Distinct expression and prognostic value of OTU domain-containing proteins in non-small-cell lung cancer

JINGJING DENG[1], GOUXIN HOU[2], ZHIXIAN FANG[1], JIALIANG LIU[1] and XIAO-DONG LV[1]

[1]Department of Respiration, Key Medical Discipline of Jiaxing, Jiaxing Lung Cancer Innovation Team; [2]Department of Oncology, The First Hospital of Jiaxing, The First Affiliated Hospital of Jiaxing University, Jiaxing, Zhejiang 314000, P.R. China

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Abstract. The ubiquitin-proteasome pathway is an important protein degradation regulatory system in cells. This pathway is also a reversible process that is strictly regulated, and the regulation of deubiquitinating enzymes (DUBs) represents an important facet of the process. Ovarian tumor-associated proteases domain-containing proteins (OTUDs), as a subfamily within the DUB family, serve an important role in regulatory mechanisms of several biological processes, through the regulation of gene transcription, cell cycle, immune response, inflammation and tumor growth processes, and may be important in the diagnosis of various diseases and constitute novel drug targets. However, the role of OTUDs in non-small-cell lung cancer (NSCLC) has not been fully elucidated. In the present study, the Oncomine database was used to examine gene expression in NSCLC, and the prognostic value of each gene was analyzed by Kaplan-Meier analysis. The results indicated that high mRNA expression levels of OTUD1, OTUD3, OTUD4 and putative bifunctional UDP-N-acetylglucosamine transferase and deubiquitinase ALG13 were associated with improved prognosis in all NSCLC and adenocarcinoma, but not in squamous cell carcinoma. By contrast, high expression levels of OTUD2 mRNA were associated with poorer overall survival in patients with NSCLC. These data suggested that these OTUD isozymes may be a potential drug target for NSCLC.

Introduction

Lung cancer, as a major cause of human mortality, is considered one of the most common types of cancer. Every year, 1.6 million individuals succumb to lung cancer, accounting for one-third of all cancer-associated mortalities. It is the most common cause of cancer-associated mortalities in males and the second most common in females (1,2). Non-small-cell lung cancer (NSCLC) accounts for 85% of all cases of lung cancer; within this group, adenocarcinoma (Ade) and squamous cell carcinoma (SCC) are the major histopathological types (3). Once diagnosed with lung cancer, the 5-year survival rate of patients is only 17.8% (4,5). In the previous decade, although promising treatments have emerged, for the majority of patients with distant metastases, surgery is not a viable option and there are no radical treatments available (6,7). Therefore, it is important to explore the potential mechanism of tumorigenesis and tumor progression in NSCLC, and to identify underlying biomarkers for prognosis that may be targeted by lung cancer-specific chemotherapeutic drugs. Furthermore, the results of these investigations may be utilized for early personalized methods, individualized precise treatment strategies and improved survival cycles in patients with lung cancer.

Ubiquitylation is a widespread post-translational modification of proteins that occurs in eukaryotic cells, and constitutes an important topic of research in the post-genome era. This type of modification is considered an important complement to the regulation of the genetic central dogma (8). Ubiquitylation is achieved by the generation of an isopeptide bond, which is formed by covalent bonding between the C-terminal carboxyl groups of ubiquitin in the lysine side chain of the substrate protein. The ubiquitination level of proteins in eukaryotic cells is regulated by the E1-E2-E3 enzyme synthesis system and the deubiquitinating enzymes (DUBs) system (9). Ubiquitinating enzymes and DUBs function together to form a unique ubiquitin network through multi-level modification of the substrate (including monoubiquitin, 8 ubiquitin chains, mixed ubiquitin chains and bifurcated ubiquitin chains), to regulate cellular processes. In contrast to the ubiquitination process, DUBs modulate ubiquitin-associated processes by reverse-modifying monoubiquitin and ubiquitin chain modifications on substrate proteins (10,11).

Ubiquitination modification is involved in protein degradation, autophagy, DNA damage repair, cell cycle, signal transduction, gene expression, inflammation, immunity and other vital life processes (12,13). The dysfunction of ubiquitinating enzymes is associated with a variety of severe diseases,

Correspondence to: Dr Xiao-Dong Lv, Department of Respiration, Key Medical Discipline of Jiaxing, Jiaxing Lung Cancer Innovation Team, The First Hospital of Jiaxing, The First Affiliated Hospital of Jiaxing University, 1882 South Zhonghuan Road, Jiaxing, Zhejiang 314000, P.R. China
E-mail: 287473706@qq.com

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including cancer, and cardiovascular and neurodegenerative diseases (14-17). In particular, in malignant tumors, previous studies have demonstrated that several types of DUBs are involved in the development and progression of cancer (18), including follicular lymphoma (19), and prostate (20), colon and breast cancer (21).

A total of ~90 DUBs are encoded in the human genome (22). Ovarian tumor-associated proteases (OTUs) are a subtype of DUBs that may be classified into 4 subfamilies according to sequence similarities. OTU domain-containing proteins (OTUDs) are a subfamily of OTUs comprising eight members: OTUD1; OTUD2; OTUD3; OTUD4; OTUD5/DUBA; OTUD6A; OTUD6B; and putative bifunctional UDP-N-acetylglucosamine transferase and deubiquitinase ALG13 (ALG13) (23).

Previous studies have focused on the function of OTUDs in tumor development, invasion and metastasis; numerous studies have demonstrated that OTUD1 serves a key role in the metastasis of thyroid and breast cancer (24-26). In addition, OTUD3 (27), OTUD4 (28), OTUD5 (29) and OTUD6B (30) also serve roles in the pathogenesis of tumors. However, the knowledge concerning the roles of different OTUDs in the development of lung cancer is limited. Therefore, the present study explored large sample-based databases to determine the expression and prognostic value of each isoenzyme of the OTUD subfamily in NSCLC.

