Prognostic significance of a component of the Hippo pathway, TAZ, in human uterine endometrioid adenocarcinoma

MAOSHENG ZHAN[1], JUN-ICHIRO IKEDA[1], NAOKI WADA[1], YUMIKO HORI[1], SATOSHI NOJIMA[1], SHIN-ICHIRO TAHARA[1], YUTAKA UEDA[2], KIYOSHI YOSHINO[2], TADASHI KIMURA[2] and EIICHI MORII[1]

Departments of [1]Pathology and [2]Obstetrics and Gynecology, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871, Japan

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Abstract. Transcriptional coactivator with PDZ-binding motif (TAZ) is a crucial component of the Hippo tumor suppressor pathway, interacting with transcriptional factors to regulate cell proliferation, apoptosis and tumorigenesis. TAZ and its paralog, Yes-associated protein (YAP), are activated at high frequencies during the progression towards malignancy in various tumors. Recently, YAP has been identified to modulate oncogenic features in endometrial adenocarcinoma, and it has also been reported that the nuclear expression of YAP is correlated with the poorly-differentiated form of endometrioid adenocarcinoma. In contrast to YAP, no studies have investigated TAZ expression in endometrioid adenocarcinoma. In the present study, TAZ expression was immunohistochemically examined in 55 clinical samples of endometrioid adenocarcinoma, and the clinical implications were evaluated. The results demonstrated that TAZ was located primarily in the cell nuclei, and that high TAZ expression was significantly correlated with high tumor-factor (P=0.024), stage (P=0.041) and histological grade (P=0.001), lymph node metastasis (P=0.046), recurrence (P=0.002) and a poor prognosis (P=0.007). Furthermore, univariate analysis identified that high TAZ expression was a poor prognostic factor for overall and disease-free survival. To the best of our knowledge, the present case is the first to report the clinical implications of TAZ in endometrioid adenocarcinoma of the uterus. TAZ may become a marker of a poor prognosis in endometrioid adenocarcinoma.

Introduction

Transcriptional coactivator with PDZ-binding motif (TAZ) is a WW domain-containing transcriptional coactivator, which interacts with a number of transcription factors, including Smad and runt-related transcription factor (1,2). TAZ is a component of the Hippo tumor suppressor signaling pathway that serves a key role in the regulation of apoptosis, cell proliferation and tumorigenesis (3,4). When the Hippo pathway is activated, *Mst*II and large tumor suppressor kinase (LATS)1/2, human homologs of *Drosophila* Hippo and LATS, phosphorylate TAZ and its paralog Yes-associated protein (YAP), sequestering them to the cytoplasm (5). Conversely, the inactivation of the Hippo pathway results in dephosphorylation of TAZ/YAP. Dephosphorylated TAZ/YAP accumulate in the nucleus, and primarily function through transcription factors to promote cell proliferation. YAP is a moderately stable protein and is predominantly regulated by nuclear-cytoplasmic shuttling. By contrast, TAZ is extremely unstable with a half-life of <2 h, indicating that its degradation is the primary method of TAZ inhibition (6). TAZ/YAP are activated at high frequencies during tumorigenesis in various forms of cancer. YAP has been detected in gastric, colon, esophageal, liver and non-small cell lung cancer, and in lobular carcinoma of the breast (7-12), whilst TAZ is expressed in the cell nuclei of breast, lung and colon cancer (7,13,14).

Uterine cancer is the most common gynecological malignancy, with an incidence rate of 12.9 cases per 100,000 women and a mortality rate of 2.4 cases per 100,000 women. Endometrioid adenocarcinoma is the most prevalent invasive malignancy of all uterine cancers (15,16). Despite advances in the detection and treatment of endometrioid adenocarcinoma, the patient prognosis remains unfavorable. The clinical implications of numerous markers, including CUB domain-containing protein 1 and aldehyde dehydrogenase 1, have been previously investigated in uterine endometrioid adenocarcinoma (17-19), but few studies have analyzed the expression of Hippo pathway components in association with this tumor. Recently, Tsujiura *et al* (20) reported that YAP modulates radiation sensitivity and oncogenic features in endometrial cancer, including endometrioid and serous adenocarcinoma. The knockdown of YAP expression in the HEC-1B cell line increases sensitivity to radiation, and the nuclear expression of YAP correlates with the poorly-differentiated form of endometrioid adenocarcinoma. To date, no studies have investigated TAZ expression in endometrioid adenocarcinoma. In the present study, TAZ expression was immunohistochemically examined

Correspondence to: Professor Eiichi Morii, Department of Pathology, Osaka Univeristy Graduate School of Medicine, Yamada-oka 2-2, Suita, Osaka 565-0871, Japan
E-mail: morii@molpath.med.osaka-u.ac.jp

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Table I. Summary of characteristics of 55 patients with endometrioid adenocarcinoma.

