,5)	
	41–60	79 (57,3)	205 (57)		145 (58,7)	139 (55,4)	
	≥61	42 (30,4)	111 (30,8)	0.996	75 (30,4)	78 (31,1)	0.620
Gender	Male	86 (94,5)	227 (63)		156 (63,1)	157 (62,5)	
	Female	52 (5,5)	133 (37)	0.879	91 (36,9)	94 (37,5)	0.888
Anemia (Hb)	<12	19 (13,8)	37 (10,3)		32 (13,4)	24 (9,3)	
	≥12	119 (86,2)	323 (89,7)	0.281	207 (86,6)	235 (90,7)	0.182
Symptoms at diagnosis	No	60 (43,4)	131 (36,4)		95 (38,5)	96 (38,2)	
	Yes	78 (56,6)	229 (63,6)	0.145	152 (61,5)	155 (61,8)	0.961
ASA	1	27 (19,5)	41 (11,4)		35 (14,3)	33 (13)	
	2	82 (59,4)	262 (72,8)		164 (66,9)	181 (71,5)	
	≥3	29 (21,1)	57 (15,8)	**0.013**	46 (18,8)	39 (15,5)	0.500
Tumor size (cm)	≤4	64 (46,4)	174 (48,3)		111 (44,9)	121 (49,4)	
	>4 and ≤7	44 (31,9)	116 (32,2)		84 (34)	76 (31)	
	>7	30 (21,7)	70 (19,5)	0.862	52 (21,1)	48 (19,6)	0.493
Nephrectomy	Partial	62 (44,9)	185 (51,4)		114 (46,2)	133 (53)	
	Radical	76 (55,1)	175 (48,6)	0.234	133 (53,8)	118 (47)	0.151
Surgical Margin	Negative	127 (92,7)	335 (92,8)		222 (91,7)	239 (93,3)	
	Positive	10 (7,3)	26 (7,2)	0.883	20 (8,3)	17 (6,7)	0.496
Fuhrman grade	1 or 2	73 (52,9)	246 (68,3)		138 (55,9)	181 (72,1)	
	3 or 4	65 (47,1)	114 (31,7)	**0.001**	109 (44,1)	70 (27,9)	**<0.001**
ISUP grade classification	1 or 2	60 (43,4)	234 (65)		121 (49)	173 (68,9)	
	3 or 4	78 (56,6)	126 (35)	**<0.001**	126 (51)	78 (31,1)	**<0.001**
Tumoral necrosis	No	99 (71,7)	268 (74,4)		176 (71,3)	191 (76)	
	Yes	39 (28,3)	92 (25,6)	0.539	71 (28,7)	60 (24)	0.220
Sarcomatoid pattern	No	135 (97,8)	351 (97,5)		242 (98)	244 (97,2)	
	Yes	3 (2,2)	9 (2,5)	0.564	5 (2)	7 (2,8)	0.397
Lymphatic invasion	No	137 (98,5)	346 (96,4)		241 (97,6)	242 (96,4)	
	Yes	2 (1,5)	13 (3,6)	0.254	6 (2,4)	9 (3,6)	0.450
Vascular embolization	No	121 (87,7)	320 (88,9)		212 (85,8)	229 (91,2)	
	Yes	17 (12,3)	40 (11,1)	0.705	35 (14,2)	22 (8,8)	**0.050**
Renal vein Invasion	No	126 (90,6)	327 (91,1)		218 (88,6)	235 (93,3)	
	Yes	13 (9,4)	32 (8,9)	0.990	28 (11,4)	17 (6,7)	0.065
Perinephric fat invasion	No	113 (81,9)	313 (86,9)		210 (85)	216 (86)	
	Yes	25 (18,1)	47 (13,1)	0.151	37 (15)	35 (14)	0.743
Collecting sys. invasion	No	137 (96,5)	345 (96,9)		242 (96,8)	240 (96,8)	
	Yes	5 (3,5)	11 (3,1)	0.783	8 (3,2)	8 (3,2)	1.0
Adrenal invasion	No	141 (98,6)	349 (98,3)		248 (99,2)	242 (97,6)	
	Yes	2 (1,4)	6 (1,7)	1.0	2 (0,8)	6 (2,4)	0.175
Pathological Stage (T)	1	97 (70,3)	258 (71,7)		172 (69,6)	183 (72,9)	
	2	15 (10,9)	40 (11,1)		26 (10,5)	29 (11,6)	
	3 or 4	26 (18,8)	62 (17,2)	0.685	49 (19,9)	39 (15,5)	0.248
Pathological Stage (N)	0	91 (65,6)	271 (75,3)		173 (70)	189 (75,3)	
	1	1 (1,4)	9 (2,5)		4 (1,6)	6 (2,4)	
	X	46 (33)	80 (22,2)	**0.05**	70 (28,4)	56 (22,3)	0.342
AJCC Clinical Stage	I or II	112 (81,1)	297 (82,5)		198 (80,2)	211 (84)	
	III or IV	26 (18,9)	63 (17,5)	0.727	49 (19,8)	40 (16)	0.256
Recurrence	No	109 (79)	319 (88,6)		196 (79,4)	232 (92,8)	
	Yes	29 (21)	41 (11,4)	**0.011**	51 (20,6)	19 (7,2)	**<0.001**

