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ORIGINAL ARTICLE

Cyclin D1 and p57 expression in relation to clinicopathological characteristics and overall survival in patients with renal cell carcinoma

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Summary

Purpose: *There is a need for identifying molecular prognostic biomarkers to better predict clinical outcomes in patients with renal cell carcinoma (RCC). This study investigated the pattern of cyclin D1 and p57 expression in RCC patients and evaluated their relation with clinicopathological characteristics and overall survival (OS).*

Methods: *Immunohistochemistry was applied to paraffin-embedded tissue sections of 74 RCC patients. Two cut-off groups were defined by the fraction of positive cells as follows: ≤10% and >10% positive cells for cyclin D1, and ≤5% and >5% positive cells for p57.*

Results: *Cyclin D1 expression in >10% of positive cells was observed mostly in the clear cell RCC, while p57 expression in ≤5% of positive cells was found in 86% of chromophobe RCC specimens. The higher expression of cyclin D1 and lower expression of p57 were more frequent in grade I-II tumors. OS was associated with unfavorable clinicopathological characteristics. However, cyclin D1/p57 expression did not influence the survival rates.*

Conclusion: *Although cyclin D1 and p57 expression did not affect survival rates in RCC patients, proper validation and establishment of the qualitative cut-off point are needed for these tumors.*

Key words: *cell cycle proteins, cyclin D1, immunohistochemistry, renal cell cancer, p57, survival*

Introduction

Renal cell carcinoma (RCC) is the most common adult malignant kidney tumor and it is also the most lethal urological tumor [1]. Disease rarely develops under the age of 40 (7%) and it usually affects male patients aged 60-70 years. During the last two decades, incidence rates increased approximately by 2% per year in both Europe and worldwide [2], however, in recent years stabilizing and declining trends were observed [3]. In Serbian population, the number of patients with RCC in last 10 years has increased [4].

Cell cycle is finely tuned by mechanism consisting of protein complexes that include cyclins, cyclin-dependent kinases (CDK) and cyclin-dependent kinase inhibitors (CDKI). Impairment of the cell cycle can lead to uncontrolled cell division

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and neoplastic transformation [5,6]. Cyclin D1 is expressed through G1 phase into mitosis and in association with CDK4 and CDK6, it has a role in the regulation of the cell cycle through the retinoblastoma (Rb) tumor suppressor protein [7,8]. Increased expression of cyclin D1 was found in many malignant tumors (breast, esophagus, liver, some types of lymphoma) [9]. Approximately 75% of RCC have higher expression of cyclin D1 than normal renal tissue [10].

p57 is a member of the Cip/Kip family of CDKI molecules along with p21 and p27. Its role includes cell-cycle control, differentiation, apoptosis, tumorigenesis and angiogenesis, migration and invasion [11]. p57 has an active role in the development of lung, breast, bladder, prostate, gastrointestinal tract and pancreatic cancer [12]. This is the only CDKI that is necessary for normal progression of the cell cycle, as other inhibitors cannot compensate its effect [11].

Current clinicopathological prognostic factors such as stage, grade and performance status provide important prognostic information. However, they lack the potential to incorporate the individual biological potential and predict clinical behavior of tumors. Molecular markers have the capacity to interrogate biological heterogeneity of tumors and may ultimately help clinicians offer individualized treatment regimens.

Based on the above information, there is a need for a prognostic marker that might act as substitute and research has been focused on identification of specific and sensitive biomarkers [13]. The aim of this study was to determine the immunohistochemical expression of cyclin D1 and p57 in patients with RCC and to analyze them in relation to clinicopathological parameters and OS.

Methods

This retrospective study included 74 patients with RCC who underwent nephrectomy between 2010-2013 in the University Hospital "Dr. Dragisa Misovic" and the Clinic of Urology, Clinical Centre of Serbia, and were diagnosed at the Institute of Pathology, School of Medicine in Belgrade. The study was performed in compliance with the ethical standards of the Institutional Ethics Committee and with the 1975 Helsinki Declaration.

Clinicopathological characteristics of patients included age, gender, tumor size, nuclear grade (according to the Fuhrman grading system) [14], TNM stage, histological subtype (according to 2004 WHO classification) [15], and vital status. OS time was defined as the time from surgery to death or the last follow-up visit. Patients who were still alive at the final follow-up date were censored.

