Pathological Significance and Prognostic Role of WWC1 in Upper Urinary Tract Urothelial Cancer

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Abstract. *Background/Aim: WW and C2 domain-containing 1 (WWC1) protein is a suppressor of malignancies. However, there is no information on the pathological significance of WWC1 in upper urinary tract cancer (UTUC). Patients and Methods: In this study, WWC1 immunoreactivity was investigated in 152 non-metastatic UTUC samples. The relationships between WWC1 expression and grade, pT stage, proliferative index (using an antibody to Ki-67), and the immunohistochemical expression of matrix metalloproteinase (MMP)-2 and -9 were evaluated. Results: WWC1 expression was negatively associated with tumor grade and pT stage (p<0.001). Positive expression of WWC1 was a better predictor of the UTUC recurrence and subsequent metastasis, and the multivariate analysis showed that WWC1 expression was a significant predictor of subsequent metastasis (hazard ratio=0.29, p=0.020). WWC1 expression inversely correlated with the proliferative index (odds ratio=2.59, p=0.023) and expression of MMP9 (odds ratio=2.19, p=0.040) but not with MMP2 expression, by multivariate analyses. Conclusion: WWC1 expression was negatively associated with malignant aggressiveness via the suppression of cancer cell proliferation and MMP9 expression in patients with UTUC. This suggests WWC1 to be a useful predictor and novel therapeutic target in patients with UTUC.*

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Urothelial carcinoma (UC) is the fourth most common tumor and is subdivided into upper urinary tract UC (UTUC) originating from the pyelocaliceal cavities and ureter and lower urinary tract UC originating from the bladder and urethra (1, 2). One of the most important pathological characteristics of UC is its high frequency of recurrence. In fact, in patients with UTUC, the recurrence rates were reported to be 28-41% despite standard operations (radical nephroureterectomy with bladder cuff excision) (2-4). In addition, subsequent extra-urothelial recurrence-free survival after radical surgery was reported to be 27-33% (5-7).

WW and C2 domain-containing 1 (WWC1, also called KIBRA) is a multi-domain phosphor protein that is predominantly expressed in the kidney and brain (8). WWC1 has been reported to play important roles in various biological processes by regulating intracellular transportation, cell polarity, learning, and memory (9, 10). On the other hand, WWC1 is well known to be closely associated with the pathogenesis and progression of various pathological conditions, such as psychiatric disorders and muscular dystrophy (11, 12). In addition, WWC1 has been suggested to play a crucial role in carcinogenesis and malignant aggressiveness in a variety of cancers (13, 14). However, there is the opinion that WWC1 has oncogenic and pro-carcinogenic activities (15, 16). Thus, there is no general agreement regarding the pathological significance of WWC1 in cancer.

The pathological functions of WWC1 are regulated by various molecules and systems including the Hippo pathway, and the Hippo pathway is associated with cancer cell proliferation, invasion, and metastasis in various types of malignancies (12, 14, 17, 18). Furthermore, the WWC1 and Hippo pathways are associated with the activities of matrix metalloproteinases (MMPs), which play important roles in tumor growth, invasion, and progression of malignant cells (17, 19). Thus, the pathological significance of WWC1 is speculated to be modulated by complex mechanisms.

Several investigators have shown that cancer cell proliferation and invasion regulated by MMP2 and -9 are

Table I. *Relationships between expression of WW and C2 domain-containing 1 (WWC1) and clinicopathological features in patients with upper urinary tract cancer (n=152).*

		Overall	WWC1 expression		*p*-Value
			Negative	Positive	
Age, years	Mean±SD	67.2±10.7	68.9±10.0	65.6±11.4	0.066
Sex, n (%)	Male	112 (73.7)	52 (46.4)	60 (53.6)	0.229
	Female	40 (26.3)	23 (57.5)	17 (42.5)	
Location, n (%)	Renal pelvis	63 (41.4)	27 (42.9)	36 (57.1)	0.139
	Ureter	58 (38.2)	28 (48.3)	30 (51.7)	
	Both	31 (20.4)	20 (64.5)	11 (35.5)	
Grade, n (%)	Low	90 (59.2)	33 (36.7)	57 (63.3)	**<0.001**
	High	62 (40.8)	42 (67.7)	20 (32.3)	
pT Stage, n (%)	Ta	16 (10.5)	4 (25.0)	12 (75.0)	**<0.001**
	T1	46 (30.3)	11 (23.9)	35 (76.1)	
	T2	25 (16.4)	13 (52.0)	12 (48.0)	
	T3	51 (33.6)	36 (70.6)	15 (29.4)	
	T4	14 (9.2)	11 (78.6)	3 (21.4)	
Muscle-invasive	No (pTa1)	62 (40.8)	15 (24.2)	47 (75.8)	**<0.001**
	Yes (≥pT2)	90 (59.2)	60 (66.7)	30 (33.3)	
PI	Mean±SD	21.9±9.2	27.0±10.4	16.8±8.1	**<0.001**
	>Median	75 (49.3)	45 (60.0)	30 (39.0)	
	≤Median	77 (50.7)	30 (40.0)	47 (61.0)	

