

Association of Androgen Receptor and PD-L1 Expression in Upper Urinary Tract Urothelial Carcinoma

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Abstract. *Background/Aim:* The response to immune checkpoint inhibitors (ICIs) or enfortumab vedotin is limited in patients with upper urinary tract urothelial carcinoma (UTUC), and the development of new targeted therapy for UTUC is eagerly needed. Several biomarkers, including programmed cell death-ligand 1 (PD-L1), have already been reported as predictors of response to ICIs therapy for UTUC. Recently, several studies have shown that steroid hormone receptors, including the androgen receptor (AR), are associated with progression of urothelial carcinoma. *Materials and Methods:* We prepared tissue microarrays (TMA) from paraffin blocks of UTUC specimens in 99 non-metastatic UTUC patients who underwent radical nephroureterectomy. With these TMA sections, we performed immunohistochemical staining for PD-L1 and AR and examined PD-L1 and AR expression levels in tumor cells. In addition, we analyzed the correlation between these markers and clinical prognosis in UTUC cases. *Results:* PD-L1 was positive in 24 (24%) of the 99 samples, whereas AR was positive in 20 (20%) patients. AR-negative samples had significantly higher PD-L1 expression level than that the AR-positive samples (mean value 4.70% versus 2.55%, $p=0.0324$). Among AR-positive cases, patients with absence of

PD-L1 expression had significantly lower cancer-specific survival (CSS) than that in PD-L1 expression-positive cases ($p=0.049$), although PD-L1 expression had no significant impact on CSS in AR-negative cases ($p=0.920$). *Conclusion:* Our findings suggest that AR is the promising target for UTUC treatment, especially in PD-L1-negative cases.

Upper urinary tract urothelial carcinoma (UTUC) is a subset of urothelial cancer (UC) and accounts for 5-10% of all UCs (1). Although UTUC shares many clinicopathological features with urothelial carcinoma of the bladder (UCB), recent advances in comprehensive genetic analysis have revealed a different profile of genetic abnormalities between UTUC and UCB cases (2, 3). Compared to UCBs, *TP53*, *RB1* and *ERBB2* were less frequently altered in UTUCs (26% vs. 46%, 3% vs. 20%, 8% vs. 19%, respectively), whereas *FGFR3* and *HRAS* were more frequently altered (40% vs. 26%, 12% vs. 4%, respectively) (2). In terms of the clinical features of these cancers, 60% of UTUC are initially diagnosed as muscle-invasive carcinoma due to the difficulty of early diagnosis of UTUC. In contrast, 20% of UCBs are initially diagnosed as muscle-invasive (4). Moreover, 5-year overall survival (OS) rate for patients with muscle-invasive UTUC used to be less than 40% and they had poorer prognosis than muscle-invasive UCB (5).

The advent of ICIs, led by pembrolizumab or atezolizumab, is expected to improve OS and progression-free survival in patients with UC (6, 7). Particularly, hypermutated UTUCs or a subset of *TP53/MDM2*-mutated UTUCs might benefit from ICIs because of their high tumor mutational burden or high expression of immune checkpoint molecules, respectively (3). However, response to ICI therapy is still limited in UTUCs (8). To date, several biomarkers including programmed cell death-ligand 1 (PD-L1) have been proposed to predict therapeutic response to ICIs in UCBs as well as UTUCs (9, 10).

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Key Words: Androgen receptor, AR, programmed cell death ligand 1, PD-L1, upper urinary tract urothelial carcinoma, UTUC.



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Recently, preclinical evidence has indicated the involvement of sex hormones and their receptor signals in urothelial cancer outgrowth, which may explain some of the sex disparity (11). In patients with UTUCs, it has been reported that the expression of steroid hormone receptors has prognostic significance (12). Especially, androgen receptor (AR) activation is associated with induction of urothelial tumorigenesis and UC progression (13, 14). However, little is known about the mutual association between the expression level of PD-L1 and AR in UTUCs, which led us to conduct the present study to evaluate these expression levels in UTUC samples and to analyze the association of their expression status with clinical outcomes in UTUC patients.

Materials and Methods

Patients and tissue microarrays. The UTUC tissue microarrays (TMAs) were constructed with spotted triplicate urothelial tumor samples (from dominant tumors/invasive components if present) and paired normal-appearing urothelial tissues (from the renal pelvis and ureter) obtained from 99 patients with non-metastatic UTUC, who underwent radical nephroureterectomy performed at Osaka General Medical Center from 1997 to 2011, as described previously (15, 16). We obtained appropriate approval from the local institutional review board (Osaka General Medical Center Institutional Review Board, Protocol Number: 25-2014, 19 June 2013) as well as written informed from all patients before construction and use of the TMAs.

Immunohistochemical analysis. TMA sections were stained for PD-L1 (1:100; clone E1L3N; Cell Signaling Technology, Danvers, MA, USA) and AR (1:200; clone N-20; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The extent of membranous PD-L1 expression on tumor cells was determined at each spot (0-100%). The average PD-L1 expression level for all spots in each case was calculated and a 1% positive cutoff value was used to classify PD-L1 staining as negative or positive. AR was classified as negative or positive, as we described previously (17). All stains were evaluated by a single experienced urologic pathologist (HM).

