Title: Analysis of DLL3 and ASCL1 in surgically resected small cell lung cancer (HOT1702)

Running Title: Analysis of DLL3 and ASCL1 in SCLC (HOT1702)

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Keywords: small cell lung cancer, delta-like protein 3 (DLL3), achaete-scute homolog-1 (ASCL1), immunohistochemistry, surgery

Abstract

Background: Delta-like protein 3 (DLL3) is a Notch ligand that has an important role in the tumorigenesis of small cell lung cancer (SCLC). Recently, rovalpituzumab tesirine (Rova-T), a DLL3-targeted antibody-drug conjugate, has been developed for treating SCLC. DLL3 is a transcriptional target of the achaete-scute homolog-1 (ASCL1) transcription factor, which is involved in pulmonary neuroendocrine cell development. However, the relationship between DLL3 and/or ASCL1 expression and the clinical features of SCLC remains unknown, especially for early-stage resected SCLC. This study aimed to investigate the expression of DLL3 and ASCL1 in resected SCLC samples using immunohistochemical analysis.

Materials and Methods: We collected 95 surgically resected SCLC samples, which were formalin-fixed and paraffin-embedded. Immunohistochemistry staining was performed to investigate the correlation between the expression of either DLL3 or ASCL1 and clinicopathological features of study patients.

Results: Seventy-seven (83%) of 93 immunohistochemically evaluable samples were positive for DLL3 (expression in ≥ 1% of tumor cells), and DLL3-high expression (≥ 75%) was observed in 44 samples (47%). Sixty-one (64%) of 95 samples were positive for ASCL1 (expression in ≥ 5% of tumor cells). A positive correlation was observed between DLL3 and ASCL1 expression. DLL3 and ASCL1 expression were not

associated with survival in SCLC patients. DLL3 was more prevalent in patients with advanced clinical disease.

Conclusion: DLL3 and ASCL1 were highly expressed in surgically resected SCLC patients. DLL3 and ASCL1 may be targets for the treatment of SCLC.

Implications for Practice

This study examines the relationship between Delta-like protein 3 (DLL3) and achaete-scute homolog-1 (ASCL1) protein expression with the clinical features of 95 surgically resected small cell lung cancer (SCLC). DLL3 is attracting attention, because rovalpituzumab tesirine (Rova-T), a DLL3-targeted antibody-drug conjugate has been developed recently. DLL3 and ASCL1 were highly expressed in surgically resected SCLC patients. DLL3 and ASCL1 may be targets for the treatment of early-stage SCLC, including with Rova-T.

GAP between current and best practice and learning objectives

BEST PRACTICE	**CURRENT PRACTICE**	**RESULTING GAPS**	**LEARNING OBJECTIVES**
Rovalpituzumab tesirine (Rova-T), a Delta-like protein 3 (DLL3)-targeted antibody-drug conjugate, has been developed for treating SCLC.	Most SCLC patients initially respond to chemotherapy and radiotherapy but usually relapse and acquire resistant disease. The	Rova-T is promising therapy for SCLC, there were few studies of the relationship between DLL3 expression and the clinical features of	In our 95 surgically resected SCLC tumors, Delta-like protein 3 (DLL3) and achaete-scute homolog-1 (ASCL1) were highly expressed,

DLL3 is a transcriptional target of the achaete-scute homolog-1 (ASCL1) transcription factor, which is involved in pulmonary neuroendocrine cell development. Analysis of DLL3 and ASCL1 expression will provide the potential predictive biomarkers and targeted therapies for SCLC.	prognosis of patients with SCLC remains poor, and they frequently require multiple treatments with no targeted therapies approved for treatment and no identified molecular biomarkers.	SCLC, especially for resected tumor samples which they can provide precise histologic information, but difficulty in obtaining. This study aimed to investigate the expression of DLL3 and its upstream target, ASCL1 in resected SCLC samples using immunohistochemical analysis. This study will clear the relationship between DLL3 and	and DLL3 expression was related to lymph node metastasis and advanced cStage.
			A significant correlation between DLL3 and ASCL1 protein expression was observed.
			DLL3 and ASCL1 may be mainstream targets in the treatment of early-stage SCLC, including

		ASCL1 and contribute to the development for DLL3 targeted therapies, including Rova-T.	rovalpituzumab tesirine (Rova-T).

Introduction

Lung cancer is the leading cause of cancer-related death worldwide, with small cell lung cancer (SCLC) accounting for approximately 15% of lung cancer cases [1, 2]. Most SCLC patients initially respond to chemotherapy and radiotherapy but usually relapse and acquire resistant disease. The prognosis of patients with SCLC remains poor, and they frequently require multiple treatments[3].

The Notch signaling pathway regulates tumorigenesis and can be either oncogenic or tumor-suppressive, depending on the cellular context [4, 5]. Regarding SCLC, overexpression of Notch1 induces G1 cell cycle arrest [6] and the Notch target gene *HES1* inhibits the neuroendocrine transcription factor achaete-scute homolog-1 (ASCL1) [7, 8], which suggests that Notch can act as a tumor suppressor in SCLC.