PI: Proliferative index; SD: standard deviation. Statistically significant *p*-values are shown in bold.

associated with tumor growth, progression, and survival in patients with UTUC (20, 21). However, the relationships between WWC1 and such cancer-related factors in UC, including bladder cancer and UTUC, are not clear. In addition, as far as we are aware, there are no studies on WWC1 expression in human UC tissues. In this study, we investigated the pathological significance and prognostic roles of WWC1 expression in patients with UTUC undergoing radical surgery. Furthermore, the relationships between WWC1 expression and cancer cell proliferation, MMP2 expression, and MMP9 expression were also analyzed in these patients.

Patients and Methods

Patients. This study included 152 patients with UTUC who underwent radical surgery at the Nagasaki University Hospital. Information on age during the operation and sex is shown in Table I. Patients with metastatic disease (regional lymph node/distant organ), perioperative chemotherapy or radiation therapy, squamous cell carcinoma, or adenocarcinoma were excluded from this study. In addition, we excluded patients with synchronous bladder cancer. Metastasis was evaluated using chest radiography and computed tomography. When bone metastasis and brain metastasis were suspected, bone scanning and magnetic resonance imaging were performed. All histological diagnoses, including grade and pT stage, were determined using formalin-fixed paraffin-embedded specimens obtained by radical surgery, and they were judged according to the American Joint Committee on Cancer classification (22). Extra-urothelial recurrence

was defined as local recurrence, lymph node metastasis, and distant organ metastasis after radical surgery, according to a previous report (7). In this study, 10 normal urothelial specimens were also evaluated for WWC1 immunoreactivity. The study protocol was approved by the Institutional Review Board of the Nagasaki University Hospital (no. 12052899). Written informed consent was obtained from all the patients before starting this study.

Immunohistochemical technique and evaluation. All immuno-reactivity was evaluated by immunohistochemical techniques using formalin-fixed, paraffin-embedded sections. For WWC1, the sections were incubated overnight with the primary antibody (anti-WWC1 antibody; Abcam, Cambridge, UK) at 4°C after antigen retrieval and inactivation of endogenous peroxidase. The sections were then incubated with Dako EnVision+ Peroxidase (Dako Corp., Carpinteria, CA, USA). Finally, the peroxidase reaction was visualized using a liquid 3,3'-diaminobenzidine tetrahydrochloride substrate. WWC1 immunoreactivity was evaluated according to a previous report (23). In brief, the immunoreactive score of WWC1 was calculated by multiplying the score for the percentage of positively stained cells (0: <5.0%, 1: 5.1-25.0%, 2: 25.1-50.0%, 3: 51.1-75.0%, 4: >75.1%) by the score for staining intensity (0: none, 1: weak, 2: moderate, 3: strong). Finally, specimens with immunoreactive scores of ≥4 were defined as positive (23).