Materials and methods

Oncomine analysis. Data entries from February 2018 to August 2018 in the Oncomine database (http://www.oncomine.org/) (31,32) were searched to determine the individual mRNA expression levels of the OTUD subfamily in different cancer types. The Oncomine 4.5 Research Edition is a web-based data mining database with cancer microarray information, aimed at promoting the expression of whole genome analysis and comparative transcriptome data analysis for the major types of cancer and in normal tissues. It currently contains 715 datasets and 86,733 samples. The present study compared the mRNA levels in datasets of patients with NSCLC and normal individuals. P=0.05, fold-change value 'all' and the top 10% gene rank were selected as thresholds to obtain the highest number of genes in the datasets.

Kaplan-Meier survival analysis. The prognostic values of OTUD sub-members (OTUD1, OTUD2, OTUD3, OTUD4, OTUD5, OTUD6B and ALG13) specifically expressed in NSCLC samples were evaluated by overall survival (OS) using the Kaplan-Meier plotter resource (33-35). Hazard ratios (HR) with 95% confidence intervals (CIs) and log-rank P-values were calculated subsequently. To evaluate the prognostic value of each member, the patient samples were divided into two groups (high vs. low expression group) based on the median gene expression value. Subsequently, GraphPad Prism 7 software (GraphPad Software, Inc.) was used to produce Kaplan-Meier survival curves according to these setting conditions. The Affymetrix identity of each gene in NSCLC was validated and summarized in Table I. In the present study, the 'array quality control' option was selected to 'exclude biased arrays' and the results of the figures were obtained by multivariate Cox regression analysis.

Table I. Desired Affymetrix ID of OTUD family genes in the Kaplan-Meier plotter resource.

OTUD	Affymetrix ID
OTUD1	226140_s_at
OTUD2	215150_at
OTUD3	213216_at
OTUD4	203480_s_at
OTUD5	233933_s_at
OTUD6B	222825_at
ALG13	205583_s_at

OTUD, OTU domain-containing proteins; ALG13, putative bifunctional UDP-N-acetylglucosamine transferase and deubiquitinase ALG13.

Results

Basic characteristics of 8 OTUD isoenzymes. To date, 8 OTUD isoenzymes have been identified in the human genome. Their characteristics are associated with protein data bank identification [RCSB Protein Data Bank (https://www.rcsb.org)], physiological processes and various types of cancer (23,27-30,36,37), as demonstrated in Table II.

Different expression of the OTUD subfamily in NSCLC. Analysis of the Oncomine database revealed the patterns of OTUD family genes expression in tissues from patients with NSCLC compared with normal tissues; the data are summarized in Table III. The analysis demonstrated that the mRNA expression levels of these OTUD subfamily members were different significantly over-expression or under-expression in patients with NSCLC compared with normal samples in different datasets.

Distinct prognostic value of the OTUD subfamily in NSCLC. The prognostic value of the mRNA expression of OTUDs was examined with Kaplan-Meier plotter (Kaplan Meier-plotter [Lung Cancer] 2015 version). Among all the OTUD subfamily members, only OTUD6A was not detected. The datasets are presented in Table IV. Firstly, the prognostic value of OTUD1 mRNA expression was determined. Survival curves were plotted for all patients with NSCLC (Fig. 1A), Ade (Fig. 1B) and SCC (Fig. 1C). High expression of OTUD1 was associated with significantly increased OS in all NSCLC (HR=0.60; CI, 0.51-0.71; $P=1.5x10^{-9}$) and Ade cases (HR=0.56; CI, 0.44-0.72; $P=3.6x10^{-6}$), but not in patients with SCC (HR=0.78; CI, 0.57-1.06; P=0.12).

Then, the prognostic value of OTUD2 mRNA expression was analyzed. Increased expression levels of OTUD2 mRNA were associated with decreased OS in patients with NSCLC (HR=1.27; CI, 1.12-1.45; P=0.00017; Fig. 2A). However, high expression of OTUD2 mRNA was not associated with OS in patients with Ade (HR=1.13; CI, 0.90-1.43; P=0.29; Fig. 2B) or SCC (HR=0.88; CI, 0.69-1.11; P=0.27; Fig. 2C).

As indicated in Fig. 3, the prognostic significance of OTUD3 expression was also evaluated. Increased expression

Table II. Basic characteristics of 8 OTUD isoenzymes.

Isoenzyme	PDB-ID	Physiological process	Associated diseases
OTUD1	4bop	N/A	Thyroid cancer
OTUD2	4bop	Endoplasmic reticulum degradation	Cervical cancer
OTUD3	4bop	PI3K/AKT	Breast cancer
OTUD4	N/A	DNA alkylation Damage repair	N/A
OTUD5	3pfy	p53	N/A
OTUD6A	N/A	N/A	N/A
OTUD6B	N/A	B-cells within the lymphatic system	NSCLC
ALG13	N/A	N/A	N/A

OTUD, OTU domain-containing proteins; ALG13, putative bifunctional UDP-N-acetylglucosamine transferase and deubiquitinase ALG13; PDB, protein data bank; N/A, not available; NSCLC, non-small cell lung cancer.

