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BRIEF REPORT

Effect of genetic variation in Notch regulator *DTX1* on SCLC prognosis compared with the effect on NSCLC prongosis

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Keywords
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Abstract

Deltex-1 (DTX1) is a negative regulator of the Notch signaling pathway. Here, we investigated the clinical effect of *DTX1* rs1732786A > G, which is associated with better prognosis in patients with early-stage non-small cell lung cancer (NSCLC), in 261 patients with small cell lung cancer (SCLC). *DTX1* rs1732786A > G was associated with a significantly worse chemotherapy response and lower overall survival in the codominant model (odds ratio = 0.42, 95% confidence interval [CI]: 0.26–0.66, $P = 2 \times 10^{-4}$; hazard ratio = 1.47, 95% CI: 1.17–1.84, $P = 0.001$, respectively). An in vitro luciferase assay was performed, and the 1732786G allele demonstrated significantly higher promoter activity than the 1732786A allele ($P = 2 \times 10^{-7}$). In summary, *DTX1* rs1732786A > G was associated with poor prognosis in patients with SCLC as opposed to patients with NSCLC.

Key points

Significant findings of the study: *DTX1* rs1732786A > G was associated with better prognosis in patients with early-stage non-small cell lung cancer (NSCLC) in our previous study.

What this study adds: *DTX1* rs1732786A > G was associated with a significantly worse chemotherapy response and lower overall survival in small cell lung cancer (SCLC).

Introduction

Lung cancer is classified into two main types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC, which accounts for 15% of all lung cancers, is characterized by a more rapid growth rate and earlier metastasis than NSCLC.[1] Smoking is a major risk factor for both types of cancer, and 95% of SCLC patients present with a history of smoking. Specific genetic mutations, including those in the Myc family and p53 genes, have been detected in both NSCLC and SCLC patients, although the incidence is different.[2]

The Notch signaling pathway plays important roles in cell proliferation, differentiation, development, and homeostasis.[3] Aberrant Notch signaling has been reported in many cancers, including lung cancer.[3, 4] Mutations in the Notch signaling pathway genes influence the prognosis of patients with various cancers.[5–7] Recently, we reported that genetic variation in the Notch regulator *DTX1* can predict the survival of patients with surgically resected NSCLC.[8] In our previous study, *DTX1* rs1732786A > G was associated with significantly better overall survival (OS) and disease-free survival (DFS) rates. Considering that Notch

Table 1 Univariate analysis for response to chemotherapy and overall survival by clinical variables

		Response to chemotherapy				Overall survival				
Variables	No. of cases	Responder (CR + PR)	Non-responder (SD + PD)	OR (95% CI)	*P*-value	MST (month)	95% CI	Log-rank *P*	HR (95% CI)	*P*-value
Overall	261	190 (72.8)[†]	71 (27.2)[†]			10.4	9.1–11.1			
Age (year)										
<68	129	100 (77.5)	29 (22.5)	1		11.6	10.7–13.0		1	
≥68	132	90 (68.2)	42 (31.8)	0.62 (0.36–1.08)	0.09	7.8	6.6–8.8	1×10^{-4}	1.60 (1.25–2.05)	2×10^{-4}
Gender										
Male	226	163 (72.1)	63 (27.9)	1		10.3	9.0–11.2		1	
Female	35	27 (77.1)	8 (22.9)	1.30 (0.56–3.02)	0.54	10.8	6.3–15.0	0.75	0.94 (063–1.40)	0.75
Smoking status										
Never	19	16 (84.2)	3 (15.8)	1		11.2	6.3–15.2		1	
Ever	242	174 (71.9)	68 (28.1)	0.48 (0.14–1.70)	0.26	10.1	9.0–11.1	0.82	1.03 (0.64–1.76)	0.82
Stage										
LD	66	46 (69.7)	20 (30.3)	1		12.8	10.6–15.2		1	
ED	195	144 (73.8)	51 (26.2)	1.23 (0.66–2.27)	0.51	9.4	8.1–10.7	0.001	1.67 (1.21–2.30)	0.002
ECOG performance										
0–1	216	162 (75.3)	53 (24.7)	1		10.4	9.1–11.3		1	
2	45	28 (60.9)	18 (39.1)	0.51 (0.26–0.99)	0.05	7.1	4.3–9.0	3×10^{-4}	1.81 (1.31–2.53)	4×10^{-4}
Weight loss										
No	184	139 (75.1)	46 (24.9)	1		11.1	10.0–11.9		1	
Yes	77	51 (67.1)	25 (32.9)	0.68 (0.38–1.21)	0.19	8.0	7.0–10.0	0.01	1.42 (1.07–1.89)	0.02
NSE										
<14.7	96	66 (68.8)	30 (31.2)	1		11.2	10.0–13.7		1	
≥14.7	147	109 (74.2)	38 (25.8)	1.30 (0.74–2.30)	0.36	9.2	7.5–10.3	0.02	1.41 (1.07–1.87)	0.02
Regimen										
EP	134	90 (67.2)	44 (32.8)			10.7	8.8–12.2			
IP	127	100 (78.7)	27 (21.3)	1.81 (1.04–3.16)	0.04	10.0	8.7–11.2	0.88	0.98 (0.75–1.28)	0.88
Second-line chemotherapy										
No	121					7.1	6.1–8.2		1	
Yes	140					11.9	11.0–13.5	1×10^{-5}	0.56 (0.43–0.73)	2×10^{-5}
Radiation to tumor										
No	227					9.5	8.1–10.6		1	
Yes	34					16.4	12.8–null	2×10^{-5}	0.33 (0.20–0.56)	4×10^{-5}