The expression of Ki-67, MMP2, and MMP9 was evaluated according to our previous reports (5, 24). Ki-67 antibody was purchased from Dako Corp. (Glostrup, Denmark), and antibodies to MMP2 and MMP9 were obtained from Daiichi Fine Chemical (Toyama, Japan). Briefly, regarding MMP2 and MMP9, the staining intensity was graded as weak, moderate, or intense, and
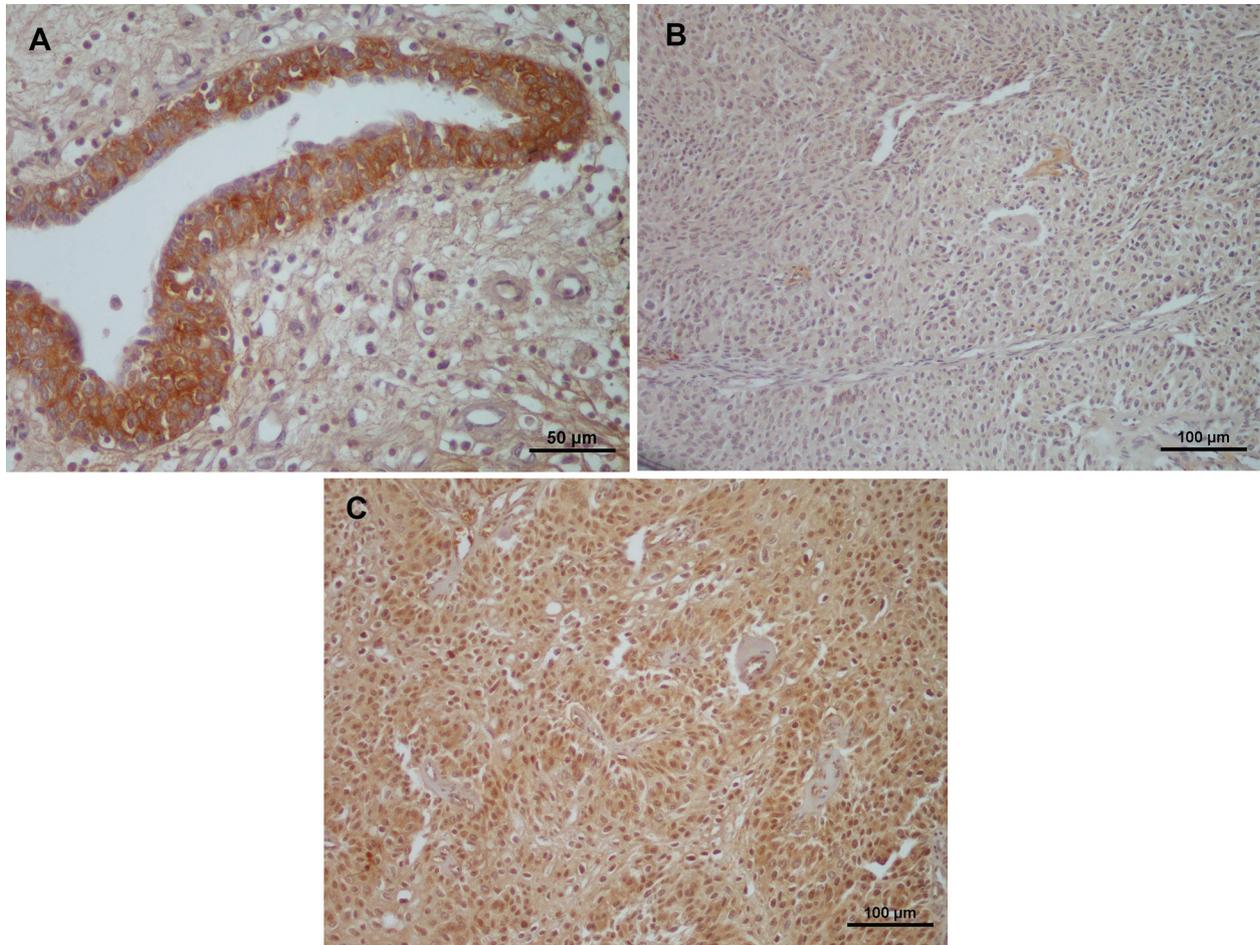
Figure 1. *A representative example of WW and C2 domain-containing 1 (WWC1) immunostaining in normal urothelium (A), upper urinary tract urothelial cancer tissue judged as negative (B) and as positive (C) (magnification ×200).*

the extent of positive staining was classified as focal (≤10%), regional (11-50%), or diffuse (≥50%). Finally, the staining patterns of moderate and diffuse, intense, and regional, or intense and diffuse were considered to be positive. The definition of the proliferative index (PI) calculated using Ki-67-stained cancer cells (number of Ki-67-positive cancer cells/total number of cancer cells ×100%) was given in our previous report (20). These evaluations were performed using a Nikon E-400 microscope, a digital imaging system (Nikon DU100, Tokyo, Japan), and a computer-aided image analysis system (Win ROOF, version 5.0; MITANI Corp, Fukui, Japan).

Statistical analyses. Student's *t*-test was used to compare continuous variables, and chi-squared tests were performed for categorical comparisons of data. Survival analyses were performed using the Kaplan–Meier survival curves with log-rank *p*-values. Multivariate analyses for recurrence and metastasis after radical surgery were performed using the Cox proportional hazard analyses [described as hazard ratios (HRs) with 95% confidence intervals (95% CIs), together with their associated *p*-values]. The crude and adjusted effects were estimated using logistic regression analysis [odds ratios (ORs) with 95% CIs, together with their associated *p*-values). All statistical analyses were performed with the statistical package StatView for Windows (version 5.0; Abacus Concept Inc, Berkeley, CA, USA), and significance was defined as *p*<0.05.