Statistical analysis. Statistical analyses were performed using JMP® Pro 14.0.0 (SAS Institute Inc., Cary, NC, USA). The Mann-Whitney *U*-test (two-tailed) was performed to detect significant differences in PD-L1 expression rates by sex, affected side of the renal pelvis or ureter, grade, age, and AR expression. Fisher's exact test was used to evaluate the association between sex and localization of the primary tumor and the expression status of AR. Cancer specific survival rates were determined using the Kaplan-Meier method, and the log-rank test was used for comparison. $p < 0.05$ was considered statistically significant, and $p < 0.1$ was considered to be a statistical trend. In the clinical course, tumor progression was defined as the development of lesions beyond the lower urinary tract, such as recurrence at the site of nephroureterectomy, lymph node metastasis, or visceral metastasis.

Results

Patients' characteristics. The clinicopathological characteristics of the 99 patients included in the study are

Table I. Clinicopathological details and outcome of 99 upper urinary tract urothelial carcinoma patients.

Variable	
Age (year), median (range)	71 (48-87)
Sex, n (%)	
Male	60 (60.6)
Female	39 (39.4)
Laterality, n (%)	
Right	43 (43.4)
Left	56 (56.6)
Tumor location, n (%)	
Renal pelvis	45 (45.5)
Ureter	50 (50.5)
Both	4 (4.0)
Tumor grade, n (%)	
Low grade	15 (15.2)
High grade	84 (85.9)
Pathological T stage, n (%)	
pTa	19 (19.2)
pT1	18 (18.2)
pT2	8 (8.1)
pT3	48 (48.5)
pT4	6 (6.1)
Lymphovascular invasion, n (%)	
No	59 (59.6)
Yes	40 (40.4)
Lymph node metastasis, n (%)	
pN0	84 (84.8)
pN+	12 (12.1)
pNx	3 (3.0)
Neoadjuvant chemotherapy, n (%)	
No	99 (100)
Yes	0 (0.0)
Adjuvant chemotherapy, n (%)	
No	73 (73.7)
Yes	26 (26.3)
Tumor progression, n (%)	
No	61 (61.6)
Yes	38 (38.4)
Follow-up (months), median (range)	37 (1-173)

shown in Table I. Of the 99 cases, the median age was 71 years at the time of surgery and 60 patients (60.6%) were male. The median follow-up period was 37 months (range=1-173). 84 patients (85.9%) had high-grade tumors, and 62 patients (62.7%) had muscle-invasive tumors (pT2, pT3, or pT4). None of the patients received neoadjuvant therapy, but 26 patients (26.3%) received MVAC (methotrexate, vinblastine, adriamycin, and cisplatin) therapy for 2 to 3 cycles as postoperative adjuvant chemotherapy. During follow-up, metachronous or synchronous recurrence in the lower urinary tract was observed in 32 patients (32.3%), and tumor progression was observed in 38 patients (38.4%).

Inverse relationship between the expression level of androgen receptor and programmed cell death-ligand 1. To

Table II. Association between androgen receptor and PD-L1 expression in upper urinary tract urothelial carcinoma patients determined by immunohistochemistry.

	PD-L1-negative, n (%)	PD-L1-positive, n (%)	Total
AR-negative, n (%)	62 (63)	17 (17)	79 (80)
AR-positive, n (%)	13 (13)	7 (7)	20 (20)
Total	75 (76)	24 (24)	99 (100)