ASA = American Society of Anesthesiologists; AJCC = American Joint Committee on Cancer; ISUP = International Society of Urological Pathology.
[a] *P* values were calculated from contingency table using the chi-square and Ficher's tests. Statistically significant results are highlighted in bold.

(IBM, São Paulo, Brazil) and R, version 3.4 (Comprehensive R Archive Network in http://cran.r-project.org). The absolute and relative frequency distributions were presented in frequency and contingency tables. To verify the association between immunohistochemical renin expression and other variables, Pearson's chi-square test was used. Fisher's exact test was applied for cases in which the expected frequencies were <5. Table 1 summarizes the clinical and pathological features of patients with CRCC, crosstabbed with the quantitative and qualitative expression of renin.

Mann-Whitney *U* test was used to compare mean renin expression between benign tissue and ccRCC.

Disease-free survival (DFS) was defined as the interval between primary surgery and the last follow-up without disease or evidence of recurrence. Ten-year DFS was estimate by Kaplan-Meier method for the survival analysis, and log-rank test was applied to compare the survival distribution between groups. A Cox semiparametric proportional hazards model (univariate and multivariate) was used to determine the variables that influenced survival. Significant variables for the multiple model were selected, based on a simple Cox model, as were those that were object of the study. The final model was obtained by backward stepwise selection.

For the quantitative analysis of renin expression, we established 2 groups with respect to a simple cutoff (≤35% or >35% renin-positive nuclei), which was estimated using the maximum of the standardized log-rank statistic, proposed by Lausen and Schumacher (Fig. 1A) [17]. We assessed the proportionally assumption on the so-called Schoenfeld residuals (Fig. 1B) [18]. In all cases, there was evidence that covariates had a constant effect over time.

A *P* value that was lower than 0.05 was considered to be a statistically significant result for all statistical analyses.

3. Results

As expected, most patients were male (62.9%). Overall, 250 (51.2%) patients underwent nephron-sparing procedures, and 248 (49.8%) were treated with radical nephrectomy. Their mean (range) age was 54.96 (21−87) years. The median postoperative follow-up was 52.46 months. No patient was lost to follow-up. A total of 221 (44.4%) patients presented with pT1a disease, and 134 (26.9%) had pT1b tumors. Fifty-seven patients (11.4%) had pT2 disease, 82 (16.5%) had pT3 disease, and 4 patients (0.8%) had pT4 disease. The mean tumor size for the entire cohort was 5.1 cm (SD = 3.09). At the end of the study, 70 (14.1%) patients experienced recurrence, and 36 (7.2%) patients died due to ccRCC.

At the end of the 10-year follow-up, 70 patients had relapsed disease, constituting 86.3% of DFS in the study population: 8 (11.4%) were local (ipsilateral kidney or renal fossa), 51 (72.9%) were distal (60% lungs), and 11 (15.7%) were local plus distal. Isolated local recurrences were proportionally higher for partial nephrectomy (26.6%) compared with radical nephrectomy (7.2%). In the univariate analysis, several clinical, demographic, anatomopathological, and staging variables had a significant effect on DFS ($P < 0.001$), as shown in Table 2.