Characteristics	Patients, n
Tumor	
T1	40
T2	5
T3	10
Stage	
I	37
II	3
III	11
IV	4
Tumor histological grade	
1	25
2	20
3	10
Lymph node metastasis	
Negative	43
Positive	12
Recurrence	
Negative	44
Positive	11
Prognosis	
Alive with no recurrence	44
Alive with recurrence	3
Succumbed to disease	8

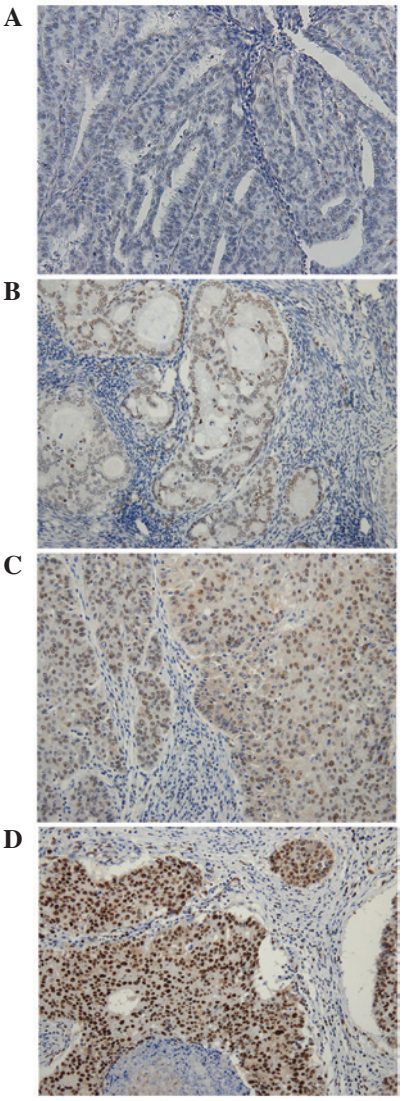

Figure 1. Varying immunohistochemical staining intensities representing TAZ expression in endometrioid adenocarcinoma tissue. Representative fields are as follows: (A) No staining, (B) weak staining, (C) moderate staining and (D) strong staining. Magnification, x400. TAZ, transcriptional coactivator with PDZ-binding motif.

in 55 clinical samples of endometrioid adenocarcinoma, and the clinical implications were evaluated.

Materials and methods

Patients and methods. A total of 55 samples were obtained from patients who underwent a hysterectomy due to endometrioid adenocarcinoma at Osaka University Hospital (Suita, Japan) between January 1999 and January 2003. No prior chemotherapy/radiotherapy was administered in any case. Each sample was examined, and the clinicopathological findings are summarized in Table I. Patient age ranged from 32-71 years (median, 56.1 years). Resected specimens were macroscopically examined to determine the location and size of the tumors. Histological specimens were fixed in 10% formalin and routinely processed for paraffin-embedding. The paraffin-embedded specimens were stored in a dark room in the Department of Pathology, Osaka University Hospital, at room temperature. The specimens were cut into 4-μm thick sections and stained with hematoxylin and eosin, and then underwent an immunoperoxidase procedure. Histological staging was determined according to the International Federation of Obstetricians and Gynecologists (FIGO) staging system (21). All patients were followed up with laboratory examinations, including routine peripheral blood cell counts at 1- to 6-month intervals, and X-ray, computed tomography and pelvic examinations at 6- to 12-month intervals. The follow-up period for survivors ranged from 7-176 months (median, 106 months). The present study was approved by the Ethical Review Board of the Graduate School of Medicine, Osaka University (Suita, Japan).

