DOI: 10.24938/kutfd.671178 Kırıkkale Üniversitesi Tıp Fakültesi Dergisi 2020;22(1):79-88
Original Article *Özgün Araştırma*

COEXISTENCE OF CD44 AND KI-67 AS THE PROGNOSTIC MARKERS IN RENAL CELL CARCINOMA

Renal Hücreli Karsinomda Prognostik Belirteçler Olarak CD44 ve Ki-67 Birlikteliği

Tuba DEVRİM[1], Mahi BALCI[2]

[1,2] *Kırıkkale University School of Medicine, Department of Medical Pathology, KIRIKKALE, TÜRKIYE*

ABSTRACT

Objective: An important feature of renal cell carcinoma (RCC) is its changeable prognosis. In recent years, several potential biomarkers have been investigated and confirmatory studies of these promising biomarkers are necessary to improve the clinical practice. In the present study, it was aimed to investigate the prognostic significance of CD44 expression by comparing it with Ki-67 and p53 in clear cell RCC.

Material and Methods: Formalin- fixed, paraffin-embedded tissue sections of clear cell RCC from 34 patients, who had undergone radical or partial nephrectomy, were included in this study. Correlations between CD44, Ki-67 and p53 immunohistochemical expressions and clinicopathological parameters were determined.

Results: CD44-high expression group (HEG) was significantly associated (p=0.035) and correlated (p=0.016, r=0.41) with a high nuclear grade. Similarly, the Ki-67-HEG was significantly associated (p=0.01) and correlated (p=0.01, r=0.436) with a high nuclear grade. We also found significant association (p<0.01) and correlation (p<0.01, r=0.621) between expressions of these markers. However, no antigen-antibody interaction was detected by p53 staining in clear cell RCC tissue sections.

Conclusion: We concluded that increased expressions of CD44 and Ki-67 in tumour tissues predict an aggressive course of RCC patients.

***Keywords**: Renal cell carcinoma, clear cell, CD44, Ki-67, p53*

ÖZ

Amaç: Renal hücreli karsinomun (RHK) önemli bir özelliği değişen prognozudur. Son yıllarda, birkaç potansiyel biyobelirteç araştırılmış olup ve bu umut verici biyobelirteçlere ait doğrulayıcı çalışmalar klinik uygulamayı geliştirmek için gereklidir. Bu çalışmada, berrak hücreli RHK'de CD44 ekspresyonunun prognostik öneminin, Ki-67 ve p53 ile karşılaştırarak araştırılması amaçlandı.

Gereç ve Yöntemler: Radikal veya parsiyel nefrektomi yapılan 34 hastanın berrak hücreli RHK'nin formalinle fikse edilmiş, parafine gömülü doku kesitleri çalışmaya dahil edildi. CD44, Ki-67 ve p53 immünohistokimyasal ekspresyonları ve klinikopatolojik parametreler arasındaki korelasyonlar belirlendi.

Bulgular: CD44-yüksek ekspresyon grubu (HEG) ile yüksek nükleer derece arasında anlamlı bir ilişki (p=0.035) ve korelasyon saptandı (p=0.016, r = 0.41). Benzer şekilde, Ki-67-HEG ile yüksek nükleer derece arasında önemli bir ilişki (p=0.01) ve korelasyon saptandı (p=0.01, r=0.436). Ayrıca bu iki belirteç arasında anlamlı ilişki (p<0.01) ve korelasyon (p<0.01, r=0.621) bulundu. Bununla birlikte, berrak hücreli RHK doku kesitlerinde p53 immünhistokimyasal boyanması saptanmadı.

Sonuç: Tümör dokularında artmış CD44 ve Ki-67 ekspresyonlarının, RHK hastalarında agresif bir seyiri öngördüğü sonucuna vardık.

***Anahtar Kelimeler**: Renal hücreli karsinom, berrak hücreli, CD44, Ki-67, p53*

Correspondence / Yazışma Adresi: **Dr. Tuba DEVRİM**
Department of Medical Pathology, Kırıkkale University School of Medicine, Kırıkkale, TÜRKİYE
Phone / Tel: +905432022088 **E-mail /** E-posta: tubadevrim@gmail.com
Received / Geliş Tarihi: 06.01.2020 **Accepted /** Kabul Tarihi: 25.03.2020
ORCID NO: [1]0000-0002-5321-2002, [2]0000-0001-5836-2344

KÜ Tıp Fak Derg 2020;22(1):79-88
Doi:10.24938/kutfd.671178

INTRODUCTION

Renal cell carcinoma (RCC) is the most common malignant kidney tumor in adults that accounts for about 3% of all adult malignant tumours. Globally, it is the 9th most common cancer in men and 14th in women (1). The incidence of RCC is increasing worldwide, with rates varying by age, race, sex and country (2). Also, it is the seventh most common cancer in Turkey (3).

