Notch signaling molecules as prognostic biomarkers for non-small cell lung cancer

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Abstract. Notch family proteins have been reported to be associated with the initiation and development of various types of tumors. The present study used a prospective design to investigate the role of Notch proteins as novel biomarkers that are capable of predicting the survival outcome for patients with non-small cell lung cancer (NSCLC). The protein expression of Notch 1, Notch 3 and their ligands, Jagged 1 and Delta-like 4, was examined using immunohistochemistry in NSCLC tissues and adjacent non-cancerous lung tissues from 101 patients who underwent surgical treatment. The expression was also correlated with clinicopathological parameters and overall survival (OS). High Notch 1 protein expression was observed in 55.4% (56/101) of NSCLC samples and high Notch 3 expression was observed in 53.5% (54/101). The nuclear expression of Notch 3 was significantly associated with the lymph node status (P=0.0026) and tumor-node-metastasis (TNM) stage (P<0.0001), while the coexpression of Notch 1 plus Notch 3 was associated with lymph node status (P=0.0056), TNM stage (P=0.0001) and the histological grading (P=0.0359). In the survival analyses, the high expression of Notch 1 and Notch 3 exhibited an additive effect toward a poorer OS compared with a subtype with low coexpression for the two proteins (P<0.001), with high nuclear Notch 3 expression in the NSCLC patients maintaining independent prognostic significance for the outcome on multivariate analysis. These data further demonstrate a central role for Notch signaling in NSCLC and the significance of Notch 3 as a prognostic indicator of a poorer survival for patients with resected NSCLC.

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Abbreviations: NSCLC, non-small cell lung cancer; JAG1, Jagged 1; DLL4, Delta-like 4; PBS, phosphate-buffered saline; HR, hazard ratio; CI, confidence interval; FVC, forced vital capacity

Key words: Notch 3, Notch 1, immunohistochemistry, non-small cell lung cancer, prognosis

Introduction

Lung cancer is the most common type of cancer worldwide (1,2). Non-small cell lung cancer (NSCLC) accounts for 80-85% of all lung cancers, and although the surgical resection of early-stage tumors confers the greatest potential for long-term survival, 30-60% of patients with disease stages IB to IIIA succumb within 5 years of surgery (3,4). Therefore, more useful prognostic factors for those patients who have undergone a resection may enable a more accurate prediction of the outcome and could identify those patient groups with poor survival who may benefit from a more precise indication of the efficacy of treatment.

In hematological malignancies, the role for Notch is well established, while more recent studies have demonstrated the significance of Notch activity in the initiation and progression of solid tumors (5-9). The mammalian Notch receptor family consists of four type I transmembrane receptors (known as Notch 1-4), all of which have been implicated in human cancer. There are also five known Notch ligands in mammals, namely Jagged 1 (JAG1), JAG2, Delta-like 1 (DLL1), DLL3 and DLL4, which undergo processing that is similar to Notch processing. The Notch receptor undergoes multiple proteolytic cleavages upon ligand binding. The final cleavage (S3 cleavage) by the γ-secretase complex results in the release of the active Notch intracellular domain from the plasma membrane and its subsequent translocation into the nucleus (10). It is the S3 cleavage that is targeted by a class of compounds known as the γ-secretase inhibitors (GSIs). Therefore, treatment with GSIs blocks the terminal cleavage and release from the plasma membrane, preventing Notch signaling.

It has been demonstrated that Notch 1, Notch 3 and their ligands, JAG1 and DLL4, may be involved in malignant transformation. The activation of Notch 1 signaling appears to sustain the motility, migration and invasion of tumor cells in esophagus squamous cell carcinomas, lingual squamous cell carcinoma, endometrial carcinoma and breast cancer (11-15). Almost all cases of T cell acute lymphoblastic leukemia (T-ALL), and colorectal, pancreatic and ovarian

cancer have been reported to exhibit aberrant Notch 3 expression (6,8,16,17). Our previous study also reported that Notch 3 overexpression was associated with a poor prognosis in NSCLC patients (18). However, the prognostic role of Notch 3 compared with other Notch family members and its association with Notch 1 in human NSCLC remains unclear. In the present study, the expression of Notch 1, Notch 3, JAG1 and DLL4 was investigated in 101 NSCLC tissue samples and association between the expression of the four Notch family members and the clinicopathological variables and prognosis in NSCLC patients was further assessed.

