Table 2 Primary tumor location and areas of nodal metastases in 127 patients with tumor limited to within the submucosa (pT1)

Area	Tumor location			Total (n = 127) (%)
	Upper (n = 22) (%)	Mid (n = 67) (%)	Lower (n = 38) (%)	
Supraclavicular	3 (13.6)	8 (11.9)	–	11 (8.7)
Upper mediastinal	12 (54.5)	15 (22.4)	5 (13.2)	32 (25.2)
Mid-mediastinal	1 (4.5)	4 (6.0)	2 (5.3)	7 (5.5)
Lower mediastinal	–	6 (9.0)	2 (5.3)	8 (6.3)
Perigastric	–	16 (23.9)	15 (39.5)	31 (24.4)
Celiac	–	2 (3.0)	–	2 (1.6)

Primary tumor location and areas of lymph node metastases in 127 patients with pT1 tumor are shown in Table 2. When tumor was limited to within the submucosal layer, frequencies of periesophageal node metastasis in the mid- and lower mediastinum were very low. Patients with tumor located in the upper esophagus showed node metastasis in the upper mediastinum most frequently as a matter of course. Even with tumors located in the mid- and lower esophagus, node metastasis was more frequent in the upper mediastinum and perigastric area than in the mid- and lower mediastinum. Supraclavicular node metastasis was also more frequent than that in the mid- and lower periesophageal area in patients with tumor located in the mid-esophagus.

Primary tumor location and areas of lymph node metastases in 229 patients with pT2-4 tumor are shown in Table 3. Frequency of lymph node metastasis in the mid- and lower mediastinum was increased dramatically compared with patients showing pT1 tumor. However, frequencies of node metastasis in the mid- and lower mediastinum were still lower than those in the upper mediastinum and perigastric area. Supraclavicular node metastasis was frequent as same as that in the lower mediastinum in patients with tumor located in the mid-esophagus. The upper mediastinal node metastasis was frequent as same as in the lower mediastinum in patients with tumor located in the lower esophagus.

Overall postoperative survival curves according to the number of involved nodes of patients with pT1 tumor and patients with pT2-4 tumor are shown in Figure 1. Overall postoperative survival curves did not differ among the areas of involved nodes (Fig. 2). More than 20% of patients with supraclavicular node metastasis showed long-term survival. The most predictive factor associated with lymph node metastasis for postoperative survival was not the area of involved nodes, but the number of involved nodes according to multivariate analyses (Table 4).

DISCUSSION

Our clinical data for the frequency of involved nodes according to the areas of dissection verified the anatomical observations[11,12] that long longitudinal extension of lymphatic drainage in the submucosa connected to the superior mediastinum along the recurrent nerve and paracardial lymphatics. Superficial tumors (pT1) obtain entry into the abundant lymph-capillary plexus in the lamina propria mucosae and submucosa of the esophagus. These lymphatic networks extend longitudinal craniocaudally and continue with the lymphatics of the proximal esophagus and cardia.[11] Superficial lymphatic vessels of the proximal part of the esophagus have abundant direct connections with the recurrent nerve nodes.[12] In patients with tumor limited to within the submucosal layer, even with tumors located in the mid- and lower esophagus, lymphatic metastasis was frequent in the upper mediastinum and perigastric area. Isolated distant lymph node involvement from superficial carcinoma is thus not necessarily a sign of advanced disease.

Another anatomical concept[11] was confirmed, with lymphatic routes to periesophageal lymph nodes usually originating from the intermuscular area of the

Table 3 Primary tumor location and areas of nodal metastases in 229 patients with tumor invading into or through the muscularis propria (pT2-4)

Area	Tumor location			Total (n = 229) (%)
	Upper (n = 33) (%)	Mid (n = 106) (%)	Lower (n = 90) (%)	
Supraclavicular	7 (21.2)	27 (25.5)	5 (5.6)	39 (17.0)
Upper mediastinal	28 (84.8)	65 (61.3)	24 (26.7)	117 (51.1)
Mid-mediastinal	2 (6.1)	52 (49.1)	21 (23.3)	75 (32.8)
Lower mediastinal	2 (6.1)	27 (25.5)	24 (26.7)	53 (23.1)
Perigastric	2 (6.1)	57 (53.8)	59 (65.6)	118 (51.5)
Celiac	–	5 (4.7)	8 (8.9)	13 (5.7)

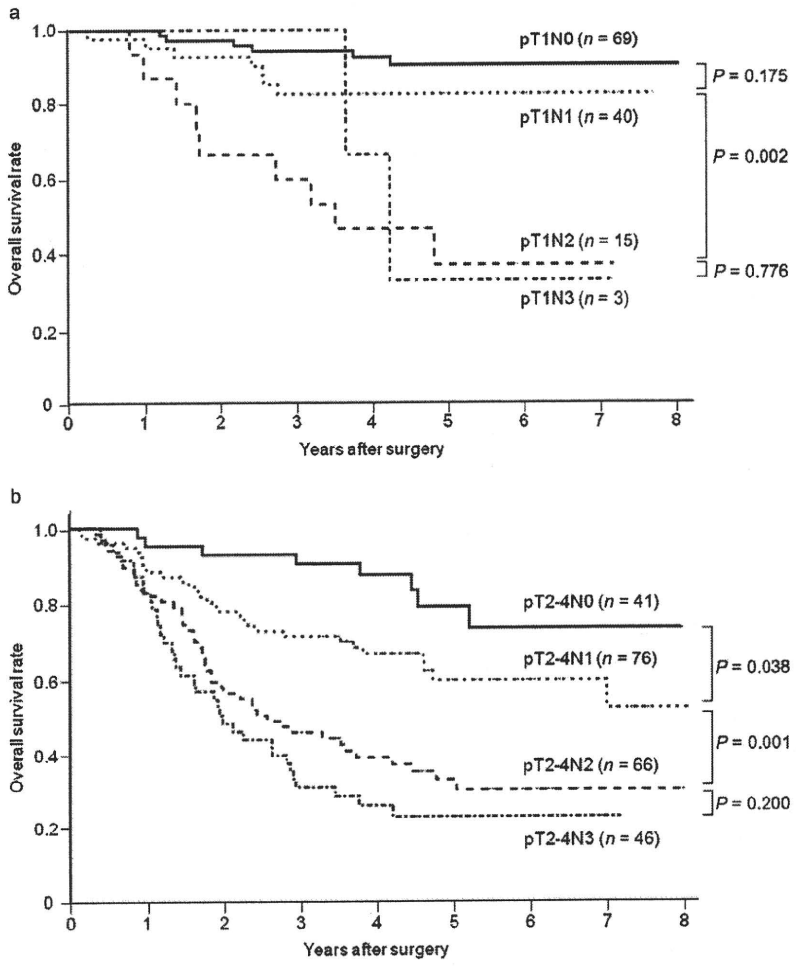

Fig. 1 Overall postoperative survival curves of patients with (a) pT1 and (b) pT2-4 tumor according to the number of involved nodes.

muscularis propria and restricted lymphatic communication between submucosal and intermuscular areas. When tumor was limited to within the submucosal layer in the mid- and lower esophagus, tumor cells had little chance to flow into lymphatic routes originating from the intermuscular area of the muscularis propria and to spread to periesophageal nodes. When tumor invaded or penetrated the muscle

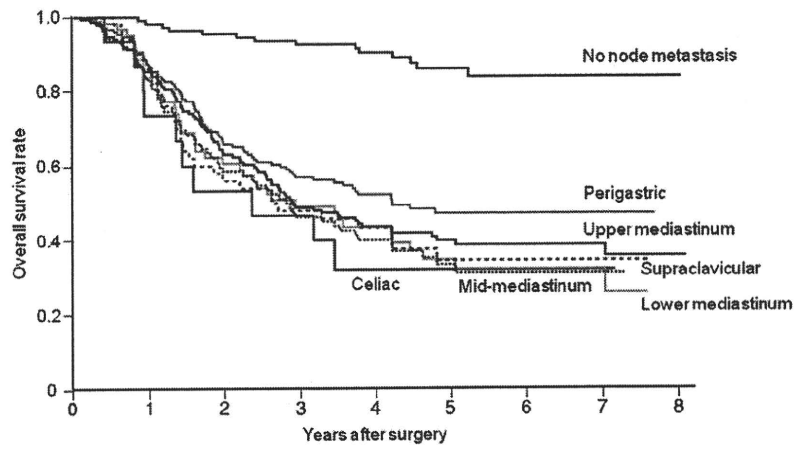

Fig. 2 Overall postoperative survival curves of patients with involved nodes according to area.

Table 4 Results from multivariate Cox-regression analysis to study association of areas and numbers of node metastasis

Categorical variable	Hazard ratio	*P*
Gender		
Female	1	–
Male	1.648 (0.884–3.075)	0.116
Age		
≤Mean	1	–
>Mean	1.631 (1.137–2.341)	0.008
Tumor location	–	0.928
Lower	1	–
Mid	0.927 (0.588–1.461)	0.745
Upper	0.930 (0.485–1.784)	0.827
T classification (pathological)	–	0.000
pT1	1	–
pT2	2.344 (1.260–4.357)	0.007
pT3	2.163 (1.312–3.567)	0.002
pT4	8.414 (3.009–23.530)	0.000
Number of nodal metastasis†	–	0.000
0	1	–
1–2	2.281 (1.070–4.864)	0.033
3–6	5.061 (2.108–12.154)	0.000
>7	5.065 (1.641–15.634)	0.005
Area of nodal metastasis		
Supraclavicular	1.097 (0.674–1.785)	0.710
Upper mediastinal	1.511 (0.909–2.509)	0.111
Mid-mediastinal	1.008 (0.629–1.614)	0.975
Lower mediastinal	1.143 (0.742–1.760)	0.545
Perigastric	0.729 (0.444–1.199)	0.213
Celiac	1.735 (0.848–3.548)	0.131

†Number of nodal metastasis included supraclavicular node metastasis.

layer, frequency of periesophageal lymph node metastasis in the mid- and lower mediastinum increased. Periesophageal lymph node metastasis in the mid- and lower mediastinum would be a sign of more advanced esophageal cancer.

