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ANATOMICAL PATHOLOGY

High Yes-associated protein 1 with concomitant negative LATS1/2 expression is associated with poor prognosis of advanced gastric cancer

Eojin Kim[1], Bokyung Ahn[1], Harim Oh[1], Yoo Jin Lee[1], Jeong Hyeon Lee[1], Youngseok Lee[1], Chul Hwan Kim[1], Yang-Seok Chae[1], Joo Young Kim[2]

[1]*Department of Pathology, Korea University Anam Hospital, Korea University College of Medicine, Seoul, South Korea;* [2]*Department of Pathology, Nowon Eulji Medical Center, Eulji University, Seoul, South Korea*

Summary

The Hippo pathway is a tumour-suppressive pathway and its inactivation is known to be associated with progression and metastasis of various cancers. LATS1/2 (large tumour suppressor homolog 1 and 2), YAP1 (Yes-associated protein 1), and TEAD4 (TEA domain-containing sequence-specific transcription factors 4) are core components of the Hippo pathway, and their prognostic roles have not yet been studied in advanced gastric cancers (AGCs). A total of 318 surgically resected AGCs were retrieved. Immunolabelling for LATS1/2, YAP1 and TEAD4 was compared with clinicopathological factors including patients' survival. High expression of YAP1 and TEAD4 was identified in 108 (34.0%) and 131 (41.2%) cases, respectively, and 223 (70.1%) cases were negative for LATS1/2 expression. High YAP1 expression was significantly correlated with the presence of perineural invasion (p=0.032). High YAP1 and high TEAD4 expressions were significantly associated with poor overall survival (p<0.001 and p=0.003, respectively), and negative LATS1/2 expression was also associated with poor overall survival (p=0.002). Combined expression of YAP1^{high}LATS1/2^{neg} showed the worst overall survival (p<0.001). Expression of YAP1^{high} (HR=2.938; 95% CI 1.726–4.998; p<0.001), LATS1/2^{neg} (HR=0.371; 95% CI 0.181–0.758; p=0.007), and combined YAP1^{high}LATS1/2^{neg} (HR=13.785; 95% CI 3.245–58.554; p<0.001) were independent poor prognostic factors of AGC patients. Combined or individual expression of YAP1, LATS1/2, and TEAD4 can be used as prognostic markers of AGC patients.

Key words: Advanced gastric cancer; hippo pathway; YAP1; LATS1/2; TEAD4; prognosis.

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INTRODUCTION

Gastric cancer is one of the most common malignancies and a leading cause of cancer-related death worldwide. Despite advances in chemotherapeutic regimens and targeted therapies in some subsets of advanced gastric cancer patients, the prognosis remains poor, with a 5-year survival rate of 20–30%.[1,2] Many molecular alterations involved in gastric carcinogenesis have been elucidated, including genetic instability, alteration of oncogenes and tumour suppressor genes, and genetic mutations.[3–6] Some of these alterations are being used for novel diagnostic methods and are targets of therapeutic agents in advanced gastric cancers.[7]

The Hippo pathway is a tumour-suppressive pathway that inhibits cell proliferation and promotes apoptosis, and its inactivation is known to be associated with progression and metastasis of various cancers.[8,9] The Hippo pathway is composed of regulatory kinases such as MST1/2 (mammalian STE20-like protein kinase 1/2) and LATS1/2 (large tumour suppressor homolog 1 and 2), and transcriptional modulators such as YAP (Yes-associated protein) and TAZ (transcriptional coactivator with PDZ-binding motif).[10] Activated kinases phosphorylate YAP and TAZ, promote their cytoplasmic sequestration and degradation. When the Hippo kinase is dysregulated, YAP and TAZ translocate into the nucleus and interact with transcription factors, such as TEAD (TEA domain-containing sequence-specific transcription factors), that modulate the transcription of various oncogenes.[10,11]

YAP1 is a nuclear transcriptional coactivator of the Hippo pathway and related to several oncogenic functions including epithelial-mesenchymal transition, growth factor-independent proliferation, suppression of apoptosis, anchorage-independent growth, and metastasis.[12,13] Nuclear overexpression of YAP1 has been reported in many kinds of cancers and is associated with unfavourable prognosis in various carcinomas including gastric cancers.[14,15] LATS1 and LATS2, serine/threonine-protein kinases, are key regulators of the Hippo pathway that generate a tumour suppressive signal via inhibiting cellular proliferation and promoting apoptosis.[16–19] Inactivation of LATS1/2 increases oncogenic properties by promoting the nuclear translocation of YAP/TAZ.[16–19] Abnormal LATS expression has been found in cancers of the cervix, breast, and liver,[20–22] and some studies showed loss of LATS expression in gastric cancers.[7,23] TEAD is a transcription factor that regulates genes related to epithelial-mesenchymal transition, as well as cell proliferation and invasion in various cancers, which promotes tumour progression and metastasis as a nuclear