Materials and methods

Lung cancer specimens. Paraffin-embedded sections were acquired from 101 patients with NSCLC who underwent surgical resection at the Department of Thoracic Surgery, The First Affiliated Hospital of Anhui Medical University (Heifei, Anhui, China) between January 2007 and December 2007. The criteria for study enrollment were as follows: Patients with histopathologically diagnosed NSCLC, no receipt of radiotherapy or chemotherapy prior to surgery, and no history of other tumors. Prior consent and approval was obtained from all NSCLC patients prior to surgery for the use of cancer tissues and adjacent non-cancerous lung tissues for research purposes, and all experiments were conducted adhering to the bioethics rules issued by the Medical Ethics Committee of The First Affiliated Hospital of Anhui Medical University.

The follow-up period ranged from 1 to 60 months, with a median time of 36 months. Informed consent was obtained from all patients for publication of this study. The age of the patients ranged from 32 to 80 years (median, 62 years), and the cohort included 78 males (77.2%) and 23 females (22.7%). The histological diagnosis was determined by hematoxylin and eosin staining according to the new pathological classification of lung cancer (19). Tumor grading and staging were classified according to the new lung cancer staging system developed by the International Association for the Study of Lung Cancer (2009) (20). The results revealed 49 adenocarcinomas (including 9 mucinous adenocarcinomas), 51 squamous cell carcinomas and 1 large cell carcinoma. Furthermore, 18 tumors were well-differentiated, 53 were moderately-differentiated and 30 were poorly-differentiated. Tumor-node-metastasis (TNM) staging revealed that 20 patients were at stage I, 48 were at stage II, 32 were at stage III and 1 was at stage IV.

Ventilatory function and small airway function were detected by the Jaeger Masterscope computer system (Jaeger-Toennies GmbH, Hoechberg, Germany). A predicted forced vital capacity (FVC) of <80% or a forced expiratory volume in 1 sec/FVC of <70% was defined as abnormal ventilatory function. A predicted maximal mid-expiratory flow curve (75/25%) value of <65% was defined as abnormal small airway function. Overall survival (OS) was defined as the interval between the date of surgery and the date of mortality. OS was censored at the date of the patient's last tumor assessment, at the date of mortality from other causes or at 5 years post-surgery. Other patient information is summarized in Table I.

Immunohistochemistry. Each tissue was fixed in formalin and embedded in paraffin, then sectioned to a thickness of 3 μm and mounted on glass slides. The sections were dewaxed in xylene and dehydrated in graded alcohol, and endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min. Next, the sections were subjected to antigen retrieval with 10 mmol/l citrate buffer solution (pH 0) for 20 min in a microwave oven at 700 W. Subsequently, the sections were incubated with 10% goat serum albumin to eliminate non-specific binding and then they were incubated overnight at 4˚C with the following primary antibodies: Rabbit polyclonal Notch 1 (1:100 dilution; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), rabbit polyclonal Notch 3 (1:50 dilution; Santa Cruz Biotechnology, Inc.,), rabbit polyclonal JAG1 (1:50 dilution; Santa Cruz Biotechnology, Inc.) and rabbit polyclonal Delta-4 (1:100 dilution; Rockland Immunochemicals, Gilbertsville, PA, USA). Sections were then incubated with the appropriate secondary antibodies (goat anti-rabbit immunoglobulin G; 1:50 dilution; ZSGB-BIO, Beijing, China) at room temperature for 60 min. After sufficient phosphate-buffered saline (PBS) rinses, diaminobenzidine was used as chromogen and the sections were counterstained with hematoxylin. The slides were counterstained with hematoxylin, then dehydrated and coverslipped. Samples incubated with PBS instead of primary antibodies were used as negative controls.

Evaluation of immunohistochemical staining. All stained slides were independently evaluated and scored by three pathologists who had no knowledge of the patients' clinical information. If a disagreement occurred, the slides were re-examined to obtain a final consensus. Positive staining was evaluated in at least five areas at x400 magnification. The mean percentage of positive cells were scored as follows: 0, 0%; 1, 1-25%; 2, 26-50%; 3, 51-75%; and 4, 76-100%. The staining intensity was scored as follows: 0, negative; 1, weak; 2, moderate; and 3, strong. A final histological score was obtained for each case by multiplying the percentage staining and intensity scores. Protein expression levels were further analyzed by classifying histological scores as low expression (histological score <5) and as high expression (histological score ≥5).

