J Gastric Cancer. 2017 Dec;17(4):363-373
https://doi.org/10.5230/jgc.2017.17.e41
pISSN 2093-582X·eISSN 2093-5641

jgc Journal of Gastric Cancer

Original Article

Check for updates

Clinicopathological Significance of Large Tumor Suppressor (*LATS*) Expression in Gastric Cancer

Myoung Won Son [1], **Geum Jong Song** [1], **Si-Hyong Jang** [2], **Soon Auck Hong** [2], **Mee-Hye Oh** [2], **Ji-Hye Lee** [2], **Moo Jun Baek** [1], **Moon Soo Lee** [1]

[1]Department of Surgery, Soonchunhyang University Cheonan Hospital, Cheonan, Korea
[2]Department of Pathology, Soonchunhyang University Cheonan Hospital, Cheonan, Korea

OPEN ACCESS

Received: Aug 1, 2017
Revised: Dec 15, 2017
Accepted: Dec 15, 2017

Correspondence to
Moon Soo Lee
Department of Surgery, Soonchunhyang University Cheonan Hospital, 31 Suncheonhyang 6-gil, Dongnam-gu, Cheonan 31151, Korea.
E-mail: msslee@schmc.ac.kr

ORCID iDs
Myoung Won Son
https://orcid.org/0000-0001-5255-9234
Geum Jong Song
https://orcid.org/0000-0002-1067-8099
Si-Hyong Jang
https://orcid.org/0000-0002-7605-086X
Soon Auck Hong
https://orcid.org/0000-0002-7902-4608
Mee-Hye Oh
https://orcid.org/0000-0002-2395-1752
Ji-Hye Lee
https://orcid.org/0000-0003-4214-1322
Moo Jun Baek
https://orcid.org/0000-0003-3567-6687
Moon Soo Lee
https://orcid.org/0000-0002-3955-1331

ABSTRACT

Purpose: The aims of this study were to evaluate the expression of the large tumor suppressor (*LATS*) genes *LATS1* and *LATS2* by immunohistochemical staining of gastric cancer, and to evaluate the clinicopathological significance of *LATS* expression and its correlation with overall survival (OS).
Materials and Methods: *LATS1* and *LATS2* expression in a tissue microarray was detected by immunohistochemistry, using 264 gastric cancer specimens surgically resected between July 2006 and December 2009.
Results: Low expression of *LATS1* was significantly associated with more advanced American Joint Committee on Cancer (AJCC) stage (P=0.001) and T stage (P=0.032), lymph node (LN) metastasis (P=0.040), perineural invasion (P=0.042), poor histologic grade (P=0.007), and diffuse-type histology by the Lauren classification (P=0.033). Low expression of *LATS2* was significantly correlated with older age (≥65, P=0.027), more advanced AJCC stage (P=0.001) and T stage (P=0.001), LN metastasis (P=0.004), perineural invasion (P=0.004), poor histologic grade (P<0.001), and diffuse-type histology by the Lauren classification (P<0.001). Kaplan-Meier survival analysis revealed significantly poor OS rates in the groups with low *LATS1* (P=0.037) and *LATS2* (P=0.037) expression.
Conclusions: Expression of *LATS1* or *LATS2* is a significant marker for a good prognosis in patients with gastric cancer.

Keywords: Stomach neoplasms; Tumor suppressor genes; *LATS1* protein, human; *LATS2* protein, human

INTRODUCTION

Gastric cancer is the fourth most common cancer and the second leading cause of cancer deaths worldwide, although its incidence and mortality rate have been decreasing for several decades [1,2]. In Korea, gastric cancer is the most frequent cancer in men, and the fourth most common cancer in women [3].

Currently, surgical resection including radical gastrectomy with lymph node (LN) dissection is the only curative treatment method for stomach cancer. In recent years, despite many remarkable advances in diagnostic and therapeutic techniques, the outcomes of patients

Funding
This work was supported by the Soonchunhyang University Research Fund.

Author Contributions
Conceptualization: S.M.W.; Data curation: S.M.W., S.G.J.; Formal analysis: B.M.J.; Funding acquisition: L.M.S., S.M.W.; Investigation: J.S.H., H.S.A.; Methodology: O.M.H., L.J.H.; Writing - original draft: S.M.W.; Writing - review & editing: S.M.W., S.G.J.

Conflict of Interest
No potential conflict of interest relevant to this article was reported.

with gastric cancer have shown only minor improvements. Patients diagnosed with locally advanced or metastatic gastric cancers have a very poor prognosis, and treatment primarily entails chemotherapy. These patients develop high-grade toxicity from aggressive chemotherapeutic regimens and experience severe deterioration in quality of life. Some patients may choose to receive only best supportive care.

The poor outcomes in patients with locally advanced or metastatic gastric cancer are thought to be associated with the heterogeneous pathogenesis of gastric cancer, which involves numerous different genetic mutations and molecular signaling pathways. In gastric cancer as well as many other malignancies, molecular signaling pathways have been a recent focus of investigation, and some of these pathways are being targeted with novel diagnostic tools and therapeutic agents. Accordingly, it is necessary to investigate the specialized molecular pathways and molecules associated with tumorigenesis and tumor progression.

