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The Prognostic Value of Sex-Determining Region Y-Box 2 and CD8+ Tumor-Infiltrating Lymphocytes in Limited-Stage Small-Cell Lung Cancer

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Keywords
Sex-determining region Y-box 2 · CD8+ tumor-infiltrating lymphocyte · Small-cell lung cancer

Abstract
Background: Sex-determining region Y-box 2 (SOX2) is a transcriptional factor that drives embryonic stem cells to neuroendocrine cells in lung development and is highly expressed in small-cell lung cancer (SCLC). However, the prognostic role of SOX2 and its relationship with tumor-infiltrating lymphocytes (TILs) has not been determined in SCLC. Herein, we assessed the expression of SOX2 and CD8+ TILs to obtain insights into the prognostic role of SOX2 and CD8+ TILs in limited-stage (LS)-SCLC. ***Methods:*** A total of 75 patients with LS-SCLC was enrolled. The SOX2 expression and CD8+ TILs were evaluated by immunohistochemistry. ***Results:*** High SOX2 and CD8+ TIL levels were identified in 52 (69.3%) and 40 (53.3%) patients, respectively. High SOX2 expression was correlated with increased density of CD8+ TILs ($p = 0.041$). Unlike SOX2, high CD8+ TIL numbers were associated with significantly longer progression-free survival (PFS; 13.9 vs. 8.0 months, $p = 0.014$). Patients with both high SOX2 expression and CD8+ TIL numbers ($n = 29$, 38.7%) had significantly longer PFS and overall survival (OS) compared to those from the other groups (median PFS 19.3 vs. 8.4 months; $p = 0.002$ and median OS 35.7 vs. 17.4 months; $p = 0.004$, respectively). Multivariate Cox regression analysis showed that the combination of high SOX2 expression and CD8+ TIL levels was an independent good prognostic factor for OS (HR = 0.471, 95% CI, 0.250–0.887, $p = 0.02$) and PFS (HR = 0.447, 95% CI, 0.250–0.801, $p = 0.007$) in SCLC. ***Conclusions:*** Evaluation of the combination of SOX2 and CD8+ TIL levels may be of a prognostic value in LS-SCLC.

Introduction

Small-cell lung cancer (SCLC) is a malignant tumor originating from pulmonary neuroendocrine cells that is characterized by fast growth and early widespread metastasis; it has a dismal prognosis [1]. Consequently, curative surgical resection, which is the gold standard treatment modality for most other cancers, is not feasible in most cases of SCLCs. SCLC treatment is relatively standard and consists of chemotherapy, including treatment with etoposide and platinum, with/without radiation and has

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been the same since the early 1990s. The overall response rate for these treatments is approximately 90%, but more than half of the patients relapse in 2 years, resulting in a median overall survival (OS) of only 20 months [2, 3]. Based on staging, SCLC is classified into 2 stages: limited and extensive stage. Limited-stage SCLC (LS-SCLC) is defined as a tumor that is confined to one hemithorax, usually with one single radiation port and no pleural effusion, while in extensive-stage SCLC (ES-SCLC) the disease extends beyond one radiation field and is generally accompanied by metastasis and pleural effusion [1]. ES-SCLC generally has a poor prognosis with a median OS of <10 months and a 5-year survival rate of <5% [4], whereas LS-SCLC has a 5-year survival rate of 20–40% [5].

Recently, etoposide and platinum with anti-programed death (PD) ligand 1 immunotherapy (atezolizumab or duvalumab) have been approved as a new standard therapy in ES-SCLC [6, 7]. However, current standard treatment in LS-SCLC is still concurrent etoposide and platinum chemoradiotherapy for decades. Combination immunotherapy such as anti-PD(L)1 and/or anti-cytotoxic T-lymphocyte-associated protein 4 has been evaluated in LS-SCLC with chemoradiotherapy or as consolidation therapy, however the role of immunotherapy in LS-SCLC has not yet defined [8].

The core concept of immunotherapy is based on the tumoricidal effect of CD8+ T-cells [9]. These findings are supported by numerous studies, showing that CD8+ tumor-infiltrating lymphocytes (CD8+ TILs) are associated with good prognosis [10, 11]. Although a complex and delicate mechanism is suggested to regulate effective CD8+ TIL response in solid tumors, tumor antigens have been suggested as regulators of antitumor immunity associated with CD8+ TILs in solid tumors [12].

Sex-determining region Y-box 2 (SOX2) is a transcriptional factor that regulates neuronal differentiation of pluripotent stem cells and differentiation of embryonic stem cells to neuroendocrine cells during lung development [13]. In SCLC, SOX2 is frequently amplified and present in approximately 25% of SCLC tumor samples [14]. However, the prognostic role of SOX2 in SCLC has not been studied extensively. Apart from the oncogenic role of SOX2 in SCLC, immunoreactive antibodies for SOX2 have been reported in the serum of patients with solid tumors, including SCLC [15–17]. Recent studies have also shown that the SOX2 tumor antigen is a target for cytotoxic T-cells [18]. However, the role of the combination of SOX2 expression and CD8+ TIL numbers in SCLC has not been studied previously.

