in vivo *37*: 2296-2305 (2023)
doi:10.21873/invivo.13332

Clinicopathological Significance of DUB3 Expression in Non-small Cell Lung Cancer and Relationship Between DUB3 Expression and LATS1 Expression

SI-HYONG JANG, HYUN DEUK CHO, JI-HYE LEE and MEE-HYE OH

Department of Pathology, College of Medicine,
Soonchunhyang University Cheonan Hospital, Cheonan, Republic of Korea

Abstract. *Background/Aim: Deubiquitinating enzyme 3 (DUB3) is a member of the ubiquitin-specific proteases family involved in regulating cell proliferation, invasion, and apoptosis. However, the biological role and clinicopathological significance of DUB3 expression have not been elucidated in non-small cell lung cancer (NSCLC). Patients and Methods: We evaluated the expression of DUB3 by immunohistochemistry using tissue microarrays and assessed the clinicopathologic significance of DUB3 expression levels in 187 patients with NSCLC, including its two major subtypes (93 cases of adenocarcinoma and 72 cases of squamous cell carcinoma). Results: In adenocarcinoma, we observed that DUB3 expression had an effect on tumor size (p=0.030), vessel invasion (p=0.038), T stage (p=0.014), and tumor recurrence (p=0.002). Kaplan-Meier curves with log-rank test showed that high DUB3 expression was correlated with significantly more favorable clinical outcomes compared to those of the low expression group in adenocarcinoma (p=0.013). Multivariate analysis of disease-free survival also demonstrated that DUB3 expression is an independent prognostic factor in lung adenocarcinoma (p=0.017). Additionally, we identified the correlation between DUB3 and the expression of large tumor suppressor kinase 1 expression, a core protein of the Hippo pathway. Conclusion: DUB3 could function as a tumor suppressor by regulating the Hippo pathway in lung adenocarcinoma and can be considered a powerful predictive factor and therapeutic target.*

Correspondence to: Mee-Hye Oh, MD, Ph.D., Department of Pathology, Soonchunhyang University Cheonan Hospital, 31, Soonchunhyang 6 Gil, Dongnam-gu, Cheonan 31151, Republic of Korea. Tel: +82 415703582, Fax: +82 415703580, e-mail: mhoh0212@hanmail.net

Key Words: Carcinoma, non-small-cell lung, DUB3 protein, LATS1 protein, prognosis.

Ubiquitination is a biologically important mechanism for the regulation of many intracellular processes by controlling protein function (1). It plays crucial roles in regulating cell cycle progression, transcription, apoptosis, receptor localization, and vesicle trafficking (2, 3). Ubiquitination can be reversed by deubiquitinating enzymes (DUBs) (1), which are composed of a large group of proteases composed of up to 90 members that remove ubiquitin from target proteins by modifying or disassembling the polyubiquitin chain (3, 4). DUBs are classified into five families: ubiquitin C-terminal hydrolases, ubiquitin-specific proteases (USPs), ovarian-tumor proteases, Machado-Joseph disease protein domain proteases, and JAMM motif proteases (5-7).

Among the members of the USP family, DUB3, also known as USP17, USP17L, or USP17L2, is involved in the regulation of cell cycle progression (8), cell migration (9), and apoptosis (10, 11). However, the biological properties of DUB3 have not yet been clearly established. The oncogenic properties of DUB3 have been reported in some studies. DUB3 is strictly regulated during the cell cycle and is an essential molecule for G1-S phase progression (12). In another oncogenic aspect, DUB3 is associated with the stabilization of SNAIL, a key factor of epithelial-mesenchymal transition, inducing cancer cell invasion and metastasis (13, 14). On the other hand, some research has suggested that DUB3 acts as a tumor suppressor by blocking growth factor-dependent proliferation (2) and regulating Hippo signaling activity (15).

The Hippo pathway has emerged as a master regulator of cell proliferation, division, apoptosis, and differentiation in embryogenesis and tumor biology (16-20). The MST1/2-SAV-MOB1-LATS1/2 signaling axis, which is the core backbone of the Hippo pathway, suppresses the activity of the downstream proto-oncogenic target proteins, yes-associated protein (YAP) and tafazzin (TAZ) (21, 22).

