

Original Article

LATS1 inhibits metastasis and epithelial-mesenchymal transition in head and neck squamous cell carcinoma

Jicheng Wu¹, Zhijuan Zhao², Huiling Zhang¹, Fanshuang Kong¹, Huamao Jiang³, Keqiang Huang⁴, Huachuan Zheng¹

¹Department of Experimental Oncology, Shengjing Hospital of China Medical University, Shenyang, China; Departments of ²Pathology, ³Urology, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou, China; ⁴Department of Stomatology, The Second Affiliated Hospital of Jinzhou Medical University, Jinzhou, China

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Abstract: LATS1 is a serine/threonine kinase of the Hippo signaling pathway that phosphorylates and inactivates transcriptional co-activators YAP1 and WWTR1. To investigate roles of LATS1 expression in head and neck squamous cell carcinomas (HNSCCs), we transfected LATS1-expressing plasmid into B88 cells and examined the phenotypes and their relevant molecules. LATS1 expression was analyzed using immunohistochemistry on tissue microarray, Oncomine, and TCGA databases. LATS1 overexpression was found to suppress growth, migration and invasion, and induce apoptosis, G2 arrest, and mesenchymal to epithelial transition (MET) ($P < 0.05$). Both increased expression of P21, Bax, and E-cadherin and decreased expression of Cyclin B1, D1, Bcl-2, and MMPs. Twist and N-cadherin were detected in B88 transfectants, in comparison to mock and control by Western blot. Nuclear LATS1 expression was weaker in primary cancers than in normal squamous tissue and dysplasia ($P < 0.05$) but versa for cytoplasmic counterpart ($P < 0.05$). Cytoplasmic LATS1 expression was positively correlated with lymph node metastasis ($P < 0.05$). Survival analysis showed that differentiation degree was an independent factor of long overall and relapse-free survival of HNSCC patients ($P < 0.05$). According to bioinformatics analysis, we found upregulated LATS1 mRNA expression in HNSCCs ($P < 0.05$). Cox proportional hazards model indicated that perineural invasion and distant metastasis were independent prognostic factors for overall survival of HNSCC ($P < 0.05$). These findings suggest nucleocytoplasmic translocation of LATS1 protein and upregulated expression of LATS1 mRNA during tumorigenesis of HNSCC. LATS1 mRNA overexpression may reverse aggressive phenotypes of HNSCC cells, as a gene therapy target.

Keywords: HNSCC, LATS1, aggressiveness, prognosis, gene therapy

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the 5th most common cancer and 8th in cancer deaths worldwide. Although early-stage head and neck cancers (especially laryngeal and oral cavity) have high cure rates, up to 50% of head and neck cancer patients present with advanced disease because it frequently transfers to neck lymph nodes earlier as the first symptom and sometimes only [1]. Therefore, recognition of original biomarkers and gene therapy targets could be conducive to increase survival rate and quality of HNSCC patients, in spite of the development of radio imaging, surgery, radiotherapy, chemotherapy, and immunotherapy.

LATS1 (large tumor suppressor 1) serine/threonine kinase is a core member of Hippo signaling, which inactivates and phosphorylates transcriptional co-activators YAP1 and WWTR1. It belongs to the Ndr/LATS subfamily of AGC (protein kinase A/PKG/PKC) kinases and is encoded by LATS1 gene [2]. The interaction of protein's N-terminal domain with CDC2 to form a complex attenuates H1 histone kinase activity, indicating a characteristic as negative regulator of CDC2/cyclin A. However, its C-terminal kinase region binds to its own N-terminal domain to negatively interfere with complex formation. LATS1 protein is localized to the mitotic organ and interacts with CDC2 kinase in early stage of mitosis [3, 4]. Further investigation has indicated that RASSF1A-LATS1 signaling stabi-

lized replication forks by restricting CDK2-mediated phosphorylation of BRCA2 [5]. CHO1 phosphorylation by LATS1 regulated centrosomal activation of LIMK1 during cytokinesis [6], but LATS1 suppressed centrosome overduplication by modulating stability of Cdc25B [7].