Table 1. Baseline characteristics of LS-SCLC

Varibles	All patients (n = 75)
Median age (range), years	70 (44–85)
Sex	
Male	64 (85.3%)
Female	11 (14.7%)
Smoking status	
Current or former smoker	64 (85.3%)
Never smoker	5 (6.7%)
Unknown	6 (8%)
ECOG PS	
0, 1	63 (84%)
2	12 (16%)
Histology type	
SCLC	62 (82.7%)
SCLC with NSCLC combined	13 (17.3%)
Serum LDH level	
<UNL (450 U/L)	30 (40%)
≥UNL (450 U/L)	45 (60%)
Treatment modality	
CCRT or sequential CT and RT	46 (61.3%)
Surgery and CT±RT	18 (24%)
CT or RT alone	11 (14.7%)
Median RT dose (range), Gy	45 (24–55)
PCI	
Yes	31 (41.3%)
No	44 (58.7%)
Subsequent CT	
Yes	38 (50.7%)
No	37 (49.3%)

ECOG PS, Eastern Cooperative Oncology Group Performance status; LDH, lactate dehydrogenase; PCI, prophylactic cranial irradiation; CT, chemotherapy; RT, radiation therapy; CCRT, concurrent chemoradiation therapy; SCLC, small-cell lung cancer; LS-SCLC, limited-stage small-cell lung cancer; NSCLC, non-small cell lung cancer.

In this study, we assessed the combined effect of SOX2 expression in tumor cells and CD8+ TIL numbers in 75 patients with LS-SCLC. Further, we investigated the correlation between SOX2/CD8+ TIL expression, clinicopathologic factors, and survival of patients with LS-SCLC.

Materials and Methods

Study Population

We retrospectively reviewed data from histologically confirmed LS-SCLC patients treated between January 1, 2010, and August 31, 2018, at the Seoul St. Mary's Hospital, Korea. Among the total of 245 patients treated for LS-SCLC, 41 patients were referred to our hospital after diagnosed with SCLC at outside institutions, and 21 patients refused cancer therapy. Among the 183 patients who received treatment of LS-SCLC, 25 patients were di-

Table 2. Correlation between SOX2, CD8+TIL, and clinicopathologic findings of LS-SCLC

Variable	Total (n = 75) (%)	SOX2			CD8+ TIL		
		high (n = 52) (%)	low (n = 23) (%)	p value	high (n = 40) (%)	low (n = 35) (%)	p value
Age							
≥65 years	51 (68)	34 (65.3)	17 (73.9)	0.465	30 (75.0)	21 (60.0)	0.165
<65 years	24 (32)	18 (34.6)	6 (26.1)		10 (25.0)	14 (40.0)	
Sex							
Male	64 (85.3)	44 (84.6)	20 (87.0)	0.792	33 (82.5)	31 (88.6)	0.458
Female	11 (24.7)	8 (15.4)	3 (13.0)		7 (17.5)	4 (11.4)	
Smoking status							
Current or former smoker	64 (85.3)	44 (84.6)	20 (87.0)	0.681	35 (87.5)	29 (82.9)	0.577
Never smoker	5 (6.7)	3 (5.8)	2 (8.7)		3 (7.5)	2 (5.7)	
Unknown	6 (8)	5 (9.6)	1 (4.3)		2 (5)	4 (11.4)	
ECOG PS							
0,1	63 (84)	46 (88.5)	17 (73.9)	0.113	33 (82.5)	30 (85.7)	0.705
2	12 (16)	6 (11.5)	6 (26.1)		7 (17.5)	5 (14.3)	
Serum LDH							
<UNL (450 U/L)	30 (40)	23 (44.2)	7 (30.4)	0.261	24 (60.0)	21 (60.0)	1
≥UNL (450 U/L)	45 (60)	29 (55.8)	16 (69.6)		16 (40.0)	14 (40.0)	
Treatment modality							
CCRT or surgery and CT ± RT	59 (78.7)	39 (75.0)	20 (87.0)	0.390	32 (80.0)	27 (77.1)	0.785
Sequential CT and RT or CT/RT alone	16 (21.3)	13 (25.0)	3 (13.0)		8 (20.0)	8 (22.9)	
PCI							
Yes	31 (41.3)	21 (40.4)	10 (43.5)	0.802	15 (37.5)	16 (45.7)	0.471
No	44 (58.7)	31 (59.6)	13 (56.5)		25 (62.5)	19 (54.3)	
Subsequent CT							
Yes	38 (50.7)	25 (48.1)	13 (56.5)	0.5	17 (42.5)	21 (60.0)	0.13
No	37 (49.3)	27 (51.9)	10 (43.5)		23 (57.5)	14 (40.0)	

ECOG PS, Eastern Cooperative Oncology Group Performance status; LDH, lactate dehydrogenase; PCI, prophylactic cranial irradiation; CT, chemotherapy; RT, radiation therapy; CCRT, concurrent chemoradiation therapy; LS-SCLC, limited-stage small-cell lung cancer; TIL, tumor-infiltrating lymphocyte; SOX2, sex-determining region Y-box 2.

agnosed with LS-SCLC by cytologic examination of endoscopic bronchial ultrasonography-fine needle aspiration or bronchoscopic evaluation. Only 75 of 158 patients were finally enrolled in this study due to paraffin blocks' availability for immunohistochemistry. Clinical data, including age, sex, Eastern cooperative oncology group performance status, smoking status, the serum LDH level, treatment modality, survival time, and survival status were retrieved from their medical records. This study was approved by the Institutional Review Board of the Seoul St. Mary's Hospital, and informed consent was waived due to the retrospective nature of the study.