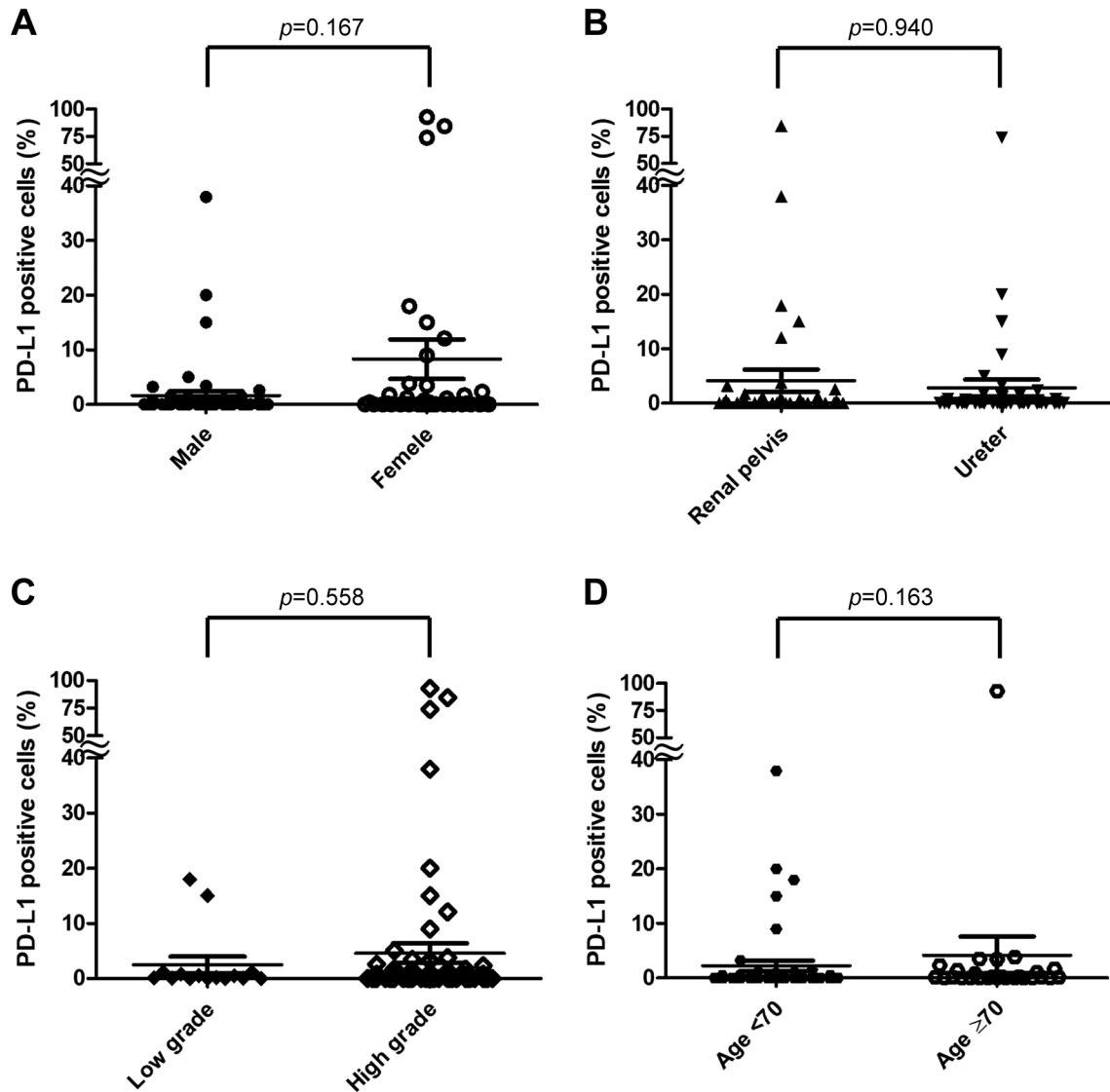


Figure 1. PD-L1 expression by sex, affected side, tumor grade, and age. There was no significant difference of PD-L1 positivity between (A) male and female, (B) renal pelvis and ureter, (C) low and high grade, and (D) age <70 and ≥70.

investigate the relationship of PD-L1 and AR expression in UTUC, we evaluated the positivity of PD-L1 and AR on cancer cells by immunohistochemical analysis. As shown in Table II, PD-L1 was negative in 75 (76%) and positive in 24

(24%) patients, whereas AR was negative in 79 (80%) and positive in 20 patients (20%). No significant difference in PD-L1 expression was observed by sex, affected side renal pelvis or ureter, tumor grade, or age ($p=0.167$, 0.121 , 0.558

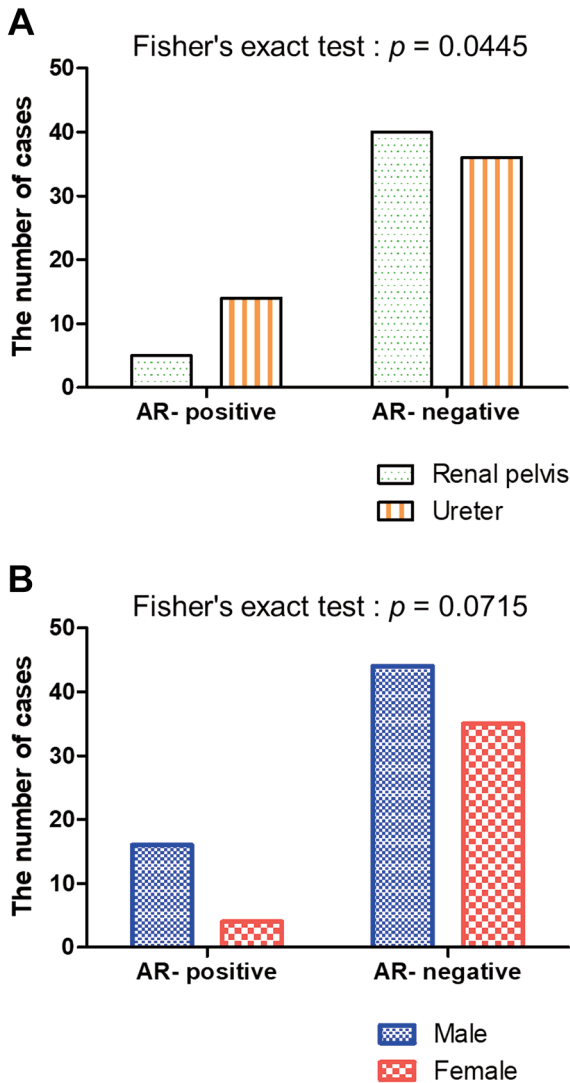


Figure 2. The positive incidence of androgen receptor expression by affected side renal pelvis or ureter and sex. (A) The positive incidence of androgen receptor (AR) expression in ureteral cancer cells was significantly higher than that in renal pelvis cancer cells ($p=0.0445$). (B) The positive incidence of AR expression on cancer cells in males tended to be higher than that in females ($p=0.0715$). Fisher's exact test was performed to evaluate the association between localization of the primary tumor and the expression status of AR. AR: Androgen receptor.

and 0.163, respectively) (Figure 1). Regarding the expression status of AR, the positive incidence of AR expression in ureteral cancer was significantly higher than that in renal pelvis cancer ($p=0.0445$). On the other hand, there was a trend of higher positive incidence of AR expression on cancer cells in males compared to females ($p=0.0715$) (Figure 2). Remarkably, the AR negative group had significantly higher PD-L1 expression levels (mean value=4.70%) compared with that in the AR-positive group

