

## A Patient with Scirrhus Stomach Cancer Treated with Combination of Hyperthermotherapy and 5-Aminolevulinic acid (ALA)

MASASHI SAITO<sup>1,2,3</sup>, KAZUHITO YANO<sup>4</sup>, TAKASHI KAMIGAKI<sup>1</sup> and SHIGENORI GOTO<sup>1</sup>

<sup>1</sup>*Seta Clinic Group, Chiyoda-ku, Tokyo, Japan;*

<sup>2</sup>*Second Opinion Institute Tokyo, Chiyoda-ku, Tokyo, Japan;*

<sup>3</sup>*Second Opinion Institute NYC, New York, NY, U.S.A.;*

<sup>4</sup>*Kasumigaura Medical Center, Tsuchiura-shi, Ibaragi-ken, Japan*

**Abstract.** A 35-year-old female with scirrhus stomach cancer (stage IV) was treated with a combination of 5-aminolevulinic acid (ALA), sodium dichloroacetate (DCA), hyperthermotherapy, and immunotherapy as terminal care. The patient survived for one year and seven months, during which her quality of life was markedly improved and she returned to work. The patient was diagnosed with poorly-differentiated adenocarcinoma and progressive signet-ring cell carcinoma, accompanied by left ovarian metastasis, peritoneal dissemination, and right hydronephrosis stage IV, and treated with combination chemotherapy with tegafur-gimeracil-oteracil potassium (TS-1) and docetaxel. Oral ALA and DCA were concomitantly administered at 50 mg each three times a day (150 mg/day, respectively). In addition, hyperthermotherapy using thermotron was concomitantly performed at 2- to 3-week intervals. Cellular immunotherapy with  $\alpha\beta$  T- and immature dendritic cells was also performed. The disease did not progress for 11 months, her quality of life was markedly improved, and she was able to return to work. However, the signs of enlargement of the ovarian metastatic lesion were noted later, for which chemotherapy with four cycles of second-line paclitaxel and a half dose of irinotecan and cisplatin as third-line treatment were performed. Combination of ALA/DCA, hyperthermotherapy, and cellular immunotherapy may be a low-invasive palliative therapy superior in maintaining quality of life of tumor-bearing terminally ill individuals.

Early discovery and treatment have become increasingly important in cancer therapy. Multidisciplinary treatment has recently been markedly promoted due to development of molecular-targeted therapy, including antibody therapy, and the healing rate has been increased by early discovery and treatment. However, for terminal cancer, no radical solution has been achieved, although improvement of palliative therapy has been attempted (1, 2).

5-Aminolevulinic acid (ALA) is a natural  $\delta$  amino acid synthesized in mitochondria in animals and plants, and it is a precursor of porphyrin, heme, and bile pigments (3, 4). An intermediate product of ALA synthesis from heme, protoporphyrin IX (PpIX), is selectively accumulated in tumors, and emits purplish red fluorescence upon excitation with blue light (5-7). Cytotoxic singlet oxygen is also produced upon excitation of PpIX. This phenomenon is applied for ALA-induced photodynamic diagnosis (PDD) and photodynamic therapy (PDT). ALA has been reported to independently induce apoptosis without irradiation and to exhibit an antitumor effect in combination with radiochemotherapy (8, 9). Chibazakura *et al.* found that ALA enhanced heat stress-induced tumor cell death, and the cell death rate was correlated with the level of PpIX which selectively accumulated in the tumor (10). Takahashi *et al.* reported enhancement of the antitumor effect by combination of ALA and hyperthermotherapy in a murine-transplanted tumor model (11). Accordingly, combination with hyperthermotherapy is expected. Combination of PDT including ALA and dendritic cell (DC) therapy is considered to induce antitumor immunity (12, 13). Saji *et al.* reported that transfer of DCs after PDT induced strong antitumor immunity (14). Therefore, not only ALA-PDT but also combination of ALA and hyperthermotherapy may induce strong antitumor immunity through presentation of antigens of dead tumor cells by immature DCs.