Survival after curative three-field dissection did not differ among patients with involved nodes in the upper, mid-, and lower mediastinum, perigastric area, and even supraclavicular area. This lack of difference in survival among patients with involved nodes according to area suggested that these nodes should be staged equivalently. The conventional strategy of dissection is based on the hypothesis that thoracic esophageal tumor cells involve the nearby periesophageal nodes first. The mid- and lower esophageal tumors involve the mid- and lower periesophageal nodes first. Then tumor cells spread to nodes a little further into the upper mediastinum and perigastric area. Finally, tumor cells reach distant nodes in the supraclavicular and celiac areas. Radical resection of those distant node metastases required extended dissection. However, the efficacy of extended dissection appears slight for this advanced situation.[13,14] This concept was not demonstrated in the present study. From the time the tumor is limited to within submucosal layer, tumor cells have the chance to spread craniocaudally via the submucosal lymphatic plexus and involve nodes in the upper mediastinum, along the recurrent nerve and perigastric area. The lymphatics channels connect the recurrent nerve node to supraclavicular node and the left gastric artery node to celiac node.

The present study supported the recent change of TNM classification[15] for esophageal cancer that the N category is classified by the number of regional lymph node metastasis. The most predictive factor for lymph node metastasis was not the area of involved nodes, but the number of involved nodes.[16,17] Patients with lymph node metastasis in supraclavicular area still show good survival after esophagectomy with lymph node dissection. Lymph node metastasis in supraclavicular area does not mean systemic disease and should not be classified as M1.

The TNM classification for esophageal cancer defines the minimum number of lymph nodes examined necessary for accurate nodal staging as 6, but does not define the areas of lymph nodes sampled.[15] Even with tumors located in the mid- and lower esophagus, node metastasis was more frequent in the upper mediastinum and perigastric area than in the mid- and lower mediastinum. Node metastasis was also more frequent in supraclavicular area than in the mid- and lower periesophageal area in patients with tumor in the mid-esophagus. For adequate nodal staging, these areas should be dissected even for patients with tumor limited to within the submucosal layer.

We understand a limitation in the present study that all analyses in the present study were based on patients with squamous cell carcinoma. In recent decades, a dramatic rise in the incidence of adenocarcinoma has been seen in Western patients. In Asian patients, including Japanese patients, squamous cell cancer remains the predominant type. No epidemiological data have yet suggested any obvious increase in adenocarcinoma in Japan.[18] During the 5-year study period, the incidence of adenocarcinoma was <3% in our institution. The proposed concept of lymphatic metastasis should be assessed for applicability to Western patients with adenocarcinoma.

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Forkhead box A1 transcriptional pathway in KRT7-expressing esophageal squamous cell carcinomas with extensive lymph node metastasis

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Received September 7, 2009; Accepted October 18, 2009

DOI: 10.3892/ijo_00000503

Abstract. Prognosis of cancers with lymph node metastasis is known to be very poor; however, it is still controversial whether metastatic potential can be evaluated by expression profiles of primary tumors. Therefore, to address this issue, we compared gene expression profiles of 24 esophageal squamous cell carcinomas (ESCCs) with extensive lymph node metastasis and 11 ESCCs with no metastatic lymph node. However, there was no gene cluster distinguishing these two groups, suggesting that lymph node metastasis-associated genes are varied depending on cases or subgroups. Therefore, we applied a recently developed filtering method (S2N') to identify such genes, and successfully extracted 209 genes associated with node status. Among them, over-expression of *CALB1*, *KRT7/CK7*, *MUC1* and *CEA/CEACAM5* in poor prognostic cases with metastatic lymph nodes was confirmed in two sets of ESCCs by RT-PCR. Each often seemed to have glandular cell type-characteristics in both the gene expression and morphology. It was also revealed that *FOXA1* siRNA treatment of esophageal cancer cells reduced the mRNA level of both *KRT7* and a stabilizer of epithelial-mesenchymal transition (EMT) regulator *LOXL2*, and that both *FOXA1* and *LOXL2* siRNAs reduced invasion and migration of ESCC cells. In 15 *KRT7*-expressing ESCCs with metastatic lymph nodes, 60% expressed *FOXA1* and 33% expressed both *FOXA1* and *LOXL2*. These results suggest that FOXA1 induces not only *KRT7* but also *LOXL2* in a subset of poor prognostic ESCCs with metastatic lymph nodes, and it is also plausible, that other FOXA1 downstream genes could be therapeutic targets of poor prognostic ESCCs.

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Key words: microarray, FOXA1, KRT7/CK7, LOXL2, esophageal cancer

Introduction

Cancer is a major cause of human deaths world-wide. Gene expression data from DNA microarrays are individualized and useful in the diagnosis and prognosis of diseases (1). Esophageal cancer is the eighth most common cancer and the sixth most common cause of cancer-related mortality (2). Esophageal cancer in East Asian countries and some parts of Europe consists mainly of squamous cell carcinomas. Chemoradiotherapy (CRT) followed by surgery is the standard therapy in Western countries. In Japan, CRT or neoadjuvant chemotherapy followed by surgery and definitive CRT are the standard therapies (3). For locally advanced esophageal cancers, surgery is still the standard therapy in Japan. A recent improvement in surgical resection following radical node dissection has been reported with 5-year survival rates of 31-55% (4). Yet also in this cancer, lymph node metastasis has been reported to be a most strong marker for poor prognosis in patients with the improved surgery (4), especially, those patients with >5 metastatic lymph nodes did very poorly compared with patients with no metastatic lymph nodes. Thus, lymph node metastasis is known to be tightly associated with a poor prognosis in many surgically resectable solid tumors. Identification of genes associated with lymph node metastasis is very important for

establishing a molecular diagnosis and also for understanding the malignant phenotype; however, it is still controversial whether metastatic potential can be evaluated by expression profiles of primary tumors. Esophageal cancer provides an opportunity to address such an important issue.

For a marker gene selection from mRNA expression profiles, the use of one of the many filtering methods is necessary, such as Mann-Whitney's U-test, Student's t-test, Welch's t-test, signal-to-noise (S2N) (5), significance analysis of microarrays (SAM) (6), and nearest shrunken centroids (NSC) (7). In our previous study, we developed the projective adaptive resonance theory (PART) filtering method (8) and reported that the PART filtering method showed a better performance than conventional methods such as S2N and NSC (9-14). By the PART method, the genes that have a low variance in the gene expression level in either of two classes or subgroups can be selected. We further developed another simple and practical filtering method, modified S2N (S2N) (12), based on the concept underlying the PART filtering method. The S2N' filtering method was statistically superior to the conventional methods such as Mann-Whitney's U-test, Student's t-test, Welch's t-test, S2N, SAM and NSC (12).

In the present study, we applied S2N' to genome-wide gene expression profiles to identify marker genes for poor prognostic esophageal squamous cell carcinomas (ESCCs) with lymph node metastasis, and successfully identified FOXA1 transcriptional pathways for cell invasion or migration in a subset of such ESCCs.

Materials and methods

Tissue samples. All cases of esophageal cancers examined in this study were diagnosed as squamous cell carcinoma. All esophageal squamous cell carcinoma (ESCC) patients underwent surgical resection following two- or three-field node dissection between 1994 and 2000 at the Central Hospital of the National Cancer Center. ESCC tissues were provided after obtaining informed consent from each patient and approval by the Center's Ethics Committee.

Microarray analysis. Gene expression profile data were obtained from 35 surgical specimens from ESCC patients: 24 patients with >5 metastatic lymph nodes (N5 group) and 11 patients with no metastatic lymph node (N0 group). For RNA extraction, trained pathologists carefully excised bulk tissue samples from the main tumor, leaving a clear margin from the surrounding normal tissue. Total RNAs extracted from the bulk tissue samples were biotin-labeled and hybridized to high-density oligonucleotide microarrays (Human Expression Array U95A version 2, Affymetrix, Santa Clara, CA, USA) according to the manufacturer's instructions. The scanned data of the arrays were processed by GeneChip Analysis Suite version 4.0, which scaled the average intensity of all the genes on each array to a target signal of 1,000 to reliably compare variable multiple arrays.

S2N' filtering of gene expression profiles. The S2N' filtering method for gene selection from microarray data was originally developed by us (12). This method allows us to extract as a marker gene even in the case that only a single gene (or a few genes) was expressed specifically in one of the two groups. By this method, we selected the top 209 marker gene candidates which were expressed preferentially in one of the two groups (N0 and N5) in this study.

Survival analysis and hierarchical clustering analysis. The Kaplan-Meier metastases analysis plots were formulated using WinSTAT Statistics for Windows ver. 3.1 (Light Stone, Tokyo, Japan). The significance of the difference in survival rates was analyzed using a log-rank test (Mantel-Cox method). Hierarchical clustering is widely used as one of the unsupervised learning methods. In this study, hierarchical clustering of microarray data of 35 ESCCs was performed by the use of CLUSTER software (15).

Semi-quantitative and quantitative RT-PCR. Surgical specimens were snap-frozen in liquid nitrogen. Total RNA was isolated by suspending the cells in Isogen lysis buffer (Nippon Gene, Toyama, Japan) followed by precipitation with isopropanol. RT-PCR was carried out using primer sets designed for detecting the 3' side of cDNA of each gene: for *CALB1*, 5'-AATCAAAATGTGTGGGAAAG-3' and 5'-CCCAG CACGAGAATAAGAG-3', for *TFF3*, 5'-CTGAGGCACCT CCAGCTGCCCCCG-3' and 5'-GGAGCATGGGACCTT TTTCG-3', for *KRT7*, 5'-CTGAAGGCTTATTCCATCCG-3' and 5'-CCTCAGAATGAGGCTGCTTT-3', for *CLDN10*, 5'-ACGGCTAACTGGATAACTGA-3' and 5'-TGTCTCACT CACTCCTCACA-3', for *MUC1*, 5'-AATTGACTCTGG CCTTCCGA-3' and 5'-GCCACCATTACCTGCAGAAA-3', for *CEA*, 5'-AGACTCTGACCAGAGATCGA-3' and 5'-GGT GGACAGTTTCATGAAGC-3', for *PGC*, 5'-CCAGCTTGA CCTTCATCATC-3' and 5'-GCTGAATCCAGAGTGGA AAG-3', for *LIPF*, 5'-ACGAGTCGCTTGGATGTGTA-3' and 5'-CGTTCCACACTGCAATTGGT-3', for *LOXL2*, 5'-CACC ATGTGTCATCACAGAC-3' and 5'-CTCCTTAGATTGCTT CTCCC-3', for *FOXA1*, 5'-GGTCATGTCATTCTGAG GTC-3' and 5'-TAACACCATGTCCAACTGTG-3', for *ACTB* (ß-actin), 5'-TCATCACCATTGGCAATGAG-3' and 5'-CAC TGTGTTGGCGTACAGGT-3'.