Statistical analysis. All statistical analyses were performed with R software for Windows (version 2.15.3; http://cran.r-project.org/web/packages/dlnm/vignettes/dlnmOverview.pdf). The associations between various clinicopathological parameters and the expression of Notch 1, Notch 3, JAG1 or DLL4 was evaluated using χ^2 or Fisher's exact tests. Kaplan-Meier and log-rank methods were used to draw and evaluate the significance of survival curves. Multivariate Cox proportional hazards regression analysis was used to identify independent prognostic factors for survival rates following univariate survival analysis. P<0.05 was used to indicate a statistically significant difference.

Results

Notch 1 expression and the association with clinicopathological parameters. Notch 1 immunoreactivity is shown in Fig. 1. Notch 1 protein expression was localized mainly in the cytoplasm, with certain tumor cells showing membranous

Table I. Correlations between the expression of Notch 1, Notch 3, Notch 1 plus Notch 3, JAG1 and DLL4 proteins and clinicopathological parameters in patients with non-small cell lung cancer.

Characteristic	Cases, n	Notch 1 expression, n			Notch 3 expression, n			Notch 1 and Notch 3 coexpression, n			JAG1 expression, n			DLL4 expression, n		
		Low	High	P-value	Low	High	P-value	Both low	Both high	P-value	Low	High	P-value	Low	High	P-value
Gender				0.4041			1.0000			0.3953			0.3203			0.9401
Male	78	37	41		36	42		22	27		43	35		30	48	
Female	23	8	15		11	12		5	9		16	7		8	15	
Age, years				1.0000			0.8433			0.9131			0.8005			0.8937
<60	43	19	24		21	22		14	17		24	19		17	26	
≥60	58	26	32		26	32		13	19		35	23		21	37	
Tumor histology				0.2661			0.2695			0.1347			0.7750			1.0000
Squamous cell carcinoma	51	26	25		27	24		18	16		31	20		19	32	
Adenocarcinoma + large cell carcinoma	50	19	31		20	30		9	20		28	22		19	31	
T status				0.3023			0.0856			0.0800			0.4710			0.4604
T1+T2	77	37	40		40	37		24	24		47	30		31	46	
T3+T4	24	8	16		7	17		3	12		12	12		7	17	
Lymph node status				0.1875			0.0026[a]			0.0056[a]			0.8213			0.9752
N0	41	22	19		27	14		17	9		25	16		16	25	
N+	60	23	37		20	40		10	27		34	26		22	38	
TNM stage				<0.0001[a]			<0.0001[a]			0.0001[a]			0.2319			0.0313[a]
I+II	68	36	32		42	26		25	15		43	25		31	37	
III+IV	33	9	24		5	28		2	21		16	17		7	26	
Grading				0.0330[a]			0.1309			0.0359[a]			0.0754			0.4218
G1+G2	71	37	34		37	34		22	19		46	25		29	42	
G3	30	8	22		10	20		5	17		13	17		9	21	
Smoking history				0.4404			0.8855			0.9707			1.0000			0.7273
No	39	15	24		19	20		11	16		23	16		16	23	
Yes	62	30	32		28	34		16	20		36	26		22	40	
Ventilatory function				1.0000			0.2931			0.5363			1.0000			0.9399
Normal	54	24	30		22	32		12	20		32	22		21	33	
Abnormal	47	21	26		25	22		15	16		27	20		17	30	

Table I. Continued.

Characteristic	Cases, n	Notch 1 expression, n		P-value	Notch 3 expression, n		P-value	Notch 1 and Notch 3 coexpression, n		P-value	JAG1 expression, n		P-value	DLL4 expression, n		P-value
		Low	High		Low	High		Both low	Both high		Low	High		Low	High	
Small airway function				0.5277			0.8991			0.9642			1.0000			0.9117
Normal	22	8	14		11	11		5	8		13	9		9	13	
Abnormal	79	37	42		36	43		22	28		46	33		29	50	
Anatomical classification				1.0000			0.9041			1.0000			0.9601			1.0000
Peripheral	52	23	29		25	27		14	18		31	21		20	32	
Central	49	22	27		22	27		13	18		28	21		18	31	

[a]P<0.05. JAG1, Jagged 1; DLL, Delta-like 4.

expression. The staining was absent in the adjacent non-cancerous normal lung tissues (Fig. 1A). High Notch 1 protein expression was observed in 55.4% (56/101) of the NSCLC samples (Fig. 1C) and low expression levels were detected in 44.6% (45/101) of the tumor sections (Fig. 1B). As summarized in Table I, the high expression of Notch 1 was significantly correlated with TNM stage (I+II vs. III+IV) (P=0.0264) and histological grading (G1+G2 vs. G3) (P=0.0330) in the NSCLC tissues.

