Recently, some surgical teams have demonstrated the efficacy of postoperative immediate extubation (IE) following an esophageal resection, most of which are Ivor-Lewis, transhiatal, or left thoracoabdominal esophagectomies with limited lymph node dissection [18–22]. However, very few reports have showed the feasibility and safety of this practice for patients with thoracic esophageal cancers who underwent transthoracic esophagectomy with extended radical 3FLND. The current study compared the early postoperative clinical course and morbidity and mortality rates between the patients with and without IE following this kind of radical surgery in this institution and evaluated the feasibility and safety of IE.

Patients and methods

Patients

During the period from 2002 to 2007, 165 patients with esophageal cancer underwent surgery in the National Kyushu Cancer Center, Japan. All cancers were pathologically diagnosed to be squamous cell carcinoma. Among them, 117 patients with thoracic esophageal cancer underwent transthoracic esophagectomy with 3FLND, basically by the same surgical procedures. Thirty-eight patients up to 2003 were managed under MV shortly after their esophagectomy (MV group). The average length of MV was 9.9 h. To avoid possible risks related to MV, the time length of postoperative MV in the surgical ward was gradually shortened during a period of 4 years from 2000 to 2003, and the policy of postoperative MV was finally changed to IE in the operating room in January 2004. Among the 79 patients after 2004, 75 were thus immediately extubated after the esophagectomy in the operating room (IE, immediate extubation group), unless there were specific contraindications. These 38 patients of the MV group and 75 patients of the IE group were included in this study.

Preoperative assessment and management

All patients underwent a full blood count, serum biochemistry, electrocardiogram, chest X-ray, and pulmonary function tests including forced vital capacity (FVC, %VC) and forced expiratory volume in the first second (FEV1.0, % FEV1.0). A blood gas analysis was also performed.

Preoperative staging of the thoracic esophageal cancer was conducted by esophagography, upper gastrointestinal endoscopy, computed tomography (CT) of the cervix, chest, and abdomen, ultrasonography of the cervix and abdomen, and bone scintigraphy. ^{18}F-Fluorodeoxyglucose-positron emission tomography (FDG-PET) was also used for some of the patients.

For the patients whose tumor was suspected to have directly invaded adjacent organs such as the aorta, trachea, and bronchus (i.e., T4 tumor), neoadjuvant therapy including chemotherapy by CDDP + 5-FU and concurrent irradiation of 30 Gy was administered. Preoperative nutritional support was given to patients with severe dysphagia and malnutrition by intravenous hyperalimentation.

Anesthesia

General anesthesia using oxygen and an inhalational agent, combined with an epidural anesthesia sited in the low thoracic position (T9–T10 to T11–T12), was performed. Just before the start of the surgical procedure, 250 mg hydrocortisone was administered intravenously.

During the procedures through right thoracotomy, one-lung mechanical ventilation with a double-lumen tracheal tube was undertaken with occasional assistance of high-frequency jet ventilation, if necessary.

The main criteria for IE were based on the judgment of the anesthesiologists when (1) the patient had no major preoperative and/or intraoperative cardiopulmonary complications, (2) the neuromuscular blockade was resolved and the patient was awake, (3) spontaneous respiration and tidal volume were adequate (respiratory rate < 30/min, tidal volume > 5 ml/kg), (4) blood gas analysis was good (>98% of SpO_2 at FiO_2 <0.4) just before extubation, and (5) adequate cough during suctioning.

Surgical procedure

All 117 patients underwent transthoracic esophagectomy through a right-side thoracotomy. The alimentary tract was reconstructed using a gastric tube made of the greater curvature of the stomach, with cervical esophagogastric anastomosis by hand-sewn or instrumental anastomosis through a retrosternal or posterior mediastinal route. For the thoracotomy, a skin incision about 10–12 cm long was made and the thoracic cavity was entered through the fourth or fifth intercostal space using a muscle-preserving splitting method and without fracture of any ribs. The transthoracic procedures were performed with thoracoscopic assistance.

An extended radical lymph node dissection was then performed in three fields. Through the right thoracotomy, complete dissection of the middle and lower mediastinal nodes included the periesophageal, parahiatal, subcarinal, and aortopulmonary window nodes. Dissection of the lymph nodes in the upper mediastinum included the nodes along the bilateral recurrent laryngeal nerves by carefully exposing them, from the level of the aortic arch to the thoracic inlet for the left nerve and near the origin at the base of the right subclavian artery for the right nerve. Through a cervical U-shaped incision, the reminder of the nodes along the recurrent laryngeal nerves, which were anatomically inseparable chains extending from the upper mediastinum to the lower neck, were also dissected, together with the lower deep cervical nodes located posterior and lateral to the carotid sheath. Lymph node dissection in the abdomen included the nodes along the celiac, left gastric, and common hepatic arteries, the nodes along the lesser curvature of the stomach, and the parahiatal nodes.

Postoperative management

After surgery, the patients were transferred to the surgical ward (until November 2005) or the newly equipped intensive care unit (after December 2005) of the hospital. Postoperative analgesia was continued with an epidural infusion using a patient-controlled analgesia system for several days. Most patients intravenously received pentazocine and/or nonsteroidal analgesia, if not contraindicated, depending on the patients' wishes. Also, 125 mg hydrocortisone was intravenously administered on postoperative day (POD) 1 and POD 2.

The patients were urged to be mobilized in the hall with assistance of nurses several times on POD 1. The thoracic drain was removed when the amount of the discharge became less than 100 ml per day.

Oral diet intake was resumed on POD 7. Parenteral nutritional support was continued until adequate oral intake was achieved. Postoperative mandatory nutrition via feeding tube was not performed in either group.

Data analyses and statistics

All the statistical analyses were performed by using the StatView software program (version 5.0; Abacus Concepts, Berkeley, CA, USA). The early postoperative clinical course was compared between the MV and IE groups. Data included body temperature, pulse rate, urine volume, peripheral white blood cell and lymphocyte counts, C-reactive protein (CRP), and PaO_2; differences were analyzed by Student's t test or the chi-square test. The occurrence of postoperative complications, timing of mobilization and removal of the thoracic drain, and the length of the postoperative hospital stay were also compared between the two groups; differences were evaluated by Mann–Whitney's U test. $P < 0.05$ was considered statistically significant.

Results

The clinical characteristics of both MV and IE groups are shown in Table 1. Among the 79 patients after 2004 who underwent transthoracic esophagectomy with 3FLND, 75 (94.9%) were immediately extubated after the esophagectomy in the operating room. The remaining 4 patients were managed under postoperative MV, including (1) a patient whose right lung was seriously injured during surgery because of marked adhesion of the pleura, (2) a patient who showed severe laryngeal spasm and choking 15 min after IE and was then reintubated, and (3) 2 patients who did not clear the respiratory function criteria described above.

There were no differences in age, sex, performance status, preoperative comorbidities, percentage of patients receiving neoadjuvant chemoradiotherapy, preoperative respiratory function (%VC and FEV1.0%), and pathological stage between the MV and IE groups. However, the average operation time, one-lung ventilation time, and anesthesia time were significantly shorter in the IE group than those in the MV group. These differences were attributed to the gradual and steady improvement in the technical skills of both the surgeons and the surgical team, because the surgical procedures, such as the methods for thoracotomy and extent of lymph node dissection, were basically the same between the two groups investigated in this study. All patients were preoperative ASA grade II or less, and no differences existed between the two groups.

Figure 1 shows comparisons of early postoperative clinical course values between the MV and IE groups. There were no significant differences in the changes in the vital clinical parameters during the first 72 h after surgery, including body temperature, pulse rate, urine volume, and PaO_2. Although the changes of the peripheral white blood cell and lymphocyte counts and CRP value during the 7 days after the operation were not significantly different between the two groups, the lymphocyte count recovered more rapidly

Table 1. Patient characteristics

Characteristic	IE ($n = 75$)	MV ($n = 38$)	P
Age (years)	63.2 ± 7.3	63.1 ± 9.0	NS
Sex (male/female)	59/16	33/5	NS
PS (0/1/2)	50/24/1	27/11/0	NS
Comorbidity (%)	41 (54.7)	21 (55.3)	NS
NeoCRTx (%)	17 (22.7)	13 (34.7)	NS
Respiratory function			
%VC	112.7 ± 14.2	111.4 ± 14.0	NS
FEV 1.0%	74.9 ± 8.0	75.6 ± 8.7	NS
Operation time (min)	531 ± 77	615 ± 74	<0.0001
OLV time (min)	213 ± 52	256 ± 68	<0.0005
Anesthesia time (min)	639 ± 74	699 ± 68	<0.0001
Blood loss (g)	507 ± 343	824 ± 545	<0.0005
pStageI/II/III/IV (n)	23/29/14/9	10/12/8/8	NS

IE, immediate extubation; MV, mechanical ventilation; NS, not significant; PS, ECOG Performance Status; neoCRTx, neoadjuvant chemoradiotherapy; VC, ventilatory capacity; OLV, one-lung ventilation
The pStage is according to the Japan Esophageal Society [29]
Some data are presented by mean ± standard deviation

Table 2. Postoperative morbidity and mortality after transthoracic esophagectomy with three-field lymph node dissection

	IE ($n = 75$)	MV ($n = 38$)	P
Morbidity			
Pulmonary complication	9 (12.0)	9 (23.7)	NS
Pneumonia/ARDS	4 (5.3)	7 (18.4)	NS
Reintubation	2 (2.7)	1 (2.6)	NS
CTN insertion	2 (2.7)	1 (2.6)	NS
Usage of bronchoscopy	9 (12.0)	9 (23.7)	NS
Pleural effusion	3 (4.0)	1 (2.6)	NS
Chylothorax	1 (1.3)	-	NS
Pulmonary embolism	-	1 (2.6)	NS
RLNP	16 (21.3)	9 (23.7)	NS
Anastomotic leakage	10 (13.3)	4 (10.5)	NS
Cardiac complication	5 (6.7)	2 (5.3)	NS
Liver complication	6 (8.0)	2 (5.3)	NS
Other	11 (14.7)	5 (13.2)	NS
Any complication	29 (38.7)	18 (47.4)	NS
Mortality	1 (1.3)	-	NS