For semi-quantitative RT-PCR, we showed data within linear range by performing 25-35 cycles of PCR. Quantitative real-time PCR was performed by a Bio-Rad iCycler with iQ Syber Green Supermix (Bio-Rad, Hercules, CA, USA) as directed by the manufacturer. The value of 1/2N (N: the number of PCR cycles corresponding to the onset of the linear amplification of each gene product) was calculated as a relative mRNA expression level of each gene normalized to *ACTB*. The data from 2 independent analyses for each sample were averaged.

Immunohistochemistry. All resected specimens were fixed in either 10% formalin or methanol and embedded in paraffin. Tissue sections of 4 μm in thickness were cut from a paraffin-embedded block including the most representative area of the tumor, and were used for immunohistochemical staining. The sections were deparaffinized in xylene, dehydrated in a graded series of ethanol, and immersed in methanol with 0.3% hydrogen peroxide for 15 min to inhibit endogenous peroxidase activity. The slides were heated at 95°C for 20 min

in a microwave oven in a citrate buffer with pH 6.0 for antigen retrieval, and allowed to cool to room temperature. Non-specific binding was blocked by preincubation with 2% normal swine serum in phosphate-buffered saline (PBS) for 60 min at room temperature. The slides were then incubated overnight at 4°C with respective primary antibodies. The slides were washed three times with PBS and incubated with EnVision (Dako, Carpinteria, CA, USA) for 1 h at room temperature. The sections were visualized using 3, 3'-diaminobenzidine tetrahydrochloride in 50 mM Tris-buffer (pH 7.6) containing 0.3% hydrogen peroxide as the chromogen, and counterstained by hematoxylin. Antibodies used in this study were anti-CK7 (mouse monoclonal, clone OV-TL, Dako, 1:50).

siRNA transfection. The esophageal cancer cell line TE3 was used in this study because among 6 cell lines (TE1, TE3, TE5, TE6, TE8 and TE10), TE3 has been reported to maintain the expression profile of primary esophageal cancers and to most mimick the basal cell (12). Three or four small interfering (si)RNA fragments were used for suppressing *FOXA1*, *KRT7* and *LOXL2* mRNA expression respectively, and the most effective one was selected by quantitative real-time RT-PCR analysis; for *FOXA1* mRNA target sequence: 5'-GGAGAGATAAGTTATAGGG-3' (siRNA ID: 107428, Ambion, Austin, TX, USA); for *KRT7*: 5'-GGCTGAGATC GACAACAT-3' (siRNA ID: 215171, Ambion); for *LOXL2*: 5'-CCCTCCAGTCTATTATAGT-3' (siRNA ID: 114218, Ambion). The siRNAs including control siRNA (1022076, Qiagen, Valencia, CA, USA) were introduced to TE3 using DharmaFECT (Dharmacon, La Fayette, CO, USA) following the procedure recommended by the manufacturer. The RT-PCR and Matrigel invasion assay were carried out after siRNA treatment of TE3 cells.

Matrigel invasion and wound healing assays. Invasion of the esophageal cancer TE3 cells *in vitro* was measured by BD BioCoat™ Matrigel™ Invasion Chamber (BD Biosciences, San Jose, CA, USA) according to the manufacturer's protocol. After siRNA transfection, the cells were trypsinized and transferred into triplicate wells. After 24-h incubation, the cells that passed through the filter into the lower wells were fixed, stained and counted. For the wound healing assay, TE3 cells were grown till they were 90% confluent. A lineal scratch wound was made on the plate by a plastic tip. Images were taken every 12 h for 2 days.

Results

Survival analysis. The Kaplan-Meier method was performed on two groups: the N0 group consisting of 11 esophageal squamous cell carcinoma (ESCC) patients with no metastatic lymph node and the N5 group consisting of 24 ESCC patients with more than 5 metastatic lymph nodes. All patients underwent surgical resection following two- or three-field node dissection. These two groups showed a significant difference ($p<0.001$) in survival rates after surgical resection (Fig. 1).

Calculation of S2N' values and ranking of each gene probe. First, we obtained mRNA expression profiles of ESCC tissues from a total of 35 patients of the two groups (N0 and N5). By unsupervised hierarchical clustering using various types of data processing, the two groups were not separated clearly (data not shown). These results implied that no large gene clusters associated with each group were present or that subgroups were present in each group. In this situation, the conventional filtering methods, such as Mann-Whitney's U-test, Student's t-test and Welch's t-test, were thought to be ineffective. In fact, only a few marker genes were selected by these t- or u-test methods (data not shown). The number of selected gene was too small to show significance. Therefore, we applied our previously developed S2N' method for marker gene selection in the two groups of ESCCs. The S2N' values were calculated for each gene probe and we successfully extracted 209 genes which were expressed preferentially in one of two groups. The top 50 genes were shown in Table I. However, this method allows us to extract a marker gene even in the case that only a single gene was expressed specifically in one of the two groups (12). Therefore, we have to further select genes with regards to the frequency of specific expression in one of the two groups. Of the top 50 genes, we selected 8 genes (Rank 1: *CALB1*; Rank 2: *TFF3*; Rank 3: *KRT7*; Rank 9: *CLDN10*; Rank 13: *FOXA1*; Rank 15: *MAL*; Rank 20: *CEACAM5*, and Rank 21: *MUC1*) that were found to be expressed preferentially and frequently in the N5 group. Their signal levels are shown in Fig. 2. These results suggest that our S2N' is very useful as a new method of candidate gene selection for genome-wide gene expression profiles obtained from microarrays.

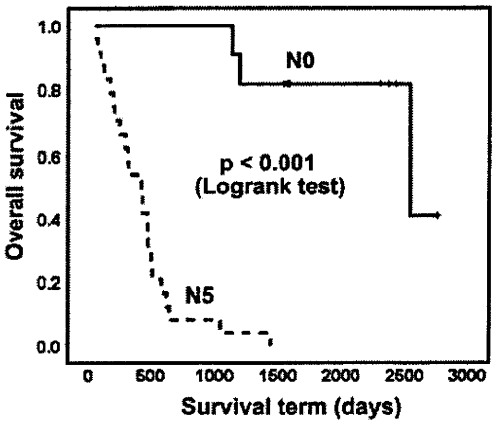

Figure 1. The Kaplan-Meier curve for each group. The N0 group with no metastatic lymph node consisted of 11 patients, and the N5 group with >5 metastatic lymph nodes consisted of 24 patients. The p-value was calculated by log-rank test.

RT-PCR analysis of 6 candidate marker genes in two sets of ESCCs with more than 5 metastatic lymph nodes or with no metastatic lymph node. Of the 8 genes, 6 (*CALB1*, *TFF3*, *KRT7/CK7*, *CLDN10*, *MUC1* and *CEA/CEACAM5*) were first analyzed by semi-quantitative RT-PCR in two independent sets of ESCCs: in the first set are 42 samples (12 patients with no metastatic lymph node including 11 N0 patients, and 30 patients with more than 5 metastatic lymph nodes including 24 N5 patients); in the second set are 22 samples

Table I. The top 50 marker gene candidates for the N0 or N5 group selected by the S2N' filtering method.

Probe set ID	GenBank accession	Gene symbol	S2N' rank	S2N'	Marker type
36570_at	AF068862	CALB1	1	14.6	N5
31477_at	L08044	TFF3	2	8.2	N5
41294_at	AJ238246	KRT7	3	6.3	N5
39220_at	T92248	SCGB1A1	4	5.1	N5
38430_at	AA128249	FABP4	5	4.9	N0
174_s_at	U61167	ITSN2	6	4.5	N5
37149_s_at	U95626	LOC728320 /// LTF	7	4.3	N5
34637_f_at	M12963	ADH1A	8	4.0	N5
39579_at	U89916	CLDN10	9	3.7	N5
1497_at	L04270	LTBR	10	2.9	N0
38173_at	AB028999	SETD1B	11	2.7	N5
40193_at	X51956	ENO2	12	2.7	N0
37141_at	U39840	FOXA1	13	2.6	N5
35314_at	D63880	NCAPD2	14	2.6	N0
38051_at	X76220	MAL	15	2.5	N5
34873_at	Y16241	NEBL	16	2.5	N5
1890_at	AB000584	GDF15	17	2.3	N5
38648_at	U80760	ZNF384	18	2.3	N0
1389_at	J03779	MME	19	2.2	N5
1582_at	M29540	CEACAM5	20	2.1	N5
38784_g_at	J05581	MUC1	21	2.1	N5
41308_at	U37408	CTBP1	22	2.0	N5
37576_at	U52969	PCP4	23	2.0	N5
37687_i_at	M31932	FCGR2A	24	1.9	N5
1006_at	X07820	MMP10	25	1.9	N5
37242_at	U79260	FTO	26	1.9	N0
36123_at	D87292	TST	27	1.9	N5
1105_s_at	M12886	TRBV3-1 /// TRBV5-4 /// TRBV7-2	28	1.9	N5
1096_g_at	M28170	CD19	29	1.9	N5
36280_at	U26174	GZMK	30	1.9	N5
35425_at	AJ243512	BARX2	31	1.8	N5
37405_at	U29091	SELENBP1	32	1.8	N5
35227_at	U72066	RBBP8	33	1.7	N5
239_at	M63138	CTSD	34	1.7	N5
39249_at	AB001325	AQP3	35	1.7	N5
1020_s_at	U85611	CIB1	36	1.7	N5
41814_at	M29877	FUCA1	37	1.7	N5
37009_at	AL035079	CAT	38	1.7	N5
37272_at	X57206	ITPKB	39	1.7	N5
31637_s_at	X72631	NR1D1 /// THRA	40	1.7	N5
35693_at	AF070616	HPCAL1	41	1.7	N5
36804_at	M34455	INDO	42	1.7	N5
700_s_at	HG371-HT26388	-[a]	43	1.7	N5
32561_at	D63480	KIAA0146	44	1.6	N0
36023_at	AI864120	-	45	1.6	N0
38783_at	J05581	MUC1	46	1.6	N5
927_s_at	J05582	MUC1	47	1.6	N5
36851_g_at	U42360	TUSC3	48	1.6	N0
35337_at	AL050254	FBXO7	49	1.6	N5
40541_at	X01630	ASS1	50	1.6	N5

[a]An official gene name is not given.

