CD155 immunohistochemical expression in upper tract urothelial carcinoma predicts poor prognosis

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Abstract. A novel immune checkpoint, CD155/T-cell immunoreceptor with Ig and ITIM domains, has been recognized as a new therapeutic target in addition to conventional immune checkpoints, such as anti-programmed cell death protein 1/programmed cell death ligand 1 (PD-L1), for urothelial carcinoma (UC). Fibroblast growth factor receptor (FGFR) is considered another new therapeutic target for UC. As FGFR3-mutant UC may be associated with decreased T-cell infiltration, FGFR3 inhibition may facilitate lymphocyte invasion into the tumor microenvironment. Although a combined effect of immune checkpoint inhibitors and FGFR inhibition is expected, the combined expression profiles of CD155, PD-L1 and FGFR3 have not been evaluated in upper tract UC (UTUC). The present study aimed to investigate the association between CD155 expression and clinicopathological factors in 208 patients with UTUC undergoing radical nephroureterectomy. Furthermore, the expression profiles of CD155, PD-L1 and FGFR3 were compared. Immunohistochemical analysis was performed using tissue microarray specimens and survival analyses were performed using the Kaplan-Meier method and the Cox proportional hazards model. High immunohistochemical expression of CD155 was observed in 177 patients (85.1%) and it was associated with advanced pathological stage and lymphovascular invasion. The survival rate was lower among patients with tumors exhibiting high CD155 expression than among those with tumors with low CD155 expression. In addition, multivariate survival analysis revealed that high CD155 expression was an independent prognostic factor for recurrence (hazard ratio=7.32, 95% CI=1.01-53.35, P=0.049). FGFR3 and immune checkpoint signaling molecules, such as CD155 and PD-L1, had a weak negative correlation. The present results indicated that the expression of CD155 is a useful marker for predicting the recurrence of UTUC. In addition, the immunohistochemical expression profiles of CD155, PD-L1 and FGFR3 may further the understanding of the role of FGFR-targeted therapies in immunotherapy for UTUC.

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Abbreviations: TIGIT, T cell immunoreceptor with Ig and ITIM domains; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; UC, urothelial carcinoma; FGFR, fibroblast growth factor receptor; ICI, immune checkpoint inhibitor; UTUC, upper tract urothelial carcinoma

Key words: CD155, FGFR3, immunohistochemistry, PD-L1, upper tract urothelial carcinoma

Introduction

Upper tract urothelial carcinoma (UTUC) derived from the renal calyces, renal pelvis or ureters is a relatively rare tumor type, accounting for 5-6% of all cases of UC (1). At the time of diagnosis, two-thirds of UTUCs have developed local invasion (2). For patients with metastatic UTUC, first-line platinum-based chemotherapy is prescribed, but they are considered incurable and have demonstrated a poor prognosis so far. Recently, programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1) inhibitors, such as pembrolizumab, nivolumab, atezolizumab, avelumab and durvalumab, were approved for second-line therapy (3). Subsequently, atezolizumab and pembrolizumab were approved in the first-line setting for cisplatin-ineligible patients with PD-L1-positive tumors. Thus, PD-1/PD-L1 inhibitors have been recognized as key drugs to control the progression of malignant tumors (4). The treatment effects of immune checkpoint inhibitors (ICIs) have been indicated to be limited (objective response rate, 11-27%) (3) and the treatment effects of emerging ICIs, such as anti-T-cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT) drugs, lymphocyte activation gene-3 inhibitors, and T-cell immunoglobulin and mucin-domain containing-3 inhibitors, are promising (4,5).

TIGIT was reported to be associated with the immune checkpoint in NK cells and T cells in 2013 (6). CD155, which interacts with TIGIT, is expressed in numerous types of tumor and is recognized to be a poor prognostic factor (7-10). With regard to UC, high expression of CD155, which is associated with poor prognosis, has been confirmed in bladder cancer, but has not been demonstrated in UTUC (8). Mechanistic analysis revealed that binding of CD155 and TIGIT facilitates tumor invasion and suppresses antitumor immunity (11,12). Thus, CD155/TIGIT is recognized as a new treatment target and clinical trials of anti-TIGIT drugs have been launched (4,13).

