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Selection of Patients by Membrane Transporter Expressions for Aminolevulinic Acid (ALA)-Guided Photodynamic Detection of Peritoneal Metastases

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Running Title; ALA-guided photodynamic diagnosis

ABSTRACT: Background. Photodynamic diagnosis (PDD) with 5-aminolevulinic acid (ALA) was used to detect peritoneal metastasis (PM). This study was done to verify the roles of the expressions of the peptide transporter PEPT1 and ATP-binding cassette transporter ABCG2 genes on the selection of patients in ALA PDD. **Methods.** The study group comprised 138 patients with PM. After oral administration of 5-ALA, PM was evaluated by PDD. Tissue protoporphyrin IX (PpIX) levels, and PEPT1/ABCG2 mRNA expressions were determined. **Results.** The tumor detection rate on PDD was 45.6% (63/138). PDD is a safe technique for the detection of PM from ovarian cancer,

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mesothelioma, and colorectal cancer. PpIX levels of ALA-PDD-positive PM were significantly higher than those of ALA-PDD-negative PM. The PpIX levels in PM that simultaneously expressed PEPT1 and ABCG2 mRNA were significantly higher than those in PM that expressed either PEPT1 or ABCG2 mRNA, as well as PM that expressed neither PEPT1 nor ABCG2 mRNA. PM with PEPT1 mRNA up-regulation showed simultaneous up-regulation of ABCG2 mRNA. PpIX accumulates in PM with PEPT1 up-regulation. At the same time, PpIX may be excreted into stromal tissue through ABCG2 transporter, resulting in the accumulation of excess PpIX in the stromal tissue near cancer cells. **Conclusions.-**Preoperative evaluations of the expressions of the PEPT1 and ABCG2 genes by RT-PCR methods might facilitate the selection of patients most likely to benefit from ALA-PDD.

Keywords: aminolevulinic acid; photosensitizer; photodynamic diagnosis; peritoneal surface malignancies; PEPT1; ABCG2, ferrochelatase

1. INTRODUCTION

Peritoneal metastasis (PM) has been considered a terminal disease, and palliative chemotherapy and/or best supportive care wass widely used for treatment. In the late 1990s, a comprehensive treatment combining cytoreductive surgery (CRS) with perioperative intraperitoneal chemotherapy was developed for selected patients with curative inten[1, 2, 3]. In the treatment, completeness of cytoreduction of PM is the most important predictors of survival. Therefore, the detection of small PM invisible on white light inspection before CRS is mandatory to perform complete cytoreduction. The recent interest in this field has resulted in the development of new techniques that enable better identification of tumor extension into the abdominal cavity and thereby improve the diagnosis of peritoneal seeding associated with PM.

Initially, photodynamic diagnosis (PDD) was proposed for the evaluation of peritoneal malignancy on the basis of animal models[4]. The detection of small PM was significantly enhanced by administration of 5-aminolevulinic acid (ALA) followed by fluorescence assessment in these studies[5]. ALA is a natural precursor of protoporphyrin IX (PpIX) and heme. After high-dose administration of ALA, PpIX accumulates in cancer cells and emits red fluorescence under violet light excitation at 405 nm[6]. Recently, PDD is used to detect PM from gastric cancer and serous carcinoma[7,8].

We previously reported that the peptide transporter PEPT1 (SLC15A1, ALA influx transporter) and ATP-binding cassette (ABC) transporter ABCG2 (porphyrin efflux transporter) genes play pivotal roles in the accumulation of PpIX in gastric cancer cell lines[9].

We now present the results of a prospective study evaluating PDD for the detection of PM from various primary sites. A major aim was to clarify the key molecules in PpIX accumulation after oral administration of ALA.

2. PATINTS AND METHODS

The study group comprised 138 patients with PM from 48 appendiceal mucinous neoplasms, 20 ovarian cancers, 7 mesotheliomas, 34 colorectal cancers, 5 pancreas cancers, and 24 gastric cancers (52 men and 86 women; mean age, 56.9 ± 11.9 years). The local ethics committee in our hospitals approved the study protocol, and written informed consent was obtained from all patients. Patients were informed about the adverse effects of

ALA-PDD, such as nausea, vomiting and skin photosensitivity in accordance with the Common Terminology Criteria for Adverse Events, version 4.0.

