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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