Immunohistochemistry for TAZ. Subsequent to deparaffinization with xylene and rehydration with graded alcohol treatment, sections were heated to 121˚C in the Pascal Pressurized Heating Chamber (Dako, Glostrup, Denmark). After cooling, the sections were washed in phosphate-buffered saline, blocked with blocking solution (cat no. X0909; Dako) and incubated with the primary rabbit anti-TAZ polyclonal antibody (dilution 1:500 cat no. ab110239; Abcam, Cambridge, UK). Next, the sections were treated with the ChemMate™ Envision™ Detection kit (Dako) that contains a polymerized secondary antibody to increase detection sensitivity for the primary antibody. 3,3'-Diaminobenzidine (Dako) was used as

Table II. Association between TAZ expression level and clinicopathological parameters.

Characteristics	TAZ histological score[a]
Tumor	
T1	113±13
T2	190±32[b]
T3	198±25[b]
Stage	
I	109±13
II	213±52[b]
III	175±24[b]
IV	211±36[b]
Tumor histological grade	
1	82±13
2	176±14[b]
3	187±31[b]
Lymph node metastasis	
Negative	120±13
Positive	191±20[b]
Recurrence	
Negative	110±11
Positive	235±14[b]
Prognosis	
Alive with no recurrence	112±11
Alive with recurrence	250±21[b]
Succumbed to disease	230±18[b]

[a]Data are represented as the mean ± standard error. [b]P<0.05. TAZ, transcriptional coactivator with PDZ-binding motif.

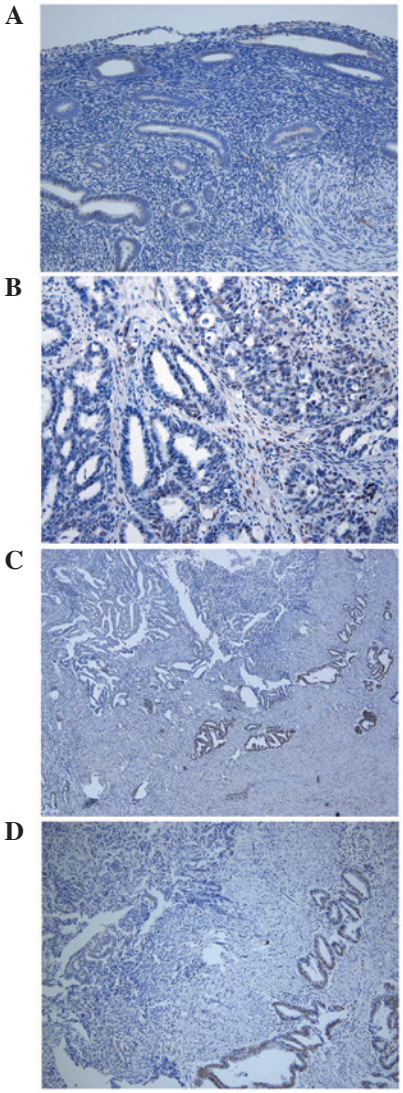

Figure 2. TAZ expression in normal endometrial and endometrioid adenocarcinoma tissues. (A) Limited signals representing TAZ expression were detected in non-cancerous endometrial tissues (magnification, x200). (B) Tumor cells with a solid structure (right of image) expressed high levels of TAZ compared with cells with a glandular structure (left of image) (magnification, x400). (C) Portions invading into the myometrium expressed high levels of TAZ (magnification, x40). (D) High-power field of (C) (magnification, x200). Invading tumor cells (right of image) expressed high levels of TAZ compared with non-invading cells (left of image). TAZ, transcriptional coactivator with PDZ-binding motif.

a chromogen. As the negative control, staining was carried out in the absence of the primary antibody. Sections were counterstained with hematoxylin and observed by microscopy (BX50; Olympus, Tokyo, Japan).

Evaluation of immunohistochemical staining. TAZ staining was scored independently by two pathologists who examined the samples in a blinded manner with respect to the clinical information of the subjects. The intensity of the signal was divided into 4 grades as follows: None, 0; weak, 1; moderate, 2; and strong, 3 (Fig. 1). The area percentage of each grade was determined. The sum of multiplying the area percentage of each grade by the signal intensity was termed the TAZ histological score, where (% of 0 x 0) + (% of 1 x 1) + (% of 2 x 2) + (% of 3 x 3). The minimum score was 0 and the maximum was 300. Cases with a TAZ histological score of <100 were categorized as TAZ-low and cases with a score of >100 were categorized as TAZ-high.