Large tumor suppressor (*LATS*) is a serine/threonine-protein kinase originally isolated from Drosophila [4,5]. The *LATS* gene family, comprising *LATS1* and *LATS2*, is a core component of the Hippo pathway, which is an essential regulator of homeostasis [6]. In mammals, the Hippo pathway has been reported to generate a tumor suppressor signal that inhibits cell proliferation and promotes apoptosis. Inactivation of the Hippo pathway — which is regulated by MST1/2, SAV1, *LATS1/2*, MOB, and yes-associated protein (YAP) — results in cell growth, enlargement of organ size, and malignant tumor formation [7-10]. *LATS* is involved in several important lives sustaining processes, including cell proliferation, apoptosis, cell migration, transcriptional regulation, and maintenance of genetic stability [11,12].

Abnormal expression or mutation of *LATS* has been found to be involved in malignant transformation and pathological progression of cervical squamous cell carcinoma, breast cancer, and hepatic malignancies [13-15]. However, the expression of *LATS* in gastric cancer and its implications have received little attention. *LATS1* expression is decreased in gastric cancer than in normal gastric epithelium and adenomas, and its expression is significantly lower in gastric cancer with LN metastasis than that without LN metastasis [16].

The aims of the present study were to confirm the expression of *LATS1* and *LATS2* in gastric cancer, to assess the association between expression of *LATS1/2* and clinicopathological factors as well as the overall survival (OS) of patients, and to evaluate whether *LATS* can be used as a potential prognostic factor in patients with gastric cancer.

MATERIALS AND METHODS

Patient selection and tissue samples

Data from 264 patients who underwent surgical resection for gastric cancer at Soonchunhyang University Cheonan Hospital between July 2006 and December 2009 were retrospectively analyzed. Patient medical records were reviewed for clinicopathological information, including age; sex; tumor location; tumor, node, metastasis (TNM) stage; tumor differentiation; presence of lymphatic, vascular, or perineural invasion; and Lauren classification. Survival data were obtained from patients' medical records. All hematoxylin and eosin (H&E) stained slides were independently re-examined by 2 pathologists to confirm the diagnosis and other pathological features. The pathologists selected the most representative sections from each gastric cancer sample. Tumor stages and grades were re-classified according to the Seventh Edition of the

https://doi.org/10.5230/jgc.2017.17.e41

jgc Journal of Gastric Cancer

American Joint Committee on Cancer (AJCC) Staging Manual. We excluded patients who presented with other critical medical conditions or had received neoadjuvant chemotherapy, or cases in which tissue blocks were unavailable.

Construction of tissue microarrays (TMAs)

TMAs were constructed by reviewing H&E stained slides and selecting one representative formalin-fixed paraffin-embedded archival block for each case. The most representative tumor area was carefully marked on the H&E stained slide. Tissue cores (2-mm thick) were extracted from individual formalin-fixed paraffin-embedded blocks (donor blocks) and re-arranged into recipient paraffin blocks (TMA blocks), using a trephine apparatus (Super Bio Chips Laboratories, Seoul, Korea). In addition, normal gastric mucosa specimens were included in 26 cases, using the same procedure. One section from the TMA block was stained with H&E for tissue confirmation.

Immunohistochemistry (IHC) for expression of *LATS1* and *LATS2*

Expression of *LATS1* and *LATS2* was detected by IHC. Tissue sections 4 μm thick extracted from the TMA blocks were transferred to poly-L-lysine-coated glass slides and incubated in a dry oven at 60°C for 1 hour. These sections were then de-waxed in xylene (3 changes), re-hydrated in a graded series of decreasing ethanol concentration, and rinsed in Tris-buffered saline solution (pH 7.4). Endogenous peroxidase activity was inactivated with 5% hydrogen peroxide in methanol at 37°C for 15 minutes. For antibody staining, antigen retrieval was performed using a microwave treatment in an epitope retrieval solution (pH 6.0) for 20 minutes. The tissue sections were incubated with a primary antibody in a humidified chamber at 4°C for 16 hours. The primary antibodies were a rabbit polyclonal antibody against *LATS1* (1:100 dilution; Abcam, Cambridge, UK), and a rabbit polyclonal antibody against *LATS2* (1:200 dilution; Abcam). A secondary antibody was then applied using a Bond Polymer Refine Detection kit (Leica, Wetzlar, Germany). Diaminobenzidine was used as the chromogen and the tissue sections were counterstained using Mayer's hematoxylin solution. Positive controls, consisting of cases with known reactivity for the antibody, and negative controls obtained by omitting the primary antibody, were also included.

Immunohistochemical assessments

IHC staining was separately evaluated by 2 pathologists, and in the rare instances in which there was a discrepancy in their judgments, the 2 investigators reviewed the slides together using a multi-head microscope and reached a consensus. Semi-quantitative IHC scores were assigned for assessment of both the intensity and extent of staining. The intensity of staining was scored on a scale of 0 to 3, corresponding to negative, weak, moderate, and strong positivity (**Figs. 1** and **2**). The extent of staining was also scored on a scale of 0 to 3 according to the percentage of cells (0%, ≤10%, >10% and ≤50%, or >50%, respectively) that stained positive for each protein. The product of the intensity and extent scores was used as the final score (i.e., 0, 1, 2, 3, 4, 6, or 9). The IHC results were classified as follows: scores of 0–1 indicated low expression of *LATS1*, and scores of 2–9 indicated high expression of *LATS1*. For *LATS2*, scores of 0–3 were defined as low expression and others were defined as high expression. Similar semi-quantitative scoring systems have been successfully used for other TMA evaluations [17].