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*Correspondence to:* Masashi Saito, MD, Seta Clinic Group 3-6-5 Iidabashi, Chiyoda-ku, Tokyo 102-0072, Japan, Tel: +81 352150086, Fax: +81 352150890, e-mail: m-saito@toushikai.com

**Key Words:** ALA, DCA, hyperthermotherapy, cellular immunotherapy, stomach cancer.

We report the case of a 35-year-old female with scirrhous stomach cancer (stage IV) treated with combination of ALA, DCA, hyperthermotherapy, and cellular immunotherapy.

## Case Presentation

Approval was obtained from the Institutional Review Board (IRB), and combination of hyperthermotherapy, ALA and DCA administration, and cellular immunotherapy was administered in accordance with the Declaration of Helsinki.

The patient was a 32-year-old female whose general condition markedly deteriorated in July 2010. She consulted us when she became unable to ingest food due to vomiting and markedly lost weight (5.0 kg).

On CT at the first examination, stomach tumors of the small curvature, metastatic left ovarian cancer, and peritoneal dissemination were noted (Figure 1A). On endoscopy, the pyloric region of the stomach was severely stenosed, and macroscopic type 4 (diffuse infiltrative type) progressive gastric carcinomas were noted in the upper gastric body, middle gastric small curvature, and its posterior wall (Figure 1B). Samples were collected at the same time, and the cytodiagnosis was poorly-differentiated adenocarcinoma and progressive signet-ring cell carcinoma (Figure 1C). Based on these findings, the patient was diagnosed with stage IV (T3N1H0P1M1) scirrhous stomach cancer accompanied by left ovarian metastasis, peritoneal dissemination, and right hydronephrosis.

**Hyperthermotherapy.** In hyperthermotherapy, heat at 43°C was applied for 20 minutes at 2-to 3-week intervals using Thermotron RF-8 (Yamamoto Vinita, Osaka, Japan).

**Oral ALA and DCA administration.** ALA phosphate (food-grade NatuALA, SBI ALApromo, Tokyo, Japan) and DCA (sodium dichloroacetate, hospital preparation) were orally administered at 50 mg, three times a day (total 150 mg/day), respectively.

**Immunotherapy.**  $\alpha\beta$  T-cells were prepared from mononuclear cells isolated from peripheral blood using the specific gravity centrifugal method (15, 16). Peripheral blood mononuclear cells were cultured with interleukin-2 (IL2) and an antibody to cluster of differentiation-3 (CD3) for 14 days. The purity of  $\alpha\beta$  T-cells was higher than 95%. A total of about  $5 \times 10^9$   $\alpha\beta$  T-cells were intravenously or intra-tumorally injected per dosing nine times at 4-week intervals. Immature DCs were prepared from the monocyte fraction by peripheral blood apheresis. The collected monocyte fraction was cultured with IL4 and granulocyte macrophage colony-stimulating factor (GM-CSF) for about seven days following the standard method. A total of about  $2.3 \times 10^6$  immature DCs were injected into tumors (17, 18).

**Treatment course.** The treatment course is outlined in Figure 2. Since no surgery was indicated and chemotherapy selectivity was very low, palliative therapy was considered to be the indication. This judgment was supported by a second opinion, but chemotherapy was selected due the insistent request by the patient. First-line chemotherapy was initiated on August 19th 2010: 40 mg/m<sup>2</sup> (54 mg/day) of docetaxel were administered by intravenous drip infusion on day 1 and 50 mg of oral tegafur-gimeracil-oteracil potassium (TS-1) was administered twice a day for 14 days, followed by a one-week withdrawal as one cycle. As an adjuvant therapy, hyperthermotherapy was performed at 2-to 3-week intervals using Thermotron RF-8. In addition, the patient concomitantly ingested 5-ALA phosphate and DCA as supplements. From September 6th,  $\alpha\beta$  T-cell therapy was performed nine times at 4-week intervals as cellular immunotherapy. On October 25th, a total of 1 ml of immature DCs was injected into three sites of tumors and a total of 10 ml of concentrated  $\alpha\beta$  T-cells were injected into the submucosal layer at five sites (2 ml each) near tumors using a gastroscope (Figure 3B).

The patient was unable to ingest food at the initiation of treatment, but her general condition had improved after one month, her body weight returned to its previous value (42 kg), and she was able to return to work in November the same year.