Immunohistochemistry

The numbers of CD8+ TILs and expression levels of SOX2 were evaluated by immunohistochemistry. Immunohistochemistry analysis was performed using 4-μm-thick paraffin-embedded tissue sections, using the following primary antibodies: anti-SOX2 (1:50, SP76, Abcam, Cambridge, UK) and anti-CD8 (1:100, C8/144B, Dako, Cambridge, UK), and Ventana BenchMark XT stainer (Ventana, Tucson, AZ, USA), according to the manufacturer's instructions. CD8+ TIL numbers and SOX2 expression were evaluated independently by 2 board-certified pathologists (S.A.H. and H.W.H.) in a blinded manner. The SOX2 expression was graded as the staining intensity and proportion of stained cells. Staining intensity was scored as follows: weak = 1; moderate = 2; strong = 3; and the proportion of stained cells was evaluated as the percentage of stained cells. Various scores, derived from multiplying the staining intensity by the proportion of stained tumor cells, were tested to determine a cut value for the high and low expression. High SOX2 expression was defined as a score above 100 that was best predicted the patient's survival, using the Kaplan-Meier method and log-rank test. The evaluation of CD8+ TIL was performed according to a previous study [19]. The number of CD8+ TILs was counted under high magnification (×400) in 5 fields in the intratumoral area, and the mean number of CD8+ TILs was calculated. The cutoff value for high CD8+ TIL numbers was above the median values of the total cases.

Statistical Analysis

SOX2 and CD8+ TIL levels were compared with baseline factors using the χ^2 test. OS was defined as the duration between the date of the start of treatment for LS-SCLC and that of death from