Statistical analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) software for Windows version 19.0 (IBM Corp., Armonk, NY, USA). Associations

https://doi.org/10.5230/jgc.2017.17.e41

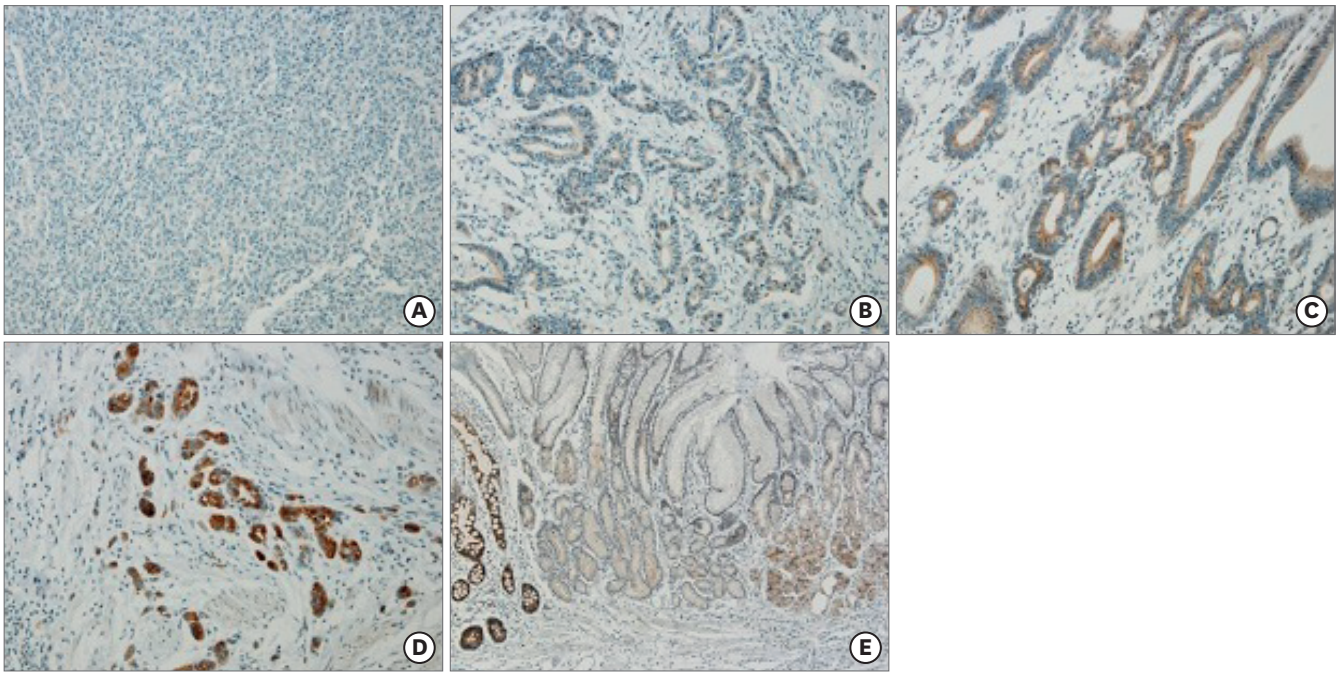

Fig. 1. Immunohistochemical staining of *LATS1* expression in gastric cancer. (A) Negative (0) staining intensity (×200). (B) Weak (1+). (C) Moderate (2+). (D) Strong (3+). (E) *LATS1* expression in normal gastric mucosa (×100).
LATS = large tumor suppressor.

