

Notch Signaling Components

Diverging Prognostic Indicators in Lung Adenocarcinoma

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Abstract: Non-small-cell lung cancer (NSCLC) is a lethal and aggressive malignancy. Currently, the identities of prognostic and predictive makers of NSCLC have not been fully established. Dysregulated Notch signaling has been implicated in many human malignancies, including NSCLC. However, the prognostic value of measuring Notch signaling and the utility of developing Notch-targeted therapies in NSCLC remain inconclusive. The present study investigated the association of individual Notch receptor and ligand levels with lung adenocarcinoma (ADC) and squamous cell carcinoma (SCC) prognosis using the Kaplan-Meier plotte database. This online database encompasses 2437 lung cancer samples. Hazard ratios with 95% confidence intervals were calculated. The results showed that higher Notch1, Notch2, JAG1, and DLL1 mRNA expression predicted better overall survival (OS) in lung ADC, but showed no significance in SCC patients. Elevated Notch3, JAG2, and DLL3 mRNA expression was associated with poor OS of ADC patients, but not in SCC patients. There was no association between Notch4 and OS in either lung ADC or SCC patients. In conclusion, the set of Notch1, Notch2, JAG1, DLL1 and that of Notch3, JAG2, DLL3 played opposing prognostic roles in lung ADC patients. Neither set of Notch receptors and ligands was indicative of lung SCC prognosis. Notch signaling could serve as promising marker to predict outcomes in lung ADC patients. The distinct features of lung cancer subtypes and Notch components should be considered when developing future Notch-targeted therapies.

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Abbreviations: 95%CI = 95% confidence intervals, ADC = adenocarcinoma, CT = chemotherapy, DLL1 = Delta-like ligand 1, DLL3 = Delta-like ligand 3, DLL4 = Delta-like ligand 4, EGA =

European Genome-phenome Archive, EGFR = epidermal growth factor receptor, GEO = Gene Expression Omnibus, HR = Hazard ratios, JAG1 = Jagged1, JAG2 = Jagged2, NA = not available, NICD = intracellular domain of the Notch receptor, NSCLC = Non-small-cell lung cancer, OS = overall survival, RT = radiotherapy, SCC = squamous cell carcinoma, TCGA = The Cancer Genome Atlas, TKIs = tyrosine kinase inhibitors.

INTRODUCTION

Lung cancer is a lethal and aggressive malignancy with high incidence and mortality worldwide; it is also a leading cause of cancer death globally.^{1,2} NSCLC accounts for the majority of lung tumors, with lung squamous cell carcinoma (SCC) and adenocarcinoma (ADC) representing the major histological subtypes.³ Surgical resection remains the cornerstone of therapy in most NSCLC cases; however, many NSCLC patients experience relapse, metastasis, and death despite surgery. Thus, the clinical outcome is still disappointing, resulting in a dismal 5-year survival rate as low as 17%.^{4,5} For advanced or distant metastatic lung cancer, platinum-based systemic chemotherapy offers the frontline treatment of choice. However, treatment response falls below expectation because of multiple resistance mechanisms.⁶ Molecularly targeted therapies are attractive alternatives for advanced NSCLC. In particular, tyrosine kinase inhibitors (TKIs) have revolutionized the treatment of these patients.³ Unfortunately, mutations in the epidermal growth factor receptor (EGFR) only occur in 10% to 30% of NSCLC patients.⁷ Acquired resistance to EGFR TKIs eventually also ensues. Therefore, it is of great clinical urgency to identify new prognostic and predictive markers that enable develop effective individual treatment strategies and improve prognosis in NSCLC patients.

The Notch signaling pathway has a complex and multifaceted nature, influencing diverse cellular functions such as cell fate specification, proliferation, apoptosis, “stemness”, differentiation, and survival.⁸ The pathway encompasses 4 Notch receptor paralogues (Notch1–4) and 5 Notch ligands (Jagged1 [JAG1], Jagged2 [JAG2], Delta-like ligand 1 [DLL1], Delta-like ligand 3 [DLL3] and Delta-like ligand 4 [DLL4]) in mammals.⁹ The pathway is initially activated by occupation of the representative Notch receptor. Subsequently, transmembrane domain cleavage of the Notch receptor is mediated by a γ -secretase complex, resulting in the release of the intracellular domain of the Notch receptor (NICD). The released NICD then moves to the nucleus and initiates transcription of Notch downstream targets.^{9–11} Alteration of Notch signaling is implicated in the genesis of several human solid tumors,^{12,13} for example, liver cancer,^{14,15} breast cancer,¹⁶ and lung cancer.^{17,18} Unsurprisingly, notch signaling has become a major potential therapeutic target for cancer.¹⁹ Various Notch modulators, including gamma-secretase inhibitors, neutralizing antibodies, soluble decoys, and blocking peptides, have been moved from

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theoretical basic research into preclinical and early clinical trials.^{20,21} Notably, recent research showed that different Notch components may have opposing roles in breast cancer and head and neck cancer.^{22,23} Therapeutic modulation of Notch components has not been applied in the clinic, perhaps because of the multifaceted roles of Notch signaling. Efforts to clarify the precise roles of Notch components in NSCLC should be made to facilitate clinical application of such modulation.