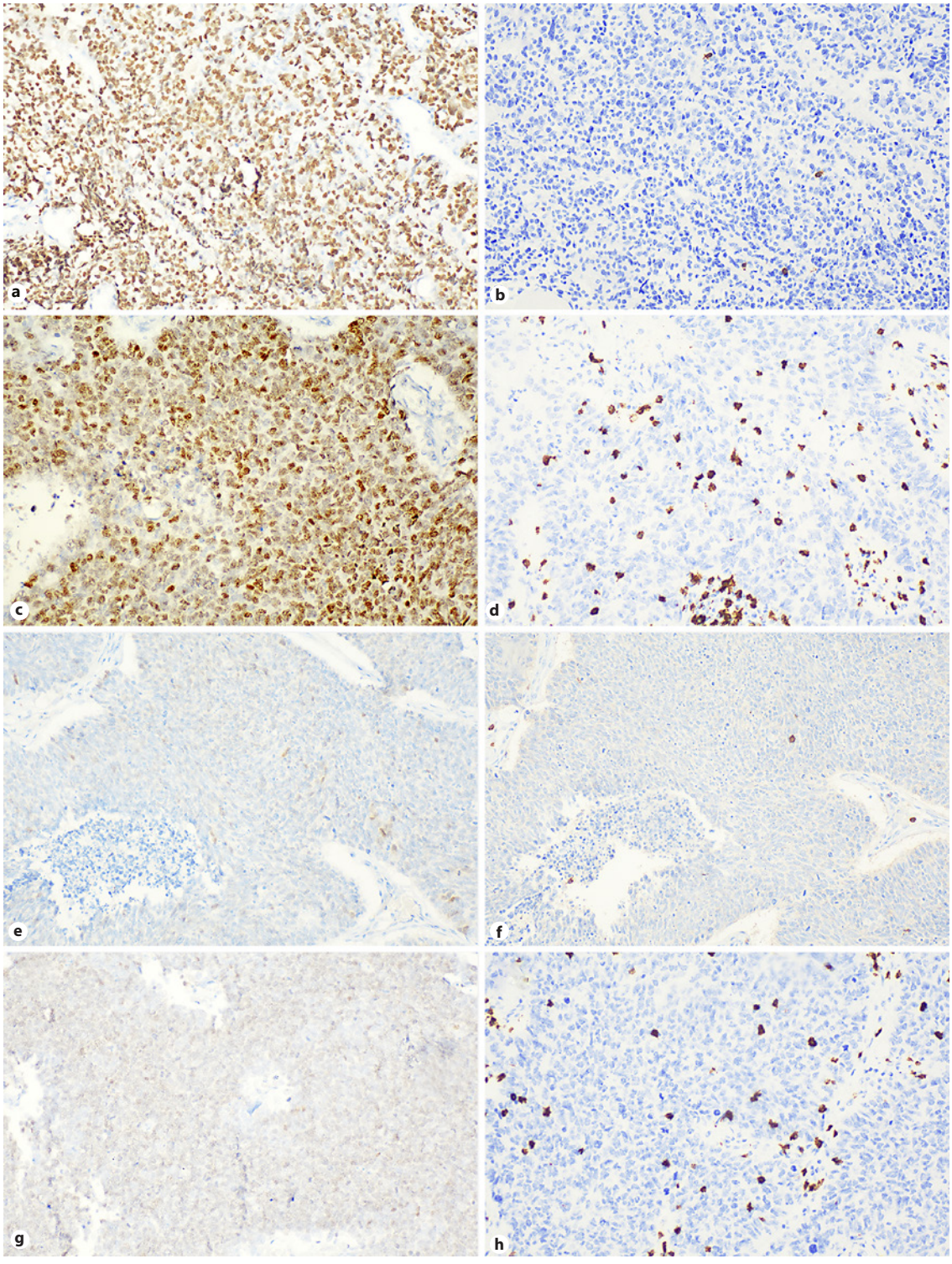

Fig. 1. Representative images of the immunohistochemical staining of SOX2 in tumor cells and CD8+ TILs. High SOX2 expression in tumor cells (**a**) and low levels of CD8+ TILs (**b**). High SOX2 expression in tumor cells (**c**) and high levels of CD8+ TILs (**d**). Low SOX2 expression in tumor cells (**e**) and low levels of CD8+ TILs (**f**). Low SOX2 expression of tumor cells (**g**) and high numbers of CD8+ TILs (**h**). TILs, tumor-infiltrating lymphocytes; SOX2, sex-determining region Y-box 2.

any cause, while the progression-free survival (PFS) was defined as the duration between the date of start of treatment and that of the detection of disease progression on radiologic exam. The OS and PFS of patients based on the expression of SOX2 and CD8+ TILs were estimated using the Kaplan-Meier method, and the log-rank test was used to compare survival curves. Univariate and multivariate analyses using Cox proportional hazard regression models were performed to examine prognostic factors for PFS and OS. $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS version 24.00 (IBM Corp, Armonk, NY, USA) and R statistical programming version 3.4.1 (http://www.r-project.org).

Results

Patient Characteristics

The baseline characteristics of patients are summarized in Table 1. The median age was 70 years (range, 44–85 years), and 85.3% of patients were male. A total of 46 (61.3%) patients were treated with etoposide/platinum concurrent chemoradiation or chemotherapy with sequential radiation therapy, while 18 (24%) patients received surgical resection with chemotherapy with/without radiation therapy. The median OS of the entire patient population was 19.3 months; the median PFS was 10.3 months.

SOX2 Expression and CD8+ TIL Numbers in LS-SCLC

Table 2 shows the patient characteristics according to the SOX2 expression and CD8+ TIL levels detected using immunohistochemistry. High SOX2 expression was observed in 52 patients (69.3%). The median number of CD8+ TILs was 13 (range, 1–115). SOX2 expression and CD8+ TIL levels did not differ with regard to any clinical variable. Based on the cut-off value for high and low CD8+ TIL numbers, a high CD8+ TIL level was identified in 40 (53.3%) patients. Based on the SOX2 expression and CD8+ TIL numbers, the patients were classified into the following 4 groups: high SOX2/low CD8+ TIL, high SOX2/high CD8+ TIL, low SOX2/low CD8+ TIL, and low SOX2/high CD8+ TIL (Fig. 1a–h). The number of CD8+ TILs in the high SOX2 group was significantly higher than that in the low SOX2 group ($p = 0.041$) (Fig. 2). High SOX2/high CD8+ TIL levels were found in 29 patients (38.7%) and were not significantly related to any clinicopathologic features (Table 3).

Effects of SOX2 Expression and CD8+ TIL Numbers on Survival

The OS and PFS of the high SOX2 group were longer than those of the low SOX2 group, but the differences were not statistically significant (median OS 21.7 vs. 17.1 months, $p = 0.118$; median PFS 12.7 vs. 9.0 months, $p = 0.11$) (Fig. 3a, b). Patients with high CD8+ TIL numbers had a longer OS and PFS than the low CD8+ TIL group. Although the difference in OS was not significant ($p = 0.058$) (Fig. 3c), the difference in PFS was statistically significant (13.9 vs. 8.0 months, $p = 0.014$) (Fig. 3d).

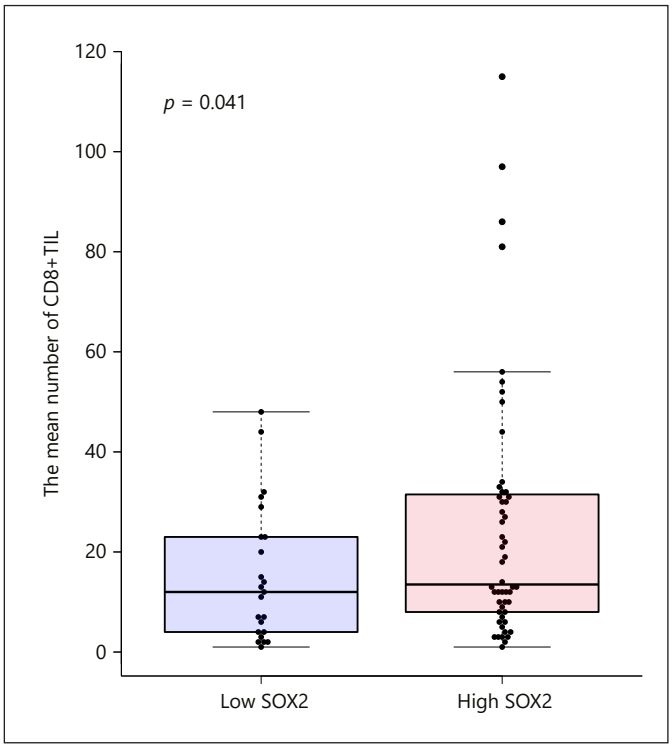

Fig. 2. Differences in the numbers of CD8+ TILs. The mean number of CD8+ TILs in the group with high SOX2 expression in the tumor is significantly higher than those in the group with low SOX2 expression ($p = 0.041$). TILs, tumor-infiltrating lymphocytes; SOX2, sex-determining region Y-box 2.