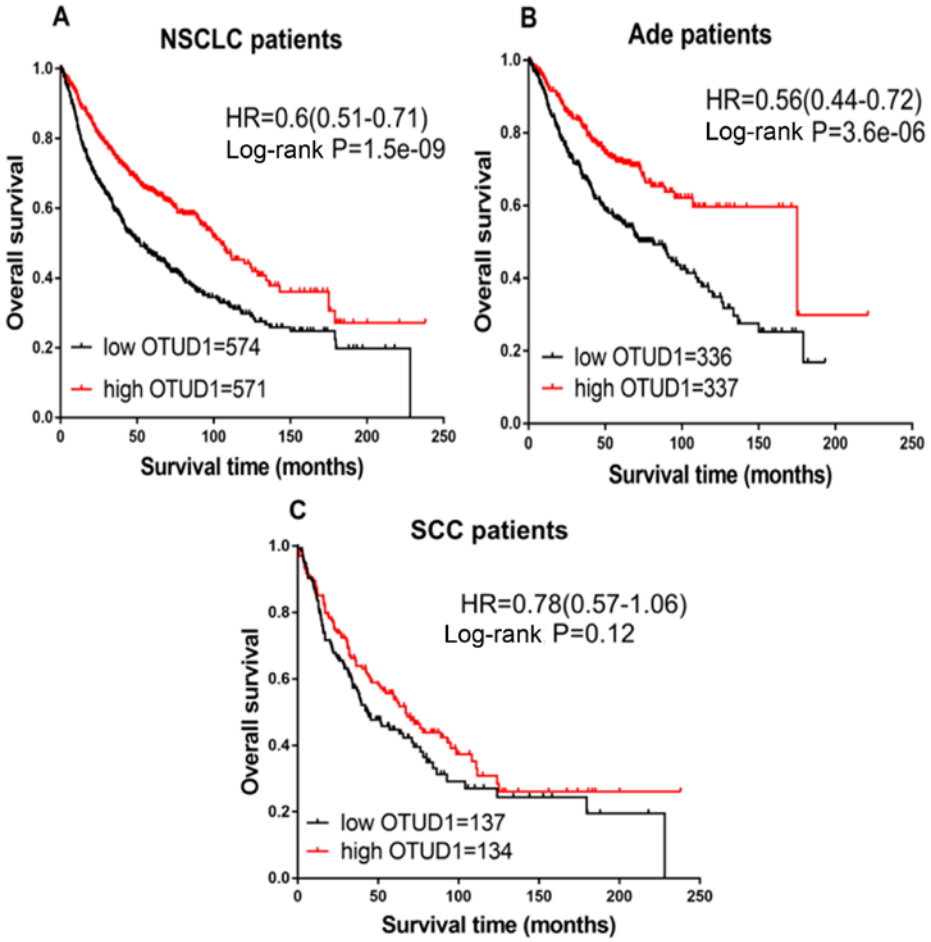

Figure 1. Prognostic value of OTUD1 mRNA expression. (A) Survival curves were plotted for all patients with NSCLC (n=1,145). (B) Survival curves were plotted for patients with Ade (n=673). (C) Survival curves were plotted for patients with SCC (n=271). Data was analyzed using Kaplan-Meier analysis. Patients with expression levels above the median are indicated in red, and patients with expression levels below the median are represented by the black line. OTUD1; NSCLC, non-small cell lung cancer; Ade, adenocarcinoma; SCC, squamous cell carcinoma; HR, hazard ratio.

of OTUD3 mRNA was associated with good OS in all patients with NSCLC (HR=0.84; CI, 0.74-0.96; P=0.0091; Fig. 3A) and Ade (HR=0.65; CI, 0.51-0.82; P=0.00035; Fig. 3B), but not with SCC (HR=0.93; CI, 0.73-1.18; P=0.55; Fig. 3C).

For OTUD4, high mRNA expression was associated with favorable OS in all patients with NSCLC (HR=0.78; CI, 0.69-0.88; P=9.6x10^{-5}; (Fig. 4A) and Ade (HR=0.47; CI, 0.37-0.60; P=6.6x10^{-10}; Fig. 4B), but not with SCC (HR=1.01; CI, 0.80-1.28; P=0.94; Fig. 4C).

Fig. 5 demonstrates the prognostic effect of OTUD5 mRNA expression. High or low expression of OTUD5 did not elicit an effect on the prognosis of patients with NSCLC (HR=1.02; CI,

Table III. OTUD family genes expression in lung cancer (Oncomine database).

A, Lung adenocarcinoma vs. normal

			Sample number			
Gene	Fold change	Dataset	Normal	Cancer	Total	P-value
OTUD1	-2.678	Hou *et al*	65	45	110	$2.79x10^{-19}$
	-3.761	Garber *et al*	5	40	45	$2.99x10^{-4}$
OTUD2	1.161	TCGA	390	261	651	$6.30x10^{-43}$
	1.093	Weiss *et al*	59	77	136	$3.73x10^{-11}$
OTUD5	1.302	Hou *et al*	65	45	110	$3.15x10^{-7}$
OTUD6B	1.135	TGCA	390	261	651	$1.72x10^{-24}$
	1.108	Weiss *et al*	59	77	136	$6.99x10^{-10}$
ALG13	-1.465	Yamagata *et al*	3	9	12	0.002

B, Squamous cell lung carcinoma vs. normal

			Sample number			
Gene	Fold change	Dataset	Normal	Cancer	Total	P-value
OTUD1	-2.921	Hou *et al*	65	27	92	$4.39x10^{-16}$
	-3,206	Garber *et al*	5	12	17	$4.55x10^{-4}$
OTUD2	1.109	TGCA	390	348	738	$5.77x10^{-14}$
OTUD4	-1.350	Yamagata *et al*	3	11	14	0.007
	-1.128	TGCA	390	348	738	$1.83x10^{-40}$
	-1.078	Weiss *et al*	59	155	214	$3.18x10^{-15}$
OTUD6B	1.100	Weiss *et al*	59	155	214	$1.36x10^{-16}$
ALG13	-1.672	Yamagata *et al*	3	11	14	$2.23x10^{-5}$

C, Large cell lung carcinoma vs. normal

			Sample number			
Gene	Fold change	Dataset	Normal	Cancer	Total	P-value
OTUD1	-4.192	Hou *et al*	65	19	84	$1.57x10^{-13}$
ALG13	-1.548	Yamagata *et al*	3	5	8	0.004

D, Small cell lung carcinoma vs. normal

			Sample number			
Gene	Fold change	Dataset	Normal	Cancer	Total	P-value
OTUD1	-3.625	Garber *et al*	5	4	9	0.002
OTUD3	2.205	Bhattacharjee *et al*	17	6	23	0.005

OTUD, OTU domain-containing proteins; ALG13, putative bifunctional UDP-N-acetylglucosamine transferase and deubiquitinase ALG13; TCGA, The Cancer Genome Atlas (https://www.cancer.gov/). Different subtypes of lung cancer were identified with the following thresholds: P=0.05, fold change value 'all', gene rank: Top 10%.