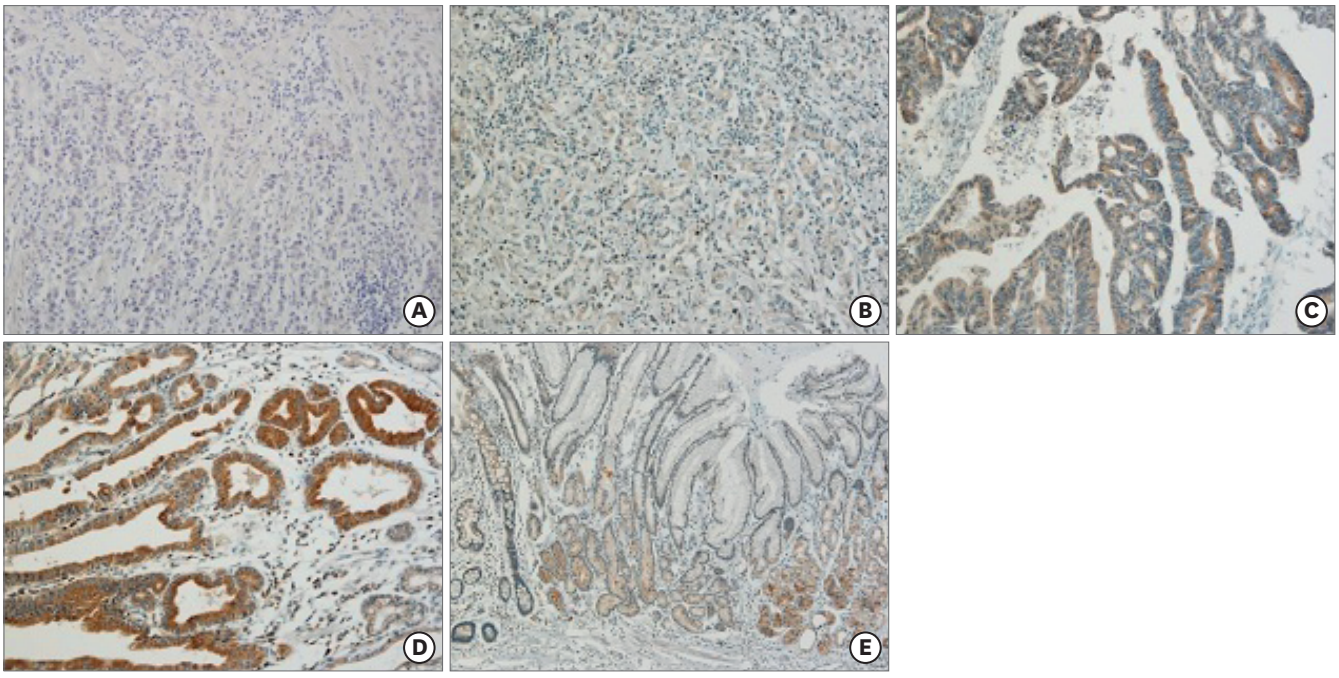

Fig. 2. Immunohistochemical staining of *LATS2* expression in gastric cancer. (A) Negative (0) staining intensity (×200). (B) Weak (1+). (C) Moderate (2+). (D) Strong (3+). (E) *LATS2* expression in normal gastric mucosa (×100).
LATS = large tumor suppressor.

between *LATS1* and *LATS2* expression and patient clinicopathological parameters were assessed using Pearson's χ^2 and Fisher's exact tests. OS was defined as the duration from the date of surgery to the date of death or last follow-up. OS rates in relationship to *LATS1*

https://doi.org/10.5230/jgc.2017.17.e41

jgc Journal of Gastric Cancer

and *LATS2* expression were calculated using the Kaplan-Meier method. To assess the differences between Kaplan-Meier curves, a log-rank test was performed. Cox proportional hazards modeling was used to investigate the significance of prognostic factors. Statistical significance was defined as a P-value of less than 0.05.

RESULTS

Clinicopathological characteristics of gastric cancer patients

Of the 264 patients with gastric cancer included in this study, 184 were men and 80 were women. The age at diagnosis (mean±standard deviation) was 60.20±12.59 years (range, 25–85 years). The cohort comprised 103 patients with early stage and 161 patients with advanced gastric cancer. In total, there were 121 for stage I, 44 for stage II, 91 for stage III, and 8 for stage IV tumors. At the time of diagnosis, 126 patients showed signs of LN metastasis and an additional 8 patients showed signs of distant metastasis.

LATS1/2 expression was evaluated in 26 normal gastric mucosa samples and 264 gastric cancer samples. In normal gastric mucosa, weak or moderate expression of *LATS1/2* was observed. However, no *LATS1/2* expression was seen in foveolar epithelium. Interestingly, gastric mucosa with intestinal metaplasia demonstrated strong expression of *LATS1*.

Expression of *LATS1* was observed in 204 patients with gastric cancer (77.3%) and expression of *LATS2* was observed in 77 patients with gastric cancer (29.2%) (**Table 1**). The correlations between *LATS1/2* expression and the clinicopathological factors of the patients are presented in **Tables 2** and **3**. Low expression of *LATS1* was significantly associated with more advanced AJCC stage (P=0.001) and T stage (P=0.032), LN metastasis (P=0.040), perineural invasion (P=0.042), poor histologic grade (P=0.007), and diffuse-type histology by the Lauren classification (P=0.033). Weak correlations between low *LATS1* expression and lymphatic invasion (P=0.061) were also observed, although these did not reach formal statistical significance.

Low expression of *LATS2* was significantly correlated with older age (≥65, P=0.027), more advanced AJCC stage (P=0.001) and T stage (P=0.001), LN metastasis (P=0.004), perineural invasion (P=0.004), poor histologic grade (P<0.001), and diffuse-type histology by the Lauren classification (P<0.001). Low *LATS2* expression was also associated with lymphatic invasion (P=0.067) and distant metastasis (P=0.065), although these correlations did not reach statistical significance.

OS of gastric cancer patients with *LATS1* and *LATS2* expression

The follow-up period for patients ranged from 5 to 113 months (median interval, 60.7 months). During the follow-up period, 85 patients died. Low expression of *LATS1* was significantly associated with poor OS rates, according to Kaplan-Meier analysis (P=0.037). The 5-year OS rate for patients with high expression of *LATS1* was 71.2% compared with 56.9% for patients with low *LATS1* expression. Patients with low *LATS2* expression had significantly shorter OS rates than patients with high *LATS2* expression (P=0.037). For *LATS2*, the 5-year OS rate was 76.5% vs. 64.4% for high expression vs. low expression, respectively (**Fig. 3**). In the Cox regression analysis, *LATS1/2* expression was a significant factor in univariate OS analysis (*LATS1*, P=0.038; *LATS2*, P=0.038), but no significant difference was observed in multivariate OS analysis (**Table 4**).

