

A retrospective study of laryngeal squamous cell carcinoma and the significance of the PIK3CA mutation for survival

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Abstract. The aim of this study was to assess clinical features, outcomes and survival in patients with laryngeal squamous cell carcinoma (LSCC) and to associate mutations in cancer-related genes with clinical outcomes. A total of 88 patients with LSCC who underwent curative treatment between April 2008 and May 2024 at Hokuto Hospital (Obihiro, Japan) were included. Mutations in targeted regions of 160 cancer-related genes were analyzed using next-generation sequencing (NGS). LSCC was of glottic type in 65 patients (74%) and supraglottic type in 23 patients (26%). As initial treatment, laryngomicrosurgery, radiotherapy (RT) alone, RT with transoral administration of S-1, concurrent chemoradiotherapy with cisplatin, and total laryngectomy were performed in 6 (7%), 48 (55%), 13 (15%), 8 (9%), and 13 (15%) patients, respectively. Of the 88 patients studied, 25 (28%) died of various causes, including LSCC in 6 (7%), carcinoma other than LSCC in 11 (13%), and other causes in 8 (9%). The 5-year survival rates among all 88 patients with LSCC were 78.7% for overall survival (OS), 91.4% for disease-specific survival (DSS), 77.3% for relapse-free survival (RFS) and 67.5% for laryngectomy-free survival (LFS). OS, DSS, RFS and LFS in patients with early stage LSCC were similar to rates reported in other studies.

Actionable mutations were detected in 21 (88%) of 24 patients who underwent NGS-based cancer panel testing. *TP53* mutations were detected in 18 (75%), *KMT2D* in 5 (21%), *PTEN* in 3 (13%) and *PIK3CA* in 2 (8%) of these 24 patients. Multivariate Cox proportional hazard analysis revealed that *PIK3CA* mutation was an independent prognostic factor for RFS (P=0.011). Overall, detection of mutations in cancer-related genes could enhance understanding of clinical outcomes in LSCC.

Introduction

Laryngeal squamous cell carcinoma (LSCC) constitutes 1.0% of the total cancer cases worldwide; there are approximately 184,600 new cases diagnosed and 99,800 deaths annually (1). In Japan, LSCC is the fourth common type of head and neck squamous cell carcinoma (HNSCC) (2), with approximately 5,100 new cases and 800 deaths annually and a relative 5-year survival rate of 81.8% (3). The etiology of LSCC is multifactorial, and tumorigenesis is affected by both genetic and environmental factors including a history of smoking and alcohol consumption (4). For patients with LSCC, both complete cure and preservation of voice function are vital.

Possible treatments for LSCC include resection by laryngomicrosurgery (LMS), transoral laser microsurgery, radiotherapy (RT), chemotherapy, total laryngectomy, and various combination treatments depending on clinical stages, institutional guidelines, and the patient's choice (5). Helical tomotherapy, an advanced form of image-guided, intensity-modulated radiation therapy (IMRT), has been used for patients with HNSCC, however, comprehensive and long-term clinical outcome data that substantiate its efficacy in LSCC are limited (6).

Next-generation sequencing (NGS)-based cancer panel testing is often used to assist histologic diagnosis and can aid in identifying useful molecular-targeted agents, as well as predicting clinical outcomes in human cancers (7). Several large studies have identified genomic alterations in patients

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with HNSCC (8-10). However, these studies did not distinguish between differing sites of HNSCC, despite the differences in etiology, clinical behaviors and treatment methods for oropharyngeal, hypopharyngeal, and laryngeal carcinomas. In addition, any correlations between oncogenic mutations identified by NGS-based cancer panel testing and clinical outcomes in LSCC patients have not been fully understood.

The aim of the present study was to characterize the clinical features, outcomes, and survival rates of patients with LSCC at a single institution and to compare these data with other reports (11,12). We also examined the associations between various clinical features, disease outcomes and mutation data to highlight the potential impact of identified mutations on clinical decision-making.

Patients and methods

Patients and study design. The study group consisted of 88 Japanese patients with LSCC who underwent curative treatment between April 2008 and May 2024 at Hokuto Hospital, Hokkaido, Japan (Table I). All patients had histologically proven as SCC with biopsy specimens by LMS. Data regarding patient characteristics and clinical parameters were retrospectively registered and collected from the patients' records and included age at diagnosis; sex; Eastern Cooperative Oncology Group performance status (PS; scores ranging from 0 to 5, with higher scores indicating worse functional status); smoking status, defined as pack-years (packs/day x years); comorbidities, including history of ischemic heart disease and cerebral apoplexy and presence of hypertension, diabetes mellitus, and chronic obstructive pulmonary disease (COPD); development of any other carcinomas throughout lifetime; and cause of death, if deceased. All patients were examined via laryngeal fiberscope, computed tomography (CT) scan, and fluorodeoxyglucose-positron emission tomography/CT before treatment. SCC originated from the true vocal cords and the anterior and posterior commissures was defined as the glottic type LSCC. SCC originated from the epiglottis, false vocal cords, ventricles, aryepiglottic folds, and arytenoids was defined as the supraglottic type LSCC (13). All patients were classified according to the 8th edition of the American Joint Committee on Cancer (AJCC) tumor, node, and metastases (TNM) classification system (14). The most recently available visit date in the database was August 31, 2024.