Statistical analysis. Statistical analyses were performed using JMP Pro 10.0.2 software (SAS Institute Inc., Cary, NC, USA). The χ^2 test was used to analyze the association between TAZ expression and clinicopathological factors in patients with endometrioid adenocarcinoma. Overall survival (OS) was measured from the time between diagnosis and mortality, and disease-free survival (DFS) was measured as the time between diagnosis and disease recurrence. Kaplan-Meier survival analysis was used to calculate the OS and DFS rates, and differences in survival curves were evaluated with the log-rank test. Cox's proportional hazards regression model with a stepwise manner was used to analyze the independent prognostic factors. P≤0.05 was considered to indicate a statistically significant difference.

Results

Immunohistochemical analysis. Limited signals representing TAZ expression were detected in non-cancerous endometrial tissues (Fig. 2A), whereas cancerous endometrial tissues

Table III. Association between TAZ histological score and clinicopathological parameters.

Characteristics	TAZ histological score		P-value
	Low	High	
Tumor			0.024
T1	21	19	
T2	0	5	
T3	2	8	
Stage			0.041
I	20	17	
II	0	3	
III	3	8	
IV	0	4	
Histological grade			0.001
1	17	8	
2	3	17	
3	3	7	
Lymph node metastasis			0.046
Negative	21	22	
Positive	2	10	
Recurrence			0.002
Negative	23	21	
Positive	0	11	
Prognosis			0.007
Alive with no recurrence	23	21	
Alive with recurrence	0	3	
Succumbed to disease	0	8	

TAZ, transcriptional coactivator with PDZ-binding motif.

expressed TAZ at varied intensities. TAZ was primarily detected in the cell nuclei. Generally, cells with a solid structure expressed high levels of TAZ when compared with cells with a glandular structure (Fig. 2B). Portions invading into the myometrium expressed high levels of TAZ (Fig. 2C and D).

Association between TAZ expression and clinicopathological features. The associations between TAZ expression level (TAZ histological score) and clinicopathological features were evaluated (Table II). Cases with low tumor (T)-factor exhibited a lower TAZ histological score (T1 vs. T2 and T3). Similarly, the histological score was significantly lower in cases with low stage and low histological grade (stage I vs. II, III and IV; grade 1 vs. 2 and 3), and without lymph node metastasis and recurrence. Cases with a poor prognosis were associated with a high TAZ histological score.

Subsequently, the cases were divided into TAZ-high and TAZ-low groups using a cut-off histological score of 100, as this score was the most optimal for discriminating between disease characteristics. A total of 23 cases were classified as TAZ-low and 32 were classified as TAZ-high. The association between TAZ expression and clinicopathological features was re-evaluated (Table III). TAZ-high cases were significantly

Table IV. Univariate and multivariate analyses of prognostic factors for overall and disease-free survival.

Characteristics	Overall survival				Disease-free survival			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Tumor	1.66 (1.14-2.74)	0.007	1.11 (0.61-2.87)	0.77	1.86 (1.24-3.33)	0.002	1.62 (0.84-6.56)	0.176
Stage	3.81 (1.57-17.4)	0.002	0.61 (0.02-7.77)	0.72	4.00 (1.65-18.2)	0.001	0.35 (0.01-6.79)	0.504
Histological grade	1.55 (0.53-4.57)	0.412	0.77 (0.24-2.91)	0.67	1.66 (0.57-4.78)	0.339	0.91 (0.28-3.27)	0.869
Lymph node metastasis	3.07 (1.15-6.04)	0.002	25.98 (0.73-148549.7)	0.09	3.05 (1.14-6.03)	0.002	18.98 (0.34-108330.1)	0.188
TAZ histological index	3.42 (1.22-12.9)	0.018	2.85 (0.65-18.9)	0.17	3.94 (1.37-15.1)	0.009	3.00 (0.72-22.6)	0.139

HR, hazard ratio; CI, confidence interval; TAZ, transcriptional coactivator with PDZ-binding motif.