0.87-1.20; P=0.81; Fig. 5A), Ade (HR=1.22; CI, 0.96-1.55; P=0.11; Fig. 5B) or SCC (HR=1.09; CI, 0.80-1.49; P=0.59; Fig. 5C).

Next, the prognostic value of OTUD6B expression was examined. In patients with NSCLC, no significant differences

Table IV. Association between OTUD isoforms and pathology subtype in patients with NSCLC.

Gene	Pathology subtype	Expression group (n)		HR (95% CI)	P-value
		Low	High		
OTUD1	NSCLC	574	571	0.60 (0.51-0.71)	$1.5x10^{-9}$
	Ade	336	337	0.56 (0.44-0.72)	$3.6x10^{-6}$
	SCC	137	134	0.78 (0.57-1.06)	0.120
OTUD2	NSCLC	966	960	1.27 (1.12-1.45)	$1.7x10^{-4}$
	Ade	366	354	1.13 (0.9-1.43)	0.290
	SCC	263	261	0.88 (0.69-1.11)	0.270
OTUD3	NSCLC	964	962	0.84 (0.74-0.96)	0.009
	Ade	361	359	0.65 (0.51-0.82)	$3.5x10^{-4}$
	SCC	262	262	0.93 (0.73-1.18)	0.550
OTUD4	NSCLC	963	963	0.78 (0.69-0.88)	$9.6x10^{-5}$
	Ade	360	360	0.47 (0.37-0.6)	$6.6x10^{-10}$
	SCC	263	261	1.01 (0.8-1.28)	0.940
OTUD5	NSCLC	578	567	1.02 (0.87-1.2)	0.810
	Ade	339	334	1.22(0.96-1.55)	0.110
	SCC	136	135	1.09 (0.8-1.49)	0.590
OTUD6B	All	574	571	1.05 (0.89-1.24)	0.560
	Ade	336	337	0.62 (0.48-0.79)	$9.2x10^{-5}$
	SCC	136	135	1.41 (1.03-1.93)	0.029
ALG13	All	965	961	0.76 (0.67- 0.87)	$3.2x10^{-5}$
	Ade	360	360	0.47 (0.37-0.6)	$7.2x10^{-10}$
	SCC	262	262	0.8 (0.63-1.02)	0.068

P-values were calculated using the log-rank test. OTUD, OTU domain-containing proteins; ALG13, putative bifunctional UDP-N-acetylglucosamine transferase and deubiquitinase ALG13; NSCLC, non-small-cell lung cancer; Ade, adenocarcinoma; SCC, squamous cell carcinomas; HR, hazard ratio; CI, confidence interval.

in prognoses were observed between the high or low OTUD6B expression groups (HR=1.05; CI, 0.89-1.24; P=0.56; Fig. 6A). However, the increased transcriptional expression of OTUD5 was associated with favorable OS in patients with Ade (HR=0.62; CI, 0.48-0.79; $P=9.2x10^{-5}$; Fig. 6B), while patients with SCC exhibited worse OS (HR=1.41; CI, 1.03-1.93; P=0.029; Fig. 6C).

Finally, the prognostic effect of the expression of ALG13 was explored. The prognoses in the high and low ALG13 expression groups were different in patients with NSCLC (HR=0.76; CI, 0.67-0.87; $P=3.2x10^{-5}$; Fig. 7A) and Ade (HR=0.47; CI, 0.37-0.60; $P=7.2x10^{-10}$; Fig. 7B). Patients with high expression of ALG13 exhibited longer OS. However, no difference was observed in patients with SCC (HR=0.80; CI, 0.63-1.02; P=0.068; Fig. 7C).

The associations between OTUDs and clinicopathological features in the NSCLC patients, including tumor stages (NCCN Non-Small Cell Lung Cancer, Version 5.2017) (38), lymph node status (NCCN Non-Small Cell Lung Cancer, Version 5.2017), smoking status, sex and chemotherapy, were also examined. Tumor stage, lymph node status and chemotherapy were not demonstrated to be associated with OTUD expression (data not shown). As demonstrated in Table SI, OTUD1 expression was identified to be associated with a significantly poorer OS in all patients with stage I and II NSCLC, while high expression of OTUD5 was associated with significantly improved OS in patients with stage II NSCLC. As indicated in Table SII, OTUD1, OTUD6B and ALG13 were significantly associated with lymph node status of patients with NSCLC. All OTUDs, with the exception of OTUD3 and OTUD5, were significantly associated with smoking status of NSCLC patients (Table SIII). Meanwhile, all OTUDs with the exception of OTUD5 and OTUD6B were significantly associated with gender of NSCLC patients (Table SIV). Only OTUD3 expression was significantly associated with chemotherapy treatment in patients with NSCLC (Table SV).

Discussion

Among the cancer markers identified to date, OTUDs have been extensively studied. However, few studies have analyzed the expression of OTUDs in lung cancer, in particular the different OTUD isoforms. Therefore, to the best of knowledge, the present study was the first to analyze and discuss the different roles of OTUD isoenzymes in the prognosis of NSCLC.