Initial treatments were as follows: i) LMS by tumor resection with 2 mm of safety margin followed by argon plasma coagulation (APC) of the surrounding area using ICC 200 and APC 300 modes (Erbe Elektromedizin GmbH, Tübingen, Germany); ii) RT conducted by helical tomotherapy (Accuray, Madison, WI) for 5 days each week, with a single daily fraction of 2 Gy to a total dose of 66-70 Gy; iii) RT with concurrent 80 mg/day of oral S-1 (tegafur/gimeracil/oteracil, Taiho Pharmaceutical, Japan) for 2 weeks, followed by 1 week of rest (abbreviated as RT+S-1) (15); iv) concurrent chemoradiotherapy (CCRT) administered via RT with tri-weekly intravenous cisplatin (80 mg/m², three times); or v) total laryngectomy.

Adverse events, including grade 3 or higher [Common Terminology Criteria for Adverse Events (CTCAE) version 4.0] were monitored for dermatitis, mucositis, nausea, blood toxicity, and renal dysfunction. After initial treatment,

all patients were examined via laryngeal fiberscope and CT scan at fixed intervals. For patients with local recurrence after the initial treatment, total laryngectomy was primarily employed.

NGS-based cancer panel testing. NGS-based cancer panel testing started in 2017 in our hospital and was not performed for the patients between 2008 and 2016. Therefore, only 24 (27.3%) of the 88 patients with LSCC treated between April 2017 and May 2024 were tested for mutations using NGS. Genetic analyses were performed as previously described (16). Amplicon sequencing of targeted regions of 160 cancer-related genes (Table SI) was carried out using a GeneRead DNAseq Targeted Panel V2 Human Clinically Relevant Tumor Panel (NGHS-101X; Qiagen). Library quality was assessed using an Agilent 2100 bioanalyzer (Agilent, Santa Clara, CA) and GeneRead Library Quant kit (Qiagen). The libraries were sequenced using an MiSeq platform (Illumina, San Diego, CA) to produce 150 bp paired-end reads. Raw read data obtained from amplicon sequencing were processed using online analytical resources from the GeneRead DNAseq Variant Calling Service and a bioinformatics pipeline called GenomeJack (Mitsubishi Space Software, Tokyo, Japan) for analysis of mutations as previously described (17). Somatic gene alterations such as single nucleotide variations and insertions/deletions were identified.

Statistical analysis. Results are expressed as number (%) or median $\pm$ standard deviation. Fisher's exact test was used for statistical analysis. Survival rates were calculated using the Kaplan-Meier method and compared using log-rank test. Overall survival (OS) was calculated from the date of diagnosis to the date of death by any cause. Disease-specific survival (DSS) was calculated from the date of diagnosis to the date of death due to LSCC or surgery-related death. Relapse-free survival (RFS) was calculated from the date of diagnosis to the date of any recurrence or metastasis of LSCC. Laryngectomy-free survival (LFS) was calculated from the date of diagnosis to the date of total laryngectomy, including an initial treatment and salvage total laryngectomy due to local recurrence, or to the date of death due to LSCC. For patients who were still alive and had not experienced recurrence or metastasis, the endpoint was defined as the last date of follow-up. Cox proportional hazard modeling was used to identify factors associated with survival. A P-value of <0.05 was considered indicative of statistical significance. BellCurve for Excel (Social Survey Research Information, Tokyo, Japan) statistical software was used for all analyses. Informed consent was obtained from all patients at the time of enrollment in the study. This study was approved by the Ethics Committee of Hokuto Hospital (no. 1078).

Results

Clinicopathologic features. A total of 88 patients with LSCC, including 82 males (93%) and 6 females (7%), were included in the study. The median age of participants was 70 ± 9.7 years (range, 41-91 years), and LSCC consisted of glottic type in 65 (74%) and supraglottic type in 23 (26%) patients (Table I). Among the 88 patients with LSCC, 66 (75%) and 22 (25%)

Table I. Clinical features and outcomes in 88 patients with LSCC.