IE, immediate extubation; MV, mechanical ventilation; NS, not significant; ARDS, acute respiratory distress syndrome; CTN, cricothyroidotomy needle; RLNP, recurrent laryngeal nerve palsy (%)

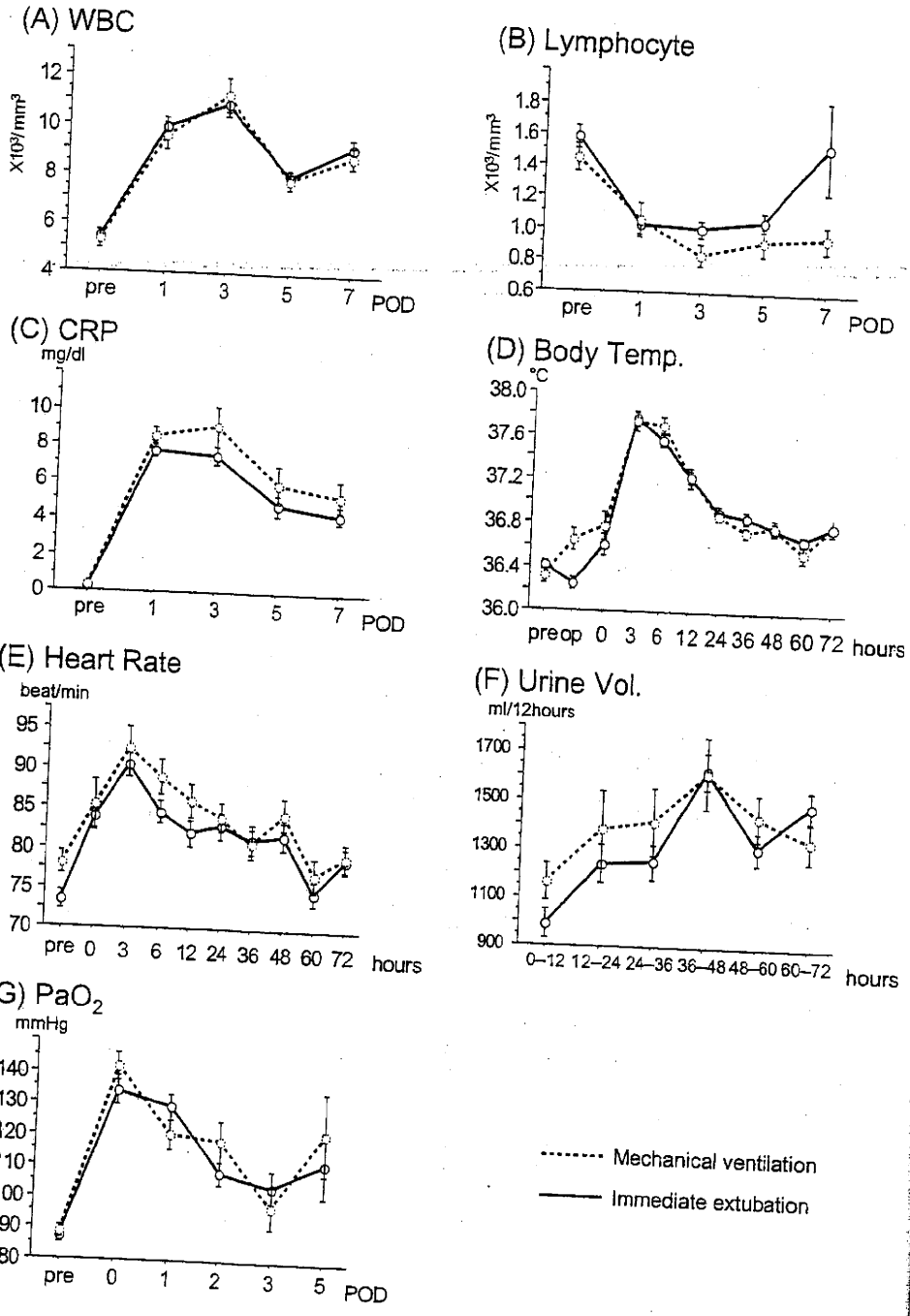

Fig. 1. Comparison of early postoperative clinical parameters between the IE (immediate extubation) and MV (mechanical ventilation) groups: white blood cell count (**A**); lymphocyte count (**B**); CRP (C-reactive protein) (**C**); body temperature (**D**); heart rate (**E**); urine volume (**F**); and PaO_2 (**G**). *Solid and dashed lines* represent the IE and MV groups, respectively; *vertical bars* indicate standard error. There were no statistical differences between the two groups in any parameter

and the CRP value was consistently lower in the IE group than in the MV group during those 7 days.

Table 2 shows the postoperative complications for which any medical and/or surgical treatments were required. Postoperative complications occurred in 38.7% (29/75) of the IE group and in 47.4% (18/38) of the MV group; this was not statistically significant. Pulmonary complications tended to occur more frequently in the MV group (9/38: 23.7%) than in the IE group (9/75: 12.0%), although the difference was not statistically significant ($P = 0.10$). Among them, reintubation or the insertion of a cricothyroidotomy needle (Trahelper; Top, Tokyo, Japan) was required to treat severe pneumonia or ARDS for 2 (2.7%) or 2 (2.7%) in the IE group, respectively, whereas these procedures were required for 1 each (2.6% each) in the MV group. Bronchoscopy for aspiration of the sputum was applied to 12.0% (8/75) and 23.7% (9/38) in the IE and MV groups, respectively. Recurrent laryngeal nerve palsy was observed in 21.3% (16/75) and 23.7% (7/38) in the IE and MV groups, respectively. Frequency of anastomotic leakage was 13.3% (10/75) and 10.5% (4/38) in the IE and MV groups, respectively, all of which could be treated conservatively. One death occurred in the IE group (1.3%), which was caused by a severe chylothorax followed by massive unexpected intracranial bleeding at POD 26. (This case was included in the patients requiring reintubation.)

Table 3. Mobilization, duration of thoracic drainage, and postoperative hospital stay

	IE ($n = 75$)	MV ($n = 38$)	P
Mobilization (POD)	1.1 ± 0.72	2.1 ± 0.39	<0.0001
Removal of TD (POD)	5.9 ± 5.8	5.8 ± 2.4	NS
Hospital stay (POD)	31.3 ± 20.3	35.8 ± 36.2	NS

Data are represented by mean ± standard deviation
IE, immediate extubation; MV, mechanical ventilation; POD, postoperative day; TD, thoracic drain

Mobilization of the patients (standing up and walking in the hall) was possible on average POD 1.1 in the IE group (95% of the patients could be mobilized on POD 1) whereas on average POD 2.1 in the MV group ($P < 0.0001$). Duration of drainage through a thoracic tube and the length of hospital stay were 5.9 and 31 days in the IE group, respectively, and 5.8 and 36 days in the MV group, respectively; the differences were not statistically significant (Table 3).

Discussion

This study demonstrated that the patients with IE after transthoracic esophagectomy with radical 3FLND showed quite a similar postoperative course to those with MV, and there were no significant differences in postoperative morbidity and mortality between the two groups. Some reports have already shown that IE after esophagectomy is safe and effective for postoperative management [18–22]. However, the esophagectomies in those studies are mostly Ivor-Lewis, transhiatal, or left thoracoabdominal esophagectomies with limited lymph node dissection. Moreover, few reported the results of comparisons in the early postoperative clinical course between patients with MV and IE. Therefore, there has been no report on this subject for more extensive transthoracic esophagectomy with radical 3FLND. In this type of surgery, more emphasis is put on the radical dissection of the lymph nodes along the recurrent laryngeal nerves, which anatomically consist of an inseparable chain extending from the upper mediastinum to the lower neck [6–8], possibly resulting in enhancement of the risks of postoperative pulmonary complications. In fact, quite high morbidity and mortality rates still continue to be reported after a 3FLND for esophageal cancer, even at specialized institutes [6–8,23,24]. Therefore, this is the first study to show the outcome of IE for patients undergoing transthoracic esophagectomy with radical 3FLND.

This study was not a prospective randomized trial, and some differences in background such as the surgical time, one-lung ventilation time, and the amount of blood loss exist between the IE and MV groups, which are thought to be the results of gradual and steady technical evolution, especially in the surgical procedures during the thoracotomy. Therefore, it is difficult to draw any definitive conclusions regarding the superiority of IE to MV in safety and feasibility. However, the morbidity and mortality rates in the IE group themselves were comparable to those from most of the institutes with a policy of elective postoperative MV [1,5,9]. Furthermore, the data in the IE group presented here are also comparable to the previous reports on IE after esophagectomies that were less extensive than the procedure with 3FLND. Those reports showed that the morbidity and mortality rates ranged from 35% to 45% and 0.3% to 8.2%, respectively, and the reintubation rate ranged from 2% to 16% [19–22]. Thus, it is unlikely that IE will increase the risk of postoperative complications, and we believe that IE can be safely performed with permissible risks. In addition, the merits of IE described below should be fully utilized, because reliable predictive indices for successful weaning after prolonged postoperative MV do not exist.

The possible merits of IE in comparison to MV after esophagectomy include avoiding the risks of pulmonary damage induced by MV and of the difficulties of weaning caused by prolonged MV. In fact, no increase in pulmonary complications was observed in the IE group in this study. Second, an emergent reintubation or tracheostomy can be more easily and safely performed in the operating room immediately after extubation, in the event of airway obstruction caused by vocal cord edema or laryngeal spasm such as in the case described in the Results. Third, IE allows mobilizing patients at an earlier stage of the postoperative course, thus leading to a decrease in morbidity such as pulmonary complications. In this institute, 95% of the patients could stand up and walk in the hall with some help on the next day after the operation, and no adverse events were observed in association with early mobilization. To facilitate IE and early mobilization, it is necessary to achieve effective postoperative analgesia [25–27]. Finally, IE after esophagectomy may help to reduce the demand on overburdened intensive care units, and subsequently the medical costs may also be reduced.

Conclusions

IE is safe and feasible even after transthoracic esophagectomy with extended radical 3FLND. To avoid the possible disadvantages of MV after surgery, IE should thus become a standard strategy for postoperative management after esophagectomy. Standardized clinical care pathways should be established, including IE after esophagectomy. Therefore, an organized institution-wide infrastructure is indispensable to optimize the outcomes in perioperative management for patients with esophageal cancer [22,28].