On combined analysis of SOX2 expression and CD8+ TIL numbers, patients with high SOX2/high CD8+ TIL demonstrated significantly longer OS ($p = 0.004$) (Fig. 4a) and PFS ($p = 0.001$) (Fig. 4b). The differences in survival among the 4 groups (high SOX2/high CD8+ TIL, high SOX2/low CD8+ TIL, low SOX2/high CD8+ TIL, and low SOX2/low CD8+ TIL), were compared and OS ($p = 0.032$) (Fig. 3c) and PFS ($p = 0.024$) (Fig. 3d) were found to be significantly different among the 4 groups.

Univariate and Multivariate Analyses

Univariate and multivariate analyses of the baseline characteristics of OS and PFS were performed (Table 4). In the univariate analysis, Eastern cooperative oncology group

Table 3. Association between combined high SOX2/CD8+ TIL and clinicopathologic findings of LS-SCLC

Variable	Combined high SOX2/CD8+ TIIL		
	yes (n = 29; 38.7%)	no (n = 46; 61.3%)	p value
Age, years			
≥65	23 (79.3%)	28 (60.9%)	0.158
<65	6 (20.7%)	18 (39.1%)	
Sex			
Male	24 (82.8%)	40 (87.0%)	0.869
Female	5 (17.2)	6 (13.0%)	
Smoking status			
Current or former smoker	25 (86.2%)	39 (84.8%)	
Never smoker	2 (6.9%)	3 (6.5%)	0.961
Unknown	2 (6.9%)	4 (8.7%)	
ECOG PS			
0, 1	26 (89.7%)	37 (80.4%)	0.461
2	3 (10.3%)	9 (19.6%)	
Serum LDH level			
<UNL (450 U/L)	12 (41.4%)	18 (39.1%)	1
≥UNL (450 U/L)	17 (58.6%)	28 (60.9%)	
Treatment modality			
CCRT or surgery and CT±RT	23 (79.3%)	36 (78.3%)	1
Sequential CT and RT or CT/RT alone	6 (20.7%)	10 (21.7%)	
PCI			
Yes	9 (31.0%)	22 (47.8%)	0.231
No	20 (69.0%)	24 (52.2%)	
Subsequent CT			
Yes	11 (37.9%)	27 (58.7%)	0.13
No	18 (62.1%)	19 (41.3%)	

ECOG PS, Eastern Cooperative Oncology Group Performance status; LDH, lactate dehydrogenase; PCI, prophylactic cranial irradiation; CT, chemotherapy; RT, radiation therapy; CCRT, concurrent chemoradiation therapy; LS-SCLC, limited-stage small-cell lung cancer; TIL, tumor-infiltrating lymphocyte; SOX2, sex-determining region Y-box 2.

performance status, the serum LDH level, and the combination of SOX2 expression and CD8+ TIL numbers were statistically significant factors for OS and PFS. In the multivariate analysis of OS, high expression levels of SOX2 and CD8+ TIL numbers were a good prognostic factor (HR 0.471, 95% CI 0.250–0.887, p = 0.02). In the multivariate analysis of PFS, the combination of high SOX2 expression levels and CD8+ TIL numbers were independent favorable variables (HR 0.447, 95% CI 0.250–0.801, p = 0.007).

Discussion

SCLC is a highly aggressive neuroendocrine tumor, characterized by rapid progression and distant metastasis; LS-SCLC is a relatively rare and curable disease [20]. Prognostic factors for LS-SCLC have not been extensively studied until recently. In our study, we analyzed the expression of SOX2 and CD8+ TILs in LS-SCLC. Although the SOX2 expression was not associated with OS and PFS, patients with concomitantly high SOX2 and CD8+ TIL levels demonstrated longer OS and PFS. Notably, high SOX2 and CD8+ TIL levels were independent prognostic factors for PFS and OS.

CD8+ TILs are the most important effector immune cells that eradicate tumor cells in various solid tumors and are associated with favorable patient prognosis. In a previous study, increased T-cell density and CD3 and CD8 immunostaining were related to favorable prognosis in ES-SCLC [21]. High CD8+ TIL number was also recognized as a good prognostic factor, with the combined analysis of either galectin-9 or PD-L1 expression in SCLC [22, 23]. The above result of the prognostic role for CD8+ TILs has been obtained from ES-SCLC cases with/without LS-SCLC. However, the prognostic role of CD8+ TILs in LS-SCLC has not been well defined. Thus, the finding