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target of YAP.[9,24] Increased YAP and TEAD has a critical role in cancer development and metastasis.[24] Upregulated TEAD is observed in various cancers including breast, colon, gastric and renal cell carcinoma and TEAD shows prognostic significance for some of these cancers.[24] A few studies reported TEAD expression in gastric cancer tissues with a limited number of cases and no clinicopathological correlation has been studied.[25,26]

Hence, in advanced gastric cancers we evaluated the expression of YAP1, LATS1/2, and TEAD4, which are core components of the Hippo pathway, and assessed their correlation with other clinicopathological factors, and their prognostic significance.

MATERIALS AND METHODS

Case selection

A total of 318 advanced gastric cancers, which are defined as gastric cancers invading the muscularis propria or deeper, regardless of lymph node and distant metastasis, resected between January 2009 and December 2012, were retrieved from the Department of Pathology at the Korea University Anam Hospital. Clinical data including age, sex, survival time, and survival status were reviewed. Pathological data such as tumour location, Lauren classification, size, grade, and pT classification of tumour, lymphovascular invasion, perineural invasion, lymph node status, distant metastasis, and resection marginal status were reviewed. The TNM stages were adjusted to the 8th American Joint Committee on Cancer (AJCC) Staging Manual. The Institutional Review Boards of Korea University Anam Hospital approved this study (AN17287-003).

Tissue microarray construction and immunohistochemistry

All slides were reviewed and representative slides and blocks for each case were selected. Tissue microarrays (TMAs) were constructed from formalin fixed, paraffin embedded tissue blocks with a tissue microarrayer. Two cores with a diameter of 3.0 mm were extracted from selected tumour blocks and rearranged to recipient blocks.

From each TMA, 4-μm thick sections were cut. Immunohistochemical staining was performed as previously described.[27] Briefly, sections were deparaffinised and hydrated in serially diluted alcohol. Sections were heated with citrate buffer for 15 min for antigen retrieval, and incubated in Hydrogen Peroxide Block (Cell Marque, USA) for 10 min to block endogenous peroxidase. Sections were incubated with a primary antibody for YAP1 (1:500; Cell Signaling, USA), LATS1/2 (1:100; Abcam, UK), and TEAD4 (1:300; Abcam) and a secondary antibody, followed by counterstaining with haematoxylin (Scytec, USA).

Assessment of immunohistochemistry

The nuclear expression of YAP1 and TEAD4 was scored by the multiplying staining intensity (0, none; 1+, weak; 2+, moderate; 3+, strong) and the proportion of stained cells (0, none; 1, 1–25%; 2, >25–50%; 3, >50–75%; 4, >75%), ranging from 0 to 12. The cases were categorised into low (score 0–5) and high (score 6–12) expression groups.[14,28,29] LATS1/2 staining was considered positive if immunoreactivity of any intensity was observed in at least 10% of tumour cells, as previously described.[30] The scoring was independently performed by two pathologists (JYK and EK).

Statistical analyses

Statistical analyses were performed using chi-square test and Fisher's exact test to assess the correlation between YAP1, LATS1/2 and TEAD4 expressions and other clinicopathological factors. The overall survival rates were analysed by the Kaplan–Meier method with a log-rank test. The significance of those expressions as prognostic factors was evaluated using the Cox proportional hazards regression model. The analysis was tested for whether Cox proportional hazard assumptions were met using time-dependent Cox proportional hazard regression analysis. $p<0.05$ was considered statistically significant, and variables with $p<0.05$ in univariate analyses were included in multivariate analyses. All statistical analyses were performed with SPSS version 18.0 (SPSS, USA).

RESULTS

Characteristics of cases

Patients' characteristics are shown in Table 1. The mean age of the patients was 60 years (range 26–84 years). There were 205 (64.5%) males and 113 (35.5%) females. There were 29 (9.1%), 107 (33.6%), and 182 (57.3%) cases of well, moderately, and poorly differentiated adenocarcinomas, respectively. According to the Lauren classification, 151 cases (47.5%) were intestinal type, and 167 (52.5%) cases were diffuse type. The mean tumour size was 6.3 cm (range 1.5–29.0 cm). There were 71 (22.3%), 155 (48.8%), and 92 (28.9%) cases of pT2, pT3, and pT4 classifications of cancers, respectively. Lymphovascular invasion was exhibited in 188 cases (59.1%), and 88 cases (27.7%) had perineural invasion. Lymph node metastasis was identified in 230 cases (72.3%), and distant metastasis occurred in 11 cases (3.5%). According to the 8th AJCC staging, 43 (13.5%), 89 (28.0%), 175 (55.0%), and 11 (3.5%) cases were I, II, III, and IV stages, respectively. The median follow-up time was 43 months (range 1–99 months).

