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Original Article

The macroscopic and histological effects of argon plasma coagulation followed by subepithelial ablation on early esophageal squamous cell carcinoma using magnifying endoscopy with blue laser imaging

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Abstract

Background Argon plasma coagulation (APC) followed by subepithelial ablation (termed APC-SEA) is an effective means of treating early esophageal squamous cell carcinomas (EESCCs). However, the mechanism of tissue exfoliation after the first ablation was unknown. This study aimed to determine the characteristics of the exfoliated esophageal mucosa after the first ablation.
Methods We examined 13 EESCC lesions. We then marked them by APC. We removed the epithelium exfoliated by the initial ablation procedure and performed magnifying endoscopy with blue laser imaging for the exfoliated area and a second ablation procedure for subepithelial degeneration. The exfoliated tissue was examined histopathologically.
Results The basement membrane was not visible on the surface of the exfoliated samples. Among the 13 exfoliated specimens, 9 were confirmed to have viable carcinoma. The basal cell layer was exposed homogeneously on most of the surface of the exfoliated esophagus. In the intraepithelial papillae, basement membrane was dissected at the same level as the exfoliated surface surrounding it, or preserved intact.
Conclusion The basal cell layer containing carcinoma *in situ* was exposed homogeneously on most of the surface of the esophagus just before the second ablation procedure. A second ablation procedure was found to be essential for treating EESCC.

Keywords: early esophageal squamous cell carcinoma, argon plasma coagulation, epithelial exfoliation, subepithelial ablation, basement membrane, intraepithelial papilla.

Introduction

In 1991, Farin and Grund[1] and Grund et al[2]. invented a device that could be inserted through a gastrointestinal endoscope to enable the use of argon plasma coagulation (APC) in flexible endoscopy. Currently, APC is used for treating early esophageal squamous cell carcinomas (EESCCs) that are not amenable to endoscopic submucosal dissection (ESD)[3].

Previously, blackening of the affected region was used as a marker for complete ablation. However, this marker requires a relatively long time for ablation per unit area, which may ablate the muscularis propria. Furthermore, it may also result in incomplete ablation of the submucosa because the carbonized tissue obstructs electrocoagulation[4]. In order to improve this conventional type of APC, we developed and reported the usefulness of APC followed by subepithelial ablation (APC-SEA) for EESCC[5-7]. APC-SEA consists of an initial ablation procedure followed by exfoliation of the ablated epithelium and a second ablation procedure. We started

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performing this treatment procedure in 2002. However, the histological effect of the first ablation procedure has not been examined because we discarded the exfoliated samples.

Blue laser imaging (BLI), developed by Fujifilm (Kanagawa, Japan) in 2012, is a new method of image-enhanced endoscopy that can observe the superficial mucosal pattern and microvessels as well as narrow-band imaging[8, 9]. Using magnifying endoscopy, intraepithelial papillary capillary loops (IPCLs) were classified by Inoue et al[10]. If the target area had only IPCL type V-1 vessels, defined as IPCL with dilation, meandering, irregular caliber, and form variation, the lesions were likely carcinoma *in situ*[10].

We previously used magnifying endoscopy with BLI to determine the area to be treated by identifying the IPCLs before the first ablation procedure. In this study, we have also used it to observe the treated area after the first ablation procedure in order to recognize the remnant tissues or microvessels on the surface of the exfoliated area.

Aim

The aim of this study was to determine the characteristics of the exfoliated esophageal mucosa after the first ablation procedure using magnifying endoscopy with BLI and to examine the exfoliated sample after the first ablation procedure histopathologically.

Materials and methods

We collected data from 13 lesions in 11 patients (median age 66 years old, 9 males and 2 females) who underwent APC-SEA using magnifying endoscopy with BLI from March 2013 to July 2015 at the Department of Gastrointestinal Surgery, Tokyo Medical and Dental University. As the necessary condition for applying APC-SEA treatment, we selected the cases containing the area where IPCL type V-1 vessels were observed within a 2 cm diameter.

We set such condition in light of our previous results which showed effective local control by APC-SEA. The previous study revealed that the APC-SEA treatment could effectively control the small EESCCs (≤2 cm) which were carcinoma in situ or invaded only the lamina propria mucosa[7].

Among the cases meeting the above-mentioned condition, we applied APC-SEA to the following cases: the case which was difficult to be treated by ESD itself because the recurrence or metachronously occurred lesion was observed near the scar previously treated with endoscopy and the cases showing poor general condition because ESD was inappropriate for such cases due to the ESD's potential risk of perforation.

