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Expression of coproporphyrinogen oxidase is associated with detection of upper gastrointestinal carcinomas by 5-aminolevulinic acid-mediated photodynamic diagnosis

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ABSTRACT

Background: 5-Aminolevulinic acid is a precursor of photosensitizing protoporphyrin IX and has been applied for photodynamic diagnosis of brain and bladder tumors with few side effects. Although most upper gastrointestinal tumors can be detected during photodynamic diagnosis, some tumors containing signet-ring cells cannot be visualized. Here, we aimed to assess whether proteins involved in the absorbance, activation, and turnover of protoporphyrin IX altered the fluorescence signal in gastric cancer.

Methods: Aminolevulinic acid-mediated photodynamic diagnosis was performed in 23 lesions from 20 patients using an endoscope equipped with a blue laser light that caused red fluorescence emission of photosensitizing protoporphyrin IX. Red fluorescence signal and intensity was assessed during photodynamic diagnosis procedures. Lesions were resected by endoscopic and/or laparoscopic surgery, and specimens were immunostained and assessed for the expression of ATP-binding cassette sub-family G member 2, oligopeptide transporter-1, and coproporphyrinogen oxidase.

Results: Photodynamic diagnosis was negative in four cases (17.4%). Three cases of photodynamic diagnosis-negative lesions were signet-ring cell carcinomas, and only one case was differentiated adenocarcinoma (intestinal type). Twenty intestinal type, photodynamic diagnosis-positive lesions showed high expression of coproporphyrinogen oxidase, whereas signet-ring cell carcinomas were all negative. Oligopeptide transporter-1 immunoreactivity was significantly higher in tumors of intestinal type. ATP-binding cassette sub-family G member 2 expression tended to be higher in luminal surface tumors than in intestinal type tumors.

Conclusion: Aminolevulinic acid-mediated photodynamic diagnosis provided good detection of upper gastrointestinal tumors of intestinal type but not diffuse type tumors, such as signet-ring cell carcinomas, possibly owing to coproporphyrinogen oxidase expression.

1. Introduction

Upper gastrointestinal tumors, including gastric cancer, are one of the leading causes of cancer-related deaths worldwide [1]. Gastric cancer of intestinal type is associated with *Helicobacter pylori* infection [2]. Although *H. pylori* has been eradicated in many regions worldwide, the incidence of gastric cancers remains high [1,2]. Resection is still the only curative treatment; however, most patients cannot undergo curative resection because early cancers are sometimes difficult to detect, and cancers are usually diagnosed at an advanced stage [1]. Therefore, it is necessary to develop novel diagnostic modalities for identifying tumors at an early stage.

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Previous experience by our group has shown that 5-aminolevulinic acid-mediated photodynamic diagnosis can be useful for detection of upper gastrointestinal tumors [3]. Photodynamic diagnosis is an imaging technology utilized in the field of brain tumors and bladder tumors [4,5]. During photodynamic diagnosis, following administration of photosensitive drugs, metabolites as luminescent substances selectively accumulate in malignant tumors and cause drug-induced fluorescence by irradiation [4–6]. 5-Aminolevulinic acid is a natural amino acid that is metabolized to protoporphyrin IX in the heme biosynthesis pathway [7]. Exogenously administered aminolevulinic acid increases the intracellular levels of photosensitizing protoporphyrin IX, resulting in stronger emission of red fluorescence at around 635 nm in certain lesions than in surrounding tissues, with blue light excitation at around 405 nm [3,5,8,9]. Nakamura et al.[10] reported the usefulness of aminolevulinic acid-mediated photodynamic diagnosis with an irradiation probe for gastric cancers. Additionally, Isomoto et al. reported the efficacy of a novel endoscopic system for aminolevulinic acid-mediated photodynamic diagnosis to detect upper gastrointestinal tumors [3]. However, some types of tumors do not exhibit red fluorescence emission. In particular, all gastric cancers of diffuse type, such as signet-ring cell carcinoma, show no emission.

Coproporphyrinogen III oxidase is a synthetic enzyme involved in the sixth step of porphyrin metabolism in the mitochondria. This enzyme mediates the oxidative decarboxylation of coproporphyrinogen III to proto-porphyrinogen IX [11]. Therefore, coproporphyrinogen oxidase and other key molecules may regulate the intracellular levels of photosensitizing protoporphyrin IX.

Accordingly, in this study, we aimed to detect the factors that cause differences in red fluorescence in aminolevulinic acid-mediated photodynamic diagnosis.