Screening of the Oncomine database identified that OTUD1, OTUD2, OTUD6B and ALG13 met the filter criteria of the present study. The analysis revealed that different OTUD subfamily mRNA expression levels were significantly different

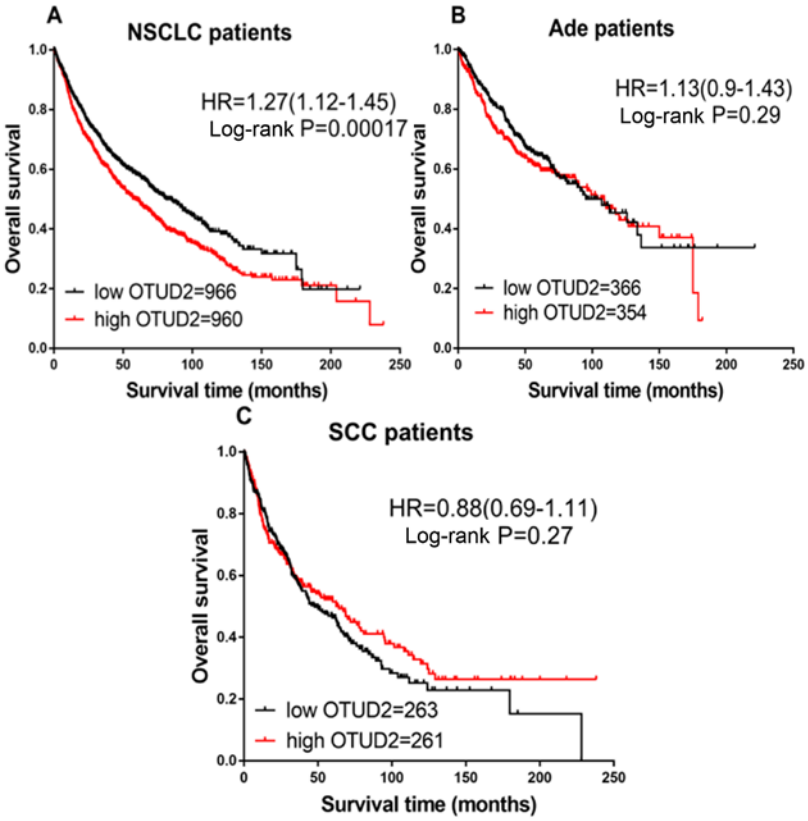

Figure 2. Prognostic values of OTUD2 mRNA expression. (A) Survival curves were plotted for all patients with NSCLC (n=1,926). (B) Survival curves were plotted for patients with Ade (n=720). (C) Survival curves were plotted for patients with SCC (n=524). Data was analyzed using Kaplan-Meier analysis. Patients with expression levels above the median are indicated in red, and patients with expression levels below the median are represented by the black line. OTUD2; NSCLC, non-small cell lung cancer; Ade, adenocarcinoma; SCC, squamous cell carcinoma; HR, hazard ratio.

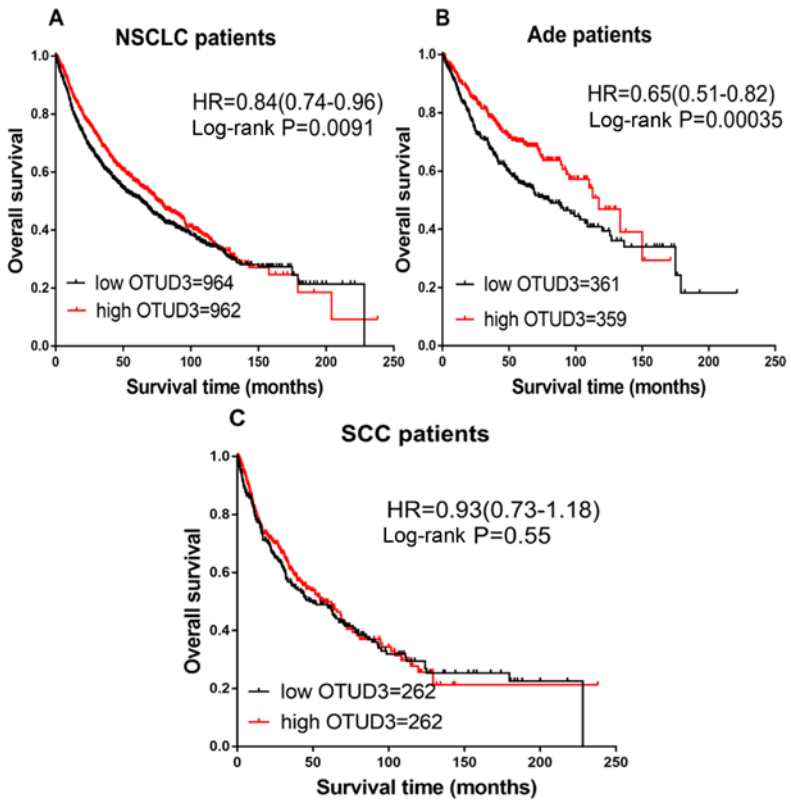

Figure 3. Prognostic value of OTUD3 mRNA expression. (A) Survival curves were plotted for all patients with NSCLC (n=1,926). (B) Survival curves were plotted for patients with Ade (n=720). (C) Survival curves were plotted for patients with SCC (n=524). Data was analyzed using Kaplan-Meier analysis. Patients with expression levels above the median are indicated in red, and patients with expression levels below the median are represented by the black line. OTUD3; NSCLC, non-small cell lung cancer; Ade, adenocarcinoma; SCC, squamous cell carcinoma; HR, hazard ratio.