RCC tumors may range from benign to highly aggressive and metastatic so that a particular contradiction in the treatment is due to the diversity of RCC types (4). Biomarkers provide an original occasion to enhance the maintenance in cancer patients (5). However, RCC cases currently lack clinically advantageous biomarkers determinative of early diagnosis or patient status. Due to lack of diagnostic alternatives, RCC has mostly been diagnosed depending on imaging result (6).

CD44 is a family of glycoproteins that exists on embryonic stem cells and in different values on various cell types, such as bone marrow and connective tissues (7). CD44 is also expressed in cancer cells and is admitted as a molecular marker for cancer stem cells (8). As a protein intervened in cell motility, CD44 has been utilized as a marker for tumor aggressiveness in a number of malignancies such as liver, stomach and breast. Nevertheless, there are contradictory reports in RCC. There are studies promoting the potential of CD44 as a prognostic marker, while in different series its significance as a predictor of survival was not confirmed (9).

Ki-67 is a DNA binding protein which is widely utilized as a marker of proliferation. It is used for grading tumors and is detected by immunohistochemical staining (10). Numerous reports about the cell cycle analysis in the nuclei has showed its relationship with cell proliferation and its co-expression with other reputed markers of proliferation point out a main role in cell division. There is a growing interest in Ki-67 as an attractive prognostic prediction and potential therapeutic target in malignant neoplasms related with bladder, lung, breast, cervix, and upper urinary tract (11). However, some studies reported that Ki-67 immunohistochemistry is prognostically irrelevant in RCC patients and the function of Ki-67 in the prognosis of RCC remains inconsistent (12,13).

P53, also recognized as "tumor suppressor p53", plays important roles in apoptosis, genomic stability, and anti-angiogenesis (14). Loss of p53 function is a critical event in tumor evolution (15). P53 mutations and/or deletions can be detected in 50% of human cancer cases and related with disease aggressiveness and worse prognosis (16,17). Its function in tumor growth was recently related to its influences on cancer stem cells, although the underlying molecular mechanisms remain unknown (15). Many investigations have reported the prognostic value of p53 immunohistochemistry in RCC, but the results were conflicting and the prognostic significance of p53 in RCC remains to be defined (14). The purpose of this study was to provide a better insight of the course of RCC by comparing CD44 levels with Ki-67 and p53 expressions and with the usual prognostic factors (pT stage, nuclear grade, and sex).

MATERIALS AND METHODS

Paraffin-embedded specimens from 34 patients (22 men and 12 women; male/female ratio 1.8) with primary clear cell RCC submitted to our laboratory between January 2010 and June 2019 were included in our study. Ethical approval was given by the Kırıkkale University Ethics Committee (Date: 15.05.2019, decision number: 2019.04.11). The median patient age was 58 years (range: 33 to 80). Tumors were grouped

KÜ Tıp Fak Derg 2020;22(1):79-88
Doi:10.24938/kutfd.671178

as ≤7cm and >7 cm in diameter according to literature (18). All specimens were re-evaluated with regard to pT stage and nuclear grade which were determined according to the World Health Organization (WHO) classifications (19).

Tissue microarray technique was used for immunohistochemical evaluation. Cylindrical core biopsies with a diameter of 0.3 mm were sampled from the highest nuclear grade area. Immunohistochemistry was performed on formalin- fixed, paraffin-embedded tumor specimens by using a Roche BenchMark® XT autostainer (Roche, Switzerland). Anti-CD44, anti-Ki-67 and anti-p53 antibodies were used according to the manufacturer's instructions (Table 1).

Table 1: Details of the Primary Antibodies Used for Immunohistochemistry.

Antibody	Clone	Source	Dilution	Incubation (min)	Positive Control
CD44	Monoclonal	ROCHE 790-4537	1/100	60	Tonsil
Ki67	Monoclonal	ROCHE 790-4286	1/150	60	Tonsil
P53	Monoclonal	ABCAM ab1101	1/75	60	Colon adenocarcinoma

The immunohistochemistry staining results were independently scored by two pathologists who were blinded for medical and pathological data of patients. Inconsistencies were unraveled by contemporaneous re-examination of the slides by both researchers handling a double-headed microscope.

Tumor cell nuclei that exhibited a distinct brown staining were considered as positive immunoreactivity for Ki-67 and documented in categories as follows: 0, no reactivity; 1, less than 15% (low expression group = LEG); and 2, ≥15% (high expression group = HEG) (20). CD44 staining was classified by a score system dividing tumors into four type of grades (0=none; 1=weak; 2=intermediate; and 3=strong) (9). Cases with staining intensity scores of 0-1 were placed in the CD44-LEG, and those with staining intensity scores of 2-3 were placed in the CD44-HEG.

Statistical Analysis

Statistical analysis was performed using a SPSS (IBM, USA) program. pT stage, grade, sex, and age groups were analyzed to find probable differences in immunohistochemistry scores using the chi-square test or Fisher's exact test. The immunohistochemistry scores and histopathological parameters were correlated using the Spearman rank test. At 95% confidence interval, $p<0.05$ was considered significant.