In mammals, there are 4 Notch receptors (Notch1 to Notch4) and 2 families of ligands, jagged (JAG1 and JAG2) and delta-like ligands (DLL1, DLL3, and DLL4) [9]. Unlike the other activating DLL ligands, DLL3 does not bind or activate Notch receptors when presented in trans, but instead inhibits Notch signaling in cis [10]. Moreover, DLL3 is regulated directly by ASCL1 [11, 12], which is essential for development in several types of neuroendocrine cells [13-15] and correlates to tumorigenicity in SCLC [16, 17]. Altogether, ASCL1-induced DLL3 expression might be associated with neurogenesis and SCLC carcinogenesis through modulating the Notch pathway.

Rovalpituzumab tesirine (Rova-T; AbbVie Inc., North Chicago, IL), a recently developed DLL3-targeted antibody-drug conjugate, comprises a humanized anti-DLL3 monoclonal antibody conjugated to a DNA-damaging pyrrolobenzodiazepine dimer toxin. It has anti-tumor efficacy *in vivo* [18], and a recent phase I study found that patients with high DLL3 expression had better objective responses than those with low expression, suggesting that DLL3 expression is a potential predictive biomarker for SCLC outcomes [19].

Although growing evidence suggests that DLL3 has a pivotal role in SCLC, little is known about the prognostic influence of DLL3 and the association between protein expression of DLL3 and ASCL1, especially in early-stage SCLC, due to the difficulty in obtaining surgical samples. Furthermore, obtaining tumor specimens is important as they can provide precise histologic information, such as pure SCLC or combined SCLC.

Therefore, the aim of this study was to investigate the expression of DLL3 and ASCL1 in resected SCLC samples using immunohistochemical analysis.

Materials and methods

Patient data

We included patients with primary SCLC who had undergone complete surgical resection of a primary lung tumor between January 2003 and January 2013 at institutions participating in either the Fukushima Investigative Group for Healing Thoracic Malignancy (FIGHT) or the Hokkaido Lung Cancer Clinical Study Group Trial (HOT).[20] Patients were centrally re-reviewed for a confirmed pathological diagnosis of pure SCLC or combined SCLC, according to the 2004 World Health Organization classification [21]. Written informed consent was obtained from patients who were still alive at the time of data accrual (from February 2013 through January 2014). The study was registered with the University Hospital Medical Information Network Clinical Trials Registry (identification number, UMIN000010117) and was approved by the Institutional Review Board of each participating institution. All individual data were obtained from medical records and de-identified. An unidentifiable code number was assigned to each tissue sample. Stages were determined or reclassified according to the 7th edition of the TNM staging system [22].

Tissue samples

Between January 2003 and January 2013, 156 patients were enrolled from 17 institutions. Ninety-five of 156 samples from 11 institutions had sufficient material to assess protein expression of DLL3 and ASCL1 by immunohistochemistry (IHC), along with patient metadata and pathology reports.

IHC

DLL3 expression was assessed in 5 µm sections cut from formalin-fixed and paraffin-embedded SCLC tissue blocks using anti-DLL3 antibody (SP347 Spring Bioscience, Pleasanton, CA). Briefly, SP347 was used at 0.24 µg/mL, followed by OptiView DAB IHC kit (Ventana, Tucson, AZ) to visualize DLL3 expression. ASCL1 expression was detected by the Vectastain ABC HRP kit (Vector Laboratories, Burlingame, CA) with a mouse monoclonal antibody (SC72.201, AbbVie Stemcentrx, South San Francisco, CA). Stained slides were then observed under a light microscope to assess positivity. DLL3 positivity was scored at 4× magnification for any cytoplasmic or membranous staining at any intensity in total tumor cells as previously described.[19] DLL3-high was defined as greater than or equal to 75% stained tumor cells, as used in the ongoing Rova-T clinical trial and DLL3-low was defined as less than 75% positivity. DLL3-positive was defined as greater than or equal to 1% stained tumor cells. ASCL1 positivity was defined as nuclear staining in greater than or equal to 5% of all tumor cells.[23, 24] DLL3 and ASCL1 scoring was only assessed in histologically SCLC cells, not in cancer cells histologically characterized as squamous cell carcinoma, adenocarcinoma, or large cell carcinoma components. Notch1 protein expression from previously reported data was used for analysis [25].

Statistical analysis

The correlation between DLL3 or ASCL1 expression and categorical variables were analyzed using the chi-square test or Fisher's-exact test, as appropriate. Survival curves were estimated using the Kaplan-Meier method, and differences in survival distributions were evaluated using the log-rank test. The inter-relationship between DLL3 and ASCL1 or Notch1 were analyzed using Spearman's rank analysis. The level of significance was set at $P < 0.05$. Statistical analyses were conducted with JMP software (JMP® Pro version 11.0.0, SAS Institute Inc, Cary, NC).