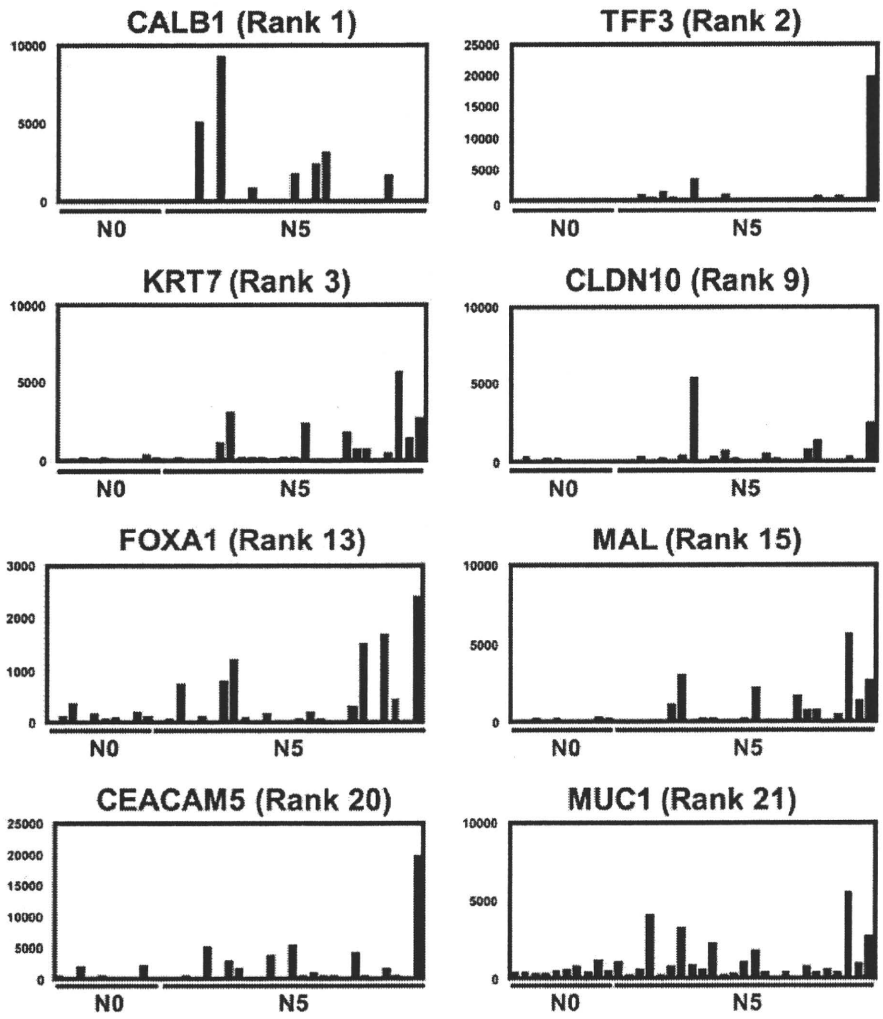

Figure 2. Each signal of microarray for 8 representative top genes whose level was highly correlated with N5 group, respectively.

(10 patients with no metastatic lymph node and 11 patients with >5 metastatic lymph nodes). Similar to the 11 N0 patients and the 24 N5 patients (Fig. 1), Kaplan-Meier analyses of the two groups in each set showed a significant difference in survival after surgical resection (data not shown). In correspondence with microarray data, preferentials expression of all the 6 genes in the N5 ESCC patients with >5 lymph nodes was confirmed in the first set (Fig. 3A). Among these 6 genes, 4 (*CALB1*, *KRT7*, *MUC1* and *CEA*) showed a reproducible expression pattern in the second set (Fig. 3B). These 4 genes preferentially expressed in patients with >5 metastatic lymph nodes in both of the two sets of samples. These results suggest that *CALB1*, *KRT7*, *MUC1* and *CEA* are potential molecular markers for ESCC with poor prognosis, and that the present gene list should provide other marker genes. Among the 6 candidate marker genes identified here, *KRT7* seemed to be one of the two best markers, *KRT7* and *CEA* for ESCC with poor prognosis, because it expressed in a few of the 22 patients with no metastatic lymph node, but in >50% of 42 patients with >5 metastatic lymph nodes (semi-quantitative RT-PCR in Fig. 3A and B). These results were confirmed by quantitative real-time RT-PCR (Fig. 3C and D).

Biological implications of the candidate marker genes for ESCC with poor prognosis. Among the 6 genes, *CEA* and *TFF3* were known not to be expressed in the squamous epithelium of the esophagus, skin and uterine cervix, but in the non-malignant or malignant glandular epithelium of the stomach and colon (17). Accordingly, a subset of poor prognostic ESCC is thought to express glandular epithelial cell markers. To confirm this hypothesis, we investigated the expression of *PGC*-encoding pepsinogen C and *LIPF*-encoding gastric lipase that are well-known as typical markers for glandular epithelium of the stomach. Three patients with >5 metastatic lymph nodes (2 in the first set and 1 in the second set) expressed both *PGC* and *LIPF*, clearly (Fig. 4A and B). The percentage in 42 patients with >5 metastatic lymph nodes was 7% (3/42). Interestingly, a patient positive with these two stomach markers (Fig. 4A, lane 42) had the most metastatic lymph nodes (49 positive nodes) among the 64 ESCC patients examined.

KRT7 has been reported to be expressed in the ducts of many tissues including the liver, kidney, pancreas and mammary gland (18); however, relating to esophagus, little is known. As shown in Fig. 4C, immunohistochemical analyses showed that KRT7 was expressed in the ducts, but not in the

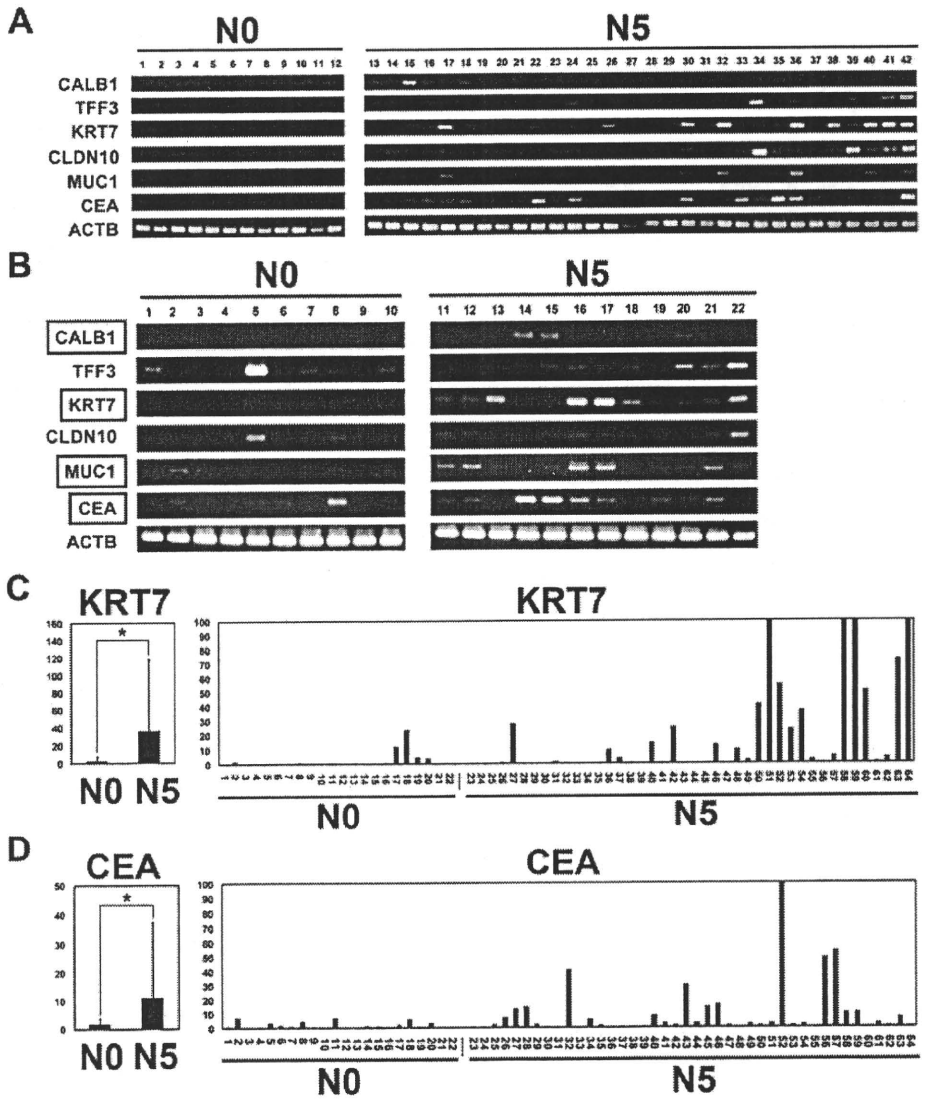

Figure 3. RT-PCR analyses of 6 candidate marker genes for poor prognostic ESCCs with >5 metastatic lymph nodes. (A) Semi-quantitative RT-PCR of 6 poor prognosis marker gene candidates (*CALB1*, *TFF3*, *KRT7*, *CLDN10*, *MUC1* and *CEA*, respectively) in 12 ESCCs with no metastatic lymph node (N0) and in 30 ESCCs with >5 metastatic lymph nodes (N5). (B) Semi-quantitative RT-PCR of these 6 genes in another set of ESCCs consisting of 10 ESCCs with no metastatic lymph node (N0) and in 12 ESCCs with >5 metastatic lymph nodes (N5). Three genes that showed preferential expression in patients with N5 in both the sets of ESCCs are indicated by boxes. (C) Quantitative real-time RT-PCR of *KRT7* in a total of 64 ESCCs consisting of 22 ESCCs with no metastatic lymph node (N0) and in 42 ESCCs with >5 metastatic lymph nodes (N5). (D) Quantitative real-time RT-PCR of *CEA* in these 64 ESCCs. The mean in all samples is indicated by a dotted line.