Characteristic	Group	Total	Glottic	Supraglottic
No. of patients		88	65	23
Age, years ^a		70.4±9.7	70.1±10.2	70.9±8.3
Sex (male:female), n (%)		82 (93):6 (7)	63 (97):2 (3)	19 (84):4 (16)
T, n (%)	T1	5 (6)	-	5 (22)
	T1a	28 (32)	28 (43)	-
	T1b	17 (19)	17 (26)	-
	T2	22 (25)	12 (19)	10 (43)
	T3	8 (9)	4 (6)	4 (17)
	T4a	8 (9)	4 (6)	4 (17)
N, n (%)	N0	72 (82)	63 (97)	9 (39)
	N1	5 (6)	1 (2)	4 (17)
	N2b	5 (6)	1 (2)	4 (17)
	N2c	6 (7)	-	6 (26)
Stage, n (%)	I	49 (56)	45 (77)	4 (17)
	II	14 (16)	11 (17)	3 (13)
	III	8 (9)	4 (6)	4 (17)
	IVA	17 (19)	5 (8)	12 (52)
PS, n (%)	1, 2	22 (25)	14 (22)	8 (35)
	0	66 (75)	51 (78)	15 (65)
Pack-years ^{a,b}		50.6±31.9	50±30	52.4±37.1
Comorbidities, n (%)	+	59 (67)	45 (69)	14 (61)
	-	29 (33)	20 (31)	9 (39)
Other carcinomas, n (%)	+	33 (38)	22 (34)	11 (48)
	-	55 (62)	43 (66)	12 (52)
Follow-up period, months ^a		61.2±50.1	65.1±49.2	33.8±49.4
Outcome, n (%)	Alive	63 (72)	49 (75)	14 (61)
	Died, LSCC	6 (7)	3 (5)	3 (13)
	Died, other cancer	11 (13)	7 (11)	4 (17)
	Died, other causes	8 (9)	6 (9)	2 (9)

^aMean ± standard deviation. ^bPack-years=tobacco packs/day x years. PS, performance status according to Eastern Cooperative Oncology Group; LSCC, laryngeal squamous cell carcinoma; Comorbidities including past history of ischemic heart disease and cerebral apoplexy and presence of hypertension and diabetes mellitus, and chronic obstructive pulmonary disease; Other carcinomas indicated any carcinoma developed throughout lifetime except for laryngeal squamous cell carcinoma; -, not present; +, present.

patients had a score of PS0 or PS1 and PS2, respectively. Current/ex-smokers and never-smokers totaled 85 (97%) and 3 (3%) patients, respectively, with 42.5±31.9 pack-years (range, 0-150 pack-years). Comorbidities were present in 59 (67%) and absent in 29 (33%) patients. Carcinomas other than LSCC occurring at some point in life were reported by 33 (38%) patients and included cancer of the colon in 13, primary lung in 5, oral cavity in 4, prostate in 4, stomach in 3, pancreas in 2, and other carcinomas in 6 patients. Among all 88 patients, LSCC was stage I in 49 (56%), stage II in 14 (16%), stage III in 8 (9%), and stage IVA in 17 (19%) patients. T classification was T1a in 28 (43%), T1b in 17 (26%), T2 in 12 (19%), T3 in 4 (6%), and T4a in 4 (6%) of the 65 patients with glottic type LSCC and T1 in 5 (22%) patients, T2 in 10 (43%), T3 in 4 (17%), and T4a in 4 (17%) of the 23 patients with supraglottic type LSCC. N classification was N0 in 63 (97%), N1 in 1 (2%), and N2b in 1 (2%) of the 65 patients with glottic type LSCC and N0 in 9 (39%), N1 in 4 (17%), and N2b in 4 (17%), and N2c in 6 (26%) of the 23 patients with supraglottic type LSCC.

With regard to initial treatment, of the 28 patients with T1aN0 glottic type LSCC, 4 (14%) were treated using LMS and 24 (86%) with RT (66-70 Gy) (Table II). Seventeen

patients with T1bN0 glottic type LSCC were treated with RT alone at 70 Gy. Of 12 patients with T2N0 glottic type LSCC, 11 (92%) patients were treated with RT+S-1. Of 5 patients with T1 supraglottic type LSCC, 2 (40%) were treated using LMS and 3 (60%) with 70-Gy RT alone. CCRT was conducted for 2 (3%) of 65 patients with glottic type LSCC and 6 (26%) of 23 patients with supraglottic type LSCC.

Total laryngectomy was performed in 6 (9%) of 65 patients with glottic type LSCC and 7 (30%) of 23 patients with supraglottic type LSCC. After total laryngectomy, postoperative pharyngocutaneous fistula was observed in 2 (15%) of 13 patients (Table III), and postoperative cardiac arrest was observed in 1 patient. Regarding adverse events, grade 3 or higher mucositis, as based on CTCAE (ver. 4.0), was observed in 3 (6%) of 48 patients who were treated with RT alone, in 3 (27%) of 13 patients who were treated with RT+S-1, and in 4 (50%) of 8 patients who were treated with CCRT. Nausea and blood toxicity were observed in 2 (25%) and 1 (13%) patient, respectively, who were treated with CCRT.

Clinical outcomes and survival rates. The follow-up period was 61±50 months (range, 1-91 months) for all 88 patients

Table II. Initial treatment according to T classification.