With regard to nuclear renin staining in the 498 immunostained ccRCC specimens, 138 (27.7%) lacked expression, and 360 (72.3%) were positive (Fig. 2). When categorized by expression level, the expression in 247 (49.6%) was ≤35, compared with 251 (50.4%) that were >35. All benign samples were positive. Although staining occurred in the cytoplasm, the expression was predominantly nuclear. The distribution of the staining was even and diffuse; no punctae were seen. We noted no significant impact on staining intensity ($P = 0.83$) on division of the cases according to storage time (≤10 years and >10 years). In the qualitative and quantitative analyses, patients who were negative ($P = 0.013$) and whose expression was ≤35 ($P < 0.001$) experienced more relapses, as shown in Table 3 and Fig. 3.

The variables that reached statistical significance in the univariate analysis for DFS were selected for the multivariate analysis. By Cox (backward) logistic regression, using 10 steps, including qualitative (Table 4) and quantitative renin expression (Table 5), the variables of interest were independent risk factors for disease relapse ($P < 0.001$). Negativity for renin increased the likelihood of tumor recurrence by nearly 3-fold (HR = 2.923, $P < 0.001$), and at <35, this risk rose nearly 4-fold (HR = 4.085, $P < 0.001$).

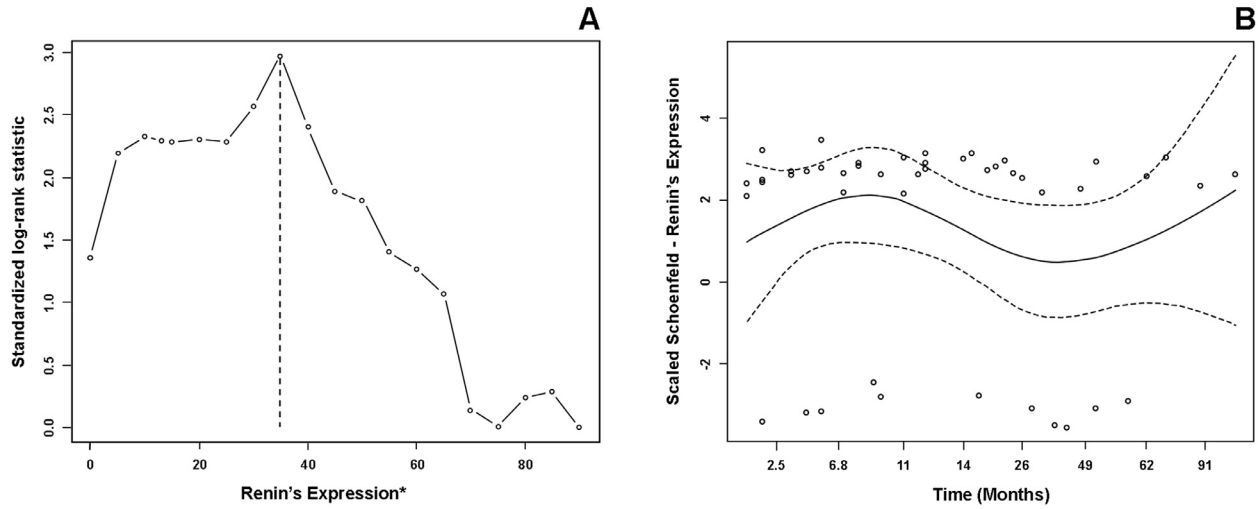

Fig. 1. Determination of the cutoff of renin expression per Lausen & Schumacher (A) and Schoenfeld (B).*Percentage of positive nuclei per 1000 cells.

Table 2
Univariate analysis (Cox proportional-hazards model) of disease-free survival at 10 years, according to variables of ccRCC patients—Significant findings.