Compelling evidence indicates Notch receptors and ligands could be variously tumor-suppressive or oncogenic in different neoplasms including NSCLC.^{24,25} Some studies found that inactivation of Notch1 in mouse models of NSCLC abrogated tumorigenesis and inhibited cell growth,^{26,27} whereas others showed that NICD1 or Notch1 inhibited NSCLC cell and xenograft tumor growth.^{28,29} Similarly, several studies have shown that high Notch1 or low DLL4 expression is a negative prognostic indicator^{30,31}; however, another study indicated that higher Notch1 predicts better survival.³²

Currently, the role of Notch seems to be determined by the genetic and pathological context.²⁶ The association of Notch components with the prognosis of NSCLC remains obscure. The Kaplan–Meier plotter database contains a large number of cancer samples, which can be used as a valuable tool of biomarker assessment. A number of genes have been identified as predictive markers in cancer using this database.³³ The current article examines the impact of individual Notch receptors and ligands on overall survival (OS) of NSCLC patients using the Kaplan–Meier plotter database.

MATERIAL AND METHODS

The Kaplan–Meier plotter database includes gene expression and clinical information downloaded from the Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.nih.gov/geo/>), the European Genome-phenome Archive (EGA; <https://www.ebi.ac.uk/ega/>), and The Cancer Genome Atlas (TCGA; <http://cancergenome.nih.gov/>).^{34,35} Currently, the Kaplan–Meier plotter database encompasses 22,277 genes and their effect on survival using 10,188 cancer samples (including 2437 lung cancer samples). The database is used here to assess the association of mRNA expression of components of the Notch signaling pathway with OS of NSCLC patients. Briefly, the Notch receptors and ligands (Notch1, Notch2, Notch3, Notch4, JAG1, JAG2, DLL1, DLL3, and DLL4) were entered into the database of lung cancers (<http://kmplot.com/analysis/index.php?p=service&cancer=lung>) to obtain Kaplan–Meier survival plots. In cases wherein these genes were detected by multiple probe sets, the best specific probes were chosen (JetSet probes).³⁶ The Multivariate Cox regression model was used and hazard ratio (HR), 95% confidence interval (95% CI), and log-rank *P* value were calculated. We used *P* < 0.01 as a threshold to reduce false-positive rate. This study used a public dataset with access to patient information. Therefore, the requirement for ethical approval was waived by our institutional ethical review board.

RESULTS

Notch Receptors

The prognostic role of mRNA expression levels of Notch receptors on OS of NSCLC patients is shown in Figures 1 and 2 (<http://kmplot.com/analysis/index.php?p=service&cancer=lung>).

The following Affymetrix IDs are valid: 218902_at (Notch1), 212377_s_at (Notch2), 203238_s_at (Notch3), and 205247_at (Notch4). Kaplan–Meier analysis revealed that high expression of Notch1 correlated with better OS in total NSCLC (*n* = 1926, HR = 0.78 [95% CI: 0.69–0.89], *P* < 0.001; Figure 1A). NSCLC was subgrouped to consider lung ADC and lung SCC. The data showed that elevated Notch1 was linked to favorable prognosis in lung ADC patients (*n* = 720, HR = 0.59 [95% CI: 0.46–0.75], *P* < 0.001; Figure 1B), but not in lung SCC patients (*n* = 524, HR = 0.78 [95% CI: 0.62–0.99], *P* = 0.044; Supplementary Figure 1, <http://links.lww.com/MD/A984>). Kaplan–Meier analysis in total NSCLC and in lung ADC patients showed correlation between high expression of Notch2 and better OS (HR = 0.7 [95% CI: 0.61–0.79], *P* < 0.001; HR = 0.6 [95% CI: 0.48–0.76], *P* < 0.001, respectively; Figure 1C and D), but not in lung SCC (HR = 0.77 [95% CI: 0.61–0.97], *P* = 0.029; Supplementary Figure 1, <http://links.lww.com/MD/A984>). Interestingly, the curves revealed that high expression of Notch3 predicted worse OS in total NSCLC and in lung ADC patients (HR = 1.48 [95% CI: 1.31–1.68], *P* < 0.001; HR = 2.07 [95% CI: 1.63–2.63], *P* < 0.001; Figure 2A and B), respectively, but not in lung SCC (HR = 1.17 [95% CI: 0.93–1.49], *P* < 0.001; Supplementary Figure 1, <http://links.lww.com/MD/A984>). Enhanced expression of Notch4 was not associated with OS in total NSCLC patients (HR = 1.02 [95% CI: 0.9–1.16], *P* = 0.72; Figure 2C), lung ADC patients (HR = 1.16 [95% CI: 0.92–1.47], *P* = 0.2; Figure 2D), and in lung SCC (HR = 0.92 [95% CI: 0.72–1.17], *P* = 0.49; Supplementary Figure 1, <http://links.lww.com/MD/A984>).