The favorable general condition was maintained thereafter with no body weight change and stable disease condition. Lesions exhibited mild redness on endoscopy, but the disease condition was stable on full-thickness evaluation of lesions, and the metastatic ovarian tumor was evaluated as long stable disease (SD) on CT (Figure 3A). Furthermore, the primary lesions were also stable on endoscopy (Figure 3C, right), and the tumor marker levels were within the normal ranges (Figure 4).

At this time point, the cell counts of lymphocyte subsets CD45<sup>+</sup> mononuclear cells (MNC), CD45<sup>+</sup> CD3<sup>+</sup>MNC, CD45<sup>+</sup> CD3<sup>+</sup>TCR $\gamma\delta$ <sup>+</sup>MNC, CD45<sup>+</sup> CD3<sup>+</sup>CD4<sup>+</sup>MNC, and CD45<sup>+</sup> CD3<sup>+</sup>CD8<sup>+</sup>MNC cells were 2736 (1636), 2016 (926), 386 (25), 988 (559), and 694 (240) cells/ $\mu$ l, respectively, higher than the means in patients with cancer given in the parentheses (19, personal communication), strongly reflecting the improvement in the patient's quality of life. Since the disease did not progress and the disease condition was stabilized, the possibility of radical surgery was considered, but colonic stenosis was observed on enema in April 2011, based on which application of radical surgery was considered difficult.

After 10 months, in June 2011, a sign suggesting relative enlargement of the metastatic ovarian tumor was noted on CT, although it was not clear and no increase was detected in the tumor markers (Figure 3D). Thus, chemotherapy was changed to second-line treatment with four cycles of weekly paclitaxel. After 4 months, the tumor markers apparently