Expression of YAP1, LATS1/2, and TEAD4 in advanced gastric cancers

The expression profiles of YAP1, TEAD4, and LATS1/2 are depicted in Fig. 1 and 2. YAP1 was highly expressed in 108 cases (34.0%) while 210 cases (66.0%) showed low expression. High YAP1 expression was significantly correlated with the presence of perineural invasion ($p=0.032$). LATS1/2 was positive in 95 cases (29.9%), and TEAD4 was highly expressed in 131 cases (41.2%). Both LATS1/2 and TEAD4 expressions were not significantly associated with any clinicopathological factors (Table 1). The high expression of YAP1 was significantly correlated with high TEAD4 expression ($p<0.001$). Positive LATS1/2 expression was significantly associated with high expression of YAP1 ($p=0.045$) and TEAD4 ($p=0.007$) (Table 2).

Survival analysis of YAP1, LATS1/2, and TEAD4 expressions in advanced gastric cancers

The overall survival of patients with $YAP1^{high}$ expression was significantly worse than that in patients with $YAP1^{low}$ expression [HR=2.777; 95% confidence interval (CI) 1.709–4.514; $p<0.001$, Fig. 3A]. The patients with LATS1/2 expression had significantly better overall survival than those without expression (HR=0.337; 95% CI 0.167–0.681; $p=0.002$, Fig. 3B). $TEAD4^{high}$ expression was significantly associated with poor overall survival (HR=1.206; 95% CI 1.067–1.362; $p=0.003$, Fig. 3C). The cases were subdivided into four groups in association with YAP1 and LATS1/2 expressions as follows: $YAP1^{low}LATS1/2^{pos}$, $YAP1^{high}LATS1/2^{neg}$, $YAP1^{low}LATS1/2^{neg}$, and $YAP1^{high}LATS1/2^{pos}$. There were 55 (17.3%), 68 (21.4%), 155 (48.7%), and 40 (12.6%) cases of $YAP1^{low}LATS1/2^{pos}$, $YAP1^{high}LATS1/2^{neg}$, $YAP1^{low}LATS1/2^{neg}$, and $YAP1^{high}LATS1/2^{pos}$, respectively, and the 5-year survival rates of those groups were 96.3%, 44.3%, 76.8%, and 74.7%, respectively ($p<0.001$, Fig. 3D). The survival of patients in $YAP1^{low}LATS1/2^{neg}$ (76.8%) and $YAP1^{high}LATS1/2^{pos}$ (74.7%) groups did not significantly differ ($p=0.766$), thus we combined $YAP1^{low}LATS1/2^{neg}$ and $YAP1^{high}LATS1/2^{pos}$ into

Table 1 Correlations between YAP1, LATS1/2 and TEAD expressions and clinicopathological factors