Treatment	Total patients	Glottic (n=65)					Supraglottic (n=23)			
		T1a	T1b	T2	T3	T4a	T1	T2	T3	T4a
No. of patients	88	28	17	12	4	4	5	10	4	4
LMS, n (%)	6 (7)	4 (14)	-	-	-	-	2 (40)	-	-	-
RT alone, n (%)	48 (55)	24 (86)	17 (100)	-	-	-	3 (60)	3 (30)	1 (25)	-
RT + S-1, n (%)	13 (15)	-	-	11 (92)	1 (25)	-	-	1 (10)	-	-
CCRT, n (%)	8 (9)	-	-	-	1 (25)	1 (25)	-	4 (40)	2 (50)	-
Total laryngectomy, n (%)	13 (15)	-	-	1 (8)	2 (50)	3 (75)	-	2 (20)	1 (25)	4 (100)

LMS, laryngomicrosurgery; RT, radiotherapy; S-1, tegafur/gimeracil/oteracil; CCRT, concurrent chemoradiotherapy with tri-weekly cisplatin 80 mg/m² three times.

Table III. Acute adverse events and complications after initial treatment.

Adverse events/complications	RT alone	RT + S-1	CCRT	Total laryngectomy
No. of patients, n	48	13	8	13
Dermatitis, n (%)	0 (0)	0 (0)	2 (25)	-
Mucositis, n (%)	3 (6)	3 (23)	4 (50)	-
Nausea, n (%)	0 (0)	0 (0)	2 (25)	-
Blood toxicity, n (%)	0 (0)	0 (0)	1 (13)	-
Renal dysfunction, n (%)	0 (0)	0 (0)	0 (0)	-
Fistula, n (%)	-	-	-	2 (15)

CCRT, concurrent chemoradiotherapy with tri-weekly cisplatin 80 mg/m² three times; adverse events including grade 3 or higher based on CTCAE ver. 4.0. S-1, tegafur/gimeracil/oteracil. The total does not add up because of multiple events.

with LSCC. At the end of the study, 63 (72%) patients were alive without LSCC, and the remaining 25 (28%) patients were deceased (Table I). The causes of death for these 25 patients included LSCC in 6 (7%), including local recurrence in 3, lung metastasis in 2, and surgery-related death in 1 patient, carcinomas other than LSCC in 11 (13%) patients, including in primary lung cancer in 4, oropharyngeal cancer in 2, pancreatic cancer in 2, colon cancer in 1, gallbladder cancer in 1, and liver cancer in 1 patient, and other causes in 8 (9%) patients. After initial treatment with RT, RT+S-1, or CCRT, 13 (15%) of the 88 LSCC patients underwent total laryngectomy due to local recurrence.

Five-year survival rates for all 88 LSCC patients were 78.7% for OS, 91.4% for DSS, 77.3% for RFS, and 67.5% for LFS (Table SII). Kaplan-Meier analysis revealed that OS was significantly worse in patients with supraglottic type LSCC than in patients with glottic type LSCC ($P=0.019$; Fig. 1A). There were significant differences by T-classification for RFS ($P=0.024$; Fig. 1H) and LFS ($P<0.001$; Fig. 1K) in patients with glottic type LSCC, and OS ($P<0.001$; Fig. 1C), DSS ($P=0.01$; Fig. 1F), RFS ($P=0.012$; Fig. 1I) and LFS ($P=0.005$; Fig. 1L) in patients with supraglottic type LSCC. Considering the 41 patients with glottic type T1a and T1b LSCC who were treated with RT alone, the 5-year survival rates were 85.0% for OS, and 97.1% for DSS, 79.0%. The locoregional control

rates at 5 years were 80.8% for total laryngectomy and 30% for primary CCRT in advanced-staged LSCC patients.

Univariate Cox proportional hazard analysis for all 88 LSCC patients revealed that PS1 and PS2 were significantly associated with poorer OS ($P=0.014$), DSS ($P=0.016$), and LFS ($P=0.005$) in patients with LSCC (Table SIII). Supraglottic type LSCC was significantly associated with poorer OS ($P=0.022$). T3 and T4a LSCC were significantly associated with poorer OS ($P=0.003$), DSS ($P=0.038$), RFS ($P=0.023$), and LFS ($P<0.001$). N1, 2b and 2c LSCC were significantly associated with poorer DSS ($P=0.005$) and LFS ($P=0.003$). Stage IVA LSCC was significantly associated with poorer DSS ($P=0.013$) and LFS ($P<0.001$). In terms of treatment, RT alone was significantly associated with poorer LFS ($P<0.001$), whereas total laryngectomy was significantly associated with poorer OS ($P=0.035$). There was a trend for the presence of comorbidities with poorer DSS ($P=0.083$). There was a trend for the presence of other carcinomas with poorer OS ($P=0.073$).