2. Materials and methods

2.1. Patient population and selection

A total of 23 upper gastrointestinal tumors in 20 consecutive patients who underwent aminolevulinic acid-mediated photodynamic diagnosis and conventional upper gastrointestinal endoscopy at Nagasaki University Hospital from December 2013 to November 2014 were included in this study. The tumors consisted of 22 gastric lesions, including gastric cancers and adenomas. One tumor was Barrette's esophageal cancer at the esophageal-gastric junction. The patients included 13 men and seven women. The median age of patients was 72 years (range, 42–84 years). Twenty tumors were from 17 patients treated with endoscopic submucosal dissection, whereas three were from patients who underwent laparoscopic surgery. The endoscopic submucosal dissection procedure was chosen in accordance with Japanese inclusion criteria, as described previously [12]. One patient underwent surgery after endoscopic submucosal dissection because the results were discordant with the criteria set for the invasion depth by pathological diagnosis. Two cases were of signet-ring cell carcinoma, which did not meet the endoscopic submucosal dissection criteria, and these patients underwent surgery with removal of lymph nodes. Adverse events related to aminolevulinic acid administration and photodynamic diagnosis procedures were assessed in each patient, and laboratory data were examined between before and after photodynamic diagnosis. This study was approved by Nagasaki University Hospital Ethics Committee (approval no. 11032827), and written informed consent was obtained from all patients before the procedure. The procedures used in this study were in accordance with the Declaration of Helsinki.

2.2. Procedure for aminolevulinic acid-mediated photodynamic diagnosis

From 3–6 h before photodynamic diagnosis, aminolevulinic acid (Cosmo Bio Co., Tokyo, Japan) in water was administered orally at a dose of 20 mg/kg, which is identical to the dose employed for brain tumors [4]. Patients were shielded from strong light, such as direct sun light, for 24 h to avoid phototoxic reactions. A novel endoscopic system (Sie-P1; Fuji Film Medical Co., Tokyo) was developed for *in vivo* fluorescence detection of photosensitizing protoporphyrin IX accumulation. The system consisted of a processor (VP-0001), a light source (LL-4450-P1), and a scope (XG-0002-P1) and enabled the blue light excitation of photosensitizing protoporphyrin IX to emit red fluorescence. This photodynamic diagnosis system could instantly switch between the blue light mode for fluorescent navigation and the white light mode for conventional observation. The endoscope (XG-0002-P1) was developed as prototype for aminolevulinic acid-mediated photodynamic diagnosis and is not currently commercially available. The lesions were identified using white light endoscopy, and the tumor extent was then successively demarcated using chromoendoscopy with indigo-carmine solution. Aminolevulinic acid-mediated photodynamic diagnosis was performed, and the concordance of diagnosis was determined. Each patient was sedated by intravenous injection of diazepam and/or pethidine before and during photodynamic diagnosis.

2.3. Immunohistochemistry and scoring

Immunohistochemical staining was carried out using antibodies targeting oligopeptide transporter-1, ATP-binding cassette sub-family G member 2, ferrochlelatase, porphobilinogen deaminase, and coproporphyrinogen oxidase. However, the antibodies for ferrochelatase and porphobilinogen deaminase proved to not be useful because inflammatory cells were stained stronger than epithelial cells, including tumor cells. Therefore, the other three targets were evaluated, as described below.

Immunohistochemical analysis was performed on 23 specimens from 20 patients who underwent photodynamic diagnosis and surgical therapy, i.e., endoscopic submucosal dissection or operation. After deparaffinization, antigen retrieval was performed with KN9 reagent (KN-09001; Pathology Institute Corp., Japan) at 95 °C for 40 min. Peroxidase activity was subsequently blocked with 3% H_2O_2 in methanol at room temperature for 10 min. The sections were then washed with distilled water and equilibrated at room temperature for 5 min with KN buffer (IN 09002; Pathology Institute Corp.). All sections were incubated for 20 min with normal horse serum to eliminate nonspecific staining and were incubated with anti-human coproporphyrinogen oxidase polyclonal antibodies (dilution, 1:200; ProteinTech Group, Chicago, IL, USA), anti-human oligopeptide transporter-1 polyclonal antibodies (dilution, 1:400; ProteinTech Group), and anti-human ATP-binding cassette transporter ATP-binding cassette sub-family G member 2 polyclonal antibodies (dilution, 1:200; ProteinTech Group). This step was followed by incubation with secondary antibodies (1:1000; Envision + Dual Link HRP labeled polymer; Dako, Denmark) for 30 min. All sections were then incubated in diaminobenzidine (Dako) diluted in distilled water. Finally, the sections were counterstained with hematoxylin for 3 min. The intensity of staining was independently assessed by two pathologists and two endoscopists and then scored as follows: 0 = negative, 1 = moderate, positive with minimal to moderate immunoreactivity; 2 = strong, strongly positive with intense immunoreactivity. The positive (scores of 1 or 2) criteria were based on comparisons with staining of fundic glands or crypt epithelium in nontumor areas. Concordance rates among evaluators were evaluated with Kendall W tests, and the average scores were calculated.

2.4. Histopathological evaluation

Clinicopathological features of cancer cases were classified and recorded in accordance with the third edition of the Japanese Classification of Gastric Carcinoma, which has been widely used in Japan and the other countries [13]. The location of tumors was

classified into upper, middle, and lower thirds of the stomach. The macroscopic tumor type was identified as elevated or flat/depressed type. Histology was classified into differentiated adenocarcinoma (well or moderately differentiated adenocarcinoma or papillary adenocarcinoma) or undifferentiated adenocarcinoma (poorly differentiated adenocarcinoma or signet-ring-cell carcinoma). Differentiated type was almost equal to intestinal type; undifferentiated type was equal to diffuse type. The tumor sizes, tumor invasion depth, and the presence of ulcerative changes, lymphatic infiltration, and vascular infiltration were examined by independent pathologists.