https://doi.org/10.5230/jgc.2017.17.e41

jgc Journal of Gastric Cancer

Table 1. *LATS1* and *LATS2* expression in gastric cancer according to the scoring system

Scores	*LATS1*	*LATS2*
Intensity score		
0	30 (11.4)	18 (6.8)
1	142 (53.8)	169 (64.0)
2	83 (31.4)	73 (27.7)
3	9 (3.4)	4 (1.5)
Extent score		
0	31 (11.7)	14 (5.3)
1	33 (12.5)	9 (3.4)
2	57 (21.6)	25 (9.5)
3	143 (54.2)	216 (81.8)
Final score		
0	31 (11.7)	18 (6.8)
1	29 (11.0)	9 (3.4)
2	45 (17.0)	25 (9.5)
3	71 (26.9)	135 (51.1)
4	15 (5.7)	0 (0.0)
6	65 (24.6)	73 (27.7)
9	8 (3.0)	4 (1.5)
Low expression	60 (22.7)	187 (70.8)
High expression	204 (77.3)	77 (29.2)
Total	264 (100)	264 (100)

Values are presented as number (%).
LATS = large tumor suppressor.

DISCUSSION

The *LATS* gene family is one of the core components of the Hippo pathway, an emerging signaling pathway that is an essential regulator of homeostasis. In mammals, the Hippo pathway has been reported as a tumor suppressor signal that inhibits cell proliferation and promotes cell apoptosis. Inactivation of the Hippo pathway results in cell growth, enlargement of organ size, and tumorigenesis. In 1995, *lats* was first identified by Xu et al. [5] as a tumor suppressor gene of *Drosophila melanogaster*. Subsequently, Xu's study group also isolated mouse (*Lats1*) and human (*LATS1*) genes [18]. In addition, human *LATS2* was isolated after the discovery of mouse *Lats2* by Yabuta et al. [19], and the researchers suggested that *LATS2* may also be a tumor suppressor gene like *LATS1*. *LATS1* and *LATS2*, members of the family of *LATS* proteins, play an important role in maintaining cellular homeostasis [6] including cell proliferation, cell apoptosis, cell migration [11], transcriptional regulation, and maintenance of genetic stability [12].

LATS1 and *LATS2* have been implicated in a number of human malignant tumors. Abnormal expression or gene mutation of *LATS1* contributes to malignant transformation and histologic progression in cervical squamous cell carcinoma [13]. Down-regulation of *LATS1* and *LATS2* is correlated in breast cancer with alterations of p53 function and cell migration [20]. *LATS2* expression is lower in human prostate tumors than in normal prostate tissue, and *LATS2* is a negative regulator of the androgen receptor [21]. Overexpression of *LATS1* promotes YAP phosphorylation and inhibits tumorigenesis in human renal cell carcinoma [22]. It has also been reported that *LATS1* contributes to better prognosis through negative regulation of YAP in non-small cell lung cancer (NSCLC) [23].

However, the expression of *LATS* in gastric cancer and its implications have received little investigation. In a Chinese study, *LATS1* expression was found to be downregulated and negatively associated with YAP in human gastric cancer [16]. The same study reported that

https://doi.org/10.5230/jgc.2017.17.e41

jgc Journal of Gastric Cancer

Table 2. Association with *LATS1* expression and clinicopathological factors

Variables	Cases	*LATS1* expression		P-value
		Low	High	
Total	264	60 (22.7)	204 (77.3)	
Sex				0.706
Male	184	43 (71.7)	141 (69.1)	
Female	80	17 (28.3)	63 (30.9)	
Age				0.384
<65	141	35 (58.3)	106 (52.0)	
≥65	123	25 (41.7)	98 (48.0)	
Location				0.633
Upper	36	9 (15.0)	27 (13.2)	
Middle	36	6 (10.0)	30 (14.7)	
Lower	192	45 (75.0)	147 (72.1)	
AJCC stage				0.001
I/II	165	27 (45.0)	138 (67.6)	
III/IV	99	33 (55.0)	66 (32.4)	
Tumor depth				0.032
T1/T2	142	25 (41.7)	117 (57.4)	
T3/T4	122	35 (58.3)	87 (42.6)	
LN metastasis				0.040
Absent	132	23 (38.3)	109 (53.4)	
Present	132	37 (61.7)	95 (46.6)	
Distant metastasis				0.876
Absent	256	58 (96.7)	198 (97.1)	
Present	8	2 (3.3)	6 (2.9)	
Lymphatic invasion				0.061
Absent	138	25 (41.7)	113 (55.4)	
Present	126	35 (58.3)	91 (44.6)	
Vascular invasion				0.676
Absent	237	53 (88.3)	184 (90.2)	
Present	27	7 (11.7)	20 (9.8)	
Perineural invasion				0.042
Absent	198	39 (65.0)	159 (77.9)	
Present	66	21 (35.0)	45 (22.1)	
Histologic grade				0.007
WD/MD	101	14 (23.3)	87 (42.6)	
PD/other	163	46 (76.7)	117 (57.4)	
Lauren classification				0.033
Intestinal	102	17 (28.3)	85 (41.7)	
Diffuse	154	43 (71.7)	111 (54.4)	
Mixed	8	0 (0)	8 (3.9)	

Values are presented as number only or number (%).
LATS = large tumor suppressor; AJCC = American Joint Committee on Cancer; LN = lymph node; WD = well differentiated; MD = moderately differentiated; PD = poorly differentiated.