	Univariate *P* value[a]
Age (≤ 40/41–60/≥61 y)	0.071/ **0.022/ 0.027**
Anemia (Hb <12/Hb ≥12)	**<0001**
Symptoms at diagnosis (no/yes)	0.050
ASA (1/2/≥3)	**0.029/** 0.089/ **0.014**
Tumor size (≤4cm/>4 and ≤7/>7)	**<0001/ 0.002/ <0.001**
Surgical margin (negative/positive)	**<0001**
Vascular embolization (no/yes)	**<0001**
Renal vein invasion (no/yes)	**0.010**
Perinephric fat invasion (no/es)	**<0.001**
Pathologic stage (pT) (1/2/3 or 4)	**<0.001/ <0.001/ <0.001**
Pathologic stage (pN) (0 or X/1)	**<0.001**
AJCC Clinical Stage (I or II/III or IV)	**<0.001**

ASA = American Society of Anesthesiologists; AJCC = American Joint Committee on Cancer.
Statistically significant results are highlighted in bold.
[a] Nonparametric Log-Rank test was used.

Symptoms at diagnosis, tumor size, pathological stage (N), and AJCC clinical stage remained independent predictors of DFS in the multivariate analysis.

4. Discussion

There are many reasons why angiotensinogenase is the subject of recent interest. There are few publications on the value of this protease as a prognostic factor, and most existing research has focused solely on angiotensin-regulating peptidases and the drugs that act on them. Further, there is evidence of the coexpression of tumor proteins and renin, suggesting that genetic mutations alter the expression of both. The tumor shares its origin in the renal cortical region, where the proximal convoluted tubules (ccRCC site) and juxtaglomerular apparatus (granular cell site) are located.

Our aim was to determine the value of renin expression in benign and neoplastic tissue and its association with variables and clinical outcomes. Based on our results, there was strong renin expression in benign samples, compared with weak levels and occasional absence in the ccRCC spots ($P < 0.001$). The reason for this decline can be explained by: (1) the direct structural derangement of the juxtaglomerular apparatus due to tumor progression, driven by adaptive advantages that are based on growth factors, angiogenesis, and apoptosis inhibitory proteins [19]; and (2) the functional dysregulation of granular cells, related to alterations in mammalian target of rapamycin (mTOR) and Von Hippel-Lindau (VHL) signaling.

In collaboration with centers in the United States, the Institute of Physiology, German University of Regensburg reported a relationship between VHL, erythropoietin (EPO), and renin. Mimicking chronic hypoxia using a conditional knockout mouse model, the demonstrate experimentally that the deletion of VHL down-regulates renin in granular cells and their descendants. By creating renal hypoxia models, the demonstrate experimentally that the deletion of VHL down-regulates renin in granular cells and their descendants. In an environment in which hypoxia-inducible factor 1-alpha (HIF-1α) accumulates, unactivated juxtaglomerular cells undergo a phenotypic transition and begin to produce EPO [20]. Recently, high EPO expression was reported to be proportionally greater in patients with adrenal invasion and associated with high-grade tumors, by Fuhrman classification [21]. We identified patients with grade 3 and 4 Fuhrman tumors, as well as other factors of a poor prognosis (microscopic venous and renal vein invasion)—that is, renin is poorly expressed ($P < 0.001$), constituting a dynamic imbalance in EPO and renin.

In addition to the kidneys, renin is expressed to a lesser extent in the adrenal glands, lungs, gonads, brain, heart, and liver. Even on deletion of VHL, EPO and renin expression remains unchanged in these sites, demonstrating the specificity of these biomolecular markers to renal tissue, favoring their use in clinical applications with greater specificity.

Because the tumor environment is associated with low renin expression, we hypothesized that this property would be associated with a worse prognosis. Initially, we performed a crosstab-based qualitative analysis, simply evaluating negativity or positivity (at any intensity). We found that cases that were negative for renin, compared with positive cases, had a greater prevalence of high-grade tumors, by Fuhrman and ISUP classification ($P < 0.001$ for both); disease recurrence ($P = 0.011$); and the presence of local ($P = 0.05$) or distance dissemination ($P = 0.003$).