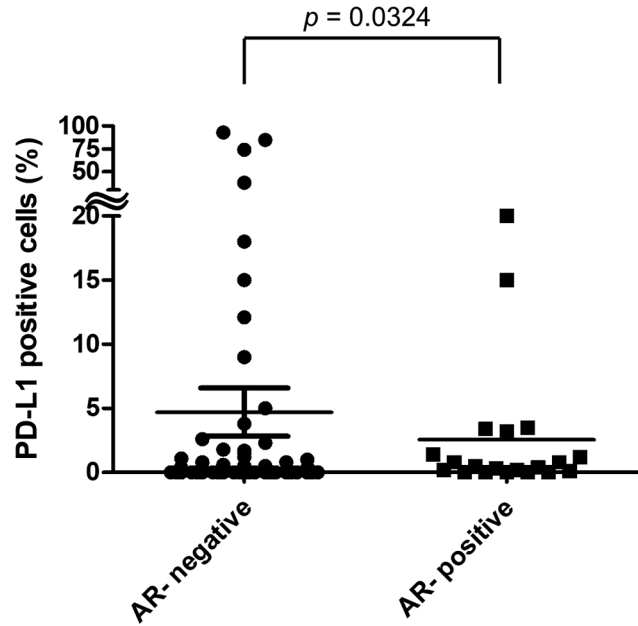


Figure 3. Correlation of the expression level of androgen receptor and PD-L1 in upper urinary tract urothelial carcinoma. Androgen receptor (AR)-negative patients had significantly higher PD-L1 expression levels (mean value; 4.70%) compared with AR-positive group (mean value; 2.55%) ($p=0.0324$). AR: Androgen receptor.

(mean value=2.55%) ($p=0.0324$, Figure 3), indicating an inverse relationship between AR and PD-L1 expression in UTUC patients.

PD-L1 negativity is associated with poor cancer-specific survival in AR-positive UTUC. We then stratified our patient cohort into four groups depending on the presence or absence of AR and PD-L1 expression and compared cancer-specific survival (CSS) rates (Figure 4). Interestingly, the AR-positive/PD-L1-positive group had the best prognosis, while the AR-positive/PD-L1-negative group had the worst prognosis. Statistical analyses showed that among patients with AR-positive tumors, negative PD-L1 status was associated with significantly shorter CSS, compared to those with positive PD-L1 expression ($p=0.0493$). On the other hand, PD-L1 expression status in AR-negative patients had no significant prognostic impact ($p=0.920$).

Discussion

While ICIs have significantly changed the treatment paradigm in metastatic UCs, the response rates of ICIs still remain around 20% of all patients (6, 18). Although recent advances, including an antibody drug conjugate, enfortumab vedotin (EV), showed promising activity after the refractory to prior platinum-based chemotherapy and ICIs (19), there is

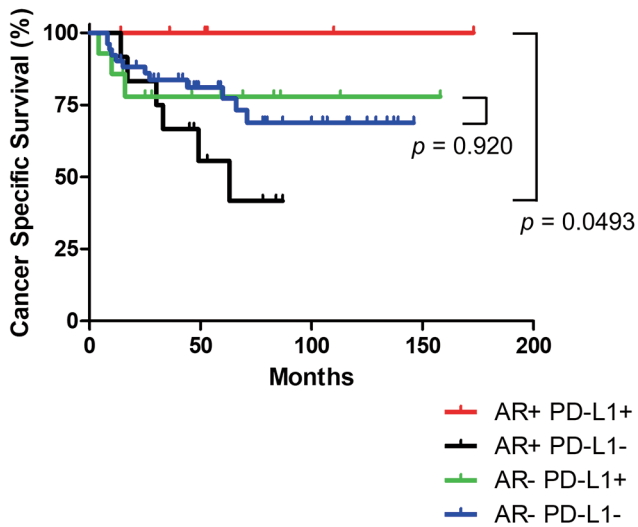


Figure 4. Kaplan-Meier curves for cancer-specific survival stratified by the status of androgen receptor and PD-L1 expression. Among patients with androgen receptor (AR)+ tumors, negative PD-L1 expression was significantly associated with shorter cancer specific survival compared to those with positive PD-L1 expression ($p=0.0493$). On the other hand, PD-L1 expression in patients with AR-tumors had no significant prognostic impact ($p=0.920$). Differences between the two groups were assessed using the log-rank test. AR: Androgen receptor.

no optional treatment after the resistance to EV in clinical settings. In addition, the subgroup analysis of overall survival in the trial showed the hazard ratio for UTUC cases [0.85 (95% CI=0.57-1.27)] was worse than that for UCB cases [0.67 (95% CI=0.51-0.88)] (19).

Recently, several studies reported that targeting the AR has therapeutic effect in bladder cancer cells by the decrease of key gene expression in oncogenic pathways (20-22). However, the lack of UTUC-specific cell lines and animal models is a major hurdle in evaluating the association of AR and other key molecules. Hence, in this study, we investigated the association between AR and PD-L1 expression using clinical samples, since the PD-1/PD-L1 axis has been shown to be therapeutically relevant in UCs and found some evidence that may support the putative role of AR signaling in UTUCs and its role as novel therapeutic target in UTUCs patients.