Before undergoing APC-SEA, all patients were given a full explanation of the procedure, and their written informed consent was obtained. The study protocol was in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. This study has been approved by the Tokyo Medical and Dental University Hospital Ethics Committee (M2016-050).

We used a magnifying endoscope (EG-L590ZW; Fujifilm, Kanagawa, Japan) and endoscopic light source (LL-4450; Fujifilm). At the beginning of the procedure, the endoscopic diagnosis of the depth of the tumor was estimated by magnifying endoscopy. All of the IPCLs in the 13 lesions were classified as type V-1 vessels, meaning that all of them were considered to be carcinoma *in situ*. A transparent hood (MAJ-1990; Olympus, Tokyo, Japan) was placed at the tip of the endoscope. APC was performed using a high-frequency oscillator unit (APC2; Erbe Elektromedizin, Tuebingen, Germany) and a flexible APC probe (FiAPC probe 3000A, axial beam, straight fire, 2.3 mm in diameter, 2.2 m in length, No. 20132-223; Erbe Elektromedizin). We used the forced APC mode and an argon gas flow rate of 2.0 L/min at a power setting of 60 W. The APC probe was inserted through the working channel of the endoscope and positioned 2–3 mm from the target tissue, and APC ablation was performed[5, 6].

The method for performing APC-SEA, analyzing the specimens histologically, and analyzing the endoscopic findings was as follows: The endoscope was inserted into the esophagus of the patient using midazolam as a sedative and pethidine hydrochloride as an analgesic. The presence of no abnormal microvessels other than IPCL type V-1 vessels in the lesion was confirmed via magnifying endoscopy with BLI. A diluted iodine-potassium solution (0.5% Lugol's solution) was sprayed onto the mucosa to assess the lesion margins, and the circumference of the lesion, which was 1 mm away from the iodine-unstained border, was marked via APC. The first ablation procedure was performed inside the marked area; the ablated epithelium turned cloudy and whitish and floated on the surface and was easily exfoliated by rubbing it with the transparent hood on the tip of the endoscope (Fig. 1a–e).

After removing the exfoliated epithelium, the exfoliated area of the esophagus was observed via magnifying endoscopy with BLI. We checked the exfoliated area for the presence of remnant tissue. The second ablation procedure was performed while the exfoliated