Notch 3 expression and the association with clinicopathological parameters. Notch 3 immunoreactivity is shown in Fig. 2. Notch 3 was predominantly localized in the nucleus of the tumor cells. The staining was absent in the adjacent non-cancerous normal lung tissues (Fig. 2A). High Notch 3 protein expression was observed in 53.5% (54/101) of the NSCLC samples (Fig. 2C) and low expression levels were detected in 46.5% (47/101) (Fig. 2B). As summarized in Table I, the high expression of Notch 3 was significantly correlated with lymph node status (N0 vs. N+) (P=0.0026) and TNM stage (I+II vs. III+IV) (P<0.0001) in the NSCLC samples.

Association between Notch 1 plus Notch 3 coexpression and clinicopathological characteristics. High coexpression of Notch 1 and Notch 3 was detected in 36 (35.6%) samples, and low expression was observed in 27 samples (26.7%). Table I shows that the high coexpression of Notch 1 plus Notch 3 was significantly correlated with lymph node status (N0 vs. N+) (P=0.0056), TNM stage (I+II vs. III+IV) (P=0.0001) and histological grading (G1+G2 vs. G3) (P=0.0359).

JAG1 and DLL4 expression and the association with clinicopathological findings. JAG1 and DLL4 immunoreactivity is shown in Fig. 3. JAG1 and DLL4 protein expression was localized mainly in the cytoplasm, with membranous staining. High JAG1 protein expression was observed in 41.6% (42/101) (Fig. 3B) and low expression levels were detected in 58.4% (59/101) (Fig. 3A). High DLL4 protein expression was observed in 62.4% (63/101) (Fig. 3D) and low expression levels were detected in 37.6% (38/101) (Fig. 3C). Table I shows no association between high JAG1 expression and the clinicopathological features in the NSCLC patients (P>0.05), while the high expression of DLL4 was significantly correlated with TNM stage (I+II vs. III+IV) (P=0.0313).

Survival analysis. Follow-up was discontinued for all patients in December 2012. The median follow-up time was 36 months (range, 1-60 months). The overall 5-year survival rate was 45%, with a median survival time of 56 months. The data indicated that there was a significant difference in OS between the patients with high and low expression of all four Notch family members (P<0.05), which indicated that the high expression of Notch 1, Notch 3, JAG1 or DLL4 was correlated with a shorter survival time. Furthermore, the subtype with high coexpression of Notch 1 and Notch 3 exhibited a worse outcome than other subtypes (log-rank, 21.227; P<0.001) (Fig. 4).

Results of the univariate analysis with regard to OS for the clinicopathological prognostic factors are shown in Table II. The high expression of the four Notch proteins was found to be a significant indicator of a poor OS (P<0.05) (Table II). With regard to other parameters, TNM stage, lymph node status and

Table II. Univariate and multivariate Cox proportional hazards analyses for prognostic factors in patients with non-small cell lung cancer.

	Overall survival		
Variable	HR	95% CI	P-value
Univariate analysis			
Gender (female vs. male)	0.8090	0.4026-1.6260	0.5508
Age (≥60 vs. <60 years)	0.7849	0.4453-1.3830	0.4011
Tumor histology (adenocarcinomas vs. quamous cell carcinoma)	1.0610	0.6021-1.8690	0.8383
T status (T3+T4 vs. T1+T2)	1.7960	0.9609-3.3570	0.0627
Lymph node status (N+ vs. N0)	3.5700	1.8140-7.0260	<0.0001[a]
TNM stage (III+IV vs. I+II)	6.4060	3.5500-11.560	<0.0001[a]
Grading (G3 vs. G1+G2)	1.9020	1.0480-3.4490	0.0315[a]
Smoking history (yes vs. no)	1.3560	0.7435-2.4730	0.3187
Ventilatory function (abnormal vs. normal)	1.2590	0.7149-2.2190	0.4237
Small airway function (abnormal vs. normal)	1.7240	0.8064-3.6840	0.1550
Anatomical classification (central vs. peripheral)	1.5630	0.8829-2.7680	0.1222
Notch 1 expression (high vs. low)	2.0800	1.1370-3.8040	0.0151[a]
Notch 3 expression (high vs. low)	3.7220	1.9590-7.0720	<0.0001[a]
JAG1 expression (high vs. low)	1.9760	1.1180-3.4940	0.0170[a]
DLL4 expression (high vs. low)	1.9620	1.0370-3.7130	0.0348[a]
Multivariate analysis			
Gender (female vs. male)	0.8756	0.3004-2.5523	0.8076
Age (≥60 vs. <60 years)	1.0026	0.5048-1.9913	0.9940
Tumor histology (adenocarcinomas vs. quamous cell carcinoma)	1.0097	0.5309-1.9202	0.9766
T status (T3+T4 vs. T1+T2)	0.8430	0.3717-1.9121	0.6827
Lymph node status (N+ vs. N0)	1.7543	0.7431-4.1411	0.1997
TNM stage (III+IV vs. I+II)	5.1447	2.1070-12.5621	0.0003[a]
Grading (G3 vs. G1+G2)	1.5180	0.7615-3.0263	0.2357
Smoking history (yes vs. no)	0.8977	0.3527-2.2850	0.8209
Ventilatory function (abnormal vs. normal)	1.4652	0.6636-3.2350	0.3445
Small airway function (abnormal vs. normal)	1.7839	0.6867-4.6345	0.2347
Anatomical classification (central vs. peripheral)	1.8915	0.9343-3.8291	0.0765
Notch 1 expression (high vs. low)	0.9105	0.3855-2.1510	0.8308
Notch 3 expression (high vs. low)	2.5126	1.1383-5.5460	0.0226[a]
JAG1 expression (high vs. low)	1.1646	0.4850-2.7970	0.7331
DLL4 expression (high vs. low)	1.2515	0.5477-2.8600	0.5947