Genomic alternations in fibroblast growth factor receptor 3 (FGFR3) have been well described in UC and have been recognized as therapeutic targets (14). FGFR3 belongs to the super-family of receptor tyrosine kinases and is involved in transmitting FGF signals. FGFR3 signaling may be associated with UC development, angiogenesis and lower T-cell infiltration (14-16). Furthermore, FGFR inhibition may activate the immune environment and is expected to benefit patients who do not respond to ICIs (17). Although the association between PD-L1 and FGFR3 has been investigated in UC (18), the association between CD155 and FGFR3 has not been explored.

The present study aimed to evaluate the association of CD155 expression with clinicopathological factors in UTUC and examine whether CD155 is a prognostic factor when compared with existing pathological factors. In addition, the correlation of immunohistochemical expression was analyzed among CD155, PD-L1 and FGFR3, all of which have been recognized as new therapeutic targets.

Materials and methods

Case selection. After receiving institutional review board approval (nos. 2018036 and 2019209), the medical records of 222 patients underwent radical nephroureterectomy for UTUC at Kansai Medical University Hospital (Hirakata, Japan) between January 2006 and December 2017 were retrospectively reviewed. An opt-out approach was used to obtain informed consent on the website of Kansai Medical University Hospital (Hirakata, Japan). A total of 14 patients were excluded from this study for the following reasons: Synchronous bilateral tumors (n=2), presence of metastasis (n=4), simultaneous radical cystectomy (n=3) and insufficient pathological material (n=5). Thus, the data of a total of 208 patients (pTa-4Nx-2M0) who underwent radical nephroureterectomy for UTUC were extracted from our institutional database for this study. Clinicopathological characteristics, including grade, pathological stage, lymphovascular invasion (LVI), surgical margin and divergent differentiation/subtypes were reviewed. Slides stained with H&E were re-evaluated by a urologic pathologist (CO) using the 2016 World Health Organization classification (19) and the 2017 Union for International Cancer Control TNM staging system (20).

Histological evaluation and tissue microarray (TMA) construction. Two representative tumor locations showing tumor invasion (if a variant existed, that area was included as well) were selected for TMA construction of radical nephroureterectomy specimens. A total of 10 TMA blocks were built from representative tumor areas, including normal urothelium samples from formalin-fixed paraffin-embedded (FFPE) tumor material. Each FFPE tissue block was sampled with 2.0-mm cores using a tissue arraying instrument (Azumaya Corporation). To validate the expression of CD155 in the preoperative biopsy specimens, other TMA sections from 14 biopsy cases which were included in the 208 patients that underwent radical nephroureterectomy were evaluated. Biopsies were performed on patients whose tumors were not detected on imaging or urine cytology.

Immunohistochemical analysis of TMAs. Immunohistochemical staining was performed on TMA sections (4-μm thick) using a Ventana Discovery Ultra Autostainer (Roche Diagnostics) or

Table I. Association between CD155 expression and clinicopathological factors (n=208).