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ORIGINAL ARTICLE

Yasushi Toh · Eiji Oki · Kazuhito Minami
Takeshi Okamura

Follow-up and recurrence after a curative esophagectomy for patients with esophageal cancer: the first indicators for recurrence and their prognostic values

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Abstract
Background. No standardized methods exist for the follow-up and treatment of recurrence after a curative esophagectomy for patients with thoracic esophageal cancers.
Methods. One hundred seventy-five patients with thoracic esophageal cancer underwent a curative resection and were followed up during a median period of 3.0 years (3 months–18 years). The time to recurrence, the first indicators (FIs) to suspect recurrence, and the factors predictive of prognosis after recurrence were investigated.
Results. Recurrence occurred in 72 (41.1%) of 175 patients. Forty, (55.6%) and 22 (30.6%) of 72 cases presented with recurrences in the first and second year after the initial operation, respectively. Clinical visit (anamnesis and physical examination), tumor markers, and imaging were FIs in 39 (54.2%), 33 (45.8%), and 49 (68.1%) of 72 patients with recurrence, respectively. Imaging was the exclusive FI in 19 (26.4%) cases. A multivariate analysis showed the favorable prognostic factors after recurrence to be recurrence later than 1 year after the initial operation and a case in which the FI was only imaging.
Conclusions. Intensive follow-up is required in the first 2 years after surgery, and early detection of recurrence is important. The accumulation of clinical data based on a fixed schedule with consensus is necessary to obtain more definite evidence for the diagnosis and treatment of recurrent esophageal cancer.

Key words Esophageal cancer · Esophagectomy · Postoperative follow-up · First indicators for recurrence · Prognostic factors after recurrence

Y. Toh (✉) · E. Oki · K. Minami · T. Okamura
Department of Gastroenterological Surgery, National Kyushu Cancer Center, 3-1-1 Notame, Minami-ku, Fukuoka 811-1395, Japan
Tel. +81-92-541-3231; Fax +81-92-542-8503
e-mail: ytoh@nk-cc.go.jp

Introduction

Despite the recent improvement in the treatment outcome for the patients with esophageal cancer by multimodality therapy, including extensive lymph node dissection [1], postoperative recurrence is observed in a considerable number of patients [2–4]. Curative treatment of patients with recurrence is necessary to further improve the prognosis after an esophagectomy.

The guidelines for diagnosis and treatment of carcinoma of the esophagus as stated by the Japan Esophageal Society [5] separately describe methods of follow-up after the initial treatments and the treatment strategies for recurrences of each initial treatment, i.e., endoscopic resection, curative esophagectomy, and definitive chemoradiation. However, critical evidence to justify these guidelines is very limited for both the follow-up method and treatment of recurrences, and no definite guiding principles have been established in Japan. This limitation is also true in Western countries. A few recommendations for follow-up observation after surgery are noted in the guidelines of the National Comprehensive Cancer Network (NCCN) and the European Society for Medical Oncology (ESMO) [6,7], although no references showing evidence are cited. Large-scale clinical studies addressing the methods of follow-up observation after treatment seem difficult to design, because the choice of the initial treatment for esophageal cancer varies markedly depending on the stage of the disease and the general condition of the patient at the time of diagnosis. Moreover, it appears to be difficult to directly adapt the data from Western countries to Japanese patients with esophageal cancers because there are large differences in the proportions of the predominant histology, in the surgical methods used, and in survival rates after surgery between Japan and the Western countries [1].

Many reports have shown the rate, timing, and mode of recurrence after a curative esophagectomy and the treatment outcomes of recurrent esophageal cancers, some of which also note the predictive factors of recurrence [2–4,8,9]. However, very few articles describing effective follow-up

methods, first clinical indicators to suspect recurrence, or factors predictive of the prognosis after the treatment of recurrence have so far been published for esophageal cancers. This study investigated the time to recurrence and predictive factors of recurrence after a curative esophagectomy with an extended lymph node dissection for esophageal cancer. Furthermore, in our study we tried to clarify the first clinical indicators to suspect recurrence and their prognostic values, using retrospective data obtained by a fixed schedule of follow-up observation in this institute. The effective postoperative follow-up strategy for patients who undergo a curative esophagectomy with an extended lymph node dissection for esophageal cancers is discussed based on the results of this study.

Patients and methods

Patients

One hundred seventy-five patients with thoracic esophageal cancer underwent a transthoracic esophagectomy with a three-field lymph node dissection with no pathological residual tumor (R0) between 1989 and 2006 in the National Kyushu Cancer Center, Japan. All cancers were pathologically diagnosed to be squamous cell carcinoma. The characteristics of the patients with and without recurrence are shown in the Results section. The median follow-up period was 3.0 years (range, 3 months–18 years).

Surgical procedure

All 175 patients underwent transthoracic esophagectomy through a right-side thoracotomy. The alimentary tract was reconstructed using a gastric tube made of the greater curvature of the stomach, with cervical esophagogastric anastomosis by hand-sewn or instrumental anastomosis [10] through a retrosternal or posterior mediastinal route.

An extended radical lymph node dissection was then performed in three fields. A complete dissection of the middle and lower mediastinal nodes was performed via a right thoracotomy, including the periesophageal, parahiatal, subcarinal, and aortopulmonary window nodes. The dissection of the lymph nodes in the upper mediastinum included the nodes along the bilateral recurrent laryngeal nerves by carefully exposing them, from the level of the aortic arch to the thoracic inlet for the left nerve and near the origin at the base of the right subclavian artery for the right nerve. The remaining nodes along the recurrent laryngeal nerves, which were anatomically inseparable chains extending from the upper mediastinum to the lower neck, were also dissected through a cervical U-shaped incision, together with the lower deep cervical nodes located posterior and lateral to the carotid sheath. The lymph node dissection in the abdomen included the nodes along the celiac, left gastric, and common hepatic arteries, the nodes along the lesser curvature of the stomach, and the parahiatal nodes.

Follow-up after surgery

The patients with a pathological stage II or higher stage [11,12] were followed up every 2 months for the first 2 years and every 3 months thereafter in the fixed schedule shown in Fig. 1. A detailed anamnesis for history and a physical examination were performed on every clinical visit. Serum levels of tumor markers including carcinoembryonic antigen (CEA: normal range, <5 ng/ml) and squamous cell carcinoma antigen (SCC-Ag: normal range, <2 ng/ml) were measured at every clinical visit. Radiologic imaging tests including cervical, chest and abdominal computed tomography (CT), and cervical and abdominal ultrasonography (US) were performed every 4 months for the first 2 years and every 6 months thereafter. CT and US were performed at the same time to complement the limitations of each imaging modality. The follow-up for the patients with pathological stage I [11,12] was less intensively performed for the first 2 years with a clinical visit and monitoring of serum levels of tumor markers at every 3 months and radiologic imaging tests at every 6 months. In addition, bone scintigraphy and gastrointestinal endoscopy were performed once a year. Positron emission tomography with ^{18}F-fluorodeoxyglucose (FDG-PET) was indicated when recurrence was suspected. The duration of follow-up observation is set for 5 years because of the extremely low rate of recurrence later than 5 years after the initial operation.

In total, 28 patients failed to be followed up by the regular schedule. Sixteen of these patients died of other diseases during the regular follow-up and 7 dropped out of

Modality	Months after esophagectomy																							
	1st year						2nd year						3rd year				4th year				5th year			
	2	4	6	8	10	12	14	16	18	20	22	24	27	30	33	36	39	42	45	48	51	54	57	60
CV, Tumor marker	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Cervical US		•		•		•		•		•		•		•		•		•		•		•		•
Cervical–thoracic CT		•		•		•		•		•		•		•		•		•		•		•		•
Abdominal–pelvic CT		•		•		•		•		•		•		•		•		•		•		•		•
Abdominal–pelvic US		•		•		•		•		•		•		•		•		•		•		•		•
Upper GI endoscopy						•						•				•				•				•
Bone scintigraphy						•						•				•				•				•
Ba enema or Colonoscopy												•								•				

Fig. 1. Schematic representation of the follow-up schedule after a curative esophagectomy for thoracic esophageal cancer at our institute. CV, clinical visit; US, ultrasonography; CT, computed tomography; GI, gastrointestinal

Table 1. (a) Mode and rate of recurrence after a curative esophagectomy for cancer and treatment for recurrence

Mode of recurrence	No. of recurrences	Treatment (no. of patients)
Lymph node	39 (22%)	CRT (23), surgery (3) RT (5), CT (4), none (4)
Distant organ	15 (9%)	CRT (2), surgery (2) RT (5), CT (5), none (1)
Pleural dissemination	4 (2%)	CT (3), none (1)
Combined	14 (8%)	CRT (7), surgery (1) RT (1), CT (4), none (1)
Total	72 (41%)	

CRT, chemoradiotherapy; RT, radiotherapy; CT, chemotherapy

a regular follow-up schedule for personal reasons. The median follow-up periods were 2.2 years (0.7–6.5 years) for the former and 2.0 years (0.5–2.5 years) for the latter. Only 5 among 72 cases with recurrence were found to have recurrences before the prefixed next timing of the schedule because they showed some symptoms and signs and spontaneously visited our hospital. Their first indicators were judged to be "clinical visit." All these patients were included in the analysis.

Data analyses and statistics

All statistical analyses were performed using the StatView software program (version 5.0; Abacus Concepts, Berkeley, CA, USA). The relationship between recurrence and the clinicopathological features was determined using a Student's *t* test, Fisher's exact test, and a logistic regression analysis. Survival rates after recurrence were calculated by the Kaplan–Meier method for the analysis of censored data. The significance of differences in survival was analyzed with a log-rank test and a generalized Wilcoxon test in a univariate analysis and a Cox's proportional hazards model in a multivariate analysis. A P value < 0.05 was considered to be statistically significant.

Results

Recurrence occurred in 72 (41.1%) of 175 patients. Lymph node recurrence, organ metastasis, pleural dissemination, and a combination of these were observed in 39 (22.3%), 15 (8.6%), 4 (2.3%), and 14 (8.0%) patients, respectively (Table 1a). In total, 51 cases showed lymph node recurrence, 17 of which were found within the dissected area. The first choice of treatment for recurrence is also shown in Table 1a. Various kinds of treatment were indicated for each mode of recurrence, which clearly showed that there was no definite strategy for treatment of recurrence, depending on the extent of recurrent diseases, the presence or absence of pervious neoadjuvant and/or adjuvant treatments, and the patient's general status at the diagnosis of recurrence.