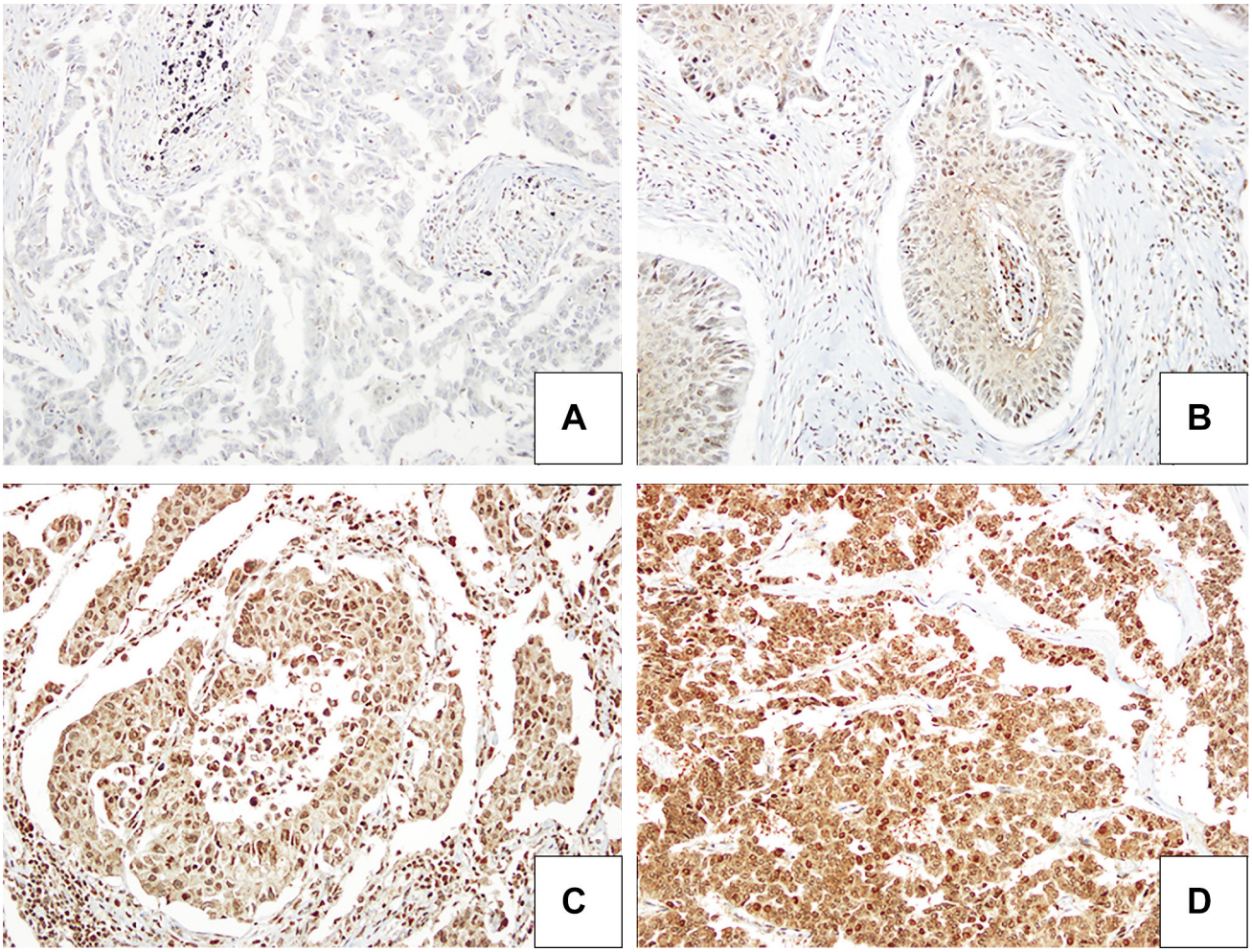

Figure 1. *Representative deubiquitinating enzyme 3 (DUB3) staining (×400). (A) Negative staining, (B) weak staining, (C) moderate staining, and (D) strong staining.*

Dysregulations in the major components of the Hippo signaling pathway have been described in tumor development and progression in various organs, including the lungs (23-26). We previously reported that the downregulations of large tumor suppressor kinase 2 (LATS2) expression might predict aggressive clinical course and poor prognosis in non-small cell lung cancer (NSCLC) (17).

Studies have reported the clinicopathological significance of aberrant DUB3 expression in malignancies including ovarian cancer (1), osteosarcoma (27), breast cancer (14), and NSCLC (3, 28). In a study of a NSCLC cell line, DUB3 was shown to induce cell cycle progression and cell proliferation by stabilizing cyclin A (28). Another study involving immunohistochemical staining of clinical samples demonstrated that high DUB3 expression is related to NSCLC recurrence and metastasis (3). However, to date, no specific research has been conducted on the subtypes of lung cancer, and the number of subjects remains limited. Furthermore, clinicopathological factors have not been considered in detail.

The aim of this study was to investigate the biological mechanism and clinicopathologic significance of DUB3 in lung adenocarcinoma and squamous cell carcinoma, the major subtypes of NSCLC, *via* immunohistochemical analysis of clinical samples. We found that loss of DUB3 expression was related to aggressive clinicopathologic parameters and unfavorable outcomes, and DUB expression could be considered as an independent prognostic factor in lung adenocarcinoma. Additionally, we found that DUB3 expression is closely related to the expression of LATS1, a core protein of the Hippo Pathway, and acts as tumor suppressor.

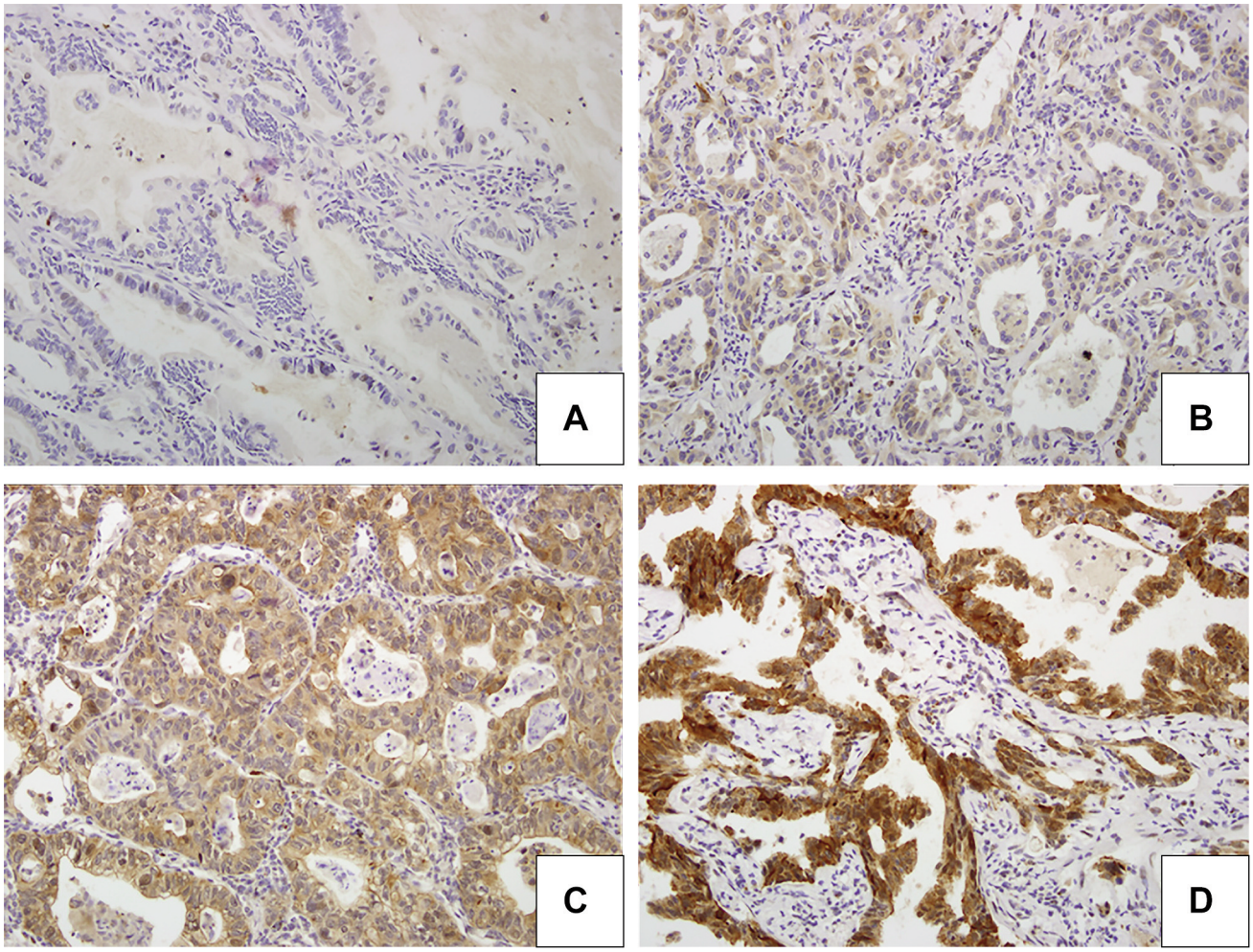

Figure 2. *Representative large tumor suppressor kinase 1 (LATS1) staining (×400). (A) Negative staining, (B) weak staining, (C) moderate staining, and (D) strong staining.*