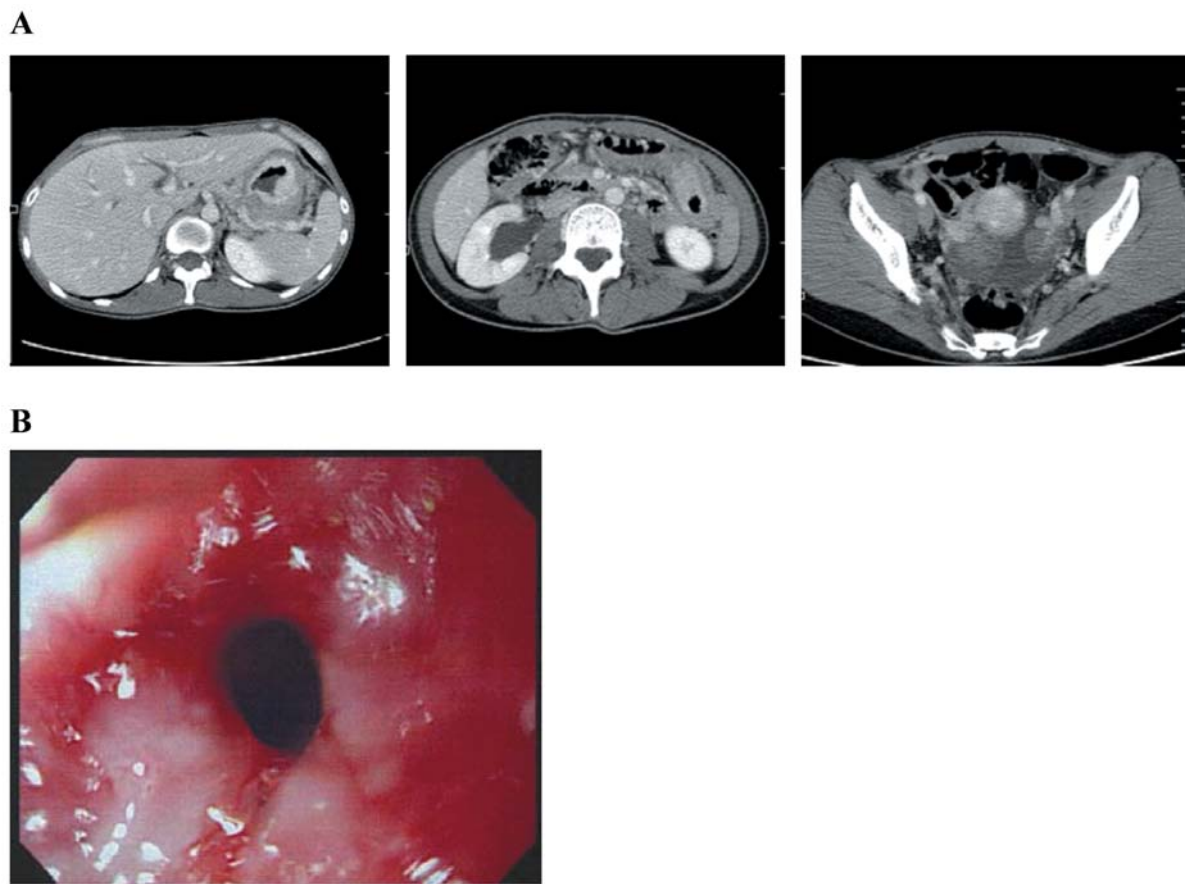


Figure 1. Images on the first examination. A: Computed tomography (CT) images on August 6th, 2010. Primary gastric cancer lesion (left), right hydronephrosis (center), and metastatic left ovarian tumor (right) were noted. B: Endoscopic images on August 18th, 2010. Type-4 diffuse infiltrative tumor was noted in the pyloric region of the stomach.

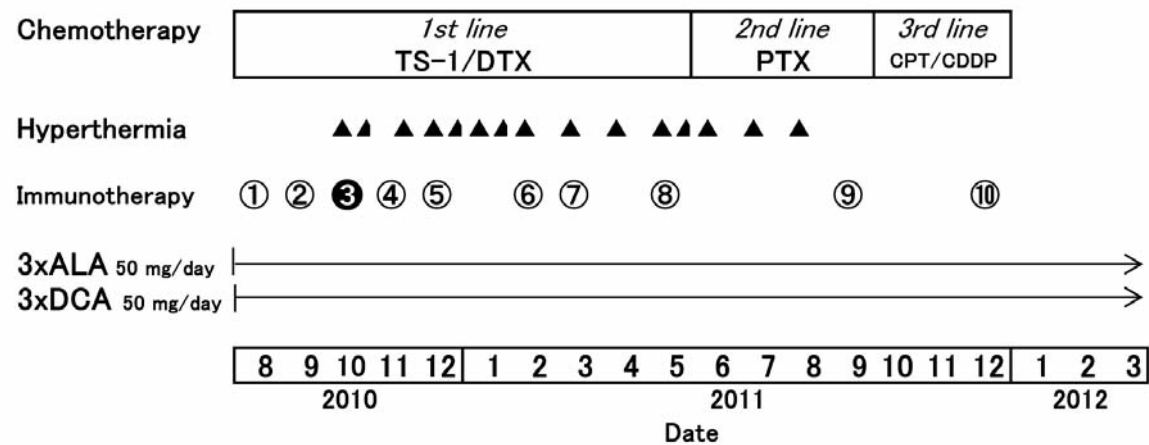


Figure 2. Summary of the treatment course of a 32-year-old female with stage IV scirrhou. TS-1/DTX: Tegafur-gemeracil-oteracil potassium/docetaxel, 10 cycles; PTX: paclitaxel, four cycles; CPT/CDDP: irinotecan/cisplatin; Immunotherapy ①:αβ T-cells alone, ③: intra-tumoral injection of αβ T-cells and immature dendritic cells. DCA: Dichloroacetic acid, ALA: aminolevulinic acid. The patient survived for one year and seven months after the diagnosis of stage IV scirrhou stomach cancer.