Clinicopathological factors	No.	YAP1 expression			LATS1/2 expression			TEAD4 expression		
		Low (%) n=210	High (%) n=108	p value	Negative (%) n=223	Positive (%) n=95	p value	Low (%) n=187	High (%) n=131	p value
Age (years)										
≤60	161	111 (68.9%)	50 (31.1%)	0.268	108 (67.1%)	53 (32.9%)	0.230	92 (57.1%)	69 (42.9%)	0.542
>60	157	99 (63.1%)	58 (36.9%)		115 (73.2%)	42 (26.8%)		95 (60.5%)	62 (39.5%)	
Sex										
Male	205	139 (67.8%)	66 (32.2%)	0.370	150 (73.2%)	55 (26.8%)	0.110	115 (56.1%)	90 (43.9%)	0.186
Female	113	71 (62.8%)	42 (37.2%)		73 (64.6%)	40 (35.4%)		72 (63.7%)	41 (36.3%)	
Differentiation										
Well	29	20 (69.0%)	9 (31.0%)	0.650	21 (72.4%)	8 (27.6%)	0.614	19 (65.5%)	10 (34.5%)	0.996
Moderate	107	67 (62.6%)	40 (37.4%)		71 (66.4%)	36 (33.6%)		59 (55.1%)	48 (44.9%)	
Poor	182	123 (67.6%)	59 (32.4%)		131 (72.0%)	51 (28.0%)		109 (59.9%)	73 (40.1%)	
Lauren classification				0.946			0.643			0.386
Intestinal	151	100 (66.2%)	51 (33.8%)		104 (68.9%)	47 (31.1%)		85 (56.3%)	66 (43.7%)	
Diffuse	167	110 (65.9%)	57 (34.1%)		119 (71.3%)	48 (28.7%)		102 (61.1%)	65 (38.9%)	
Size										
≤6 cm	193	127 (65.8%)	66 (34.2%)	0.913	129 (66.8%)	64 (33.2%)	0.112	114 (59.1%)	79 (40.9%)	0.906
>6 cm	125	83 (66.4%)	42 (33.6%)		94 (75.2%)	31 (24.8%)		73 (58.4%)	52 (41.6%)	
pT classification										
pT2	71	50 (70.4%)	21 (29.6%)	0.141	48 (67.6%)	23 (32.4%)	0.282	39 (54.9%)	32 (45.1%)	0.708
pT3	155	105 (67.7%)	50 (32.3%)		106 (68.4%)	49 (31.6%)		99 (63.9%)	56 (36.1%)	
pT4	92	55 (59.8%)	37 (40.2%)		69 (75.0%)	23 (25.0%)		49 (53.3%)	43 (46.7%)	
Lymphovascular invasion										
Absence	130	88 (67.7%)	42 (32.3%)	0.604	88 (67.7%)	42 (32.3%)	0.430	79 (60.8%)	51 (39.2%)	0.554
Presence	188	122 (64.9%)	66 (35.1%)		135 (71.8%)	53 (28.2%)		108 (57.4%)	80 (42.6%)	
Perineural invasion										
Absence	230	160 (69.6%)	70 (30.4%)	0.032[a]	165 (71.7%)	65 (28.3%)	0.310	139 (60.4%)	91 (39.6%)	0.340
Presence	88	50 (56.8%)	38 (43.2%)		58 (65.9%)	30 (34.1%)		48 (54.5%)	40 (45.5%)	
Lymph node metastasis										
Absence	88	62 (70.5%)	26 (29.5%)	0.304	60 (68.2%)	28 (31.8%)	0.639	58 (65.9%)	30 (34.1%)	0.111
Presence	230	148 (64.3%)	82 (35.7%)		163 (70.9%)	67 (29.1%)		129 (56.1%)	101 (43.9%)	
Distant metastasis										
Absence	307	202 (65.8%)	105 (34.2%)	0.633	215 (70.0%)	92 (30.0%)	0.848	179 (58.3%)	128 (41.7%)	0.340
Presence	11	8 (72.7%)	3 (27.3%)		8 (72.7%)	3 (27.3%)		8 (72.7%)	3 (27.3%)	
AJCC stage				0.113			0.264			0.934
I	43	26 (60.5%)	17 (39.5%)		27 (62.8%)	16 (37.2%)		27 (62.8%)	16 (37.2%)	
II	89	54 (60.7%)	35 (39.3%)		62 (69.7%)	27 (30.3%)		53 (59.6%)	36 (40.4%)	
III	175	122 (69.7%)	53 (30.3%)		126 (72.0%)	49 (28.0%)		97 (55.4%)	78 (44.6%)	
IV	11	8 (72.7%)	3 (27.3%)		8 (72.7%)	3 (27.3%)		10 (90.9%)	1 (9.1%)	

[a]Significant at the level of p<0.05.