LATS1 expression was lower in gastric cancer than in normal gastric epithelium and adenoma, and its expression was significantly decreased in gastric cancer with LN metastasis than that without LN metastasis [16].

In the present study, we hypothesized that decreased expression of *LATS1* and *LATS2* would be associated with a poor prognosis in patients with gastric cancer and that overexpression of *LATS1* and *LATS2* would be correlated with a better prognosis through inhibition of tumor progression. We found that overexpression of *LATS1* and *LATS2* was significantly associated with factors indicating a good prognosis. Positive expression of *LATS1* was associated with lower AJCC stage, negative LN metastasis, absence of perineural invasion, well/moderately differentiated grade, and intestinal-type histology based on the Lauren classification. *LATS2* expression was also associated with a number of clinicopathological factors. In addition, the

https://doi.org/10.5230/jgc.2017.17.e41

jgc Journal of Gastric Cancer

Table 3. Association with *LATS2* expression and clinicopathological factors

Variables	Cases	*LATS2* expression		P-value
		Low	High	
Total	264	187 (70.8)	77 (29.2)	
Sex				0.694
Male	184	129 (70.6)	55 (71.4)	
Female	80	58 (31.0)	22 (28.6)	
Age				0.027
<65	141	108 (57.8)	33 (42.9)	
≥65	123	79 (42.2)	44 (57.1)	
Location				0.614
Upper	36	26 (13.9)	10 (13.0)	
Middle	36	23 (12.3)	13 (16.9)	
Lower	192	138 (73.8)	54 (70.1)	
AJCC stage				0.001
I/II	165	105 (56.1)	60 (77.9)	
III/IV	99	82 (43.9)	17 (22.1)	
Tumor depth				0.001
T1/T2	142	88 (47.1)	54 (70.1)	
T3/T4	122	99 (52.9)	23 (29.9)	
LN metastasis				0.004
Absent	132	83 (44.4)	49 (63.6)	
Present	132	104 (55.6)	28 (36.4)	
Distant metastasis				0.065
Absent	256	179 (95.7)	77(100)	
Present	8	8 (4.3)	0 (0)	
Lymphatic invasion				0.067
Absent	138	91 (48.7)	47 (61.0)	
Present	126	96 (51.3)	30 (39.0)	
Vascular invasion				0.402
Absent	237	166 (88.8)	71 (92.2)	
Present	27	21 (11.2)	6 (7.8)	
Perineural invasion				0.004
Absent	198	131 (70.1)	67 (87.0)	
Present	66	56 (29.9)	10 (13.0)	
Histologic grade				<0.001
WD/MD	101	56 (29.9)	45 (58.4)	
PD/other	163	131 (70.1)	32 (41.6)	
Lauren classification				<0.001
Intestinal	102	55 (29.4)	47 (61.0)	
Diffuse	154	124 (66.3)	30 (39.0)	
Mixed	8	8 (4.3)	0 (0)	

Values are presented as number only or number (%).
LATS = large tumor suppressor; AJCC = American Joint Committee on Cancer; LN = lymph node; WD = well differentiated; MD = moderately differentiated; PD = poorly differentiated.

expression of *LATS1* and LATS2 was associated with T stage, and the proportion of *LATS2*-positive cases increased as the T stage decreased (P=0.007). *LATS2* expression was observed in 40/103 cases with pT1 (51.9%), 15/42 with pT2 (19.5%), 14/75 with pT3 (18.2%), and 8/44 with pT4 (10.4%).

Expression of *LATS1* and *LATS2* was also significantly associated with stage (*LATS1*/AJCC stage, p=0.014; *LATS2*/AJCC stage, P=0.002). *LATS1* overexpression was observed in 102 of 121 stage I patients (84.3%), and lack of expression of *LATS2* was found in 74 of 91 stage III patients (81.3%), but of 8 patients with stage IV, none showed expression of *LATS2*.

In the present study, we used different cut-off values for positive expression of *LATS1* and *LATS2*. Because the differences in the cut-off values are presumably the result of the different