Patients and Methods

Patients and specimens. We enrolled a series of 187 patients with NSCLC (93 cases of adenocarcinoma, 72 cases of squamous cell carcinoma, 13 cases of large cell neuroendocrine carcinoma, 7 cases of pleomorphic carcinoma, and 2 cases of adenosquamous carcinoma) who underwent resection at the Soonchunhyang University Cheonan Hospital (Cheonan, Republic of Korea) between January 2001 and December 2012. All cases were confirmed by histology and immunohistochemistry. Samples that were subjected to hematoxylin-eosin (H&E) staining were reviewed, and representative paraffin-embedded blocks were chosen for the construction of tissue microarrays by two pathologists (MH Oh and SH Jang). Medical records including pathological reports were reviewed to collect clinicopathological information on the patients including age, sex, smoking history, tumor size, pleural invasion, lymphovascular invasion, lymph node metastasis, distant metastasis, American Joint Committee on Cancer (AJCC) stage, recurrence, and survival. Tumors were staged according to the 8th edition of the AJCC cancer staging manual (stage I, 108; stage II, 48; stage III, 25; stage IV, 6). The enrolled patients included 127 men (67.9%) and 60 women (32.1%), with ages ranging from 39 to 83 years (mean of 63.4 years). All patients were monitored every six months or one year after surgery, and the mean follow-up period was 40 months (range of 0-157 months). This study was approved by the Institution Review Board of Soonchunhyang University Cheonan Hospital, and informed consent was waived (Institution Review Board no. 2017-10-014). This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Tissue microarray construction and immunohistochemistry. Representative, non-necrotic, and hypercellular tumor areas were carefully selected and 2-mm single-tissue cores were obtained from formalin-fixed, paraffin-embedded donor blocks using a tissue array instrument and assembled into tissue microarray (TMA) blocks. For immunohistochemical staining, 4-mm-thick sections were cut from

Table I. *Correlation between deubiquitinating enzyme 3 (DUB3) expression and clinicopathologic parameters in 187 non-small cell lung cancer patients.*

Variables	N (%)	DUB3 expression		*p*-Value
		Low expression	*High expression*	
Sex				
Male	127 (100)	58 (45.7)	69 (54.3)	0.639
Female	60 (100)	25 (41.7)	35 (58.3)	
Age (years)				1.000
<65	89 (100)	40 (44.9)	49 (55.1)	
≥65	98 (100)	43 (43.9)	55 (56.1)	
Tumor type				0.078
Adenocarcinoma	93 (100)	34 (36.6)	59 (63.4)	
Squamous cell carcinoma	72 (100)	39 (54.2)	33 (45.8)	
Other types	22 (100)	10 (45.5)	12 (54.5)	
Tumor size				0.141
≤3	91 (100)	35 (38.5)	56 (61.5)	
>3	96 (100)	48 (50.0)	48 (50.0)	
Pleural invasion				0.555
Absent	102 (100)	43 (42.2)	59 (57.8)	
Present	85 (100)	40 (47.1)	45 (52.9)	
Vascular invasion				0.306
Absent	170 (100)	73 (42.9)	97 (57.1)	
Present	17 (100)	10 (58.8)	7 (41.2)	
Lymphatic invasion				0.829
Absent	163 (100)	73 (44.8)	90 (55.2)	
Present	24 (100)	10 (41.7)	14 (58.3)	
Tumor status				0.121
T1	64 (100)	23 (35.9)	41 (64.1)	
T2, T3 and T4	123 (100)	60 (48.8)	63 (51.2)	
Lymph node metastasis				1.000
Absent	133 (100)	59 (44.4)	74 (55.6)	
Present	54 (100)	24 (44.4)	30 (55.6)	
Metastasis				1.000
Absent	181 (100)	80 (44.2)	101 (55.8)	
Present	6 (100)	3 (50.0)	3 (50.0)	
Relapse				0.100
No	112 (100)	44 (39.3)	68 (60.7)	
Yes	75 (100)	39 (52.0)	36 (48.0)	
Pathological stage				0.655
Stage I	108 (100)	46 (42.6)	62 (57.4)	
Stage II, III and IV	79 (100)	37 (46.8)	42 (53.2)	
Smoking				0.259
Non-smoker	72 (100)	37 (51.4)	35 (48.6)	
Smoker	111 (100)	45 (40.5)	66 (59.5)	
No response	4 (100)	1 (25.0)	3 (75.0)	
EGFR mutation				0.616
Absent	42 (100)	15 (35.7)	27 (64.3)	
Present	26 (100)	11 (42.3)	15 (57.7)	

DUB3: Deubiquitinating enzyme 3; EGFR: epidermal growth factor receptor.

Table II. *Correlation between deubiquitinating enzyme 3 (DUB3) expression and clinicopathologic parameters in 93 adenocarcinoma patients.*