CI, confidence interval; CR, complete response; ECOG, Eastern Cooperative Oncology Group; ED, extensive disease; EP, etoposide-cisplatin; HR, hazard ratio; IP, irinotecan-cisplatin; LD, limited disease; MST, median survival time; NSE, neuron specific enolase; OR, odds ratio; PD, progressive disease; PR, partial response; SD, stable disease.

[†]Row percentage.

mutations are frequently detected in patients with SCLC,[9–11] this variant might also affect SCLC prognosis. Therefore, we explored the association of *DTX1* rs1732786A > G with survival outcomes of patients with SCLC.

Methods

Patients

We enrolled 261 patients diagnosed with SCLC in Kyungpook National University Hospital (KNUH) in Daegu, Korea, between March 2001 and November 2017. All patients were of Korean ethnicity. Tumor staging was determined as either limited or extensive stage, in accordance with the system of the Veterans Administration Lung Study Group.[12] Chemotherapy response was evaluated after every two cycles of treatment using the response evaluation criteria in solid tumors (RECIST).[13] To avoid the confounding effect of radiation on chemotherapy response, we excluded patients who had undergone concurrent chemoradiotherapy. However, patients who received radiotherapy after chemotherapy were enrolled. This study was approved by the institutional review board of KNUH, and written informed consent was obtained from all patients.

Polymorphism and genotyping

DTX1 rs1732786A > G, which influenced the clinical outcomes of patients with NSCLC in our previous study, was selected. Blood samples for genotyping were provided by the National Biobank of KNUH, which was supported by the Ministry of Health, Welfare and Family Affairs. Blood samples were obtained prior to the initiation of cancer treatment, when the patients were diagnosed with SCLC. Genotyping was performed blindly using MassARRAY iPLEX assay (Sequenom Inc., San Diego, CA, USA).

Promoter-luciferase constructs and luciferase assay

An in vitro luciferase assay was performed, and a 618 bp fragment including rs1732786A > G was synthesized by polymerase chain reaction using genomic DNA from a donor-carrying heterozygote. The primer sequence was as follows: forward primer with KpnI restriction site, 5′-GGGGTACCGACGCAGTTGGGAGTGCAAA-3′, and reverse primer with XhoI restriction site, 5′-CCGCTC GAGCGTTCTCAATGTGGTGGCAC-3′. The pGL3-basic vector (Promega, Madison, WI, USA) was used to make pGL3-Basic-DTX1 constructs containing either the rs1732786 A or G allele. The SCLC cell line (H146) was transfected with pRL-SV40 (Promega) and pGL3-basic

Table 2 Genotypes of rs1732786 polymorphisms and their associations with the response to chemotherapy and overall survival in SCLC

		Response to chemotherapy				Overall survival			
Polymorphism	No. of cases (%)†	Responder (%)‡	Nonresponder (%)‡	OR (95% CI)§	*P*-value§	MST (95% CI)¶	Log-rank P	HR (95% CI)¶	*P*-value¶
rs1732786									
AA	107 (41.6)	87 (82.9)	18 (17.1)	1.00		11.6 (10.6–13.9)		1.00	
AG	115 (44.8)	81 (70.4)	34 (29.6)	0.42 (0.21–0.85)	0.02	9.1 (7.4–11.6)		1.18 (0.86–1.62)	0.30
GG	35 (13.6)	19 (61.3)	13 (38.7)	0.18 (0.07–0.45)	3×10^{-4}	7.5 (5.8–10.7)	0.004	2.61 (1.65–4.14)	5×10^{-5}
Dominant				0.34 (0.18–0.67)	0.002	9.0 (7.4–10.7)	0.007	1.37 (1.02–1.84)	0.04
Recessive				0.29 (0.13–0.66)	0.004	11.0 (9.6–12.0)	0.007	2.41 (1.56–3.73)	7×10^{-5}
Codominant				0.42 (0.26–0.66)	2×10^{-4}			1.47 (1.17–1.84)	0.001

CI, confidence interval; HR, hazard ratio; MST, median survival time (months); OR, odds ratio.