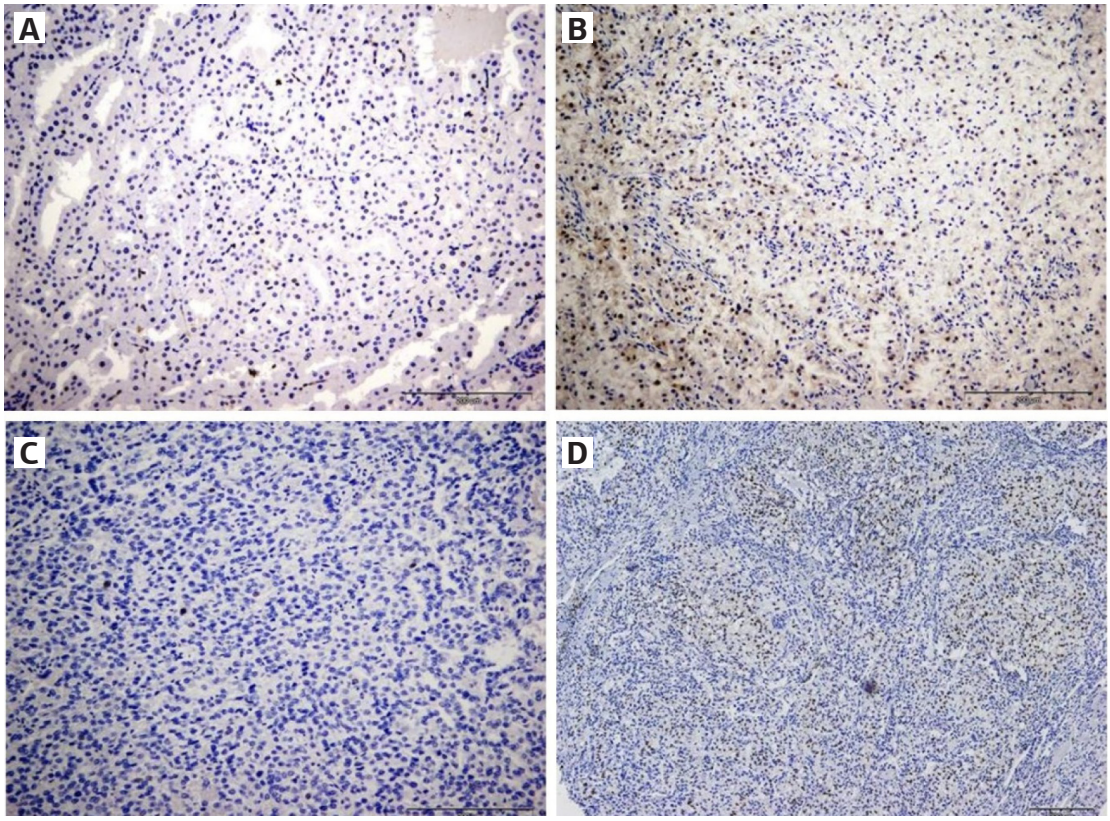

Figure 1. Immunohistochemical staining of cyclin D1 and p57 in renal cell carcinoma. **(A)** cyclin D1 expression in ≤10% of tumor cells; **(B)** cyclin D1 expression in>10% of tumor cells; **(C)** p57 expression in ≤5% of tumor cells; and **(D)** p57 expression in >5% of tumor cells. Original magnification x 20.