A great number of evidences indicate that biological functions of LATS1 depend on phosphorylation and ubiquitination [8-21]. hMOB1 was reported to recruit LATS1 to the plasma membrane for multi-site phosphorylation and activation [8]. Although Han et al. [9] found that dissociation of WWC3 from LATS1 reduced LATS1 phosphorylation to suppress lung cancer invasion and metastasis, the interaction between TNFAIP8 and LATS1 decreased phosphorylation of the latter, finally promoting aggressiveness of hepatocellular carcinoma cells [10]. On the other hand, LATS1-mediated YAP phosphorylation at HX(R/H/K)XX(S/T) sequence facilitated dissociation of the YAP-TEAD4 complex and association of YAP-RUNX3 complex [11, 12], controlled nephron progenitor epithelialization, and inhibited myofibroblast formation [13]. LATS1 phosphorylated CDC26 to modulate assembly of the tetratricopeptide repeat subcomplex of APC/C [14], Angiomotin to inhibit YAP transcription and cell growth due to serum deprivation [15], and FOXL2 to repress StAR mRNA expression [16]. LATS1 ubiquitination by NEDD4, E3 ubiquitin ligase CRL4, and WWP1 E3 ligase leads to proteasomal degradation and promotes nuclear localization and transcriptional activity of YAP [17-19]. Ni et al. [20] found that a novel lncRNA uc.134 repressed hepatocellular carcinoma progression by inhibiting CUL4A-mediated ubiquitination of LATS1 and increasing YAP (S127) phosphorylation to silence target genes. Additionally, LATS1 ablation promotes the luminal phenotype and increases number of bipotent and luminal progenitors, the proposed cells-of-origin of most human breast cancers, by targeting ubiquitination and DCAF1-dependent proteasomal degradation of ER α [21].

LATS1 is downregulated in various human cancers such as breast cancer, lung cancer, colorectal cancer, astrocytoma, and glioma [22-26]. Sun et al. [27] found that LATS1 deletion increased germ cell apoptosis and follicular cysts in mouse ovaries. LATS1 knockout mice showed low neonate survival, a lack of mam-

mary gland development, infertility and growth retardation, development of soft-tissue sarcomas, ovarian stromal cell tumors, pituitary dysfunction, and a high sensitivity to carcinogenic treatments [28]. Yabuta et al. [29] found that mouse embryonic fibroblasts (MEFs) from LATS1 knockout mice displayed mitotic defects, centrosomal overduplication, chromosomal misalignment, multipolar spindle formation, chromosomal bridging, and cytokinesis failure. They also showed anchorage-independent growth and continued cell cycles and cell growth, by passing cell-cell contact inhibition similar to tumor cells. In our study, the effects of ectopic LATS1 overexpression on aggressive phenotypes of HNSCC cells were found and relevant mechanisms were analyzed. Moreover, LATS1 expression in squamous epithelium, cancer of head and neck, and dysplasia was examined. We also examined the correlation of LATS1 expression with clinicopathological and prognostic parameters of cancers by immunohistochemistry and bioinformatics analysis.

Materials and methods

Cell culture and transfection

Tongue cancer, B88, was bought from ATCC and cultured in MEM (HyClone, Logan, UT, USA) along with fetal bovine serum (10%) (FBS; HyClone), penicillin (100 U/ml; Sigma, St-Louis, MO, USA), and streptomycin (100 μ g/ml; Sigma) in a humidified atmosphere of 5% CO₂ at 37°C. B88 cells were transfected with pcDNA3.1-LATS1 at 24 hours using Attractene Transfection Reagent (QIAGEN, USA) and subjected to monocloning selection with pcDNA3.1 as a mock. Cells were collected by centrifugation, washed twice with phosphate buffered saline (PBS), and finally used for RNA and protein extraction.

Proliferation assay

Cells were seeded in triplicate in 96-well plates at a density of 3×10^3 cells/well. Every 24 hours after cell adhesion, cell counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) was employed to determine number of viable cells by detecting absorbance at 450 nm using a FL600 fluorescence plate reader (Bio-Rad, Hercules, CA, USA).

Table 1. Primary antibodies used in the present study

Name	Source	Company	Dilution
N-cadherin	Rabbit	Cell Signaling Technology	1:1000
E-cadherin	Rabbit	Cell Signaling Technology	1:1000
Twist	Rabbit	Abcam	1:1000
ZEB2 (E-11)	Mouse	Santa Cruz Biotechnology	1:500
LATS1	Mouse	Santa Cruz Biotechnology	1:500
CyclinB 1	Mouse	Santa Cruz Biotechnology	1:500
CyclinD 1	Rabbit	Santa Cruz Biotechnology	1:500
p21	Rabbit	Santa Cruz Biotechnology	1:500
Bcl-2	Mouse	Santa Cruz Biotechnology	1:500
MMP-9	Mouse	Santa Cruz Biotechnology	1:500
MMP-2	Rabbit	Wanleibio	1:1000
MMP-1	Rabbit	Wanleibio	1:1000
Survivin	Mouse	Santa Cruz Biotechnology	1:300
14-3-3 (H-8)	Mouse	Santa Cruz Biotechnology	1:300
XIAP (H-202)	Rabbit	Santa Cruz Biotechnology	1:300
Bax (B-9)	Mouse	Santa Cruz Biotechnology	1:300
β-catenin	Rabbit	Cell Signaling Technology	1:1000
GAPDH	Rabbit	Wanleibio	1:1000

Cell cycle analysis

Cells were harvested by trypsinization, rinsed twice with PBS, and fixed in 5 mL 70% ice-cold ethanol for more than 2 hours. Then, cells were washed with PBS again and incubated with 1 mL RNase (Sigma, 0.25 mg/mL) at 37°C. One hour later, the tube with cells was incorporated with propidium iodide (Sigma) at a concentration of 50 µg/mL and incubated in the dark at room temperature for 30 minutes. Cell cycle analysis was performed by PI staining and flow cytometry.