2.1 Fluorescence detection of peritoneal metastases, tumor sampling, and CRS

Each patient received 20 mg/kg body weight of 5-ALA (Cosmo Bio Co., Ltd, Tokyo, Japan) dissolved in 50–100 ml of orange juice. The mixture was given orally 2 hours before surgery. After treatment with 5-ALA, patients were kept away from direct sunlight for 24 hours.

After laparotomy, standard evaluations of the distribution and size of PM were done under white light. Tumor tissues and normal peritoneum were discriminated under white light. Then, all lights in the operation room were turned off, and PDD was performed using a xenon lamp (300 W) emitting violet light with a wavelength of 375-445 nm for fluorescence excitation, and the abdominal cavity was examined[5].

Two specimens were taken from red fluorescent-positive peritoneal nodules or from fluorescent-negative peritoneal nodules, and two specimens were collected from red fluorescent-negative normal peritoneum. The red fluorescence intensity of each specimen was evaluated and categorized, and the results were recorded. Half of each sampled specimen was examined histopathologically to confirm the presence of cancer cells. The obtained specimens were immediately stored at -80℃ in the dark for further analysis. All patients with PM received cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy[8].

2.2 ***High-performance liquid chromatographic (HPLC) analysis of protoporphyrin IX (PpIX)***

Samples were treated as described previously, with some modifications[9]. Porphyrins were then separated using an HPLC system (Type Prominence, Shimadzu, Kyoto, Japan) equipped with a reversed-phase C18 column (CAP-CELL PAK, C18, SG300, 5 □m, 4.6 mm × 250 mm; Shiseido, Tokyo, Japan)[9]. Porphyrins were continuously detected with a fluorospectrometer (excitation at 404 □m, detection at 624 nm). The porphyrin concentrations in samples were estimated from calibration curves obtained with standard porphyrins[9].

2.3 Reverse transcription-polymerase chain reaction (RT-PCR) analysis of mRNA expressions of PEPT1, ABCG2, and ferrochelatase in tumors and normal peritoneum

Total RNA was extracted from tissue samples using ISOGEN (Nippon Gene, Tokyo, Japan) according to the manufacturer's protocols, and first-strand cDNA was prepared from total RNA by reverse transcriptase reaction, as described previously[9]. Then, the first-strand cDNA preparations encoding PEPT1, ABCG2, ferrochelatase, and □-actin were individually amplified by PCR in a Thermal Cycler Dice Mini (TaKaRa Bio, Otsu, Japan). The sense and antisense primers of PEPT1 (NM_005073) are 5'-TGACGCCAATTCTCGGAGCTCTTATC-3', and 5'-CAGGAACATCACCCTCGTAACCATCT-3', and the product size is 665 bp.

The specific primer sets of ABCG2 (NM_004827) are 5'-TTCGGCTTGCAACAACTATGACGAA-3' (sense) and 5'-ATAGGCCTCACAGTGATAACCAGCTGATTC-3 (antisense)', with a product size of 425 bp.

The sense and antisense primers of ferrochelatase (NM_001012515) are 5'-CAACCGCAGAAGAGGTATGAGTCTAACATC-3',

and 5'-TGGGCTTCCGTCCCACTTGATTATAGTATC-3', and the product size is 490 bp.

□-actin (NM_001101) was used as the internal control, and the sense and antisense primers were 5'-GAAAATCTGGCACCACACCTT-3', and 5'-TTGAAGGTAGTTTCGTGGAT-3'. The product size is 591 bp.

The PCR reaction consisted of hot-start incubation at 95°C for 3 min and 33 cycles each of 94°C for 30 seconds, 62°C for 40 sec, and 72°C for 60 sec. The resulting amplicons were separated by 1.2% agarose gel electrophoresis and detected with ethidium bromide under ultraviolet light.