†Column percentage.

‡Row percentage.

§OR, 95% CI, and their corresponding *P*-values were calculated by multivariate regression analysis, adjusted for age, gender, smoking status, stage, ECOG performance status, weight loss, NSE level, and first chemotherapy regimen.

¶HRs, 95% CIs and their corresponding *P*-values were calculated using multivariate Cox proportional hazard models, adjusted for age, gender, smoking status, stage, ECOG performance status, weight loss, NSE level, first chemotherapy regimen, second-line chemotherapy, and radiation to primary tumor.

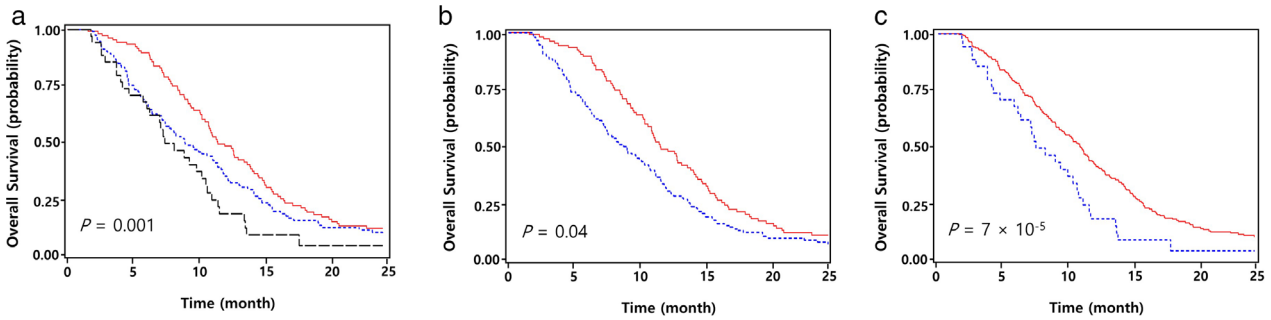

Figure 1 (**a**) Kaplan-Meier plots of overall survival in the codominant, AA; ——, AG; ------, GG ------; (**b**) dominant, AA; ——, AG + GG ------; and (**c**) recessive ——, AA + GG; ------, GG models. The *P*-value was calculated using multivariate Cox proportional hazard models adjusted for age, sex, smoking status, stage, Eastern Cooperative Oncology Group performance status, weight loss, neuron-specific enolase level, first chemotherapy regimen, second-line chemotherapy, and radiation to primary tumor.

vectors using lipofectamine 3000 transfection reagent (Thermo Fisher, Waltham, MA, USA). The cells were harvested after 48 hours of transfection. We used the Synergy HTX Multi-Mode Microplate Reader (BioTek Instruments, Winooski, VT, USA) to measure luciferase activity. The results were compared with pRL-SV40 Renilla luciferase activity.

Statistical analysis

We analyzed the association between the rs1732786A > G genotype and clinical outcomes of patients with SCLC. Patients' response to chemotherapy was calculated using unconditional logistic regression. OS was determined from the date of the first chemotherapy treatment to death or last follow-up and was analyzed by the Kaplan–Meier method and log-rank test. To estimate the hazard ratio (HR) and 95% confidence interval (CI), we used multivariate Cox proportional hazards models. Student's *t*-test was used to compare promotor activity of DTX1 rs1732786 A or G allele. The Statistical Analysis System for Windows, version 9.4 (SAS Institute, Cary, NC, USA), was used for analysis.

Results

Clinical characteristics and univariate analyses of clinical outcomes are shown in Table 1. Age, stage, Eastern Cooperative Oncology Group performance status, weight loss, neuron-specific enolase level, second-line chemotherapy, and radiation to tumor were associated with OS. *DTX1* rs1732786A > G was associated with both chemotherapy response and OS of patients with SCLC. rs1732786A > G was associated with significantly worse chemotherapy response and lower OS in the codominant model (odds ratio = 0.42, 95% CI: 0.26–0.66, $P = 2 \times 10^{-4}$; HR = 1.47, 95% CI: 1.17–1.84, $P = 0.001$, respectively; Table 2 and Fig 1). When stratified by clinical variables, the effects of rs1732786A > G on chemotherapy response and OS did not differ in each subgroup (*P*-values for homogeneity test >0.05; Table S1), except for chemotherapy response by weight loss.