Characteristic	CD155 low (n=31)	CD155 high (n=177)	P-value
Age, years	76 (68.5-79)	72 (67-78)	0.20
Sex			0.84
Female	10 (32.3)	54 (30.5)	
Male	21 (67.7)	123 (69.5)	
Grade			0.40
Low	6 (19.4)	23 (13)	
High	25 (80.6)	154 (87)	
pT stage			0.04
pTa	14 (45.2)	33 (18.6)	
pTis	0 (0.0)	2 (1.1)	
pT1	5 (16.1)	32 (18.1)	
pT2	3 (9.7)	21 (11.9)	
pT3	9 (29.0)	74 (41.8)	
pT4	0 (0.0)	15 (8.5)	
pN stage			0.10
pNx	0 (0.0)	4 (2.3)	
pN0	30 (96.8)	137 (77.4)	
pN1	0 (0.0)	17 (9.6)	
pN2	1 (3.2)	19 (10.7)	
LVI			0.001
Absent	22 (71)	68 (38.4)	
Present	9 (29)	109 (61.6)	
Surgical margin			0.22
Absent	31 (100)	164 (92.7)	
Present	0 (0)	13 (7.3)	
Divergent differentiation/ subtype			0.54
Absent	29 (93.5)	157 (88.7)	
Present	2 (6.5)	20 (11.3)	
Neoadjuvant chemotherapy			0.22
No	31 (100)	165 (93.2)	
Yes	0 (0)	12 (6.8)	
Adjuvant chemotherapy			0.007
No	30 (96.8)	134 (75.7)	
Yes	1 (3.2)	43 (24.3)	
PD-L1			0.03
Low	26 (86.7)	117 (66.5)	
High	4 (13.3)	59 (33.5)	
FGFR3			0.32
Low	1 (3.3)	18 (10.2)	
High	29 (96.7)	158 (89.8)	
Median follow-up, months	72.9 (51.1-94.9)	70.3 (46.3-96.8)	0.93

Values are expressed as n (%) or median (interquartile range). LVI, lymphovascular invasion; PD-L1, programmed death-ligand 1; FGFR3, fibroblast growth factor receptor 3.

