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# The role of COL6A1and PD–1 expressions in renal cell carcinoma

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## ABSTRACT

**Objective:** The COL6A1 is a gene encoding the alpha 1 polypeptide subunit of collagen 6 (COL6A1), an extracellular matrix protein subunit. Programmed cell death receptor-1 (PD-1) and its ligand, programmed cell death receptor ligand-1 (PD-L1) have been shown to have a prognostic significance in clear cell renal cell carcinomas (RCCs). In this study, we evaluated the expressions of COL6A1 and PD-1 in four different RCC subtypes.

**Materials and methods:** A total of 161 radical nephrectomy and nephron-sparing surgery cases with RCCs from five different health care centers were included in this study. Clinical data of the cases were taken from electronic records of the institutions. The pathological data were collected by an expert uropathologist and re-evaluated with slides obtained from paraffin blocks of the cases. The correlation of COL6A1 and PD-1 expression with sex, age, tumor type, lymphovascular invasion (LVI), World Health Organization/International Society of Urological Pathology (WHO/ISUP) grade, and tumor stage (pT) was analyzed with the Pearson chi-squared test.

**Results:** Patients with sarcomatoid RCC and clear cell RCC had significantly higher COL6A1 scores and intensities than in other types of RCC ($p=0.004$ and $p=0.002$, respectively). WHO/ISUP grade and, COL6A1 and PD-1 staining scores also showed positive correlation ($r=0.230$, $p=0.004$ and $r=0.277$, $p=0.001$, respectively for COL6A1 and $r=0.191$, $p=0.018$ and $r=0.166$, $p=0.041$, respectively for PD-1). The staining scores and intensities of COL6A1 and PD-1 were not different between the patients with positive and negative LVI ($p>0.05$).

**Conclusion:** In high-grade RCCs, we found the relationship between immunohistochemical staining scores of COL6A1 and PD-1 proteins and clinical, demographic, and histopathological parameters. Our results proved that COL6A1 and PD-1 are really promising proteins as prognostic parameters and for targeted immunotherapy.

**Keywords:** Renal cell carcinoma; COL6A1; PD-1.

## Introduction

Renal cell carcinoma (RCC) is a carcinoma originating from the renal cortex and accounts for 80-85% of all primary renal tumors.[1] It is the seventh most common cancer in men and ninth in women.[2] RCC actually represents a group of malignancies that may have very different histopathological and clinical features. Because the clinical features and treatments of different subtypes may be diverse, new studies are published at an increasing rate to find out new histopathological markers to differentiate these subtypes. Programmed cell death receptor-1 (PD-1), one of the prominent substances in this context, has been shown to be a useful prognostic marker in clear cell RCCs.[3]

PD-1 and its ligand (PD-L1) were discovered while investigating the tumor microenvironment.[4] Nivolumab, an anti-PD-1 antibody was recently approved by the U.S. Food and Drug Administration for the treatment of metastatic RCC. Because PD-1 and its ligands are known to be involved in inhibiting the recognition of certain tumors by the immune system[4],

Karabulut et al. COL6A1and PD–1 in renal cell carcinoma

283

Nivolumab and other anti-PD-1, anti-PD-L1 antibodies which are currently in clinical investigation[5-8], are thought to act by modifying the immune response. To date, in some of the studies on PD-1 expression in RCC cases, increased expression of PD-1 was associated with increased risk of cancer-related death[9,10] and recurrence.[11]

*COL6A1* is a gene that encodes the alpha 1 polypeptide of collagen 6 (COL6A1), an extracellular matrix protein. Collagen 6 has been shown to play a role in malignant tumor progression.[12] In addition, a study by Chiu et al. [13] found that the metastatic ability of non-COL6A1 lung cancer cells was suppressed, but lung cancer cells overexpressing COL6A1 had increased metastatic ability. The only study found in the literature regarding the potential role of COL6A1 in RCCs was conducted by Wan et al.[14] in 2015, which found an increase in COL6A1 expression was correlated with poor prognosis in cases of clear cell RCC. COL6A1 was also shown to promote tumor growth in vivo.