Variables	N (%)	DUB3 expression		*p*-Value
		Low expression	*High expression*	
Sex				1.000
Male	42 (100)	15 (35.7)	27 (64.3)	
Female	51 (100)	19 (37.3)	32 (62.7)	
Age (years)				0.830
<65	47 (100)	18 (38.3)	29 (61.7)	
≥65	46 (100)	16 (34.8)	30 (65.2)	
Tumor size				**0.030**
≤3	55 (100)	15 (27.3)	40 (72.7)	
>3	38 (100)	19 (50.0)	19 (50.0)	
Pleural invasion				0.087
Absent	47 (100)	13 (27.7)	34 (72.3)	
Present	46 (100)	21 (45.7)	45 (54.3)	
Vascular invasion				**0.038**
Absent	88 (100)	30 (34.1)	58 (65.9)	
Present	5 (100)	4 (80.0)	1 (20.0)	
Lymphatic invasion				0.250
Absent	83 (100)	32 (38.6)	51 (61.4)	
Present	10 (100)	2 (20.0)	8 (80.0)	
Tumor status				**0.014**
T1	35 (100)	7 (20.0)	28 (80.0)	
T2, T3 and T4	58 (100)	27 (46.6)	31 (53.4)	
Lymph node metastasis				0.809
Absent	68 (100)	24 (35.3)	44 (64.7)	
Present	25 (100)	10 (40.0)	15 (60.0)	
Metastasis				0.568
Absent	89 (100)	32 (36.0)	57 (64.0)	
Present	4 (100)	2 (50.0)	2 (50.0)	
Relapse				**0.002**
No	53 (100)	12 (22.6)	41 (77.4)	
Yes	40 (100)	22 (55.0)	18 (45.0)	
Pathological stage				1.000
Stage I	57 (100)	21 (36.8)	36 (63.2)	
Stage II, III and IV	36 (100)	13 (36.1)	23 (63.9)	
Smoking				0.372
Non-smoker	55 (100)	23 (41.8)	32 (58.2)	
Smoker	37 (100)	11 (29.7)	26 (70.3)	
No response	1 (100)	0 (0.0)	1 (100.0)	
EGFR mutation				0.577
Absent	27 (100)	9 (33.3)	18 (66.7)	
Present	26 (100)	11 (42.3)	15 (57.7)	

DUB3: Deubiquitinating enzyme 3; EGFR: epidermal growth factor receptor. Significant *p*-values are shown in bold.

the tissue microarray blocks and transferred onto adhesive-coated slides. The slides were deparaffinized by heating at 60˚C for one hour and washed three times for 5 min each with xylene. Endogenous peroxidase activity was eliminated with 5% hydrogen peroxide in methanol for 15 min at 37˚C. Antigen retrieval was performed by microwave treatment in an epitope retrieval solution (pH 6.0) for 20 min. The sections were incubated with primary antibodies against DUB3 (1:100, ab188236, Abcam, Cambridge, UK) or LATS1 antibody (1:200, ab111206, Abcam) in a humidified

Table III. *Correlations between deubiquitinating enzyme 3 (DUB3) expression and clinicopathologic parameters in 72 squamous cell carcinoma patients.*

Variables	N (%)	DUB3 expression		*p*-Value
		Low expression	*High expression*	
Sex				0.390
Male	68 (100)	36 (52.9)	32 (47.1)	
Female	4	3 (75.0)	1 (25.0)	
Age (years)				1.000
<65	31	17 (54.8)	14 (45.2)	
≥65	41	22 (53.7)	19 (46.3)	
Tumor size				0.812
≤3	30	17 (56.7)	13 (43.3)	
>3	42	22 (52.4)	20 (47.6)	
Pleural invasion				0.474
Absent	43	25 (58.1)	18 (41.9)	
Present	29	14 (48.3)	15 (51.7)	
Vascular invasion				0.531
Absent	63	35 (55.6)	28 (44.4)	
Present	9	4 (44.4)	5 (55.6)	
Lymphatic invasion				0.762
Absent	60	33 (55.0)	27 (45.0)	
Present	12	6 (50.0)	6 (50.0)	
Tumor status				0.802
T1	24	14 (58.3)	10 (41.7)	
T2, T3 and T4	48	25 (52.1)	23 (47.9)	
Lymph node metastasis				0.225
Absent	47	28 (59.6)	19 (40.4)	
Present	25	11 (44.0)	14 (56.0)	
Metastasis				
Absent	72	39 (54.2)	33 (45.8)	
Present	0	0 (0.0)	0 (0.0)	
Relapse				0.430
No	52	30 (57.7)	22 (42.3)	
Yes	20	9 (45.0)	11 (55.0)	
Pathological stage				1.000
Stage I	38	21 (55.3)	17 (44.7)	
Stage II, III and IV	34	18 (52.9)	16 (47.1)	
Smoking				**0.007**
Non-smoker	12	11 (91.7)	1 (8.3)	
Smoker	58	28 (48.3)	30 (51.7)	
No response	2	0 (0.0)	2 (100.0)	
EGFR mutation				
Absent	9	4 (44.4)	5 (55.6)	
Present	0	0 (0.0)	0 (0.0)	

DUB3: Deubiquitinating enzyme 3; EGFR: epidermal growth factor receptor. Significant *p*-values are shown in bold.

chamber at 4˚C for 16 h. Subsequently, the sections were treated with secondary antibodies in Bond Polymer Refine Detection kit (Leica Biosystems, Wetzlar, Germany). Diaminobenzidine was used as the chromogen.

Immunohistochemical analysis of DUB3 and LATS1. Immunoreactivity of DUB3 and LAT1 was evaluated by two independent pathologists (SH Jang and MH Oh) using a light microscope in a blinded fashion. In the case of disagreement, consensus was obtained through reassessment and discussion by two pathologists with the use of a multi-head microscope. DUB3 expression level was graded by assessing the intensity of cytoplasmic and nuclear staining as follows (28): 0 (negative), 1 (weak staining), 2 (moderate staining in more than half of the tumor cells), and 3 (strong staining in most tumor cells) (Figure 1). We then classified the patients into two groups: low DUB3 expression (grade 0 and 1) and high DUB3 expression (grade 2 and 3). LATS1 immunoreactivity was evaluated semi-quantitatively by a grading system based on tumor cytoplasmic/nuclear staining intensity and proportion, as previously described (26). The intensity score was graded as follows: 0 (negative), 1 (weak staining), 2 (moderate staining), and 3 (strong staining) (Figure 2). The proportion score was graded as 0 (negative), 1 (lower than 10%), 2 (11% to 50%), 3 (51% to 80%), and 4 (higher than 80%). The immunoreactive score of LATS1 was calculated by multiplying the intensity score with the proportion score. A score of 0 to 3 was defined as low LATS1 expression and a score of higher than 4 (including 4) was defined as high LATS1/2 expression.

Table IV. *Univariate analyses of disease-free survival and overall survival in 187 patients with non-small cell lung cancer.*

	Univariate analyses (*p*-Value)	
	Disease-free survival	*Overall survival*
Low DUB3 expression	0.307	0.235

DUB3: Deubiquitinating enzyme 3.