2.5. Statistical analysis

Fisher's exact test, chi-squared tests, Mann-Whitney *U* tests, Student's *t*-tests, and Kendall's coefficient of concordance were employed as appropriate. Results with p values of less than 0.05 were considered statistically significant.

3. Results

3.1. Aminolevulinic acid-mediated photodynamic diagnosis for fluorescence detection of upper gastrointestinal tumors

As we reported previously [4], laser-equipped endoscopy via switching to the blue light excitation mode could be used for detection of upper gastrointestinal tumors with red fluorescence emission during ongoing endoscopic procedures. Table 1 shows the clinicopathological characteristics of subjective tumors with respect to photodynamic diagnosis results. When the red fluorescence signal was visualized and confined to the tumors but not the surrounding nontumorous tissue, the tumor was referred as photodynamic diagnosis-positive. Although intestinal metaplasia showed slight fluorescence, the fluorescence of these lesions was significantly weaker than that of tumors. Among the 23 lesions, 19 were photodynamic diagnosis-positive. All the intestinal type (differentiated type) adenocarcinomas including Barrett's adenocarcinomas, except for a minute well-differentiated intramucosal adenocarcinoma measuring 6 mm in size and two adenomas, were clearly identified by photodynamic diagnosis. In contrast, the three signet-ring cell carcinomas (undifferentiated type or diffuse type on macroscopic findings) showed no red fluorescent signal (photodynamic diagnosis-negative). There were two differentiated-type cases that exhibited a mixed poorly differentiated component, whereas no signet-ring cell carcinomas were mixed with intestinal type tumors. Mixed cases were all positive in photodynamic diagnosis. With regard to tumor size, photodynamic diagnosis-positive tumors were significantly larger in diameter than negative tumors ($p = 0.0001$). In contrast, the four photodynamic diagnosis-negative lesions were smaller and consisted of three undifferentiated and one small differentiated type.

Table 1
Total patients' characteristics.

	Patients (n = 20) Lesions (n = 23)
Age (year, range)	72 (42–84)
Gender (male:female)	14:9
Tumor location	
Upper third	2
Middle third	11
Lower third	9
Barrett's esophagus	1
Tumor size (mm)	23(6–35)
Macroscopic finding	
Elevated	9
Flat/Depressed	14
Depth of tumor invasion	
Intramucosal	17
Submucosal	4
Adenoma	2
Lymph node metastasis, positive	1
Stage (IA:IB)	22:1
Histopathology	
Differentiated	18
Undifferentiated	3
Adenoma	2
Lymphatic invasion, positive	1
Ulcerative changes, positive	3

Clinicopathological characteristics of 23 tumors in 20 patients.

Factors associated with photodynamic diagnosis positivity were analyzed using logistic regression analysis. However, there were no significant factors identified from multivariate analysis.

Figs. 1 and 2 show two representative cases. The photodynamic diagnosis-positive case shown in Fig. 1 was a 56-year-old man with a macroscopic type IIc tumor at the greater coverture. The tumor was observed with conventional chromoendoscopy with indigo-carmine on the white light mode of XG-0002-P1. The lesion was relatively detectable at the oral side, whereas the anal side was not as clear. The aminolevulinic acid-mediated photodynamic diagnosis system clearly showed intense and diffuse red fluorescent signal consistent with the lesion *in vivo*. Complete *en bloc* endoscopic submucosal dissection was performed for the lesion in *en bloc* manner. The tumor was diagnosed as differentiated type, but invaded into the submucosal layer with venous invasion and was not concordant with the criteria for curative resection; thus, the patient underwent an additional operation. Fig. 2 shows a photodynamic diagnosis-negative case. Aminolevulinic acid-mediated photodynamic diagnosis was carried out for a macroscopic type IIc tumor in a 51-year-old man. The lesion was observed as an unclear, depressive, pale lesion using the white light mode of XG-0002-P1. Aminolevulinic acid-mediated photodynamic diagnosis with blue laser light revealed no emission of red fluorescence. The biopsy showed that this tumor was a tiny signet-ring cell carcinoma; therefore, endoscopic submucosal dissection was performed. The histological result was concordant with the criteria for curative resection.

3.2. Immunostaining analysis of specimens

Tables 2 and 3 show the intensities of immunostaining. Twenty differentiated type, photodynamic diagnosis-positive specimens showed high expression of coproporphyrinogen oxidase, whereas signet-ring cell carcinomas were all negative (concordance rate: $k = 1$; $p < 0.001$). Oligopeptide transporter-1 immunoreactivity was higher in tumors of differentiated type, but the concordance rate of the score was relatively low ($P < 0.05$, Kendall W test: 0.74295). The total score of ATP-binding cassette sub-family G member 2 was not significantly different between intestinal type and signet-ring cell carcinoma. We next analyzed the expression of ATP-binding cassette sub-family G member 2 for the luminal side of the cell membrane (luminal surface), which could be related more significantly to exportation of ATP-binding cassette sub-family G member 2 expression was higher in the luminal surface of tumors in intestinal type tumors than signet-ring cell carcinoma($P < 0.05$, Kendall W test: 0.70918). Taken together, these findings suggested that coproporphyrinogen oxidase expression may be related to photodynamic diagnosis positivity in upper gastrointestinal tumors and that the localization of ATP-binding cassette subfamily G member 2 may be an important factor Fig. 3–5 .