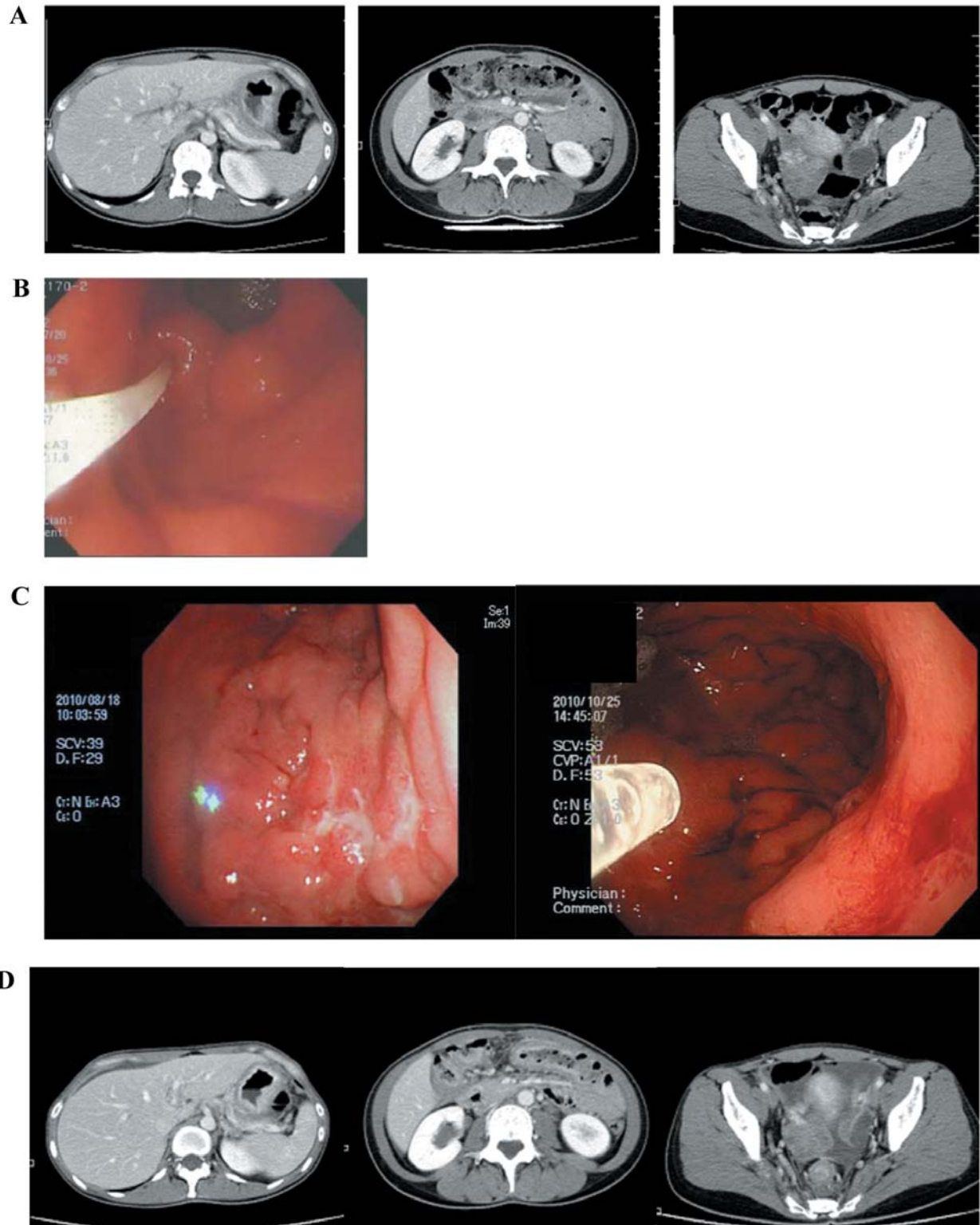


Figure 3. Images during therapy. A: Computed tomography (CT) images on January 5th, 2011. Primary gastric cancer lesion (left), right hydronephrosis (center), and metastatic left ovarian tumor (right). No tumor enlargement was observed compared to those on the first examination. B: Endoscopic image on October 25th, 2010. Immature dendritic cells were infused. C: August 16th, 2010. Hardness and ulceration of the gastric wall were noted from small curvature and its posterior wall to large curvature of the upper gastric body (left). October 25th, 2010. The sites injected with immature dendritic cells were noted to ulcerate and scar (right). D: CT images in June 2011.