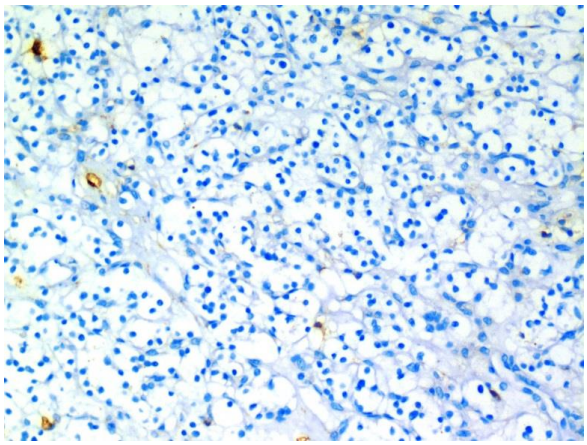
RESULTS

Median age of the patients (n=34) was 58.0±13.5 ranging from 33 to 80 and the male/female ratio was 22/12. The median tumor size was 4.5±2.3 cm, ranging from 1.5 to 10.5 cm. Distribution of the tumor stages were determined as follows; pT1a in 15 (44.1%), pT1b in 13 (38.2%), pT2a in 1 (3%), pT2b in 2 (5.8%), and pT3 in 3 (8.8%) cases. Additionally, the tumor grades were detected as follows; G1 in 10 (29.4%), G2 in 10 (29.4%), G3 in 9 (26.4%), and G4 in 5 (14.7%) cases.

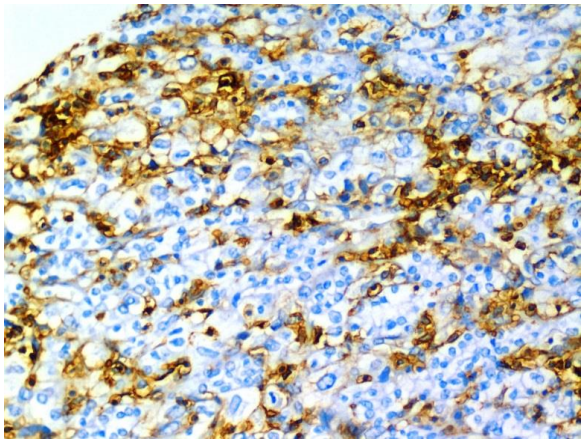
CD44 staining was intensive especially in the membrane and cytoplasm of the RCC cells. Subsequently, CD44 expression levels were assessed by examining the positively stained tumor cells (Figure 1). Low CD44 expression was detected in 21 of cases (61.7%) while high CD44 expression was determined in 13 of cases (38.2%). The patient characteristics with CD44 are summarized in Table 2. There was a significant relation between nuclear grade and CD44 (p=0.035). No significant difference was detected between CD44 and age, gender, pT or tumor size.

Of the 34 RCC cases, Ki-67 was positive in 32 (94.1%) cases while ≥15% positivity for Ki-67 was observed in 18 (52.9%) specimens (Figure 2).

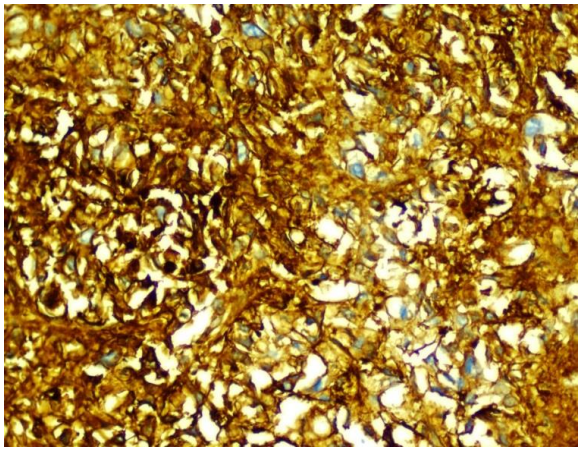
KÜ Tıp Fak Derg 2020;22(1):79-88
Doi:10.24938/kutfd.671178