Firstly, we revealed an inverse correlation between AR and PD-L1 expression on cancer cells with large scale use of UTUC samples. Our findings partially align with those reported by Necchi and colleagues that described a similar inverse association between AR and PD-L1, in patients with advanced UCBs (23). Sun *et al.* also reported that AR negatively regulated PD-L1 expression by directly binding to AR response elements on the PD-L1 promoter region using a human UCB cell line (24). This inverse relationship

has actually been observed in other types of cancer. A significant inverse correlation between AR levels and PD-L1 has been found using RNA-sequencing of papillary thyroid carcinoma (25), and AR-mediated transcriptional repression of PD-L1 has been observed in papillary thyroid and hepatocellular carcinoma cell lines (25, 26). These findings may also be applicable to the understanding of these two molecules in UTUCs.

Secondly, we identified that among AR-positive patients, PD-L1-negative status was associated with a poorer prognosis compared with PD-L1-positive status. Interestingly, PD-L1 expression status had no influence on clinical prognosis in AR-negative patients. These results suggest that AR inhibition may improve the prognosis of AR-positive UTUC patients, particularly when PD-L1 expression on tumor tissue is negative. Indeed, clinical studies of AR inhibitor-based therapy for metastatic UCB have been initiated to assess if it increases the rate of responders and rescue PD-L1-negative patients (27).

Study limitations. Firstly, the present study is observational and retrospective in nature. Secondly, only the clinical data entered in the medical records were available for analysis, which may lead to lack of information about post-treatment.

Conclusion

The expression of AR and PD-L1 on cancer cells in UTUC was inversely correlated. AR-positive and PD-L1-negative UTUC patients had a poor prognosis. This study describes a novel role of AR in regulating the immune response in UTUC patients, providing potential therapeutic strategies especially in patients with PD-L1-negative tumors.

Conflicts of Interest

The Authors declare that they have no conflicts of interest or financial ties related to this study.

Authors' Contributions

Y. O. and T. K. were responsible for the study conception and design, conducted the study, collected and analyzed the data, and drafted the manuscript. H. F. stained the TMA sections and evaluated their staining scores. K. F., H. M., G. N., and N. N. conducted the study, collected and analysed the data, drafted and revised the manuscript. All Authors have read and approved the final manuscript for submission.

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Materials and Methods

Patients and tissue microarrays. The UTUC tissue microarrays (TMAs) were constructed with spotted triplicate urothelial tumor samples (from dominant tumors/invasive components if present) and paired normal-appearing urothelial tissues (from the renal pelvis and ureter) obtained from 99 patients with non-metastatic UTUC, who underwent radical nephroureterectomy performed at Osaka General Medical Center from 1997 to 2011, as described previously (15, 16). We obtained appropriate approval from the local institutional review board (Osaka General Medical Center Institutional Review Board, Protocol Number: 25-2014, 19 June 2013) as well as written informed from all patients before construction and use of the TMAs.

Immunohistochemical analysis. TMA sections were stained for PD-L1 (1:100; clone E1L3N; Cell Signaling Technology, Danvers, MA, USA) and AR (1:200; clone N-20; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The extent of membranous PD-L1 expression on tumor cells was determined at each spot (0-100%). The average PD-L1 expression level for all spots in each case was calculated and a 1% positive cutoff value was used to classify PD-L1 staining as negative or positive. AR was classified as negative or positive, as we described previously (17). All stains were evaluated by a single experienced urologic pathologist (HM).

Statistical analysis. Statistical analyses were performed using JMP® Pro 14.0.0 (SAS Institute Inc., Cary, NC, USA). The Mann-Whitney *U*-test (two-tailed) was performed to detect significant differences in PD-L1 expression rates by sex, affected side of the renal pelvis or ureter, grade, age, and AR expression. Fisher's exact test was used to evaluate the association between sex and localization of the primary tumor and the expression status of AR. Cancer specific survival rates were determined using the Kaplan-Meier method, and the log-rank test was used for comparison. $p < 0.05$ was considered statistically significant, and $p < 0.1$ was considered to be a statistical trend. In the clinical course, tumor progression was defined as the development of lesions beyond the lower urinary tract, such as recurrence at the site of nephroureterectomy, lymph node metastasis, or visceral metastasis.

Results

Patients' characteristics. The clinicopathological characteristics of the 99 patients included in the study are

Table I. *Clinicopathological details and outcome of 99 upper urinary tract urothelial carcinoma patients.*