Apoptosis assay

1×10⁵ cells in 6-well plates were harvested by trypsinization, washed with PBS, and resuspended in 1 mL PBS. Flow cytometry was performed following staining with fluorescein isothiocyanate (FITC)-Annexin V (KeyGEN Biotech, Nanjing, China) and PI (Sigma). Phosphatidylserine externalization, as an endpoint indicator of apoptosis in the cells, was detected immediately by flow cytometry.

Transwell assay

24-well Transwell chambers with 8-µm pore size (BD Biosciences, USA) were used to evaluate cell invasion and migration potential. For

the invasion assay, 2.5 × 10⁵ cells in 100 µl serum-free culture medium were seeded into the top chamber of matrigel-coated Transwell inserts (BD Bioscience). The lower compartment of the chamber was filled up with corresponding media containing 10% v/v FBS as a chemoattractant. After incubation for 24 hours, non-invading cells on the upper surface of the membrane were wiped away and invading cells on the bottom were washed with PBS, fixed in 100% methanol, and stained with 0.1% crystal violet to quantify the extent of invasion. For migration assay, we performed the abovementioned experiment, except for non-coating inserts.

Selection of patient samples

This study was approved by the Ethics Committee of China Medical University and Kanagawa Cancer Center Hospital (KCCH). Informed consent was obtained from all subjects, according to the Declaration of Helsinki. The study included a total of 509 HNSCC patients that underwent curative resection at KCCH between April 1995 and January 2009. All patients had been treated according to protocol for head and neck cancer treatment at KCCH, based on TNM staging system. None of these patients had undergone chemotherapy or radiotherapy before surgery. In addition, 113 normal and 99 dysplasia samples from the head and neck were also gathered and used for comparison with HNSCC. Average age at surgery was 51.2 years (range 20-81 years). We followed up patient's survival informations by consulting their case documents, out-patient review, and by mail and telephone.

Pathology and tissue microarray (TMA)

All specimens were subjected to routine block preparation, cut into thin slides, and pathologies were confirmed by hematoxylin-and-eosin staining. TMA was prepared using a Tissue Microarray kit (AZUMAYA KIN-1, Tokyo, Japan). Clinicopathological staging was evaluated for the samples according to TNM staging system.