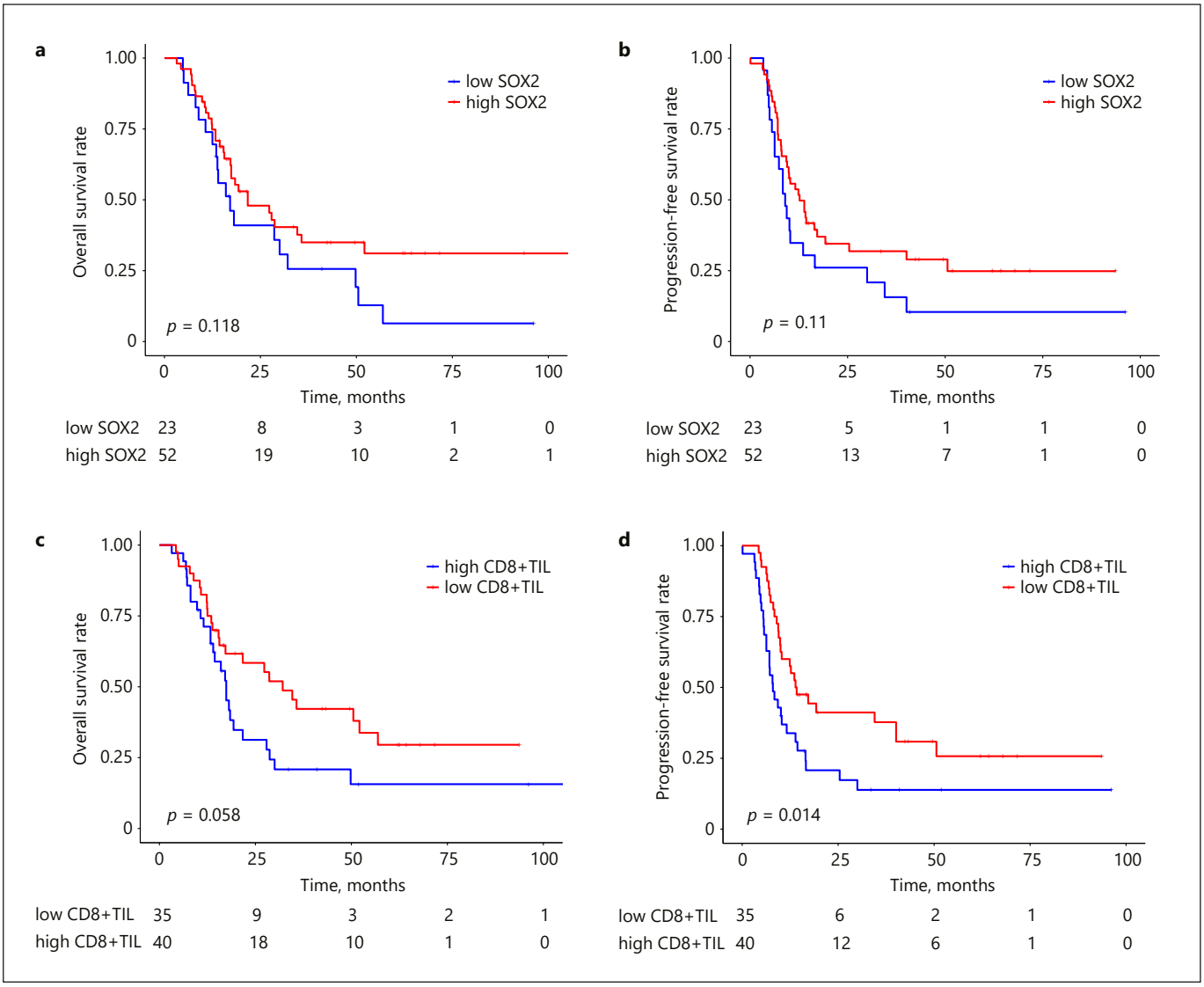

Fig. 3. Kaplan-Meier curves and log-rank analysis. OS (**a**) and PFS (**b**) curves according to SOX2 expression. OS (**c**) and PFS (**d**) curves according to the level of CD8+ TILs. TILs, tumor-infiltrating lymphocytes; PFS, progression-free survival; OS, overall survival; SOX2, sex-determining region Y-box 2.

that a high number of intratumoral CD8+ TILs is significantly related with longer PFS in LS-SCLC ($p = 0.014$) in our study could be valuable. Additionally, this finding suggests that some LS-SCLCs may have a favorable tumor microenvironment characterized by preexisting CD8+ T-cells; this has been linked to the increased efficacy of immune checkpoint inhibitors [24]. In addition, Ishii et al. [25] reported that the aberrant expression of PD-L1 in tumor cells is frequently observed with LS-SCLC, compared to ES-SCLC. Taken together, these findings suggest that antitumor immunotherapy mediated through CD8+ TILs may have a crucial role in LS-SCLC.

SOX2 is implicated in the carcinogenesis in SCLC. Amplification for SOX2 was found in 27% of SCLCs [14]. SOX2 is known to play a role in maintenance of the proliferative potential and stem cell function in cancer cells. In an in vitro study, suppression of the SOX2 gene blocked the proliferation of a SCLC cell line [14]. In a clinical study, high SOX2 expression in SCLC was observed in 55.9% of patients and was significantly associated with a poor prognosis [26]. However, in this study, we found that SOX2 expression itself was not significantly related to patient survival in SCLC ($p = 0.118$). These differences may be due to the difference in the patient population