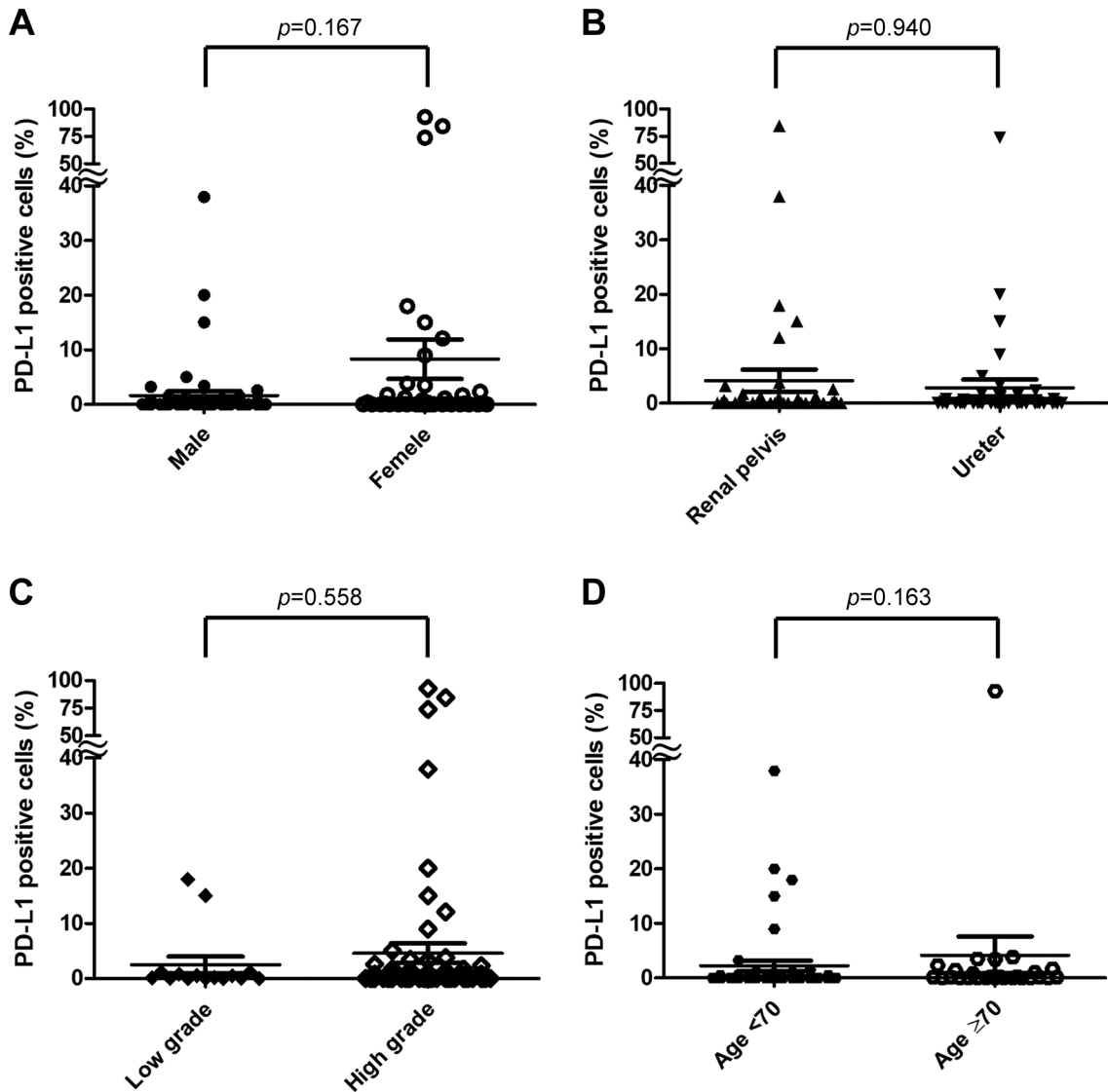
Variable	
Age (year), median (range)	71 (48-87)
Sex, n (%)	
Male	60 (60.6)
Female	39 (39.4)
Laterality, n (%)	
Right	43 (43.4)
Left	56 (56.6)
Tumor location, n (%)	
Renal pelvis	45 (45.5)
Ureter	50 (50.5)
Both	4 (4.0)
Tumor grade, n (%)	
Low grade	15 (15.2)
High grade	84 (85.9)
Pathological T stage, n (%)	
pTa	19 (19.2)
pT1	18 (18.2)
pT2	8 (8.1)
pT3	48 (48.5)
pT4	6 (6.1)
Lymphovascular invasion, n (%)	
No	59 (59.6)
Yes	40 (40.4)
Lymph node metastasis, n (%)	
pN0	84 (84.8)
pN+	12 (12.1)
pNx	3 (3.0)
Neoadjuvant chemotherapy, n (%)	
No	99 (100)
Yes	0 (0.0)
Adjuvant chemotherapy, n (%)	
No	73 (73.7)
Yes	26 (26.3)
Tumor progression, n (%)	
No	61 (61.6)
Yes	38 (38.4)
Follow-up (months), median (range)	37 (1-173)

shown in Table I. Of the 99 cases, the median age was 71 years at the time of surgery and 60 patients (60.6%) were male. The median follow-up period was 37 months (range=1-173). 84 patients (85.9%) had high-grade tumors, and 62 patients (62.7%) had muscle-invasive tumors (pT2, pT3, or pT4). None of the patients received neoadjuvant therapy, but 26 patients (26.3%) received MVAC (methotrexate, vinblastine, adriamycin, and cisplatin) therapy for 2 to 3 cycles as postoperative adjuvant chemotherapy. During follow-up, metachronous or synchronous recurrence in the lower urinary tract was observed in 32 patients (32.3%), and tumor progression was observed in 38 patients (38.4%).

Inverse relationship between the expression level of androgen receptor and programmed cell death-ligand 1. To

Table II. Association between androgen receptor and PD-L1 expression in upper urinary tract urothelial carcinoma patients determined by immunohistochemistry.