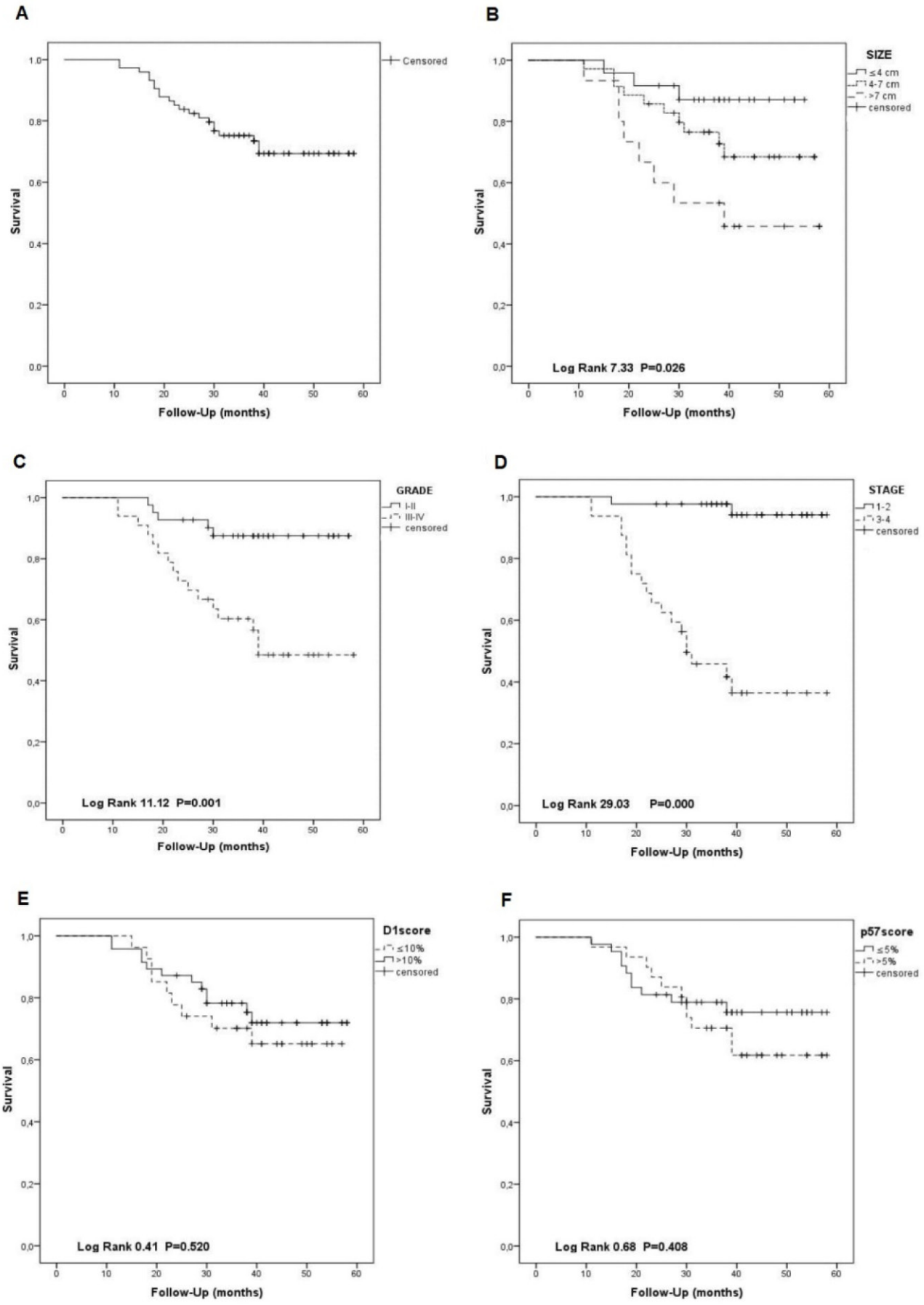

Figure 2. Kaplan-Meier curves for overall survival in patients with renal cell carcinoma. **(A)** Survival rate of the cohort. Survival rate according to **(B)** tumor size, **(C)** Fuhrman grade, **(D)** stage, **(E)** cyclin D1expression and **(F)** p57 expression.

Immunohistochemistry

For immunohistochemical detection, rabbit polyclonal antibodies to cyclin D1(diluted 1:50, Thermo Scientific, USA) and to p57 (diluted 1:600, Santa Cruz Biotechnology, USA) were used. The whole procedure has been described in detail elsewhere [16].

Based on different literature cut-off data, we classified the expression into 2 groups defined by the fraction of positive cells in the tumor section as follows: ≤10% positive cells and >10% positive cells for cyclin D1 (overexpression), and ≤5% positive cells and >5% positive cells for p57. The groups were considered as tumors exhibiting low expression of the cyclin D1 and p57 protein, and tumors showing high protein expression, respectively (Figure 1).

Statistics

Statistical analyses were performed using SPSS 23.0 for Windows (SPSS Inc., Chicago, IL, USA). In order to test the association between categorical variables, the contingency tables (Chi-square and Fisher exact test) were used. The 5-year OS rate was calculated by Kaplan-Meier method and groups were compared using Log-rank test. Univariate and multivariate Cox proportional hazards regression model was used to analyze the independent factors related to prognosis. P values <0.05 were considered significant.