Mutational analysis. Actionable cancer-related gene mutations were detected in 21 (88%) of 24 patients who underwent NGS-based panel testing between April 2017 and May 2024. The genome sequencing data was deposited in the URL (<https://ddbj.nig.ac.jp/search/entry/bioproject/PRJDB19720>) under the accession number PRJDB19720. Mutations were

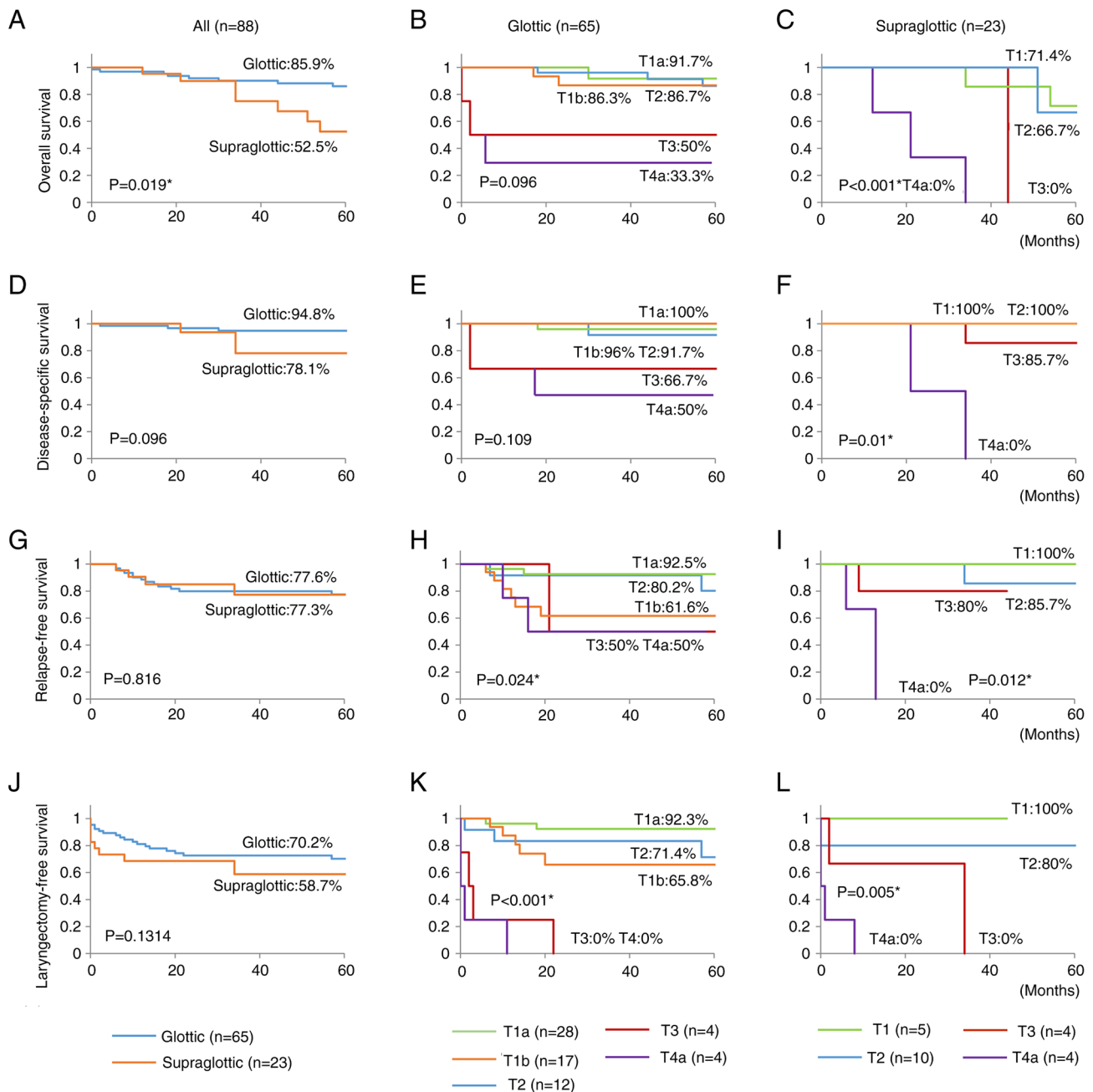


Figure 1. Kaplan-Meier curves for (A, B and C) overall survival, (D, E and F) disease-specific survival, (G, H and I) relapse-free survival and (J, K, and L) laryngectomy-free survival (A, D, G and J) among all 88 patients with LSCC by subsite, (B, E, H and K) among 65 patients with glottic type LSCC by T-classification and (C, F, I and L) among 23 patients with supraglottic type LSCC by T-classification. Differences in patients' survival were analyzed using a log-rank test. *P<0.05 was considered statistically significant.

detected in *TP53* mutation in 18 (75%), *KMT2D* in 5 (21%), *PTEN* in 3 (13%), *PIK3CA* in 2 (8%), *FBXW7* in 2 (8%), and *CDKN2A* in 1 (4%) of 24 patients with LSCC who were screened for mutations (Table SIV). The presence of any actionable mutations and mutations in *TP53*, *KMT2D* and *PTEN* were not associated with clinical features and outcomes (Table SV). Univariate Cox proportional hazard analysis for 24 LSCC patients revealed that *PIK3CA* mutation was significantly associated with poorer RFS (P=0.011) (Table SVI). Multivariate Cox proportional hazard analysis revealed that *PIK3CA* mutation was an independent prognostic factor for RFS (hazard ratio: 35.115; 95% confidence interval: 2.291-538.323, P=0.011) (Table IV).