Notch Ligands

The relationship between Notch ligand expression and clinical outcomes in NSCLC was assessed (<http://kmplot.com/analysis/index.php?p=service&cancer=lung>). The following Affymetrix IDs are valid: 216268_s_at (JAG1), 209784_s_at (JAG2), 224215_s_at (DLL1), 219537_x_at (DLL3), and 223525_x_at (DLL4). As shown in Figures 3 and 4, Kaplan–Meier analysis suggested that high JAG1 was not linked with OS in total NSCLC or in lung SCC patients (HR = 0.95 [95% CI: 0.84–1.08], *P* = 0.45 [Figure 3A]; HR = 0.88 [95% CI: 0.7–1.12], *P* = 0.3 [Supplementary Figure 2, <http://links.lww.com/MD/A984>] respectively). However, higher expression of JAG1 predicted better OS in lung ADC patients (HR = 0.54 [95% CI: 0.42–0.69], *P* < 0.001; Figure 3B). The curves showed that high JAG2 mRNA expression was associated with poor OS in total NSCLC and in lung ADC patients (HR = 1.24 [95% CI: 1.09–1.4], *P* = 0.001; HR = 1.5 [95% CI: 1.19–1.89], *P* < 0.001, respectively; Figure 3C and 3D), but not in lung SCC patients (HR = 0.87 [95% CI: 0.68–1.1], *p* = 0.23; Supplementary Figure 2, <http://links.lww.com/MD/A984>). Enhanced expression of DLL1 was a marker for favorable lung ADC outcome (HR = 0.63 [95% CI: 0.49–0.8], *P* < 0.001; Figure 4B); enhanced expression of DLL1 was not a prognostic indicator in NSCLC or lung SCC (HR = 0.99 [95% CI: 0.84–1.17], *P* = 0.92 [Figure 4A]; HR = 1.02 [95% CI: 0.75–1.38], *P* = 0.92 [Supplementary Figure 2, <http://links.lww.com/MD/A984>]), respectively. High DLL3 mRNA expression correlated with poor OS in total NSCLC and lung ADC patients (HR = 1.31 [95% CI: 1.15–1.48], *P* < 0.001 [Figure 4C]; HR = 1.63 [95% CI: 1.29–2.06], *P* < 0.001 [Figure 4D], respectively). High DLL3 mRNA expression did not correlate with poor OS in lung SCC (HR = 1.18 [95% CI: 0.93–1.49], *P* = 0.18; Supplementary Figure 2, <http://links.lww.com/MD/A984>). From Figure 4E,

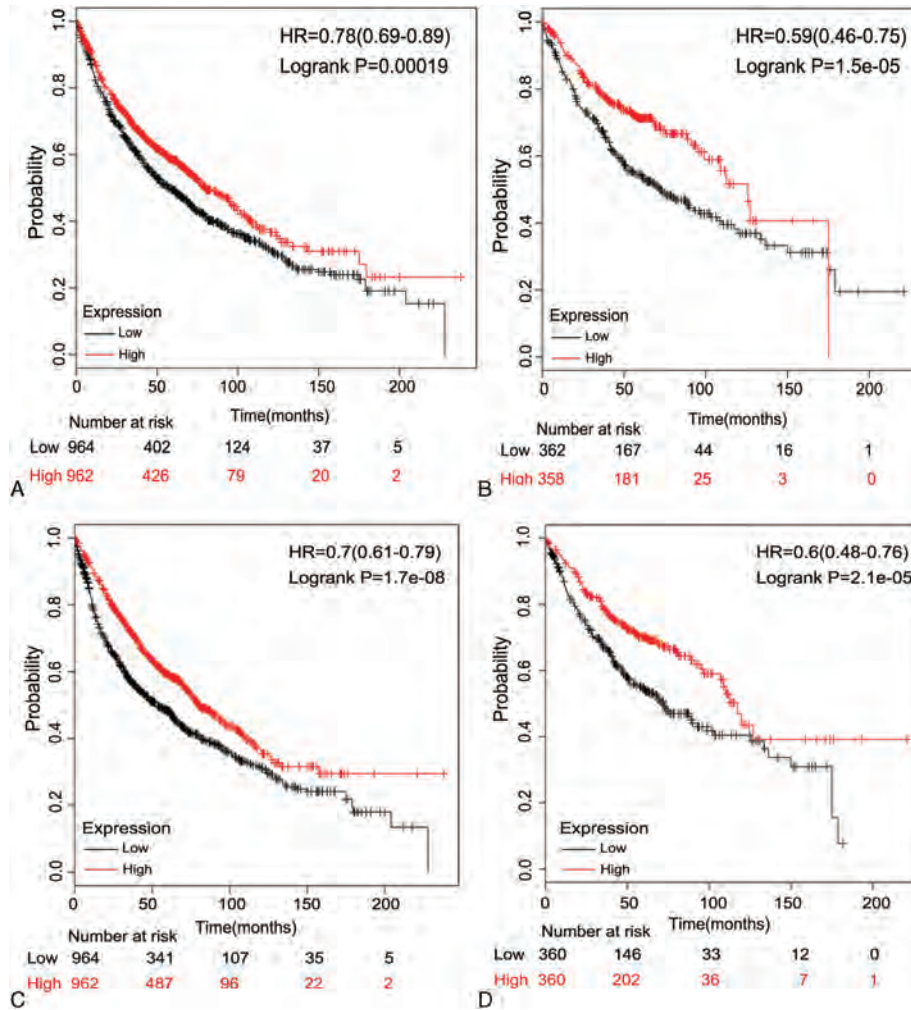


FIGURE 1. The prognostic value of Notch1 and Notch2 expression. The following Affymetrix IDs are valid: 218902_at (Notch1), 212377_s_at (Notch2). The Kaplan-Meier plots show that prognostic role of Notch1 in total NSCLC patients (A), in lung ADC patients (B). The prognostic value of Notch2 in total NSCLC patients (C), in lung ADC patients (D).

total NSCLC patients with elevated DLL4 expression had better OS (HR = 0.75 [95% CI: 0.64–0.88], $P < 0.001$). Nonetheless, high expression of DLL4 was not linked with OS in lung ADC (HR = 0.97 [95% CI: 0.76–1.24], $P = 0.81$; Figure 4F) and SCC patients (HR = 0.97 [95% CI: 0.71–1.32], $P = 0.85$; Supplementary Figure 2, <http://links.lww.com/MD/A984>).