According to the World Health Organization (WHO) classification revised in 2016, RCC has four main histological subgroups.[15] Clear cell RCC accounts for the majority of all RCCs (75-83%)[16] and therefore the primary renal malignancies are divided into clear cell RCC and non-clear cell RCC.[15] The common subtypes in the non-clear cell RCC group were papillary RCC (11.3%), chromophobe RCC (4.3%) and collecting duct carcinoma (0.3%), respectively.[15,16]

Sarcomatoid differentiation is considered to be a poor prognostic factor and includes RCCs that cover a small amplification site (×40) and have the following conditions for sarcomatoid differentiation; the presence of carcinoma in the adjacent area or the presence of epithelial differentiation in spindle cells.[15] In this study, RCCs carrying sarcomatoid differentiation conditions were evaluated as a different entity called sarcomatoid RCC. We evaluated the expressions of COL6A1 and PD-1 in four different RCC subtypes.

**Main Points:**

- To our knowledge, our study is the first one in which, the relationship between PD-1 and COL6A1 expressions and prognostic parameters are evaluated specifically.

- Patients with sarcomatoid RCC and clear cell RCC had significantly higher COL6A1 scores and intensities than in other types of RCC.

- WHO/ISUP grade and, COL6A1 and PD-1 staining scores also showed positive correlation.

- COL6A1 and PD-1 are really promising proteins as prognostic parameters and for targeted immunotherapy.

## Materials and methods

This study was approved by the Clinical Research Ethics Committee of Mersin University. The study included 161 radical nephrectomy and nephron-sparing surgery cases with RCCs from five different health care centers. Clinical data of the

**Table 1. Distribution of patients included in the study according to parameters**

| Group | Parameter | N | % |
|---|---|---|---|
| Diagnosis | Clear cell RCC | 116 | 72.05 |
| | Papillary RCC | 26 | 16.15 |
| | Chromophobe RCC | 9 | 5.59 |
| | Sarcomatoid RCC | 10 | 6.21 |
| pT | 1b | 10 | 6.21 |
| | 2a | 19 | 11.80 |
| | 2b | 10 | 6.21 |
| | 3a | 100 | 62.11 |
| | 3b | 7 | 4.35 |
| | 4 | 15 | 9.32 |
| LVI | Present | 120 | 74.53 |
| | Absent | 41 | 25.47 |
| WHO/ISUP grade | 1 | 1 | 0.62 |
| | 2 | 51 | 31.68 |
| | 3 | 68 | 42.24 |
| | 4 | 32 | 19.88 |
| Sex | Male | 112 | 69.57 |
| | Female | 49 | 30.43 |
| COL6A1 score | 0 | 74 | 45.96 |
| | 1 | 39 | 24.22 |
| | 2 | 39 | 24.22 |
| | 3 | 9 | 5.59 |
| PD-1 score | 0 | 71 | 44.10 |
| | 1 | 34 | 21.12 |
| | 2 | 38 | 23.60 |
| | 3 | 18 | 11.18 |

RCC: renal cell carcinoma; pT: tumor stage; LVI: lymphovascular invasion; WHO/ISUP: World Health Organization/International Society of Urological Pathology; PD-1: Programmed cell death receptor-1
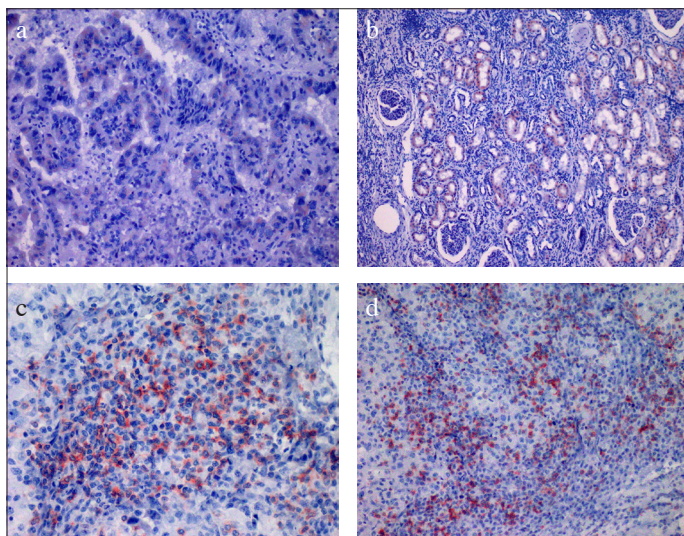
Figure 1. a-d. PD-1 staining. Score 0, there is no PD-1 expression (H&E; ×400) (a). Score 1, staining of 1–25% of tumor (H&E; ×100) (b). Score 2, staining of 25–50% of tumor (H&E; ×400) (c). Score 3, staining >50% of tumor (H&E; ×200) (d)
PD-1: programmed cell death receptor-1; H&E: hematoxylin and eosin