Forty (55.6%) and 22 (30.6%) of the 72 cases presented with recurrences in the first and second year after the initial operation, respectively, thus indicating that more than 86% of recurrences occurred within 2 years after surgery (Table 1b). However, 4 of the remaining 10 cases presented their recurrences later than 4 years after the operation (data not shown).

Table 1. (b) Time to recurrence after a curative esophagectomy for cancer

Months after surgery	Number of cases	Cumulative ratio
Earlier than 6 months	20	
From 6 to 12 months	20	56%
From 12 to 18 months	13	
From 18 to 24 months	9	86%
Later than 24 months	10	100%
Total	72	100%

The relationship between recurrence and clinicopathological features at surgery is shown in Table 2a. A univariate analysis showed statistically significant associations between recurrence and the pathological depth of tumor invasion (pT), pathological lymph node metastasis (pN), pathological stage (pStage), permeation to lymphatic vessels and venous invasion, the number of fields (cervical, mediastinal, or abdominal) where lymph node metastasis was observed, and the number of metastasized lymph nodes (0–4 vs. 5 and more: this way of division yielded the statistically largest difference). The average numbers of metastasized lymph nodes were 3.84 and 0.74 in the recurrent and nonrecurrent patients, respectively, which showed a statistically significant difference ($P < 0.0001$) (data not shown). A logistic regression analysis including these factors indicated that only the presence of permeation to lymphatic vessels ($P < 0.05$, odds ratio = 5.11, 95% confidence interval = 1.34–19.45) and lymph node metastasis when observed in more than two fields ($P < 0.001$, odds ratio = 4.78, 95% confidence interval = 1.99–11.47) were selected as statistically significant factors that would predict recurrence after surgery (Table 2b).

The surveillance tools that first indicated a suspicion of recurrence (first indicator, FI) were investigated. Table 3 shows that 39 (54.2%) of 72 patients with recurrence were suspected to have recurrence by a clinical visit including anamnesis of history (symptoms) and signs observed during a physical examination. Symptoms most frequently observed were pain at metastasized sites, general fatigue, dysphagia, and appetite loss. Signs most frequently observed were fever, cough and sputum caused by pneumonia, hoarseness,

Table 2. Relationship between recurrence and clinicopathological factors after a curative esophagectomy for esophageal cancer
(a) Univariate analysis

Variables	Recurrence (+) (n = 72)	Recurrence (–) (n = 103)	P value
Age (years)	61.8 ± 8.4	62.0 ± 7.8	N.S.
Gender (male/female)	64/8	84/19	N.S.
Tumor location:			
Upper/Middle/Lower	10/32/30	13/58/32	N.S.
Depth of tumor invasion			
pT 0, 1/2, 3	16/56	47/56	<0.0001
Lymph node metastasis			
pN 0, 1, 2/3, 4	31/41	76/25	<0.0001
Pathological stage			
pStage 0, I, II/III, IV	27/45	81/22	<0.0001
Lymph vessel permeation			
ly (–)/(+)	26/46	83/20	<0.0001
Vascular invasion			
v (–)/(+)	41/31	85/18	<0.0001
No. of fields of LNM			
0, 1/2, 3	40/32	94/9	<0.0001
No. of metastasized LN			
0–4/5 and more	54/18	99/4	<0.0001

pT, pN, pStage are according to references 11, 12
N.S., not significant; LN, lymph node; LNM, LN metastasis

(b) Multivariate analysis (logistic regression analysis)

Variables	P values	Odds ratio	95% CI
pT 0, 1/2, 3	0.16	1.87	(0.78–4.46)
pN 0, 1, 2/3, 4	0.35	0.54	(0.15–1.94)
pStage 0, I, II/III, IV	0.37	1.83	(0.49–6.88)
ly (–)/(+)	<0.05	5.11	(1.34–19.45)
v (–)/(+)	0.27	2.71	(0.46–15.91)
No. of fields of LNM			
0, 1/2, 3	<0.001	4.78	(1.99–11.47)
No. of metastasized LN			
0–4/5 and more	0.98	1.10	(0.39–2.63)

CI, confidence interval

Table 3. First indicators to suspect recurrence and its frequency

First indicator	No. of patients
Clinical visit	39 (54%)
Symptoms	36 (50%)
Signs	22 (31%)
Tumor marker	33 (46%)
CEA	9 (13%)
SCC-Ag	28 (39%)
Imaging	49 (68%)
CT	45 (63%)
US	8 (11%)
Imaging only[a]	19 (26%)

CEA, carcinoembryonic antigen; SCC-Ag, squamous cell carcinoma
[a]Imaging only means the cases in which imaging was exclusive first indicator without any other first indicators such as clinical visit or tumor marker

and abnormal neurological findings such as paralysis. The FI in 33 cases (45.8%) was monitoring of tumor markers of CEA and/or SCC-Ag. Imaging including CT and/or US indicated a suspected recurrence in 49 cases (68.1%). Imaging was the exclusive FI in 19 cases (i.e., no symptoms or signs, normal levels of tumor marker; 26.4%).

The FIs were compared between 40 patients within 12 months after surgery and 32 patients more than 12 months after surgery among the recurrent patients. The FIs of them were clinical visit (65.0% and 40.6%, P = 0.039), tumor marker abnormalities (55.0% and 34.4%, P = 0.081), and imaging (70.0% and 65.6%, P = 0.69), respectively. The rate of patients whose recurrences were found by exclusively imaging abnormalities without any symptoms, signs, or abnormal tumor marker levels was less frequent in the former group (17.5%) than in the latter one (37.5%), although this difference was statistically not significant (P = 0.056).

The overall survival rates were compared by the time to recurrence, mode of recurrence, and various FIs. The overall 1- and 3-year survival rates of all cases after the diagnosis of recurrence were 29% and 14%, and the median survival time (MST) was 7 months (data not shown). The MSTs of cases with lymph node recurrence, organ metastasis, and combined recurrence were 9, 6, and 6 months, respectively, showing no significant differences. There are 4 patients who are still alive 30 months after recurrence. The mode of recurrence, treatment modalities, and prognosis of each of these cases are solitary brain metastasis, gamma-knife radiotherapy, and 43 months (case 1); cervical lymph node metastasis, surgical resection, and 34 months (case 2); solitary lung metastasis, surgical resection, and 32 months (case

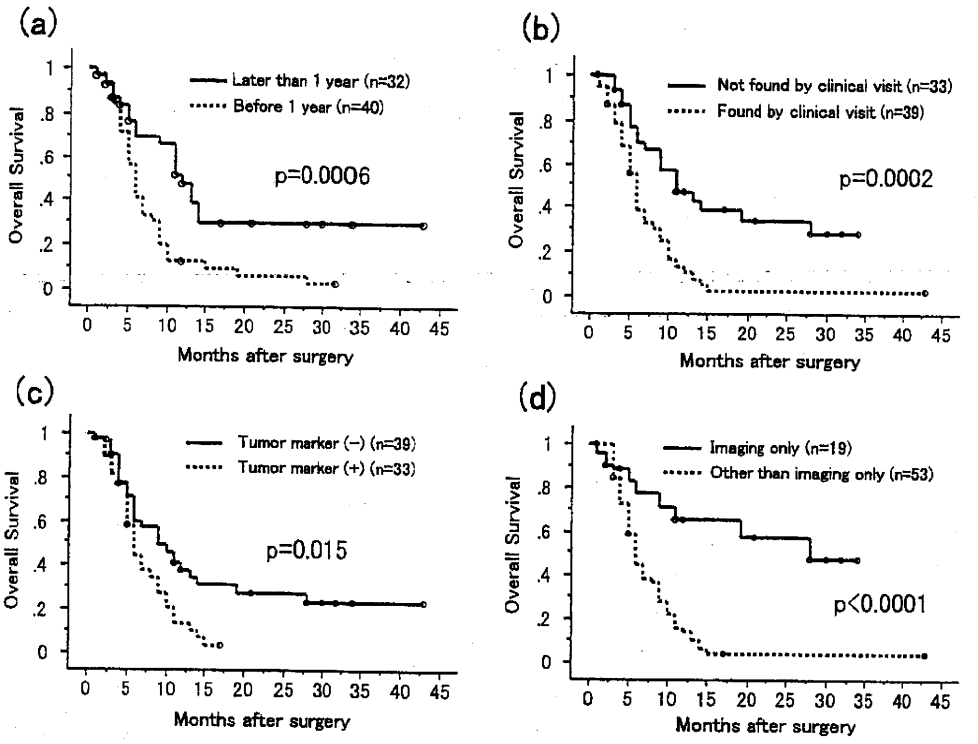

Fig. 2. Overall survival rates after recurrence of esophageal cancers were compared by time to recurrence [*later than 1 year* versus less than (*before*) *1 year* after surgery] (**a**), presence versus absence of symptoms and/or signs on *clinical visit* (**b**), presence versus absence of *tumor marker* abnormalities (**c**), and the cases in which *imaging* was the exclusive first indicator versus the cases that presented with any other first indicators (FIs) with or without imaging (**d**)

3); and lower mediastinal lymph node recurrence, chemoradiotherapy, and 30 months (case 4).

The patients whose recurrences were found later than 1 year after surgery showed significantly better survival rate than those within 1 year; 1- and 2-year survival rates were 47.3 % and 30.1%, respectively, in the former group and 12.8% and 6.4%, respectively, in the latter group (P = 0.0006) (Fig. 2a). When the recurrence was found by a clinical visit (symptom and/or signs), the prognosis was significantly worse than in those who showed no symptoms or signs (P = 0.0002; Fig. 2b). The patients who showed symptoms had a significantly poorer prognosis than those without (P = 0.0008). Similarly, the prognosis of the patients who showed any signs was significantly worse than those without signs (P = 0.036; data not shown). Abnormal serum tumor marker level (CEA and/or SCC-Ag) at the diagnosis of recurrence was also an unfavorable prognostic indicator (Fig. 2c). The prognosis of the patients whose FI was exclusively imaging (that is, patients who showed no symptom, sign, or abnormal tumor marker level) was significantly better than that of the patients who presented with any other FIs with or without imaging (P < 0.0001) (Fig. 2d).