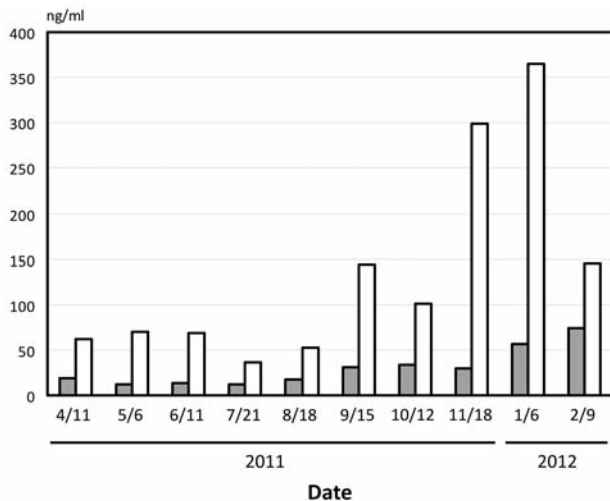


Figure 4. Time-course of tumor markers Carbohydrate 125 (CA125: white bar) and CA19-9 (dark grey bar). Carcinoembryonic antigen (CEA) was also measured at the same time and it was under 2.5 ng/ml in peripheral blood of the patient.

increased, for which third-line chemotherapy with irinotecan and cisplatin (one-day administration followed by two days off) was administered. Unfortunately, sufficient cellular immunotherapy could not be performed after chemotherapy was changed from first- to second-line, 5-ALA and DCA administrations became irregular, and hyperthermotherapy could not be performed after September 2011.

The possibility of cancer peptide vaccine was always considered as a treatment option, but the patient's HLA-A type was A31 and HLA-B was B62 B52. Since this HLA type is rare in Japanese, no HLA-competent cancer antigen peptide for DC cancer vaccine was available. For DC vaccine therapy using autologous tumor, partial tumorectomy (left ovarian tumor) was performed in December 2011, although radical surgery was not indicated. During surgery, cancerous peritonitis was noted, but no apparent dissemination was observed in the pelvic cavity. On palpation of the upper abdominal region, the upper gastric body was relatively soft, and the greater omentum was atrophied and hard due to dissemination. On postoperative pathological examination, both tumors of the bilateral ovaries and oviducts were T3N1H0P1M1 poorly-differentiated adenocarcinoma and signet-ring cell carcinoma (Figure 5A). Initially, the ovarian lesions were assumed to be double-cancer, not metastatic, but later by histopathological examination their metastatic nature was confirmed. MHC-class 1 expression was 2+ on immunohistological staining after surgery, strongly supporting the indication of cancer vaccine administration (Figure 5B). The tumor antigen was Wilms' tumor gene-1 (WT-1) 1+, mucin-1(MUC-1) -, and melanoma antigen family A-1(MAGE-1) - (Figure 5C).