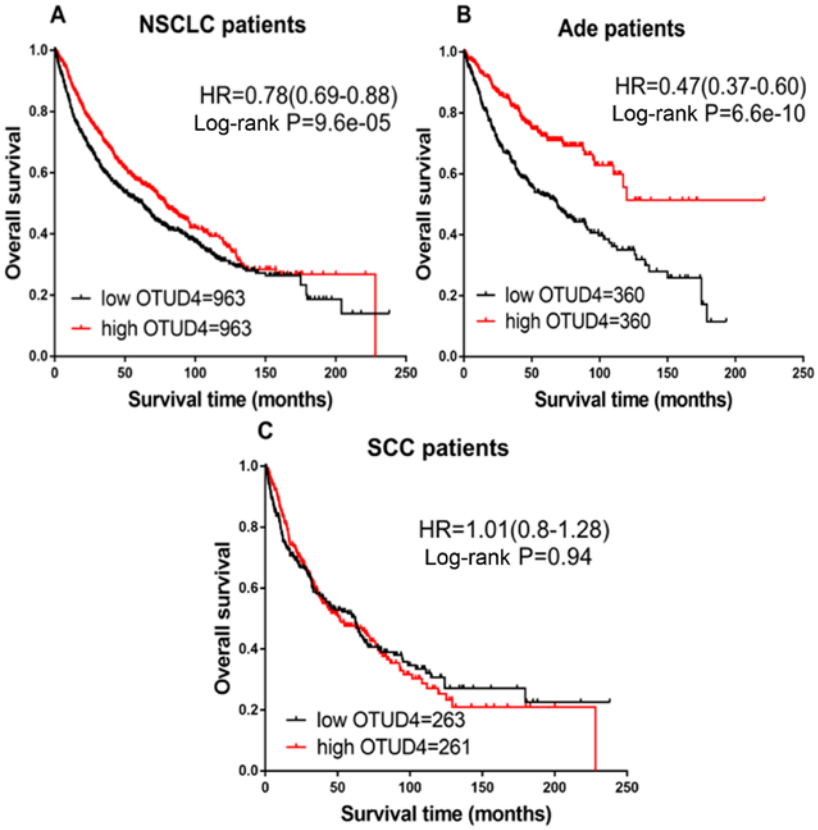

Figure 4. Prognostic value of OTUD4 mRNA expression. (A) Survival curves were plotted for all patients with NSCLC (n=1,926). (B) Survival curves were plotted for patients with Ade (n=720). (C) Survival curves were plotted for patients with SCC (n=524). Data was analyzed using Kaplan-Meier analysis. Patients with expression levels above the median are indicated in red, and patients with expression levels below the median are represented by the black line. OTUD4; NSCLC, non-small cell lung cancer; Ade, adenocarcinoma; SCC, squamous cell carcinoma; HR, hazard ratio.

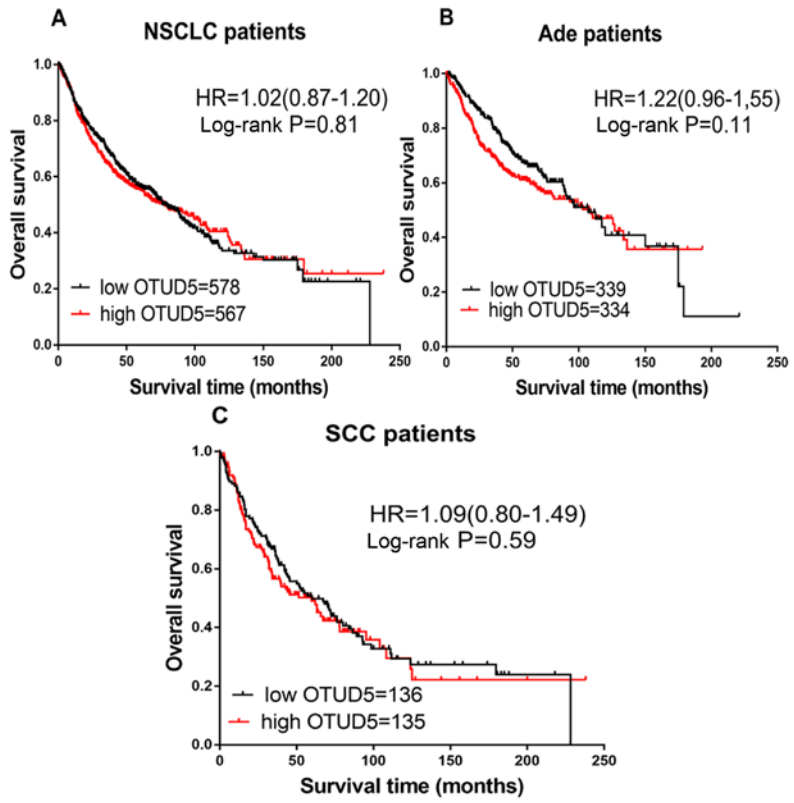

Figure 5. Prognostic value of OTUD5 mRNA expression. (A) Survival curves were plotted for all patients with NSCLC (n=1,145). (B) Survival curves were plotted for patients with Ade (n=673). (C) Survival curves were plotted for patients with SCC (n=271). Data was analyzed using Kaplan-Meier analysis. Patients with expression levels above the median are indicated in red, and patients with expression levels below the median are represented by the black line. OTUD5; NSCLC, non-small cell lung cancer; Ade, adenocarcinoma; SCC, squamous cell carcinoma; HR, hazard ratio.