Table 1. Clinicopathological characteristics of patients with RCC (n=74)

Characteristics	*Occurrence n (%)*
Age, years	
≤50	13 (17.6)
51-60	25 (33.8)
61-70	27 (36.5)
>70	9 (12.2)
Gender	
male	48 (64.9)
female	26 (35.1)
Size (cm)	
≤4	24 (32.4)
4.1-7	35 (47.3)
>7	15 (20.3)
Grade	
I-II	41 (55.4)
III-IV	33 (44.6)
Stage	
1-2	42 (56.8)
3-4	32 (43.2)
Histology	
clear cell	49 (66.2)
papillary	18 (24.3)
chromophobe	7 (9.5)
Vital status	
alive	53 (71.6)
dead	21 (28.4)

Results

Clinicopathological characteristics of patients are given in Table 1. Patients' age ranged between 33 and 85 years (mean 59.29) with male predominance (64.9%). The median tumor size was 4.83 cm (IQR=3.5-6.73). The majority of tumor diameters (47.3%) ranged between 4.1 and 7 cm. The rates of low (nuclear grade I and II) and high-grade RCC (nuclear grade III and IV) were 55.4% and 44.6%, respectively. Stages pT1 and pT2 were classified as low-stage tumors (56.8%) and pT3 and pT4 as high-stage tumors (43.2%). At the time of diagnosis, the clear cell RCC (ccRCC) was the most frequent histological subtype (66.2%), followed by papillary (pRCC) (24.3%) and chromophobe (chRCC) (9.5%).

Tables 2 and 3 show the associations among clinicopathological variables, cyclin D1 and p57 expressions. The analysis showed a statistically significant difference only between the histological subtypes and cyclin D1 expression (p=0.000, Chi-square test). The cyclin D1 overexpression was predominantly found in patients with ccRCC (95.7%). There was no statistical significance between the p57 expression and any of the clinicopathological parameters.

Survival analysis

The 5-year OS for all patients was 69.3% (Figure 2A). Statistically significant correlation was found between survival and tumor size (Log-rank 7.33, p=0.026), grade (Log-rank 11.12, p=0.001), and stage (Log-rank 29.03, P=0.000) (Figure 2, B-D).

The 5-year OS rates for each size were as follows: 87.1% for patients with RCC size ≤4 cm; 68.4% and 45.7%, for patients with tumor size 4.1-7 cm and >7cm, respectively (Figure 2B). After 5 years, the survival rate among subjects with low-grade tumors was 87.5%. In the high-grade tumor group, the survival rates were 93.9, 60.3 and 48.5% after 1, 3, and 5 years, respectively (Figure 2C). Patients with low (1-2) stage tumors had survival rate of 94.1%, while 36.4% of patients with stages 3-4 were alive after a 5-year follow-up period. There was no significant association between gender, histological subtype and survival rate (Log-rank 0.68, p=0.408; Log-rank 0.58, p=0.748, respectively). The 5-year OS rates were 73.6% and 61.2% for men and women, respectively, and 69.9, 64.8 and 85.7% for ccRCC, pRCC and chRCC, respectively.

The OS analysis based on cyclin D1/p57 expression level (Figure 2 E-F) revealed no statistically significant difference between the survival rates

for these two parameters (Log-rank 0.41, p=0.520; Log-rank 0.68, p=0.408, respectively). Taking into consideration only the ccRCC histological subtype, no significant difference in OS according to cyclin D1 and p57 expression was observed (Log-rank 1.96, p=0.161; Log-rank 0.01, p=0.916, respectively).

Cox's univariate regression analysis showed that larger tumor size, higher grade and tumor stage significantly affected survival. Multivariate Cox regression analysis identified higher grade (p=0.035) and stage (p=0.001) as the independent predictors of survival (Table 4).

Discussion

Despite the advances in clinical treatment, immunotherapy, targeted cancer therapies, and chemo- or radiotherapy, the prognosis of RCC patients is still very poor. Renal tumorigenesis is a complex multistep process which also involves aberrations in cell cycle control through cyclins and their regulators [7].