A

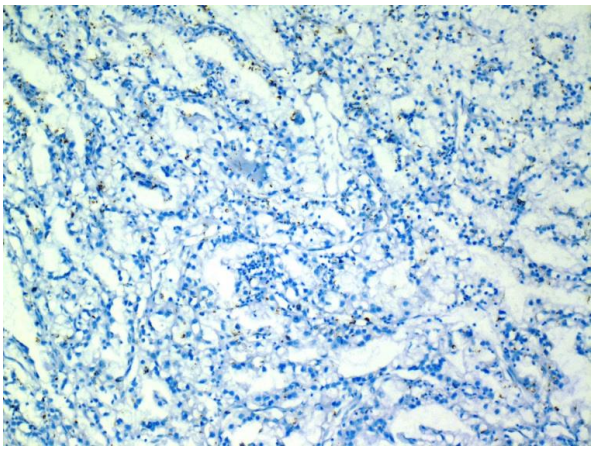


B

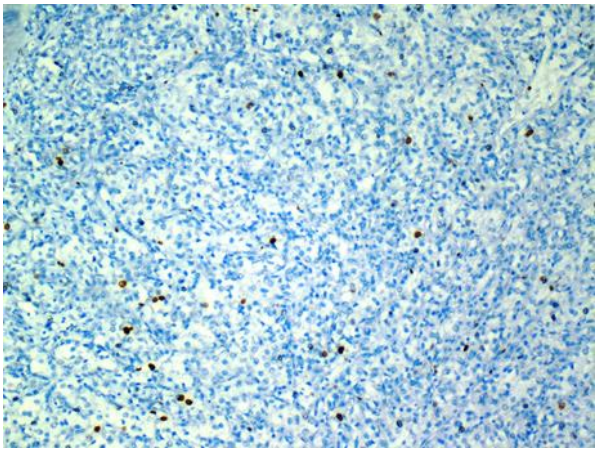


C

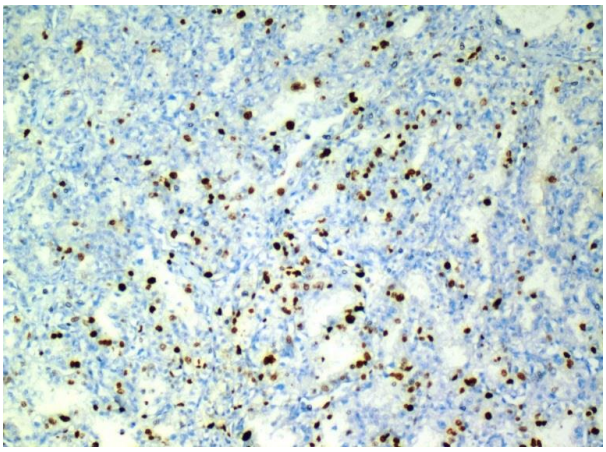
Figure 1: Representative photomicrographs of immunohistochemical staining for CD44 in renal cell carcinoma tissues (*x200*). No staining intensity (A), intermediate staining intensity (B) and strong staining intensity (C).



A



B



C

Figure 2: Representative photomicrographs of immunohistochemical staining for Ki-67 in renal cell carcinoma tissues (*x100*). No staining (A), low expression (B) and high expression (C).

The patient characteristics with Ki-67 are summarized in Table 3. There was a significant (p<0.01) relation between Ki-67 and CD44. Associations of the expression of Ki-67 with CD44 expression are summarized in Table 4. We found a significant (p=0.01) association between nuclear grade and Ki-67 expression. Also, there was a significant relation between Ki-67 and CD44 expressions (p<0.01). However, no significant difference was detected between Ki-67 and age, gender, pT or tumor size.

Table 2: CD44 Immunoreactivty of RCC.

Variable	Total (n)	CD44-Low (n)	CD44-High (n)	*p* value
Total	34			
Sex				
female	12	8 (38.1%)	4 (30.8%)	0.478
male	22	13 (61.9%)	9 (69.2%)	
Tumor diameter, cm				
≤ 7	31	19 (90.5%)	12 (92.3%)	0.678
> 7	3	2 (9.5%)	1 (7.7%)	
Stage				
pT1a	15	11 (52.4%)	4 (30.8%)	0.565
pT1b	13	7 (33.3%)	6 (46.2%)	
pT2a	1	1 (4.8%)	0 (0%)	
pT2b	2	1 (4.8%)	1 (7.7%)	
pT3	3	1 (4.8%)	2 (15.4%)	
Grade				
1	11	8 (38.1%)	3 (23.1%)	0.035
2	10	8 (38.1%)	2 (15.4%)	
3	9	5 (23.8%)	4 (30.8%)	
4	4	0 (0%)	4 (30.8%)	

Table 3: Ki-67 Immunoreactivty of RCC.

Variable	Total (n)	Ki67-Low (n)	Ki67-High (n)	*p* value
Sex				
female	12	6 (37.5%)	6 (33.3%)	0.541
male	22	10 (62.5%)	12 (66.7%)	
Stage				
pT1a	15	9 (56.3%)	6 (33.3%)	0.642
pT1b	13	5 (31.3%)	8 (44.4%)	
pT2a	1	0 (0%)	1 (5.6%)	
pT2b	2	1 (6.3%)	1 (5.6%)	
pT3	3	1 (6.3%)	2 (11.1%)	
Grade				
1	11	6 (37.5%)	5 (27.8%)	0.01
2	10	9 (56.3%)	1 (5.6%)	
3	9	1 (6.3%)	8 (44.4%)	
4	4	0 (0%)	4 (22.2%)	