The prognostic values of FIs for 40 patients who showed recurrences within 12 months after surgery were also analyzed. Importantly, the patients whose recurrences were found by exclusively imaging abnormalities without any symptoms, signs, or abnormal tumor marker levels (7 cases) showed a significantly longer survival rate than the remaining 33 cases (P = 0.0027 by log-rank test) (MST: 19.0 months vs. 6.0 months, P = 0.037 by a generalized Wilcoxon test).

Table 4 summarizes the results of a multivariate analysis to identify independent prognostic factors using a Cox's proportional hazards model. Subsequently, recurrences later than 1 year after surgery and when imaging was the exclusive FI were indicated to be independent factors for a favorable prognosis after recurrence (Table 4).

Discussion

The primary aim of the follow-up after a curative resection of an esophageal cancer is to detect local recurrence, distant metastasis, or metachronous primary cancers at an early stage when curative treatments are still possible, thus leading to an improvement of the prognosis. Follow-up is also important to evaluate and administrate the general condition and the quality of life of the patients, because an esophagectomy is associated with a significant level of surgical stress. However, achieving a successful cure of patients with recurrence is extremely rare even after multimodality therapies. The MST after a diagnosis of recurrence is about 5–8 months [2–4,8,9]. Nevertheless, it is also obvious that there are a few patients who could be cured if their recurrence were diagnosed at an early stage [13–15]. Furthermore, even when a curative treatment is impossible, early detection of recurrence could possibly provide patients with a better compliance for various treatments and with an opportunity to obtain a more prolonged survival and a better quality of life. The fact that patients whose FI was exclusively imaging (that is, patients who showed no symptoms, signs, or abnormal tumor marker levels) had a significantly longer survival rate clearly indicates the usefulness of a regular follow-up, and this is also true among the patients whose recurrences were found within 12 months after surgery. Thus, these data strongly suggest that the

Table 4. A Cox's proportional hazards model for factors predictive of prognosis after recurrence

Variables	*P* value	Hazards ratio	95% CI
Time of recurrence			
<1 year vs. >1 year	0.002	3.04	(1.51–6.12)
Symptoms (–) vs. (+)	0.27	2.08	(0.57–7.59)
Signs (–) vs. (+)	0.12	1.87	(0.85–4.10)
Clinical visit[a]			
(–) vs. (+)	0.28	0.39	(0.071–2.19)
Tumor marker[b]			
(–) vs. (+)	0.78	1.18	(0.36–3.86)
Imaging[c]			
(–) vs. (+)	0.30	0.49	(0.13–1.86)
Imaging only[d]			
(–) vs. (+)	0.011	5.22	(1.46–18.68)

CI, confidence interval
[a] Clinical visit: symptom and/or signs
[b] Tumor marker: CEA and/or SCC-Ag
[c] Imaging: CT and/or US
[d] Imaging only: the cases in which imaging was exclusive first indicator without any other first indicators such as clinical visit or tumor marker

patients whose recurrences could be found before appearance of any symptoms, signs, or tumor marker abnormalities can expect a better chance of longer survival.

No standard method for postoperative follow-up observation after a curative esophagectomy for esophageal cancer has been established. The clinical practice guidelines for esophageal cancer established by NCCN [6] state a brief follow-up: (1) for asymptomatic patients, complete history and physical examination every 4 months for 1 year, then every 6 months for 2 years, and annually thereafter, and (2) circulating blood cell count and serum chemistry evaluation, endoscopy, and imaging studies as clinically indicated. However, no evidence or references are cited in this guideline. The clinical recommendations for esophageal cancer by ESMO show no method for postoperative follow-up and note that there is no evidence that regular follow-up after initial therapy influences the outcome [7]. The Japanese guidelines [5] briefly discuss the follow-up procedures, including imaging modalities, to be used, but again no definite data or evidence is presented.

This report documented the follow-up method used in this institute. This method identified the FIs that suggested recurrence and the factors predictive of prognosis after recurrence. More than half (54%) of the recurrences were suspected merely by clinical visits (symptom and/or sign), indicating that complete anamnesis and the history and physical examination of the patient are extremely important on every clinical visit. Measurement of the serum level of tumor markers, including CEA and SCC-Ag, is also effective to find recurrences. In particular, the SCC-Ag level was increased in about 40% of the patients with recurrence. Imaging including CT and/or US was also shown to be effective for follow-up. CT and US were performed at the same time because the use of both these imaging methods sometimes complemented the deficiencies of the other. Four patients were suspected to have recurrence by only US but not by CT (data not shown).

These FIs could therefore be factors predictive of the prognosis after recurrence. A univariate analysis indicated the presence of symptoms and/or signs, and abnormal tumor maker levels at the diagnosis of recurrence would predict more unfavorable prognosis after recurrence. In contrast, the patients whose recurrences were identified by imaging only (i.e., no symptoms or signs, and normal level of tumor markers) could therefore expect a significantly better prognosis after recurrence. A multivariate analysis also demonstrated that this factor could be an independent predictor of a favorable prognosis. This finding clearly showed that recurrence should be found as early as possible before appearance of any symptoms, signs, or tumor marker abnormalities. Furthermore, patients with recurrence later than 1 year after the initial operation were shown to have significantly better prognosis than those before 1 year in both the univariate and multivariate analyses, which may mean that recurrent lesions found within a year after surgery consisted of tumor cells with more aggressive potential than those after 1 year. However, even in such cases, earlier detection of recurrence would give a greater possibility for cure by multimodality treatments including surgery and chemoradiotherapy. Considering that most recurrences occurred within 2 years after the operation, postoperative follow-up should be more intensive for the first 2 years and less intensive for the following 3 years.

Recently, FDG-PET has been shown to be effective in detecting recurrence of esophageal cancer after surgical resection. FDG-PET seems to be more accurate that conventional CT for detection of both locoregional recurrence and distant metastases, except small lung metastasis [16,17]. The fact that FDG-PET has a larger field of imaging than CT can be another merit for detecting recurrences. However, FDG-PET is not always facilitated in most hospitals, including this one, and is reserved for patients with suspected recurrence detected by the conventional follow-up system.

It is also mandatory to check for the development of either asynchronous remnant esophageal cancer or asynchronous multiple cancers of other organs such as of the stomach (gastric tube used for reconstruction) or head and neck region. Sato et al. reported that a second malignancy was the major cause of death among the patients without any lymph node metastasis who underwent an esophagectomy for thoracic esophageal cancer [18]. Therefore, endoscopic examinations are conducted for the head and neck region, remnant esophagus, stomach, and colorectum (see Fig. 1).

Conclusions

No standard follow-up method after a curative esophagectomy for esophageal cancer has yet been established. Furthermore, so far few studies have investigated the effectiveness of any follow-up schedules including the frequency and modalities used. The efficacy and suitability of the schedule shown in this article for the cure of patients with recurrence of esophageal cancer are not known. A nationwide accumulation of larger-scale clinical data based on a fixed schedule with a consensus is necessary to obtain evidence for the diagnosis and treatment of recurrent esophageal cancer. In the future, the performance of meta-analyses using the findings of many reports on postoperative follow-up are absolutely required.

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REVIEW ARTICLE

Alcohol drinking, cigarette smoking, and the development of squamous cell carcinoma of the esophagus: molecular mechanisms of carcinogenesis

Yasushi Toh · Eiji Oki · Kippei Ohgaki · Yasuo Sakamoto · Shuhei Ito · Akinori Egashira · Hiroshi Saeki · Yoshihiro Kakeji · Masaru Morita · Yoshihisa Sakaguchi · Takeshi Okamura · Yoshihiko Maehara

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Abstract Esophageal cancer is the eighth most common incident cancer in the world and ranks sixth among all cancers in mortality. Esophageal cancers are classified into two histological types; esophageal squamous cell carcinoma (ESCC), and adenocarcinoma, and the incidences of these types show a striking variety of geographic distribution, possibly reflecting differences in exposure to specific environmental factors. Both alcohol consumption and cigarette smoking are major risk factors for the development of ESCC. Acetaldehyde is the most toxic ethanol metabolite in alcohol-associated carcinogenesis, while ethanol itself stimulates carcinogenesis by inhibiting DNA methylation and by interacting with retinoid metabolism. Cigarette smoke contains more than 60 carcinogens and there are strong links between some of these carcinogens and various smoking-induced cancers; these mechanisms are well established. Synergistic effects of cigarette smoking and alcohol consumption are also observed in carcinogenesis of the upper aerodigestive tract. Of note, intensive molecular biological studies have revealed the molecular mechanisms involved in the development of ESCC, including genetic and epigenetic alterations. However, a wide range of molecular changes is associated with ESCC, possibly because the esophagus is exposed to many kinds of carcinogens including alcohol and cigarette smoke, and it remains unclear which alterations are the most critical for esophageal carcinogenesis. This brief review summarizes the general mechanisms of alcohol- and smoking-induced carcinogenesis and then discusses the mechanisms of the development of ESCC, with special attention to alcohol consumption and cigarette smoking.

Keywords Esophageal squamous cell carcinoma · Carcinogenesis · Alcohol · Acetaldehyde · Smoking · Carcinogen · Molecular alterations

Y. Toh (✉) · E. Oki · K. Ohgaki · Y. Sakamoto · Y. Sakaguchi · T. Okamura
Department of Gastroenterological Surgery, National Kyushu Cancer Center, 3-1-1 Notame, Minami-ku, Fukuoka 811-1395, Japan
e-mail: ytoh@nk-cc.go.jp

S. Ito · A. Egashira · H. Saeki · Y. Kakeji · M. Morita · Y. Maehara
Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Introduction

The International Agency for Research on Cancer (IARC) has concluded from epidemiological data that the occurrence of malignant tumors of various organs, including the head and neck region, esophagus, liver, colorectum, and breast, is causally related to chronic alcohol consumption [1–3]. Cigarette smoking also causes more than one million cancer deaths per year in the world. About 90% of lung cancer is attributed to smoking [4–6]. The molecular mechanisms involved in alcohol- and smoking-related carcinogenesis have been clarified, although they are not fully understood [3, 5].