2.4 Immunohistochemical analyses

The expressions of PEPT1, ABCG2, and ferrochelatase in paraffin-embedded tumor or normal tissue sections were detected by immunohistochemical staining with the DAKO LSAB+system-HRP as described previously[13]. As primary antibodies, anti-PEPT1 rabbit polyclonal antibody H-235 (1:200 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-ABCG2 mouse monoclonal antibody BXP-21 (1:200 dilution; Convance Research 152 Products, Emeryville, CA), and anti-ferrochelatase antibody (C-20) (1:200 dilution; Santa Cruz Biotechnology) were used.

2.5 ***Statistical Analysis***

Data were collected for all patients included in the study. The primary outcome variable of statistical analysis was the metastatic lesions detected by PDD. For statistical analysis, the SPSS version 17.0 statistical software package (SPSS, Chicago, Ill) was used.

3. RESULTS

3.1 Diagnostic value of 5-ALA for PM and side effects

Typical examples of metastatic nodules on PDD are shown in Figures 1 and 2. Figure 1 shows PM from ovarian cancer. Figure 2 shows the PDD findings of metastasis from an appendiceal mucinous neoplasm to the small bowel mesentery. The stroma of the appendiceal mucinous neoplasm contained large amounts of mucinous material (Figure 3), which emitted strong red fluorescence on irradiation with violet light.

The diameter of the smallest PM detected by PDD was about 0.5 mm. The overall detection rate of PM by PDD was 44.2% (61/138 patients). PDD had high detection rates for metastatic nodules from ovarian cancer (95%, 19/20), pancreatic cancer (80%, 4/5), mesothelioma (71.4%, 5/7), and colorectal cancer (56.7%, 19/34). In contrast, red fluorescence was detected in only 7 (29.2%) of 24 gastric cancers and 7 (14.6%) of 48 appendiceal mucinous neoplasms.

Nausea and vomiting occurred as adverse effects after administration of 5-ALA in only 1 patient (0.7%). No patient had symptoms of porphyria. There was no postoperative mortality. Grade 3 or 4 complications occurred in 21 patients (15.2%). The complications were related to cytoreductive surgery and were not attributed to treatment with 5-ALA. The complication rate of cytoreductive surgery after ALA administration was lower than that reported previously[10].

3.2 Expressions of porphyrin biosynthesis pathway-related genes: PEPT1, ABCG2, and ferrochelatase mRNA-expression in ALA-PDD positive/negative specimens

Expressions of the PEPT1, ABCG2, and ferrochelatase genes were examined in peritoneal nodules from 73 patients. The mRNA expressions of the PEPT1, ABCG2, and ferrochelatase genes are shown according

to the ALA PDD fluorescence status in Figure 4.

Table 1 shows the correlation between PEPT1 mRNA expression and ALA PDD fluorescence status. Among 21 PEPT1 mRNA-positive tumors, 18 (85.7%) emitted red fluorescence. In contrast, 37 (71.2%) of the 52 PEPT1 mRNA-negative tumors showed no fluorescence on ALA PDD. The PEPT1 mRNA status significantly correlated with the ALA-PDD status (P<0.0001, Table 1).

Seventeen (68.0%) of the 25 PM that expressed ABCG2 mRNA showed positive red emission on ALA PDD. In contrast, 32 (66.7%) of the 48 PM without ABCG2 mRNA expression did not emit red fluorescence (P=0.002, Table 1).

Table 1 Correlation between PEPT1 and ABCG2 mRNA expression and ALA PDD fluorescence status.

		PDD negative	PDD positive	Total	
PEPT1 mRNA Expression	negative	37	15	52	P<0.0001
	Positive	3	18	21	
ABCG2 mRNA Expression	negative	32	16	48	P<0.002
	positive	8	17	25	
		40	33	73	

Sixteen (55.1%) of the 29 PM that expressed ferrochelatase mRNA showed positive red emission on ALA PDD. In contrast, 27 (61.6%) of the 44 PM without ferrochelatase mRNA expression did not emit red fluorescence. There was no correlation between ferrochelatase mRNA expression and ALA PDD fluorescence status (P=0.165) .