LATS1 in head and neck squamous cell carcinoma

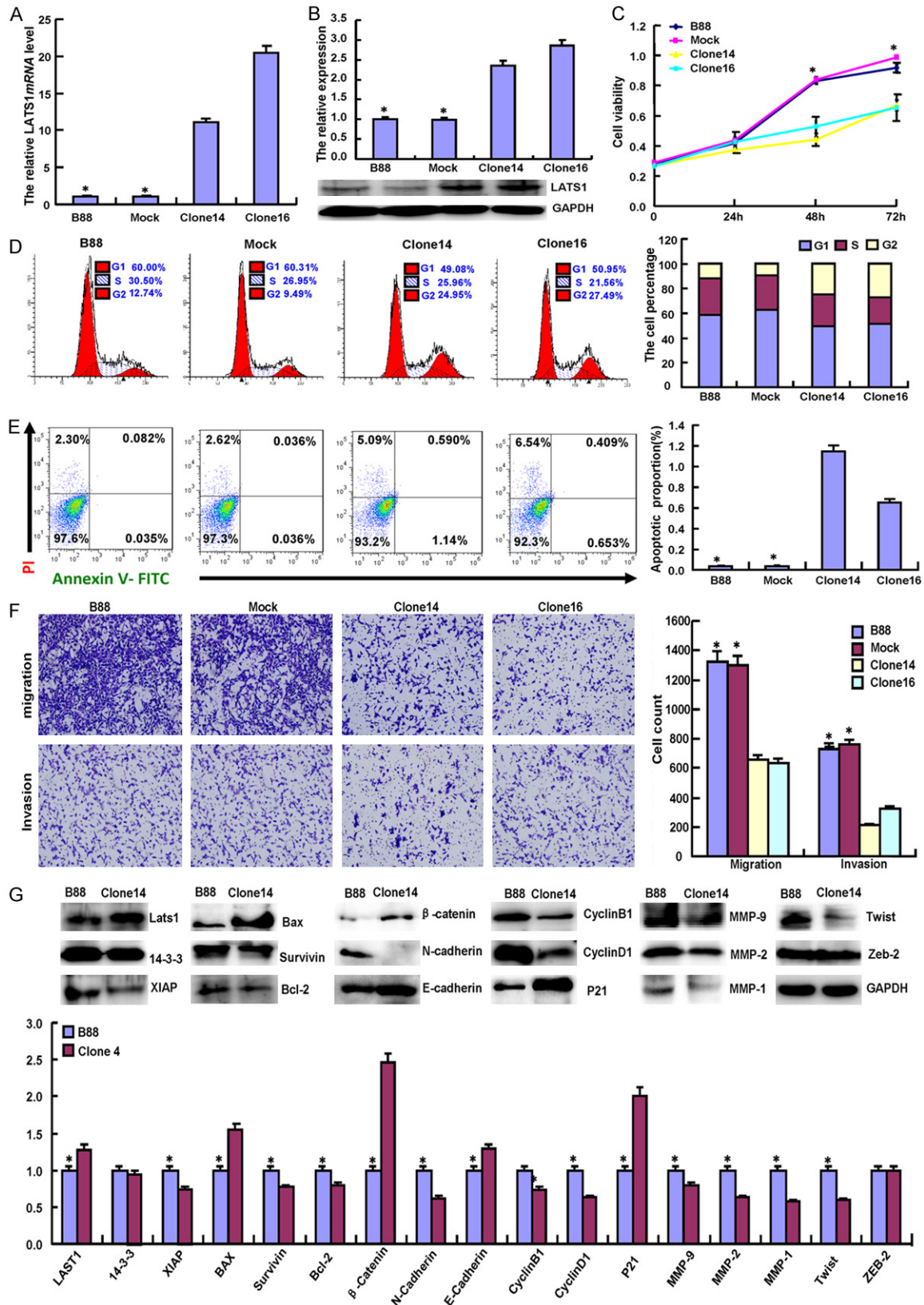


Figure 1. LATS1 expression altered the phenotypes of HNSCC cells. After transfection of pCDNA3.1-LATS1, its expression became strong in B88 cells, by RT-PCR (A) and Western blot (B). The cell viability was measured using CCK-8 assay (C). The apoptosis, cell cycle, migration, and invasion were examined by Annexin-V staining (D), PI staining (E), and Transwell chamber assay (F). The phenotype-associated molecules were screened by Western blot (G). * $P < 0.05$, compared with the transfectant.

Real-time reverse transcriptase-polymerase chain reaction (real-time RT-PCR)

Total RNA was extracted from cells using TRIzol (Takara, Kyoto, Japan) and reverse-transcribed into cDNA. Real-time PCR was conducted using SYBR Premix Ex Taq II (Takara), using cDNA as the template in an iCycler iQ real-time PCR system (Bio-Rad). Reactions started at 95°C for 1 minute, followed by 59 cycles of 5 seconds at 95°C alternating with 30 seconds at 60°C. The threshold cycle (C_T) of each sample was determined and then normalized to values for GAPDH using the following equation: $\Delta C_T = C_T$ (gene of interest) - C_T (GAPDH). The relative level was calculated as $2^{-\Delta C_T}$. The primers were as follows: LATS1 Forward: 5'-GGGTCCTCGGCAAAGTTTA-3'; LATS1 Reverse: 5'-TTTCTTGGCACAAACACC-AT-3'; GAPDH Forward: 5'-CAATGACCCCTTCATGACC-3'; GAPDH Reverse: 5'-TGGAAGATGGTGATGGGATT-3'.

Western blot analysis

Protein assays were performed by Bradford method using the Bio-Rad protein assay kit (Bio-Rad, USA). Western blot was carried out as previously described [9]. The primary antibodies are summarized in **Table 1**.

Immunohistochemistry

Consecutive sections of TMA samples (4 μ m) were dewaxed, rehydrated with alcohol, and subjected to intermittent irradiation immunohistochemistry, according to manufacturer protocol as described previously [30]. Normal rabbit serum was used as a negative control. Results were evaluated by three observers (WJC, ZZJ, and ZHC) independently.

Bioinformatics analysis

LATS1 expression level was analyzed using Oncomine database (www.oncomine.org) and differences of LATS1 mRNA levels between normal squamous tissue and carcinoma of head and neck were compared. All data were log-transformed, median centered per array, and standard deviation normalized to one per array. In addition, a total of 519 primary HNSCC patients with clinicopathological data and detailed expression of LATS1 were downloaded from the updated Cancer Genome Atlas (TCGA) database by TCGA-assembler in R software. We integrated the raw data, analyzed LATS1

expression in HNSCC, and compared it with clinicopathological features and prognostic data of patients with HNSCC.