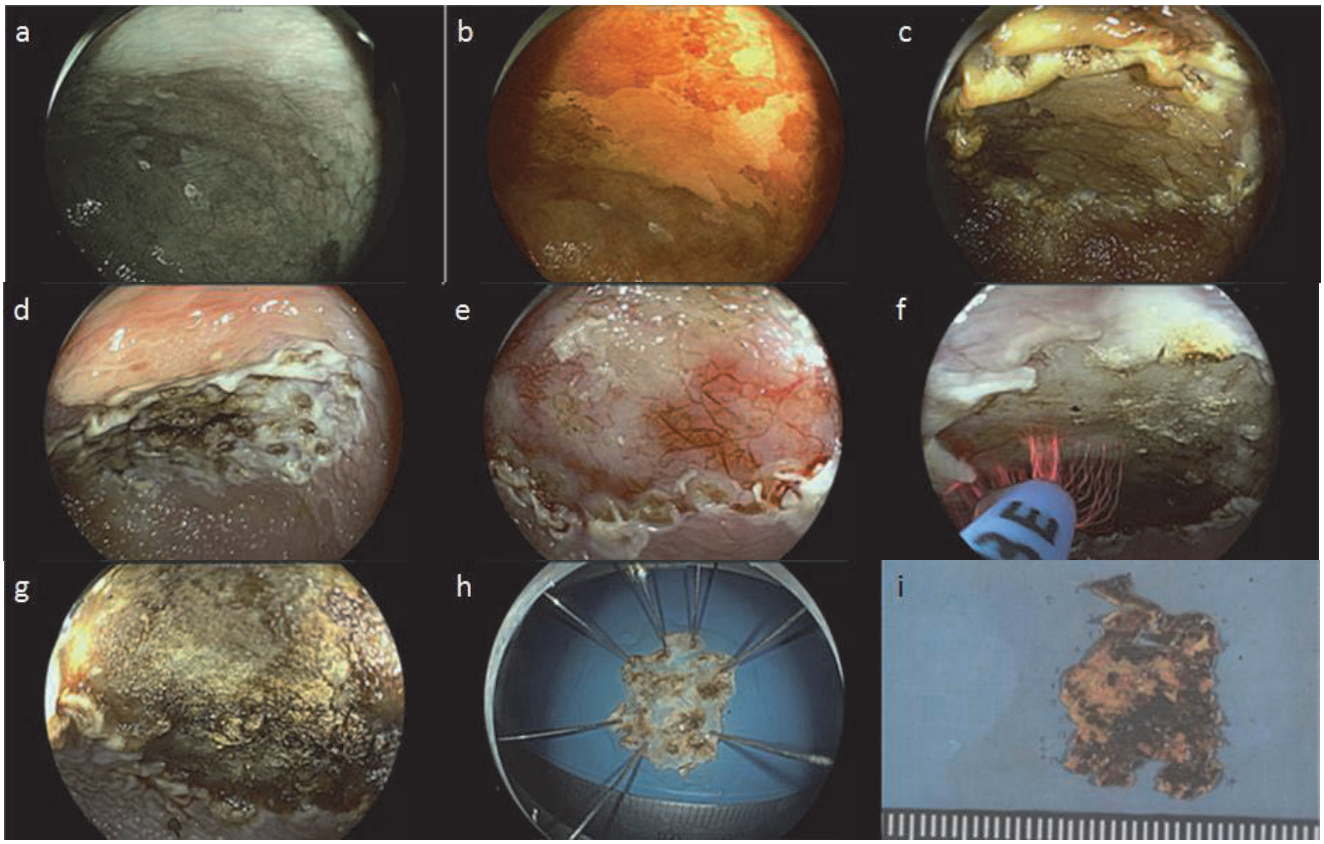

Figure 1. a Brownish area identified via blue laser imaging; **b** The same area as in panel **a** with iodine staining; **c** Marking the circumference of the area via ablation; **d** Cloudy and whitish epithelium after the first ablation procedure; **e** Area after removing the ablated epithelium; **f** The second ablation procedure after the removal of the ablated epithelium; **g** Area after the second ablation procedure; **h** Removed epithelium after the first ablation procedure; **i** A 2-mm section after formalin fixation.

area could still be observed and proceeded until the subepithelial tissue in the exfoliated area turned brown throughout. The ablation depth was assessed via magnified endoscopy (Fig. 1f, g)[5, 6], and the endoscopic findings were compared with the histological findings. After the treatment, we pinned the exfoliated epithelium on the setting board and prepared a formalin-fixed sample using a procedure similar to that used for the preparation of ESD samples (Fig. 1h). Next, 2-mm-thick sections were sliced (Fig. 1i) and stained with hematoxylin and eosin. A pathologist (T. Ito) familiar with the diagnosis of esophageal cancer who was also a member of the Japanese Society of Pathology subjectively determined the percentage of degenerated area by observing the exfoliated tissue in a low-power field. He also evaluated the presence of carcinoma.

After the pathological examination, the exfoliated tissue samples were immunohistochemically stained to determine whether or not they contained the basement membrane or IPCLs. Doing so allowed us to accurately assess the layer from which the sample originated as well as to clarify which layers were exfoliated in the first ablation procedure. We used anti-type-IV collagen antibody and anti-CD31 antibody to detect the basement membrane and vascular endothelial cells, respectively.

Results

Observation of the exfoliated area with magnifying endoscopy with BLI

We removed the epithelium that had turned whitish and had a texture different from that of the non-treated area after the initial ablation; the exfoliated areas corresponding to the 13 lesions were examined before the second ablation procedure. Almost no petechiae were observed over the entire exfoliated area via conventional endoscopy. There were whitish and reddish areas. The whitish area was slightly raised above the