[a]P<0.05. JAG1, Jagged 1; DLL, Delta-like 4; CI, confidence interval; HR, hazard ratio; TNM, tumor-node-metastasis.

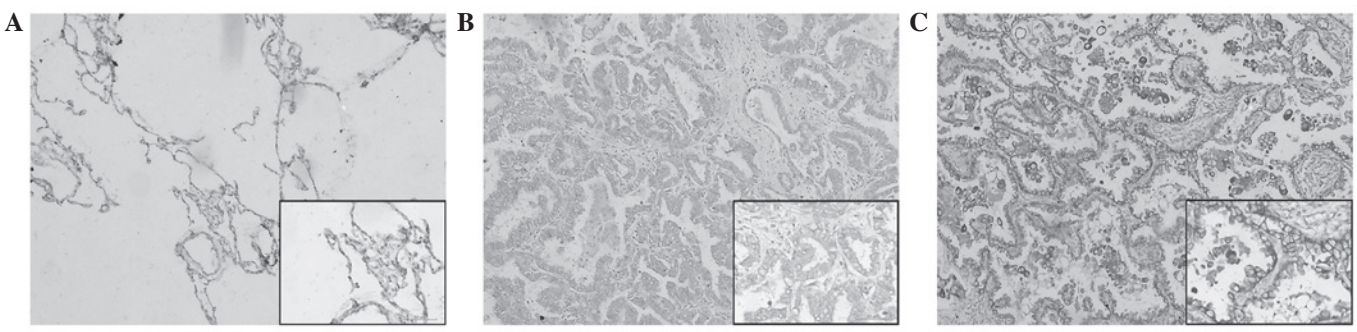

Figure 1. Representative immunohistochemical staining for Notch 1 protein in primary non-small cell lung cancer (NSCLC) tissues and adjacent non-cancerous lung tissues. (A) Negative expression of Notch 1 protein in adjacent non-cancerous lung tissues. (B) Low expression of Notch 1 in primary NSCLC specimens. (C) High expression of Notch 1 protein in primary NSCLC specimens. Magnification, x100; inset images, x400.