Figure 2. a-d. COL6A1 staining. Score 0, there is no COL6A1 expression (H&E; ×400) (a). Score 1, staining of 1–25% of tumor (H&E; ×400) (b). Score 2, staining of 25–50% of tumor (H&E; ×400) (c). Score 3, staining >50% of tumor (H&E; ×400) (d)
COL6A1: alpha 1 polypeptide of collagen 6; H&E: hematoxylin and eosin

cases were taken from electronic records of the institutions. The pathological data were collected by an expert uropathologist and re-evaluated with slides obtained from paraffin blocks of the cases. COL6A1 and PD-1 expression levels were examined immunohistochemically.

COL6A1 and PD-1 expression intensities were examined immunohistochemically through antibodies, and the staining area percentages of these immunohistochemical stains were scored between 0-3 (≤1%, 0; 1-25%, 1; 25-50%, 2; >50%, 3) (Figures 1 and 2).[14] Positive areas of stroma and walls of vascular structures were excluded from the staining area percentage.
**Statistical analysis**

Possible correlation among the staining score of COL6A1 and PD-1, gender, RCC subtypes, WHO/International Society of Urological Pathology (ISUP) grade, lymphovascular invasion (LVI), and tumor stage (pT) were investigated with the Pearson chi-squared test. In addition, *t*-test was used to investigate the relationship between the staining scores of COL6A1 and PD-1 in the same tissues. Statistical analysis was performed with Statistical Package for the Social Sciences software (version 17.0, IBM SPSS Corp., Armonk, NY, USA) and a p-value of less than 0.05 was considered statistically significant.

## Results

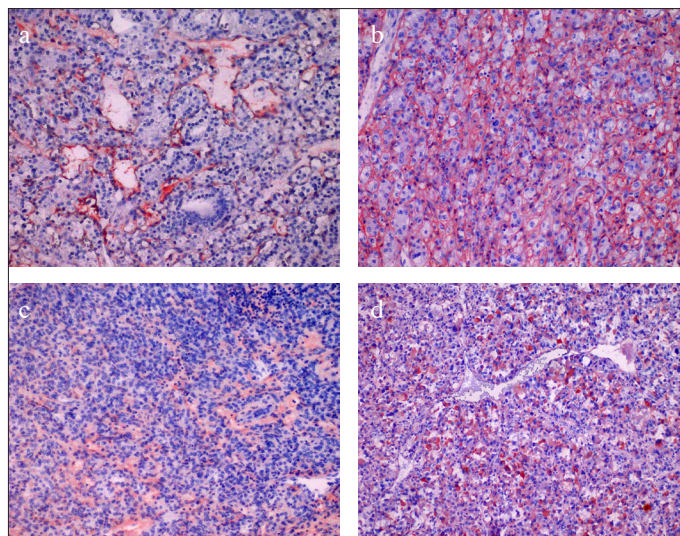A total of 161 patients, including 112 males (69.5%) and 49 females (30.5%), were included in the study. The mean age of the patients was 61 years (38-92 years). The patients were diagnosed with clear cell RCC (n=116, 71.2%), papillary RCC (n=26, 16%), chromophobe RCC (n=9, 5.5%), and sarcomatoid RCC (n=10, 6.1%). The parameters of the patients were summarized in Table 1.