Results

WWC1 expression and correlation with clinicopathological features. Representative examples of WWC1 expression in normal urothelium, UTUC tissues judged as negative, and those as positive were showed in Figure 1A-C, respectively. WWC1 expression was mainly detected in the cytoplasm and nucleus. All normal urothelial tissues were judged as exhibiting positive expression for WWC1. The relationship between WWC1 expression and clinicopathological features is shown in Table I. The proportion of WWC1-positive specimens in patients with high-grade tumors (32.3%) was significantly lower (*p*<0.001) than that in those with low-grade tumors (63.3%). Similarly, the proportion of pT4
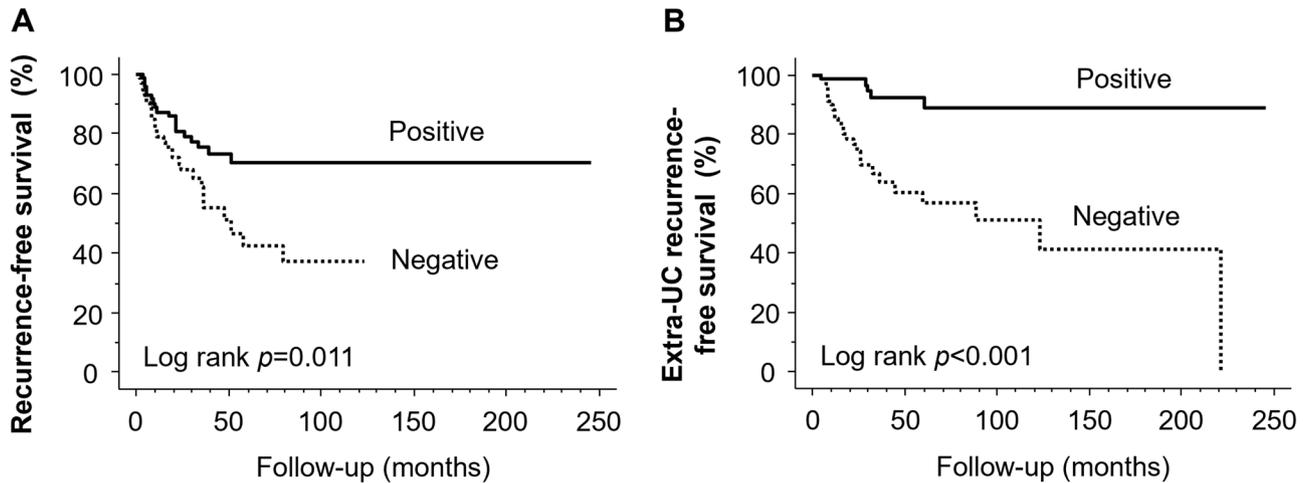
Figure 2. *The Kaplan–Meier survival curves according to WW and C2 domain-containing 1 (WWC1) expression for urinary tract cancer recurrence (A) and subsequent metastasis (B) after radical surgery.*

tumors (21.4%) was remarkably lower ($p<0.001$) than that of the pTa tumors (75.0%). Thus, WWC1 expression was negatively associated with all the pathological features (Table I). On the other hand, the mean age at surgery in patients with WWC1-positive tumors tended to be lower than in those with WWC1-negative tumors (65.6 *versus* 68.9 years, respectively); however, this difference was not significant ($p=0.066$). There was no significant difference in sex and tumor location between the two groups ($p=0.229$ and 0.139, respectively).

Predictive values. The Kaplan–Meier survival curves showed that UC recurrence-free survival for patients with WWC1-positive status was significantly higher ($p=0.011$) than for those with WWC1-negative status (Figure 2A). Similarly, positive expression of WWC1 was a better predictor of extra-UC recurrence-free survival ($p<0.001$) compared to negative expression of WWC1 (Figure 2B). However, the multivariate Cox proportional hazard model including grade, pT stage, and WWC1 expression showed that positive expression of WWC1 was not an independent predictive factor for urinary tract UC recurrence-free survival (Table II). In contrast, multivariate analyses did demonstrate that positive expression of WWC1 was a significant predictor of extra-UC recurrence-free survival (HR=0.29, 95% CI=0.10-0.82, $p=0.020$; Table II).

Correlation of WWC1 with cancer cell proliferation and expression of MMP2 and MMP9. The relationships between WWC1 expression and PI, MMP2, and MMP9 expression are shown in Table III. The univariate logistic regression analyses showed that WWC1 expression was negatively associated with all of these factors (Table III). On the other hand, in the

multivariate analysis models, WWC1 expression was independently associated with PI (OR=0.39, 95% CI=0.17-0.88, $p=0.023$) and MMP9 expression (OR=0.46, 95% CI=0.22-0.97, $p=0.040$), but not with MMP2 expression.

Discussion

Our results showed that WWC1 expression was negatively associated with grade, pT stage, and metastasis in patients with UTUC. In general, WWC1 is recognized as a tumor suppressor through *in vivo* and *in vitro* studies in many cancer types, including lung cancer, cholangiocarcinoma, hepatocellular carcinoma, and renal cell carcinoma (25, 26). Thus, our results were in unison with the expectation regarding the relationship between WWC1 expression and pathological features in UTUC. WWC1 has also been reported to act as a tumor promoter in a variety of cancers, such as gastric and prostate (15, 27). These reports suggest that the pathological significance of WWC1 may depend on the type of cancer. On the other hand, regarding this speculation, it should be noted that in breast cancer, some investigators support the opinion that WWC1 has anti-oncogenic activities (14, 25, 28, 29) but others have shown that WWC1 has pro-carcinogenic activities (8, 16, 30). To summarize, the pathological significance of WWC1 was different for the same type of cancer. Although the methodology and patient backgrounds were not the same in these studies, this finding supports the opinion that the pathological roles of WWC1 in cancer are regulated by complex mechanisms besides the type of cancer.