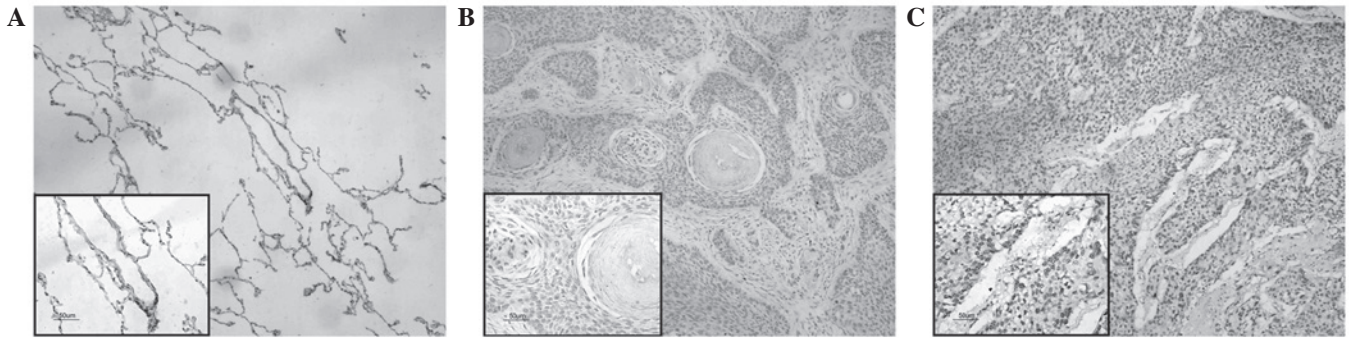

Figure 2. Representative immunohistochemical staining for Notch 3 protein in primary non-small cell lung cancer (NSCLC) tissues and adjacent non-cancerous lung tissues. (A) Negative expression of Notch 3 protein in adjacent non-cancerous lung tissues. (B) Low expression of Notch 3 in primary NSCLC specimens. (C) High expression of Notch 3 protein in primary NSCLC specimens. Magnification, x100; inset images, x400.

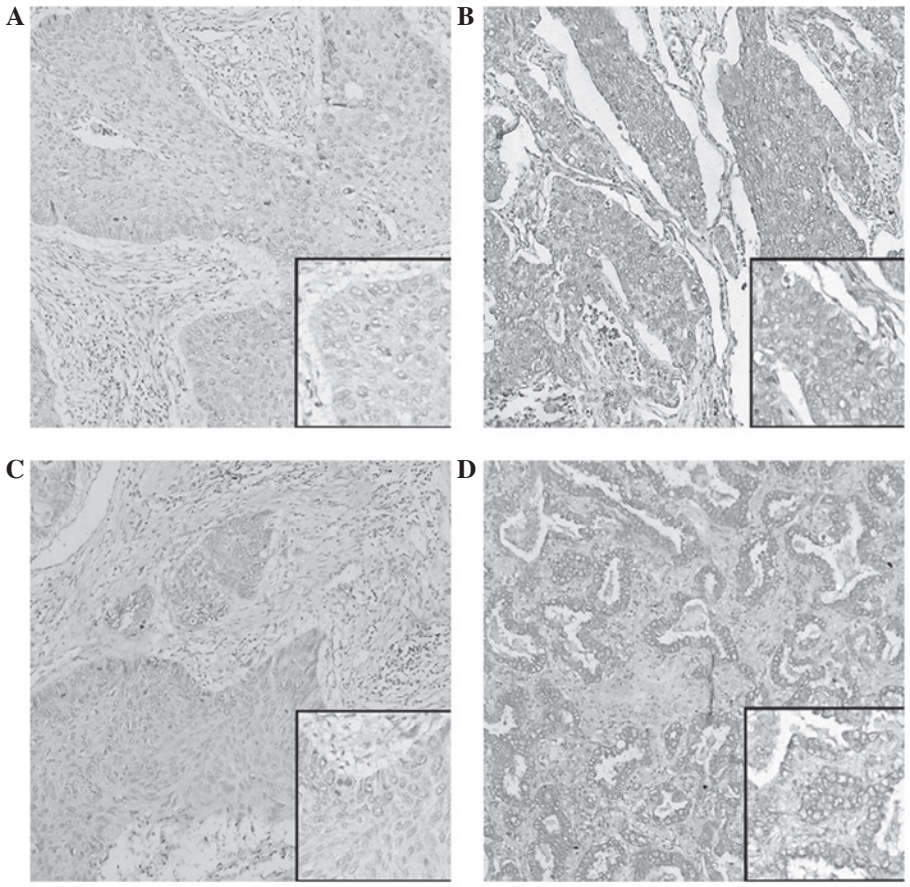

Figure 3. Representative immunohistochemical staining for Jagged 1 (JAG1) and Delta-like 4 (DLL4) protein in primary non-small cell lung cancer (NSCLC) tissues and adjacent non-cancerous lung tissues. (A) Low expression of JAG1 in primary NSCLC specimens. (B) High expression of JAG1 protein in primary NSCLC specimens. (C) Low expression of DLL4 in primary NSCLC specimens. (D) High expression of DLL4 protein in primary NSCLC specimens. Magnification, x100; inset images, x400.

histological grading were determined as positive significant prognostic factors for OS (P<0.05) (Table II). In the multivariate analysis, Notch 3 expression was found to be an independent prognostic factor of OS in patients with NSCLC compared with the other three Notch family members (P=0.0226) (Table II).