Stratification Survival Analysis According to Clinicopathological Features and Treatments

Analysis was then considered according to various clinicopathological features and treatment groups. More specifically, in assessing the relationship between Notch signaling and OS of NSCLC patients, the data were stratified according to the tumor stage, pathological grade, smoking status, chemotherapy received, and radiotherapy received. As shown in Table 1, higher Notch1 mRNA expression corresponded with better prognosis in stage I and stage II of NSCLC patients. In contrast, elevated Notch2, JAG2, and DLL3 expression was associated with an unfavorable effect on OS in stage I NSCLC patients. The opposite was observed for Notch3 (Table 2). All

Notch receptor and ligand RNA levels were not correlated with OS in different grades of NSCLC patients. Higher Notch1 and Notch2 mRNA expression predicted better OS in patients with a history of smoking. Conversely, high Notch3 mRNA expression was linked with worse OS in patients with and without smoking history. From Table 2, high expression of JAG2 was associated with favorable outcome in patients who had never smoked. For NSCLC patients with a history of smoking, DLL3 overexpression predicted worse OS, which was in line with the unfavorable prognostic value of Notch3. There was no correlation between Notch receptor and ligand mRNA expression and OS in patients with NSCLC, based upon whether the patients received chemotherapy or radiotherapy or not.

DISCUSSION

A wealth of studies has focused on the progression and metastasis of NSCLC. Clinically, metastasis renders most NSCLC inoperable resulting in a dismal prognosis. From a clinical perspective, identifying new biomarkers of prognosis to

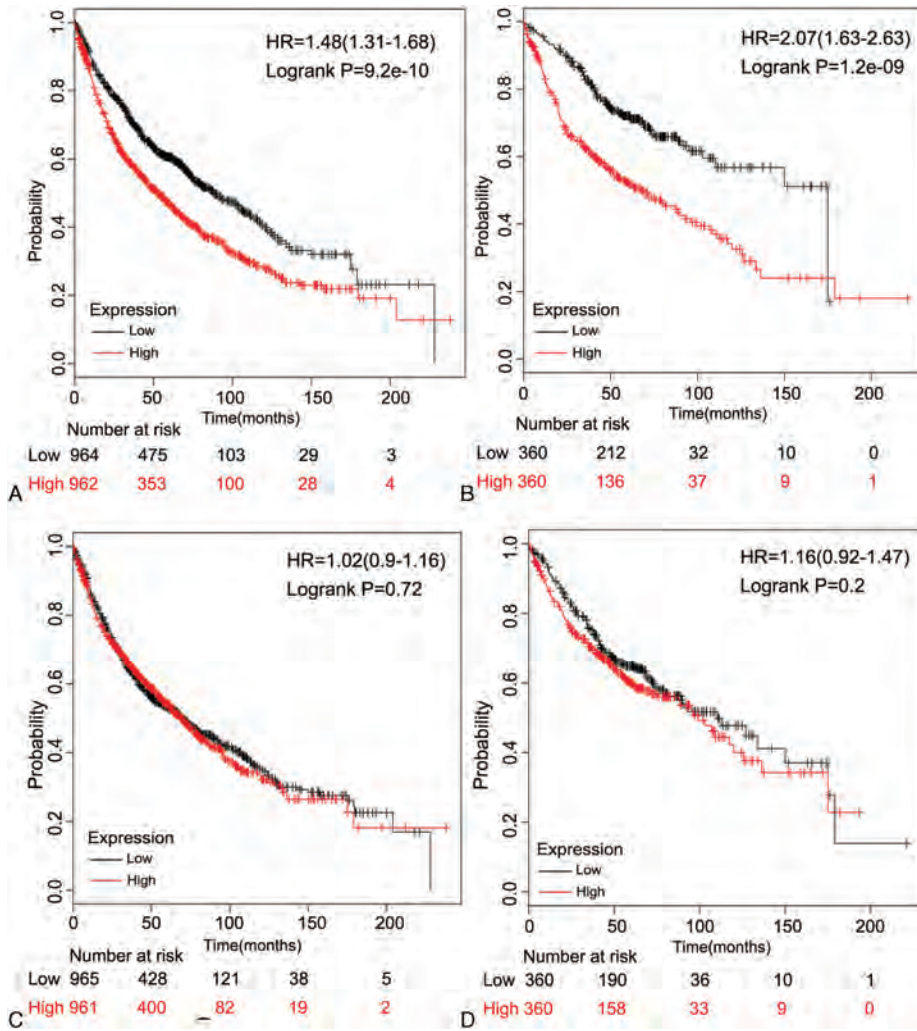


FIGURE 2. The prognostic value of Notch3 and Notch4 expression. The following Affymetrix IDs are valid: 203238_s_at (Notch3) and 205247_at (Notch4). The Kaplan-Meier plots show that prognostic role of Notch3 in total NSCLC patients (A), in lung ADC patients (B). The prognostic value of Notch4 in total NSCLC patients (C), in lung ADC patients (D).