stratified epithelia of the esophagus. Therefore, KRT7 could be a marker of the esophageal ducts. Those results suggested that a small subset of ESCCs with poor prognosis expressed some markers of the glandular epithelial cell of the stomach, and that a large subset of the ESCCs with poor prognosis showed a feature of the ducts in the esophagus. We next carefully compared the morphological differences between 13 node negative cases and 19 node positive cases (>5 metastatic lymph nodes). In this morphological study, two expert pathologists examined the slides that were prepared by the other pathologist without information on the nodal status. Percentages of the cases with partial glandular structure in each group were averaged. Interestingly, in accordance with the above-mentioned glandular-epithelial cell marker expression in poor prognostic node-positive cases, the partial glandular structure was found preferentially in node-positive cases (74%) compared with node-negative cases (38%) (Fig. 4D).

Forkhead box A1 (FOXA1) is an upstream regulator of KRT7 and LOXL2 is expressed in a subset of poor prognostic ESCC patients. Although *KRT7* was found to be one of the useful markers for poor prognostic ESCCs, the reason why *KRT7*-overexpressing ESCCs are poor is still unknown. We next searched the upstream transcriptional regulator of *KRT7* in the gene list as shown in Table I and found FOXA1 as a transcriptional factor co-expressed with *KRT7* (Fig. 2). To investigate whether *FOXA1* is an upstream regulator for *KRT7*, we introduced siRNA of *FOXA1* and *KRT7* into an esophageal cancer cell line, TE3, that has been confirmed to

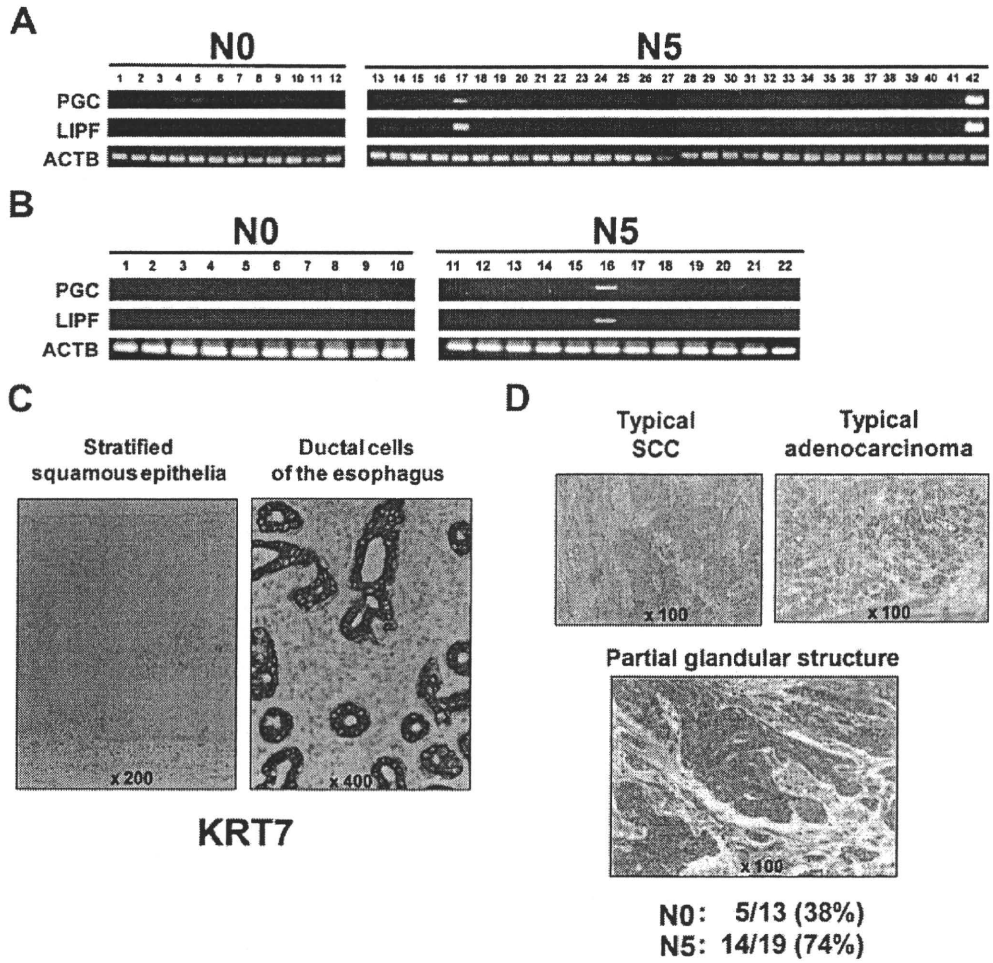

Figure 4. Poor prognostic ESCCs showed characteristics of glandular and/or ductal epithelial cells. (A) RT-PCR of 2 stomach glandular epithelial cell markers (*PGC* and *LIPF*) in the first set of ESCCs. (B) RT-PCR of these 2 stomach glandular epithelial cell markers in the second set of ESCCs. (C) Immunohistochemical staining of KRT7 in normal stratified squamous epithelia and ductal cells in the esophagus. KRT7 is expressed only in ductal cells of the esophagus. (D) Comparison of morphology between N0 and N5 cases. Partial glandular structure was observed preferentially in N0 cases (38%) compared with N5 cases (74%). A typical squamous cell carcinoma (SCC) and adenocarcinoma in the esophagus are shown as a reference (upper).

maintain the expression profile of primary ESCCs the most among 5 cell lines (16) and to express these two genes (data not shown). In this *in vitro* culture, *FOXA1* siRNA successfully reduced the mRNA level of both *FOXA1* and *KRT7*, while *KRT7* siRNA decreased only its own mRNA level (Fig. 5A). We next investigated whether *in vivo* co-expression between *FOXA1* and *KRT7* is observed in primary ESCCs by semi-quantitative RT-PCR. Nine (lanes 16, 17, 22, 34, 36, 39, 40, 41 and 42, respectively) (60%) out of 15 *KRT7* expressing ESCCs with >5 metastatic lymph nodes showed *FOXA1* expression (Fig. 5B). These results suggest that *FOXA1* is an upstream regulator for *KRT7* in a subset of poor prognostic ESCCs.

We next performed genome-wide screening of *FOXA1* downstream genes by microarray analysis of *FOXA1* siRNA-transfected TE3 cells, and found that reduced LOXL2 expression corresponded to a decrease of *FOXA1* mRNA. This result suggests that FOXA1 regulates *LOXL2* expression, which was recently reported as a new poor prognosis marker of laryngeal SCCs (19). To confirm the microarray result, we performed quantitative real-time RT-PCR after transfection of control and *FOXA1* siRNAs into TE3 cells. A significant decrease of *LOXL2* mRNA was found by *FOXA1* siRNA compared with control siRNA (Fig. 5C, left panel). In accordance with the initial *in vitro* study of breast cancer cells (20,21), *LOXL2* siRNA inhibited TE3 cell invasion in Matrigel (Fig. 5C, right panel). We examined the *LOXL2* mRNA expression in primary ESCCs by quantitative real-time RT-PCR. Overexpression of *LOXL2* was observed preferentially in ESCCs with >5 metastatic lymph nodes (Fig. 5D). Five (No. 17, 36, 40, 41, 42) (33%) out of 15 *KRT7* expressing ESCCs with more than 5 metastatic lymph nodes showed both *FOXA1* and LOXL2 expression (Fig. 5B and D). To this end, we investigated whether FOXA1 is also involved in cell migration by wound healing assay. As shown in Fig. 6, *FOXA1* siRNA strongly inhibited TE3 cell migration compared with control siRNA. This effect on of the *FOXA1* siRNA treatment was confirmed not to be dependent on growth inhibition of the treatment (data not shown).