Results

DLL3 and ASCL1 expression in SCLC

Patient characteristics are shown in Table 1. The median patient age was 70 years, 74 patients (77.9%) were male, and 81 patients (85.2%) were current or former smokers. The numbers of patients with pure SCLC and combined SCLC were 66 (69.5%) and 29 (30.5%), respectively. Seventy-one, 13, and 11 patients had clinical disease stage (cStage) I, II, and III (TNM, 7th edition), respectively.

Ninety-three and 95 samples were evaluable for DLL3 and ASCL1 expressions by IHC staining, respectively. Representative images of DLL3 and ASCL1 staining and histograms detailing the distribution of expression are shown in Figure 1. DLL3 was localized in the cytoplasm and membrane of tumor cells, while ASCL1 was localized in

the nucleus (Figure 1C). Seventy-seven out of 93 patients (83%) with any stage and 66 of 82 patients (80%) with cStage I/II were positive for DLL3 staining in at least 1% of tumor cells. Forty-four patients (47%) with any stage and 35 patients (43%) with cStage I/II showed DLL3-high expression (expression ≥75% of tumor cells) (Table 2). Sixty-one of 95 patients (64%) with any stage and 52 of 84 patients (62%) with cStage I/II showed positive expression for ASCL1 in at least 5% of tumor cells (Table 2).

A positive correlation was observed between DLL3 and ASCL1 expression ($R = 0.723$, $P < 0.0001$) (Figure 2). As shown in Figure 1A–C, DLL3 and ASCL1 were expressed in similar regions of the tumor specimen and co-expressed in the majority of tumor cells. Although DLL3 is a Notch ligand and ASCL1 is suppressed by Notch target genes,[7, 8] there were no correlations between DLL3 or ASCL1 expression with Notch1 expression using the tumor specimens from our previous study (Supplementary Figure S1).[25] Moreover, we obtained data regarding the expression of neuroendocrine (NE)-specific proteins, including chromogranin A, synaptophysin, and CD56, based on pathological reports of each surgical specimen when possible. There was significant correlation between the expression of ASCL1 and synaptophysin ($P = 0.001$), but there was no correlation between the expression of DLL3 and NE-specific proteins (Supplementary Table S1).

Correlation between DLL3 or ASCL1 expression and clinicopathological features

Next, we evaluated the association of clinicopathological features with high expression of DLL3 and expression of ASCL1. DLL3-high expression was significantly more prevalent in patients with clinical lymph node status (cN) 2–3 than in those with cN 0–1 (P = 0.006; Fisher's-exact test) and in patients with cStage III/IV than in those with cStage I/II (P = 0.022; Fisher's-exact test) (Table 3). No other clinicopathological features were correlated with DLL3 expression. ASCL1 expression was correlated with pure SCLC histology (P = 0.003) (Table 3).

Prognostic value of DLL3 and ASCL1 expression

In the analysis of all study patients, the overall survival was 24.4 months in the DLL3-high group and 33.3 months in the DLL3-low group, with no significant difference existing between these 2 groups in terms of survival (P = 0.160) (Figure 3A). To exclude any bias of cStage in the DLL3 expression analysis, we analyzed patients with cStage I/II or cStage III/IV separately. Similar to the analysis of all patients, there was no correlation between DLL3 positivity and overall survival in patients with cStage I/II (P = 0.182) or with cStage III/IV (P = 0.641) (Figure 3C and Supplementary Figure S2A). ASCL1 expression was not associated with survival in the entire study population (P = 0.096), in patients with pure SCLC (P = 0.111), and in patients with cStage I/II (P = 0.139) (Figure 3B and D and Supplementary Figure S2B).

Discussion

In this study, we demonstrated that both DLL3 and ASCL1 were highly expressed in patients with SCLC, with approximately half of these patients having DLL3-high expression levels. Moreover, there was positive correlation between the expression of DLL3 and ASCL1. This is the first study to analyze the DLL3 and ASCL1 expression levels in surgically resected, early-stage SCLC tumor specimens.

In our study, DLL3-high expression levels were observed in 44 (47%) of the surgically resected specimens, which was similar to the expression rates of 67%, which was determined in a phase I trial of Rova-T [19], and 32%, which was shown in a DLL3 IHC study from Japan [26]. In these studies, an anti-DLL3 mouse monoclonal antibody was used and the cut-off rate was 50%, whereas in our study an anti-DLL3 rabbit monoclonal antibody was used and the cut-off rate was 75%, which was in-line with the protocol being used in several ongoing Rova-T clinical trials. One study [18] evaluated DLL3 expression of normal lung samples, lung squamous cell carcinoma, lung adenocarcinoma, large cell neuroendocrine cell carcinoma (LCNEC), and SCLC. No normal lung specimen or lung squamous cell carcinoma tumor cells stained positively and 3 of 82 (3.7%) lung adenocarcinoma had DLL3 expression, whereas 37 of 57 (65%) LCNEC, 120 of 167 (72%) treatment-naïve SCLC, and 17 of 20 (85%) recurrent and treatment-refractory SCLC had DLL3 expression, respectively. Furthermore, aside from SCLC, DLL3 protein expression was also strongly correlated with ASCL1 protein