Seventeen (81.0%) of the 21 PEPT1 mRNA expression-positive tumors expressed ABCG2 mRNA. In contrast, 44 (84.6%) of the 52 PEPT1 mRNA expression-negative tumors did not express ABCG2 mRNA. PEPT1 mRNA expression correlated with ABCG2 mRNA expression (P<0.0001)

Next we studied the relation between ALA PDD status and PEPTI mRNA expression according to the ABCG2 mRNA expression status. In 48 PM negative for ABCG2 mRNA expression, red fluorescence was found on PDD in 15 (93.8%) of 16 PEPT1 mRNA expression-positive PM and in 1 (3.1%) of 32 PEPT1 mRNA expression-negative PM (P<0.0001).

In 25 PM for ABCG2 mRNA expression, red fluorescence was found on PDD in 15 (88.2%) of 17 PEPT1 mRNA expression-positive PM and in 2 (25.0%) of 8 PEPT1 mRNA expression-negative PM. There was a significant correlation between ALA-PDD status and PEPT1 mRNA expression (P=0.0036) in PM positive for ABCG2 mRNA expression.

Ferrochelatase mRNA was expressed by 20 (95.2%) of 21 PEPT1 mRNA expression-positive PM, and was not expressed in 43 (82.6%) of 52 PEPT1 mRNA negative nodules (P<0.0001).

Ferrochelatase mRNA was expressed by 25 (100%) of 25 ABCG2 mRNA expression-positive PM, and was not expressed in 44 (91.7%) of 48 PEPT1 mRNA negative nodules (P<0.0001).

In normal peritoneal tissues, the detection rates of PEPT1, ABCG2, and ferrochelatase mRNA expression were 25.0% (10/40), 47.5% (19/40), and 70.0% (28/40), respectively. In normal peritoneal tissues, 8 (80.0%) of 10 PEPT1 mRNA expression-positive specimens expressed ABCG2 mRNA. In contrast, 19 (63.3%) of 30 PEPT1 mRNA expression-negative normal tissue specimens did not express ABCG2 mRNA. PEPT1 mRNA expression was significantly related to ABCG2 mRNA expression (P=0.044). Ten (47.6%) of 21 normal peritoneum specimens without ABCG2 mRNA expression did not express ferrochelatase mRNA. In contrast, 18 (94.7%) of 19 normal peritoneum with ABCG2 mRNA expression expressed ferrochelatase mRNA (P<0.0001). However, there was no significant relation between PEPT1 mRNA expression and ferrochelatase mRNA expression in normal peritoneum.

3.3 PpIX concentrations in peritoneal tissue and PM

The PpIX level was significantly higher in PM (0.0079±0.0070 nm/mg-protein, N=73) than in normal peritoneum (0.0036±0.0024 nm/mg-protein, N=40) (P=0.0145). In the PM, the PpIX level of 33 ALA-PDD-positive nodules (0.0146±0.0212nm/mg-protein) was significantly higher than that of 40 ALA-PDD-negative nodules (0.0027±0.00191 nm/mg-protein) (P=0.0124). PpIX levels in PM of ovarian, pancreas, colorectal, gastric cancer, appendiceal neoplasm and mesothelioma were 0.0185±0.0017 (N=10), 0.0104±0.0108 (N=4), 0.0107±0.0009 (n=29), 0.0016±0.0017 (n=10), 0.0025±0.0016 (n=15) and 0.0156±0.0105 (n=5) nm/mg-protein, respectively.

3.4 ***PEPT1, ABCG2, and ferrochelatase mRNA expressions and PpIX levels in PM***

The PpIX level was significantly higher in PEPT1 mRNA expression-positive PM (0.0101±0.0082 nm/mg/protein) than in PEPT1 mRNA expression-negative PM (0.0023±0.0019 nm/mg/protein) (P=0.022). There was no significant difference in the PpIX level between ABCG2 mRNA expression-positive PM (0.0080±0.0078 nm/mg/protein) and ABCG2 mRNA expression-negative PM (0.0068±0.0075 nm/mg/protein).

The PpIX level did not differ significantly between ferrochelatase mRNA expression-negative PM (0.0067±0.0076) and ferrochelatase mRNA expression-positive PM (0.0079±0.0071 nm/mg/protein).

The PpIX level was 0.0059±0.0069 nm/mg-protein in ALA-PDD-negative PM without expression of ABCG2 mRNA and 0.0113±0.0082 nm/mg-protein in ALA-PDD-positive PM without expression of ABCG2 mRNA (NS).