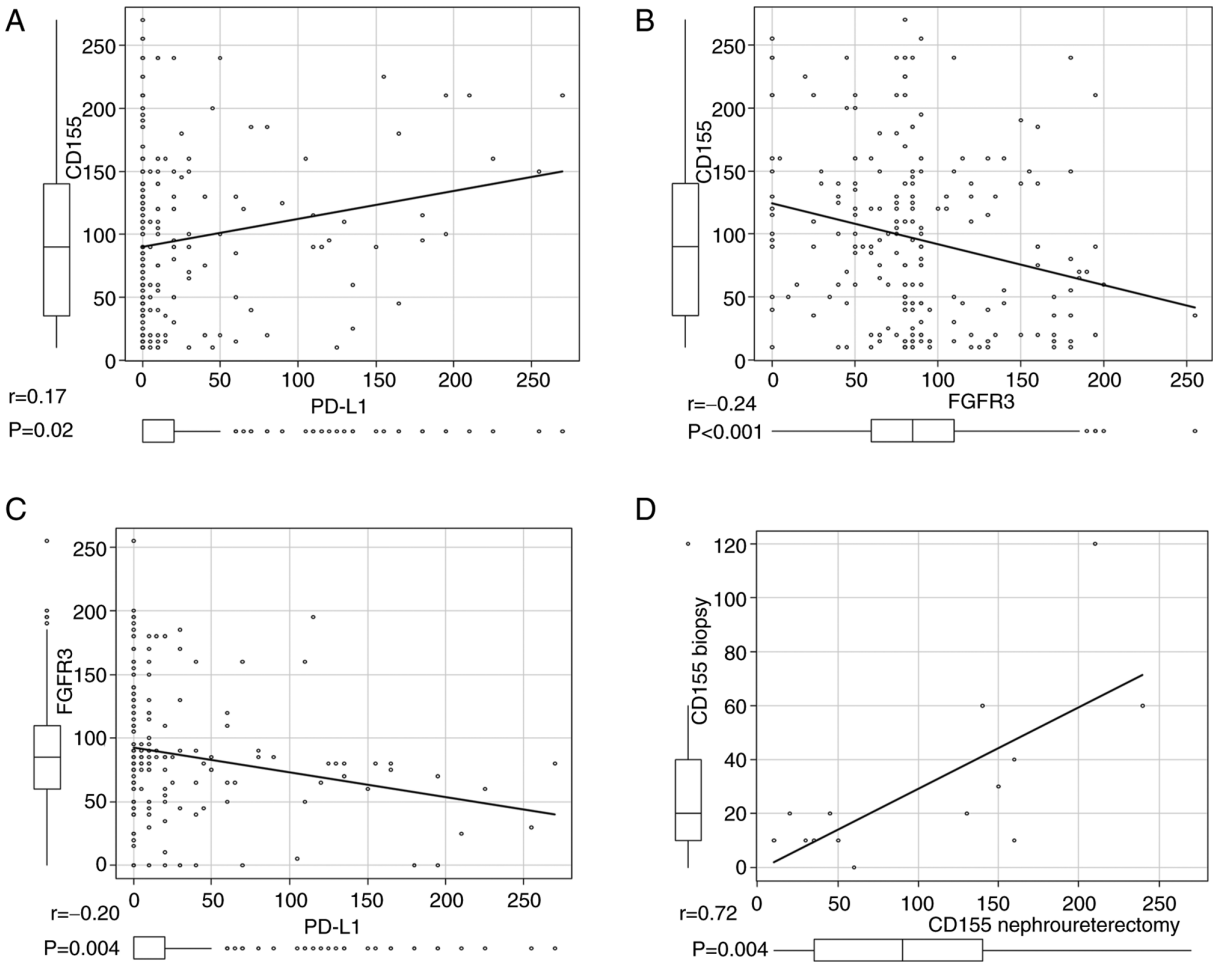

Figure 1. Pearson's product-moment correlation coefficient determined for the immunohistochemical expression of two markers or two specimens. (A) CD155 and PD-L1, (B) CD155 and FGFR3, and (C) FGFR3 and PD-L1. (D) CD155 expression in radical nephroureterectomy specimens and CD155 expression in biopsy specimens. PD-L1, programmed death-ligand 1; FGFR3, fibroblast growth factor receptor 3.

Leica Bond-III (Leica Microsystems, Ltd.). Primary antibodies against CD155 (#81254 rabbit monoclonal; 1:200 dilution; Cell Signaling Technology, Inc.) and FGFR3 (sc-13121; mouse monoclonal; 1:50 dilution; Santa Cruz Biotechnology, Inc.) were visualized using the OptiView DAB IHC Detection Kit (Ventana Medical Systems) according to the manufacturer's instructions. Anti-PD-L1 primary antibodies (PA0832; rabbit monoclonal; prediluted; Leica Microsystems, Ltd.) were visualized using BOND Polymer Refine Detection (Leica Microsystems, Ltd.) according to the manufacturer's instructions. The cell membrane and cytoplasmic expression patterns of CD155 in tumor cells were semi-quantitatively assessed by using the H-score. The H-score was determined by multiplying the staining intensity (0, none; 1, weak; 2, moderate; 3, strong) and the percentage of positive cells (range, 0-300), as previously described (21). Representative CD155 immunohistochemical expression patterns in normal urothelium and tumor cells are presented in Fig. S1. The final scores (average H-score for the two cores) were classified into two categories (low, H-score <20; high, H-score ≥20), with the cutoff determined by a receiver operating characteristic curve for 5-year recurrence. PD-L1 and FGFR3 were also evaluated by the H-score and divided into two categories (low, H-score <20; high, H-score ≥20). Immunohistochemical evaluation was independently performed by two pathologists (JI and CO) blinded to clinical outcomes and discordant patterns were resolved by consensus.

Statistical analysis. Continuous data were presented as the median and interquartile range (IQR) and count data as n (%). Fisher's exact test and the Mann-Whitney U-test were used for comparisons between two groups. Pearson's product-moment correlation coefficient was measured between two immunohistochemical expression patterns. Recurrence-free survival (RFS), cancer-specific survival (CSS) and overall survival (OS) were assessed using the Kaplan-Meier method and a univariate Cox proportional hazards model. Bladder relapse was not defined as recurrence in the present study. Logistic regression analysis using the multivariate Cox proportional hazards model was performed to determine the hazard ratio (HR). All statistical analyses were performed using EZR version 1.55 (Saitama Medical Center) (22). P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. Of the 208 patients included, 64 (30.8%) were female and 144 (69.2%) were male, with a median age of 72 years (IQR, 68-78 years) (Table SI). A total of 22 patients had