guide surveillance is important and urgent. Recent data show that Notch signaling is an essential player in lung cancer growth, invasion, angiogenesis, and metastasis.^{37,38} Specifically, Notch1 inhibits lung tumor formation and JAG2 mediates ADC epithelial-mesenchymal transition and metastasis in mice.^{24,26} DLL4 is involved in angiogenesis in NSCLC.²⁹ Notch1 and Notch3 have been extensively studied in lung cancer. Little is known about the role of Notch2, Notch4, or Notch ligands in NSCLC.^{18,24} The present study introduces Notch receptors and ligands as candidate biomarkers for prognosis of NSCLC.

Notch1 signaling regulates cellular behavior in lung carcinoma.³⁹ Nonetheless, its role in lung cancer remains a controversial topic. Some studies found that overexpression of Notch1 inhibited lung carcinogenesis,^{28,40} or that DLL4 activated Notch1, which reduced lung tumor cell growth via PTEN signaling.²⁹ Others showed that high Notch1 activity maintained tumor growth. Licciulli et al⁴¹ reported that Notch1 was required for Kras-driven ADC through its regulation of p53 at a posttranslational level, whereas activation of Notch1

contributed synergistically with Myc to the genesis of ADC.⁴² Different NSCLC genetic contexts and microenvironmental conditions may lead to these contradictory findings.²⁶ Considering its crucial role in lung cancer development, it is not surprising that Notch1 modulation may be a target for lung cancer therapy. In a preclinical study, inhibitors of Notch blocked lung cancer cells growth.⁴³ Recent studies showed that activation of Notch1 conferred gefitinib resistance on lung cancer cells and that targeting Notch1 with inhibitors or siRNA restored sensitivity to gefitinib.⁴⁴ A few studies found that Notch1 overexpression correlated with worse prognosis in NSCLC patients.^{32,45} In the present study, enhanced Notch1 was associated with better outcome in lung ADC, consistent with the report by Wael et al³⁹ that enhanced Notch1 had an inhibitory effect on ADC but not on SCC cells.

Few studies have delineated a specific role for Notch2. A very recent article indicated that Notch2 deletion increased carcinogenesis in a Kras(G12D)-driven endogenous NSCLC mouse model, in which it functioned as a tumor suppressor.⁴⁶ The presently reported study, in line with the previous reports,

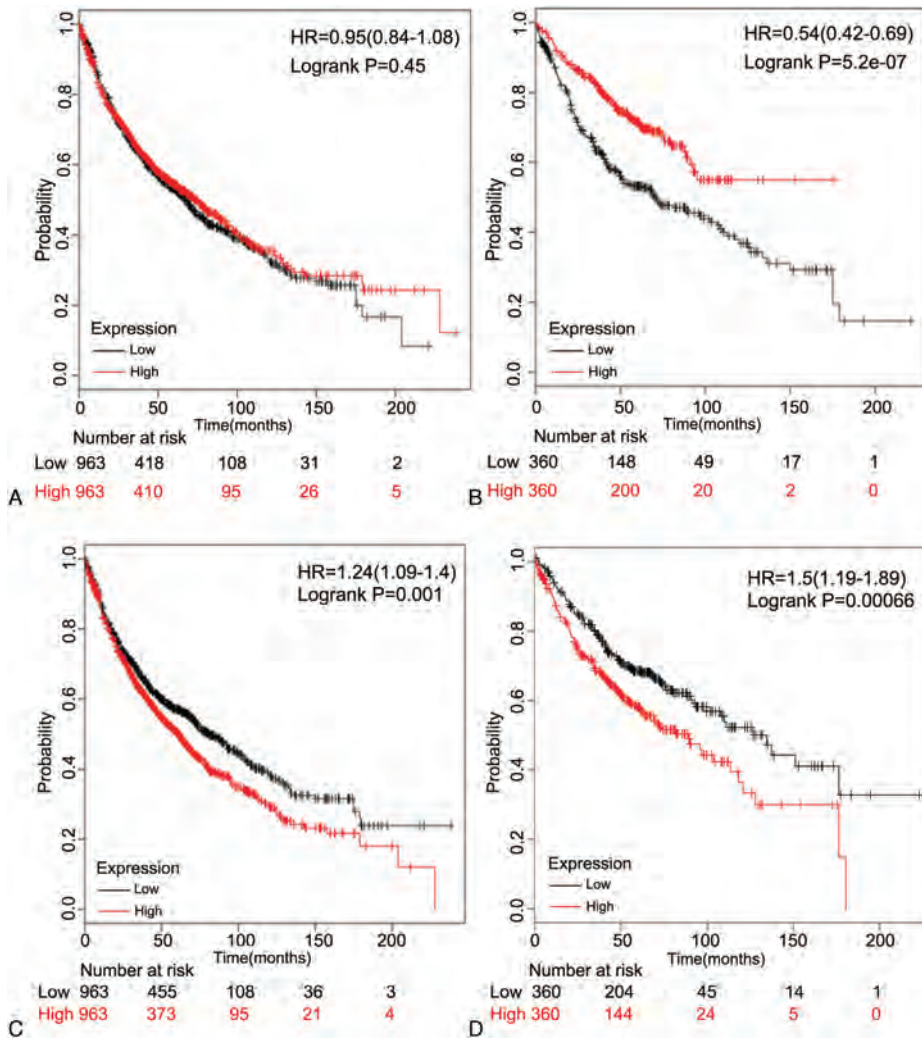


FIGURE 3. The prognostic value of JAG1 and JAG2 expression. The following Affymetrix IDs are valid: 216268_s_at (JAG1), 209784_s_at (JAG2). The Kaplan-Meier plots show that prognostic role of JAG1 in total NSCLC patients (A), in lung ADC patients (B). The prognostic value of JAG2 in total NSCLC patients (C), in lung ADC patients (D).