https://doi.org/10.5230/jgc.2017.17.e41

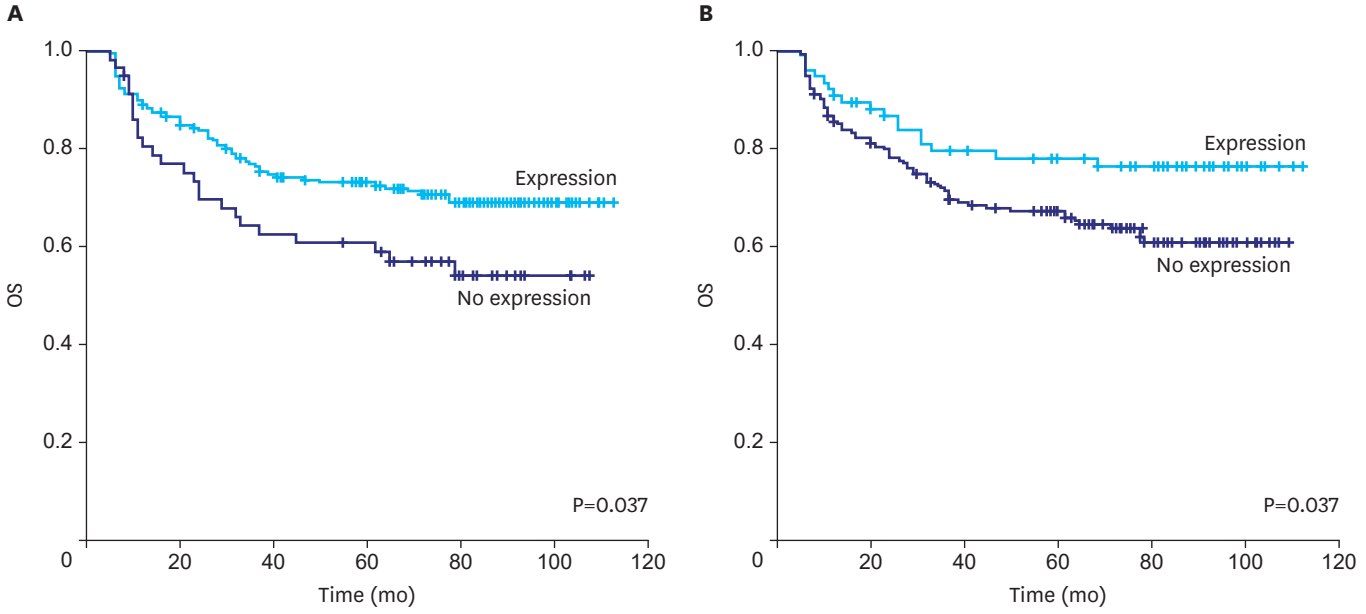

Fig. 3. Kaplan-Meier survival analysis with log-rank test. Low expression of *LATS1* (A) and *LATS2* (B) were significantly associated with poor OS, respectively.
LATS = large tumor suppressor; OS = overall survival.

Table 4. Univariate and multivariate analysis of factors in patients with gastric cancer by Cox regression analysis

Parameters	Overall survival		
	Univariate analysis	Multivariate analysis	
	P-value	Hazard ratio (95% CI)	P-value
Gender (female vs. male)	0.302	0.980 (0.586–1.638)	0.938
Age (<65 vs. ≥65)	0.021	1.688 (1.052–2.709)	0.030
Tumor depth (T1/T2 vs. T3/T4)	<0.001	8.314 (2.973–23.247)	<0.001
LN metastasis (absent vs. present)	<0.001	1.510 (0.511–4.460)	0.456
Distant metastasis (absent vs. present)	<0.001	3.016 (1.356–6.709)	0.007
Histologic grade (WD/MD vs. PD/other)	0.002	1.071 (0.407–2.820)	0.890
Lauren classification (intestinal vs. diffuse/mixed)	0.003	1.535 (0.572–4.119)	0.395
Lymphatic invasion (absent vs. present)	<0.001	2.363 (0.905–6.170)	0.079
Vascular invasion (absent vs. present)	<0.001	1.784 (1.051–3.030)	0.032
Perineural invasion (absent vs. present)	<0.001	2.735 (1.572–4.761)	<0.001
LATS1 expression (high vs. low)	0.038	1.039 (0.638–1.691)	0.879
LATS2 expression (high vs. low)	0.038	0.586 (0.306–1.119)	0.105

LATS = large tumor suppressor; LN = lymph node; WD = well differentiated; MD = moderately differentiated; PD = poorly differentiated; CI = confidence interval.

dilutions of the antibodies, it is possible that there is a difference between the degree of *LATS1* and *LATS2* expression.

Most normal gastric mucosal tissues were weakly positive for *LATS1* and *LATS2*. However, the expression of *LATS* varies in gastric cancer; as shown in the previous results, overexpression was associated with good prognostic factors as well as with tumor suppressor function. However, with loss of expression, the tumor suppressor function is weakened, and it is associated with factors indicative of a poor prognosis. These findings suggest that activation of *LATS* acts as a tumor suppressor in gastric cancer. Further studies will be needed to confirm the tumor suppressive activity of these proteins in non-neoplastic gastric epithelial cells or to explain the manner in which expression of *LATS1* and *LATS2* plays an important role.

In addition, we investigated whether the expression of *LATS* was associated with survival in patients with both *LATS1* and *LATS2* expression. The number of patients with expression of

https://doi.org/10.5230/jgc.2017.17.e41

jgc Journal of Gastric Cancer

both *LATS1* and *LATS2* was 74/264 (28.0%), and there was a statistically significant correlation between survival and simultaneous *LATS1/2* expression (P=0.016).

A potential limitation of this study was the lack of significant results in the subgroup and multivariate analyses. Thus, additional studies should be performed to support the recognition of *LATS* expression as an important prognostic factor.

In conclusion, the expression of *LATS1* and *LATS2* in patients with gastric cancer was found to be related to a number of clinicopathologic factors, and was associated with a good prognosis, including a higher survival rate. If detailed mechanisms underlying the loss or overexpression of *LATS* can be identified, this knowledge may have a positive effect on the treatment and clinical outcomes of various malignant tumors, including gastric cancer.