To our knowledge, this is the first study on the prognostic roles and proliferative effects of WWC1 expression in patients with UTUC. We found that positive expression of WWC1 was

Table II. *Multivariate analysis for location of recurrence after radical surgery.*

		UT			Extra-urothelial organs		
		HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Grade	High	2.15	1.13-4.08	0.020	1.94	0.85-4.44	0.118
pT Stage	MI (≥pT2)	1.15	0.57-2.32	0.696	4.83	1.35-17.22	**0.015**
WWC1	Positive	0.65	0.33-1.28	0.212	0.29	0.10-0.82	**0.020**

CI: Confidence interval; HR: hazard ratio; MI: muscle-invasive; UT: urinary tract. Statistically significant *p*-values are shown in bold.

Table III. *Pathological associations of WW and C2 domain-containing 1 (WWC1) expression.*

	Univariate analyses			Multivariate analyses*		
	OR	95% CI	*p*-Value	OR	95% CI	*p*-Value
PI >Median	0.19	0.09-0.38	<0.001	0.39	0.17-0.88	**0.023**
MMP2-positive	0.32	0.17-0.62	0.001	0.67	0.31-1.47	0.320
MMP9-positive	0.34	0.17-0.65	0.001	0.46	0.22-0.97	**0.040**

CI: Confidence interval; MMP: matrix metalloproteinase; OR: odds ratio associated with WWC1 positivity with WWC1 negativity as reference; PI: proliferative index. Statistically significant *p*-values are shown in bold. *Adjusted for high grade and high pT stage (≥pT2).

a better predictor of extra-urothelial recurrence in the multivariate analysis. Similar results were also observed in patients with breast cancer (26, 28). On the other hand, a previous study reported that WWC1 expression was not associated with any prognostic parameters in patients with gastric cancer (27). However, that study also showed that high expression of WWC1/low expression of atypical protein kinase Cλ/ι correlated with disease-specific and relapse-free survival (27). Unfortunately, except for these, there are few reports on the predictive value of WWC1 expression in patients with cancer. Thus, information on the relationship between WWC1 expression and outcomes is not sufficient to discuss the prognostic roles in patients with cancer. Our results also showed that WWC1 expression was significantly associated with lower cancer cell proliferation in a multivariate analysis model including pathological features. Several investigators have reported that WWC1 suppressed cancer cell proliferation in lung and breast cancer (28, 31). The present study supports these findings. However, it has been reported that overexpression of WWC1 stimulated the proliferation of prostate cancer cell lines (15). In addition, other *in vivo* and *in vitro* studies on breast cancer demonstrated that WWC1 plays an oncogenic role in cancer cell proliferation and tumor growth (16). Thus, there is no general agreement regarding the proliferative effects of WWC1 in cancer.

One of the most interesting results of the present study is that WWC1 expression was closely associated with MMP9 expression. MMP9 has been reported to be positively associated with malignant aggressiveness and poor prognosis

in patients with UTUC (5, 32). Unfortunately, direct correlation between WWC1 and MMP9 is not clear in UTUC. In contrast, *in vivo* and *in vitro* studies have shown that MMP9 expression is negatively associated with Hippo pathway-related molecules in malignant cells. For example, knockdown of YES1-associated transcriptional regulator (YAP1), which is the most well-known effector of the Hippo pathway, significantly inhibited cell proliferation and invasion *via* down-regulation of MMP9 expression in lung cancer and gastric cancer cells (33). In addition, other investigators showed that knockdown of tafazzin (TAZ), which is an important signaling molecule in the Hippo pathway, led to down-regulation of MMP9 expression in a glioma cell line and its orthotopic animal model (34). Moreover, in several cancer types, WWC1 plays an important role in anti-carcinogenic behavior *via* regulation of YAP/TAZ activity (14, 35). This substantiates the belief that WWC1 may inhibit tumor progression through the suppression of MMP9 expression, and the Hippo pathway may be correlated with such WWC1-related tumor-suppressive effects in UTUC. At the same time, further studies are necessary to determine the pathological role of WWC1 in patients with UTUC.

Our study has several limitations. For example, our study population did not include patients with metastatic UTUC. In metastatic UTUC, patients are often treated with systematic chemotherapy without collecting tumor tissues because the diagnosis is clinically confirmed by cytology and imaging examinations. Therefore, we analyzed WWC1 expression only in patients with non-metastatic UTUC treated with radical

surgery. Another limitation is that there were no *in vitro* data about the pathological roles of WWC1. Unfortunately, a versatile UTUC cell line is not yet established. We initially planned to examine WWC1 using bladder cancer cell lines. However, the opinion that bladder cancer has different genetic, biological, and molecular features from UTUC is widely accepted as a result of improvements in genomic engineering and molecular biology (36, 37). Therefore, in this study, we performed only *in vivo* studies.

In conclusion, in patients with non-metastatic UTUC, WWC1 expression was negatively associated with grade and pT stage. In addition, its expression is a useful marker predictive of subsequent metastasis after radical surgery. The molecular mechanism of WWC1-related anticancer effects suggests the suppression of cancer cell proliferation and MMP9 expression. There is the opinion that WWC1 is a novel therapeutic target for malignant cells (38). We suggest that WWC1 may also be a potential therapeutic target, as well as a novel useful predictive marker, in patients with UTUC.