Although the precise etiology of cancers in the upper aerodigestive tract (UADT) (i.e., oral cavity, pharynx, larynx, and esophagus) still remains unclear, dietary and environmental factors are strongly implicated. Cigarette smoking and alcohol consumption are considered to be significant risk factors for esophageal squamous cell carcinoma (ESCC) [7–9]. Various kinds of genetic abnormalities have been investigated in ESCC, including the

activation of oncogenes and inactivation of tumor-suppressor genes, and a large body of knowledge has been obtained concerning esophageal carcinogenesis [10, 11]. However, direct evidence showing a causal relationship of alcohol consumption or cigarette smoking with the genetic abnormalities observed in ESCC is insufficient. This brief review discusses the general molecular mechanisms of alcohol- and smoking-related carcinogenesis and then addresses the genetic alterations in ESCC, with attention to alcohol and cigarette smoke.

Molecular mechanism of alcohol-related carcinogenesis (Fig. 1)

Well-established carcinogens in alcohol and its metabolites

The mechanisms of ethanol-induced carcinogenesis are closely related to the metabolism of ethanol [3]. Ethanol is oxidized by alcohol dehydrogenase (ADH) in the liver, which results in the generation of acetaldehyde (AD). AD is then metabolized to acetate by aldehyde dehydrogenase-2 (ALDH2). AD is a carcinogen in various animals, because AD may induce gene mutations [3, 12]. Epidemiological studies clearly demonstrate that the inactive ALDH2 encoded by the *ALDH2*1/2*2* genotype, which causes an increased accumulation of AD following alcohol consumption, is a strong risk factor for the development of

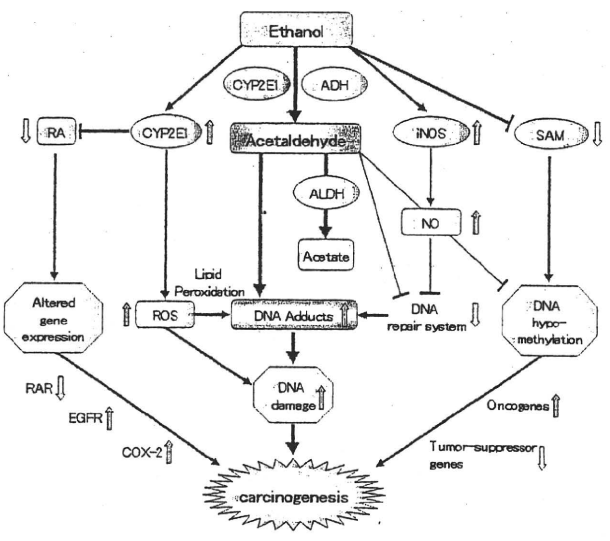

Fig. 1 Schematic presentation of ethanol metabolism and its role in carcinogenesis. *ADH* alcohol dehydrogenase, *CYP2E1* cytochrome P450 2E1, *ALDH* aldehyde dehydrogenase, *RA* retinoic acid, *RAR* RA receptor, *EGFR* epidermal growth factor receptor, *COX-2* cyclooxygenase-2, *ROS* reactive oxygen species, *iNOS* inducible nitric oxide synthase, *NO* nitric oxide, *SAM* *S*-adenosyl-L-methionine

UADT cancers, in particular esophageal cancer [13, This fact indicates the carcinogenicity of AD.

There are unique mechanisms of topical AD produc from local ethanol in the UADT [14]. Increasing alc consumption results in increasing AD concentration in saliva which is higher than that in blood. The normal microflora can oxidize ethanol to AD, and this contrib to the AD level in the saliva. Moreover, because fur metabolism of AD to acetate by oral bacteria is limited, AD concentration in the saliva can be 10–100 times hig than that in the blood [15]. It is possible that AI involved in UADT carcinogenesis, including carcinog esis in the esophagus, because AD in saliva comes direct contact with the mucosa of the UADT [3, 14].

General mechanisms of alcohol-induced carcinogenesis

AD interacts with DNA to form stable DNA adducts [3] AD-induced DNA adducts escape cellular repair mec nisms and persist, they may lead to miscoding, resulting permanent gene mutations [16]. For example, AD cau point mutations in the hypoxanthine phosphoribos transferase (HPRT) 1 gene locus in human lymphocy and induces sister chromatic exchanges and gross ch mosomal aberrations [17, 18]. In fact, a high level of A DNA adducts has been found in lymphocyte DNA fr alcoholic patients [19].

One of the important AD-induced DNA adducts is methyl-γ-OH-propano-deoxyguanosine (Cr-PdG). Cr-P is highly mutagenic and the formation of this DNA add can be facilitated in the presence of polyamines [3, 2 Relevant polyamine concentrations are present in tiss that are in hyper-regenerative environments. Chro alcohol consumption causes such an environment in mucosa of the UADT [21], brought about by the h concentration of AD in the saliva [22]; thus leading to generation of highly mutagenic Cr-PdG adducts in th tissues. This process may be related to carcinogenesis the UADT, including the esophagus [3]. AD also binds various proteins involved in DNA repair and DNA me ylation and causes structural and functional alterations these proteins [3, 23].

Reactive oxygen species (ROS) such as superox anion (O_2^-) and hydrogen peroxide (H_2O_2) are genera by several enzyme systems, including cytochrome P4 2E1 (CYP2E1)-dependent microsomal monooxygena Chronic alcohol consumption in animals and huma induces the hepatic CYP2E1 enzyme at concentrations 1 20 times higher than those in subjects without chro alcohol consumption [24, 25]. CYP2E1 induction alcohol has also been confirmed in the gastrointesti mucosa of animals [26]. CYP2E1 has a high rate nicotinamide adenine dinucleotide phosphate, reduc

(NADPH) oxidase activity and produces a large amount of O_2^- and H_2O_2. Furthermore, chronic alcohol consumption also induces nitric oxide synthase and increases nitric oxide production, leading to the generation of highly reactive peroxynitrite ($ONOO^-$) [3, 27]. The ROS cause oxidative injury, inflammation, and lipid peroxidation [24]. Lipid peroxidation leads to the production of 4-hydroxynonenal, which reacts with DNA bases such as deoxyadenosine and deoxycytidine and forms exocyclic DNA adducts, which are highly mutagenic and induce a point mutation at the hotspot of the *p53* gene [3, 28]. Therefore, the oxidative stress caused by ROS is accepted as a critical pathophysiological mechanism in various human diseases, including cancer. In fact, malignant tumors often show increased levels of DNA base oxidation and mutations [29].

Ethanol itself may also stimulate carcinogenesis by inhibiting DNA methylation and by interacting with retinoid metabolism [3]. The methylation and the demethylation of genes are among the most important mechanisms for the regulation of gene transcription [30]. *S*-Adenosyl-L-methionine (SAM) is a universal methyl group donor and enzyme activator in methyl transfer reactions, and alcohol consumption inhibits SAM synthesis [31]. For example, the inhibition of SAM synthesis by alcohol leads to global hypomethylation of hepatic DNA but not of the *p53* gene, resulting in the upregulation of oncogenes and downregulation of tumor-suppressor genes [32]. Therefore, aberrant methyl transfer caused by the inhibition of SAM synthesis may be important for alcohol-mediated carcinogenesis [3]. Retinoic acid (RA) regulates the transcription of many genes that are important for cellular growth and differentiation by signaling through its nuclear receptors (RARs) [7]. Chronic alcohol consumption decreases RA concentrations in the liver by inducing CYP2E1 [33]. The disruption of RA metabolism and signaling may have an important role in carcinogenesis. For example, in the rat liver, the decrease in the RA level induced by alcohol results in the downregulation of RARs and the enhancement of AP-1 (c-Jun and c-Fos) expression, thus resulting in the hyperproliferation of hepatic cells and a decrease of apoptosis [3, 34].

Molecular mechanism of smoking-related carcinogenesis (Fig. 2)

Well-established carcinogens in cigarette smoke

Cigarette smoke is a cause of lung cancer, as well as being a cause of esophageal, oral, pharyngeal, laryngeal, pancreatic, and other cancers [5]. Cigarette smoke contains more than 60 carcinogens that have been evaluated by the IARC [4, 5]. Fifteen of these compounds are carcinogenic

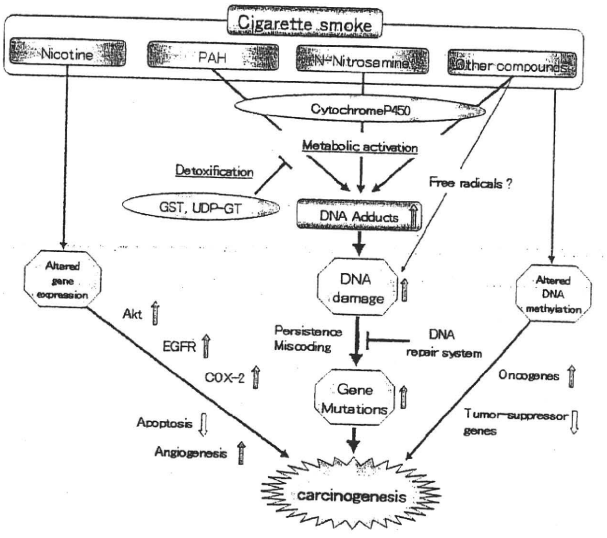

Fig. 2 Schematic presentation of compounds in cigarette smoke and their roles in carcinogenesis. *PAH* polycyclic aromatic hydrocarbons, *GST* glutathione-*S*-transferases, *UDP-GT* uridine diphosphate–glucuronosyl transferases, *EGFR* epidermal growth factor receptor, *COX-2* cyclooxygenase-2

in humans, with polycyclic aromatic hydrocarbons (PAHs) and *N*-nitrosamines being the most important carcinogens in cigarette smoke. There are strong links between these carcinogens and various types of smoking-induced cancers [35]. The mechanisms of their actions are believed to be the induction of DNA adducts, gene methylation and mutation, and chromosomal translocation in target organs [5, 7].