Statistical analysis. All statistical analyses were performed using the SPSS software (SPSS Inc., version 18.0, Chicago, IL, USA). The relationship between DUB3 expression and clinicopathologic variables was evaluated using Fisher's exact test and Pearson's chi-square test. A Kaplan-Meier analysis with a log-rank test was performed to analyze survival data. Multivariate analysis was conducted using the Cox proportional hazards regression method to evaluate the independent prognostic significance of DUB3 expression. To analyze the correlation between DUB3 and LATS1, Pearson's Chi-square test and Spearman correlation coefficient were used. *p*-Value <0.05 was considered statistically significant.

Results

Clinicopathological significance of DUB3 expression in NSCLC. Out of the 187 NSCLC specimens, 104 (55.6%) showed high DUB3 expression. To explore the impact of DUB3 expression, we examined its relationship with clinicopathologic factors. There was no significant difference between DUB3 and all variables among the 187 patients with NSCLC (Table I). Next, we selected patients grouped in two major NSCLC subtypes, adenocarcinoma (93 cases) and squamous cell carcinoma (72 cases), and evaluated the clinicopathologic significance of DUB3 expression in each subtype. In lung adenocarcinoma, 59

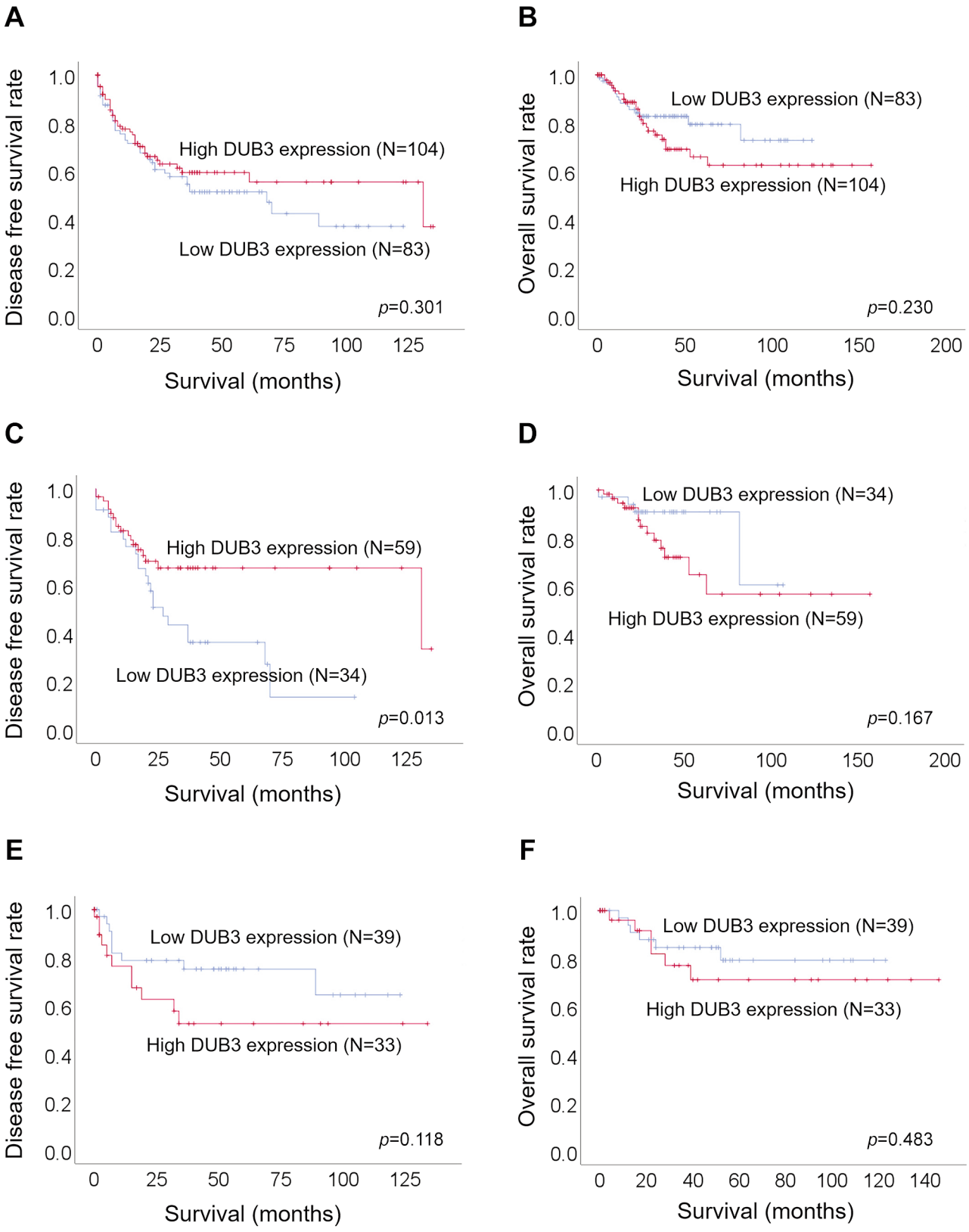

Figure 3. *Kaplan-Meier curves for disease-free survival (DFS) and overall survival (OS) of patients with non-small cell lung cancer (NSCLC) (A, B), adenocarcinoma (C, D) and squamous cell carcinoma (E, F) with low and high deubiquitinating enzyme 3 (DUB3) expression.*

Table V. *Univariate analyses of disease-free survival and overall survival in 93 patients with adenocarcinoma.*

	Univariate analyses (*p*-Value)	Multivariate analyses (*p*-Value)	HR	95%CI
Disease-free survival				
Low DUB3 expression	**0.017**	0.048	0.502	0.253-0.995
Sex (female *vs*. male)	0.394			
Age (≥65 *vs*. <65)	0.735			
Pleural invasion	**0.011**	0.051	2.046	0.997-4.198
Vascular invasion	0.364			
Lymphatic invasion	**0.01**	0.032	2.538	1.085-5.937
Stage (II, III, IV *vs*. I)	**<0.001**	**<0.001**	3.389	1.764-6.509
Smoking	0.621			
EGFR mutation status	0.97			
Overall survival				
Low DUB3 expression	0.177			

DUB3: Deubiquitinating enzyme 3; EGFR: epidermal growth factor receptor. Significant *p*-values are shown in bold; HR: hazard ratio; CI: confidence interval.