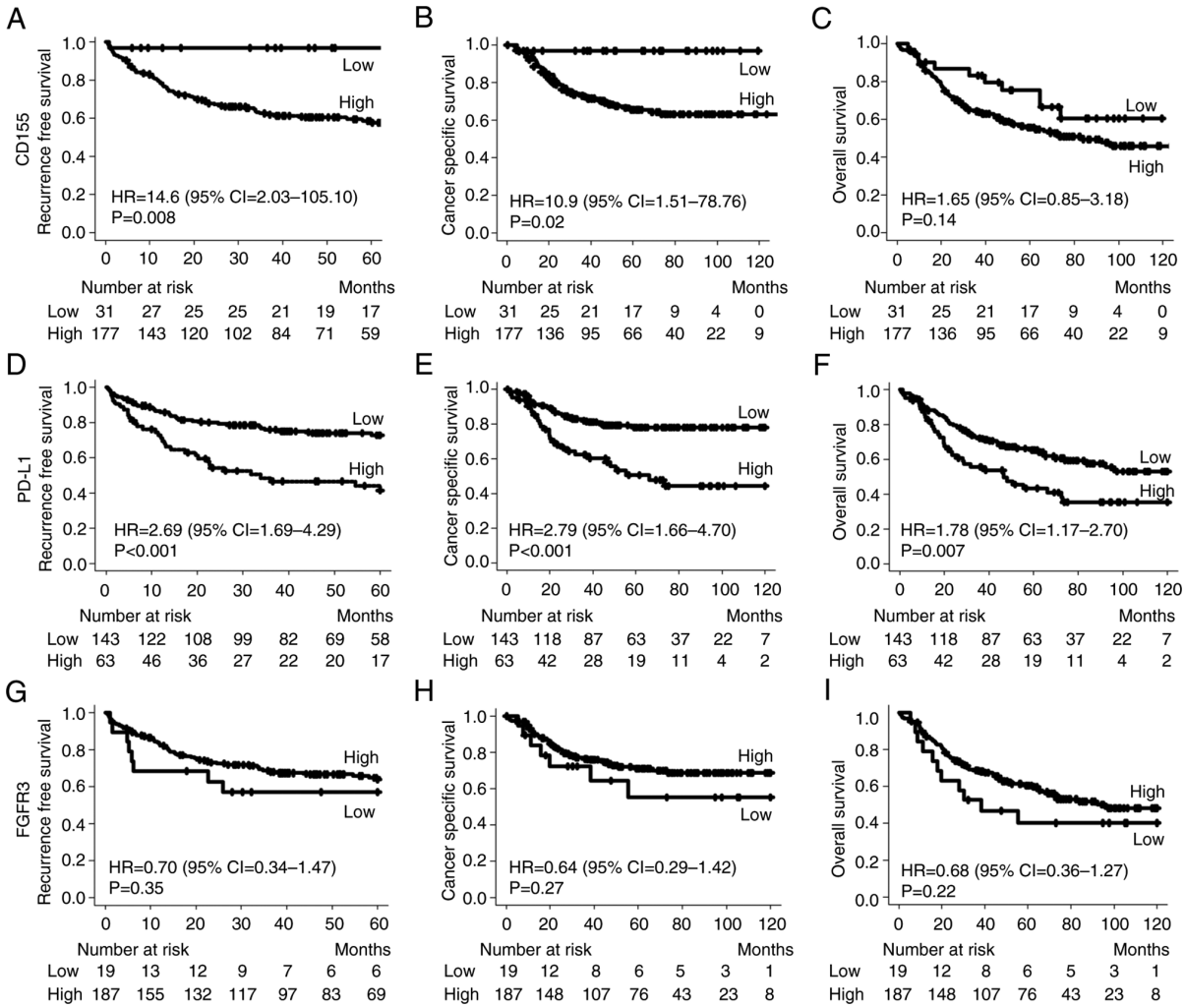

Figure 2. Comparison of the survival curve and HR for the immunohistochemical expression of CD155, PD-L1 and FGFR3. Kaplan-Meier curves of (A) recurrence-free survival for CD155 staining, (B) cancer-specific survival for CD155 staining, (C) overall survival for CD155 staining, (D) recurrence-free survival for PD-L1 staining, (E) cancer-specific survival for PD-L1 staining, (F) overall survival for PD-L1 staining, (G) recurrence-free survival for FGFR3 staining, (H) cancer-specific survival for FGFR3 staining and (I) overall survival for FGFR3 staining. PD-L1, programmed death-ligand 1; FGFR3, fibroblast growth factor receptor 3; HR, hazard ratio.