Statistical analysis

Spearman's rank correlation coefficient was employed to analyze ranked data for statistical evaluation. Association between continuous variables and categorical variables was evaluated using Mann-Whitney U-tests for two groups. Log-rank test and the Kaplan-Meier method were applied to examine the effects of LATS1 on overall survival and relapse-free survival. Cox proportional hazards model was performed for multivariate analysis. A *P*-value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS 17.0 software.

Results

Regulation of LATS1 expression contributes to tumor aggressive phenotypes

We successfully overexpressed LATS1 in B88 cells by transfecting the plasmid. The effect of transfection was evaluated by real-time PCR (**Figure 1A**, *P* < 0.05) and Western blot (**Figure 1B**, *P* < 0.05). Compared with mock and control, LATS1 overexpression showed a lower growth evidenced by CCK-8 assay (**Figure 1C**, *P* < 0.05), a higher G2-phase cell proportion (**Figure 1D**, *P* < 0.05), and apoptosis (**Figure 1E**, *P* < 0.05) by flow cytometry. Cell invasion assay showed that the number of invading cells was significantly lower in LATS1 transfectants (**Figure 1F**, *P* < 0.05). Western blot revealed that overexpression of LATS1 promoted expression of p21, Bax, and E-cadherin and inhibited expression of XIAP, Cyclin B1, Cyclin D1, MMP-1, MMP-2, MMP-9, Twist, and N-cadherin (**Figure 1G**, *P* < 0.05).

Correlation between LATS1 protein expression and clinicopathological parameters of HNSCCs

Immunostaining revealed the positive staining of LATS1 expression in the cytoplasm and/or nucleus of normal squamous epithelial cells and dysplasia, but weakly or negative in primary or metastatic squamous cell carcinomas (**Figure 2A**). As shown in **Table 2**, cytoplasmic LATS1 expression was detectable in normal squamous epithelium (9.7%, 11/113), dysplasia (15.6%, 15/99), and primary cancer (62.5%,

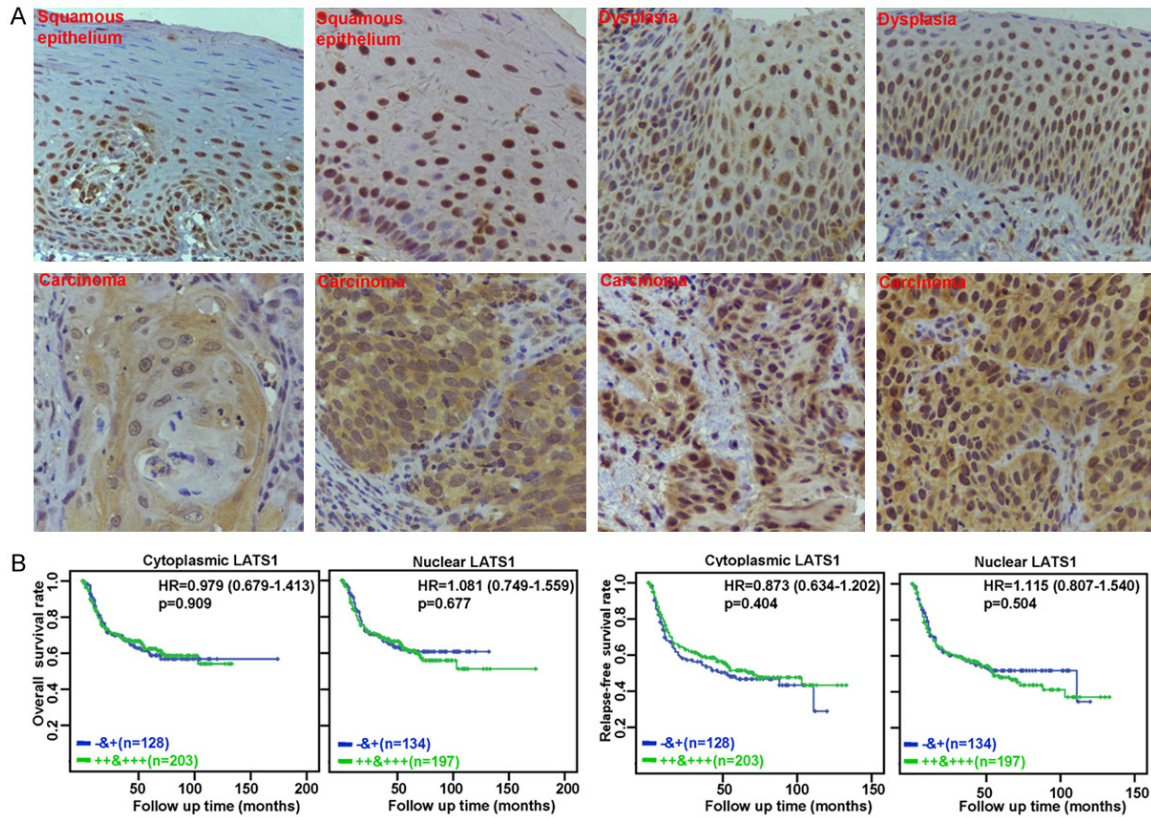


Figure 2. Clinicopathological prognostic significances of LATS1 protein expression in HNSCC. LATS1 was strongly expressed in the cytoplasm and/or nucleus of normal squamous epithelium, dysplasia, and carcinoma of head and neck (A). Kaplan-Meier curves were made for cumulative and relapse-free survival rates of patients with head and neck squamous cell carcinoma according to LATS1 expression (B).