expression in small cell bladder cancer, and DLL3 was positive (expression in ≥ 1% of tumor cells) in 68% of patients, with 58% having expression in > 10% of tumor cells [27]. Collectively, these findings indicate that DLL3 is highly expressed in small cell cancers, whereas its expression is low in the normal lung or non-small cell lung cancer. Because DLL3-high expression was correlated to the response to Rova-T treatment in a phase 1 trial [19], the results presented in several studies might be helpful for the development of DLL3-targeted therapy. However, a phase 3 trial assessing the efficacy of Rova-T versus topotecan as a second-line treatment in advanced SCLC patients with high levels of DLL3 resulted in stopping enrollment due to the shorter survival in the Rova-T arm [28]. Other clinical studies for Rova-T as first-line maintenance therapy (NCT03033511) or combination therapy with immunotherapy (NCT03026166) are also ongoing. We need further data from ongoing studies to discuss the efficacy of Rova-T in SCLC.

We showed that the expression of DLL3 was not related to SCLC patient survival in our study population, which is consistent with another report from Japan that evaluated DLL3 expression in SCLC patients with limited disease or extensive disease [26]. DLL3 itself may have little impact on the survival of SCLC patients based on those data; however, SCLC patients with DLL3-high or ASCL1-positive expression tended to have worse survival. Therefore, adjuvant therapy with Rova-T might have potential for patients with DLL3-high or ASCL1-positive expression. Our study showed that DLL3-high expression was more prevalent in patients with lymph node metastasis and advanced

cStage. However, the numbers of patients with advanced cStage was small; therefore, more investigation into this topic is required.

ASCL1 has been shown to promote tumor growth and is a therapeutic target for lung cancer with NE features [11, 12]. Moreover, DLL3 promotes the growth of murine lung cancer cells [29]; however, there are few analyses regarding the role of DLL3 in the tumorigenesis of human lung cancer. It has already been reported that DLL3 is a downstream target of ASCL1 [11, 12] and that the mRNA expression of both genes is highly correlated [18]. We also found that there was a positive correlation between DLL3 and ASCL1 protein expression, which supports the above previous results.

SCLC can be pathologically divided into pure SCLC and combined SCLC.[21] In this study, ASCL1 was expressed more in pure SCLC samples than in combined SCLC samples. To the best of our knowledge, there are no reports analyzing differences in ASCL1 expression between pure SCLC and combined SCLC samples, partly due to the difficulty in obtaining large tissue samples from patients, since surgery is rarely performed in SCLC cases. The genetic or molecular difference between pure SCLC and combined SCLC remains unknown. Moreover, the cell origin of combined SCLC remains unclear. SCLC components and non-SCLC components in combined SCLC have been reported to share almost 75% common mutations and showed similar genetic background, suggesting that the SCLC components and non-SCLC components are derived from common precursors [30]. Moreover, this also implies that one component of combined

SCLC arises from other components with subsequent acquisition of oncogenic change and microenvironment in combined SCLC [30]. In our study, ASCL1 expression was lower in combined SCLC, and we hypothesize that ASCL1 expression may be lost during oncogenic change and through different microenvironments in combined SCLC. Recent molecular studies on SCLC cell lines suggest that SCLC could comprise 2 distinct subgroups with different expression patterns of ASCL1 and NEUROD1, which are associated with classic and variant SCLC, respectively, and which may distinguish SCLC heterogeneity using different genetic programs [31-34]. These differences may influence therapeutic outcomes. For example, there have been reports that ASCL1- enriched tumors were more sensitive to Bcl2 inhibition, whereas MYC-driven variant tumors were more sensitive to an Aurora kinase inhibitor [32, 34, 35]. Pathological classification has no impact on the current treatments for SCLC, the relationship between pathological classification and subtypes in human SCLC cell lines remains unknown. However, the correlation between pure SCLC pathology and ASCL1 in this study indicates that pure SCLC possesses features of classic SCLC and may allow for multiple therapeutic options based on the expression levels of ASCL1.

DLL3 protein expression was significantly higher in *Myc* wild-type genetically engineered mouse models than that in *Myc* overexpressing model. TTF-1 is also highly expressed in DLL3-positive human SCLC samples [35]. ASCL1 expression is higher in *Myc* wild-type models and in human SCLC samples with MYC-low and TTF-1 high

expression [34, 35]. Moreover, inactivating Notch mutations were detected in human SCLC samples and induced ASCL1 activation [36]. These studies show that molecules, such as MYC, TTF-1, Notch, modulate DLL3 or ASCL1 and might contribute to treatment response or survival in SCLC.

This study has some limitations, including the small sample size (n = 95), and its retrospective, non-global design. Moreover, there was a limited number of deaths (n = 57, 60%). Various treatment regimens were used in a heterogeneous patient population, and it could introduce another bias.

Conclusion

DLL3 and ASCL1 were highly expressed in SCLC tumors, and DLL3 was related to lymph node metastasis and advanced cStage. A statistically significant correlation between DLL3 and ASCL1 expression was observed in our IHC analysis. Our data suggest that DLL3 and ASCL1 may be mainstream target in the treatment of SCLC.

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Conflicts of interest

All authors declare no conflicts of interest.