KÜ Tıp Fak Derg 2020;22(1):79-88
Doi:10.24938/kutfd.671178

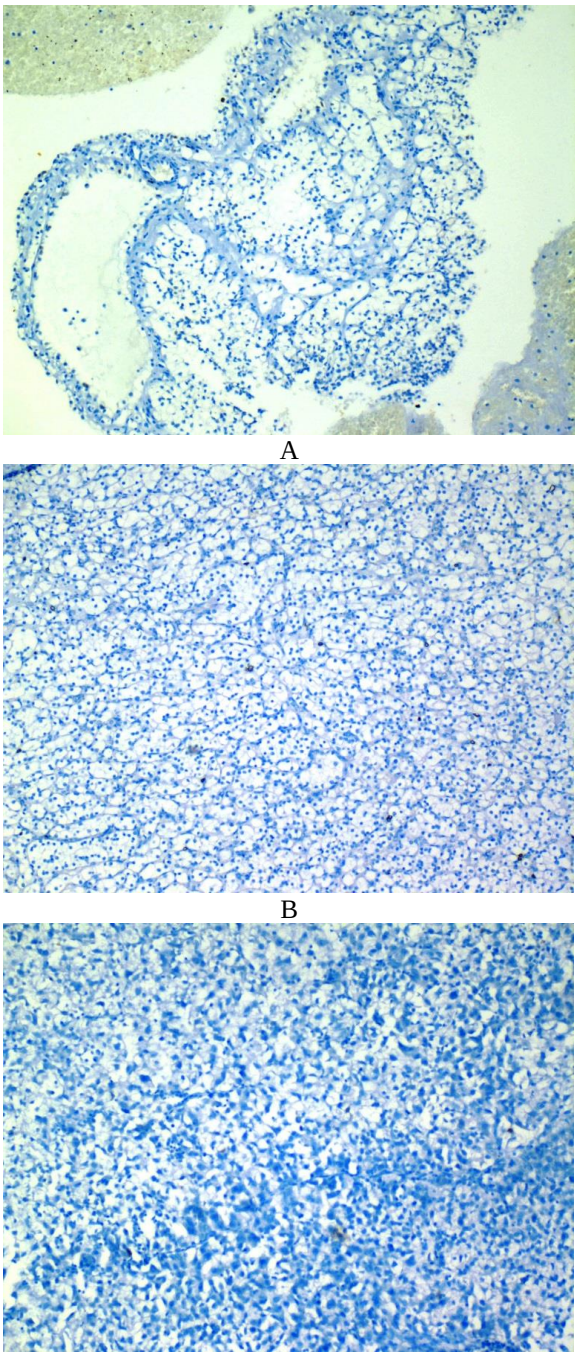
Table 4: Associations of the expression of Ki-67 with CD44 expression.

	Ki67	
	LEG (n = 16)	HEG (n = 18)
CD44 low	15 (93.8%)	6 (33.3%)
CD44 high	1 (6.3%)	12 (66.7%)

LEG = <%15 immunohistochemical expression, HEG = ≥15% immunohistochemical expression, (p<0.01).

Our study showed the concomitant upregulation of CD44 and Ki-67 in 66.7% of clear cell RCC specimens. Although we used positive controls and applied immunohistochemical staining three times, no antigen-antibody interaction was detected by the p53 staining (Figure 3).

Subsequently the relation between CD44 expression and clinicopathological factors were analyzed. There were not any meaningful differences in pT, tumor size, sex, and age parameters. CD44-HEG group was correlated positively with a higher nuclear grade (p=0.016, r=0.41). Additionally, we evaluated the relation of Ki-67 expression and clinicopathological factors. However, there were no statistically significant differences in age, gender, pT or tumor size. Furthermore, the Ki67-HEG group was positively correlated with a higher nuclear grade (p=0.01, r=0.436). We also found a positive correlation between Ki-67 and CD44 expressions (p<0.01, r=0.621).



A

B

C

Figure 3: Representative photomicrographs of immunohistochemical staining for P53 in renal cell carcinoma tissues. (*x100*). No staining intensity. Grade 1 (A), grade 2 (B), and grade 4 (C).

KÜ Tıp Fak Derg 2020;22(1):79-88
Doi:10.24938/kutfd.671178

DISCUSSION

Considering the recent accomplishments in genetic and molecular investigation, the prognostic assessment of RCC is still relying on clinicopathological and molecular factors (18). Inadequate comprehension on the biological heterogeneity of the RCC can cost time and treatment potential of patients (21). Recently no valid and potent immunohistochemistry markers are known which are routinely utilized for RCCs. In the current age of novel therapy choices, there are requirements of first priority for better prognostic markers to follow-up RCC patients (22). Ki-67 behaves as an effective molecular agent of the aggressive behaviour displayed by tumors and therapy response for survival outcome assessment in several cancers including RCC (21). In this respect, Ki-67 is recently suggested as a powerful independent predictor in patients with non-metastatic localized clear cell RCC (22,23).