(63.4%) out of 93 cases showed high DUB3 expression. Increased DUB3 expression was significantly correlated with favorable prognostic parameters including small tumor size (≤3 cm) (p=0.030), absence of vascular invasion (p=0.038), low tumor status (p=0.014), and no disease recurrence (p=0.002). Although the difference was not significant, high DUB3 expression tended to be associated with the absence of pleural invasion (p=0.087) (Table II). In lung squamous cell carcinoma, high DUB3 expression was more frequently observed in tumors of patients with smoking history (p=0.007). However, there was no correlation between DUB3 expression and the other clinicopathologic factors (Table III).

Correlation between DUB3 expression and disease-free survival (DFS) or overall survival (OS). The impact of DUB3 expression on patient survival was evaluated. In total, for NSCLC patients and squamous cell carcinoma patients, Kaplan-Meier curves with log-rank test revealed that there was no correlation between DUB3 expression level and either DFS or OS (Figure 3A, B, E, and F). In adenocarcinoma patients, the high DUB3 expression group exhibited significantly longer DFS than that of the low DUB3 expression group (Figure 3C) (p=0.013, log-rank test). However, there were no statistical differences in OS between the groups (Figure 3D).

We also performed univariate and multivariate analyses for all NSCLC patients (Table IV) and for those with adenocarcinoma (Table V) and squamous cell carcinoma (Table VI). DUB3 expression, sex, age, tumor size, pleural invasion, vascular invasion, lymphatic invasion, stage, smoking history, and epidermal growth factor receptor mutation status were evaluated. Consistent with the aforementioned results of the Kaplan-Meier curve with log-rank test, univariate analysis showed that DUB3 expression was not correlated with DFS among all NSCLC patients and the squamous cell carcinoma subtype. However, in adenocarcinoma patients, pleural invasion (p=0.011), lymphatic invasion (p=0.010), high pathologic stage (p<0.001), and DUB3 expression (p=0.017) were significantly correlated with worse DFS based on univariate analysis. We conducted a multivariate analysis using a Cox regression hazard model to test whether DUB3 expression could be an independent prognostic factor of disease recurrence in lung adenocarcinoma. We demonstrated that high DUB3 expression is an independent predictive factor for DFS (p=0.048, HR=0.502) (Table V).

Table VI. *Univariate analyses of disease-free survival and overall survival in 72 patients with squamous cell carcinoma.*

	Univariate analyses (*p*-Value)	
	Disease-free survival	*Overall survival*
Low DUB3 expression	0.129	0.486

DUB3: Deubiquitinating enzyme 3.

Correlation between DUB3 and LATS1 expression in lung cancer tissues. Immunohistochemical staining of LATS1 was performed to investigate the correlation between the protein expression of LATS1 and DUB3 (Figure 2). LATS1 expression showed a statistically significant positive correlation with DUB3 expression in 163 NSCLC patients (r=0.268, p=0.001) and in 88 patients with lung adenocarcinoma (r=0.235, p=0.037) (Table VII).

Table VII. *Correlation between deubiquitinating enzyme 3 (DUB3) and large tumor suppressor kinase 1 (LATS1) expression in 158 non-small cell lung cancer (NSCLC) and 88 adenocarcinoma patients.*

	LATS1-low	LATS1-high	Total
NSCLC*			
DUB3-low	50	21	71
DUB3-high	38	49	87
Adenocarcinoma#			
DUB3-low	16	17	33
DUB3-high	14	41	55

*p=0.001, r=0.268; #p=0.037, r=0.235.

Discussion

The ubiquitin-proteasome system (UPS) is an attractive therapeutic target for the treatment of various types of malignancies (29, 30). Some UPS-active agents (bortezomib and lenalidomide) have been developed and are especially effective against hematologic malignancies (31). Moreover, a study was published, demonstrating the effectiveness of UPS targeted therapy in bladder cancer cells that have developed resistance to conventional chemotherapy (32). USPs, which are a main component of the UPS, control the ubiquitination of target proteins and more than 30 USPs linked to cancer have been identified (30). Studies using breast cancer cell lines demonstrated that the oncogenic trait of DUB3 promotes cell cycle progression and regulates cancer metastasis and invasion *via* SNAIL1. In a lung cancer cell line study, Hu *et al*. found that DUB3 acts as a tumor promoter that regulates cell cycle promotion through cyclin A stabilization. On the other hand, experiments with lymphocytes and HeLa cells have revealed the antitumor activity of DUB3 (2, 15). We investigated the role of DUB3 in NSCLC in this study. Since the carcinogenesis of lung adenocarcinoma and squamous cell carcinoma is distinctly distinguished, we separately evaluated and compared the correlation between DUB3 expression and clinicopathological factors in two subtypes of NSCLC. Notably, high DUB3 expression was associated with favorable prognosis in terms of several parameters, including tumor size (p=0.030), vascular invasion (p=0.038), tumor status (p=0.014), and recurrence (p=0.002) in lung adenocarcinoma (Table II). Small tumor size and low T stage might indicate low proliferative capacity, whereas low vascular invasion, low T stage, low recurrence, and low frequency of pleural invasion could reflect reduced invasiveness. However, there was no significant difference between DUB3 expression and clinicopathologic factors when evaluated among all NSCLS cases and among only squamous cell carcinoma patients, except for smoking history (p=0.007) in squamous cell carcinoma.

The Hippo signaling pathway and its downstream effectors, including YAP and TAZ, play important roles in lung cancer development and progression (16, 20, 33, 34). Our previous studies have shown that LATS2 and AMOT p130, which are components of Hippo signaling, are potentially independent prognostic indicators in lung adenocarcinoma and NSCLC (17, 19). Furthermore, Nguyen *et al*. (15) reported that DUB3 regulates the Hippo pathway by stabilizing the protein expression of ITCH, LATS, and AMOT. In this study, we examined the relationship between DUB3 expression and LATS1 expression in NSCLC *via* immunohistochemistry. The results revealed that DUB3 expression exhibited a significant positive correlation with LATS1 expression in both total NSCLC and adenocarcinoma cases. Although the precise mechanism and interaction between DUB3 and the Hippo pathway need to be clarified in follow-up *in vitro* studies, the present study suggests the relevance of these molecules in clinical tissue samples of lung cancer.