4. Discussion

We previously demonstrated the efficacy of aminolevulinic acid-mediated photodynamic diagnosis for upper gastrointestinal tumors in patients undergoing endoscopy with a novel endoscopic system [3]. This novel system was developed based on the LASEREO video endoscopic system, which uses a semiconductor laser light source (Fuji Film Medical Co.) [14]. When used in the blue light mode, the laser

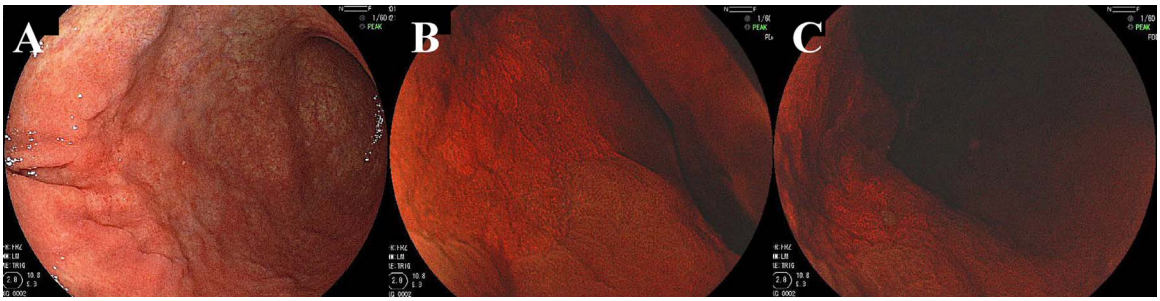

Fig. 1. A case of a 56-year-old man with a macroscopic type IIc tumor in the body of the stomach. A: Features of white light visualization. B: After direct spraying of indigo carmine dye, though the lesion was more detectable, the demarcation line was difficult to visualize. C: During aminolevulinic acid-mediated photodynamic diagnosis, the boundary of the lesion was clear, with strong red fluorescence.

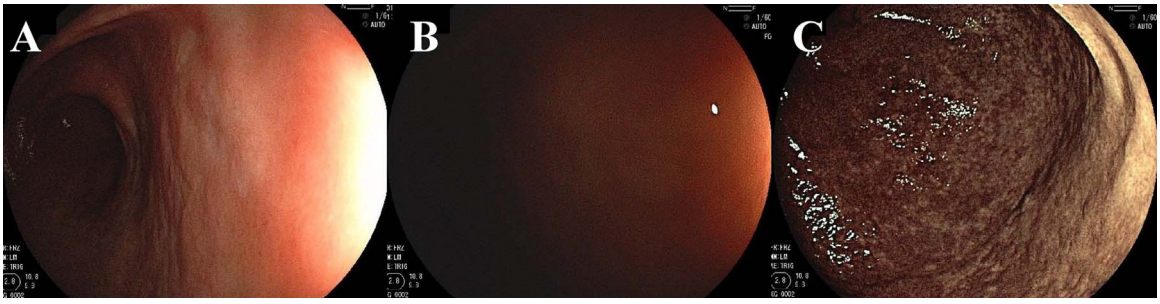

Fig. 2. A case of a 51-year-old man with a macroscopic type IIc tumor in the body of the stomach. A: Features of white light visualization. The lesion was slightly depressed, and the color was pale. The demarcation line was unclear. B: During aminolevulinic acid-mediated photodynamic diagnosis, the boundary of the lesion was unclear, and there was no red fluorescence. C: The lesion was also unclear when visualized in FICE mode.

Table 2
Patients' characteristics who underwent ALA-PDD.

	PDD-positive	PDD-negative	p-value
	(n = 19)	(n = 4)	
Age (year, range)	76 (42–84)	63(51–70)	NS
Gender (male:female)	12:7	2:2	NS
Tumor location			
Upper third	2	0	NS
Middle third	9	2	
Lower third	7	2	
Barrett's esophagus	1	0	
Tumor size (mm)	23.21 (6–35)	8 (6–10)	0.0001
Macroscopic finding			
Elevated	9	0	NS
Flat/Depressed	10	4	
Depth of tumor invasion			
Intramucosal	14	3	NS
Submucosal	3	1	
Adenoma	2	0	
Lymph node metastasis, positive	1	0	NS
Stage (IA:IB)	18:1	4:0	NS
Histopathology			
Differentiated	17	1	0.0001
Undifferentiated	0	3	
Adenoma	2	0	
Lymphatic invasion, positive	1	0	NS
Ulcerative changes, positive	2	1	NS

Clinicopathological characteristics of 23 tumors in 20 patients with who underwent 5-amilevulinic acid (ALA)-mediated photodynamic diagnosis (PDD). NS; not significant.

Table 3
Scoring of Immunostaining: CPOX and PEPT1.