In PM positive for ABCG2 mRNA expression, the PpIX level was significantly higher in ALA-PDD-positive PM (0.0098±0.0081 nm/mg-protein) than in ALA-PDD-negative PM (0.0019±0.0015 nm/mg-protein) (P=0.0095).

3.5 ***Immunohistochemical expressions of PEPT1, ABCG2 protein, and ferrochelatase***

Figures 5 show the PEPT1 immunoreactivity on PM from colorectal cancer (Figure 5). PEPT! and ABCG2 immunoreactivities were detected on the cell membrane, and ferrochelatase immunooreactivity was found on the cytoplasm of cancer cells.

4. DISCUSSION

Experimental and clinical studies have demonstrated that ALA PDD can detect PM from different cancers[5, 6, 8]. In gastric cancer, the accuracy of fluorescence imaging by laparoscopy was higher than that of white light imaging[7]. The false-positive rate on white light imaging was 14.2% (2/14), but no false-positive or false-negative results were obtained on fluorescence imaging[7]. Liu et al reported that the specificity of ALA PDD in 40 ovarian cancer specimens was 100%, and the false-negative rate was 7.5% in the 40 non-fluorescent areas[5]. That study provided promising results in the detection of metastatic nodules by ALA PDD.

Although the overall detection rate was 45.6% (63/138) in the present study, PDD enhanced the detection of metastatic nodules in most ovarian cancers (95.0%), mesotheliomas (71.4%) and pancreatic cancers (80%). In addition, metastatic tumor nodules were detected in 56.7% of PM from colorectal cancer. In PM from gastric cancer and appendiceal mucinous neoplasms, however, the detection rates by PDD were low. ALA PDD positivity depends on the tumor-specific accumulation of photosensitizing PpIX after the administration of ALA. The present study demonstrated that the PpIX levels in PM from gastric cancer and appendiceal mucinous neoplasms were significantly lower than those in PM from ovarian cancer, pancreas cancer, or mesothelioma, which had high positive detections rates on PDD. PM from gastric cancer and appendiceal mucinous neoplasms have abundant stromal tissue and mucinous material as compared with PM from ovarian cancer and mesothelioma. The amount of stromal tissue in PM nodules may be related to the PpIX level.

The present study demonstrated that ALA PDD could detect small tumors 0.5 mm in diameter, which could not be detected under white light. These results suggest that ALA-PDD is an accurate and reliable technique for the detection of small PM in selected patients.

On the contrary, PM in 75 (54.4%) of 138 cases did not emit red fluorescence on irradiation with violet light. False-negative results on ALA PDD have been reported in brain tumors and ovarian cancer[11, 12]. Red fluorescence emitted after irradiation with violet light on ALA PDD depends on the amount of photosensitizing PpIX.

The reasons why PpIX excessively accumulates in cancer cells remain unclear. The following two hypotheses have been proposed: 1) PpIX accumulates because of decreased ferrochelatase activity in cancer cells[11]. 2) ALA has a high affinity for malignant cells[12]. Kaneko et al. reported that the ferrochelatase activity in malignant glioma cells was lower than that in control cells[13]. In control brain cells, a large amount of PpIX is biosynthesized in mitochondria, and an excessive amount of PpIX is metabolized into heme by ferrochelatase, resulting in a decreased PpIX level. In contrast, PpIX accumulates in glioma cells because such cells have significantly decreased ferrochelatase activity. In bladder cancer, Hagiya et al. reported that ferrochelatase was down-regulated, and the PpIX level of tumor tissue increased (17).

Intrinsic ALA is synthesized from succinyl-CoA and glycine by ALA synthase, and ALA synthase is controlled by a heme-regulated feedback mechanism. In the heme synthesis pathway, ALA is converted to porphobilinogen by ALA dehydratase as the rate-limiting enzyme and is metabolized to heme by 7 processes. After excess administration of ALA, however, intracellular PpIX synthesis increases without intervention by the feedback mechanism. In cells that

express ferrochelatase, PpIX is rapidly metabolized into heme by ferrochelatase as the rate-limiting enzyme.