Discussion

Regarding clinical features of patients with LSCC, the sex ratio of 93% males and 7% females in the present study was consistent with the 89.9% male and 10.2% female ratio in patients with laryngeal carcinoma reported in the head and neck cancer registry of Japan in 2020 (2). Regarding subsites of LSCC, the glottic type in 74% and supraglottic type in 26% of patients in the present study was consistent with the glottic type in 66.3% and supraglottic type in 26% of patients reported previously (2).

In the present study, 85 (97%) of the 88 LSCC patients were current/ex-smokers, whereas never-smokers accounted

Table IV. Multivariate Cox proportional hazards analysis of relapse-free survival in 24 patients with laryngeal squamous cell carcinoma treated between April 2017 and May 2024.

Characteristic	Category	N (%)	HR (95% CI)	P-value
Age	≥70	12 (50)	0.775 (0.035-17.16)	0.872
	<69	12 (50)		
Subsite	Supraglottic	9 (38)	0.984 (0.168-5.761)	0.986
	Glottic	15 (62)		
T	3, 4a	3 (13)	24.562 (0.803-751.695)	0.067
	1a, 1-2	21 (87)		
TP53 mutation	+	18 (75)	0.129 (0.008-2.081)	0.149
	-	6 (25)		
PIK3CA mutation	+	2 (8)	35.115 (2.291-538.323)	0.011 ^a
	-	22 (92)		

HR, hazard ratio; CI, confidence interval; -, not present; +, present. ^aP<0.05.

for only 3 patients (3%). A strong association exists between LSCC development and cigarette smoking, as historically, 95% of patients with LSCC have a history of smoking (18). Several reports have revealed that heavy smoking is associated with poorer survival in LSCC (12,19). Smoking is strongly related to the development of various comorbidities, including hypertension, diabetes mellitus, COPD, ischemic heart disease, and cerebral apoplexy (20). These comorbidities per se are closely associated with diminished survival rates in healthy individuals (21). These comorbidities have also been associated with increased frequencies of postoperative complications such as infection and ruptured sutures in LSCC patients who underwent laryngectomy (22) and with increased frequencies of adverse events in LSCC patients who underwent RT (23). A study has shown an association between coexisting comorbidities and lower OS and progression-free survival in patients with LSCC (24). In the present study, comorbidities were observed in 67% of LSCC patients and tended to be associated with lower DSS rates.

Cigarette smoking is the most established risk factor for the development of most human cancers (25). In the present study, 33 (38%) of the 88 LSCC patients had a history of other carcinomas other than LSCC. A total of 11 (13%) patients died due to other carcinomas despite having been completely cured of LSCC, and 6 (7%) patients died due to LSCC. The presence of carcinomas other than LSCC tended to be associated with lower OS rates in LSCC patients in the present study. Therefore, cessation of cigarette smoking is beneficial not only as a means of avoiding LSCC treatment failure but also the development of comorbidities and other carcinomas, thereby leading to improvement of prognosis.

RT is vital for treating LSCC because it provides local tumor control, facilitates organ preservation, preserves voice quality, and can be combined effectively with chemotherapy. In the present study, we used helical tomotherapy for 69 (78%) LSCC patients treated with RT alone, RT+S-1 and CCRT. Helical tomotherapy is a modality for delivering IMRT plans using a rotating linear accelerator mounted on a continuously moving slip ring gantry in synchrony with the couch motion (6). Helical tomotherapy was confirmed to be superior

to other IMRT techniques, specifically in terms of minimizing exposure to surrounding healthy tissues (26). A prospective study revealed favorable outcomes using helical tomotherapy with a 5-year RFS rate of 75.1% for early stage laryngeal carcinoma (27). Another study reported that the 3-year OS and DFS rates of 71.7 and 60.4%, respectively, with acceptable radiation-induced toxicity in 45 patients with laryngeal carcinoma at any clinical stage, all of whom were treated with the helical tomotherapy (28). Our study demonstrated favorable 5-year survival rates of 85.0% for OS and 97.1% for DSS in 41 patients with T1a and T1b of glottic type LSCC who were treated with RT alone. Only 6% of grade 3 mucositis occurred in those patients.