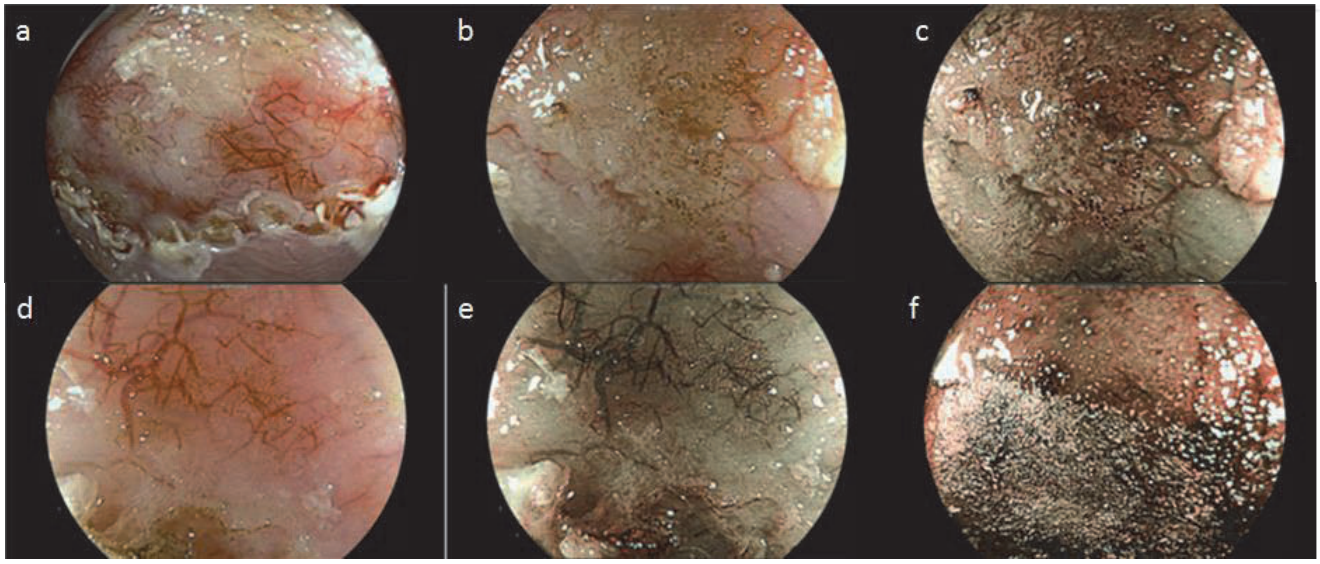

Figure 2. **a** Removed area after the first ablation procedure; **b** Magnified image of the exfoliated area shows loop-like vessels in the white degenerated epithelium and over the reddish branching vessels near the surface of the muscularis mucosa; **c** Blue laser imaging of the area shown in panel b shows the loop vessels more clearly; **d** A magnified image of another exfoliated area shows the subepithelial capillary networks (SECNs) over the reddish branching vessels near the surface of the muscularis mucosa; **e** Blue laser imaging of the area shown in panel **d** shows the SECNs more clearly; **f** A magnified image after the second ablation procedure.

surrounding area. Its surface was not smooth and contained few blood vessels. In contrast, the surface of the reddish area was smooth, and branching vessels could be easily observed throughout the upper layers (Figure 2a).

We examined the 13 lesions via magnifying endoscopy with BLI. In the whitish area, magnifying endoscopy revealed the presence of looped microvessels resembling IPCL type V-1 vessels[10] in some portions with a rough surface (Fig. 2b, c). No other blood vessels could be seen in this area, as remnant whitish epithelium covered the muscularis mucosa. In contrast, in the reddish area, magnifying endoscopy revealed that almost all of the epithelium had been removed, and we could clearly see the branching vessels on mascularis mucosa (Fig. 2d). We were also able to see the subepithelial capillary network (SECN)[11] covering the branching vessels (Fig. 2e).

We immediately diagnosed the tissue resembling IPCLs as the remaining tissue and easily performed the secondary ablation procedure.

Endoscopic observation after the second procedure showed that the treated area had been ablated homogeneously and turned brown throughout the area. No blood vessels were observed, nor was the muscular layer exposed (Fig. 2f).

Histological findings for the epithelium exfoliated by the first ablation procedure

The characteristics of the 13 lesions in 11 patients (median age were 66, male were 9, female were 2) selected for a histological evaluation are shown in Table 1. The median tumor size was 10 mm (range 5-20 mm). At the evaluation, 54% (range, 30%–85%) of the hematoxylin and eosin-stained exfoliated epithelium samples contained cells or nuclear material fused by heat degeneration in each sample (Table 2, Fig. 3a). We determined the presence of carcinoma in the areas with the least heat damage. The cells in 9 of the 13 lesions (69.2%) had obvious atypical nuclei or an increase in the nuclear-to-cytoplasmic ratio at the surface of the specimen, indicating the presence of carcinoma after the first ablation procedure (Table 2, Fig. 3h). Thus, among these nine specimens, the vertical margin was positive for carcinoma. We had the impression that the visible IPCL diagnosed as carcinoma was shorter and had slightly higher density compared with the IPCL in non-carcinoma area.