We recently reported that PEPT1 and ABCG2 are key players in regulating intracellular PpIX levels. In our *in vitro* study using gastric cancer cell lines, up-regulation of PEPT1 (ALA influx transporter) gene and down-regulation of the ABCG2 (porphyrin efflux transporter) gene were suggested to play pivotal roles in ALA-induced tumor-specific PpIX accumulation.

The oligopetide transporters (i.e., PEPT1) are reportedly involved in the cellular uptake of ALA, coupled with the co-transporter of H^+/H_3O^+ [12, 14]. The present study also demonstrated that a significant increase in PpIX levels in PM was associated with up-regulation of PEPT1, and that PEPT1 mRNA-positive tumors had higher PpIX levels.

ABCG2 is a known ALA efflux transporter [15, 16]. Accordingly, PpIX accumulation in bladder cancer strongly correlates with the PEPT1 up-regulation and ABCG2 down-regulation[13]. In contrast to bladder cancer[13] and brain tumor[12], the present study showed that simultaneous upregulation of the PEPT1 and ABCG2 genes have a significant role in the PpIX contents in PM tissues.

After an excess amount of ALA is administered, ALA is transported into cancer cells through PEPT1. Then, the heme synthesis pathway is activated by ALA dehydratase, which is a rate-limiting enzyme, and the intracellular PpIX levels increase. The present study showed that most PM with overexpression of PEPT1 had up-regulated expression levels of ferrochelatase and ABCG2 mRNA. Accordingly, a part of PpIX may be rapidly metabolized to heme by ferrochelatase. At the same time, PpIX might be excreted from cancer cells into the stromal tissue through ABCG2 transporter, resulting in the accumulation of excess PpIX in stromal tissue near cancer cells. Typical findings were observed in the case of an appendiceal mucinous neoplasm that expressed ABCG2 and PEPT1 mRNA (Figure 2). In this case, PpIX that had accumulated in mucinous material emitted strong red fluorescence on PDD. Unlike brain tumors[11], PpIX accumulation in PM nodules apparently does not depend on the ferrochelatase activity, becasuse no relation was found between ferrochelatase expression and the PpIX level..

These results suggest that up-regulation of PEPT1 and ABCG2 gene expression increases the PpIX levels in PM tissue. However, 1 (25%) of 4 ALA-PDD-positive PM did not express either PEPT1 or ABCG2 mRNA, and 2 (11.8%) of 17 ALA-PDD-negative PM simultaneously expressed PEPT1 and ABCG2 mRNA. Other unknown influx or efflux transporters of ALA may be related to the PDD status in such cases.

In normal peritoneal tissues, no red fluorescence was emitted on irradiation with violet light. The PpIX level in normal peritoneal tissue was significantly lower than that in PM. Accordingly, the PpIX levels in normal peritoneal tissues are not high enough to emit red fluorescence on exposure to violet light. In normal peritoneal tissues, the detection rates of PEPT1, ABCG2, and ferrochelatase mRNA expression were 25.0% (10/40), 47.5% (19/40), and 70.0% (28/40), respectively. PpIX synthesized from ALA might be excreted into the peritoneal cavity by ABCG2 transporter and then be metabolized to heme by ferrochelatase, resulting in low PpIX levels in the normal peritoneum.

The present study confirmed that the oral administration of ALA is associated with very few adverse reactions because ALA is an intrinsic molecule and is rapidly

metabolized to PpIX through the porphyrin/heme pathway.

In conclusion, ALA-PDD-guided surgery may improve the complete cytoreduction rate by facilitating the detection of small PM nodules and may reduce the risk of recurrence after complete cytoreduction of PM. PDD is safe and feasible for the detection of PM from ovarian cancer, mesothelioma, and colorectal cancer. Simultaneous expression of the PEPT1 and ABCG2 genes might play a pivotal role in the accumulation of PpIX in cancer tissues. Preoperative examinations to assess the expressions of the PEPT1 and ABCG2 genes by histological or RT-PCR methods may therefore facilitate the selection of patients most likely to benefit from ALA-PDD.

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FIGURE LEGENDS

Figure 1: Peritoneal metastasis from ovarian cancer emitted strong red fluorescence, under irradiation of violet light of 405 nm wave length.