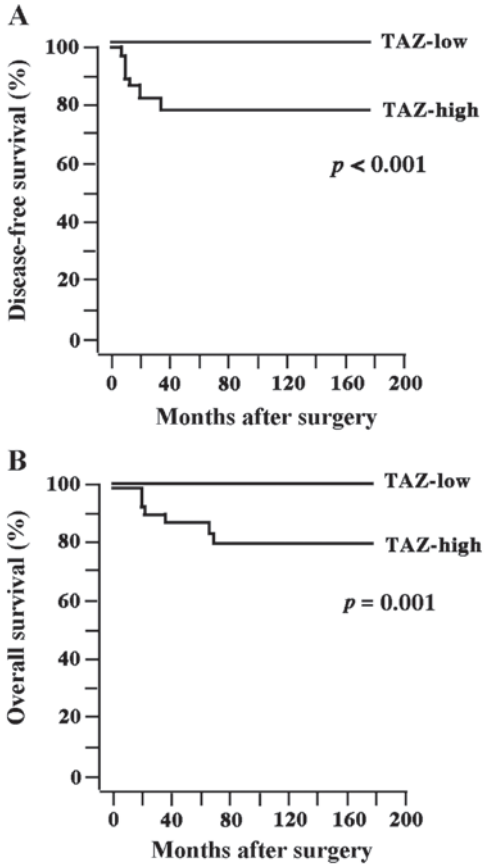

Figure 3. Kaplan-Meier survival plots. (A) Disease-free and (B) overall survival curves are shown. TAZ-high cases demonstrated less favorable disease-free and overall survival. TAZ, transcriptional coactivator with PDZ-binding motif.

correlated with high T-factor (P=0.024), stage (P=0.041) and histological grade (P=0.001), lymph node metastasis (P=0.046), recurrence (P=0.002) and a poor prognosis (P=0.007). The 5-year DFS and OS rates were 80.0 and 85.5%, respectively. Tumors recurred in 11 patients, and 8 of these patients succumbed to the disease. There was a significant difference in DFS (P<0.001) and OS rates (P=0.001) between the TAZ-high and TAZ-low groups (Fig. 3).

Univariate analysis demonstrated that T-factor, stage, lymph node metastasis and TAZ expression were significant factors for OS and DFS rate (Table IV). Multivariate analysis was subsequently performed on these four factors. The results indicated that none of the factors significantly affected OS or DFS rate (Table IV).

Discussion

As major downstream effectors of the Hippo signaling pathway, TAZ and YAP are not only similar in terms of their structures, but also with regard to their functions (1). Overexpression of YAP has been reported to be associated with a poor prognosis in several types of human cancer, including ovarian, hepatocellular and breast cancer, and malignant mesothelioma (3,9,12,22,23). Tsujiura *et al* (20) reported that high levels of YAP expression in the cell nuclei of endometrioid adenocarcinoma correlates with the poorly-differentiated histological type and a poor prognosis. To date, no studies have investigated TAZ expression in endometrioid adenocarcinoma. In the present study, it was demonstrated that patients with endometrioid adenocarcinoma and high TAZ expression had a significantly shorter survival time, and that high TAZ expression was associated with high clinical stage. In addition, the expression level of TAZ was higher in grade 2 and grade 3 tumors than in grade 1 tumors, indicating that poorly-differentiated histological type was correlated with TAZ expression. These results were consistent with the functional similarities between TAZ and YAP.

YAP/TAZ is primarily regulated by nuclear-cytoplasmic shuttling. The activation of the Hippo pathway results in phosphorylation of YAP/TAZ by LATS1/2, which sequesters YAP/TAZ to the cytoplasm, where it is subsequently degraded (5). YAP/TAZ nuclear accumulation is a key determinant of their function, as YAP/TAZ is a transcription factor that functions in the nucleus (1,2). In endometrioid adenocarcinoma, YAP has been reported to be located in the cytoplasm and nucleus (20). By contrast, the present study demonstrated that TAZ was predominantly located in the nucleus. The half-life of TAZ is known to be shorter than that of YAP. Thus, when TAZ is sequestered to the cytoplasm it may be easily degraded, which may possibly explain why limited TAZ expression was detected in the cytoplasm of the endometrioid adenocarcinoma tissues.

In conclusion, to the best of our knowledge, the present study has demonstrated for the first time that TAZ expression is correlated with a poor prognosis, and that it serves as an independent prognostic factor for survival in patients with uterine endometrioid adenocarcinoma. TAZ may become a future clinical marker of a poor prognosis in this disease.

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