Regarding the mechanism of exfoliation, most of the epithelium was exfoliated at the basal cell layer or the squamous cell layer close to the basal cell layer. The basement membrane was not visible on the surface of the exfoliated samples, and the SECN located just below the basement membrane was not visible in the samples.

Table 1. Characteristics of the patients and lesions selected for the histological evaluation

Sex (male/female)	9 / 2
Median age, year (range)	66 (54 – 90)
Median diameter of the tumor, mm (range)	10 (5 – 20)
Tumor location	
Cervical esophagus, n (%)	0 (0)
Upper thoracic esophagus, n (%)	2 (15)
Middle thoracic esophagus, n (%)	4 (31)
Lower thoracic esophagus, n (%)	6 (46)
Abdominal esophagus, n (%)	1 (8)
Previous endoscopic resection, n (%)	12 (92)
Reason for choosing APC-SEA	
Tumor located on the scar from a previous treatment, n (%)	12 (92)
Poor general health status, n (%)	1 (8)

Eleven patients from 12 cases with a total of 13 lesions were pathophysiological analyzed.
APC, argon plasma coagulation

The histological evaluation also revealed the presence of several dissected intraepithelial papillae in the samples (Fig. 3b, c) but could not definitively confirm whether or not they contained the basement membrane or IPCLs. We therefore selected the three samples with the least amount of heat damage for immunostaining with anti-type-IV collagen antibody and anti-CD31 antibody for the detection of the basement membrane and capillary vessels, respectively. It was thought that structures would be most clearly visualized in the areas with the least heat damage. The samples that were immunostained are described in Table 2.

Immunostaining revealed the presence of the basement membrane, which encompassed the outside of two blood vessels, only in the intraepithelial papillae in which capillary vessels were also present (Fig. 3d, e–g). The basement membrane was not observed on the surface of the exfoliated samples (Fig. 3d). These findings suggest that the basement membrane is preserved almost intact at the treated site and that the epithelium is exfoliated at the basal cell layer or slightly higher. Our findings also revealed two basement membrane patterns in the intraepithelial papillae: dissection of the basement membrane at the same level as the exfoliated surface surrounding it, and preservation of the basement membrane intact.

Short-term treatment results

In this study, all cases were treated safely without serious adverse events including postoperative hemorrhage, perforation, and stenosis. The median follow-up period was 17 months. EESCC recurred in the peripheral zone of the original carcinoma in one patient (8%). Recurrence was controlled via follow-up APC-SEA. Lymph node and distant metastasis were not observed. All patients in this study are currently alive without EESCC.

Table 2. Characteristics of and pathological findings for 13 lesions

No.	Age (yr)	Sex	Tumor diameter (mm)	Heat degenerated area (%)	Viable carcinoma	ICH
1	62	M	5	50	-	-
2	54	F	10	55	+	-
3	65	M	10	50	+	-
4	46	M	20	50	-	-
5	68	M	5	50	-	-
6	59	M	10	50	-	-
7	77	M	15	65	+	-
8	90	F	10	30	+	+
9	70	M	10	70	+	-
10	70	M	10	55	+	-
11	66	M	10	85	+	-
12	65	M	10	40	+	+
13	67	M	10	50	+	+