In the present study, patients with T1b glottic type LSCC had unexpectedly poorer survival rates than those with T2 glottic type LSCC, as evidenced by 5-year RFS and LFS rates of 61.6 and 65.8% for T1b, respectively, versus 80.2 and 71.4% for T2, respectively. One possible explanation for the poorer survival rates for T1b glottic type LSCC is the difficulty in distinguishing between T1b and T2 glottic tumors (i.e., risk of misdiagnosis regardless of the use of modern imaging), leading to possible understaging and undertreatment in some cases. It is noteworthy that most of the patients with glottic type T2 tumors in the present study received RT and transoral administration of S-1 as their initial treatment. Therefore, patients with T1b glottic type LSCC will need to be treated with RT plus some type of chemotherapy.

For treatment for advance-stage patients with LSCC, either total laryngectomy, CCRT, or induction chemotherapy followed by RT has been indicated as an initial treatment (29). We firstly recommended total laryngectomy for advance-stage patients with LSCC. If the patient refused total laryngectomy, CCRT was performed. The effectiveness of CCRT with cisplatin as an organ preservation approach for advance-stage LSCC was studied in the Radiation Therapy Oncology Group (RTOG) 91-11 trial and its update (11,30). This study reported that the 10-year laryngeal preservation rate was significantly higher in the CCRT arm compared with the induction chemotherapy followed by RT or RT alone arms. However, in the long-term follow-up study of

RTOG 91-11, Forastiere *et al* (30) stressed the importance of recognizing that although CCRT can improve cure rates, it is also associated with a higher frequency of late-staged complications affecting the voice and swallowing functions, resulting in non-cancer-related deaths. In the present study we treated 8 patients (9%) (T2: 4, T3: 3 and T4a: 1) with CCRT and 13 patients (15%) (T2: 3, T3: 3 and T4a: 7) with total laryngectomy as initial treatment. The locoregional control rates at 5 years were 80.8% for total laryngectomy and 30% for primary CCRT in advanced-staged LSCC patients. The locoregional control rates at 5-years were reportedly 67.7-95% for primary surgery and 50-76% for primary CCRT in advanced-staged LSCC patients (31-33). Although the differences of patient and tumor characteristics, surgical procedures, and regimen of CCRT may vary the locoregional control rates, the locoregional control rates by total laryngectomy were considered to be superior to those by CCRT. Patients with LSCC with cartilage destruction or dysfunctional larynx were not ideal candidates for organ preservation (34). Therefore, total laryngectomy is still considered a reasonable and curative treatment for advanced-stage LSCC patients. As the result of the present study, 5-year LFS was 0% in patients with T3 and T4a LSCC. Five-year DSS of T3 and T4a glottic type LSCC were 66.7 and 50% and that of T3 and T4a supraglottic type LSCC were 85.7 and 0%, respectively. To improve laryngeal preservation and survival rates at our hospital, we further need to introduce new treatments such as transoral robotic surgery (35), super-selective intra-arterial chemoradiation therapy (36), and administration of molecular-targeted drugs (37) or immune checkpoint inhibitors (38).

A variety of factors acting in conjunction must be considered in predicting survival after a diagnosis of LSCC. Survival rates depend primarily on patient age, tumor subtype, clinical stage, and cervical lymph node and distant metastasis (39-41). Five-year OS rates have been reported for Europe (55%) (42) and Japan (77.6%) (43) and by large population-based studies in the United States (52%) (44) and India (48.7%) (12). In the present study, the 5-year OS rate for the 88 LSCC patients was 78.7%, which is considerably higher than the rates shown above. One of the reasons for this discrepancy may be the higher frequency of early stage patients. In the present study, the 5-year OS and DSS rates of 52.5 and 78.1%, respectively, for the patients with supraglottic type LSCC were lower than the 85.9 and 94.8% rates, respectively, for glottic type LSCC. Stage I was 45 (77%) and Stage IVA was 5 (8%) of 65 patients with glottic type LSCC, whereas Stage I tumors were observed in 4 (17%) and Stage IVA in 12 (52%) of 23 patients with supraglottic type LSCC. Neck lymph node metastasis was present in only 2 (3%) of 65 patients with glottic type LSCC, whereas neck lymph node metastasis was present in 14 (61%) of 23 patients with supraglottic type LSCC. This is probably the result of the supraglottic type tumors being diagnosed at advanced stages at presentation, whereas glottic tumor becomes clinically symptomatic much earlier due to speech difficulties (40).

Although numerous cancer-related genetic alterations associated with HNSCC have been described (8,10), the specific molecular effects of these alterations in driving onset and progression of LSCC remain unclear (45). With regard

to the frequency of cancer-related gene mutations associated with LSCC, one study identified mutations in *TP53* in 68 (92%), *KMT2D* in 17 (23%), *PTEN* in 2 (3%), *FBXW7* in 4 (5%), *CDKN2A* in 17 (23%), and *PIK3CA* in 19 (26%) of 74 patients with LSCC (10). The NGS-based cancer panel testing conducted in the present study detected mutations in *TP53* in 18 (75%), *KMT2D* in 5 (21%), *PTEN* in 3 (13%), *PIK3CA* in 2 (8%), *FBXW7* in 2 (8%), and *CDKN2A* in 1 (4%) of the 24 LSCC patients tested. The types and frequencies of the mutations we identified were similar to those described in the previous report. Mutations in *TP53*, *KMT2D*, *PTEN*, *FBXW7*, and *CDKN2A* were not associated with clinical features and outcomes in the present study.