The use of suitable molecular prognostic markers, including Ki-67 which represents the tumor proliferative index, appears very promising (24). Similar to previous and recent studies the current research found that Ki-67 proliferative index increased in nuclear grade (p=0.01). (12,20,24-26). Also, Ki-67 was detected as positive in 94.1% of our cases. However, different studies have utilized different cut-off values for Ki-67 labelling index. Zheng et al. investigated clear cell RCC cases and determined Ki-67 positivity as 47.7% (12). In another article by Amouian and colleagues, 25 out of 30 tumors were diagnosed and 20 (66.6%) were positive for Ki67 (26). Besides, Delahunt and colleagues investigated 206 cases, and detected a high Ki-67 expression of 83% in RCC cases which is similar to our findings (27). Chakraborty et al. (2019) reported the tumors (54.0%) had Ki-67 labelling index ≥15% while the rest 46% of cases had Ki-67 <15% (20). When we take the 15% rate into account, we found a 52.9% Ki-67 positivity in 18 cases in our evaluation. When all these data are evaluated together with our results, we can conclude that WHO/ISUP grading in clear cell RCC would be associated with Ki-67 (p=0.01).

Cancer stem cells are proposed to have high tumor-initiating potential and to be competent to self-renew. In this way, they are hypothesized to drive cancer upkeep, progression and metastasis (28). In recent years, the emphasis given to various stem cell markers such as CD44 in RCCs has increased. Zanjani et al. reported higher CD44 expression associated with more aggressive behavior, tumor progression and worse prognosis in clear RCC cases (29). Furthermore, CD44 expression was related with more advanced tumor stage and higher tumor grade (30,31). In a breast cancer study, Uchino et al. reported that anti-CD44 antibody treatment significantly inhibited cell migration and invasion and they suggested CD44 as a potantial novel molecular target (32). Li et al. reported that high CD44 expression correlated with poor 5-year progression free survival, disease-specific survival, and overall survival. Also, CD44 expression also correlated with WHO/ISUP grade (33). Therefore, they suggested CD44 as a prognostic marker in RCC (33,34). We strongly detected that WHO/ISUP nuclear grade (p=0.035) as well as Ki-67 expression (p<0.01) are associated with CD44 expression also. Considering the results of the present study and in compliance with the report of Chakraborty et al. which is one of the most recent studies on this subject, it can be deduced that the significant increase in CD44 expression and Ki-67 labelling index with tumor grade suggest that these two markers are associated with tumor growth and progression in RCCs (20).

Previous studies proposed that loss of p53 function is a critical event in the evolution of RCC (35-37). Kang et al. (2019) reported that transglutaminase 2 (TGase-2) crosslinks p53 in autophagosomes, resulting in p53 depletion and the decrement of apoptosis in tumor cells (38). Inhibition of TGase-2 stabilizes p53 and induces tumor cells to enter apoptosis and proposed the

KÜ Tıp Fak Derg 2020;22(1):79-88
Doi:10.24938/kutfd.671178

mechanism of p53 degradation (38). Knezović Florijan et al. (2019) reported that p53 isoforms can be differentially expressed due to p53 mutational status and they emphasized to consider both p53 mutation and p53 isoforms expression in RCC clinical studies (39). In addition to that, p53 protein expressions in RCC subtypes were reported by Noroozinia et al., and high p53 negativity was noted in clear cell RCC (40). The authors found p53 to be positive in only two of 39 cases. Thus, 37 cases were identified significantly ($p = 0.000$) as *p53-negative* in clear cell RCC. Authors identified *p53-positive* cases as papillary or chromophobe type RCC. In agreement with Noroozinia and colleagues, Mombini et al. reported 79.4% p53-negativity in clear cell type of RCC (41). As for our study, it revealed no antigen-antibody interaction detected by the p53 staining, possibly exhibiting the depletion of p53 in our cases.

It is a fact that there is a lack of prognostic immunohistochemistry markers which are routinely used for RCCs. Nuclear grade is one of the reliable prognostic factors in RCC, and tumors with a high WHO/ISUP grade have a more aggressive phenotype. We determined significant relations between nuclear grade, Ki-67 and CD44. We also noted the importance of both Ki-67 proliferation index and CD44 immunohistochemistry in RCC prognosis and these two correlated with each other. Ki-67 is easily utilizable by most immunohistochemistry-equipped pathology laboratories. In addition to the use of Ki-67, we also recommend CD44 for predicting the prognosis of RCC. Moreover, our results underline the p53 depletion in clear cell RCC cases. When all these data are evaluated together, we concluded that increased expression of CD44 and Ki-67 in tumor tissues predicts an aggressive course of RCC patients and they can be used concominantly for the prognosis.

Acknowledgments: None.

Funding: None.

Conflict of Interest: None.