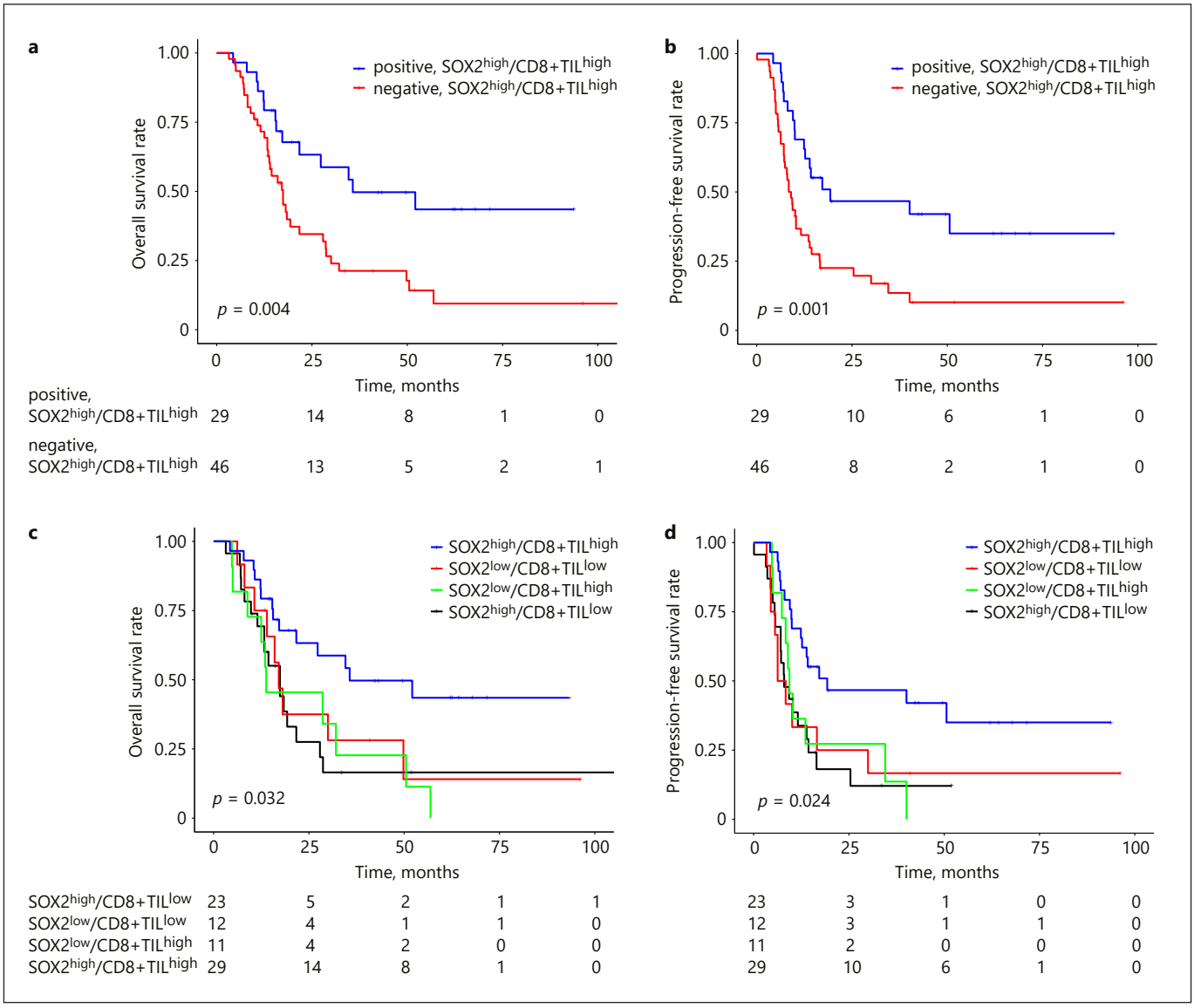

Fig. 4. Kaplan-Meier curves were plotted based on the combined analysis of SOX2 expression and CD8+ TIL levels. The patients with the combination of high SOX2 expression and levels of CD8+ TILs demonstrated longer OS (**a**) and PFS (**b**). OS (**c**) and PFS (**d**) in the 4 subgroups were plotted based on the combination of SOX2 expression and CD8+ TIL levels. TILs, tumor-infiltrating lymphocytes; PFS, progression-free survival; OS, overall survival; SOX2, sex-determining region Y-box 2.

used in this study, which was limited to LS-SCLC cases. Another reason for the different roles of SOX2 may be due to differences in the immunogenic antigens derived from tumor cells in various cancers, especially SCLC [27, 28]. Antibodies against the SOX2 protein were observed in the serum samples of 22% of the patients with SCLC [29]. The rate of detection of SOX2 antibodies was high at 67% in patients with concomitant SCLC and Lambert-Eaton myasthenia syndrome, a rare autoimmune disease characterized by muscle weakness [16]. Neurologic paraneoplastic syndromes, including Lambert-Eaton myasthenia syndrome, may be related to a favorable prognosis in SCLC that is induced by high CD8+ TIL numbers and levels of tumor antigens, including SOX2 [30, 31]. In our study, LS-SCLC with high SOX2 expression demonstrated a significantly increased density of CD8+ TILs (p = 0.041). According to these findings, further studies are needed to elucidate the actual role of SOX2 through inte-

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Table 4. Univariate and multivariate analyses of factors associated with OS of LS-SCLC