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Figure Legends

Figure 1. DLL3 and ASCL1 expression in SCLC.

(A) DLL3 protein expression levels, as determined by immunohistochemistry (IHC), in representative samples of positive (98%) and negative (0%) tumor specimens. (B) ASCL1 positive (95%) and negative (0%) tumor specimens. SCLC specimens were stained with an anti-DLL3 or anti-ASCL1 antibody (scale bars, 100 μm, right corner inset, lower magnification images). (C) Subcellular location of DLL3 and ASCL1 expression (red arrow, positive cell; yellow arrow, negative cell). (D) Histogram illustrating the distribution of DLL3 expression of any intensity in the 93 SCLC tumor specimens (arrow, proposed cut-off value for high DLL3 expression). (E) Histogram illustrating the distribution of ASCL1 expression of any intensity in the 95 SCLC tumor specimens (arrow, proposed cut-off value for positive expression of ASCL1).

DLL3, delta-like protein 3; SCLC, small cell lung cancer; ASCL1, achaete-scute homolog-1

Figure 2. Relationship between the expression of DLL3 and ASCL1.

DLL3 and ASCL1 expression levels in SCLC tumor specimens showed a significant correlation ($R = 0.723$, $P < 0.0001$).

DLL3, delta-like protein 3; SCLC, small cell lung cancer; ASCL1, achaete-scute homolog-1

Figure 3. Kaplan-Meier survival curves of all study patients.

Survival curves for all SCLC patients who underwent surgical resection, stratified by the expression of (A) DLL3 and (B) ASCL1 (n = 93 and n = 95, respectively). (C) Survival curves for SCLC patients with cStage III/IV who underwent surgical resection were stratified by the expression of DLL3 (n = 11). (D) Survival curves for pure SCLC patients who underwent surgical resection were stratified by the expression of ASCL1 (n = 41).

DLL3, delta-like protein 3; SCLC, small cell lung cancer; ASCL1, achaete-scute homolog-1

Supplementary Figure Legends

Figure S1. Relationship between the expression of DLL3 or ASCL1 and Notch1.

(A) DLL3 or (B) ASCL1 and Notch1 expressions in SCLC tumor specimens showed no correlation (R = 0.106, P = 0.314 and R = 0.140, P = 0.276, respectively).

DLL3, delta-like protein 3; SCLC, small cell lung cancer; ASCL1, achaete-scute homolog-1

Figure S2. Kaplan-Meier survival curves of patients with clinical disease stage (cStage) I/II in this study.

Survival curves for SCLC patients with cStage I/II who underwent surgical resection were stratified by the expression of (A) DLL3 and (B) ASCL1 (n = 82 and n = 84, respectively). DLL3, delta-like protein 3; SCLC, small cell lung cancer; ASCL1, achaete-scute homolog-1

Table 1. Clinical characteristics of patients included in this study.

Characteristics	Patients (n = 95)	
	No.	**%**
Age, median (range in years)	70 (52-83)	NA
Sex		
Male	74	77.9
Female	21	22.1
Smoking status		
Never-smoker	7	7.4
Smoker (current or former)	81	85.2
Unknown	7	7.4
ECOG PS[a]		

0	60	63.2
1	28	29.5
Unknown	7	7.4
Histology		
SCLC[b]	66	69.5
Combined SCLC	29	30.5
cStage[c]		
I	71	74.7
II	13	13.7
III	11	11.6
Adjuvant chemotherapy		
Yes	60	63.2
No	35	36.8

[a] Eastern Cooperative Oncology Group performance status

[b] Small cell lung cancer

[c] Clinical disease stage

Table 2. Data are number of patients according to rate of DLL3 or ASCL1 expression.

	All patients	Patients with cStage[a] I/II
	No. of patients (%)	
DLL3 positive cells/total cancer cells		
≥ 1%	77/93 (83%)	66/82 (80%)
≥ 75%	44/93 (47%)	35/82 (43%)
ASCL1 positive cells/total cancer cells		
≥ 5%	61/95 (64%)	52/84 (62%)

[a]Clinical disease stage

Table 3. Relationship between expression of DLL3 or ASCL1 and clinical and clinicopathological characteristics.

Characteristics	DLL3 expressions (n = 93)			ASCL1 expressions (n = 95)		
	No. of Patients			No. of Patients		
	Low	High	*P*	Negative	Positive	*P*
Age (years)						
<65	13	17	0.268	13	18	0.494
≥65	36	27		21	43	
Sex						
Male	37	35	0.805	27	47	1.000
Female	12	9		7	14	
Smoking (pack-years)						
≥ 20	38	37	0.526	26	51	0.198

< 20	7	4		6	5	
Unknown	4	3		2	5	
ECOG PS[a]						
0	31	28	1.000	22	38	0.812
1	14	13		9	19	
Unknown	4	3		3	4	
Histology						
SCLC[b]	33	31	0.824	17	49	0.003
Combined SCLC	16	13		17	12	
cT[c]						
T1-2	44	43	0.208	30	59	0.183
T3-4	5	1		4	2	

cN[d]						
N0-1	48	35	0.006	32	53	0.323
N2-3	1	9		2	8	
cStage[e]						
I-II	47	35	0.022	32	52	0.317
III-IV	2	9		2	9	
Adjuvant chemotherapy						
Yes	33	26	0.518	21	39	0.829
No	16	18		13	22	