Table 2. LATS1 protein expression in head and neck squamous carcinogenesis

Groups	n	Cytoplasmic LATS1 expression					Nuclear LATS1 expression				
		-	+	++	+++	%	-	+	++	+++	%
Squamous epithelium	113	102	8	3	0	9.7	21	15	26	51	81.4
Dysplasia	99	84	11	2	2	15.6	22	12	18	47	77.8
Primary cancer	509	191	93	140	85	62.5*	197	46	104	162	61.3*

Abbreviation: PR, positive rate. *, compared with squamous epithelium and dysplasia, $P < 0.05$.

318/509), respectively. Positive rates of nuclear LATS1 expression were 81.4% (92/113), 77.8% (77/99), and 61.3% (312/509) in normal squamous epithelium, dysplasia, and primary cancer. According to its expression frequency and density, cytoplasmic LATS1 expression was stronger in primary cancer than dysplasia and normal epithelium ($P < 0.05$), while versa for nuclear counterpart ($P < 0.05$). As summarized in **Table 3**, cytoplasmic LATS1 expression was positively correlated with lymph node metastasis of HNSCC ($P < 0.05$) but not with age, gender, tumor size, distant metastasis, TNM stag-

ing, or differentiation ($P > 0.05$). However, nuclear LATS1 expression was not related to the abovementioned parameters ($P > 0.05$). There was a significant positive correlation between cytoplasmic and nuclear LATS1 expression in primary HNSCC samples (**Table 4**, $P < 0.01$). Following up information was available on 334 HNSCC patients for periods ranging from 7 to 96 months (median = 28.1 months). Univariate analysis using Kaplan-Meier method indicated no relationship between cytoplasmic or nuclear LATS1 expression and cumulative or relapse-free survival rate of

Table 3. Relationship between LATS1 expression and clinicopathological features of HNSCC

Clinicopathological features	n	Cytoplasmic LATS1 expression						Nuclear LATS1 expression					
		-	+	++	+++	%	P	-	+	++	+++	%	P
Age (years)							0.621						0.195
< 65	193	72	35	54	32	62.7		82	14	43	54	57.5	
≥ 65	273	97	55	72	49	64.5		97	24	54	98	64.5	
Sex							0.792						0.530
Male	314	119	61	81	53	62.1		127	25	57	105	59.6	
Female	77	31	14	19	13	59.7		30	7	22	18	61.0	
Tumor status							0.463						0.362
T ₁₋₂	227	91	42	55	39	59.9		89	16	45	77	60.8	
T ₃₋₄	155	54	32	43	24	65.2		64	15	32	44	58.7	
Lymph node metastasis							0.008						0.259
-	184	81	32	50	21	56.0		73	11	33	67	60.3	
+	201	65	42	49	45	67.7		81	20	45	55	59.7	
Distant metastasis							0.541						0.845
-	378	142	74	98	64	62.4		151	31	77	119	60.1	
+	6	4	0	0	2	33.3		3	0	1	2	50.0	
TNM staging							0.375						0.084
I-II	181	65	33	47	36	64.1		64	14	42	61	64.6	
III-IV	180	70	36	44	30	61.1		80	15	34	51	55.6	
Differentiation							0.170						0.081
Well-differentiated	122	55	18	26	23	54.9		47	7	21	47	61.5	
Moderately-differentiated	134	43	29	37	25	67.9		50	15	31	38	62.7	
Poorly-differentiated	80	26	14	28	12	67.5		36	8	17	19	55.0	

Table 4. Relationship between cytoplasmic and nuclear LATS1 expression in HNSCC

Nuclear LATS1 expression	n	Cytoplasmic LATS1 expression				
		-	+	++	+++	%
-	179	125	30	17	7	30.2
+	38	12	12	8	6	68.4
++	97	11	22	43	21	88.7
+++	152	21	26	58	47	86.2
Total	466	169	90	126	81	63.7

$P < 0.001$.

patients with HNSCC (**Figure 2B**, $P > 0.05$). Multivariate analysis using Cox risk proportional model showed that differentiation was an independent prognostic factor for HNSCC patients (**Table 5**, $P < 0.05$).