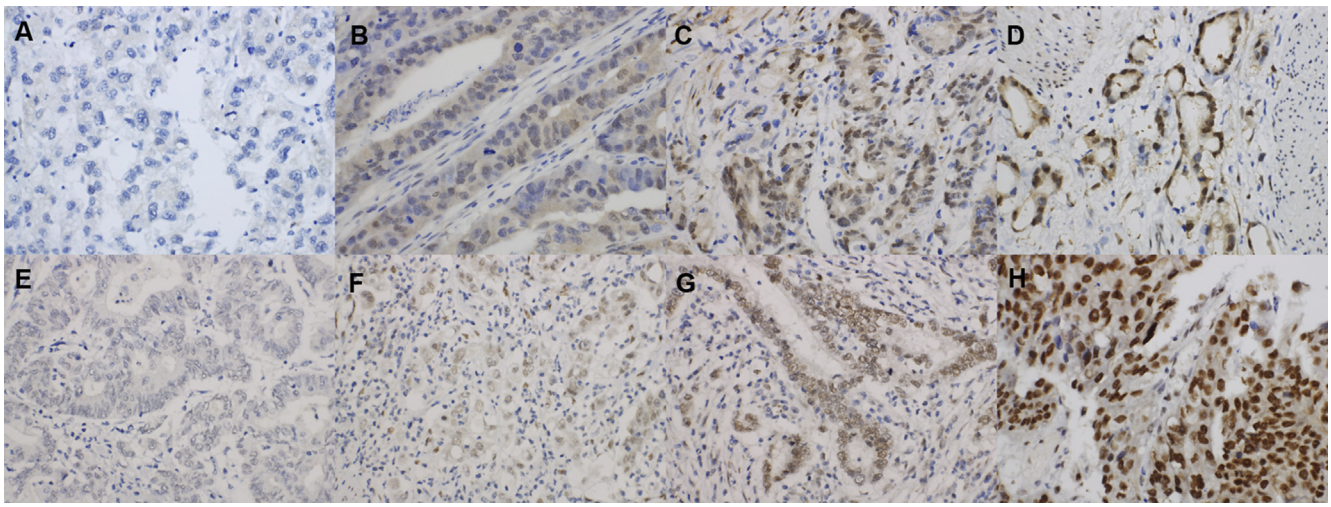

Fig. 1 Representative images of immunostainings. (A) YAP1 negative, (B) YAP1 weak, (C) YAP1 moderate, (D) YAP1 strong, (E) TEAD4 negative, (F) TEAD4 weak, (G) TEAD4 moderate, and (H) TEAD4 strong.

one group. The patients with YAP1^{low}LATS1/2^{pos} expression showed the best overall survival rate while those with YAP1^{high}LATS1/2^{neg} expression showed the worst overall survival rate (p<0.001, Fig. 3E). Other clinicopathological factors correlated with poor overall survival were poor differentiation (p=0.031), diffuse type of Lauren classification (p=0.005), large size (p<0.001), high pT stage (p<0.001), and presence of lymphovascular invasion (p<0.001), perineural

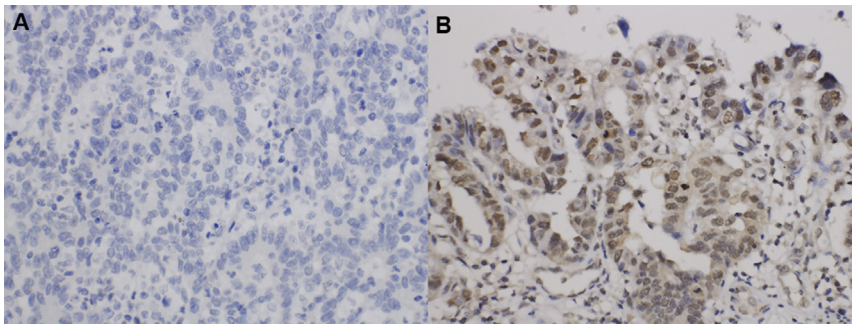

Fig. 2 Representative images of immunostainings. (A) LATS1/2 negative, and (B) LATS1/2 positive.

Table 2 Correlations between YAP1, LATS1/2 and TEAD expressions

	YAP1			TEAD4		
	Low	High	*p* value	Low	High	*p* value
LATS1/2						
Negative	155 (69.5%)	68 (30.5%)	0.045[a]	142 (63.7%)	81 (36.3%)	0.007[a]
Positive	55 (57.9%)	40 (42.1%)		45 (47.4%)	50 (52.6%)	
TEAD4						
Low	148 (79.1%)	39 (20.9%)	<0.001[a]			
High	62 (47.3%)	69 (52.7%)				

[a] Significant at the level of $p<0.05$.

invasion ($p=0.006$), lymph node metastasis ($p=0.003$), and distant metastasis ($p=0.001$).

Variables with $p<0.05$ in univariate analysis were included in multivariate analyses, including differentiation, Lauren classification, size, pT classification, lymphovascular invasion, perineural invasion, lymph node and distant metastasis, expression of YAP1, LATS1/2, and TEAD4, and combined expression of YAP1 and LATS1/2. Each expression of YAP1, LATS1/2, and TEAD4 and combined expression of YAP1 and LATS1/2 were separately evaluated. In multivariate analysis, $YAP1^{high}$ expression (HR=2.938; 95% CI 1.726–4.998; $p<0.001$) and negative LATS1/2 expression (HR=0.371; 95% CI 0.181–0.758; $p=0.007$) were independent poor prognostic factors in patients with advanced gastric cancer, while TEAD4 expression was not (HR=1.596; 95% CI 0.935–2.725; $p=0.086$). When performing multivariate analysis with combined YAP1 and LATS1/2 expressions, $YAP1^{high}LATS1/2^{neg}$ expression (HR=13.785; 95% CI 3.245–58.554; $p<0.001$) was also an independent poor prognostic factor in advanced gastric cancer. Other clinicopathological factors including large tumour size, high pT classification, and presence of lymphovascular invasion, and distant metastasis were independent poor prognostic factors (Table 3).