Conflicts of Interest

The Authors declare that they have no conflicts of interest.

Authors' Contributions

Yasuyoshi Miyata designed the study. HK, KM, TK, Yuta Mukae, JH, TM, Yasuyoshi Miyata performed all experiments and prepared the figures and tables. KM, TM, KO, and Yasushi Mochizuki collected, analyzed, and interpreted the clinical data. TK, KM, Yasuyoshi Miyata, and HS wrote and edited the article. All Authors read and approved the final article.

Acknowledgements

References

1 Siegel RL, Miller KD and Jemal A: Cancer statistics, 2019. CA Cancer J Clin *69(1)*: 7-34, 2019. PMID: 30620402. DOI: 10.3322/caac.21551

2 Rouprêt M, Babjuk M, Burger M, Capoun O, Cohen D, Compérat EM, Cowan NC, Dominguez-Escrig JL, Gontero P, Hugh Mostafid A, Palou J, Peyronnet B, Seisen T, Soukup V, Sylvester RJ, Rhijn BWGV, Zigeuner R and Shariat SF: European Association of Urology Guidelines on upper urinary tract urothelial carcinoma: 2020 update. Eur Urol *79(1)*: 62-79, 2021. PMID: 32593530. DOI: 10.1016/j.eururo.2020.05.042

3 Iwata H, Sassa N, Kato M, Murase Y, Seko S, Kawanishi H, Hattori R, Gotoh M and Tsuzuki T: UroVysion® predicts intravesical recurrence after radical nephroureterectomy for urothelial carcinoma of the upper urinary tract: a prospective study. Int J Clin Oncol *26(1)*: 178-185, 2021. PMID: 32959230. DOI: 10.1007/s10147-020-01785-9

4 Shigeta K, Matsumoto K, Ogihara K, Murakami T, Anno T, Umeda K, Izawa M, Baba Y, Sanjo T, Shojo K, Tanaka N, Takeda T, Kosaka T, Mizuno R, Mikami S, Kikuchi E and Oya M: The clinicopathological characteristics of muscle-invasive bladder recurrence in upper tract urothelial carcinoma. Cancer Sci *112(3)*: 1084-1094, 2021. PMID: 33368857. DOI: 10.1111/cas.14782

5 Miyata Y, Watanabe S, Kanetake H and Sakai H: Thrombospondin-1-derived 4N1K peptide expression is negatively associated with malignant aggressiveness and prognosis in urothelial carcinoma of the upper urinary tract. BMC Cancer *12*: 372, 2012. PMID: 22928942. DOI: 10.1186/1471-2407-12-372

6 Dzamic Z, Milojevic B, Kajmakovic B, Grozdic Milojevic I, Bojanic N and Sipetic Grujicic S: Extraurothelial recurrence after radical nephroureterectomy: preoperative predictors and survival. Int Urol Nephrol *47(5)*: 775-779, 2015. PMID: 25772384. DOI: 10.1007/s11255-015-0946-8

7 Kawamura K, Miyai K, Asakuma J, Sato K, Matsukuma S, Tsuda H and Ito K: Tumor budding in upper urinary tract urothelial carcinoma: a putative prognostic factor for extraurothelial recurrence and overall survival. Virchows Arch *479(1)*: 45-55, 2021. PMID: 33404852. DOI: 10.1007/s00428-020-02989-0

8 Singh G, Mishra S and Chander H: KIBRA team up with partners to promote breast cancer metastasis. Pathol Oncol Res *26(2)*: 627-634, 2020. PMID: 30977035. DOI: 10.1007/s12253-019-00660-x

9 Schneider A, Huentelman MJ, Kremerskothen J, Duning K, Spoelgen R and Nikolich K: KIBRA: A new gateway to learning and memory? Front Aging Neurosci *2*: 4, 2010. PMID: 20552044. DOI: 10.3389/neuro.24.004.2010

10 Wilson KE, Yang N, Mussell AL and Zhang J: The regulatory role of KIBRA and PTPN14 in Hippo signaling and beyond. Genes (Basel) *7(6)*: 23, 2016. PMID: 27240404. DOI: 10.3390/genes7060023

11 Stepan J, Anderzhanova E and Gassen NC: Hippo signaling: Emerging pathway in stress-related psychiatric disorders? Front Psychiatry *9*: 715, 2018. PMID: 30627107. DOI: 10.3389/fpsyt.2018.00715

12 Yatsenko AS, Kucherenko MM, Xie Y, Aweida D, Urlaub H, Scheibe RJ, Cohen S and Shcherbata HR: Profiling of the muscle-specific dystroglycan interactome reveals the role of Hippo signaling in muscular dystrophy and age-dependent muscle atrophy. BMC Med *18(1)*: 8, 2020. PMID: 31959160. DOI: 10.1186/s12916-019-1478-3