divergent differentiation/subtypes including squamous differentiation, glandular differentiation and sarcomatoid subtypes. Furthermore, 72 patients (34.6%) experienced recurrence and 57 patients (27.4%) died due to UTUC. The median follow-up time was 72.2 months (IQR, 46.4-96.2 months).

Immunohistochemical expressions of CD155, PD-L1 and FGFR3. Immunohistochemical analysis of CD155 indicated that 177 patients (85.1%) had high expression and 31 patients (14.9%) had low expression (Table I). High PD-L1 expression was noted in 63 patients (30.6%) and low expression in 143 patients (69.4). High FGFR3 expression was noted in 187 patients (90.8%) and low expression in 19 patients (9.2%) (Table SI). To identify the percentage of patients eligible for combination therapy, the combined immunohistochemical expression profiles of CD155, PD-L1 and FGFR3 were reviewed, as presented in Table SII.

Association of CD155 expression with clinicopathological factors. CD155 expression was significantly and positively associated with the T stage (P=0.04), LVI (P=0.001), administration of adjuvant chemotherapy (P=0.007) and PD-L1 expression (P=0.03) (Table I).

Correlation among CD155, FGFR3 and PD-L1. A weakly positive correlation was obtained between CD155 and PD-L1 [correlation coefficient (r)=0.17, P=0.02; Fig. 1A]. A weak negative correlation was confirmed between CD155 and FGFR3, and between PD-L1 and FGFR3 (r=-0.24, P<0.001 and r=-0.20, P=0.004, respectively; Fig. 1B and C).

Correlation between CD155 expression in radical nephroureterectomy specimens and that in biopsy specimens. A positive correlation was confirmed between CD155 expression in radical nephroureterectomy specimens and that in biopsy specimens (r=0.72, P=0.004; Fig. 1D).

Association of CD155, PD-L1 and FGFR3 expression with patient prognosis. Kaplan-Meier survival analysis indicated that RFS and CSS were significantly lower in patients with tumors having high CD155 expression than in those with tumors exhibiting low CD155 expression (HR=14.6, P=0.008;

Table II. Univariate and multivariate analysis of clinicopathological factors for predicting recurrence.

	Univariate		Multivariate	
Parameter	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, years (>65 vs. ≤65)	1.82 (0.96-3.46)	0.07	-	-
Sex (male vs. female)	0.76 (0.47-1.22)	0.26	-	-
Grade (high vs. low)	7.87 (1.93-32.12)	0.004	4.34 (1.05-18.03)	0.04
pT stage (>2 vs. ≤2)	5.68 (3.29-9.82)	<0.001	2.28 (1.22-4.28)	0.01
LVI (present vs. absent)	7.83 (3.89-15.77)	<0.001	3.24 (1.48-7.09)	0.003
Surgical margin (present vs. absent)	5.17 (2.60-10.24)	<0.001	3.45 (1.69-7.05)	<0.001
CD155 (present vs. absent)	14.6 (2.03-105.10)	0.008	7.32 (1.01-53.35)	0.049
PD-L1 (high vs. low)	2.69 (1.69-4.29)	<0.001	1.24 (0.76-2.01)	0.38
FGFR3 (high vs. low)	0.70 (0.34-1.47)	0.35	-	-

LVI, lymphovascular invasion; PD-L1, programmed death-ligand 1; FGFR3, fibroblast growth factor receptor 3; HR, hazard ratio.