Taken together, this study suggests that FOXA1 induces not only *KRT7* but also *LOXL2* in a subset of poor prognostic

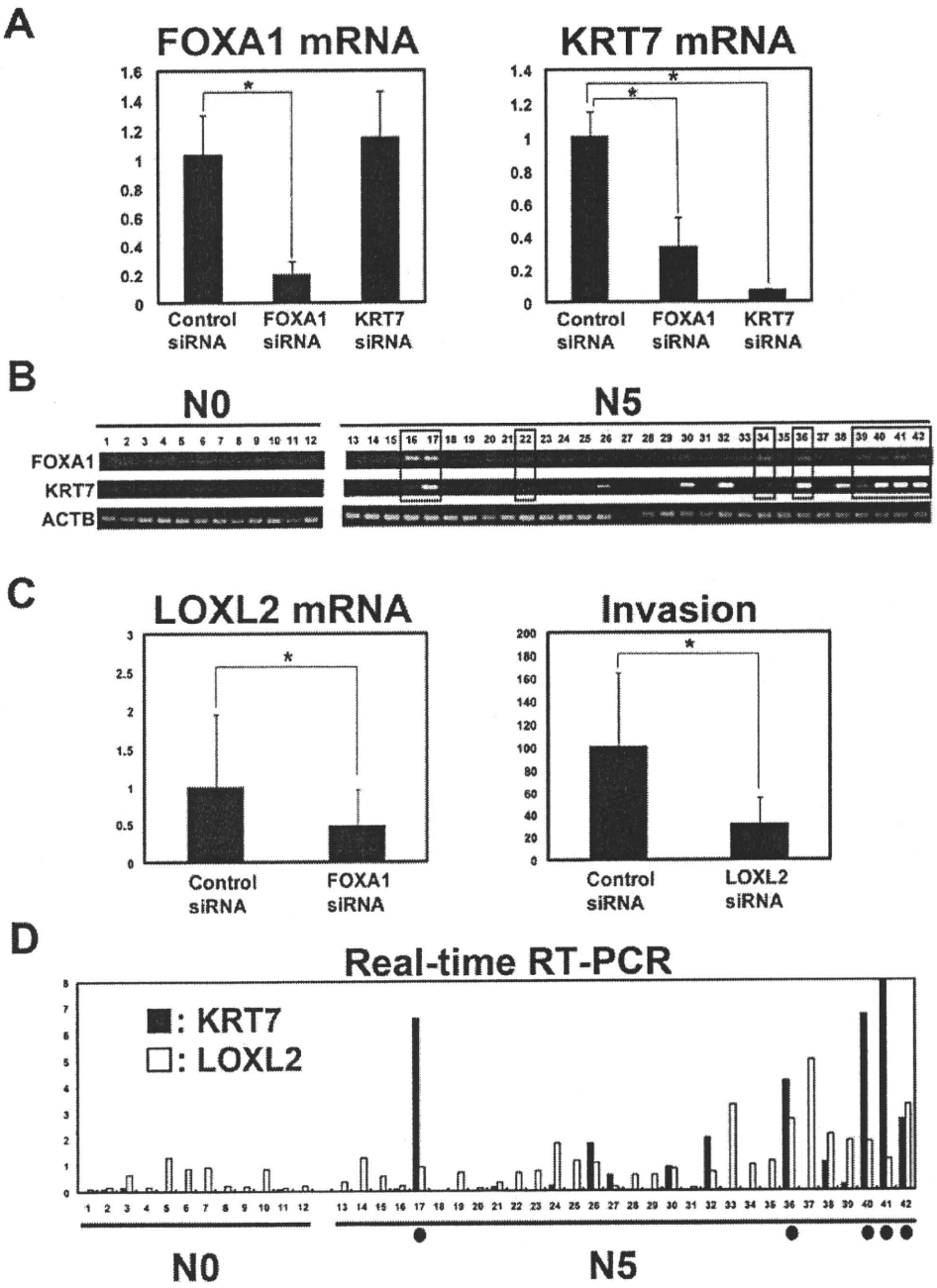

Figure 5. FOXA1 is an upstream regulator of *KRT7* and *LOXL2* in a subset of poor prognostic ESCCs. (A) *FOXA1* siRNA-transfected TE3 cells show reduction of both *FOXA1* and *KRT7* mRNA. Effects on *FOXA1* mRNA (left panel) and *KRT7* mRNA (right panel) after siRNA transfection. (B) Semi-quantitative RT-PCR for showing *in vivo* co-expression between *FOXA1* and *KRT7* in primary ESCCs with poor prognosis. Nine (60%) out 15 *KRT7*-expressing ESCCs with >5 metastatic lymph nodes show *FOXA1* expression (box). (C) *LOXL2* is another target of *FOXA1* in TE3 cells, and is involved in cell invasion. *FOXA1* siRNA-transfected TE3 cells show reduction of *LOXL2* mRNA (left panel). *LOXL2* siRNA treatment inhibits migration of TE3 cells compared with control siRNA (NC). (D) Overexpression of *LOXL2* in primary ESCCs with >5 metastatic lymph nodes is confirmed by quantitative real-time RT-PCR. Cases with co-expression between *KRT7* and *LOXL2* are indicated by closed circle.

ESCCs with metastatic lymph nodes, and that other FOXA1 downstream genes could be therapeutic targets of poor prognostic ESCCs.

Discussion

Based on our present results, we could divide *KRT7*-expressing ESCCs with >5 metastatic lymph nodes into three subgroups (Fig. 7). *KRT7* was found to be overexpressed in 15 (50%) of 30 poor prognostic ESCCs with >5 metastatic lymph nodes (Fig.3A). Out of the 15 *KRT7*-overexpressing ESCCs with poor prognosis, 9 cases (60%) showed *FOXA1* expression (Fig. 5B). Therefore, transcriptional factors other than *FOXA1* (as TFs shown in Fig. 7) must activate *KRT7* and metastasis-associated genes (as Xs shown in Fig. 7) in the 6 remaining cases with KRT7 over-

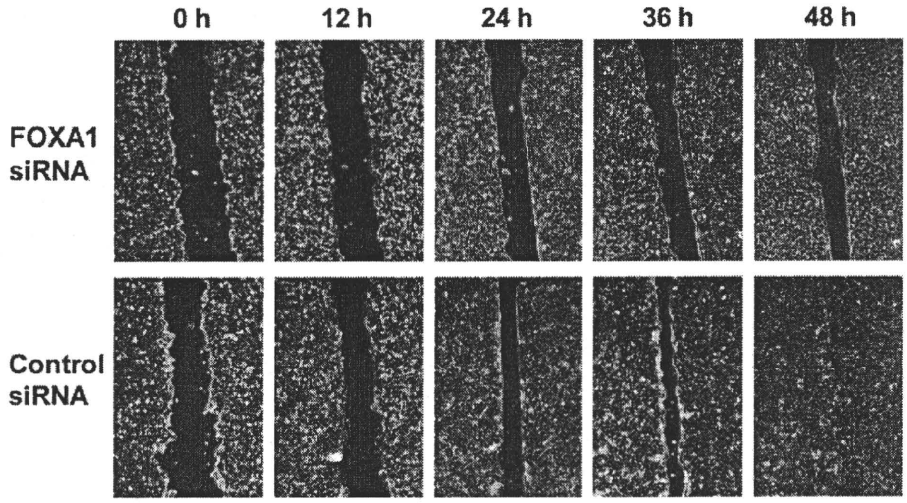

Figure 6. TE3 cell migration after *FOXA1* siRNA treatment. Scratch wound healing assays were performed on TE3 cells after *FOXA1* siRNA and control siRNA transfection. Phase contrast images (original magnification x40) of wound closure at 0, 12, 24, 36 and 48 h, respectively are shown.

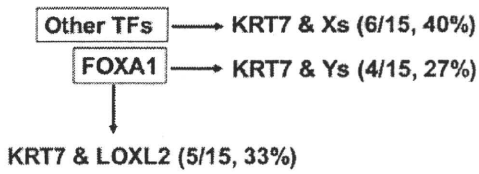

Figure 7. Three subgroups of *KRT7*-expressing ESCCs with poor prognosis. Fifteen of 30 ESCCs with >5 metastatic lymph nodes expressed *KRT7*. The *KRT7*-expressing ESCCs could be divided into three subgroups by the presence of different transcriptional cascades regulating KRT7. TFs, transcription factor regulating *KRT7* other than FOXA1, and Xs and Ys, metastasis-associated genes other than LOXL2.

expression (40%). In five (33%) of the 15 poor prognostic ESCCs, *FOXA1* may regulate both *KRT7* and *LOXL2* (Fig. 5D). Therefore, *FOXA1* must activate not only *KRT7* but also metastasis-associated genes other than *LOXL2* (as Ys shown in Fig. 7) because both FOXA1 and LOXL2 are shown to be involved in cell invasion and migration (Figs. 5 and 6).

In accordance with our results, immunohistochemical studies recently revealed that both LOXL2 and KRT7 are poor prognosis markers of squamous cell carcinomas including ESCCs (22,23). We also recently reported a map of crosstalk between Hedgehog and EMT signaling in ESCCs (16). LOXL2 has been reported to stabilize an EMT regulator SNAI1/SNAIL through physical interaction on the SLUG domain and Snail's lysine residues K98 and K137 (19). In ESCCs, the expression of this EMT regulator should be analyzed in the near future.

The presence of the variable transcriptional cascade in ESCCs may be one of the reasons why a robust gene set, which expresses in association with the prognosis of ESCC patients, could not be extracted by conventional t- or u-test. On this point, our introduced statistical method (S2N') could contribute to the extraction of some genes associated with poor prognosis. However, to understand the molecular mechanisms in lymph node metastasis and to develop a diagnostic method for all the ESCC patients with poor prognosis, the Xs and their upstream transcriptional factors and Ys should be identified. Chromatin immunoprecipitation (ChIP) -on-chip analysis is a potential tool for identifying *in vivo* direct interaction of the target gene promoter with FOXA1. Recently, genome-wide mapping of FOXA1 targets by ChIP-on-chip analysis revealed that FOXA1 translates epigenetic signatures into enhancer-driven lineage-specific transcription and its binding consensus sequences in breast and prostate cancer (24). As shown in Fig. 3, poor prognostic ESCCs often seemed to have glandular cell-type characteristics in both the gene expression and morphology. This fact of transdifferentiation from squamous cell to glandular cell may correspond with cell lineage-specific functions of FOXA1 (24). We were able to find the binding sequences of FOXA1 at -2630 to -2635 (TAGTTTG) of *KRT7* and at -4530 to -4524 (TGTTTAC), -4304 to -4299 (TGTTTGT), -4300 to -4295 (TGTTTGG), and -2642 to -2636 (TGTTTAC) of *LOXL2*. Future studies on the ChIP-on-chip assay with an anti-FOXA1 antibody may contribute to an understanding of the malignancy of ESCCs and to the identification of therapeutic targets. In addition, we showed that aside from *KRT7*, other markers for ESCCs with poor prognosis could be *CALB*, *MUC1* and *CEA* (Fig. 3). Therefore, identification of their upstream transcriptional regulators also remains for future studies.