	PD-L1-negative, n (%)	PD-L1-positive, n (%)	Total
AR-negative, n (%)	62 (63)	17 (17)	79 (80)
AR-positive, n (%)	13 (13)	7 (7)	20 (20)
Total	75 (76)	24 (24)	99 (100)

Figure 1. PD-L1 expression by sex, affected side, tumor grade, and age. There was no significant difference of PD-L1 positivity between (A) male and female, (B) renal pelvis and ureter, (C) low and high grade, and (D) age <70 and \geq 70.

investigate the relationship of PD-L1 and AR expression in UTUC, we evaluated the positivity of PD-L1 and AR on cancer cells by immunohistochemical analysis. As shown in Table II, PD-L1 was negative in 75 (76%) and positive in 24

(24%) patients, whereas AR was negative in 79 (80%) and positive in 20 patients (20%). No significant difference in PD-L1 expression was observed by sex, affected side renal pelvis or ureter, tumor grade, or age ($p=0.167$, 0.121 , 0.558

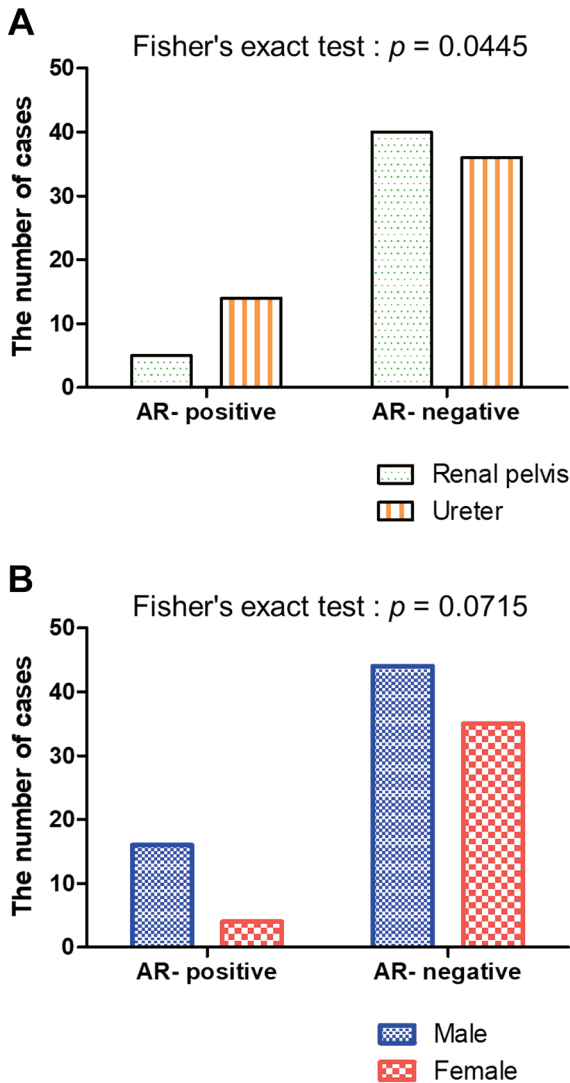


Figure 2. The positive incidence of androgen receptor expression by affected side renal pelvis or ureter and sex. (A) The positive incidence of androgen receptor (AR) expression in ureteral cancer cells was significantly higher than that in renal pelvis cancer cells ($p=0.0445$). (B) The positive incidence of AR expression on cancer cells in males tended to be higher than that in females ($p=0.0715$). Fisher's exact test was performed to evaluate the association between localization of the primary tumor and the expression status of AR. AR: Androgen receptor.

and 0.163, respectively) (Figure 1). Regarding the expression status of AR, the positive incidence of AR expression in ureteral cancer was significantly higher than that in renal pelvis cancer ($p=0.0445$). On the other hand, there was a trend of higher positive incidence of AR expression on cancer cells in males compared to females ($p=0.0715$) (Figure 2). Remarkably, the AR negative group had significantly higher PD-L1 expression levels (mean value=4.70%) compared with that in the AR-positive group

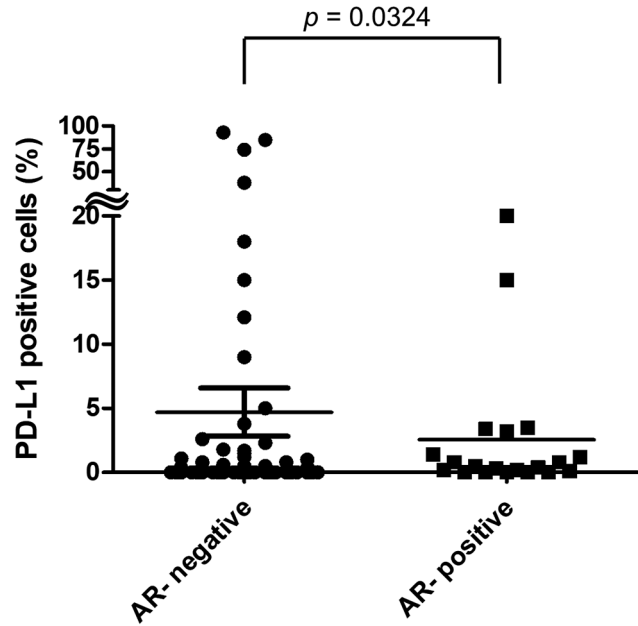


Figure 3. Correlation of the expression level of androgen receptor and PD-L1 in upper urinary tract urothelial carcinoma. Androgen receptor (AR)-negative patients had significantly higher PD-L1 expression levels (mean value; 4.70%) compared with AR-positive group (mean value; 2.55%) ($p=0.0324$). AR: Androgen receptor.

(mean value=2.55%) ($p=0.0324$, Figure 3), indicating an inverse relationship between AR and PD-L1 expression in UTUC patients.