DISCUSSION

The Hippo pathway is a tumour-suppressive kinase cascade that negatively regulates oncoproteins, such as YAP and TAZ.[31] The deregulation of the Hippo pathway has an emerging role in tumourigenesis.[32,33] LATS1/2 phosphorylates YAP1, promotes its cytoplasmic sequestration and degradation, and prevents YAP1 from entering the nucleus and its role as a transcription coactivator. When LATS1/2 kinases are inactivated, YAP1 translocates into the nucleus and interacts with the transcription factor TEAD4. The YAP1-TEAD4 complex promotes the transcription of genes related with tumour invasion, progression, and metastasis.[31] LATS1/2, YAP1, and TEAD4 are core components of the Hippo pathway, and some recent studies illustrated the expression of each component in various carcinomas including liver, lung, colon, ovary, and stomach.[7,14,15,31,34,35] To the best of our knowledge, this is the first study to evaluate the sequential expression of core molecules of the Hippo pathway, YAP1, LATS1/2, and TEAD4 in advanced gastric cancers.

High YAP1 expression was identified in 108 cases (34.0%) which was consistent with the previously reported range of high YAP1 expression in gastric cancers, from 27.4% to 48.8%.[14,15,34] High YAP1 expression was significantly correlated with the presence of perineural invasion. Previous studies also found a few factors significantly correlated with YAP1 expression, such as age or lymph node metastasis.[15,34] LATS1/2 and TEAD4 were expressed in 95 cases (29.9%) and 131 cases (41.2%), respectively, and no clinicopathological factors were significantly correlated with expression of these proteins. The previously reported expression rates of LATS1 and LATS2 in gastric cancers are 77.3% and 29.2%, respectively, and these expressions were significantly correlated with some clinicopathological factors such as tumour stage, invasion depth, and lymph node metastasis.[7] The differences of expression rate and clinicopathological correlation can be explained by heterogeneity of the previous study cohorts, which included early gastric cancers or only the intestinal type of adenocarcinomas. Using different antibodies with different cut-off values for immunohistochemical staining might also be the cause of variable results.

According to the sequence of Hippo pathway, active LATS1/2 induces YAP1 sequestration in the cytoplasm and blocks nuclear translocation of YAP1. Thus, an inverse correlation between LATS1/2 and nuclear YAP1 expression can be expected. However, we showed that LATS1/2 expression