REFERENCES

1. Moch H, Humphrey PA, Ulbright TM, Reuter VE. World Health Organization (WHO) classification of tumours. Pathology and genetics of the urinary system and male genital organs. 4th ed. Geneva. WHO Press, 2016.
2. Gorin MA, Allaf ME. Diagnosis and Surgical Management of Renal Tumors. Springer, Cham, 2019.
3. Isik U, Kostek O, Demiray G, Dirican A, Simsek M, Buyuksimsek M et al. Real-life data from Turkey regarding the impact of first-line sunitinib and pazopanib in metastatic renal cell cancer. J Clin Oncol. 2019;37(15):e16075.
4. Barrisford GW, Singer EA, Rosner IL, Linehan WM, Bratslavsky G. Familial renal cancer: molecular genetics and surgical management. Int J Surg Oncol. 2011;2011:658767.
5. Farber NJ, Kim CJ, Modi PK, Hon JD, Sadimin ET, Singer EA. Renal cell carcinoma: the search for a reliable biomarker. Transl Cancer Res. 2017;6(3):620-32.
6. Sato T, Kawasaki Y, Maekawa M, Takasaki S, Saigusa D, Ota H et al. Value of global metabolomics in association with diagnosis and clinicopathological factors of renal cell carcinoma. Int J Cancer. 2019;145(2):484-93.
7. Domev H, Amit M, Laevsky I, Dar A, Itskovitz-Eldor J. Efficient engineering of vascularized ectopic bone from human embryonic stem cell-derived mesenchymal stem cells. Tissue Eng Part A. 2012;18(21-22):2290-302.
8. Yin T, Wang G, He S, Liu Q, Sun J, Wang Y. Human cancer cells with stem cell-like phenotype exhibit enhanced sensitivity to the cytotoxicity of IL-2 and IL-15 activated natural killer cells. Cell Immunol. 2016;300:41-5.
9. Costa WH, Rocha RM, Cunha IW, Guimaraes GC, Zequi Sde C. Immunohistochemical expression of CD44s in renal cell carcinoma lacks independent

prognostic significance. Int Braz J Urol. 2012;38(4):456-65.

10. Luo Y, Zhang X, Mo M, Tan Z, Huang L, Zhou H et al. High Ki-67 immunohistochemical reactivity correlates with poor prognosis in bladder carcinoma: a comprehensive meta-analysis with 13,053 patients involved. Medicine (Baltimore). 2016;95(15):e3337.
11. Xie Y, Chen L, Ma X, Li H, Gu L, Gao Y et al. Prognostic and clinicopathological role of high Ki-67 expression in patients with renal cell carcinoma: a systematic review and meta-analysis. Sci Rep. 2017;7:44281.
12. Zheng K, Zhu W, Tan J, Wu W, Yang S, Zhang J et al. Retrospective analysis of a large patient sample to determine p53 and Ki67 expressions in renal cell carcinoma. J Buon. 2014;19(2):512-6.
13. Gontero P, Ceratti G, Guglielmetti S Andorno A, Terrone C, Bonvini D et al. Prognostic factors in a prospective series of papillary renal cell carcinoma. BJU Int J. 2008;102(6):697-702.
14. Wang Z, Peng S, Jiang N, Wang A, Liu S, Xie H et al. Prognostic and clinicopathological value of p53 expression in renal cell carcinoma: a meta-analysis. Oncotarget. 2017;8(60):102361-370.
15. Santoro A, Vlachou T, Luzi L, Melloni G, Mazzarella L, D'Elia E et al. p53 loss in breast cancer leads to myc activation, increased cell plasticity, and expression of a mitotic signature with prognostic value. Cell Rep. 2019;26(3):624-38.
16. Soussi T, Wiman KG. Shaping genetic alterations in human cancer: the p53 mutation paradigm. Cancer Cell. 2007;12(4):303-12.
17. Miller LD, Smeds J, George J, Vega VB, Vergara L, Ploner A et al. An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. Proc Natl Acad Sci USA. 2005;102(38):13550-5.
18. Zhang F, Xie Y, Ma X, Gu L, Li H, Li X et al. Preoperative apolipoprotein B/A1 ratio is an independent prognostic factor in metastatic renal cell carcinoma. Urol Oncol. 2019;37(3):184.e9-e17.
19. College of American Pathologists (CAP). Accessed date: 6 January 2020: https://documents.cap.org/protocols/cp-kidney-17protocol-4011.pdf.
20. Chakraborty B, Sarkar P, Bhattacharya P, Ghosh T, Maiti K. Correlation of vascular endothelial growth factor (VEGF) and Ki-67 expression with histological grade and stage of renal cell carcinoma. J Evolution Med Dent Sci. 2019;8(10):706-11.
21. Menon SS, Guruvayoorappanb C, Sakthivelc KM, Rasmid RR. Ki-67 protein as a tumour proliferation marker. Clinica Chimica Acta. 2019;491:39-45.
22. Mehdi MZ, Nagi AH, Naseem N. MCM-2 and Ki-67 as proliferation markers in renal cell carcinoma: A quantitative and semi - quantitative analysis. Int Braz J Urol. 2016;42(6):1121-8.
23. Gayed BA, Youssef RF, Bagrodia A, Darwish OM, Kapur P, Sagalowsky A et al. Ki67 is an independent predictor of oncological outcomes in patients with localized clear-cell renal cell carcinoma. BJU Int J. 2014;113(4):668-73.
24. Toma MI, Weber T, Meinhardt M, Zastrow S, Grimm MO, Füssel S et al. Expression of the Forkhead Transcription Factor FOXP1 is Associated with Tumor Grade and Ki67 Expression in Clear Cell Renal Cell Carcinoma. Cancer Invest. 2011;29(2):123-9.
25. Wong PK, Lee ST, Murone C, Eng J, Lawrentschuk N, Berlangieri SU et al. In vivo imaging of cellular proliferation in renal cell carcinoma using 18F-fluorothymidine PET. Asia Ocean J Nucl Med Biol. 2014;2(1):3-11.
26. Amouian S, Farzadnia M, Memar B, Attaranzadeh A, Tayyebi N. Expression of P53 and Ki67 proteins in renal cell carcinoma and its relationship with nuclear grade. Iranian J Pathol. 2008;3(1):25-9.