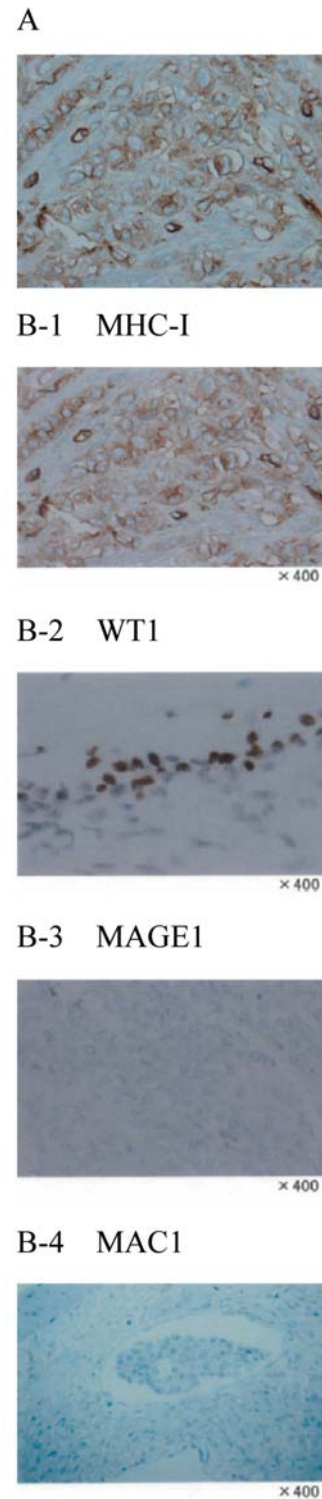


Figure 5. Histopathology (A) and immunohistology (B) of excised specimen in December 2011. Poorly-differentiated adenocarcinoma, progressive signet-ring cell carcinoma in a female patient, 32 years old, with scirrhus stomach cancer, ovarian metastasis, and peritoneal dissemination, stage IV. MHC I: Major histocompatibility complex class I (B-1); WT1: Wilms' tumor gene 1 (B-2); MAGE1: melanoma antigen family A1 (B-3); MUC1: mucin-1 (B-4).

$\alpha\beta$  T-cell therapy was performed in late December 2011 (Figure 2), but diarrhea occurred after surgery (about 10 times/day) and weight loss was marked (36 kg from 42 kg). In 2012, ascites accumulation was noted with feeling of abdominal distention and anorexia, and puncture was performed twice a week. The patient's general condition was aggravated, and the performance scores (PS), which was level 1 on the first examination, became level 2.

No 5-ALA and DCA administrations, hyperthermotherapy, DC vaccine therapy, or chemotherapy could be performed after surgery, and the patient died in March 2012. The survival time after the diagnosis of stage IV scirrhous stomach cancer was one year and seven months.

## Discussion

We present the case of a patient with scirrhous stomach cancer with stage IV ovarian metastasis and peritoneal dissemination treated with combination of hyperthermotherapy, ALA, and DCA. ALA is a precursor of porphyrin and heme, and formed by condensation of glycine and succinyl-CoA by ALA synthetase (2). In tumor cells, ALA is an abnormality present in the heme synthesis step of iron coordination from PpIX. PpIX accumulates in tumors (4), and this is considered to be due to abnormal iron metabolism or reduced activity of the catalyst, ferrochelatase (20, 21). PpIX accumulated in tumors is excreted by ATP-binding cassette transporter G2 (ABCG2), which may weaken tumor cells because this mode of excretion uses ATP.

It has been suggested that PpIX produced by ALA administration induces apoptosis of tumor cells in response to temperature stimulation and radiation, other than laser irradiation. DCA promotes glucose oxidation tricarboxylic acid (TCA) cycle in mitochondria by inhibiting pyruvate dehydrogenase kinase and enhancing pyruvate dehydrogenase activity (22, 23). Since mitochondrial function is inhibited in cancer cells, apoptosis may not readily occur. ALA is also known to potentiate mitochondrial complex IV and ATP production (24). Therefore, DCA and ALA may normalize mitochondrial activity in cancer cells, allowing for apoptosis to occur readily. Interestingly, apoptotic tumor cells are appropriate for antigen presentation in host immune responses. Thus, if antigen-presenting DCs are present, strong antitumor immunity may be induced. It has been reported that immature DCs are markedly phagocytic and appropriate for presenting antigens (25). Repasky *et al.* investigated the action of hyperthermotherapy on the immune system, and observed that production of inflammatory cytokines, such as tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) and interleukin-6 (IL6), by macrophages was enhanced by body temperature elevation. They suggested the involvement of heat shock protein 70 kD (HSP70) expression in the thermal effect of macrophages (26, 27). Coordination

of innate and adaptive immunities has recently been suggested. Combination of ALA, DCA, hyperthermotherapy, and immunotherapy may have been effective for induction of HSP-mediated specific immunity by macrophages, DCs, and T-cells.

We concomitantly administered hyperthermotherapy, ALA, DCA, and immunotherapy to a patient with scirrhous stomach cancer with stage IV ovarian metastasis and peritoneal dissemination. Although it was applied in only one patient, the quality of life of the patient was markedly improved despite the stage being terminal IV, and, surprisingly, the patient was able to return to work, although only for a short time. Correction of mitochondrial abnormality in cancer cells and induction of cancer-specific immunity may become a target of cancer therapy in the future. Since the side-effect of combination of ALA, DCA, hyperthermotherapy, and immunotherapy are mild for patients, it may be a treatment option applicable without loading stress on tumor-bearing patients. A large-scale clinical study to further investigate the safety and efficacy involving an increased number of patients is expected.

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