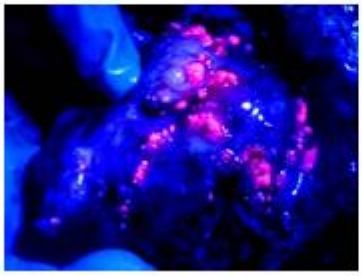

Figure 1. Peritoneal metastasis from ovarian cancer emitted strong red fluorescence on irradiation with violet light.

Figure 2: Metastasis from appendiceal mucinous neoplasm on the small bowel mesentery emitted strong red fluorescence under irradiation of violet light. Mucinous material shows strong red fluorescence..RT-PCR examination demonstrated expression of PEPT1 and ABCG2 mRNA.

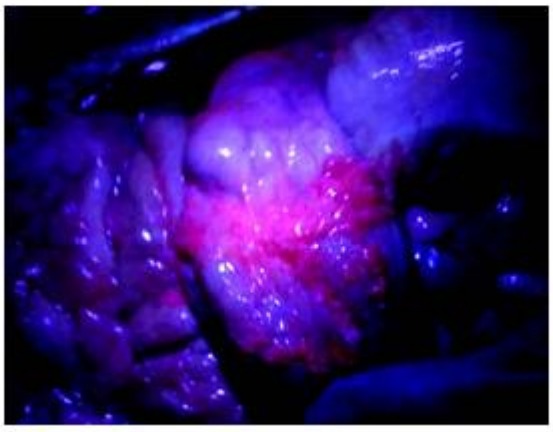

Figure 2. Metastasis from appendiceal mucinous neoplasm on the small bowel mesentery emitted strong red fluorescence on irradiation with violet light. Mucinous material shows strong red fluorescence. RT-PCR examination demonstrated expression of PEPT1 and ABCG2 mRNA.