Tumor type	PEPT1	CPOX
Differentiated type adenocarcinoma	2	2
Differentiated type adenocarcinoma	2	2
Differentiated type adenocarcinoma	1	2
Differentiated type adenocarcinoma	1	2
Differentiated type adenocarcinoma	1.75	2
Differentiated type adenocarcinoma	1.25	2
Differentiated type adenocarcinoma	2	2
Differentiated type adenocarcinoma	2	2
Differentiated type adenocarcinoma	1.5	2
Differentiated type adenocarcinoma	2	2
Differentiated type adenocarcinoma	1.25	2
Differentiated type adenocarcinoma	1.75	2
Differentiated type adenocarcinoma	1	2
Differentiated type adenocarcinoma	2	2
Differentiated type adenocarcinoma	2	2
Differentiated type adenocarcinoma	1	2
Differentiated type adenocarcinoma	2	2
Differentiated type adenocarcinoma	1.25	2
signet-ring cell carcinoma	0.25	0
signet-ring cell carcinoma	1.25	0
signet-ring cell carcinoma	0	0
Adenoma (intestinal type)	1.25	2
Adenoma (intestinal type)	1.5	2

PEPT1: Kendall W = 0.74295.
CPOX: Kendall W = 1.

activates the emission of red fluorescence, which passes through the selected cut-filter, allowing imaging of the signal *in vivo*. This is an all-in-one type endoscope equipped with a laser light source inside, enabling a switch to the blue light excitation mode during ongoing endoscopy. It is not necessary to insert the probe; thus, we could perform chromoendoscopy with direct spraying of indigo charmine. As we demonstrated in this study, almost all intestinal type upper gastrointestinal tumors, including differentiated adenocarcinoma and adenoma, were positive in aminolevulinic acid-photodynamic diagnosis. However, we encountered four photodynamic diagnosis-negative cases; three cases were diffuse type gastric cancers, and one case was differentiated type but small, measuring 6 mm in diameter. Therefore, this result showed the diagnostic ability of aminolevulinic acid-mediated photodynamic diagnosis for detecting differentiated type tumors, but not undifferentiated adenocarcinomas Table 4.

The underlying mechanism associated with differential accumulation of aminolevulinic acid-induced photosensitizing protoporphyrin IX remains uncertain [15–17]. However, this result could reflect the

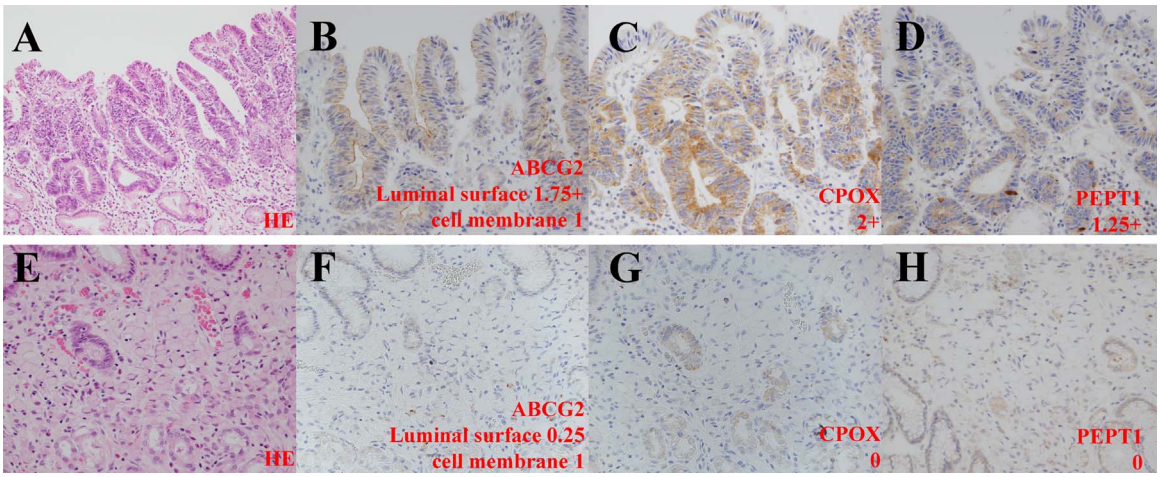

Fig. 3. Immunohistochemical analysis with antibodies targeting coproporphyrinogen oxidase, oligopeptide transporter-1, and ATP-binding cassette sub-family G member 2. Four doctors evaluated and scored each case. A: HE staining of a differentiated type lesion. B: Immunostaining of ATP-binding cassette sub-family G member 2 in a differentiated type lesion, scored as 2 at the luminal surface. Immunostaining of coproporphyrinogen oxidase in a differentiated type lesion, scored as 2. D: Immunostaining of oligopeptide transporter-1 in a differentiated type lesion; three doctors scored this staining as 2, and one doctor scored this staining as 1. E: HE staining of an undifferentiated type lesion. B: Immunostaining of ATP-binding cassette subfamily G member 2 in an undifferentiated type lesion. Tumor cells showed loss of polarity, and the score was low. C: Immunostaining of coproporphyrinogen oxidase in an undifferentiated type lesion; the score was 0. D: Immunostaining of oligopeptide transporter-1 in an undifferentiated type lesion; the score was 0.

diversity of histological types of cancers, e.g., differentiated type and undifferentiated type, particularly for signet-ring cell carcinoma.