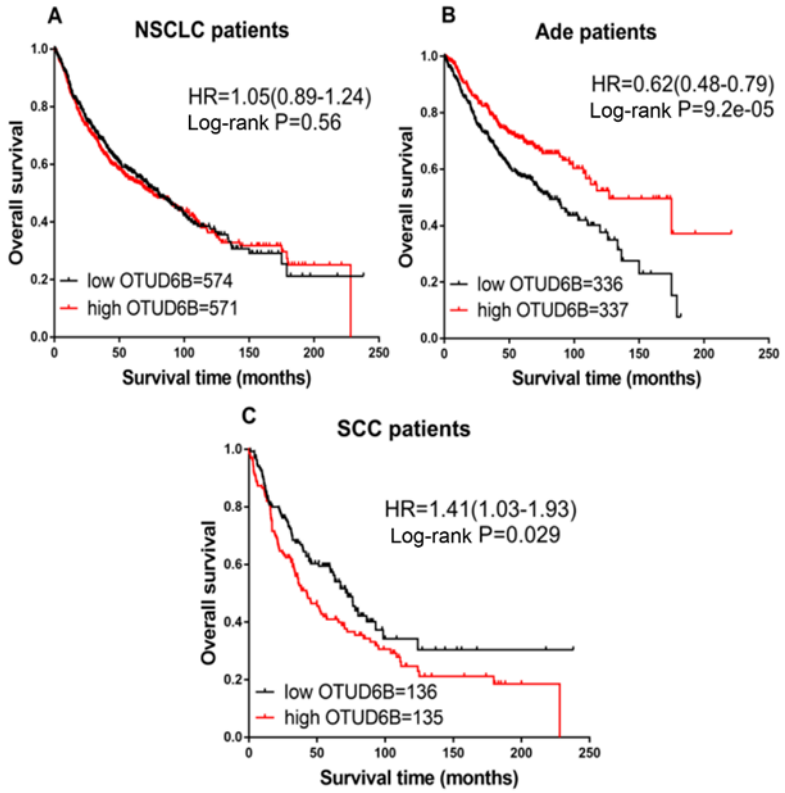

Figure 6. Prognostic value of OTUD6B mRNA expression. (A) Survival curves were plotted for all patients with NSCLC (n=1,125). (B) Survival curves were plotted for patients with Ade (n=673). (C) Survival curves were plotted for patients with SCC (n=271). Data was analyzed using Kaplan-Meier analysis. Patients with expression levels above the median are indicated in red, and patients with expression levels below the median are represented by the black line. OTUD6B; NSCLC, non-small cell lung cancer; Ade, adenocarcinoma; SCC, squamous cell carcinoma; HR, hazard ratio.

in NSCLC compared with normal samples. Hou *et al* (39) and Garber *et al* (40) have demonstrated that OTUD1 exhibits lower mRNA levels in Ade and SCC compared with normal tissue. In addition, the datasets analyzed in the studies by Yamagata *et al* (41) and Selamat *et al* (42) demonstrated that ALG13 mRNA was also expressed at decreased levels in Ade and SCC. Conversely, OTUD2 and OTUD6B mRNA were expressed at increased levels in Ade and SCC.

OTUD1 is a DUB, which belongs to the OTU family. It is an important enzyme that controls the activity or abundance of substrates by removing covalently linked ubiquitin from proteins (43). However, its substrates and its role in cells are unknown. OTUD1 directly suppresses the ubiquitination of p53 in cells to increase apoptosis and decrease cell proliferation, and a previous study by Piao *et al* (26) indicated that OTUD1 exerted a pivotal role in regulating p53 stability and activity. These results suggest that OTUD1 is a novel regulator of p53. In addition, OTUD1 has a role in the occurrence and development of thyroid carcinogenesis (24). OTUD1 is a metastasis suppressor, and its high expression may inhibit cancer stem cell (CSC) self-renewal and unlimited proliferation, and prevent metastasis. A study by Zhang *et al* (25) concluded that the absence of OTUD1 allowed breast cancer cells to undergo epithelial-mesenchymal transition and acquire CSC traits that promoted metastasis to distant organs including lungs and bone. However, the downregulation of OTUD1 expression was associated with an improved OS compared with that of patients with high OTUD1 expression in urothelial bladder carcinoma (44). In addition, the present study revealed that mRNA expression of OTUD1 had a prognostic value, as OTUD1 was downregulated in NSCLC and high mRNA expression of OTUD1 predicted a longer prognosis.

OTUD2, also termed YOD1 deubiquitinase, is a member of the OTU DUB family and contains the K11-specific OTU domain (23,45). Originally, OTUD2 was identified as a cofactor for protein processing (46). Then, Rumpf *et al* (46) suggested that it was released from tumor necrosis factor receptor-associated factor 6 upon interleukin-1 stimulation and that its depletion enhanced the canonical activation of NF-κB. Various studies have suggested that NF-κB was responsible for the malignant metastasis of lung cancer (47-50). Based on these observations, we hypothesized that OTUD2 was associated with the metastasis and prognosis of lung cancer. The results of the present study also demonstrated that high OTUD2 mRNA expression was significantly associated with poorer OS in patients with NSCLC.

OTUD3 belongs to the DUB family and contains an OTU domain with a priority hydrolyzed K6- and K11-linked distal ubiquitin (45). A *Toxoplasma gondii* deubiquitinase within the OTU family, TgOTUD3A, is the most similar ortholog of human OTUD3 in terms of structure (51). TgOTUD3A mRNA serves a key role in cell cycle regulation and exhibits decreased expression in the G1 phase of the cell cycle (52). Furthermore, as suggested by Yuan *et al* (27), OTUD3 may exhibit a tumor-suppressive role. The present study also revealed that increased expression levels of OTUD3 mRNA were significantly associated with favorable OS in patients with NSCLC. Therefore, OTUD3 may inhibit the growth of NSCLC cells.