REFERENCES

1. Bertuccio P, Chatenoud L, Levi F, Praud D, Ferlay J, Negri E, et al. Recent patterns in gastric cancer: a global overview. Int J Cancer 2009;125:666-673.
PUBMED | CROSSREF
2. Bosetti C, Bertuccio P, Malvezzi M, Levi F, Chatenoud L, Negri E, et al. Cancer mortality in Europe, 2005–2009, and an overview of trends since 1980. Ann Oncol 2013;24:2657-2671.
PUBMED | CROSSREF
3. Oh CM, Won YJ, Jung KW, Kong HJ, Cho H, Lee JK, et al. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2013. Cancer Res Treat 2016;48:436-450.
PUBMED | CROSSREF
4. Justice RW, Zilian O, Woods DF, Noll M, Bryant PJ. The Drosophila tumor suppressor gene warts encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation. Genes Dev 1995;9:534-546.
PUBMED | CROSSREF
5. Xu T, Wang W, Zhang S, Stewart RA, Yu W. Identifying tumor suppressors in genetic mosaics: the Drosophila lats gene encodes a putative protein kinase. Development 1995;121:1053-1063.
PUBMED
6. Visser S, Yang X. LATS tumor suppressor: a new governor of cellular homeostasis. Cell Cycle 2010;9:3892-3903.
PUBMED | CROSSREF
7. Oh HJ, Lee KK, Song SJ, Jin MS, Song MS, Lee JH, et al. Role of the tumor suppressor RASSF1A in Mst1-mediated apoptosis. Cancer Res 2006;66:2562-2569.
PUBMED | CROSSREF
8. Zhao B, Li L, Lei Q, Guan KL. The Hippo-YAP pathway in organ size control and tumorigenesis: an updated version. Genes Dev 2010;24:862-874.
PUBMED | CROSSREF
9. Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J, et al. Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. Genes Dev 2007;21:2747-2761.
PUBMED | CROSSREF
10. Zhou D, Conrad C, Xia F, Park JS, Payer B, Yin Y, et al. Mst1 and Mst2 maintain hepatocyte quiescence and suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene. Cancer Cell 2009;16:425-438.
PUBMED | CROSSREF
11. Xia H, Qi H, Li Y, Pei J, Barton J, Blackstad M, et al. LATS1 tumor suppressor regulates G2/M transition and apoptosis. Oncogene 2002;21:1233-1241.
PUBMED | CROSSREF
12. Zeng Q, Hong W. The emerging role of the hippo pathway in cell contact inhibition, organ size control, and cancer development in mammals. Cancer Cell 2008;13:188-192.
PUBMED | CROSSREF
13. Zhou Y, Tao F, Cheng Y, Xu F, Yao F, Feng D, et al. Up-regulation of ITCH is associated with down-regulation of LATS1 during tumorigenesis and progression of cervical squamous cell carcinoma. Clin Invest Med 2014;37:E384-E394.
PUBMED | CROSSREF

14. Morinaga N, Shitara Y, Yanagita Y, Koida T, Kimura M, Asao T, et al. Molecular analysis of the h-warts/LATS1 gene in human breast cancer. Int J Oncol 2000;17:1125-1129.
PUBMED

15. Li H, Wolfe A, Septer S, Edwards G, Zhong X, Abdulkarim AB, et al. Deregulation of Hippo kinase signalling in human hepatic malignancies. Liver Int 2012;32:38-47.
PUBMED | CROSSREF

16. Xu ZP, Zhu JS, Zhang Q, Wang XY. A breakdown of the Hippo pathway in gastric cancer. Hepatogastroenterology 2011;58:1611-1617.
PUBMED | CROSSREF

17. Kim KJ, Jung HY, Oh MH, Cho H, Lee JH, Lee HJ, et al. Loss of ARID1A expression in gastric cancer: correlation with mismatch repair deficiency and clinicopathologic features. J Gastric Cancer 2015;15:201-208.
PUBMED | CROSSREF

18. Tao W, Zhang S, Turenchalk GS, Stewart RA, St John MA, Chen W, et al. Human homologue of the Drosophila melanogaster lats tumour suppressor modulates CDC2 activity. Nat Genet 1999;21:177-181.
PUBMED | CROSSREF

19. Yabuta N, Fujii T, Copeland NG, Gilbert DJ, Jenkins NA, Nishiguchi H, et al. Structure, expression, and chromosome mapping of LATS2, a mammalian homologue of the Drosophila tumor suppressor gene lats/warts. Genomics 2000;63:263-270.
PUBMED | CROSSREF

20. Furth N, Ben-Moshe NB, Pozniak Y, Porat Z, Geiger T, Domany E, et al. Down-regulation of LATS kinases alters p53 to promote cell migration. Genes Dev 2015;29:2325-2330.
PUBMED | CROSSREF

21. Powzaniuk M, McElwee-Witmer S, Vogel RL, Hayami T, Rutledge SJ, Chen F, et al. The LATS2/KPM tumor suppressor is a negative regulator of the androgen receptor. Mol Endocrinol 2004;18:2011-2023.
PUBMED | CROSSREF

22. Yu T, Bachman J, Lai ZC. Evidence for a tumor suppressor role for the large tumor suppressor genes LATS1 and LATS2 in human cancer. Genetics 2013;195:1193-1196.
PUBMED | CROSSREF

23. Lin XY, Zhang XP, Wu JH, Qiu XS, Wang EH. Expression of LATS1 contributes to good prognosis and can negatively regulate YAP oncoprotein in non-small-cell lung cancer. Tumour Biol 2014;35:6435-6443.
PUBMED | CROSSREF