13 Schelleckes K, Schmitz B, Ciarimboli G, Lenders M, Pavenstädt HJ, Herrmann E, Brand SM and Brand E: Promoter methylation inhibits expression of tumor suppressor KIBRA in human clear cell renal cell carcinoma. Clin Epigenetics *9*: 109, 2017. PMID: 29046731. DOI: 10.1186/s13148-017-0415-6

14 Knight JF, Sung VYC, Kuzmin E, Couzens AL, de Verteuil DA, Ratcliffe CDH, Coelho PP, Johnson RM, Samavarchi-Tehrani P, Gruosso T, Smith HW, Lee W, Saleh SM, Zuo D, Zhao H, Guiot MC, Davis RR, Gregg JP, Moraes C, Gingras AC and Park M: KIBRA (WWC1) is a metastasis suppressor gene affected by chromosome 5q loss in triple-negative breast cancer. Cell Rep *22(12)*: 3191-3205, 2018. PMID: 29562176. DOI: 10.1016/j.celrep.2018.02.095

15 Stauffer S, Chen X, Zhang L, Chen Y and Dong J: KIBRA promotes prostate cancer cell proliferation and motility. FEBS J *283(10)*: 1800-1811, 2016. PMID: 27220053. DOI: 10.1111/febs.13718

16 Anuj, Arivazhagan L, Surabhi RP, Kanakarajan A, Sundaram S, Pitani RS, Mudduwa L, Kremerskothen J, Venkatraman G and Rayala SK: KIBRA attains oncogenic activity by repressing RASSF1A. Br J Cancer *117(4)*: 553-562, 2017. PMID: 28664913. DOI: 10.1038/bjc.2017.192

17 Zhou PJ, Xue W, Peng J, Wang Y, Wei L, Yang Z, Zhu HH, Fang YX and Gao WQ: Elevated expression of Par3 promotes prostate cancer metastasis by forming a Par3/aPKC/KIBRA complex and inactivating the hippo pathway. J Exp Clin Cancer Res *36(1)*: 139, 2017. PMID: 29017577. DOI: 10.1186/s13046-017-0609-y

18 Yu D, Liu H, Qin J, Huangfu M, Guan X, Li X, Zhou L, Dou T, Liu Y, Wang L, Fu M, Wang J and Chen X: Curcumol inhibits the viability and invasion of colorectal cancer cells *via* miR-30a-5p and Hippo signaling pathway. Oncol Lett *21(4)*: 299, 2021. PMID: 33732375. DOI: 10.3892/ol.2021.12560

19 Xiao H, Jiang N, Zhou B, Liu Q and Du C: TAZ regulates cell proliferation and epithelial-mesenchymal transition of human hepatocellular carcinoma. Cancer Sci *106(2)*: 151-159, 2015. PMID: 25495189. DOI: 10.1111/cas.12587

20 Miyata Y, Mitsunari K, Akihiro A, Watanabe SI, Mochizuki Y and Sakai H: Smoking-induced changes in cancer-related factors in patients with upper tract urothelial cancer. Mol Clin Oncol *3(2)*: 287-294, 2015. PMID: 25798255. DOI: 10.3892/mco.2014.471

21 Su YL, Luo HL, Huang CC, Liu TT, Huang EY, Sung MT, Lin JJ, Chiang PH, Chen YT, Kang CH and Cheng YT: Galectin-1 overexpression activates the FAK/PI3K/AKT/mTOR pathway and is correlated with upper urinary urothelial carcinoma progression and survival. Cells *9(4)*: 806, 2020. PMID: 32225123. DOI: 10.3390/cells9040806

22 Edge SB and Compton CC: The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol *17(6)*: 1471-1474, 2010. PMID: 20180029. DOI: 10.1245/s10434-010-0985-4

23 Zhang Y, Yan S, Chen J, Gan C, Chen D, Li Y, Wen J, Kremerskothen J, Chen S, Zhang J and Cao Y: WWC2 is an independent prognostic factor and prevents invasion *via* Hippo signalling in hepatocellular carcinoma. J Cell Mol Med *21(12)*: 3718-3729, 2017. PMID: 28815883. DOI: 10.1111/jcmm.13281

24 Miyata Y, Kanda S, Nomata K, Hayashida Y and Kanetake H: Expression of metalloproteinase-2, metalloproteinase-9, and tissue inhibitor of metalloproteinase-1 in transitional cell carcinoma of upper urinary tract: correlation with tumor stage and survival. Urology *63(3)*: 602-608, 2004. PMID: 15028476. DOI: 10.1016/j.urology.2003.09.035

25 Schelleckes K, Schmitz B, Lenders M, Mewes M, Brand SM and Brand E: ZFP226 is a novel artificial transcription factor for selective activation of tumor suppressor KIBRA. Sci Rep *8(1)*: 4230, 2018. PMID: 29523820. DOI: 10.1038/s41598-018-22600-6