PD-L1 negativity is associated with poor cancer-specific survival in AR-positive UTUC. We then stratified our patient cohort into four groups depending on the presence or absence of AR and PD-L1 expression and compared cancer-specific survival (CSS) rates (Figure 4). Interestingly, the AR-positive/PD-L1-positive group had the best prognosis, while the AR-positive/PD-L1-negative group had the worst prognosis. Statistical analyses showed that among patients with AR-positive tumors, negative PD-L1 status was associated with significantly shorter CSS, compared to those with positive PD-L1 expression ($p=0.0493$). On the other hand, PD-L1 expression status in AR-negative patients had no significant prognostic impact ($p=0.920$).

Discussion

While ICIs have significantly changed the treatment paradigm in metastatic UCs, the response rates of ICIs still remain around 20% of all patients (6, 18). Although recent advances, including an antibody drug conjugate, enfortumab vedotin (EV), showed promising activity after the refractory to prior platinum-based chemotherapy and ICIs (19), there is

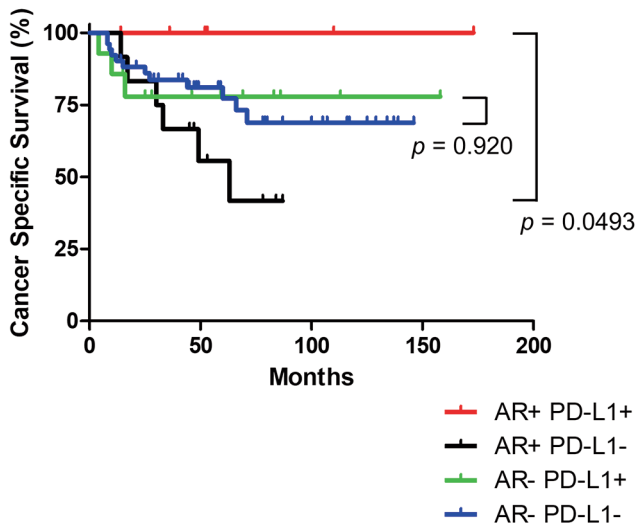


Figure 4. Kaplan-Meier curves for cancer-specific survival stratified by the status of androgen receptor and PD-L1 expression. Among patients with androgen receptor (AR)+ tumors, negative PD-L1 expression was significantly associated with shorter cancer specific survival compared to those with positive PD-L1 expression ($p=0.0493$). On the other hand, PD-L1 expression in patients with AR-tumors had no significant prognostic impact ($p=0.920$). Differences between the two groups were assessed using the log-rank test. AR: Androgen receptor.

no optional treatment after the resistance to EV in clinical settings. In addition, the subgroup analysis of overall survival in the trial showed the hazard ratio for UTUC cases [0.85 (95% CI=0.57-1.27)] was worse than that for UCB cases [0.67 (95% CI=0.51-0.88)] (19).

Recently, several studies reported that targeting the AR has therapeutic effect in bladder cancer cells by the decrease of key gene expression in oncogenic pathways (20-22). However, the lack of UTUC-specific cell lines and animal models is a major hurdle in evaluating the association of AR and other key molecules. Hence, in this study, we investigated the association between AR and PD-L1 expression using clinical samples, since the PD-1/PD-L1 axis has been shown to be therapeutically relevant in UCs and found some evidence that may support the putative role of AR signaling in UTUCs and its role as novel therapeutic target in UTUCs patients.

Firstly, we revealed an inverse correlation between AR and PD-L1 expression on cancer cells with large scale use of UTUC samples. Our findings partially align with those reported by Necchi and colleagues that described a similar inverse association between AR and PD-L1, in patients with advanced UCBs (23). Sun *et al.* also reported that AR negatively regulated PD-L1 expression by directly binding to AR response elements on the PD-L1 promoter region using a human UCB cell line (24). This inverse relationship

has actually been observed in other types of cancer. A significant inverse correlation between AR levels and PD-L1 has been found using RNA-sequencing of papillary thyroid carcinoma (25), and AR-mediated transcriptional repression of PD-L1 has been observed in papillary thyroid and hepatocellular carcinoma cell lines (25, 26). These findings may also be applicable to the understanding of these two molecules in UTUCs.

Secondly, we identified that among AR-positive patients, PD-L1-negative status was associated with a poorer prognosis compared with PD-L1-positive status. Interestingly, PD-L1 expression status had no influence on clinical prognosis in AR-negative patients. These results suggest that AR inhibition may improve the prognosis of AR-positive UTUC patients, particularly when PD-L1 expression on tumor tissue is negative. Indeed, clinical studies of AR inhibitor-based therapy for metastatic UCB have been initiated to assess if it increases the rate of responders and rescue PD-L1-negative patients (27).

Study limitations. Firstly, the present study is observational and retrospective in nature. Secondly, only the clinical data entered in the medical records were available for analysis, which may lead to lack of information about post-treatment.

Conclusion

The expression of AR and PD-L1 on cancer cells in UTUC was inversely correlated. AR-positive and PD-L1-negative UTUC patients had a poor prognosis. This study describes a novel role of AR in regulating the immune response in UTUC patients, providing potential therapeutic strategies especially in patients with PD-L1-negative tumors.

Conflicts of Interest

The Authors declare that they have no conflicts of interest or financial ties related to this study.

Authors' Contributions

Y. O. and T. K. were responsible for the study conception and design, conducted the study, collected and analyzed the data, and drafted the manuscript. H. F. stained the TMA sections and evaluated their staining scores. K. F., H. M., G. N., and N. N. conducted the study, collected and analysed the data, drafted and revised the manuscript. All Authors have read and approved the final manuscript for submission.

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