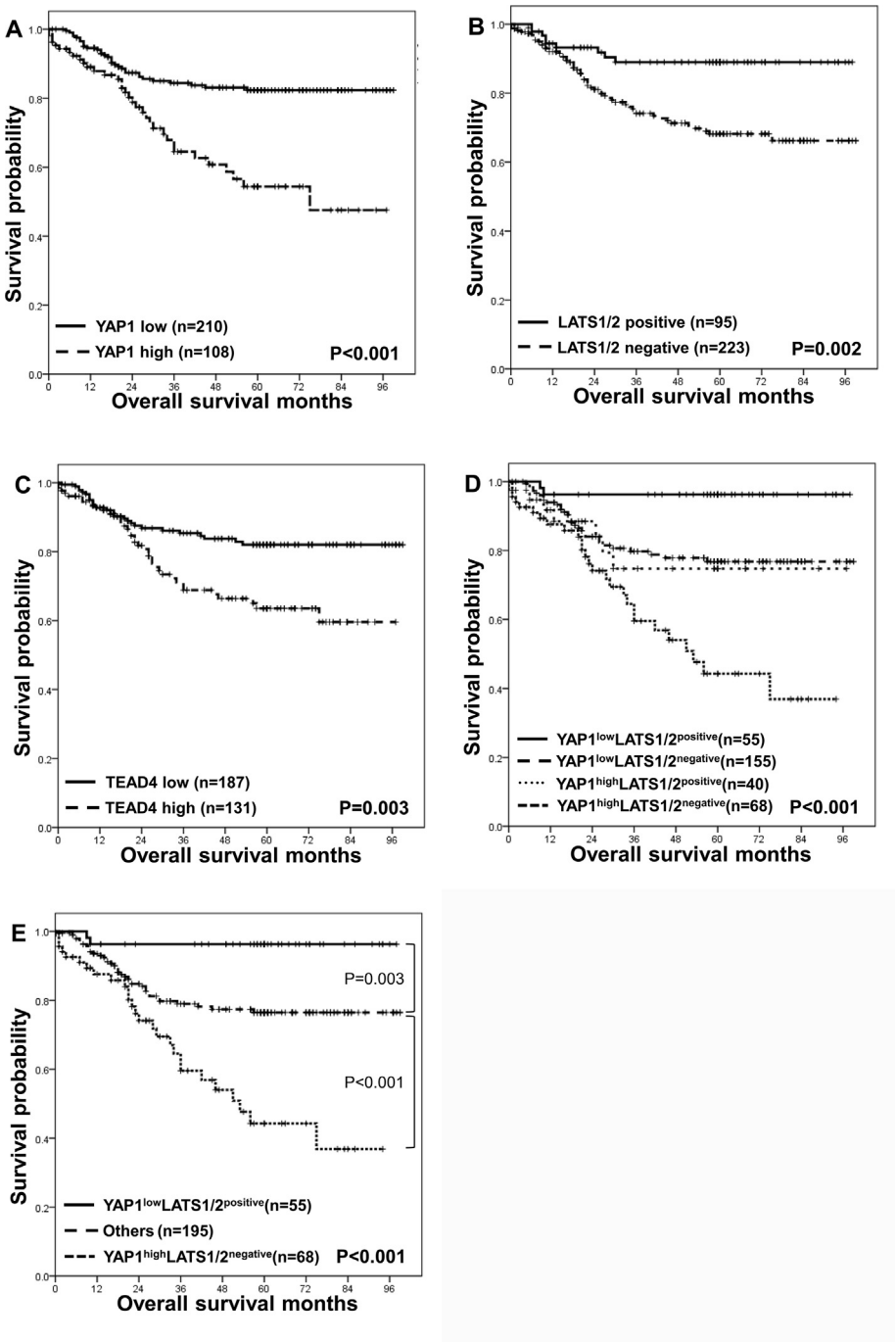

Fig. 3 Kaplan–Meier survival analyses. (A) Patients with YAP1^{high} expression showed significantly worse 5-year overall survival than those with YAP1^{low} expression (54.4% vs 82.4%, p<0.001). (B) Patients with LATS1/2 expression had significantly better overall survival than those without expression (5-year survival rate, 89.0% vs 68.2%, p=0.002). (C) The overall survival of patients with TEAD4^{high} expression was significantly worse than that in patients with TEAD4^{low} expression (5-year survival rate, 63.5% vs 82.0%, p=0.003). (D) When combining YAP1 and LATS1/2 expression, the 5-year survival rates of YAP1^{low}LATS1/2^{pos}, YAP1^{high}LATS1/2^{neg}, YAP1^{low}LATS1/2^{neg}, and YAP1^{high}LATS1/2^{pos} were 96.3%, 44.3%, 76.8%, and 74.7%, respectively (p<0.001). (E) The patients with YAP1^{low}LATS1/2^{pos} expression showed the best overall survival rate while those with YAP1^{high}LATS1/2^{neg} expression showed the worst overall survival rate. The 5-year survival rates of the YAP1^{low}LATS1/2^{pos} group, other groups, and the YAP1^{high}LATS1/2^{neg} expression group were 96.3%, 76.5%, and 44.3%, respectively (p<0.001). The other groups include cases with YAP1^{low}LATS1/2^{neg} and YAP1^{high}LATS1/2^{pos} expression.

is directly correlated with high YAP1 nuclear expression. One possible explanation is that different stimuli may activate YAP1 other than the inactivation of LATS1/2.[31,36] These include Rho GTPases,[37] mechanotransduction,[11] Wnt,[38] and mevalonate pathways.[37] Crosstalk with other signalling pathways including TGF-β, Notch, and Hedgehog pathways may also cause YAP1 activation independently of LATS1/2.[39] Thus, YAP1 expression can be regarded as a consequence of interactions of various cell signalling pathways, including the Hippo pathway. More comprehensive pathway analyses including the direct and indirect signalling between LATS1/2 and YAP1 are needed to provide more corrective evidence of the oncogenic or tumour suppressive role of the Hippo pathway.

In this study, high expression of YAP1 and TEAD4 was associated with poor overall survival, supporting their