ICH, immunohistochemistry
+, yes; -, no

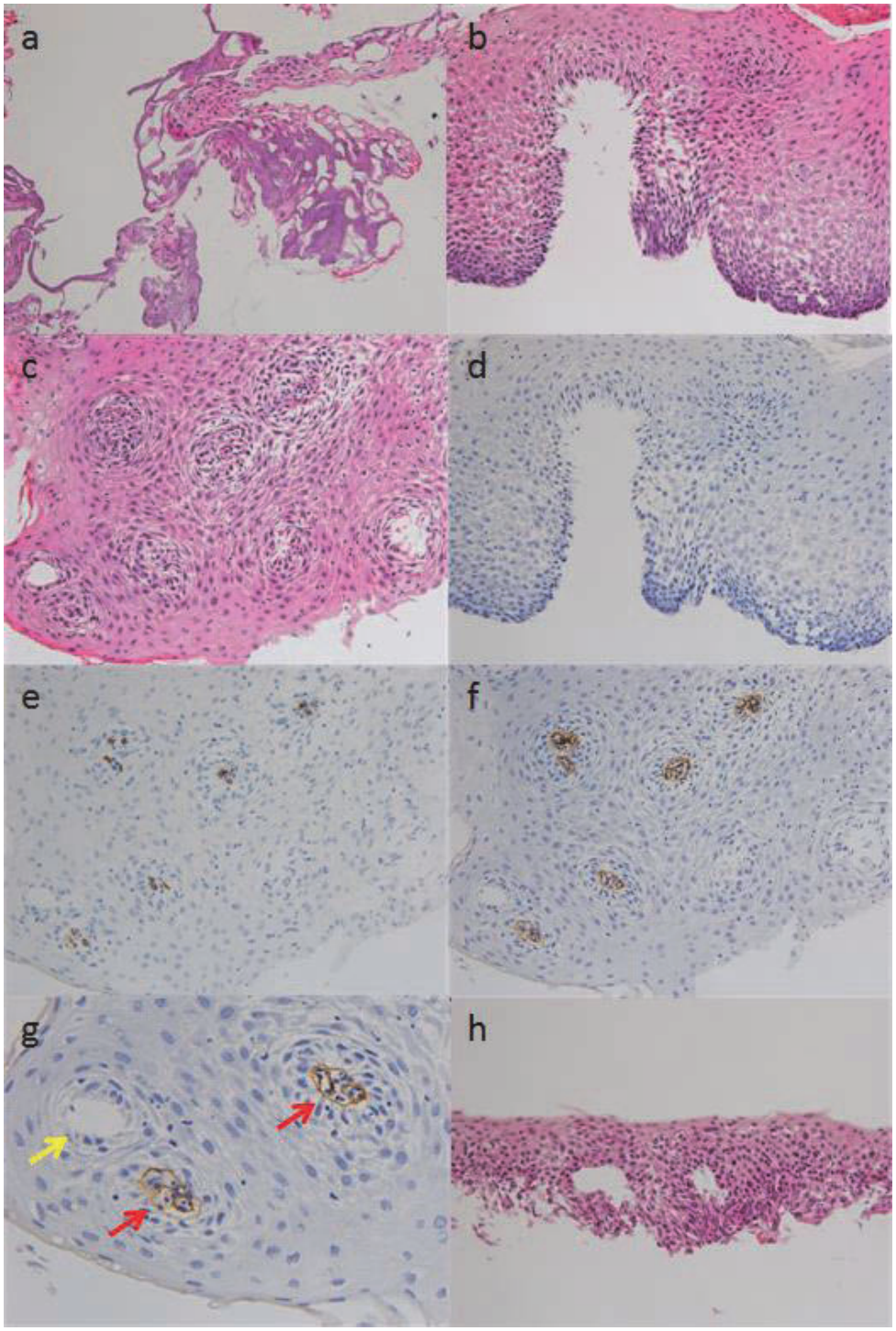

Figure 3. **a** Highly degenerated area due to ablation (x200); **b** Exfoliated epithelium almost at the level of the basal cell layer containing intraepithelial papillae vertically sectioned (x200); **c** Horizontally sectioned intraepithelial papillae with and without intraepithelial papillary capillary loops (IPCLs) (x200); **d** Immunostaining of the area shown in panel **b** using anti-type-IV collagen antibody (x200); **e** Immunostaining of the area shown in panel **c** using anti-CD31 antibody (x200); **f** Immunostaining of the area shown in panel c using anti-type-IV-collagen antibody (x200); **g** A magnified image of f (x400). The red arrows indicate the intraepithelial papillae with IPCLs and basement membrane, and the yellow arrow indicates an intraepithelial papilla without IPCLs or basement membrane; **h** Area showing characteristics of carcinoma.

Discussion

Since it was first reported by Oyama et al. in 2005, ESD has been widely used as a less-invasive procedure than surgery or chemoradiation therapy for the treatment of EESCC[12]. However, while ESD is indeed less invasive than other procedures, large-scale investigations including about 13,000 patients who underwent ESD in Japan (where ESD is most often used) have reported that an average of 3.3% of patients experience perforation-related complications[13]. The percentage of such patients differs depending on the size of the facility; for example, the percentage was found to be as high as 5% in small facilities that perform 17 cases or fewer annually[13]. Other complications after ESD are also more likely to occur in smaller facilities with fewer ESD cases than in larger ones, as well as in cases where ESD is performed close to a scar. In contrast, because APC-SEA requires less skill than does ESD, its performance presumably is safe, regardless of the facility size and physician experience.