27. Delahunt B, Bethwaite PB, Thornton A, Ribas JL. Proliferation of renal cell carcinoma assessed by fixation-resistant polyclonal Ki-67 antibody labeling. Correlation with clinical outcome. Cancer. 1995;75(11):2714-9.
28. Nguyen LV, Vanner R, Dirks P, Eaves CJ. Cancer stem cells: an evolving concept. Nat Rev Cancer. 2012;12(2):133-43.
29. Zanjani LS, Madjd Z, Abolhasani M, Rasti A, Fodstad O, Andersson Y et al. Increased expression of CD44 is associated with more aggressive behavior in clear cell renal cell carcinoma. Biomark Med. 2018;12(1):45-61.
30. Lim SD, Young AN, Paner GP, Amin MB. Prognostic role of CD44 cell adhesion molecule expression in primary and metastatic renal cell carcinoma: a clinicopathologic study of 125 cases. Virchows Archiv. 2008;452(1):49-55.
31. Qin J, Yang B, Xu BQ, Smithc A, Xu L, Yuan JL et al. Concurrent CD44s and STAT3 expression in human clear cell renal cellular carcinoma and its impact on survival. Int J Clin Exp Pathol. 2014;7(6):3235-44.
32. Uchino M, Kojima H, Wada K, Imada M, Onoda F, Satofuka H et al. Nuclear β-catenin and CD44 upregulation characterize invasive cell populations in non-aggressive MCF-7 breast cancer cells. BMC Cancer. 2010;10:414.
33. Li X, Ma X, Chen L, Gu L, Zhang Y, Zhang F et al. Prognostic value of CD44 expression in renal cell carcinoma: a systematic review and meta-analysis. Sci Rep. 2015;19(5):13157.
34. Jeong BJ, Liang Z, Huang SM, Lim JS, Kim JM, Lee HJ. CD44 is associated with tumor recurrence and is an independent poor prognostic factor for patients with localized clear cell renal cell carcinoma after nephrectomy. Exp Ther Med. 2012;3(5):811-7.
35. Warburton HE, Brady M, Vlatković N, Linehan WM, Parsons K, Boyd MT. p53 Regulation and Function in Renal Cell Carcinoma. Cancer Res. 2005;65(15):6498-503.
36. Haitel A, Wiener HG, Baethge U, Marberger M, Susani M. MDM2 expression as a prognostic indicator in clear cell renal cell carcinoma: comparison with p53 overexpression and clinicopathological parameters. Clin Cancer Res. 2000;6(5):1840-4.
37. Zigeuner R, Ratschek M, Rehak P, Schips L, Langner C. Value of p53 as a prognostic marker in histologic subtypes of renal cell carcinoma: a systematic analysis of primary and metastatic tumor tissue. Urol. 2004;63(4):651-5.
38. Kang JH, Lee SH, Cheong H, Lee CH, Kim SY. Transglutaminase 2 promotes autophagy by LC3 induction through p53 depletion in cancer cell. Biomol Ther (Seoul). 2019;27(1):34-40.
39. Knezović Florijan M, Ozretić P, Bujak M, Pezzè L, Ciribilli Y, Kaštelan Ž et al. The role of p53 isoforms' expression and p53 mutation status in renal cell cancer prognosis. Urol Oncol. 2019;37(9):578.e1-578.e10.
40. Noroozinia F, Fahmideh AN, Yekta Z, Rouhrazi H, Rasmi Y. Expression of CD44 and P53 in renal cell carcinoma: association with tumor subtypes. Saudi J Kidney Dis Transpl. 2014;25(1):79-84.
41. Mombini H, Givi M, Rashidi I. Relationship between expression of p53 protein and tumor subtype and grade in renal cell carcinoma. Urol J. 2006;3(2):79-81.