Benzo[*a*]pyrene (BaP) is one of the 10 PAH compounds listed by the IARC as carcinogens [4]. It has powerful carcinogenic activity and is considered to be carcinogenic to humans [36]. PAHs are usually locally acting carcinogens, and they also induce various kinds of cancer depending on the route of administration [5]. Although a causal link has been established between PAHs and cancer in skin, lung, and bladder cancers, evidence linking individual PAHs to ESCC is based only on ecological studies and is therefore circumstantial [37]. Evaluating the association of PAHs with ESCC has proven difficult, partly because there are no valid and reliable markers of long-term exposure to PAHs that can be used in epidemiological studies [37].

N-Nitrosamines also cause various cancers in several animal models [37]. The important *N*-nitrosamines in cigarette smoke are *N*-nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N'*-nitrosonornicotine (NNN). The tobacco-specific NNK is a potent lung carcinogen in animals and also induces pancreatic and nasal cavity tumors [38]. NNN is the most prevalent *N*-nitrosamine carcinogen in cigarette smoke, which causes tumors of the esophagus in rats [5, 39, 40].

Aromatic amines, formaldehyde, volatile hydrocarbons, organic compounds, metals, and other compounds contained in cigarette smoke are listed as carcinogens in humans by the IARC (reviewed by Hecht [5]). Cigarette smoke contains free radicals and induces oxidative damage [41]. Freshly generated cigarette smoke contains large amounts of nitric oxide and other unstable oxidants [42]. However, the role of oxidative damage as a cause of specific tobacco-induced cancers remains unclear [35].

General mechanisms of smoking-induced carcinogenesis

The major established pathway of carcinogenesis by cigarette smoke is the formation of covalent bonds between the carcinogens in smoke and DNA, which produces DNA adducts, resulting in permanent mutations in critical genes such as oncogenes and tumor-suppressor genes in somatic cells [5].

Most of the cigarette smoke carcinogens are oxygenated by cytochrome P450 enzymes and are converted to a form that is highly soluble in water [43]. However, some of the intermediates are quite reactive with DNA, resulting in the formation of DNA adducts [43, 44], which are central to the carcinogenic process [45]. This process is known as the metabolic activation of carcinogens [35]. The balance between the metabolic activation of carcinogens and detoxification by various enzymes, including glutathione-*S*-transferases [46] and uridine diphosphate (UDP)-glucuronosyl transferases [47], varies among individuals and is likely to affect cancer susceptibility. The levels of DNA adducts in the lung and other tissues are higher in smokers than in nonsmokers, and some data have demonstrated links between higher adduct levels and a higher probability of cancer development [6].

DNA adducts can be eliminated in normal cells by elaborate DNA repair systems [48]. For example, adducts of PAHs are repaired by nucleotide excision repair, and miscoding in methylated base O^6-methylguanine is repaired by a direct repair system with O^6-methylguanine DNA methyltransferase. DNA adducts persist if these repair systems are insufficient or overwhelmed by the amount of DNA damage. Mutations may arise during DNA replication if persisting DNA adducts are bypassed incorrectly by DNA polymerase, leading to dysregulation of normal cell growth and apoptosis, genomic instability, and a higher probability of cancer development [48, 49].

DNA adducts induced by different carcinogens may have significantly different mutational properties. Therefore, it is useful to identify the link between DNA damage and specific mutations in tumor cells in order to elucidate the role that environmental elements play in carcinogenesis in humans [35]. The available data indicate that many DNA adducts associated with cigarette smoke exposure may frequently produce G-to-T transversions [42]. example, the mutational spectrum of the *p53* tumor pressor gene in lung cancer cells is similar to the mut patterns induced in vitro by PAH metabolites [35, 50, The major adduct of BaP produces a G-to-T transve [52] and the frequency of the G-to-T transversion is nificantly higher in smokers than that in nonsmokers Methylated CpG dinucleotides are the preferred sites fo to-T transversion, and the striking sequence specificit benzo[*a*]pyrene-7,8-diol-9,10-epoxide (BPDE) for ducing G-to-T transversion hotspots at methylated sequences is similar to the distribution of G-to-T tr version hotspots in smoking-associated lung tumors 51, 53].

Other possible mechanisms of smoking-induced carcinogenesis

There are other pathways in which carcinogens in ciga smoke cause cancers. Nicotine, the main known addic agent in cigarette smoke, may be at least partially invo in the initiation, promotion, and even progression of tu [54, 55]. Nicotine modulates the phenotype of nor airway epithelial cells by rapidly activating Akt, a ser threonine kinase, leading to decreased apoptosis increased angiogenesis [56, 57]. Moreover, cigarette sm activates the epidermal growth factor receptor (EG tyrosine kinase in oral epithelial cells that then stimul cyclooxygenase-2 (COX-2) [58]. This leads to the inh tion of apoptosis, promotion of angiogenesis, modula of inflammation and immune function, and increased tu cell invasiveness [59]. Epigenetic changes, such as methylation of CpG islands, may therefore be ano mechanism of smoking-induced carcinogenesis [60].

Synergistic effects of smoking and alcohol in carcinogenesis (Fig. 3)

Epidemiological data have suggested that alcohol inter synergistically with cigarette smoke in the developmen ESCC [61]. Alcohol is metabolized to AD locally in oral cavity by the ADH of microbes in the normal flora. Heavy alcohol drinking, chronic smoking, and poor oral hygiene frequently observed in drinkers smokers modify the oral flora to contain a larger abunda of aerobic bacteria and yeasts which are highly capable generating AD from ethanol [3, 62]. Furthermore, ALDH enzyme in the oral mucosa in smokers is inhibi by microbial changes in the oral cavity due to smoki resulting in a significant deposition of AD in the sal [63]. Therefore, AD may link drinking alcohol, smoki and poor oral health to ESCC [37].

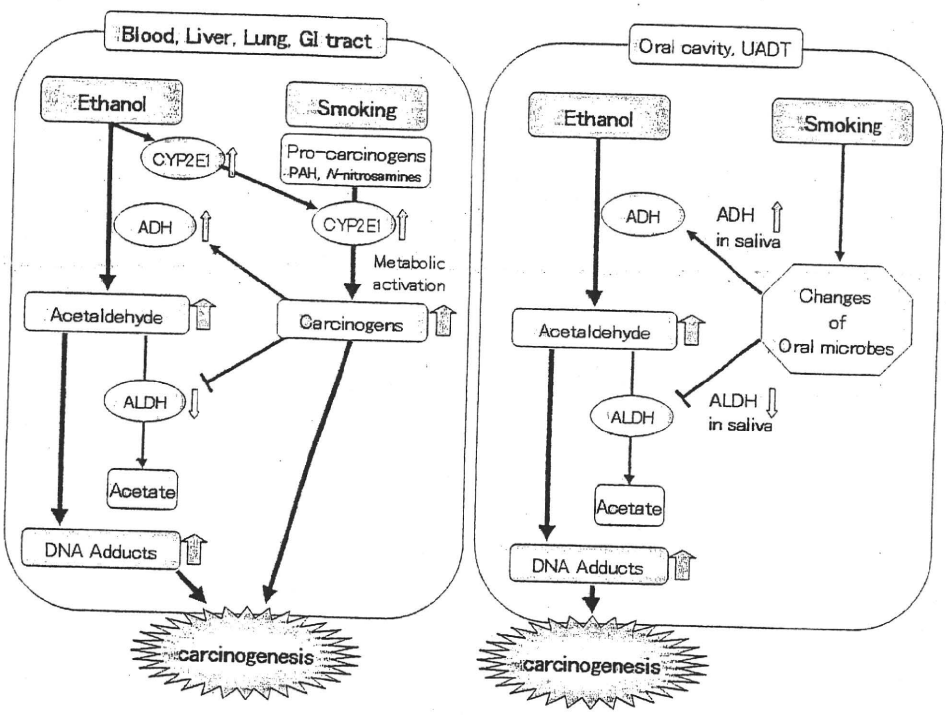

Fig. 3 Synergistic effects of smoking and alcohol in carcinogenesis. *GI* gastrointestinal, *UADT* upper aerodigestive tract, *ADH* alcohol dehydrogenase, *CYP2E1* cytochrome P450 2E1, *ALDH* aldehyde dehydrogenase

Also, chronic alcohol consumption induces cytochrome P450 enzymes in the liver and the gastrointestinal mucosa [24–26], possibly leading to acceleration of the metabolic activation of cigarette smoke-related procarcinogens to active carcinogens [35]. Furthermore, there is also evidence of inhibition of the ALDH enzyme by smoking, which leads to less efficient AD metabolism and, consequently, to higher AD concentrations in the UADTs of smokers [64]; as well, BaP increases the ADH level in bronchial epithelial cells (our data). All of these data suggest that AD derived from both ethanol and tobacco appears to act in the UADT as a local carcinogen in a synergistic way [65].

Molecular alterations in esophageal squamous cell carcinoma:review with a special focus on cigarette smoking and alcohol consumption

Numerous molecular alterations associated with the genesis of ESCC have been reported. These include alterations in cell-cycle regulation, growth factors and their receptors, and DNA repair systems. Such alterations in ESCC will be reviewed here, paying special attention to cigarette smoking and chronic alcohol consumption.

p53

p53 is a tumor-suppressor gene and its primary function is to maintain human genetic stability and DNA repair capacity [66]. The function of the *p53* gene is lost mainly by gene mutations, as well as by various other factors, including overexpression of the murine double minute gene 2 (*MDM2*); this causes acceleration of p53 degradation [67]) or inactivation of $p14^{ARF}$ [this protein suppresses MDM2 activity], leading to inhibition of cell-cycle arrest, DNA repair, and the subsequent apoptosis [68].

p53 is one of the first tumor-suppressor genes that has been shown to have undergone frequent point mutations in primary ESCC and ESCC cell lines [66]. The point mutations in this gene occur even at an early stage of ESCC and correlate with tumor progression [69], thus suggesting an important role of this abnormality in esophageal carcinogenesis. The reported frequencies of *p53* gene mutations vary from 17% to 84%, possibly because of differences in the analytical methods used [70]. Egashira et al. [70] investigated the frequency of mutation of this gene by very elaborate direct DNA sequencing and demonstrated that 47.4% of the patients with ESCC had a *p53* gene mutation. The prognostic value of *p53* gene mutations in ESCC is controversial.