Moreover, depending on the study aims and one's expertise, despite the technology that is available, a purely qualitative immunohistochemical reading is subject to bias, which can influence the results. To minimize flaws in the reading process, we also implemented a quantitative method. When we applied this technique, we found that low expression is ominous—that lower expression is associated with worse findings. We determined the most accurate cutoff in the survival analysis (above or below 35), and by crosstabbing, we noted the significance of Eastern Cooperative Oncology Group (ECOG) performance status ($P = 0.014$), microscopic venous invasion ($P = 0.05$), renal vein invasion ($P = 0.065$), and tumor recurrence ($P = 0.011$ for $P < 0.001$).

Using the same system for the survival curve, the resulting findings showed the same behavior. Thus, the univariate analysis of DFS was qualitatively significant ($P = 0.013$) and more so when quantitatively refined ($P < 0.001$). By Cox regression in a multivariate model, renin expression maintained a high predictive impact on the DFS curve ($P < 0.001$). In the presence of other factors, renin can improve the power of the model, consistent with the rationale for multiple-model analysis [22]. Thus, renin strengthens existing prognostic models in clinical practice with regard to renal tumors—for example, the UCLA Integrated Staging System for RCC, which includes such variables as TNM

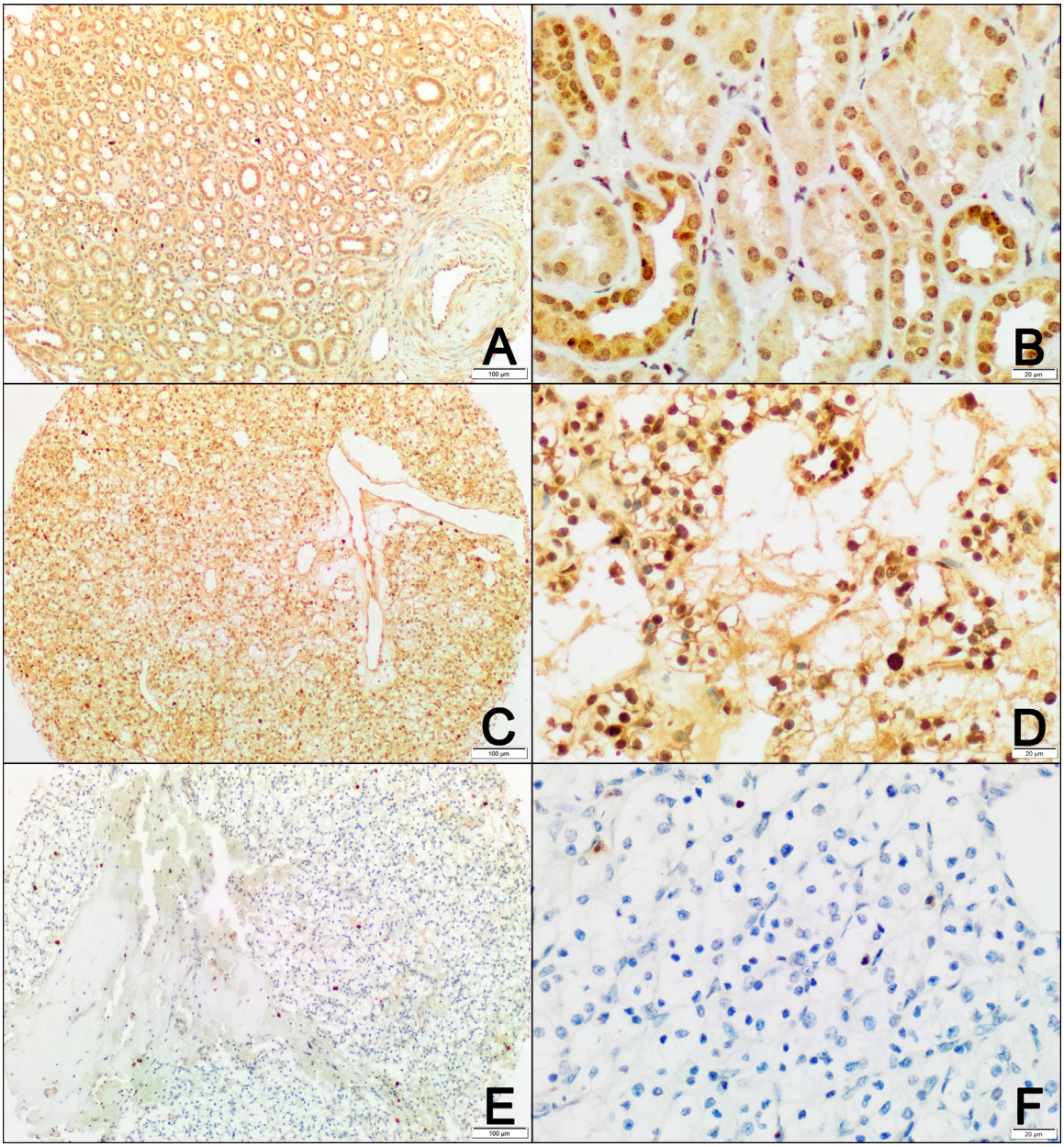

Fig. 2. Photomicrographs of immunohistochemical expression of renin. (A) Renin-stained nontumor renal parenchyma and (B) higher magnification. (C) Clear cell renal cell carcinoma positive for renin and (D) higher magnification. (E) Clear cell renal cell carcinoma negative for renin and (F) higher magnification.

Table 3
Cumulative probability of 10-year disease-free survival according to the degree of renin expression in 498 ccRCC patients.

	Category	No Recurrence—*n*(%)	Recurrence—*n*(%)	Univariate *P* value[a]
Renin's expression (qualitative)	Positive	319 (88,6)	41 (11,4)	
	Negative	109 (79,0)	29 (21,0)	**0.013**
Renin's cutoff (quantitative)	>35	232 (92,4)	19 (7,6)	
	≤35	196 (79,3)	51 (20,7)	**<0.001**

Statistically significant results are highlighted in bold.
[a] Within the variable, the comparison of the estimated curves for each category was performed by the nonparametric Log-Rank test.

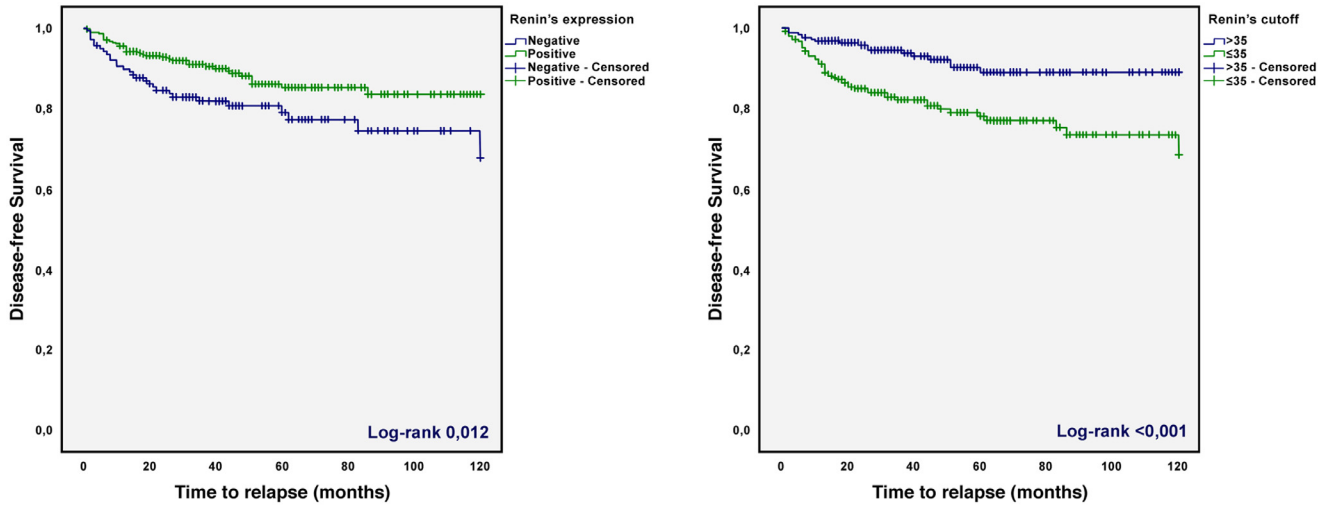

Fig. 3. Kaplan-Meier disease-free survival curves based on qualitative renin expression (A) and cutoff (B) in clear cell renal cell carcinomas. Significant differences in 10-year DFS between positive and negative cases (log-rank test $P < 0.012$) and cases above and below 35 (log-rank test $P < 0.001$).