Depending on the subtype and cancer type, USPs perform diverse biological functions. Only one report has described the prognostic significance of DUB3 in NSCLC (3). McFarlane *et al*. investigated 100 NSCLC patients (29 adenocarcinoma and 71 squamous cell carcinoma) in their study and reported that high DUB3 expression is associated with unfavorable prognosis, as indicated by increased local recurrence and distant metastasis in NSCLC and adenocarcinoma patients. For our research, we included more cases than those in previous studies to evaluate prognosis (93 adenocarcinoma and 72 squamous cell carcinoma). Through univariate and multivariate analyses, the possibility of DUB3 as an independent prognostic factor was delicately evaluated in lung adenocarcinoma, squamous cell carcinoma, and NSCLC in general. The results indicated that high DUB3 expression is associated with good prognosis in lung adenocarcinoma. The study of McFarlane *et al*. included a limited number of samples with squamous cell carcinoma being the predominant subtype (3). In our study, squamous cell carcinoma demonstrated a similar outcome to McFarlane *et al*.'s study, indicating a tendency towards a poor prognosis associated with increased DUB3 expression, albeit not statistically significant (Figure 3E and F). Conversely, increased DUB3 expression in adenocarcinoma was associated with a favorable prognosis (Figure 3C and D). In our study, more cases of adenocarcinoma were examined compared to the study of McFarlane *et al*., enabling a more comprehensive and precise evaluation of clinical, pathological, and prognostic factors.

Considering the relationship between DUB3 expression and clinicopathological factors, and between DUB3 and LATS1 expression, we propose that high DUB3 expression correlates with better clinical outcomes in lung adenocarcinoma.

Conclusion

In conclusion, we investigated the expression and clinicopathological significance of DUB3 in 187 NSCLC patients. High DUB3 expression is closely related to favorable clinicopathologic phenotypes and better prognosis in lung adenocarcinoma. In addition, we showed by immunohistochemical analysis that DUB3 expression is positively correlated with LATS1 expression. These findings indicate that DUB3 is a reliable predictor of disease recurrence and a potential therapeutic target for treatment of lung adenocarcinoma.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contribution

Conceptualization: Si-Hyong Jang, Mee-Hye Oh; Data curation: Hyun Deuk Cho; Validation: Mee-Hye Oh; Visualization: Ji-Hye Lee; Writing – original draft: Si-Hyong Jang.

Acknowledgements

This work was supported, in part, by the Soonchunhyang University Research Fund and National Research Foundation of Korea (NRF-2017R1C1B5075932).

References

1 Zhou B, Shu B, Xi T, Su N, Liu J: Dub3 expression correlates with tumor progression and poor prognosis in human epithelial ovarian cancer. Biomed Pharmacother 70: 84-89, 2015. DOI: 10.1016/j.biopha.2015.01.015

2 Burrows JF, Mcgrattan MJ, Rascle A, Humbert M, Baek K, Johnston JA: DUB-3, a cytokine-inducible deubiquitinating enzyme that blocks proliferation. J Biol Chem 279(14): 13993-14000, 2004. DOI: 10.1074/jbc.M311291200

3 McFarlane C, McFarlane S, Paul I, Arthur K, Scheaff M, Kerr K, Stevenson M, Fennell DA, Johnston JA: The deubiquitinating enzyme USP17 is associated with non-small cell lung cancer (NSCLC) recurrence and metastasis. Oncotarget 4(10): 1836-1843, 2013. DOI: 10.18632/oncotarget.1282

4 Mehić M, de Sa VK, Hebestreit S, Heldin CH, Heldin P: The deubiquitinating enzymes USP4 and USP17 target hyaluronan synthase 2 and differentially affect its function. Oncogenesis 6(6): e348, 2017. DOI: 10.1038/oncsis.2017.45

5 Braten O, Livneh I, Ziv T, Admon A, Kehat I, Caspi LH, Gonen H, Bercovich B, Godzik A, Jahandideh S, Jaroszewski L, Sommer T, Kwon YT, Guharoy M, Tompa P, Ciechanover A: Numerous proteins with unique characteristics are degraded by the 26S proteasome following monoubiquitination. Proc Natl Acad Sci U.S.A. 113(32): E4639-E4647, 2016. DOI: 10.1073/pnas.1608644113

6 Komander D, Clague MJ, Urbé S: Breaking the chains: structure and function of the deubiquitinases. Nat Rev Mol Cell Biol 10(8): 550-563, 2009. DOI: 10.1038/nrm2731

7 Nijman SM, Luna-Vargas MP, Velds A, Brummelkamp TR, Dirac AM, Sixma TK, Bernards R: A genomic and functional inventory of deubiquitinating enzymes. Cell 123(5): 773-786, 2005. DOI: 10.1016/j.cell.2005.11.007

8 Pereg Y, Liu BY, O'Rourke KM, Sagolla M, Dey A, Komuves L, French DM, Dixit VM: Ubiquitin hydrolase Dub3 promotes oncogenic transformation by stabilizing Cdc25A. Nat Cell Biol 12(4): 400-406, 2010. DOI: 10.1038/ncb2041

9 de la Vega M, Kelvin AA, Dunican DJ, McFarlane C, Burrows JF, Jaworski J, Stevenson NJ, Dib K, Rappoport JZ, Scott CJ, Long A, Johnston JA: The deubiquitinating enzyme USP17 is essential for GTPase subcellular localization and cell motility. Nat Commun 2: 259, 2011. DOI: 10.1038/ncomms1243

10 Ramakrishna S, Suresh B, Lee EJ, Lee HJ, Ahn WS, Baek KH: Lys-63-specific deubiquitination of SDS3 by USP17 regulates HDAC activity. J Biol Chem 286(12): 10505-10514, 2011. DOI: 10.1074/jbc.M110.162321

11 Vucic D, Dixit VM, Wertz IE: Ubiquitylation in apoptosis: a post-translational modification at the edge of life and death. Nat Rev Mol Cell Biol 12(7): 439-452, 2011. DOI: 10.1038/nrm3143

12 McFarlane C, Kelvin AA, De La Vega M, Govender U, Scott CJ, Burrows JF, Johnston JA: The deubiquitinating enzyme USP17 is highly expressed in tumor biopsies, is cell cycle regulated, and is required for G1-S progression. Cancer Res 70(8): 3329-3339, 2010. DOI: 10.1158/0008-5472.Can-09-4152

13 Liu T, Yu J, Deng M, Yin Y, Zhang H, Luo K, Qin B, Li Y, Wu C, Ren T, Han Y, Yin P, Kim J, Lee S, Lin J, Zhang L, Zhang J, Nowsheen S, Wang L, Boughey J, Goetz MP, Yuan J, Lou Z: CDK4/6-dependent activation of DUB3 regulates cancer metastasis through SNAIL1. Nat Commun 8: 13923, 2017. DOI: 10.1038/ncomms13923