and HR=10.9, P=0.02; Fig. 2A and B, respectively). The OS rate was not significantly different between the CD155 high and low expression groups (HR=1.65, P=0.14; Fig. 2C). The median follow-up time was 72.9 months (IQR: 51.1-94.9) in the low CD155 expression group and 70.3 months (IQR: 46.3-96.8) in the high CD155 expression group, and there were no significant differences between the two groups (P=0.93; Table I). RFS, CSS and OS were significantly worse in patients with tumors having high PD-L1 expression than in those with tumors having low PD-L1 expression (HR=2.69, P<0.001; HR=2.79, P<0.001; and HR=1.78, P=0.007; Fig. 2D-F, respectively). Although RFS, CSS and OS were better in patients with high FGFR3 expression than in those with low FGFR3 expression, the difference was not statistically significant (Fig. 2G-I).

Univariate and multivariate analyses for predicting recurrence. The association between clinicopathological factors and recurrence after radical nephroureterectomy is presented in Table II. The univariate analysis indicated that grade, pT stage, LVI, surgical margin, CD155 expression and PD-L1 expression were associated with recurrence (all P<0.05). Multivariate analysis was performed on these significantly different factors, suggesting that grade (HR=4.34, P=0.04), pT stage (HR=2.28, P=0.01), LVI (HR=3.24, P=0.003), surgical margin (HR=3.45, P<0.001) and CD155 expression (HR=7.32, P=0.049) were significant factors affecting recurrence.

Discussion

In the present study, the association between CD155 expression and clinicopathological factors in UTUC was investigated. It was indicated that high CD155 expression was significantly associated with poor prognosis. Furthermore, multivariate analysis suggested that CD155 was an independent unfavorable prognostic factor. Therefore, confirming the immunohistochemical expression of CD155 may be useful in predicting prognosis.

Previously, the expression of CD155 has been immunohistochemically evaluated in muscle-invasive bladder cancer (8), while the expression in UTUC has not been assessed, to the best of our knowledge. Thus, the present study was the first to investigate the correlation between CD155 immunohistochemical expression and clinicopathological factors in UTUC. Furthermore, as the association between the expression of CD155, PD-L1 and FGFR3 had not been previously investigated, the correlation between these three markers was investigated in the present study.

CD155, originally identified as a poliovirus receptor, is a type I transmembrane glycoprotein that belongs to the immunoglobulin superfamily, known as nectin-like 5 (NECL5) (23-25). CD155 has been reported to be overexpressed in numerous types of cancer (26) and to be associated with tumor invasion and metastasis (11). The binding of CD155 and TIGIT in NK and T cells leads to evasion of tumor immunity (5,12). TIGIT blockade has been demonstrated to enhance NK cell activity and numerous clinical trials of anti-TIGIT monoclonal antibodies, which are applied in combination with PD-1/PD-L1 inhibitors, have been performed (4,13).

The present results suggested that prognosis was unfavorable in patients with tumors with high expression of CD155 than in those with tumors having low CD155 expression. High CD155 expression was associated with high T stage, LVI and administration of adjuvant chemotherapy. As advanced pathological findings, such as a high T stage and LVI, were detected in tumors having high CD155 expression, adjuvant chemotherapy may be provided to avoid cancer recurrence. Sloan *et al* (11) showed that CD155 promotes tumor cell invasion and migration. The role of tumor angiogenesis and proliferation has been drawing attention (26). High vascular endothelial growth factor (VEGF) expression in bladder cancer was associated with advanced pathological stage and lymph node metastasis (27). Furthermore, CD155 was associated with VEGF receptor 2 and regulated VEGF-induced angiogenesis (7,28). Chauvin and Zarour (12) reported that CD155/TIGIT was associated with immune suppression. Greater infiltration of immune cells in UC has been indicated to be associated with favorable prognosis (21). The reason for the poor prognosis in the high CD155 group may be that immune cell infiltration was suppressed by the activation

of the CD155/TIGIT immune checkpoint. By investigating its association with clinicopathological factors, the present study confirmed that CD155 may promote tumor progression. However, further studies are necessary to reveal the molecular mechanism of tumor development.