Table 4
Univariate and multivariate analysis (Cox regression model) of disease-free survival at 10 years according to variables, including the qualitative expression of renin. Variables remaining at the end of the model.

	Univariate[a]	Multivariate[b]		
	P value	HR	95% CI	*P* value
Symptoms at diagnosis				
No		1		
Yes	0.050	2,226	1,059–4,678	**0.035**
Tumor size				
≤4cm	**<0.001**	1		**0.002**
>4 and ≤7	**0.002**	2,714	1,156–6,369	**0.022**
>7	**<0.001**	5,287	2,130–13,128	**<0.001**
Pathologic stage (pN)				
0 or X		1		
1	**<0.001**	3,934	1,289–12,005	**0.016**
AJCC Clinical Stage				
I or II		1		
III or IV	**<0.001**	2,063	1,000–4,255	**0.050**
Renin				
Positive		1		
Negative	**0.013**	2,923	1,633–5,235	**<0.001**

AJCC = American Joint Committee on Cancer.
Statistically significant results are highlighted in bold.
[a] Nonparametric Log-Rank test was used.
[b] Multivariate analysis showing hazard ratio, confidence interval and *P* values in the final step by backward stepwise Cox regression analysis (10 steps).

Table 5
Univariate and multivariate analysis (Cox regression model) of disease-free survival at 10 years according to variables, including quantitative renin expression. Variables remaining at the end of the model.

	Univariate[a]	Multivariate[b]		
	*P*value	HR	95% CI	*P* value
Symptoms at diagnosis				
No		1		
Yes	0.050	2,467	1,186–5,132	**0.016**
Tumor size				
≤4cm	**<0.001**	1		**<0.001**
>4 and ≤7	**0.002**	2,698	1,153–6,311	**0.022**
>7	**<0.001**	7,295	3,239–16,433	**<0.001**
Pathologic stage (pN)				
0 or X		1		
1	**<0.001**	5,922	2,001–17,525	**<0.001**
Renin				
>35		1		
≤35	**<0.001**	4,085	2,056–8,116	**<0.001**

Statistically significant results are highlighted in bold.
[a] Nonparametric Log-Rank test was used.
[b] Multivariate analysis showing hazard ratio, confidence interval and *P* values in the final step by backward stepwise Cox regression analysis (10 steps).

stage, Fuhrman nuclear grade, and ECOG performance status, all of which interact in the multivariate analysis when the presence of renin is also required, and, perhaps, the Mayo Clinic's Stage, Size, Grade, and Necrosis (SSIGN) model [23].

Serum renin levels are influenced by several individual characteristics (eg, health status, comorbidities, medications, renal function, genetic predisposition, and habits) that were not evaluated in this retrospective study. Independent of possible systemic influences (as above), intratumoral expression provided significant clinical information in this series.

5. Conclusions

The immunohistochemical expression of renin is higher in non-neoplastic renal tissue (benign) compared with ccRCC. Low renin expression is an unfavorable feature and is associated with more aggressive tumors, with high ISUP and Fuhrman grades (categories 3 and 4), which are significantly higher with the presence of microscopic venous invasion, renal vein invasion, and relapse. The rates of

neoplastic recurrence after initial treatment were higher in those with low renin expression. We observed an impact of renin on DFS for the univariate and multivariate analyses, and based on our cutoff (expression ≤35 was related to worse outcomes), it increased the prognostic accuracy.

Authors' contribution

FA Paula: Project development, data collection, data analysis, manuscript writing; SM Bezerra: data analysis; IW Cunha: data analysis; GC Munhoz: data collection; D Abreu: data management; PN Lara Jr: Project development; WH Costa: Project development, data collection; SC Zéqui: Project development, data collection.

Conflict of interest

The authors declare that they have no conflict of interest.

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