5-Aminolevulinic acid is a natural amino acid synthesized from succinyl-CoA and glycine in the mitochondria [7], and exogenous 5-aminolevulinic acid is taken up through oligopeptide transporter-1 and converted into heme via several enzymatic reactions [11,17]. Some prior reports have shown that catalytic deficiency of ferrochelatase or higher activity of porphobilinogen deaminase may be related to greater production and retention of photosensitizing protoporphyrin IX [17–19], on the other hand, other reports have demonstrated that altered expression of peptide transporter oligopeptide transporter-1 and ATP-binding cassette transporter ATP-binding cassette sub-family G member 2 could affect intracellular photosensitizing protoporphyrin IX levels in gastric cancer cells *in vitro* [15,16]. Coproporphyrinogen oxidase catalyzes the oxidative decarboxylation of coproporphyrinogen III to proto-porphyrinogen IX in the mitochondria [11]. Our results showed that this protein was highly expressed in differentiated type tumors, with red strong fluorescence under photodynamic diagnosis. The expression of oligopeptide transporter-1 was also higher, suggesting that coproporphyrinogen oxidase and oligopeptide transporter-1

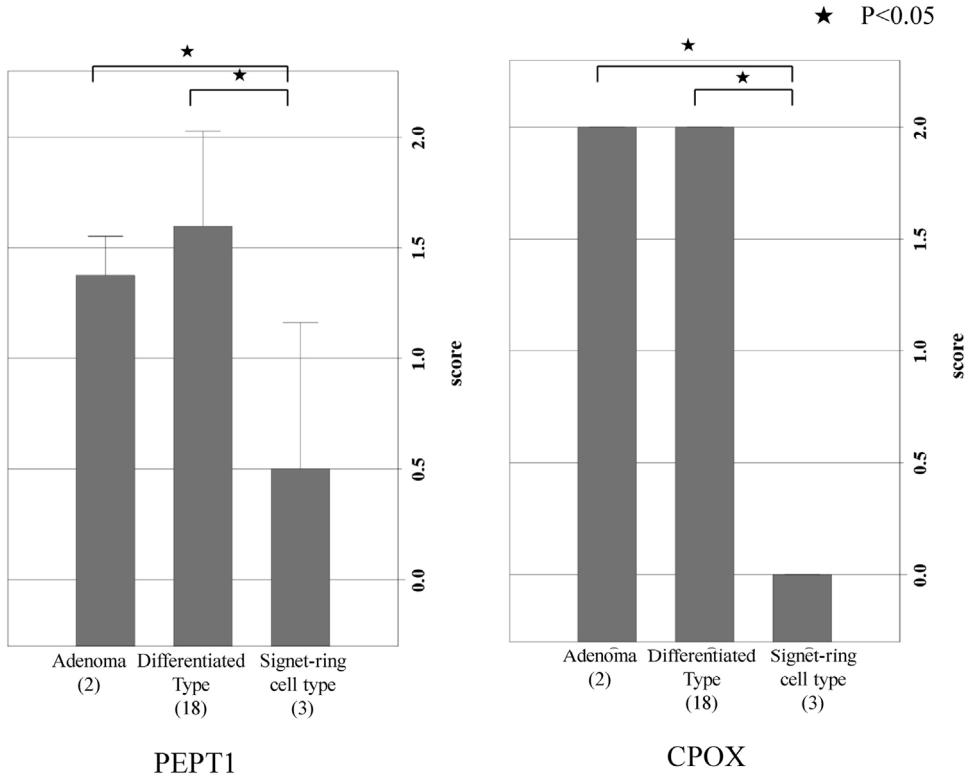

Fig. 4. Immunohistochemical analysis of oligopeptide transporter-1. Expression was significantly higher in differentiated type lesions than in signet-ring cell carcinoma. Twenty specimens that were differentiated type (all positive for photodynamic diagnosis) showed high expression of coproporphyrinogen oxidase, which is involved in the synthesis of photosensitizing protoporphyrin IX, whereas signet-ring cell carcinomas were all negative for coproporphyrinogen oxidase.