CD155 and PD-L1 are immune checkpoints expressed on the tumor surface. The present study confirmed a weakly positive correlation between the expression of CD155 and PD-L1 (r=0.17, P=0.02). CD155 and PD-L1 may suppress the cytotoxicity of tumor-infiltrating lymphocytes via interaction with ligands expressed on the lymphocytes (9).

Since the two immune checkpoints of CD155 and PD-L1 may have a similar status, a positive correlation of expression patterns was confirmed. Furthermore, the efficacy of combination therapies of TIGIT inhibitors and PD-1/PD-L1 inhibitors is expected (13). The association between co-stimulatory molecules, co-suppressive molecules and their ligands are complex (9) and further investigation is required.

In the present study, a weak negative correlation was found between the expression of CD155 and FGFR3 (r=-0.24, P<0.001). Furthermore, a negative correlation between PD-L1 and FGFR3 (r=-0.20, P=0.004) was obtained, which was in agreement with a previous report (18). The immune exclusion system is activated by the Wnt/β-catenin signal (29), which is associated with the non-T-cell-inflamed phenotype (30). An active Wnt/β-catenin signal is associated with impaired T-cell infiltration and low PD-L1 expression (30,31). On the other hand, an active FGFR3 pathway may contribute to T-cell exclusion (32). Furthermore, a study indicated an overlap of Wnt/β-catenin and FGF signaling (33). Therefore, a weak negative correlation between CD155 and FGFR3 was to be expected. As the effectiveness of the combination therapy of PD-1/PD-L1 inhibitors and FGFR inhibitors in metastatic UC has been proven (17,34), the combination therapy of TIGIT inhibitors and FGFR inhibitors may also be useful.

The present study suggested that high CD155 expression was associated with significantly reduced RFS and CSS. Univariate and multivariate survival analyses revealed that high CD155 expression was an independent risk factor. Thus, CD155 may be a robust biomarker to predict recurrence in UTUC. According to preliminary data by our group on the prediction of CD155 expression with preoperative biopsy specimens (n=14), a positive correlation was statistically confirmed between CD155 expression in radical nephroureterectomy specimens and that in preoperative biopsy specimens.

Confirming the immune checkpoint status in biopsy samples may predict the efficacy of TIGIT inhibitors in cases ineligible for surgery. Furthermore, the possibility of prognosis prediction using preoperative biopsy specimens may be further investigated.

The present study has several limitations. First, it was a retrospective single-center study. Furthermore, CD155 expression was evaluated with TMAs constructed with two representative cores. In addition, the present results should be externally validated with other cohorts. As another limitation, CD155 expression was evaluated using only TMAs and not the whole section, which may have caused unidentified bias. Furthermore, it was not possible to validate the multiplexed immunofluorescence analysis to determine CD155 PD-L1 and FGFR3 co-expressed in a cell. Further investigation by multicolor fluorescence methods and spatial gene expression analysis is required to analyze the association between cancer cells and immune cells in the tumor microenvironment. Despite these limitations, the present results add a new role to the immunohistochemical detection of CD155 expression in UTUC.

In conclusion, the present study indicated that confirming the expression of CD155 by immunohistochemistry may be useful for predicting recurrence of UTUC. In addition, FGFR3 and immune checkpoint signaling molecules, such as CD155 and PD-L1, had a weak negative correlation. The immunohistochemical expression profiles of CD155, PD-L1 and FGFR3 may help us understand the roles of FGFR-targeted therapies in immunotherapy for UTUC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JI, CO and KT designed the study. JI, CO and TY performed data collection and pathological assessments. JI performed the statistical analyses. JI, CO, TY, RS, KT and HK interpreted the data. JI and CO drafted the manuscript. All authors read and approved the final version of the manuscript. JI and CO confirmed the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board at the Kansai Medical University Hospital (Hirakata Japan; approval nos. 2018036 and 2019209). Informed consent was obtained in the form of opt-out on the website of Kansai Medical University Hospital.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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