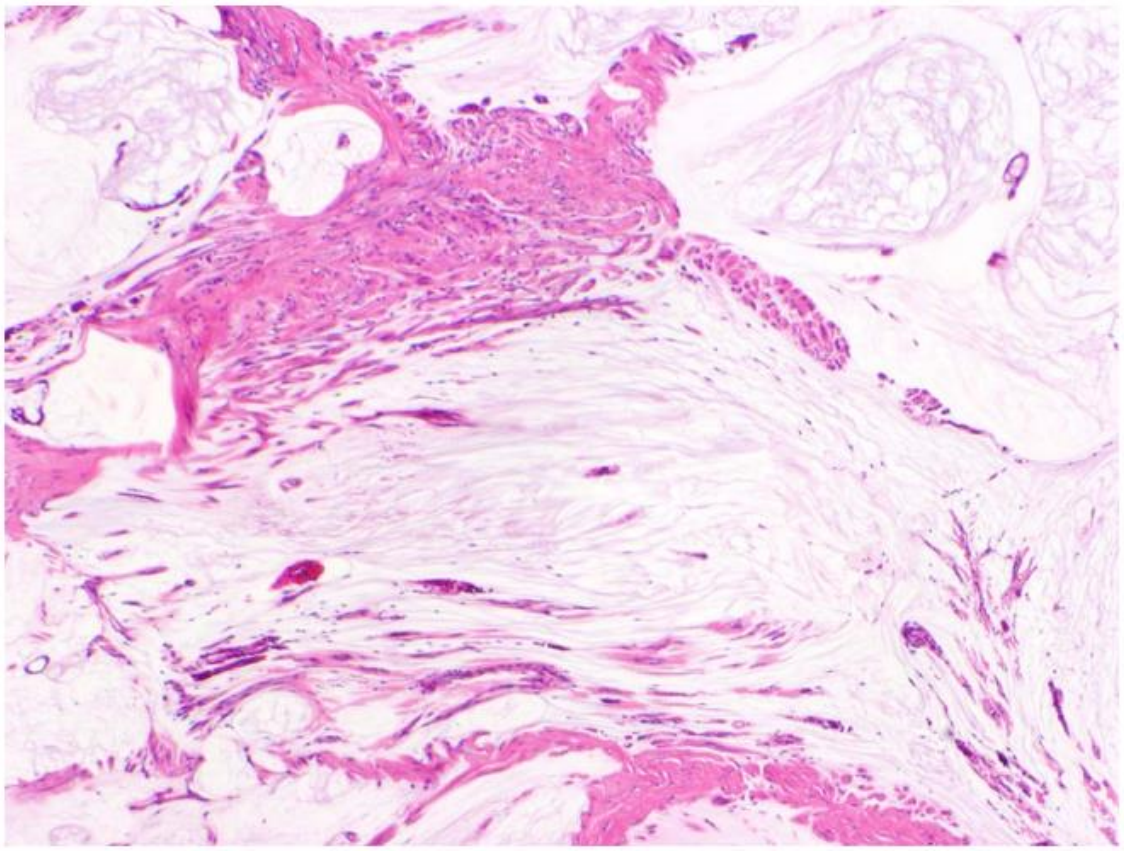

Figure 3. Histologic findings of the appendiceal mucinous neoplasm in Figure 1B (hematoxylin and eosin stain). Mucinous material occupies stromal tissue.

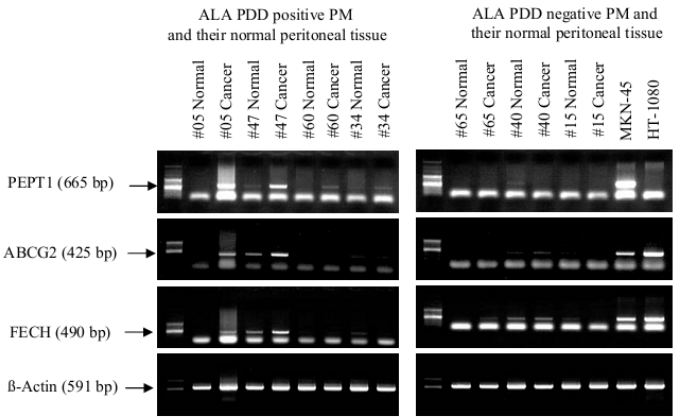

Figure 4: RT-PCR analysis of mRNA expression in PMP tissues and their normal peritoneal tissues
The mRNA expression of ALA influx transporter PEPT1, PpIX efflux transporter ABCG2, and heme biosynthetic enzyme ferrochelatase (FECH) was examined by the RT-PCR analysis. Gastric cancer cell line MKN-45 that expresses PEPT1 mRNA, and human fibrosarcoma cell line T-1080 that expresses the high level of ABCG2 mRNA are used as positive controls.

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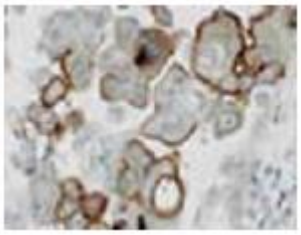

Figure 5. PEPT1 immunoreactivity on the cell membrane in colon cancer.

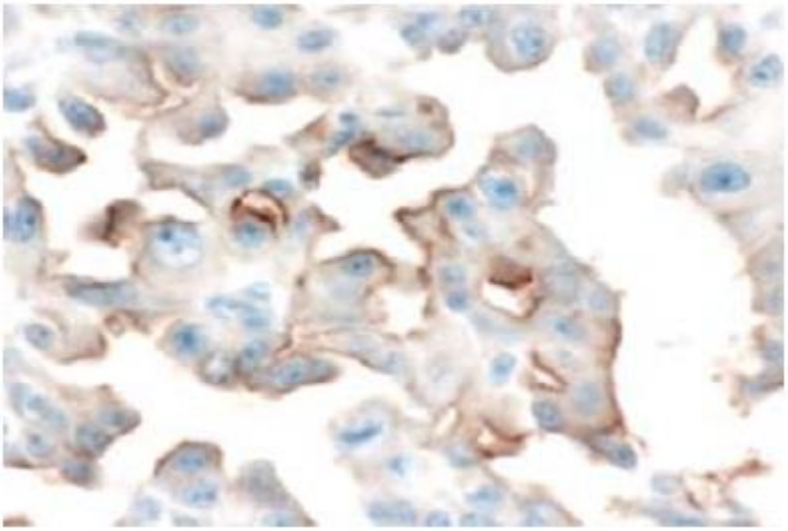

Figure 6. ABCG2 immunoreactivity on colon cancer.