In our study, for immunohistochemical staining scores and staining intensity, statistically significant correlations were found between COL6A1 and PD-1 (r=0.208, p=0.008 and r=0.260, p=0.001, respectively). Comparison of sexes revealed that male patients had slightly higher PD-1 scores, but the difference was not statistically significant. Patients with sarcomatoid RCC and clear cell RCC had higher COL6A1 scores and intensities than other RCC types significantly (p=0.004 and p=0.002, respectively) while PD-1 staining intensities were slightly higher. PD-1 staining scores were not different from other RCC types (p>0.05).

WHO/ISUP grade and scores with intensities for COL6A1 and PD-1 showed positive correlation (r=0.230, p=0.004 and r=0.277, p=0.001, respectively for COL6A1 and r=0.191, p=0.018 and r=0.166, p=0.041, respectively for PD-1). The COL6A1 scores and intensities were significantly higher in WHO/ISUP grade 4 tumors (p=0.002 and p=0.055, respectively). The mean values of immunohistochemical staining were 0.0% (grade1), 21.7% (grade 2), 20.6% (grade 3) and 46.1% (grade 4) (p=0.0001) while PD-1 scores and intensities were not different among grade groups (p>0.05).

The staining scores and intensity of COL6A1 with PD-1 were not different between LVI positive and negative patients

Karabulut et al. COL6A1and PD−1 in renal cell carcinoma

285

**Table 2. The relationship of various parameters with COL6A1 staining scores and intensity**

| | COL6A1 intensity | | | | | COL6A1 score | | | | |
| | % | | | | | % | | | | |
| Parameter | 0 | 1 | 2 | 3 | p | 0 | 1 | 2 | 3 | p |
|---|---|---|---|---|---|---|---|---|---|---|
| **Diagnosis** | | | | | | | | | | |
| Clear cell RCC | 36.2 | 30.2 | 27.6 | 6.0 | | 36.2 | 18.1 | 18.1 | 27.6 | |
| Papillary RCC | 80.8 | 7.7 | 11.5 | 0.0 | 0.002 | 80.8 | 7.7 | 7.7 | 3.8 | 0.004 |
| Chromophobe RCC | 77.8 | 11.1 | 11.1 | 0.0 | | 77.8 | 22.2 | 0.0 | 0.0 | |
| Sarcomatoid RCC | 40.0 | 10.0 | 30.0 | 20.0 | | 40.0 | 10.0 | 20.0 | 30.0 | |
| **WHO/ISUP grade** | | | | | | | | | | |
| 1 | 100.0 | 0.0 | 0.0 | 0.0 | | 100.0 | 0.0 | 0.0 | 0.0 | |
| 2 | 51.0 | 25.5 | 21.6 | 2.0 | 0.002 | 51.0 | 11.8 | 19.6 | 17.6 | 0.055 |
| 3 | 50.0 | 29.4 | 14.7 | 5.9 | | 50.0 | 19.1 | 11.8 | 19.1 | |
| 4 | 18.8 | 15.6 | 53.1 | 12.5 | | 18.8 | 15.6 | 21.9 | 43.8 | |
| **LVI** | | | | | | | | | | |
| Present | 46.3 | 24.4 | 24.4 | 4.9 | 0.997 | 46.3 | 12.2 | 17.1 | 24.4 | 0.868 |
| Absent | 45.8 | 24.2 | 24.2 | 5.8 | | 45.8 | 17.5 | 15.0 | 21.7 | |
| **pT** | | | | | | | | | | |
| 1.Group (1b, 2a, 2b) | 41.0 | 23.1 | 35.9 | 0.0 | 0.108 | 41.0 | 25.6 | 15.4 | 17.9 | 0.309 |
| 2.Group (3a, 3b, 4) | 47.5 | 24.6 | 20.5 | 7.4 | | 47.5 | 13.1 | 15.6 | 23.8 | |

RCC: renal cell carcinoma; pT: tumor stage; LVI: lymphovascular invasion; COL6A1: alpha 1 polypeptide of collagen 6; WHO/ISUP: World Health Organization/International Society of Urological Pathology

(p>0.05). Neither COL6A1 nor PD-1 scores showed any statistically significant correlation with tumor stage (pT) (p>0.05).