Cyclin D1 overexpression was demonstrated in various malignancies including colorectal cancer [17] and breast cancer [18], correlating with early cancer onset, tumor progression, shorter survival and increased metastases [19]. In a variety of kidney tumors, cyclin D1 expression demonstrated diffuse positivity in clear cell kidney sarcoma [20], as well as in RCC with Xp11.2 translocation [21]. In our cohort of patients, the expression of cyclin D1 in more than 10% of positive cells, used as a cut-off value, was observed mostly in the ccRCC histological type, while about 90% of pRCC and 100% of chRCC subjects had cyclin D1 expression in less than 10% of tumor cells. These findings are in accordance with the observations of Hedberg et al. and Lima et al. [22,23] in which the evaluated cell cycle proteins varied between the different RCC types. Studies carried out by Sukov et al. [24] and Zhao et al. [25], demonstrated absence of cyclin D1 expression in chRCC. Regarding the tumor features, the study of Hedberg et al. [22] showed that low cyclin D1 protein level in the ccRCC was associated with high nuclear grade and large tumor size; however, we found no association between the levels of cyclin D1 expression and tumor stage or grade in this study. It was reported that expression of cyclin D1 may be controlled through regulation of transcription, or proteasomal degradation [17],

Table 2. Correlation between cyclin D1 expression and clinicopathological characteristics

Characteristics	*≤ 10% positive cells (n=27) n (%)*	*>10% positive cells (n=47) n (%)*	*p value*
Age, years			
≤50	4 (14.8)	9 (19.1)	
51-60	8 (29.6)	17 (36.2)	x^2=1.816
61-70	10 (37.0)	17 (36.2)	p=0.611
>70	5 (18.5)	4 (8.5)	
Gender			
male	16 (59.3)	32 (68.1)	x^2=0.586
female	11 (40.7)	15 (31.9)	p=0.460
Size (cm)			
≤4	6 (22.2)	18 (38.3)	x^2=3.210
4.1-7	13 (48.1)	22 (46.8)	p=0.201
>7	8 (29.6)	7 (14.9)	
Grade			
I-II	12 (44.4)	29 (61.7)	x^2=2.067
III-IV	15 (55.6)	18 (38.3)	p=0.224
Stage			
1-2	15 (55.6)	27 (57.4)	x^2=0.025
3-4	12 (44.4)	20 (42.6)	p=1.00
Histology			
clear cell	4 (14.8)	45 (95.7)	x^2=50.477
papillary	16 (59.3)	2 (4.3)	p=0.000
chromophobe	7 (25.9)	0 (0.0)	
Vital status			
alive	18 (66.7)	35 (75.4)	x^2=0.514
dead	9 (33.3)	12 (25.5)	p=0.593

Table 3. Correlation between p57 expression and clinicopathological characteristics

Characteristics	*≤ 5% positive cells (n=43) n (%)*	*> 5% positive cells (n=31) n (%)*	*p value*
Age, years			
≤50	8 (18.6)	5 (16.0)	
51-60	15 (34.9)	10 (32.3)	x^2=2.630
61-70	17 (39.5)	10 (32.3)	p=0.452
>70	3 (7.0)	6 (19.4)	
Gender			
Male	25 (58.1)	23 (74.2)	x^2=2.037
Female	18 (41.9)	8 (25.8)	p=0.218
Size (cm)			
≤4	17 (39.5)	7 (22.6)	x^2=2.613
4.1-7	19 (44.2)	16 (51.6)	p=0.271
>7	7 (16.3)	8 (25.8)	
Grade			
I-II	26 (60.5)	15 (48.4)	x^2=1.064
III-IV	17 (39.5)	16 (51.6)	p=0.349
Stage			
1-2	24 (55.8)	18 (58.1)	x^2=0.037
3-4	19 (44.2)	13 (41.9)	p=1.00
Histology			
clear cell	26 (60.5)	23 (74.2)	x^2=2.771
papillary	11 (25.6)	7 (22.6)	p=0.250
chromophobe	6 (14.0)	1 (3.2)	
Vital status			
alive	33 (76.7)	20 (64.5)	x^2=1.325
dead	10 (23.3)	11 (35.5)	p=0.310

Table 4. Factors affecting overall survival in patients with renal cell carcinoma

Variables	*Coefficient b*	*HR*	*95% CI*	*p value*
Univariate analysis				
Size (cm)				0.042
≤4		1.0	-	
4.1-7	0.869	2.385	0.656-8.667	0.187
>7	1.634	5.127	1.358-19.355	0.016
Grade				
I-II		1.0	-	
III-IV	1.544	4.681	1.714-12.787	0.003
Stage				
1-2		1.0	-	
3-4	2.917	18.491	4.278-79.934	0.000
Cyclin D1				
≤10%		1.0	-	
>10%	-0.282	0.755	0.318-1.792	0.523
p57				
≤5%		1.0	-	
>5%	0.358	1.430	0.607-3.367	0.413
Multivariate analysis				
Grade	1.096	2.993	1.083-8.270	0.035
Stage	2.738	15.457	3.177-75.2	0.001

and a study of pediatric rhabdoid tumors pointed out downmodulation of cyclin D1 as crucial in the treatment [26]. As ccRCC is the most common histological subtype, it can be useful to further analyze transcriptional regulation of cyclin D1 expression.