Tumor suppressor p53, encoded by the *TP53* gene, plays a key role in many cellular anticarcinogenic processes such as apoptosis and cell-cycle control (46). Exogenous carcinogens such as benzo(a)pyrene diolepoxide, which is present in cigarette smoke, readily induces G:C>A:T alterations at highly mutable CpG dinucleotides, and this propensity could explain the high rate of transversion events in human cancers (47). The frequency of mutations in *TP53* in smokers is reportedly higher than that in non-smokers with HNSCC (48). A previous study found that smoking status was the only independent variable associated with an increased risk of *TP53* mutations in the laryngeal mucosa (49). In the present study, the higher frequency of *TP53* mutations in patients with LSCC (75%) may be associated with the observation that 97% of the patients were current/ex-smokers. Other studies have reported that *TP53* mutations are associated with advanced stages of LSCC (50) and poor survivals in the case of disruptive mutations (8). However, other studies and our results revealed no significant association between *TP53* mutations and survival in LSCC (51).

Oncogenic mutations in *PIK3CA*, the α -type isoform of the catalytic subunit of phosphatidylinositol-3-kinase (PI3K), have been reported in various human cancers (52,53). Over 90% of the mutations in the *PIK3CA* gene in human cancers occur in 4 regions: the p85-binding (exons 1 and 2), C2 (exon 7), helical (exon 9), and kinase (exon 20) domains (54). Two mutations in the *PIK3CA* gene we identified were located within the kinase domain. The frequency of *PIK3CA* mutations in patients with HNSCC is reportedly 10.4-12.6% of patients with HNSCC (55-58). In another study, *PIK3CA* mutations were identified in 6 (12.8%) of 47 patients with LSCC (59). In the present study, mutations in *PIK3CA* were identified in 2 (8%) of 24 patients tested, and these 2 patients (with T1aN0 and T1bN0 of glottic type LSCC, respectively) exhibited local recurrence within 1 year after 70-Gy RT, suggesting that *PIK3CA* mutations are associated with radiation resistance in LSCC tumor cells. Upregulated of the PI3K/Akt/mTOR signaling pathway caused by *PIK3CA* mutations is reportedly related to the development of radiation resistance (60). *PIK3CA* mutation was identified as an independent prognostic factor for RFS by multivariate Cox proportional hazard analysis. A study revealed that *PIK3CA* mutations are associated with worse disease-free survival in HNSCC patients (61). Therefore, the detection of *PIK3CA* mutations could suggest the efficacy of RT is low, even in early stage LSCC. Treatment with inhibitors of the PI3K/Akt/mTOR signaling pathway could therefore be beneficial in such cases (60).

Our study has several limitations. First, its retrospective observational study recruiting a heterogeneous population with a variety of treatments introduced inherent biases and potential confounders. Second, the sample size was small, which may reduce the statistical power of some analyses. In particular, only 24 (27.3%) of the 88 patients with LSCC enrolled were tested for mutations using NGS because we started NGS analysis in 2017. Third, the heterogeneity of the follow-up schedule could have affected the survival rates. Despite these limitations, the present study provides valuable insights into the clinical outcomes of LSCC patients.

In conclusion, the clinical outcomes of LSCC patients in the present study, including the 5-year survival rates, were consistent with those of other studies. NGS-based testing of 160 cancer-related genes in 24 of the LSCC patients revealed that mutations in *PIK3CA* are significantly associated with poorer RFS. Therefore, NGS-based cancer panel testing could be useful for enhancing our understanding of the clinical features and outcomes of patients with LSCC.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author. The genome sequencing data was deposited in the URL (<https://ddbj.nig.ac.jp/search/entry/bioproject/PRJDB19720>) under the accession number PRJDB19720.

Authors' contributions

AK conceptualized the study, performed the investigation and wrote the original draft. NB conceptualized the study, performed the formal analysis, and reviewed and edited the manuscript. TG, MK, RS, SSu, SSa, RT, SH, MH and DA analyzed the data. SB and YK designed the methodology. NB, AK, TG and SB confirmed the authenticity of all the raw data. MT, HN and HK conceptualized the study and designed the methodology. All authors have read and agreed to the final version of the manuscript.

Ethics approval and consent to participate

All procedures performed on patient tumor samples in this study were carried out in accordance with the ethical standards of the Institute Ethics Committee and the Declaration of Helsinki of 1964 and its subsequent amendments or comparable ethical standards. The present study was approved by the Ethics Committee of Hokuto Hospital (approval no. 1078; Obihiro, Japan). Informed consent to participate in this study and to publish was obtained from all patients.

Patient consent for publication

Informed consent for genetic analysis was obtained from 24 patients.

Competing interests

The authors declare that they have no competing interests.

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