The mutational spectrum of the *p53* gene in lung cancers is consistent with the mutation patterns induced by certain PAHs such as BaP in cigarette smoke [35, 50, 51]. The major adduct of BaP produces a G-to-T transversion [52], and 40%–50% of *p53* gene mutations in Japanese patients with ESCC are predominantly the transversion of G to T [70, 71]; these findings also suggest that cigarette smoke might be related to esophageal carcinogenesis. However, Pfeifer et al. [35] have noted that it is difficult to identify the unambiguous molecular signature of tobacco carcinogens in the *p53* mutational spectrum of esophageal cancer,

because the patterns of mutation are extremely heterogeneous. On the other hand, the main mutations caused by AD, the primary metabolite of alcohol, are G-to-A transitions [72]. Noori and Hou [73] demonstrated that the mutational spectrum induced in vitro by AD in the *HPRT* gene of human T lymphocytes was consistent with the predominance of G-to-A transitions and mutations at A:T base pairs in the *p53* gene in esophageal tumors. These data may indicate that various factors are related to esophageal carcinogenesis, including cigarette smoke and alcohol. Positive correlations between the ratios of heavy alcohol drinkers and cigarette smokers and a high accumulation of p53 protein, related to its gene mutations [74], have been demonstrated in ESCC [75, 76].

Multiple ESCCs frequently occur in individual patients [77]. Ito et al. [78] demonstrated that *p53* mutation profiling of multiple ESCCs was quite heterogeneous not only in the presence/absence of mutations but also in the mutational patterns if they exist. The finding of different *p53* gene mutations among multiple ESCCs suggested evidence of field carcinogenesis in the human esophagus. Furthermore, this finding may reflect the condition that the esophagus is exposed to a wide variety of carcinogens.

p21

The $p21^{WAF1/CIP1}$ gene, a cyclin-dependent kinase inhibitor (CDKI) induced by p53, mediates G1 arrest after DNA damage [79]. Although mutations or deletions in this gene are rarely reported in human cancers, polymorphisms of this gene may play some roles in esophageal carcinogenesis [80]. p53-dependent expression of p21 is observed in ESCC, while the lack of an absolute correlation between abnormal p53 protein expression and p21 protein expression suggests that p53-independent expression of p21 protein might also occur in ESCC [81]. The direct relationship between *p21* and carcinogens in cigarette smoke and alcohol has so far only seldom been studied.

$p16^{INK4a}$ and $p14^{ARF}$

p16 protein inhibits CDK 4 and 6 that bind to cyclin D1 and downregulate the pRb pathway which blocks cell-cycle progression from the G1 to S phase [60]. Inactivation of the $p16^{INK4a}$ gene is a frequent event in human cancers, and is associated with a homozygous deletion, genetic mutation, or aberrant DNA methylation [60, 82]. Losses of the *p16* gene and the subsequent protein expression occur in the early stage of ESCC carcinogenesis, either by promoter methylation or by loss of heterozygosity [8, 9, 83]. Silencing of the *p16* gene by promoter methylation plays a role in smoking-related lung cancer [60]. The radionuclides in cigarette smoke may explain the phenomenon of *p16* inactivation by promoter methylation in smoking-asso ated lung tumors [84]. Although the contribution of cig rette smoke to the inactivation of the *p16* gene in ESC remains to be elucidated, Ito et al. [85] have reported th the promoter methylation rate of $p16^{INK4a}$ was 76% a hypermethylation of this gene tended to occur more fi quently in heavy drinkers and smokers.

The $p14^{ARF}$ gene is transcribed from the same locus $p16^{INK4a}$ by alternative splicing, and the protein produ interacts with MDM2 protein, thus resulting in the stab ization of p53 [86]. The $p14^{ARF}$ promoter is aberrant methylated in 61% of patients with ESCC, leading downregulation of the expression of this gene [85].

Cyclin D1

Cyclin D1 protein is involved in the p16-pRb pathway ar induces pRb phosphorylation with CDK4/6, indicating i critical role in the progression of the cell cycle through th G1 to S phase [87]. Amplification or overexpression of th gene plays an essential role in human esophageal carcin genesis [11]. A causal relationship between tobacco ca cinogens and *cyclin D1* upregulation has been reported lung cancer, oral cancer, and ESCC. Hu et al. [88] reporte that cigarette smoke extract stimulated cell proliferation ar increased the cyclin D1 protein level in a dose-depende manner in a human ESCC cell line. A correlation betwee alcohol consumption and upregulation of *cyclin D* expression was also observed in esophageal cancer [89].

EGFR, RA, and RARs

EGFR is a receptor tyrosine kinase and plays an importa role in cell-cycle regulation and carcinogenesis. EGFR overexpressed in 29%–92% of resected ESCC specimer [11, 90]. *EGFR* gene amplification is one of the mech nisms of its activation [91], which can be a marker f predicting lymph node metastasis and unfavorable pro nosis [91, 92]. *EGFR* gene mutations in esophageal carc noma are rare, but they do exist [93].

RA can suppress EGF-associated cell proliferation ar EGFR expression by inhibiting EGFR-dependent ERK1, activation [94, 95]. Immortalized human bronchial ep thelial cells are transformed by NNK, a tobacco carcin gen, with overexpression of *EGFR* and *cyclin D1*. Retino treatment prevents this transformation by downregulatir *EGFR* and *cyclin D1* expression [94]. *EGFR* expression also inhibited in esophageal cancer cells by the induction RA and RAR-β_2. Furthermore, BPDE, a potent carcinog in cigarette smoke, can suppress RAR-β_2 expression murine lung cancer through methylation of the *RAR-* gene promoter [96]. Xu's group similarly demonstrated, immortalized esophageal epithelial cells and esophage

cancer cells, that BPDE induced methylation of the *RAR-β_2* gene promoter, thus leading to the loss of RAR-β_2 expression [7, 97, 98]. This induced the overexpression of EGFR, ERK1/2, AP-1, and COX-2 [7].

The induction of CYP2E1 by alcohol can enhance the degradation of RA. Consequently, RA levels in cells are reduced, resulting in the altered expression of different genes, such as the reduced expression of *RAR-β_2* and increased expression of *EGFR*, *ERK1/2*, *AP-1*, and *COX-2* [99].

COX-2

COX-2 is one of the two enzymes that catalyze the first step in the synthesis of prostaglandins (PGs) from arachidonic acid. Multiple lines of evidence suggest that COX-2 is associated with many of the critical steps in carcinogenesis and tumor progression. Zimmermann et al. [100] have demonstrated that COX-2 is expressed in the majority of ESCC tissues and that COX-2-derived PGs play an important role in the regulation of proliferation and apoptosis of esophageal cancer cell lines. Various animal and human esophageal tissues contain high levels of PGs in cancer [59]. The levels of COX-2 have been shown to increase in the oral mucosa of smokers in comparison to those in nonsmokers, and the activation of EGFR signaling contributes to the elevated levels of COX-2 [58]. Furthermore, nicotine enhances the migration and invasion of human ESCC cell lines, a process which is inhibited by nimesulide, a selective COX-2 inhibitor that decreases the protein level of COX-2 [55].

E-cadherin

E-cadherin belongs to the cadherin family of Ca^{2+}-dependent cell–cell adhesion molecules and is a key molecule in the suppression of the epithelial–mesenchymal transition (EMT) that occurs during the development and progression of cancers. Yoshino et al. investigated the correlation between tobacco smoking and EMT in a lung cancer cell line and found that BaP decreased the E-cadherin expression level and induced EMT [101, 102]. Furthermore, Davis et al. [103] showed that nicotine significantly reduced the expression of E-cadherin in cultured lung, breast, and pancreatic cancer cells, leading to EMT. The association of E-cadherin expression and smoking or alcohol consumption in ESCC remains to be elucidated.

BRCA1

Several studies have shown a frequent loss of heterozygosity in the region of the *BRCA1* gene locus in ESCC [104], suggesting that BRCA1 may be a candidate tumor-suppressor gene in esophageal cancer. The finding that BPDE can bind to the *BRCA1* gene after normal esophageal epithelial cells are treated with BPDE may therefore be an important phenomenon [105].

FHIT

The fragile histidine triad *(FHIT)* gene has been identified as a candidate tumor-suppressor gene localized at chromosome 3p14.2 [106]. Inactivation of *FHIT* occurs at an early stage in the development of ESCC [107] and methylation of the *FHIT* gene promoter is closely associated with transcriptional inactivation in ESCC [108], which is linked to cigarette smoking [109]. Nicotine induces methylation of the *FHIT* gene in human ESCC cell lines and attenuates Fhit protein in association with the increased expression of DNA methyltransferase 3a, which is implicated primarily in de-novo methylation [110]. Furthermore, an association of the loss of Fhit protein with alcohol consumption is also suggested in human ESCC [111].

DNA repair genes

There is no direct evidence to show a correlation between cigarette smoke or alcohol consumption and the impairment of DNA repair systems in ESCC. Several studies have indicated that abnormalities of DNA repair systems are uncommon in esophageal carcinogenesis [112]. On the other hand, Mimori et al. [113] have reported that microsatellite instability is significantly related to allelic loss in the *FHIT* region, but that mutS homologue 2 might be unrelated to progression or the oncogenic process in ESCC.

Conclusion

Despite recent advances in diagnostic and surgical techniques and multimodal treatments, esophageal cancer still remains one of the most aggressive and lethal diseases [114, 115]. Many types of epidemiological data have demonstrated that both cigarette smoking and alcohol consumption are the two major risk factors for the development of ESCC [61]. The aim of the present review was to summarize the current evidence for contributory mechanisms of alcohol- and smoking-induced carcinogenesis and to discuss the molecular mechanisms of esophageal carcinogenesis with special attention to these carcinogens. Although the most important goal in conquering ESCC is to prevent the development of this disastrous disease by enlightening the public about the risk of these carcinogens, it is equally important to clearly elucidate the underlying mechanisms of esophageal carcinogenesis. However, a comprehensive

understanding of the molecular mechanisms of esophageal carcinogenesis remains elusive. Therefore, greater effort is required to identify more genetic changes, as well as epigenetic changes such as the methylation and acetylation or deacetylation of histones and other important proteins which are observed in esophageal cancer [116, 117]. These mechanistic insights could be translated into practical approaches for the prevention and cure of alcohol- and smoking-induced esophageal cancer.

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Conflict of interest statement The authors indicated no potential conflicts of interest.

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