Discussion

In this study, Notch 1, Notch 3, JAG1 and DLL4 protein expression was examined immunohistochemically in a well-defined cohort of NSCLC patients and the expression levels were correlated to clinical parameters and patient outcome.

The phenotypic outcome of Notch signaling is often context-dependent. Different Notch receptors play different, even opposing, roles in tumor development, showing the complexity of Notch signaling in cancer. The expression of Notch 3 has been found to be significantly decreased in human tumor cell lines, and in primary human breast cancer and melanoma samples compared with normal control tissues (21). However, the present data demonstrated that Notch 3 is

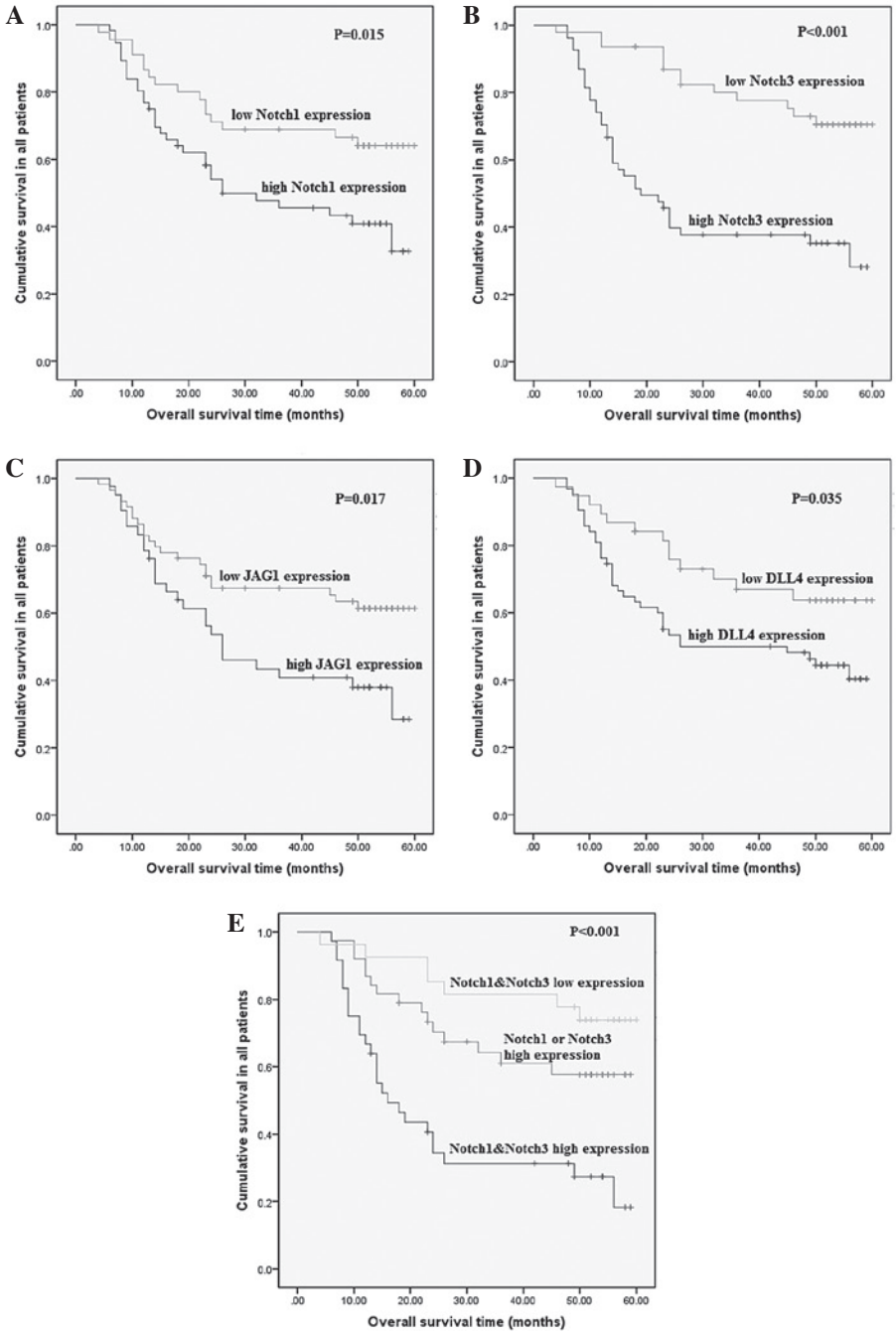

Figure 4. Kaplan-Meier curves of survival patients with (A) Notch 1, (B) Notch 3, (C) Jagged 1 (JAG1), (D) Delta-like 4 (DLL4), and (E) Notch 1 plus Notch 3 expression. The log-rank test was used to calculate P-values.