26 Wang T, Qin ZY, Wen LZ, Guo Y, Liu Q, Lei ZJ, Pan W, Liu KJ, Wang XW, Lai SJ, Sun WJ, Wei YL, Liu L, Guo L, Chen YQ, Wang J, Xiao HL, Bian XW, Chen DF and Wang B: Epigenetic restriction of Hippo signaling by MORC2 underlies stemness of hepatocellular carcinoma cells. Cell Death Differ *25(12)*: 2086-2100, 2018. PMID: 29555977. DOI: 10.1038/s41418-018-0095-6

27 Yoshihama Y, Izumisawa Y, Akimoto K, Satoh Y, Mizushima T, Satoh K, Chida K, Takagawa R, Akiyama H, Ichikawa Y, Kunisaki C, Inayama Y, Endo I, Nagashima Y and Ohno S: High expression of KIBRA in low atypical protein kinase C-expressing gastric cancer correlates with lymphatic invasion and poor prognosis. Cancer Sci *104(2)*: 259-265, 2013. PMID: 23163744. DOI: 10.1111/cas.12066

28 Mudduwa L, Peiris H, Gunasekara S, Abeysiriwardhana D, Liyanage N, Rayala SK and Liyanage T: KIBRA; a novel biomarker predicting recurrence free survival of breast cancer patients receiving adjuvant therapy. BMC Cancer *18(1)*: 589, 2018. PMID: 29793439. DOI: 10.1186/s12885-018-4491-6

29 Wang Z, Katsaros D, Biglia N, Shen Y, Fu Y, Tiirikainen M and Yu H: Low expression of WWC1, a tumor suppressor gene, is associated with aggressive breast cancer and poor survival outcome. FEBS Open Bio *9(7)*: 1270-1280, 2019. PMID: 31102318. DOI: 10.1002/2211-5463.12659

30 Yang S, Ji M, Zhang L, Chen Y, Wennmann DO, Kremerskothen J and Dong J: Phosphorylation of KIBRA by the extracellular signal-regulated kinase (ERK)-ribosomal S6 kinase (RSK) cascade modulates cell proliferation and migration. Cell Signal *26(2)*: 343-351, 2014. PMID: 24269383. DOI: 10.1016/j.cellsig.2013.11.012

31 An Y, Zhang Q, Li X, Wang Z, Li Y and Tang X: Upregulated microRNA miR-21 promotes the progression of lung adenocarcinoma through inhibition of KIBRA and the Hippo signaling pathway. Biomed Pharmacother *108*: 1845-1855, 2018. PMID: 30372890. DOI: 10.1016/j.biopha.2018.09.125

32 Chen CC, Hsieh TF, Chang CH, Ma WL, Hung XF, Tsai YR, Lin MH, Zhang C, Chang C and Shyr CR: Androgen receptor promotes the migration and invasion of upper urinary tract urothelial carcinoma cells through the upregulation of MMP-9 and COX-2. Oncol Rep *30(2)*: 979-985, 2013. PMID: 23715826. DOI: 10.3892/or.2013.2506

33 Cui ZL, Han FF, Peng XH, Chen X, Luan CY, Han RC, Xu WG and Guo XJ: YES-associated protein 1 promotes adenocarcinoma growth and metastasis through activation of the receptor tyrosine kinase Axl. Int J Immunopathol Pharmacol *25(4)*: 989-1001, 2012. PMID: 23298489. DOI: 10.1177/039463201202500416

34 Li W, Dong S, Wei W, Wang G, Zhang A, Pu P and Jia Z: The role of transcriptional coactivator TAZ in gliomas. Oncotarget *7(50)*: 82686-82699, 2016. PMID: 27764783. DOI: 10.18632/oncotarget.12625

35 Park J, Kim JS, Nahm JH, Kim SK, Lee DH and Lim DS: WWC1 and NF2 prevent the development of intrahepatic cholangiocarcinoma by regulating YAP/TAZ activity through LATS in mice. Mol Cells *43(5)*: 491-499, 2020. PMID: 32451369. DOI: 10.14348/molcells.2020.0093

36 Green DA, Rink M, Xylinas E, Matin SF, Stenzl A, Roupret M, Karakiewicz PI, Scherr DS and Shariat SF: Urothelial carcinoma of the bladder and the upper tract: disparate twins. J Urol *189(4)*: 1214-1221, 2013. PMID: 23023150. DOI: 10.1016/j.juro.2012.05.079

37 Sfakianos JP, Gul Z, Shariat SF, Matin SF, Daneshmand S, Plimack E, Lerner S, Roupret M and Pal S: Genetic differences between bladder and upper urinary tract carcinoma: implications for therapy. Eur Urol Oncol *4(2)*: 170-179, 2021. PMID: 33386276. DOI: 10.1016/j.euo.2020.12.007

38 Mavuluri J, Beesetti S, Surabhi R, Kremerskothen J, Venkatraman G and Rayala SK: Phosphorylation-dependent regulation of the DNA damage response of adaptor protein KIBRA in cancer cells. Mol Cell Biol *36(9)*: 1354-1365, 2016. PMID: 26929199. DOI: 10.1128/MCB.01004-15