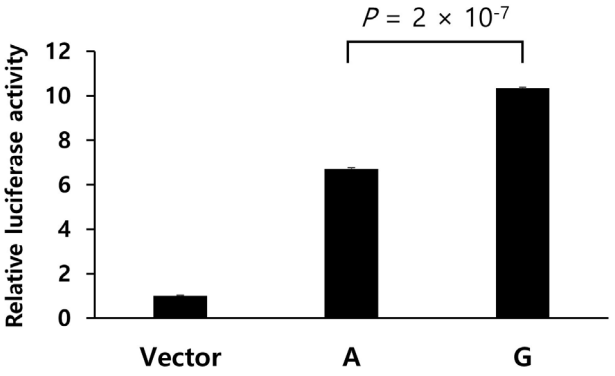

Figure 2 Promotor assay of *DTX1* rs1732786A > G. A small cell lung cancer cell line (H146) was transfected with pGL3-Basic-*DTX1* constructs containing either the rs1732786 A or G allele and pRL-SV40 vector. Luciferase activities were normalized to pRL-SV40 Renilla luciferase activity. The *P*-value was calculated using Student's *t*-test.

rs1732786A > G is located in the *DTX1* promoter region (−16 from the transcription start site). To investigate whether rs1732786A > G modulated the promoter activity of the *DTX1* gene, we conducted an in vitro functional study using a luciferase assay. The 1732786G allele showed significantly higher promoter activity than the 1732786A allele ($P = 2 \times 10^{-7}$; Fig 2).

Discussion

In this study, *DTX1* rs1732786A > G was associated with worse chemotherapy response and lower OS of patients with SCLC. However, in our previous study, *DTX1* rs1732786A > G was associated with better OS and DFS of

patients with early-stage NSCLC.[5] This contradictory result might be attributed to the different roles of the Notch pathway based on the type of cancer. The Notch signaling pathway plays pleiotropic roles during embryonic development and is important in cell-to-cell communication.[3, 14] Notch signaling dysregulation contributes to carcinogenesis, and its role seems to be mostly oncogenic.[3, 4, 15] The oncogenic role of Notch signaling has also been reported in NSCLC.[16, 17] However, Notch signaling are remarkably varied depending on the cellular context and can be either oncogenic or tumor suppressive. It has been previously determined that Notch receptors can function as cell autonomous oncoproteins, cell autonomous tumor suppressors, or microenvironment-dependent oncoproteins in different cellular contexts.[18] The role of Notch signaling as a tumor suppressor has been reported in several cancers, including SCLC.[19–22] Sriuranpong *et al.* showed that a decrease in human achaete-scute homologue-1 expression through Notch signaling could lead to cell cycle arrest and reduce tumor potential in SCLC.[20] Therefore, these different roles of Notch signaling (i.e., an oncogenic role in NSCLC and a tumor suppressing role in SCLC) may produce opposite effects, even in the same variant.

DTX1 is one of the key negative regulators of the Notch pathway.[23, 24] Recently, poor prognosis of patients with diffuse large B-cell lymphoma with *DTX1* mutations has been reported.[25] rs1732786A > G is located in the promoter region of *DTX1*, and our in vitro functional study showed that rs1732786A > G was associated with increased promoter activity. In our previous study, the variant also increased *DTX1* mRNA expression in surgically resected NSCLC.[8] Thus, rs1732786A > G could increase *DTX1* activity, which would suppress Notch signaling. However, because surgery is rarely used in SCLC, it is difficult to obtain sufficient tissue for examination, so the difference between gene polymorphism and gene expression in SCLC tumor tissue could not be investigated. Considering the tumor suppressor effect of Notch signaling in SCLC, suppression of Notch signaling would lead to poor chemotherapy response and prognosis. However, the exact biological mechanism through which the variant affects SCLC prognosis requires further study.

In summary, *DTX1* rs1732786A > G is associated with worse clinical outcomes of patient with SCLC. The opposite result previously found in NSCLC with the same variant may be attributed to the different roles of Notch signaling based on cancer subtype. Further studies are warranted to confirm this finding.

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Disclosure

The authors have declared no conflicts of interest.

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Supporting Information

Additional Supporting Informationmay be found in the online version of this article at the publisher's website:

Supplementary Table S1 Stratified Analysis of the Effects of the rs1732786 Genotypes under a Codominant Model.