showed that high Notch1 expression was associated with better OS in NSCLC or lung ADC patients. Available evidence suggested that Notch3 plays an oncogenic role in lung carcinoma.⁴⁷ Overexpressed Notch3 was found in 40% of NSCLC and correlated with unfavorable survival outcome.^{47,48} The current report showed that higher Notch3 expression predicted poorer prognosis in NSCLC patients, reinforcing that concept. Haruki et al⁴⁹ found that Notch3 inhibition induced apoptosis and facilitated sensitivity to EGFR inhibitors in lung cancer cells. Konishi et al⁵⁰ also suggested that dual inhibition of Notch3 and EGFR pathways increased apoptosis and potentially circumvented chemoresistance.

Only a handful of studies report the prognostic role of Notch4, JAG1, JAG2, DLL1, DLL3, or DLL4 in NSCLC patients.⁵¹ The data presented here indicated that high expression of Notch4 and JAG1 was not connected to OS in NSCLC patients, but high DLL4 levels indicated worse survival outcome in NSCLC. Interestingly, higher JAG1 and DLL1 mRNA levels predicted better OS in lung ADC patients, in disagreement with the findings of cervical and breast cancer.^{51,52} The

lung ADC sample size in this study is relatively limited, perhaps accounting for this discrepant result. Further studies are therefore needed. One study reported DLL4 expression was uniformly low in 70 NSCLC cell lines by using Illumina arrays.⁵³ The data presented here showed that high DLL4 levels indicated favorable survival in NSCLC, which coincides with the previous study. In addition, the present data revealed that elevated JAG2 and DLL3 mRNA expression was significantly associated with poor OS in NSCLC patients. These observations may partially support the notion that high expression of JAG1 and DLL3 contributes to malignant progression of NSCLC. In current study, JAG1 and DLL1 have opposite prognostic value to JAG2 and DLL3. The mechanism of this phenomenon can be partially explained in a study by Choi et al.⁵³ In that study, JAG1 and JAG2 were mutually suppressive and regulated through distinct mechanisms. Compared with JAG2, JAG1 was more highly expressed in EGFR mutant lung cancer cells. EGFR regulated the expression of JAG1 but not JAG2. There is very little evidence to highlight the specific role for DLL family members in NSCLC. Therefore, more researches are needed to