Acknowledgements

This study was supported in part by the program for promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation; a Grant-in-Aid for the Third Comprehensive 10-Year Strategy for Cancer Control and for Cancer Research (20-12) from the Ministry of Health, Labour and Welfare of Japan; Princess Takamatsu Cancer Research Fund, and Foundation for Promotion of Cancer Research (RRs: M.S. and T.M.).

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日消外会誌　43（9）：877～881，2010年

原　著

食道癌 salvage 手術と気道壊死に関する検討

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はじめに：食道癌に対する根治的化学放射線療法（chemoradiotherapy；以下，CRT）後の遺残・再発症例は，salvage 手術が唯一の治療法である．しかし，合併症率が高く，特に気道壊死は致死的合併症となる．**方法**：1997 年から 2007 年まで，当院で胸部食道癌に対し salvage 手術を施行された 49 例を対象．気道壊死症例の検討を通して salvage 術後の気道壊死の特徴を明らかにし，臨床学的背景，手術手技について，気道壊死症例と非気道壊死症例で比較検討した．**結果**：気道壊死は 5 例（10.2%）に認められた．気道血流障害が主因の primary necrosis（3 例）と胃管の縫合不全が先行した secondary necrosis（2 例）に分類できた．穿孔時期は術後 7～36 日と幅広く，腫瘍局在，深達度，手術時間，出血量と気道壊死との関連性は認められなかった．気管支動脈切除，頸部・気管分岐部リンパ節郭清が気道壊死に関与している傾向にあった．そして，secondary necrosis の 2 症例は，後縦隔経路再建例に認められ，胃管気道瘻へ発展した．**考察**：Salvage 手術後の致死的合併症は，気道壊死が大きく関与していた．気道血流に与える放射線照射の影響が，気道壊死を招く大きな要因と考えられた．郭清操作は血流に配慮して行い，再建経路は胸骨後経路が望ましいと考えられた．

緒　言

近年，食道癌に対する根治的化学放射線療法（chemoradiotherapy；以下，CRT）の成績は目覚ましいものがある．その高い治療効果により，CRT の適応は拡大されてきている．一方で，根治的 CRT 後の遺残・再発率は 40%～60% という報告もあり[1]，CRT 単独治療には限界がある．遺残・再発した症例に対し，唯一の救済手段が salvage 手術である[2]．しかし，salvage 手術は，致死的合併症の一因となる気道壊死の頻度が非常に高い[3][4]．今回，salvage 手術のより安全な術式を確立することを目的に，当院での手術経験に基づき，致死的合併症の原因となりうる気道壊死の特徴・要因などについて検討した．

対象と方法

1997 年 5 月から 2007 年 5 月までに，当院で salvage 手術を施行された 49 例を対象とした．このうち 19 例は，他院で CRT が行われていた．化学療法は，プラチナ製剤，5FU 製剤を併用もしくは単独で投与した症例とした．放射線療法は，総照射量 50Gy 以上で，予防照射を含めた照射野に気管気管支が含まれる胸部食道癌に限定し，頸部食道癌と腹部食道癌は除外した．その結果，すべての症例で気管分岐部周囲に少なくとも 40Gy 以上照射されていた．また，術式によるバイアスを排除するため，右開胸アプローチ，胃管再建，単一術者で施行した症例とした．

まず，気道壊死症例の検討を通して，salvage 術後における気道壊死の特徴を明らかにし，次いで患者の臨床学的背景，手術手技（郭清，再建経路）について，気道壊死症例と非気道壊死症例で比較検討した．

なお，参考文献検索は検索語として「食道癌/salvage」を用い，1983 年～2009 年まで，PubMed ならびに医学中央雑誌を用いて行った．

＜2010 年 1 月 27 日受理＞別刷請求先：田中　則光
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Table 1　Clinical parameters of patients with airway necrosis

Case	Location	Depth	Reconstruction route	Onset of airway necrosis (POD)	Emergency operation	Prognosis
1	Mt	cT3	Retro	7	−	Dead
2	Mt	cT3	Retro	36	−	Dead
3	Ut	cT1b	Retro	16	+	Alive
4	Mt	cT3	Post	7	+	Dead
5	Lt	cT2	Post	24	+	Dead

Ut : Upper thoracic esophagus　Mt : Middle thoracic esophagus　Lt : Lower thoracic esophagus
Retro : Retrosternal route　Post : Posterior mediastinal route　POD : Postoperative day

結　果

気道壊死症例の特徴

気道壊死症例の臨床経過について示す（Table 1）．気道壊死は，salvage 手術を施行した 49 例中，5 例（10.2%）に認めた．Table 1 に気道壊死を発症した 5 例の臨床経過を示す．5 例中 4 例は気道壊死が原因で死亡した．Case 1-3 の 3 例は，気管支鏡上，白苔付着の所見から，多発的にピンホールを形成し，徐々に穿孔部が拡大した．穿孔時期は，Case 2 が術後 36 日目，Case 3 が術後 16 日目であった．経過から，Case 1-3 は気道血流障害による虚血が気道壊死の主因と考えられ，primary necrosis とした．Case 1，2 の 2 例は，縦隔組織で自然に被覆されていたため手術を施行せず保存的治療を行ったが，縦隔炎による大血管からの出血で死亡した．Case 3 は，気道穿孔後，大網被覆術を施行し，救命可能であった．Case 4 は，胃管気道瘻から術後 7 日目に緊急手術を施行した．胃管小彎縫合線に沿って軽微な壊死性変化を認めたため，胸腺被覆術を施行した．しかし，その後も縦隔炎が進行し，術後 18 日目に胃管抜去と大網被覆術を行ったが，術後 57 日目に死亡した．Case 5 は，明らかな合併症を認めず，術後 16 日目に退院した．術後 24 日目に突然の吐血・喀血で再入院し，胃管気道瘻を認めたため緊急手術を施行したところ，胃管小彎切離線の縫合不全と気管膜様部が，瘻孔を形成していた．胃管抜去と大網被覆術を施行したが，術後 61 日目に死亡した．Case 4 と 5 は，縫合不全が先行しており，縫合不全に続発した縦隔炎が原因の secondary necrosis と考えられた．

Table 2　Clinical characteristics

Airway necrosis	Yes n = 5	No n = 44
Gender (Male/Female)	3/0	43/1
Age (years)*	60.6 ± 2.6	60.0 ± 1.4
Periods of esophageal preservation (day)*	242 ± 31	304 ± 30
History of diabetes mellitus	2 (40.0%)	6 (13.6%)
Location (Ut/Mt/Lt)	1/3/1	9/20/15
cT1/2/3/4	1/1/3/0	8/2/32/2
cN0/1	1/4	23/21
cM0/1	0/5	7/37
Bleeding (g)*	571.4 ± 60.0	447.7 ± 43.4
Operation time (min)*	440.2 ± 22.7	393.9 ± 10.0

CRT : Chemoradiotherapy　Ut : Upper thoracic esophagus　Mt : Middle thoracic esophagus　Lt : Lower thoracic esophagus　* : Values are means ± SD

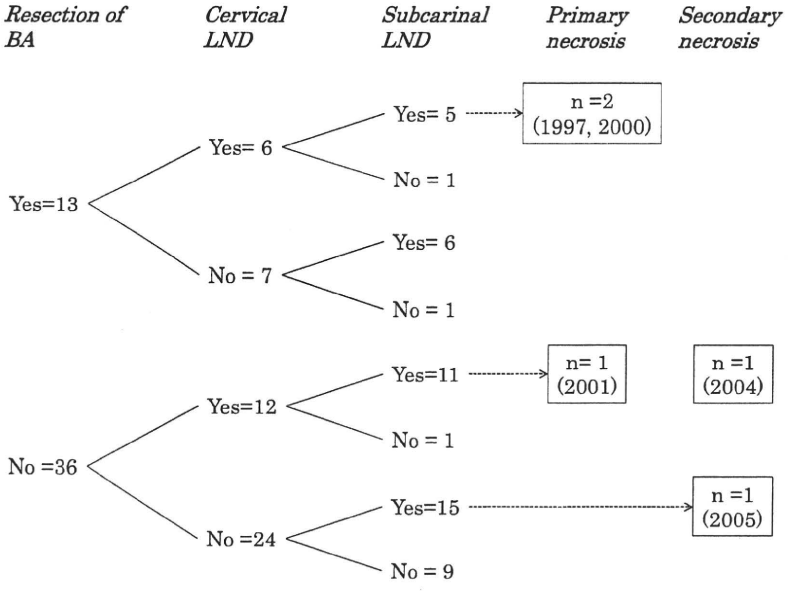
臨床学的背景の比較

臨床学的背景について，気道壊死した 5 症例と気道壊死のない 44 症例を比較した（Table 2）．患者因子として，性別・年齢・食道温存期間で両群に関連性を認めなかった．糖尿病既往歴を持つ症例に，気道壊死が多い傾向にあった（気道壊死 5 例中 2 例（40%）vs. 非気道壊死 44 例中 6 例（13.6%））．腫瘍因子としては，腫瘍局在と深達度で両群に関連性を認めなかった．手術因子も，出血量と手術時間で両群に関連性を認めなかった．

手術手技と気道壊死の検討

手術手技の検討として，郭清操作（気管支動脈切除，頸部リンパ節郭清，気管分岐部郭清）と気道壊死との関係を示す（Fig. 1）．Primary necrosis の中で，Case 1 と 2 は，気管支動脈を切離したうえに，頸部リンパ節と気管分岐部リンパ節の郭清

Fig. 1 The relationship between airway necrosis and operative procedures
BA : Bronchial artery LND : Lymph node dissection
Values in parentheses are era of incidence.