The relationship of various parameters with COL6A1 staining scores and intensity were showed Table 2. The staining patterns of tumors showed some variations. WHO/ISUP grade 4 tumors showed cytoplasmic staining of tumor cells with large cytoplasm. Also, poorly differentiated tumors had extensive staining of their sarcomatoid type stroma. Papillary RCC tumors with clear cell morphology showed more extensive staining with COL6A1 and PD-1.

## Discussion

RCCs constitutes the majority of primary renal tumors and represents a highly heterogeneous group of malignant neoplasms originating from the renal cortex. Studies on different histomorphological and clinical features of carcinomas under the heading of RCCs have recently been increasing. Although there are differ-

ent prognostic models, the prominent prognostic parameters are histological subtype, WHO/ISUP grade, tumor necrosis, perirenal adipose tissue invasion, vascular embolization and invasion, TNM stage, and the presence of metastasis.[17,18]

Studies on tumor microenvironment, which is a relatively new research focus in determining the clinical features of cancers, have enabled the development of new therapeutic targets and enabled the extraction of histopathological features with clinical implications. In this context, PD-1, one of the most frequently investigated proteins, is associated with immune system response, especially in the tumor microenvironment, and has been shown to have clinical and prognostic significance in some tumors. Antibodies developed against PD-1 and PD-1 ligands have begun to be routinely used in cancer treatments, and clinical trials of further treatment agents are currently underway. The second protein COL6A1 included in this study represents the alpha 1 subunit of collagen 6, one of the main proteins of the extracellular matrix. Collagen 6 has been shown to play an important role in tumorigenesis and tumor progression, and

changes in expression have been reported to be associated with poor prognosis in clear cell RCC.[14]

Supporting the current literature, in our study, which carried on with high WHO/ISUP grade (2 or more) tumors, significant relationship was found between the WHO/ISUP grade and COL6A1 immunohistochemical staining scores. COL6A1 has been confirmed as a TGF-β/Smad target in human dermal fibroblasts.[12] Wan et al. [14] claimed that TGF-β overactivation in cancer cells secreted and acted on surrounding stromal cells, these cells proliferate and increase TGF-β secretion. This over-abundance of TGF-β causes immunosuppression and angiogenesis and increases the invasive ability of cancer cells. Therefore, COL6A1 upregulation may be a consequence of TGF-β activation in tumors leading to poor patient prognosis. Some published data suggests that sorafenib inhibits TGF-β activity[17,18]; thus, COL6A1 expression level may also reflect patient responses to sorafenib treatment in metastatic ccRCC. Because the WHO/ISUP grade is shown to be a strong prognostic parameter, the fact that COL6A1 and PD-1 expressions are statistically significant between different WHO/ISUP grades, this may indicate these anticors also can be used as prognostic parameters.

When the relationship of COL6A1 and PD-1 expressions with sex was examined, it was seen that there was no statistically significant difference. This finding suggests that there is no expected sex difference in the efficacy in treatment agents developed against PD-1 or potentially developed against COL6A1. We found that the immunohistochemical staining scores of COL6A1 and PD-1 were significantly different in the four different RCC subtypes. This finding suggests that COL6A1 and PD-1 immunohistochemical expressions can be used as diagnostic parameters in high-grade RCCs. The majority (62.1%) of RCCs included in our study were advanced stage (pT3a) tumors. The absence of a significant relationship between the tumor stage and the expressions of COL6A1 and PD-1 indicates a low level of prognostic significance of these proteins in advanced stage tumors.

This study is mainly limited by the retrospective nature. On the other hand, there are studies that evaluate the relationship between PD-1 expression and prognosis[19-21]; to our knowledge, our study is the first one in which, the relationship between PD-1 and COL6A1 expressions and prognostic parameters are evaluated specifically.

## Conclusion

RCC represents a very heterogeneous group of diseases, and the clinical features of these diseases are also different from each other. It is important to find molecules and proteins that can be used as prognostic parameters and targets for targeted treatment. We examined the relationship between immunohistochemical staining scores of PD-1 and COL6A1 proteins and clinical, demographic, and histopathological parameters. The results proved that, PD-1 and COL6A1 are really promising proteins as prognostic parameters and for targeted immunotherapy.

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Karabulut et al. COL6A1and PD–1 in renal cell carcinoma

287

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