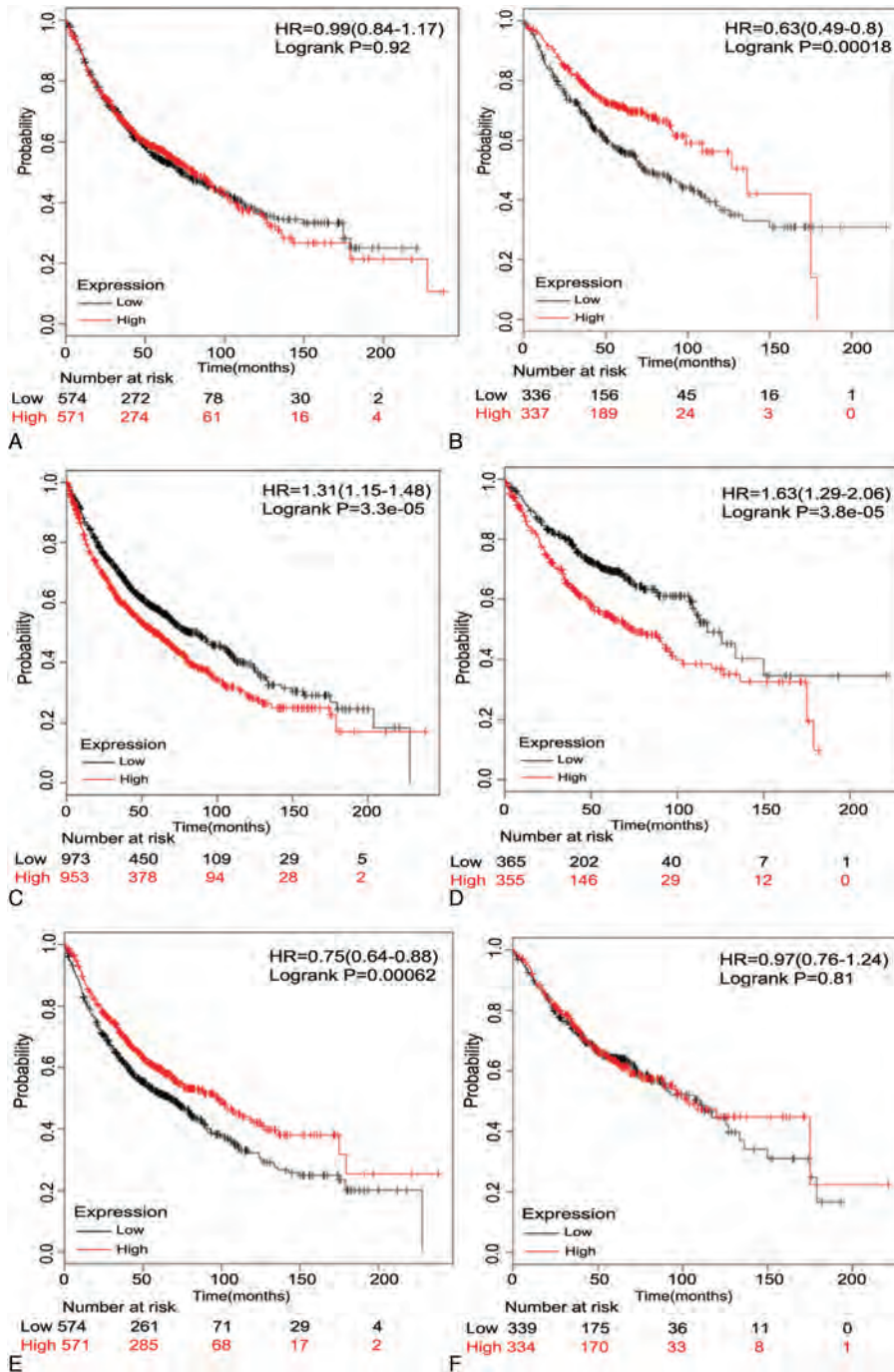


FIGURE 4. The prognostic value of DLL1, DLL3 and DLL4 expression. The following Affymetrix IDs are valid: 224215_s_at (DLL1), 219537_x_at (DLL3) and 223525_x_at (DLL4). The Kaplan-Meier plots show that prognostic role of DLL1 in total NSCLC patients (A), in lung ADC patients (B). The prognostic value of DLL3 in total NSCLC patients (C), in lung ADC patients (D). The prognostic value of DLL4 in total NSCLC patients (E), in lung ADC patients (F).

further explore the association between DLL family members and NSCLC survival and carcinogenesis. In agreement with previous meta-analysis,³¹ our results support that Notch3 and DLL3 are poor prognostic factors. However, in this study, Notch1 correlates with better prognosis, which is opposite to the previous study. Since previous meta-analysis reported that

Notch signaling protein expression level correlated with survival in patients with NSCLC, which enrolled literatures were immunohistochemistry-based. Whereas, the present study assesses the association of components of the Notch signaling with survival at the transcriptional level by using the on-line Kaplan-Meier plotter database. The contradictory prognostic

TABLE 1. Correlation of Notch Receptors With OS of NSCLC Patients Restricted by Different Clinicopathological Parameters

Clinicopathological Parameters	Notch1		Notch2		Notch3		Notch4	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Grades	I	0.82 (0.57–1.17)	1.1 (0.77–1.57)	0.6	1.42 (0.99–2.04)	0.054	1.45 (1.01–2.09)	0.044
	II	1.15 (0.84–1.57)	1.01 (0.74–1.38)	0.39	1.25 (0.91–1.71)	0.17	0.97 (0.71–1.33)	0.84
	III	0.58 (0.3–1.13)	0.57 (0.3–1.11)	0.1	1.6 (0.82–3.1)	0.16	1 (0.52–1.94)	1
Stages	I	0.54 (0.41–0.71)	0.38 (0.29–0.51)	<0.001	2.2 (1.67–2.91)	0.036	0.9 (0.71–1.21)	0.57
	II	0.52 (0.35–0.75)	0.83 (0.58–1.2)	<0.001	1.48 (1.02–2.13)	0.77	0.91 (0.63–1.31)	0.61
	III	1.04 (0.6–1.79)	1.05 (0.61–1.82)	0.89	1.08 (0.63–1.87)	0.57	0.77 (0.45–1.32)	0.34
Smoking	No	0.6 (0.34–1.06)	0.85 (0.49–1.48)	0.076	2.54 (1.39–4.67)	0.0018	1.03 (0.59–1.8)	0.9
	Yes	0.71 (0.58–0.88)	0.76 (0.62–0.94)	0.0016	1.53 (1.24–1.89)	<0.001	1.22 (0.99–1.49)	0.064
CT	No	1.08 (0.77–1.51)	1.03 (0.73–1.43)	0.66	1.34 (0.96–1.88)	0.085	1.18 (0.85–1.66)	0.32
	Yes	0.76 (0.5–1.16)	0.68 (0.45–1.04)	0.21	1.25 (0.83–1.88)	0.28	0.8 (0.54–1.2)	0.29
RT	No	1.31 (0.92–1.87)	0.95 (0.67–1.36)	0.14	1.14 (0.8–1.63)	0.47	1.28 (0.9–1.83)	0.17
	Yes	0.98 (0.57–1.69)	0.64 (0.37–1.11)	0.93	1.05 (0.62–1.79)	0.85	1.07 (0.63–1.81)	0.82

CI = confidence interval, HR = hazard ratio, CT = chemotherapy, NSCLC = non-small-cell lung cancer, OS = overall survival, RT = radiotherapy.