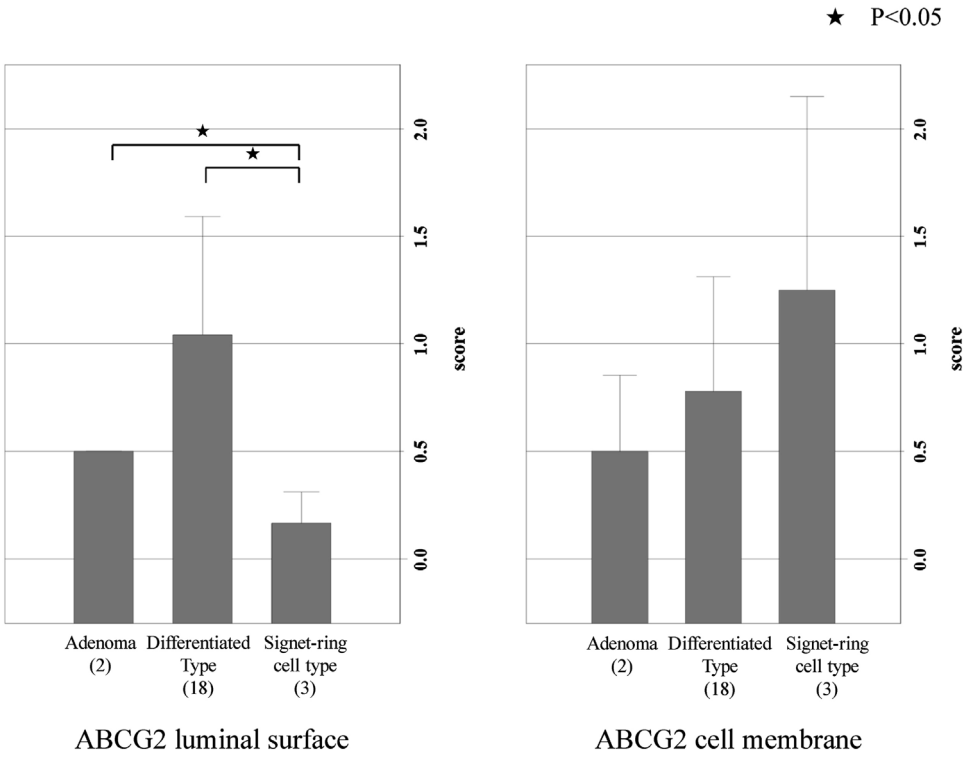

Fig. 5. Immunohistochemical analysis of ATP-binding cassette sub-family G member 2 expression. Expression was higher in the luminal surface in differentiated type lesions ($p < 0.05$, Kendall W test: 0.70918), whereas there was no significant difference in expression on the cell membrane (Kendall W test: 0.73143).

Table 4
Scoring of Immunostaining: ABCG2.

Tumor type	luminal surface	cell membrane
Differentiated type adenocarcinoma	1	1
Differentiated type adenocarcinoma	1	1
Differentiated type adenocarcinoma	2	0
Differentiated type adenocarcinoma	0	0
Differentiated type adenocarcinoma	1.25	0.25
Differentiated type adenocarcinoma	1.75	1.75
Differentiated type adenocarcinoma	0.75	0.25
Differentiated type adenocarcinoma	1.25	0.75
Differentiated type adenocarcinoma	0.5	0.75
Differentiated type adenocarcinoma	0.5	1.25
Differentiated type adenocarcinoma	0.25	1.5
Differentiated type adenocarcinoma	1.75	1.25
Differentiated type adenocarcinoma	0.5	0.75
Differentiated type adenocarcinoma	0.5	0.25
Differentiated type adenocarcinoma	1.25	1
Differentiated type adenocarcinoma	1	1
Differentiated type adenocarcinoma	2	0.5
Differentiated type adenocarcinoma	1.75	0
signet-ring cell carcinoma	0.5	1.25
signet-ring cell carcinoma	0.5	1.75
signet-ring cell carcinoma	0.25	2
Adenoma (intestinal type)	1.25	0.5
Adenoma (intestinal type)	0	0.25

PEPT1: Kendall W = 0.74295.
CPOX: Kendall W = 1.

could be related to the absorbance and accumulation of photosensitizing protoporphyrin IX. Although the expression of ATP-binding cassette transporter ATP-binding cassette sub-family G member 2 was observed in both types of lesions, loss of polarity was observed in undifferentiated type tumors; additional studies are needed to determine the meaning of this result. There were no significant differences between well-differentiated type and poorly differentiated type without signet-ring cells.

There were some limitations to this study. First, the number of diffuse type gastric cancer cases was only three, which was too small to demonstrate a significant difference. Secondly, one photodynamic diagnosis-negative case was differentiated type but was positive for coproporphyrinogen oxidase and oligopeptide transporter-1. Although the result may be related to the small size of the specimen, further studies are needed to determine the cause of this discrepancy.

In conclusion, our results showed that the expression of coproporphyrinogen oxidase and oligopeptide transporter-1 may be associated with the expression of photosensitizing protoporphyrin IX and photodynamic diagnosis positivity in upper gastrointestinal tumors. In the future, owing to the widespread of eradication of *H. pylori*, the ratio of diffuse type gastric cancers, such as signet-ring cell carcinoma, may increase. Thus, our findings may contribute to the development of aminolevulinic acid-mediated photodynamic diagnosis of upper gastrointestinal tumors, particularly diffuse type lesions, which are difficult to detect with conventional methods.