Variable	Univariate analysis						Multivariate analysis					
	OS			PFS			OS			PFS		
	HR	95% CI	*p* value	HR	95% CI	*p* value	HR	95% CI	*p* value	HR	95% CI	*p* value
Age												
<65 years	1	0.886–3.060	0.115	1	0.708–2.217	0.438						
≥65 years	1.646			1.253								
Sex												
Female	1	0.249–1.589	0.327	1	0.190–1.198	0.115						
Male	0.629			0.478								
ECOG PS												
0–1	1	2.332–9.025	<0.001	1	1.640–6.613	0.001	1	1.991–7.801	<0.001	1	1.463–5.563	0.002
≥2	4.588			3.179			3.941			2.853		
Smoking status												
Never, unknown	1	0.758–4.820	0.170	1	0.694–3.397	0.290						
Current, former	1.911			1.535								
Serum LDH level												
<UNL (450 U/L)	1	1.119–3.695	0.020	1	1.049–3.220	0.034	1	0.889–3.104	0.111	1	0.851–2.732	0.156
≥UNL (450 U/L)	2.033			1.837			1.661			1.525		
Treatment modality												
Sequantial CT and RT or CT/RT alone	1	0.325–1.249	0.189	1	0.442–1.532	0.539						
CCRT or surgery and CT±RT	0.637			0.539								
PCI												
Yes	1	0.857–2.671	0.154	1	0.785–2.303	0.281						
No	1.513			1.345								
High SOX2/CD8+ TIL												
Yes	0.410	0.219–0.766	0.005	0.414	0.232–0.739	0.003	0.471	0.250–0.887	0.02	0.447	0.250–0.801	0.007
No	1			1			1			1		

OS, overall survival; PFS, progression-free survival; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance status; LDH, lactate dehydrogenase; PCI, prophylactic cranial irradiation; CT, chemotherapy; RT, radiation therapy; CCRT, concurrent chemoradiation therapy; LS-SCLC, limited-stage small-cell lung cancer; TIL, tumor-infiltrating lymphocyte; SOX2, sex-determining region Y-box 2.

grated analysis of the oncogenic and tumor immunity-associated functions of SOX2.

We found that patients with high SOX2 expression and high CD8+ TIL numbers demonstrated a favorable prognosis, and these constitute independent prognostic factors in LS-SCLC. SOX2-specific T-lymphocytes stimulated by CD154-activated B cells and with combined immune checkpoint inhibitors have a strong tumoricidal effect on lung cancer cell lines [32, 33]. SOX2 is also an endogenous target of T-cell immunity in non-SCLC. Interestingly, patients with SOX2-targeting T-cells in non-SCLC who were treated with an immune checkpoint blocker showed a superior response rate compared to those without SOX2-targeting T-cells [18]. Altogether, the evaluation of SOX2 expression in tumor cells and the levels of CD8+ TILs can be helpful to stratify patients with LS-SCLC according to their prognosis. In addition, further studies on the efficiency of immune checkpoint blockers on the levels of SOX2 and CD8+ TILs are needed.

Our study has a few limitations. The limitation concerns the sample size and heterogeneous treatment modality, although the majority (86.7%) of patients received 4 cycles of etoposide, platinum, and definite local therapy (sequential chemo and radiotherapy or). The current standard of treatment for LS-SCLC is chemotherapy and concurrent thoracic radiotherapy. Chemotherapy plus radiotherapy showed a 5.4% improvement in OS at 2 years comparing to chemotherapy alone [34]. Compared to the homogeneous platinum based systemic chemotherapy approach in ES-SCLC, LS-SCLC is treated with varied treatment modalities based on clinical characteristics such as the lung function and TNM stage. Recently, surgical resection and concurrent chemoradiotherapy are recommended for LS-SCLC with T1-2N0M0 [35]. Moreover, the study for the radiotherapy schedule, radiation dose, and the candidate for prophylactic cranial irradiation has been continuously conducted to improve the prognosis of patients with LS-SCLC [36, 37]. Therefore, further studies using well-defined patient groups based on the patients' treatment methods are needed and can be helpful to address an additional role of SOX2 and CD8+ TIL in LS-SCLC.

Conclusion

Our study showed the prognostic role of SOX2 in association with CD8+ TILs in LS-SCLC. The SOX2 expression itself is not significantly related to patient survival. However, concomitantly high SOX2 and CD8+ TIL levels are significantly associated with longer OS and PFS and represent independent favorable prognostic factors in LS-SCLC. Studies on the interaction between SOX2 and tumor immunity in SCLC will provide a better understanding of tumor immunology and improve the efficacy of immunotherapy in SCLC.

Statement of Ethics

All procedures followed were in accordance with the ethical standards of the Responsible Committee on human experimentation and with the Helsinki Declaration of 1964 and later versions. Our study was approved by the Seoul St. Mary's Hospital's Institutional Review Board for (protocol KC18SES10552), where written informed consent or a substitute for it was obtained from all patients included in the study.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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Author Contributions

J.L.: conception and design, collection and assembly of data, data analysis and interpretation, writing, and approval of the final report. Y.Y.J., J.H.L., and H.-W.H.: collection and assembly of data, and approval of the final report. M.H.: conception and design, data analysis and interpretation, editing, and approval of the final report. S.-H.H. and S.A.H: conception and design, collection and assembly of data, data analysis, and interpretation, writing and editing, approval of the final report, and responsibility for overall content.

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