Correlation between LATS1 mRNA expression and clinicopathological parameters of HNSCCs

We then used Pyeon's, Ye's, and Peng's datasets to conduct bioinformatics analysis. It was revealed that *LATS1* mRNA expression was

higher in HNSCC than normal tissues (**Figure 3A**, $P < 0.05$). From TCGA's database, there was no association between expression of *LATS1* and age, gender, tumor status, lymph node metastasis, distant metastasis, or TNM staging of HNSCCs ($P > 0.05$, data not shown). Univariate analysis showed no significant association between *LATS1* mRNA expression and overall better prognosis of patients with HNSCC (**Figure 3B**, $P > 0.05$). Multivariate

analysis using Cox hazards proportional model indicated that perineural invasion and distant metastasis were independent prognostic factors for HNSCC (**Table 6**, $P < 0.05$).

Discussion

LATS1 phosphorylates angiotensin II to suppress cytokinesis, cell migration, and angiogenesis by inhibiting F-actin binding and polymerization via negative modulation of LIMK1 [31-33]. In glioma U251 cells, *LATS1* overexpression not only

Table 5. Multivariate analysis of hazard factors of the prognosis of patients with HNSCC, according to immunohistochemistry

Clinicopathological features	Overall survival		Relapse-free survival	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Age (< 65/≥ 65 years)	0.968 (0.491-1.909)	0.925	1.379 (0.887-2.142)	0.153
Gender (Female/Male)	1.580 (0.933-2.678)	0.089	1.602 (0.940-2.731)	0.083
Tumor status (T ₁₋₂ /T ₃₋₄)	0.983 (0.588-1.642)	0.947	0.828 (0.542-1.264)	0.381
Lymph node metastasis (-/+)	1.337 (0.731-2.444)	0.345	1.226 (0.740-2.030)	0.428
Distant metastasis (-/+)	1.187 (0.155-9.096)	0.869	0.786 (0.105-5.892)	0.814
TNM staging (I-II/III-IV)	0.842 (0.491-1.444)	0.532	1.173 (0.742-1.855)	0.494
Differentiation (Well/Moderately/Poorly)	1.451 (1.028-2.048)	0.034	1.359 (1.011-1.829)	0.042
Cytoplasmic LATS1 expression (-/+ +++)	0.859 (0.461-1.602)	0.633	0.928 (0.555-1.552)	0.775
Nuclear LATS1 expression (-/+ +++)	1.069 (0.590-1.937)	0.827	1.096 (0.670-1.792)	0.714

CI, confidence interval.

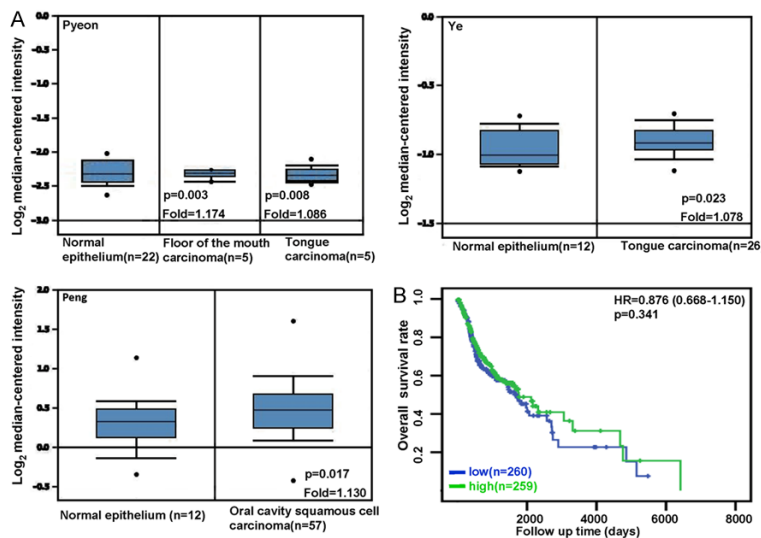


Figure 3. Clinicopathological prognostic significances of *LATS1* mRNA expression in HNSCC. Pyeon's, Ye's, and Peng's datasets were employed for bioinformatics analysis to analyze *LATS1* expression in head and neck squamous carcinoma (A). The prognostic significance of *LATS1* expression was analyzed using TCGA database (B). Note: HR, hazard ratio.

significantly inhibits cell growth, migration and invasion, but postpones cell cycle progression from G₂/M to G₁ *in vitro* [26]. Chen et al. [34] found that *LATS1* demethylation and overexpression downregulated YAP expression, inhibited cell proliferation, and induced cell apoptosis and cell cycle arrest in renal cell carcinoma cells. In cervical cancer cells, Deng et al. [35] demonstrated that *LATS1* overexpression inhibited cell proliferation and invasion with upregulated p27 expression, downregulated expression of cyclin E and MMP-9, and stimulated YAP phosphorylation. Lin et al. [23] found that