Table 3 Univariate and multivariate analyses

Clinicopathological factors	Univariate analysis				Multivariate analysis[a]			Multivariate analysis[b]		
	5YSR (%)	HR	95% CI	*p* value	HR	95% CI	*p* value	HR	95% CI	*p* value
Age, years										
≤60	70.5	1.00								
>60	79.6	0.743	0.454–1.218	0.239						
Sex										
Male	74.7	1.00								
Female	73.7	0.989	0.765–1.277	0.930						
Differentiation										
Differentiated	74.7	1.00			1.00			1.00		
Undifferentiated	50.0	1.800	1.056–3.068	0.031[c]	1.010	0.520–1.961	0.977	1.074	0.557–2.071	0.831
Lauren classification										
Intestinal	83.3	1.00			1.00			1.00		
Diffuse	66.5	2.097	1.249–3.521	0.005 [c]	1.639	0.953–2.818	0.074	1.639	0.956–2.808	0.072
Size										
≤6 cm	83.8	1.00			1.00			1.00		
>6 cm	57.2	3.107	1.896–5.091	<0.001[c]	1.970	1.141–3.402	0.015[c]	1.918	1.115–3.299	0.019[c]
pT classification										
pT2-3	83.1	1.00			1.00			1.00		
pT4	43.8	3.682	2.252–6.020	<0.001[c]	2.215	1.287–3.812	0.004[c]	2.346	1.380–3.985	0.002[c]
Lymphovascular invasion										
Absence	87.4	1.00			1.00			1.00		
Presence	64.0	2.985	1.675–5.320	<0.001[c]	1.967	1.076–3.596	0.028[c]	2.006	1.102–3.654	0.023[c]
Perineural invasion										
Absence	79.0	1.00			1.00			1.00		
Presence	60.3	2.017	1.224–3.322	0.006[c]	1.195	0.693–2.061	0.522	1.286	0.753–2.196	0.357
Lymph node metastasis										
Absence	88.6	1.00			1.00			1.00		
Presence	67.8	2.804	1.428–5.506	0.003[c]	1.379	0.644–2.950	0.408	1.332	0.619–2.868	0.464
Distant metastasis										
Absence	75.5	1.00			1.00			1.00		
Presence	35.6	4.667	1.861–11.705	0.001[c]	3.851	1.484–9.995	0.006[c]	3.490	1.362–8.942	0.009[c]
YAP1 expression										
Low	82.4	1.00			1.00					
High	54.4	2.777	1.709–4.514	<0.001[c]	2.938	1.726–4.998	<0.001[c]			
LATS1/2 expression										
Negative	68.2	1.00			1.00					
Positive	89.0	0.337	0.167–0.681	0.002[c]	0.371	0.181–0.758	0.007[c]			
TEAD4 expression										
Low	82.0	1.00			1.00					
High	63.5	1.206	1.067–1.362	0.003[c]	1.596	0.935–2.725	0.086			
YAP1 and LATS1/2										
$YAP1^{low}LATS1/2^{pos}$	96.3	1.00		<0.001[c]				1.00		<0.001[c]
$YAP1^{high}LATS1/2^{neg}$	44.3	16.885	4.006–71.171	<0.001[c]				13.785	3.245–58.554	<0.001[c]
Others	76.5	6.740	1.623–27.985	0.009[c]				4.765	1.137–19.981	0.033[c]

[a] Analysis including each expression of YAP1, LATS1/2, and TEAD4.
[b] Analysis including combined expression of YAP1 and LATS1/2.
[c] Significant at the level of $p<0.05$.

oncogenic role, and loss of LATS1/2 expression was correlated with poor survival, supporting its tumour suppressive role in gastric cancers. In addition, high YAP1 expression and loss of LATS1/2 expression were independent poor prognostic factors in gastric cancers. We suggested that loss of LATS1/2 expression induces nuclear translocation and expression of YAP1 and subsequent interaction with TEAD4. Subsequently, activated nuclear YAP1 and TEAD4 upregulated the transcription of target oncogenes, inducing poor prognosis of gastric cancers.[34,40] The combination of YAP1 and LATS1/2 expression profiles can more accurately stratify the prognosis of advanced gastric cancer patients. Patients with $YAP1^{high}LATS1/2^{neg}$ expression were significantly associated with worse overall survival while patients with $YAP1^{low}LATS1/2^{pos}$ expression were significantly correlated with better overall survival than other groups. Combined or individual expression profiles of the Hippo pathway effectors are independent prognostic factors that can predict prognosis of advanced gastric cancer patients.

The Hippo pathway has emerging roles as predictive markers to anti-cancer therapies and as therapeutic targets in some kinds of cancers.[9,41] High YAP expression has been correlated with poor response to a BRAF inhibitor in non-small cell lung cancer and melanoma patients.[42] YAP expression was also associated with low response rate to cetuximab treatment in colon cancers and incomplete response to radiotherapy or chemotherapy in head and neck squamous cell carcinomas.[43,44] Inhibiting the YAP-TEAD interaction may also be a potential target to prevent YAP-induced tumourigenesis.[41] Verteporfin (VP), clinically used in photodynamic therapy, induced a conformational change of YAP, thereby inhibiting transcriptional activity of the YAP-TEAD complex,[45] and disrupting the growth of hepatocellular carcinoma cells.[46] Vestigial-like family member 4 (VGLL4) competes with YAP to bind with TEAD, suppressing the transcriptional activity of YAP.[47] A peptide drug mimicking VGLL4 has been generated as a YAP antagonist to treat gastric cancers.[47] The expression of Hippo

pathway molecules, such as YAP and TEAD, may represent candidates for potential targeted therapy in gastric cancers.

In conclusion, high YAP1 and TEAD4 expressions and loss of LATS1/2 expression are significantly associated with poor prognosis. Combined expression of YAP1 and LATS1/2 is an independent prognostic factor in advanced gastric cancers. Expression of YAP1, LATS1/2 and TEAD4 may be used as prognostic markers and potential therapeutic targets in advanced gastric cancer patients.

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Address for correspondence: Joo Young Kim, Department of Pathology, Nowon Eulji Medical Center, Eulji University, 68, Hangeulbiseok-ro, Nowon-gu, Seoul, 01830, South Korea. E-mail: lepetit80@hanmail.net

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