In adult tissues, p57 is mainly expressed in skeletal muscles, heart, brain, lung, kidney, pancreas, testis and placenta. It is involved in the regulation of different cellular processes including apoptosis, differentiation, development, and migration in tumorigenesis [27]. Several studies demonstrated that lower p57 expression in lung, oral, oesophageal, gastric, colorectal, hepatocellular and pancreatic malignancies was associated with poor prognosis [27]; however, the role of p57 has been studied to a small extent in cardiovascular and renal pathologies [28] and it was found that p57 was among the genes which were commonly downregulated in RCC [29]. No correlation was found between the expression of p57 and tumor stage, nuclear grade, tumor size and gender in this study. We also did not find any evidence of an association between p57 expression and RCC histological subtypes; however, the low expression (in less than 5% of positive tumor cells) was mostly observed in the majority of chRCC and pRCC specimens. In cell lines, downregulation of p57 increases the expression of cyclin D1, enhancing the cellular proliferation [30], initiation and progression [31] and also accelerates the growth and invasion, as it was shown in hepatocellular carcinoma cells [32,33]. The importance of p57 downregulation in human cancers and relevance in tumor-targeted therapy is a subject of investigation. Some clinical studies suggested that absence of p57 abrogates the pro-apoptotic function of anticancer therapy and results in poor prognosis due to development of drug resistance [28].

The prognostic role of cyclin D1 and p57 has not been resolved yet. Low levels of cyclin D1 were associated with worse prognosis [22]. Migita et al. [34] found that low cyclin D1 expression had a tendency to shortened survival, but it was not confirmed as an independent prognostic factor in patients with ccRCC. The study of Aaltomaa et al. [35] also found no correlation between the expression of cyclin D1 and survival; however, high expression of cyclin D in correlation with good prognosis was demonstrated in study by Lima et al. [23]. Reduced p57 expression correlated with decreased survival [27] similarly to the recent studies of breast cancer cases which showed that decreased expression of p57 was associated with worse prognosis [36].

In this study, the overall survival was associated with standard unfavorable clinicopathological characteristics including disease stage 3-4, histological grade III-IV and tumor size over 7 cm, as shown previously [4,37], but not with the level of cyclin D1 and p57 expression according to the cut-off values we used.

The common issue with immunohistochemistry is the determination of the extent of tumor positivity for a given marker that is biologically and clinically relevant. Different results can be obtained due to lack of consensus regarding the method of evaluating the expression of cell cycle markers, especially when defining a cut-off value for a high/low proliferative index in tumor cells. Some studies set the mean value of expression as the boundary value for the entire cohort study. Other authors evaluated the expression semi-quantitatively, so the results were divided into quartiles, with a cut-off value that exceeded the expression of this protein in the normal tissue [38]. Thus, future research should focus on the definition of the optimal limit values for the evaluation of the immunohistochemical expression of cell cycle markers.

Usually, a cut-off score is set arbitrarily and it varies between different reports. As indicated in the newest reports [39,40] since there is a lack of proper validation and establishment of the qualitative cut-off point for these tumor types, no cell cycle or proliferative biomarkers are currently used in routine care to guide treatment decisions. In our study, two cores we used to determine the expression of the investigated parameters may also not have been sufficient enough to capture the biology of the whole specimen.

The heterogeneity of this tumor suggests that numerous pathobiological phenomena play an important role in its development. For the clinical outcome and therapeutic approach, it is of utmost importance to observe and interpret these differences. Therefore, in addition to already established prognostic factors, such as stage and grade, the current research focuses on the molecular biology of the RCC, as it can help to improve the therapy for these patients. Data of immunohistochemical expression of the cell cycle proteins could contribute to molecular profiling and more precise subclassification of the RCC, which might be applied in additional therapeutic modalities.

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Conflict of interests

The authors declare no conflict of interests.

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