14 Wu Y, Wang Y, Lin Y, Liu Y, Wang Y, Jia J, Singh P, Chi YI, Wang C, Dong C, Li W, Tao M, Napier D, Shi Q, Deng J, Evers BM, Zhou BP: Dub3 inhibition suppresses breast cancer invasion and metastasis by promoting Snail1 degradation. Nat Commun 8: 14228, 2017. DOI: 10.1038/ncomms14228

15 Nguyen HT, Kugler JM, Cohen SM: DUB3 deubiquitylating enzymes regulate hippo pathway activity by regulating the stability of ITCH, LATS and AMOT proteins. PLoS One 12(1): e0169587, 2017. DOI: 10.1371/journal.pone.0169587

16 Janse Van Rensburg HJ, Azad T, Ling M, Hao Y, Snetsinger B, Khanal P, Minassian LM, Graham CH, Rauh MJ, Yang X: The Hippo pathway component TAZ promotes immune evasion in human cancer through PD-L1. Cancer Res 78(6): 1457-1470, 2018. DOI: 10.1158/0008-5472.Can-17-3139

17 Jang S, Oh M, Cho H, Lee J, Lee H, Ahn H, Kim H: Low LATS2 expression is associated with poor prognosis in non-small cell lung carcinoma. Pol J Pathol 70(3): 189-197, 2019. DOI: 10.5114/pjp.2019.90395

18 Yu FX, Guan KL: The Hippo pathway: regulators and regulations. Genes Dev 27(4): 355-371, 2013. DOI: 10.1101/gad.210773.112

19 Jang S, Cho HD, Lee J, Lee HJ, Hong SA, Cho J, Kim HJ, Oh M: Reduced angiomotin p130 expression correlates with poor prognosis in lung adenocarcinoma. J Clin Pathol 70(7): 625-630, 2017. DOI: 10.1136/jclinpath-2016-204071

20 Hong SA, Son MW, Cho J, Jang S, Lee HJ, Lee J, Cho HD, Oh M, Lee MS: Low angiomotin-p130 with concomitant high Yes-associated protein 1 expression is associated with adverse prognosis of advanced gastric cancer. APMIS 125(11): 996-1006, 2017. DOI: 10.1111/apm.12750

21 Gomez M, Gomez V, Hergovich A: The Hippo pathway in disease and therapy: cancer and beyond. Clin Transl Med 3: 22, 2014. DOI: 10.1186/2001-1326-3-22

22 Ehmer U, Sage J: Control of proliferation and cancer growth by the Hippo signaling pathway. Mol Cancer Res 14(2): 127-140, 2016. DOI: 10.1158/1541-7786.MCR-15-0305

23 Steinhardt AA, Gayyed MF, Klein AP, Dong J, Maitra A, Pan D, Montgomery EA, Anders RA: Expression of Yes-associated protein in common solid tumors. Hum Pathol 39(11): 1582-1589, 2008. DOI: 10.1016/j.humpath.2008.04.012

24 Wang Y, Dong Q, Zhang Q, Li Z, Wang E, Qiu X: Overexpression of yes-associated protein contributes to progression and poor prognosis of non-small-cell lung cancer. Cancer Sci 101(5): 1279-1285, 2010. DOI: 10.1111/j.1349-7006.2010.01511.x

25 Takahashi Y, Miyoshi Y, Takahata C, Irahara N, Taguchi T, Tamaki Y, Noguchi S: Down-regulation of *LATS1* and *LATS2* mRNA expression by promoter hypermethylation and its association with biologically aggressive phenotype in human breast cancers. Clin Cancer Res 11(4): 1380-1385, 2005. DOI: 10.1158/1078-0432.Ccr-04-1773

26 Xu B, Sun D, Wang Z, Weng H, Wu D, Zhang X, Zhou Y, Hu W: Expression of LATS family proteins in ovarian tumors and its significance. Hum Pathol 46(6): 858-867, 2015. DOI: 10.1016/j.humpath.2015.02.012

27 Song C, Liu W, Li J: USP17 is upregulated in osteosarcoma and promotes cell proliferation, metastasis, and epithelial–mesenchymal transition through stabilizing SMAD4. Tumour Biol 39(7): 101042831771713, 2017. DOI: 10.1177/1010428317717138

28 Hu B, Deng T, Ma H, Liu Y, Feng P, Wei D, Ling N, Li L, Qiu S, Zhang L, Peng B, Liu J, Ye M: Deubiquitinase DUB3 regulates cell cycle progression *via* stabilizing cyclin a for proliferation of non-small cell lung cancer cells. Cells 8(4): 297, 2019. DOI: 10.3390/cells8040297

29 Pal A, Donato NJ: Ubiquitin-specific proteases as therapeutic targets for the treatment of breast cancer. Breast Cancer Res 16(5): 461, 2014. DOI: 10.1186/s13058-014-0461-3

30 Pal A, Young MA, Donato NJ: Emerging potential of therapeutic targeting of ubiquitin-specific proteases in the treatment of cancer. Cancer Res 74(18): 4955-4966, 2014. DOI: 10.1158/0008-5472.Can-14-1211

31 Chen D, Frezza M, Schmitt S, Kanwar J, Dou QP: Bortezomib as the first proteasome inhibitor anticancer drug: current status and future perspectives. Curr Cancer Drug Targets 11(3): 239-253, 2011. DOI: 10.2174/156800911794519752

32 Okubo K, Isono M, Asano T, Reßing N, Schulz WA, Hansen FK, Sato A: Ubiquitin-proteasome system is a promising target for killing cisplatin-resistant bladder cancer cells. Anticancer Res 41(6): 2901-2912, 2021. DOI: 10.21873/anticanres.15072

33 Hong SA, Jang S, Oh M, Kim SJ, Kang J, Hong S: Overexpression of YAP1 in EGFR mutant lung adenocarcinoma prior to tyrosine kinase inhibitor therapy is associated with poor survival. Pathol Res Pract 214(3): 335-342, 2018. DOI: 10.1016/j.prp.2018.01.010

34 Yu M, Chen Y, Li X, Yang R, Zhang L, Huangfu L, Zheng N, Zhao X, Lv L, Hong Y, Liang H, Shan H: YAP1 contributes to NSCLC invasion and migration by promoting Slug transcription *via* the transcription co-factor TEAD. Cell Death Dis 9(5): 464, 2018. DOI: 10.1038/s41419-018-0515-z

Received May 29, 2023
Revised July 1, 2023
Accepted July 4, 2023