TABLE 2. Correlation of Notch Ligands With OS of NSCLC Patients Restricted by Different Clinicopathological Parameters

Clinicopathological Parameters	JAG1		JAG2		DLL1		DLL3		DLL4		
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	
Grades	I	1.29 (0.9–1.84)	0.17	0.96 (0.67–1.37)	0.83	NA	NA	1.23 (0.86–1.77)	0.25	NA	NA
	II	1.32 (0.96–1.81)	0.082	1.2 (0.88–1.64)	0.26	NA	NA	0.92 (0.67–1.26)	0.61	NA	NA
	III	1.08 (0.56–2.07)	0.83	0.94 (0.49–1.81)	0.85	NA	NA	0.94 (0.49–1.81)	0.86	NA	NA
Stages	I	0.89 (0.68–1.16)	0.39	1.52 (1.16–1.99)	0.0024	0.71 (0.52–0.97)	0.03	2.32 (1.75–3.07)	<0.001	0.74 (0.54–1.01)	0.057
	II	0.71 (0.49–1.03)	0.07	1.39 (0.97–2.01)	0.075	0.92 (0.58–1.44)	0.71	1.47 (1.02–2.11)	0.039	0.94 (0.59–1.48)	0.78
	III	0.8 (0.46–1.38)	0.42	0.9 (0.52–1.54)	0.69	0.93 (0.47–1.85)	0.84	1.25 (0.73–2.16)	0.41	1.22 (0.61–2.47)	0.57
Smoking	No	0.46 (0.25–0.83)	0.0088	1.59 (0.9–2.8)	0.11	1.13 (0.75–1.69)	0.55	1.8 (1.01–3.21)	0.042	2.62 (1.11–6.16)	0.023
	Yes	0.94 (0.76–1.15)	0.54	1.17 (0.95–1.44)	0.14	0.26 (0.1–0.67)	0.0025	1.41 (1.15–1.74)	0.0011	1.31 (0.87–1.96)	0.19
CT	No	1.28 (0.91–1.78)	0.15	1.33 (0.95–1.86)	0.096	2.48 (0.45–13.72)	0.28	0.97 (0.69–1.35)	0.84	0.76 (0.15–3.81)	0.73
	Yes	1.46 (0.96–2.22)	0.079	1.18 (0.79–1.78)	0.42	0.58 (0.18–1.85)	0.35	1.08 (0.72–1.62)	0.7	1.82 (0.54–6.16)	0.33
RT	No	1.05 (0.74–1.5)	0.78	1.27 (0.89–1.82)	0.18	NA	NA	0.94 (0.66–1.35)	0.75	NA	NA
	Yes	1.11 (0.65–1.9)	0.7	1.12 (0.65–1.93)	0.67	NA	NA	0.96 (0.57–1.63)	0.88	NA	NA

CI = confidence interval, HR = hazard ratio, CT = chemotherapy, NSCLC = non-small-cell lung cancer, OS = overall survival, NA = not available, RT = radiotherapy.

findings may be because of the differences in Notch1 mRNA/protein stability or the differences in post-transcriptional regulation.⁵¹ Remarkably, restriction of the analysis to individual histological subtypes indicated that the prognostic effect of mRNA expression of Notch components in lung ADC differed from that in lung SCC patients. The results corroborate previous studies reporting that Notch1 has no significant impact on prognosis of SCC.^{30,39} The variations may be because of inherent heterogeneity of subtypes and the complex nature of Notch signaling, suggesting that the individual so the features of various subtypes should be considered when developing Notch-targeted therapies in lung cancer.

The results of present study showed Notch receptor and ligand levels could be used as prognostic predictors in NSCLC patients. Among the patients, especially in lung ADC, not in lung SCC patients, higher Notch1, Notch2, JAG1, and DLL1 mRNA expressions were associated with better OS; enhanced Notch3, JAG2 and DLL3 mRNA expressions were associated with a poor survival. Notch4 or DLL4 expression was not linked with OS for lung ADC or lung SCC patients. These findings indicated that, except for Notch4, Notch receptor and ligand levels could be used as promising new markers to predict prognosis and improve individual treatment strategies in NSCLC patients. Importantly, Notch-targeted therapy for lung cancer should be based on specific receptors and ligands. Also, the subtypes of lung cancer should be thoroughly considered.

In the present study, although the sample size was relatively large, several limitations still exist, and some findings need to be interpreted cautiously. The study was performed using the retrospective Kaplan–Meier plotter database. A future prospective and multicenter trial may be needed to validate these results. It was not possible to assess the prognostic effect of Notch signaling in stage IV patients because of the limitation of sample size. Furthermore, the role of Notch signaling in large-cell carcinoma was not determined. Information on more varied subtypes is required to confirm these data.

CONCLUSIONS

In summary, the set of Notch1, Notch2, JAG1, and DLL1 and the set of Notch3, JAG2, and DLL3 play opposite prognostic roles in lung ADC patients. Notch components are not indicators of prognosis in lung SCC. Notch receptor and ligand levels could be used as promising markers to predict prognosis in lung ADC patients. Most notably, targeting Notch receptors and ligands represent a potentially novel therapeutic strategy. The distinct prognostic association of Notch components on lung cancer outcomes warrants additional investigations to elucidate the related mechanisms.

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