ubiquitously expressed in NSCLC. The majority of tumors showed high levels of nuclear Notch 3 expression. This expression pattern closely resembles data previously recorded in pancreatic ductal adenocarcinomas (22), while certain more recent studies have demonstrated that the immunoreactivity of Notch 3 is observed mainly in the cytoplasm of tumor cells with or without nuclei staining (8,9,13). The mechanism of this phenomenon and how Notch 3 exerts its function in NSCLC remain unclear and require further research. Most notably, in the present study, the upregulated expression of Notch 3 was a predictor of different aggressive tumor behaviors, such as advanced clinical stage and lymph node metastasis. In lung cancer, Notch 1 is known to suppress tumor proliferation under normoxia, but in hypoxia, it exhibits a converse role in tumor promotion (23). In the present study, Notch 1 expression, with levels varying from low to high, was demonstrated in a number of NSCLC patients. Notch 1 expression was localized in the cytoplasm, with membranous expression, similar to the results found in studies of other tissue tumors (11-14). Furthermore, Notch 1 expression was found to be associated with TNM stage and histological grading, which also indicated the impact of Notch 1 expression on the progression of NSCLC. The results strongly suggested that Notch 1 and Notch 3 may play key roles in the advancement of NSCLC.

As Notch receptor ligands, JAG1 and DLL4 have been found to function in cancer progression and

metastasis (24-28). In the present study, the expression of the JAG1 and DLL4 notch ligands was examined in NSCLC tissues and the expression levels were compared with clinical parameters. However, no correlation was found between the JAG1 expression levels and clinical parameters in NSCLC. In addition, it was shown that in tumor tissues, high levels of DLL4 expression were correlated with TNM stage, which suggested that DLL4 may be associated with the progression of NSCLC.

Specific foci, including comborbidities, smoking history, and general clinical and demographic features have been investigated in previous analyses of prognostic factors in surgically resected NSCLC. Pathological TNM stage, age and gender were all determined to be independent prognostic factors for survival, with pathological TNM stage representing the most important prognostic factor (29). However, the outcome may vary depending on several biochemical and clinical parameters, even among those patients with clinically localized disease. In the present study, Kaplan-Meier analysis of the survival curves showed a significantly worse overall survival rate for patients with tumors that exhibited high Notch 1, Notch 3, JAG1 or DLL4 protein levels, indicating that high Notch 1, Notch 3, JAG1 and DLL4 protein levels are markers of a poor prognosis for patients with NSCLC. Furthermore, the present results showed that the high coexpression of Notch 1 and Notch 3 predicted a worse outcome compared with the Notch 1 or Notch 3 high expression subtypes. These data suggested that the coexpression of Notch 1 and Notch 3 has additive roles in the biological behavior of NSCLC. Moreover, univariate analysis showed that high Notch 1, Notch 3, JAG1 and DLL4 expression, TNM stage and tumor histological grading were risk factors for a poor prognosis in NSCLC patients, but multivariate analysis showed that a high level of Notch 3 expression was the only independent risk factor of prognosis for NSCLC patients, suggesting that the level of Notch 3 expression in NSCLC tissue samples may be used as a more useful prognostic marker compared with Notch 1 in NSCLC patients. Previous studies have demonstrated that cellular proliferation is significantly reduced by γ-secretase inhibitor (30,31) and that the apoptosis of Notch 3-expressing cells is induced. In lung cancer, the inhibition of Notch activation using a γ-secretase inhibitor is a potential novel approach for targeted therapy (32). Thus, in NSCLC patients, Notch 3 expression may represent a useful additive prognostic marker to the TNM staging system and thus, such patients are good candidates for receiving aggressive adjuvant targeted therapy.

In summary, the present findings demonstrated that high levels of Notch 1 and Notch 3 expression were significantly correlated with NSCLC progression and a poor prognosis. Furthermore, Notch 3 expression can be used as an adjunct to the TNM staging system to improve prognostication for individual patients. Additionally, it can be concluded that Notch 3 may present as a more attractive prognostic biomarker associated with NSCLC compared with Notch 1. Therefore, we hypothesize that targeting Notch 3 in specific cell types may be more useful than targeting Notch 1. In the near future, targeting the Notch 3 pathway may be used for the formation of novel preventive and therapeutic strategies for NSCLC.

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