が行われていた．Case 3は，気管支動脈を温存した上で，頸部リンパ節と気管分岐部リンパ節の郭清が行われていた．Secondary necrosisの2例は，1例が頸部リンパ節と気管分岐部リンパ節の郭清を施行し，1例が気管分岐部リンパ節郭清を施行されていた．気管支動脈温存，頸部リンパ節と気管分岐部リンパ節の郭清を省略した症例で気道壊死は認めなかった．

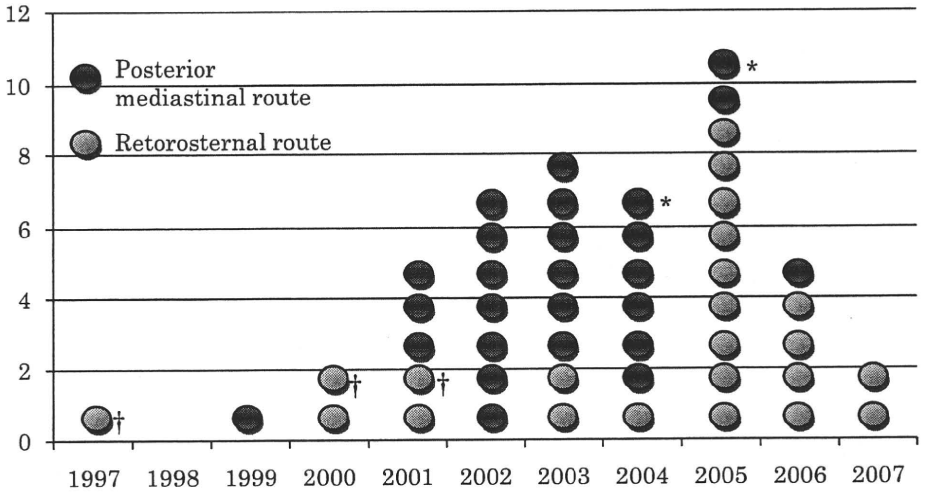
再建経路（胸骨後経路，後縦隔経路）と気道壊死との関係について示す（Fig. 2）．胸骨後経路は26例に施行され，そのうち3例でprimary necrosisを生じ，2001年以前の症例であった．また，後縦隔経路は23例に施行され，そのうち2例で胃管の縫合不全を伴うsecondary necrosisから胃管気道瘻を生じた．2005年途中から，再度胸骨後経路で再建しているが，気道壊死は経験していない．

考　察

根治的CRT後の遺残，局所再発に対するsalvage手術の致死的合併症は，術前無治療手術と比較し非常に高く，6～33%との報告がある[5)~11)]．今回の検討で，致死的合併症率は10.2%であり，その80%に気道壊死が関与していた．そして，膜様部の壊死性変化が緩徐に進行した気道壊死（primary necrosis）と，胃管の縫合不全が先行し，縦隔炎を介して2次的に発生した気道壊死（secondary necrosis）に大別された．

Primary necrosisには剥離が比較的容易なT1症例も含まれていたことから，その要因として，剥離操作よりもむしろ照射による血流障害が主因であると考えられた．放射線照射の晩期障害は，照射後8～12か月以降に生じ，組織の線維化から穿孔，瘻孔形成を起こしやすいとされている[12)]．また，Bartelsら[3)]も術前CRTを施行した症例は有意に気道壊死が多いとし，放射線照射による気道血流障害が原因であるとしている．気道の血流は，主に気管支動脈や下甲状腺動脈の分枝から，lateral longitudinal anastomosisを介し，気道全体へと供給される[13)]．根治的CRTで気管気管支も照射野に含まれた場合，lateral longitudinal anastomosisを構成する毛細血管が閉塞性内膜炎を起こす．そのため，気道全体が血流障害を生じる可能性がある．今回の検討で，気管支動脈を切除し，かつ頸部リンパ節と気管分岐部リンパ節郭清した5例のうち2例(40%)で気道壊死を認めた．しかし，気管支動脈を温存し，かつ頸部リンパ節と気管分岐部リンパ節郭清を省略した9例では，気道壊死

Fig. 2　Annual change in the reconstruction route
† : Primary necrosis * : Secondary necrosis



は認めていない．つまり，気道への供給血管である気管支動脈や下甲状腺動脈の分枝，lateral longitudinal anastomosis の温存は，気道壊死を予防する上で，非常に重要であると考えられる．

Secondary necrosis は，胃管の縫合不全が先行し，縦隔炎を介して 2 次的に発生した気道壊死である．後縦隔経路で再建した場合，胃管気道瘻を形成するため，極めて死亡率が高い．今回の検討でも胃管気道瘻を形成した 2 例とも死亡した．胃管の縫合不全が，Case 4 は軽微な壊死性変化から持続性に進行し，Case 5 は退院後の術後 24 日目と遅発性に認められた．これは，気道の primary necrosis 同様，照射による胃管の血流障害が関与している可能性がある．根治的 CRT を施行した場合，気管気管支だけでなく，胃噴門部周囲も照射野に含まれることが多い．そのため胃管の粘膜下血流が障害され，虚血から縫合不全に至るリスクが通常より高いと考えられる[4]．気道側も照射の影響で脆弱になっていると考えられ，胃管の縫合不全から致死的な胃管気道瘻を形成する可能性がある．また，気道壊死 5 症例中 2 例は糖尿病の既往歴を持つ症例であり，糖尿病による血流障害が放射線による血流障害を増強させた可能性がある．

当科では，salvage 手術導入当初，3 領域郭清を行い，胸骨後経路で再建していた．しかし，照射による瘢痕化のため食道切除後にできる気管膜様部背側の死腔が残存し，気道壊死（primary necrosis）に続発した縦隔炎が重篤化し，大血管からの出血で死亡した症例を経験した．そこで 2001 年途中から，切除後の死腔を胃管で裏打ちすることを目的に，再建経路を後縦隔経路に変更した．しかし，secondary necrosis から致死的な胃管気道瘻を 2 例経験した．そこで，2005 年途中からは，気道血流に配慮した郭清操作を行ったうえで，再び胸骨後経路で再建することとした．それ以後，気道壊死を経験していない．

Salvage 手術後の致死的合併症は，気道壊死が大きく関与していた．その主たる要因は，照射に伴う気道の血流障害と考えられた．Salvage 術後の気道壊死は極めて死亡率が高く，気道血流に配慮した手術操作が第一義的に求められる．また，胃管も照射による血流障害の影響で，通常より縫合不全を起こす危険性が高く，縫合不全に続発する致死的な胃管気道瘻を回避するためには，胸骨後経路による再建がより安全と考えられた．今後，CRT の適応が拡大するにつれて，salvage 手術の症例は増えることが予想される．より安全で確実な salvage 手術を確立することが，今後さらに食道癌治療の成績を向上させる一助になると考えられる．

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Airway Necrosis after Salvage Esophagectomy

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Introduction : Salvage esophagectomy is the sole curative intent treatment for patients with persistent or recurrent locoregional disease after definitive chemoradiotherapy (CRT) for esophageal carcinoma. However, salvage esophagectomy is a very high-risk operation, and airway necrosis is a fatal complication. **Methods** : Between 1997 and 2007, 49 patients with thoracic esophageal cancer underwent salvage esophagectomy after definitive CRT. We retrospectively compared patients with and without airway necrosis, and investigated operative procedures related to airway necrosis. **Result** : Airway necrosis occurred in five patients (10.2%), of four patients (80%) died during their hospitalization. Airway necrosis seemed to be closely related to operative procedures, such as resection of bronchial artery and cervical and subcarinal lymph node dissection. Bronchogastric fistula following necrosis of gastric conduit occured in 2 patients reconstructed through posterior mediastinal route. **Conclusions** : Airway necrosis is a highly lethal complication after salvage esophagectomy. It is important in salvage esophagectomy to take airway blood supply into consideration sufficiently and to reconstruct through retrosternal route to prevent bronchogastric fistula.

Key words : salvage esophagectomy, airway necrosis, chemoradiotherapy, esophageal cancer

〔Jpn J Gastroenterol Surg 43 : 877—881, 2010〕

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Accepted : January 27, 2010

特集　食道癌手術―困難症例・偶発症対処の秘策―

化学放射線療法後救済手術

日月　裕司*

I. 術前準備

1. 適　応

食道癌に対する救済手術（サルベージ手術）は，根治的放射線療法または根治的化学放射線療法後の癌遺残または再発に対する手術である。日本食道学会の食道癌取扱い規約第10版では，放射線線量は50 Gy以上とされている。非手術的根治治療としては化学放射線療法が第1選択であり，放射線照射単独治療の適応は化学療法併用が困難な症例に対するものと考えられるため，多くの場合にはサルベージ手術は化学放射線療法後の手術を示すと考えられる。

サルベージ手術の内容には，食道切除，リンパ節摘出（郭清），内視鏡切除などがある。あくまで根治的化学放射線療法後の癌遺残または再発に対する局所治療であり，遺残または再発が明らかではない部位の予防的切除が目的ではない。リンパ節のみに遺残または再発を認める場合は，リンパ節切除のみを行い食道切除は行わない。内視鏡粘膜切除が可能な病変は内視鏡粘膜切除を行い，病理学的検索で根治度Aであれば食道切除は行わない。

サルベージ手術の目的は癌遺残のないR0の切除である。サルベージ手術後の局所遺残に対する放射線治療によるさらなる救済治療は期待できないため，R0が得られないと判断された場合はサルベージ手術の適応とならない。そのため，内視鏡検査，CT検査に加え，PET検査による切除適応外病変の除外は必須と考える。

放射線照射による腫瘍周囲組織の線維化，瘢痕化により，術前の検査でR0切除の可否の判断がむずかしい場合は，術中所見で最終決定する。手術開始後も，まずR0切除が可能であることを確認し，可能ではないと判断されれば即座に手術を終了する。

サルベージ手術は合併症率や在院死亡率が高い手術であるが[1]，他の治療法では根治を望めない状況での治療であるため，耐術可能であればできる限り切除手術を試みており，年齢などによる制限はしていない。

2. 術前のインフォームド・コンセント

術前のインフォームド・コンセントでは，高い合併症率，在院死亡率，術中判断による中止の可能性，サルベージ術後の再再発の可能性，長期・晩期の心機能・呼吸機能・消化機能の障害について十分に説明し，納得していただいたうえで手術を受けていただく。安易な楽観的予測を説明しない。

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key words：食道癌，化学放射線療法，食道切除術