As the Japanese population is rapidly graying, the number of elderly patients who are vulnerable to ESD, albeit less so than chemotherapy or surgery, will increase. Therefore, opportunities to perform APC, which is safer and less burdensome for patients, will also increase. Given this situation, we previously reported the long-term outcomes of patients treated with APC-SEA[7]. The report showed that among 264 times APC-SEA treatments for 70 patients, no delayed bleeding and no perforation was observed. Except for the patients who had already experienced stenosis, no patients had stenosis. The median APC treatment time per lesion was 2. Among 33 cases which had carcinoma in situ or only invaded into lamina propria mucosa, 32 cases could be locally controlled only by APC-SEA. The remaining one case developed gastric cancer, which was removed with the esophagus. After the operation, only 2mm remnant of intraepithelial carcinoma was detected, which illustrated the APC treatment could almost perfectly control the legion locally.

Although this treatment was proved to be safe, of note, the APC procedure makes it difficult to obtain pathophysiological information, such the depth of carcinoma invasion. However, NBI magnified endoscopy is likely to complement this drawback due to NBI's high diagnostic capacity for IPCL type V-1 vessel[10]. IPCL type V-1 vessels are an indicator of whether or not a carcinoma is located only in the epithelium.

BLI is a newly developed method for enhancing images in order to facilitate the detection of superficial vessels over NBI[8, 9]. In this study, we performed APC-SEA along with magnifying endoscopy equipped with BLI. First, we focused on the condition of the exfoliated surface of the epithelium after the first ablation procedure. We were able to directly observe real-time changes in the IPCL-like blood vessels in the remaining whitish epithelium. The persistence of the carcinoma was inferred from the blood vessels. At the reddish site where the epithelium was uniformly exfoliated, SECN was detected. SECN is known to exist under the basement membrane from which IPCL-like blood vessels extend[11]. Thus, the detection of SECNs suggests the disappearance of IPCLs. Given these findings, we were able to determine the area that should be ablated.

We then examined to what extent the first ablation procedure had reached and the degree of degeneration obtained by examining the exfoliated tissue pathologically. The examination of the exfoliated epithelium revealed that almost all of the samples had been exfoliated at the basal cell layer. This finding shows pathophysiologically that the basement membrane and SECN were preserved on the side of the esophagus. On the surface of the exfoliated area, viable remnant carcinoma was detected in 69% cases, indicating the importance of a second ablation procedure for obtaining a more reliable treatment effect, as the first ablation procedure was shown to be insufficient.

An immunohistochemical examination showed that two conditions occurred simultaneously: one in which the basement membrane and IPCLs in the intraepithelial papillae were preserved intact on the surface of the exfoliated area in the esophagus, and another in which the intraepithelial papillae were cut and removed with the basement membrane. These conditions may reflect differences in the level of heat degeneration and suggest that the basement membrane is almost strong enough to withstand ablation.

In nearly half of the cases in our study, the basement membrane was not cut, even at the vertically rising portions corresponding to the intraepithelial papillae. This suggests that the basement membrane is significantly stronger than is the adhesion of the squamous cells above the basal cell layer. These observations provide collateral evidence of the likely spread of esophageal cancer into the adjacent epithelium.

The limitations associated with this study include its small sample size, retrospective design, and lack of controls. Despite these limitations, our results suggest that APC-SEA is a safe, reliable, and easy-to-perform method of tumor control. However, it makes the pathophysiological diagnosis of EESCC impossible and is less effective than ESD in terms of radical correction

of EESCC. Therefore, APC-SEA cannot replace ESD as the standard treatment for EESCC.

However, APC-SEA has the potential to be applied in cases that are difficult to treat with ESD. APC-SEA is also a reliable method for use in facilities with less experience in performing ESD.

Conclusion

The basal cell layer containing carcinoma *in situ* was exposed homogeneously on most of the surface of the esophagus just before the second ablation procedure. A second ablation procedure was found to be essential for treating EESCC.

Disclosures

Yuichiro Kume, Kenro Kawada, Takuya Okada, Akihiro Hoshino, Yutaka Tokairin, Yasuaki Nakajima, Takashi Ito, Yusuke Kinugasa and Tatsuyuki Kawano have no conflicts of interest or financial ties to disclose.

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