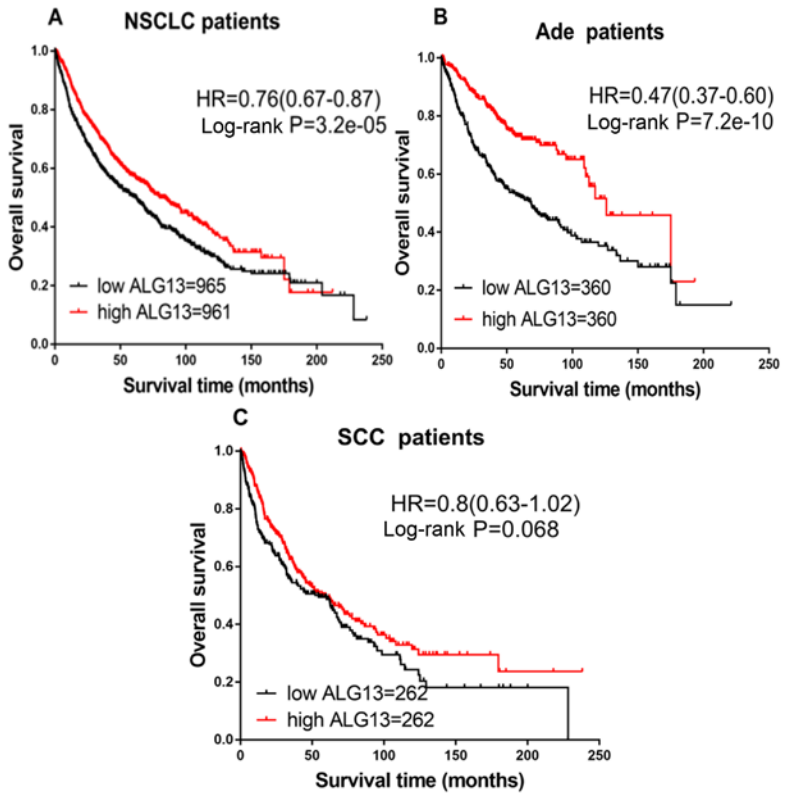

Figure 7. Prognostic value of ALG13 mRNA expression. (A) Survival curves were plotted for all patients with NSCLC (n=1,926). (B) Survival curves were plotted for patients with Ade (n=720). (C) Survival curves were plotted for patients with SCC (n=524). Data was analyzed using Kaplan-Meier analysis. Patients with expression levels above the median are indicated in red, and patients with expression levels below the median are represented by the black line. ALG13; NSCLC, non-small cell lung cancer; Ade, adenocarcinoma; SCC, squamous cell carcinoma; HR, hazard ratio.

OTUD4 is an additional novel deubiquitinase that is considered to serve a role in DNA alkylation repair (28). However, its role in cancer has not yet been explored. In the present study, it was observed that OTUD4 was highly expressed in patients with NSCLC and Ade, where it was a good prognostic marker according to Kaplan-Meier analysis, but not in SCC.

OTUD5 or DUBA is a 571-amino-acid protein (53). It is a deubiquitinase that regulates the production of type I interferon to regulate tissue factor R3 signaling (54). Park *et al* (55) identified the programmed cell death 5-OTUD5 network as a central hub for regulating p53-mediated apoptosis. p53 is an important gene in tumor growth and metastasis. However, the results of the present did not identify a significant association between OTUD5 and NSCLC prognosis.

To the best of our knowledge, OTUD6A has not been described in the relevant literature to date. OTUD6B is also a DUB. It is a cleavable ubiquitin-linked protease that has recently been demonstrated to be involved in regulating B-cell proliferation following cytokine stimulation. Santiago-Sim *et al* (55) observed that OTUD6B was associated with a severe intellectual disability syndrome. However, there is no relevant information on the role of this molecule in cancer, particularly in lung cancer. The results from the present study demonstrated that high mRNA expression of OTUD6B in Ade is associated with a good prognosis, whereas in SCC it exhibited an inverse association, as patients with increased mRNA expression of OTUD6B had poorer prognosis.

ALG13 is a highly conserved protein in the majority of eukaryotes and also belongs to the OTU family (56). De Antonellis *et al* (57) observed that ALG13 has been identified as an early target of microRNA-34a, with relevance to neuroblastoma tumorigenesis. However, to the best of our knowledge, expression of ALG13 in NSCLC has not been detected to date. In the previously described results of the present study, the mRNA expression of ALG13 was decreased in patients with Ade and SCC compared with that in normal tissues. High mRNA expression of ALG13 did not exhibit a significant association with the prognosis of patients with SCC, but did predict an improved OS in patients with NSCLC and Ade.

In conclusion, the present study evaluated the differential expression of OTUDs in NSCLC and normal tissues, and the results revealed that the expression levels of OTUD1 and ALG13 were decreased in NSCLC compared with normal lung tissue according to Oncomine analysis. In the Kapan-Meier analysis, the effect of 7 OTUDs on the prognoses of patients with NSCLC was analyzed, and it was demonstrated that increased expression levels of OTUD1, OTUD3, OTUD4 and ALG13 were associated with increased OS in patients with NSCLC and Ade, but not in patients with SCC. Similarly, increased expression of OTUD2 mRNA indicated poorer prognosis in all NSCLC cases, but no association was observed in the Ade or SCC patient cohorts. Increased mRNA expression of OTUD5 was not associated with OS in NSCLC, Ade or SCC. In addition, the increased expression of OTUD6B in patients with NSCLC was not associated with survival. These data reveal the complexity and heterogeneity of the molecular biology of lung cancer, and may provide novel

avenues for prognosis prediction, although the mechanism of its carcinogenicity and the investigation of novel drug treatment targets requires additional analysis in future studies.

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Availability of data and materials

The datasets analyzed in the present study are available in the Oncomine database (http://www.oncomine.org/) and the Kaplan-Meier plotter [Lung Cancer] (http://kmplot.com/analysis/index.php?p=service&cancer=lung).

Authors' contributions

JJD, JLL and XDL conceived the study and wrote and revised the manuscript. JJD and XDL reviewed, collected and analyzed the data. JJD,GXH and ZXF designed the study and acquired the data. All authors contributed to the writing of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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