References

[1] J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, et al., Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012, Int. J. Cancer 136 (136) (2015) E359–E386.
[2] N. Uemura, S. Okamoto, S. Yamamoto, N. Matsumura, S. Yamaguchi, M. Yamakido, et al., *Helicobacter pylori* infection and the development of gastric cancer, N. Engl. J. Med. 345 (2001) 784–789.
[3] H. Isomoto, A. Nanashima, T. Senoo, K. Ogiwara, M. Hashisako, K. Ohnita, et al., In vivo fluorescence navigation of gastric and upper gastrointestinal tumors by 5-aminolevulinic acid mediated photodynamic diagnosis with a laser-equipped video image endoscope, Photodiagnosis Photodyn. Ther. 12 (2015) 201–208.
[4] T. Ishikawa, K. Takahashi, N. Ikeda, Y. Kajimoto, Y. Hagiya, S.I. Ogura, et al., Transporter-mediated drug interaction strategy for 5-aminolevulinic acid (ALA)-based photodynamic diagnosis of malignant brain tumor: molecular design of ABCG2 inhibitors, Pharmaceutics 3 (2011) 615–635.
[5] A. Lopez, J.C. Liao, Emerging endoscopic imaging technologies for bladder cancer detection, Curr. Urol. Rep. 15 (2014) 406.
[6] Y. Inoue, R. Tanaka, K. Komeda, F. Hirokawa, M.M. Hayashi, K. Uchiyama, Fluorescence detection of malignant liver tumors using 5-aminolevulinic acid-mediated photodynamic diagnosis: principles, technique, and clinical experience, World J. Surg. 38 (2014) 1786–1794.
[7] P. Hinnen, F.W. de Rooij, E.M. Terlouw, A. Edixhoven, H. van Dekken, R. van

Hillegersberg, et al., Porphyrin biosynthesis in human Barrett's oesophagus and adenocarcinoma after ingestion of 5-aminolevulinic acid, Br. J. Cancer 83 (2000) 539–543.
[8] M. Nakamura, J. Nishikawa, K. Hamabe, A. Goto, J. Nishimura, H. Shibata, et al., Preliminary study of photodynamic diagnosis using 5-aminolevulinic acid in gastric and colorectal tumors, World J. Gastroenterol. 21 (2015) 6706–6712.
[9] I. Kausch, M. Sommerauer, F. Montorsi, A. Stenzl, D. Jacqmin, P. Jichlinski, et al., Photodynamic diagnosis in non-muscle-invasive bladder cancer: a systematic review and cumulative analysis of prospective studies, Eur. Urol. 57 (2010) 595–606.
[10] T. Nakamura, T. Oinuma, H. Yamagishi, H. Masuyama, A. Terano, Evaluation of a novel high-resolution magnifying videoendoscope that is capable of photodynamic diagnosis and therapy for gastric cancer, Photodiagnosis Photodyn. Ther. 12 (2015) 115–122.
[11] J.C. Kennedy, R.H. Pottier, Endogenous protoporphyrin IX, a clinically useful photosensitizer for photodynamic therapy, J. Photochem. Photobiol. B 14 (1992) 275–292.
[12] H. Isomoto, S. Shikuwa, N. Yamaguchi, E. Fukuda, K. Ikeda, H. Nishiyama, et al., Endoscopic submucosal dissection for early gastric cancer: a large-scale feasibility study, Gut 58 (2009) 331–336.
[13] Japanese Gastric Cancer Association, Japanese classification of gastric carcinoma: 3rd English edition, Gastric Cancer 14 (2011) 101–112, http://dx.doi.org/10.1007/s10120-011-0041-5.
[14] N. Yoshida, N. Yagi, Y. Inada, M. Kugai, T. Okayama, K. Kamada, et al., Ability of a novel blue laser imaging system for the diagnosis of colorectal polyps, Dig. Endosc. 26 (2014) 250–258.
[15] Y. Hagiya, Y. Endo, Y. Yonemura, K. Takahashi, M. Ishizuka, F. Abe, et al., Pivotal roles of peptide transporter PEPT1 and ATP-binding cassette (ABC) transporter ABCG2 in 5-aminolevulinic acid (ALA)-based photocytotoxicity of gastric cancer cells in vitro, Photodiagnosis Photodyn. Ther. 9 (2012) 204–214.
[16] H. Kobuchi, K. Moriya, T. Ogino, H. Fujita, K. Inoue, T. Shuin, et al., Mitochondrial localization of ABC transporter ABCG2 and its function in 5-aminolevulinic acid-mediated protoporphyrin IX accumulation, PLoS One 7 (2012) e50082.
[17] K. Inoue, H. Fukuhara, A. Kurabayashi, M. Furihata, M. Tsuda, K. Nagakawa, et al., Photodynamic therapy involves an antiangiogenic mechanism and is enhanced by ferrochelatase inhibitor in urothelial carcinoma, Cancer Sci. 104 (2013) 765–772.
[18] D. Ickowicz Schwartz, Y. Gozlan, L. Greenbaum, T. Babushkina, D.J. Katcoff, Z. Malik, Differentiation-dependent photodynamic therapy regulated by porphobilinogen deaminase in B16 melanoma, Br. J. Cancer 90 (2004) 1833–1841.
[19] S.L. Gibson, D.J. Cupriks, J.J. Havens, M.L. Nguyen, R. Hilf, A regulatory role for porphobilinogen deaminase (PBGD) in delta-aminolaevulinic acid (delta-ALA)-induced photosensitization? Br. J. Cancer 77 (1998) 235–242.