LATS1 markedly inhibited cell proliferation and invasion of non-small-cell lung cancer by regulating the nuclear location of YAP. Here, we found that *LATS1* overexpression inhibited proliferation, migration, and metastasis, and induced apoptosis with E-cadherin overexpression and N-cadherin under-expression, indicating that *LATS1* suppresses proliferation and EMT and might be employed as a gene therapy target in clinical practice. Additionally, the reversing effects of *LATS1* expression on aggressive phenotypes were closely linked to overexpression of p21 and Bax and hypo-expression of XIAP, Cyclin B1, Cyclin D1, MMP-1, MMP-2, MMP-9, and Twist because p21 can bind

to and inhibit Cyclin D1 to arrest G1-S transition and Cyclin B1-Cdk1 is involved in early events of mitosis [36]. XIAP might play a role as apoptotic inhibitor by binding to caspase and suppressing its activation [37]. MMPs promote degradation of extracellular matrix [38] and Twist induced N-cadherin overexpression and E-cadherin hypo-expression as a transcription factor [39].

Reportedly [22-26, 39-42], mRNA expression level of *LATS1* is down regulated in lung cancer, oral squamous cell carcinoma, astrocytoma,

Table 6. Multivariate analysis of hazard factors of the prognosis of patients with HNSCC, according to TCGA database

Clinicopathological features	Hazard ratio (95% CI)	P
Age (≤ 60 / > 60 years)	1.110 (0.778-1.582)	0.566
Gender (Female/Male)	0.926 (0.626-1.370)	0.702
Tumor status (T_{1-2}/T_{3-4})	1.498 (0.758-2.961)	0.245
Perineural invasion (-/+)	1.996 (1.383-2.881)	< 0.001
Lymph node metastasis (-/+)	1.181 (0.766-1.820)	0.452
Distant metastasis (-/+)	8.219 (1.744-38.736)	0.008
TNM staging (I-II/III-IV)	0.677 (0.287-1.596)	0.373
LATS1 mRNA expression (Low/High)	0.941 (0.663-1.335)	0.732

CI, confidence interval.

breast cancer, colorectal cancer, glioma, and pterygium tissues, due to its hyper-methylation. Zhou et al. [43] found that low membrane expression of LATS1 was strongly associated with malignant transformation of the cervical epithelium. Chen et al. [34] found that expression of LATS1 was markedly decreased in renal clear cell carcinoma tissues, compared with normal tissue. In this study, we found that nuclear LATS1 expression was downregulated and its cytoplasmic counterpart upregulated in HNSCC, but not in precancerous lesions. This suggests that nucleocytoplasmic translocation may contribute to head and neck squamous cell carcinogenesis as a late event. In contrast, upregulated mRNA expression of *LATS1* might be a reactive and negative feedback. A body of evidences has shown that LATS1 expression is negatively correlated with tumor size, lymph node metastasis, histological grading or TNM staging of breast cancer, glioma, or cervical cancer [22, 23, 26]. Zhou et al. [43] demonstrated that low membrane expression of LATS1 was strongly associated with histological grading, clinical stage, and tumor size of cervical cancer. In our present study, we found only a positive relationship between cytoplasmic LATS1 expression and lymph node metastasis of HNSCCs, despite a significantly positive association between nuclear and cytoplasmic expression of LATS1 protein. This indicates that cytoplasmic LATS1 might be employed as a molecular marker of aggressiveness of HNSCC.

Loss of *LATS1* mRNA expression has been associated with overall short survival of patients with breast cancer [22] or lung cancer [40]. Non-small-cell lung cancer [23] or glioma [26] patients with lower LATS1 expression have

had a significantly shorter overall survival time than patients with higher LATS1 expression, as an independent prognostic indicator. In contrast, no association between LATS1 expression and prognosis of HSCC patients was found at both mRNA and protein levels in our study. Multivariate analysis of immunohistochemistry showed that differentiation was an independent factor for overall or relapse-free survival of HNSCC patients while TCGA database showed that perineural invasion and distant metastasis were independent factors. This discrepancy is due to differing subjects, methodologies, and clinical parameters involved in the study.

In conclusion, there was nucleocytoplasmic translocation of LATS1 protein during HNSCC tumorigenesis, as a late event. Cytoplasmic LATS1 expression was positively correlated with lymph node metastasis. As a gene therapy target, LATS1 overexpression may reverse malignant phenotypes of HNSCC cells.

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Disclosure of conflict of interest

None.

Address correspondence to: Huachuan Zheng, Department of Experimental Oncology, Shengjing Hospital of China Medical University, Shenyang 110004, China. Tel: +86-18704067718; Fax: +86-24-96615; E-mail: zheng_huachuan@hotmail.com

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