[a] Eastern Cooperative Oncology Group performance status

[b] Small cell lung cancer

[c] Clinical tumor classification

[d] Clinical lymph node status

[e] Clinical disease stage

Fig. 1a

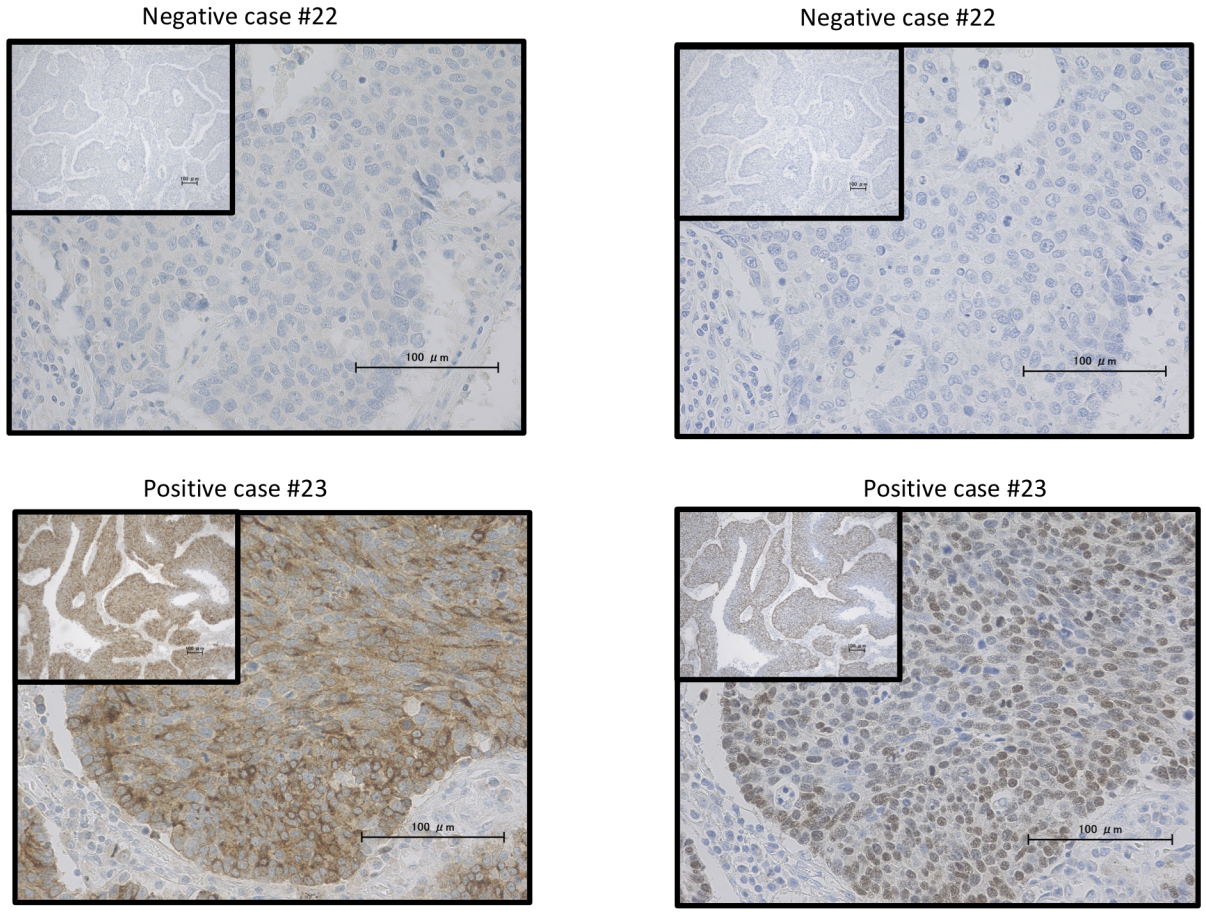


Fig. 1b

Fig. 1c

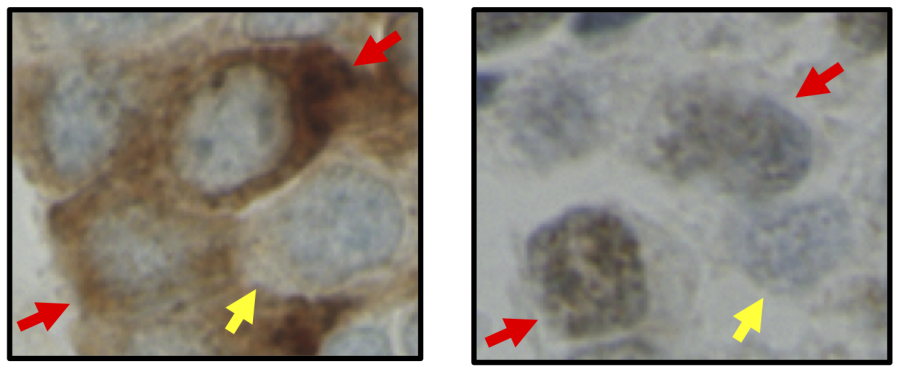


Fig. 1d

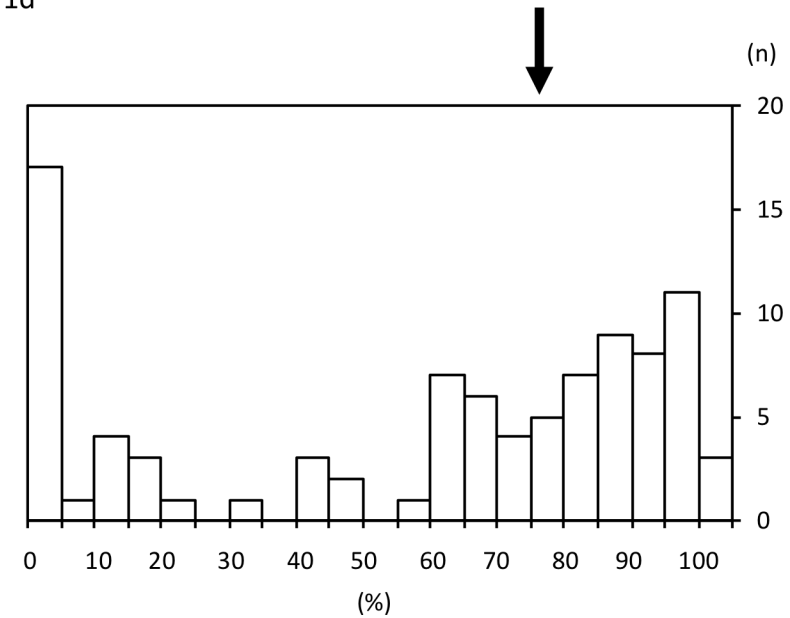


Fig. 1e

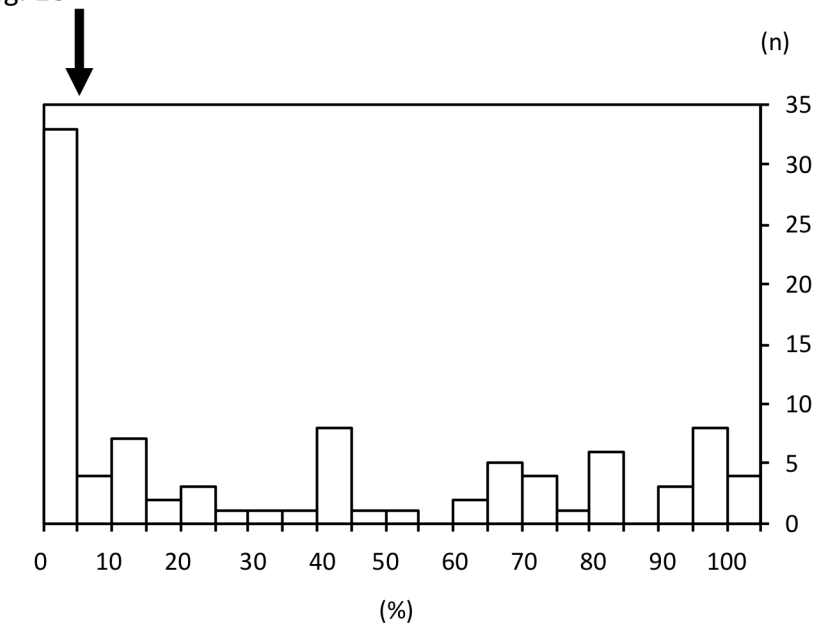


Fig. 2

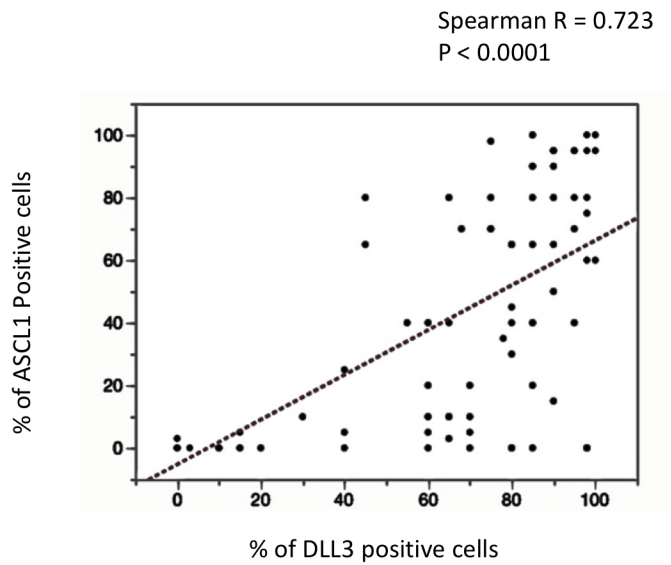
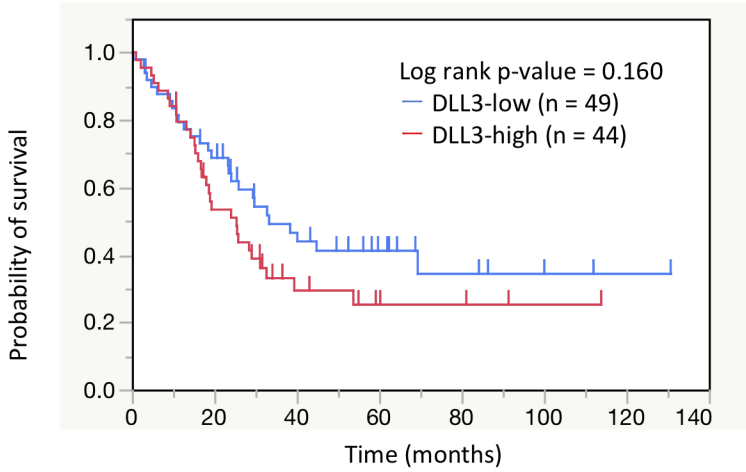
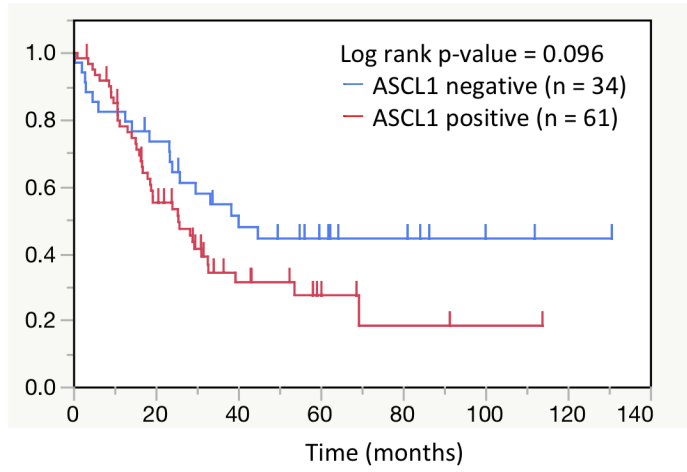

Spearman R = 0.723
P < 0.0001
% of ASCL1 Positive cells
100
80
60
40
20
0
0
20
40
60
80
100
% of DLL3 positive cells

Fig. 3a



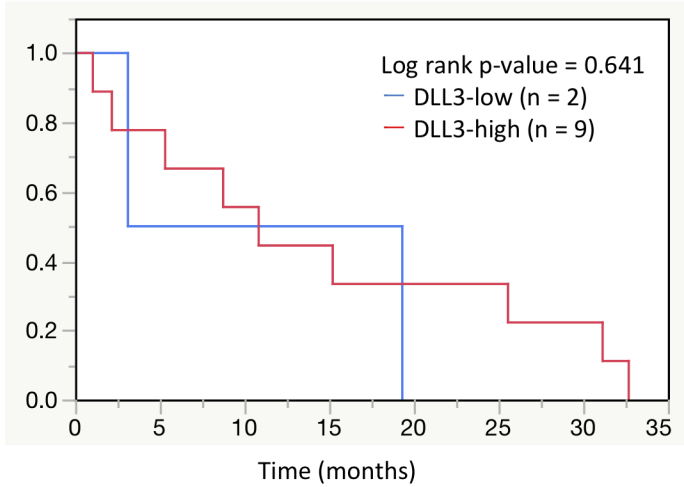
DLL3	Median OS	CI. Median
Low	33.3	23.5, NA
High	24.4	16.8, 32.7

Fig. 3b



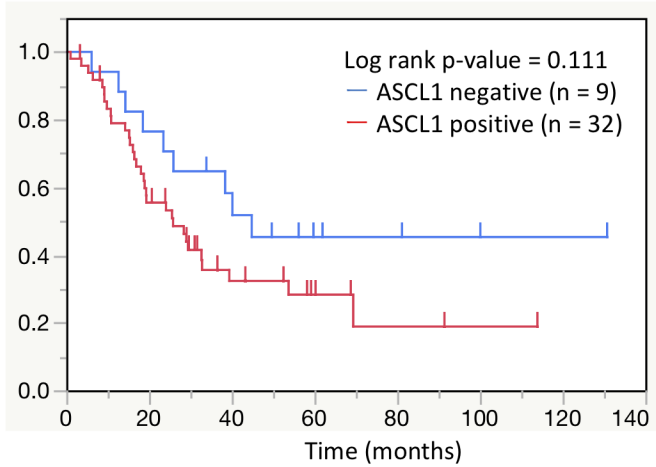
ASCL1	Median OS	CI. Median
Negative	40.2	23.5, NA
Positive	25.8	18.1, 32.7

Fig. 3c



DLL3	Median OS	CI. Median
Low	11.2	3.1, 19.3
High	10.8	1.1, 31.1

Fig. 3d



ASCL1	Median OS	CI. Median
Negative	44.8	18.5, NA
Positive	25.8	18.1, 32.8