Original Contribution

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Comparison between the Gastric Cancer Cell Line MKN-45 and the High-Potential Peritoneal Dissemination Gastric Cancer Cell Line MKN-45P

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Summary: Peritoneal metastasis is the most common form of recurrence in gastric cancer, and is associated with a poor prognosis. It is clear that many agents are involved at the various stages of this process, however, many aspects of the progression remain unclear. In the present study we compared the gastric cancer cell line MKN-45 with the high-potential peritoneal dissemination gastric cancer cell line MKN-45P, established from MKN-45. The supernatant of culture medium of MKN-45 cells or MKN-45P cells was collected, and the concentrations of interleukin-1β (IL-1β), IL-6, IL-8, hepatocyte growth factor (HGF), Transforming growth factor beta-β1 (TGF-β1), vascular endothelial growth factor (VEGF), matrix metalloproteinase-2 (MMP-2), MMP-9, and tissue inhibitor of metalloproteinase-1 (TIMP-1) proteins were measured using an enzyme-linked immuno sorbent assay (ELISA) method. Invasion, wound healing and adhesion assays were performed in vitro to examine interstitial invasion, migration and adhesion in the gastric cancer cell lines. Moreover, Western blotting was performed to determine the expression of cyclooxygenase-1 (COX-1) and COX-2 proteins in the culture media of the cell lines. The concentrations of IL-6, IL-8, VEGF and MMP-2 protein in the culture supernatant of MKN-45P were significantly higher than those of MKN-45. Percent adhesion of MKN-45P was significantly higher than that of MKN-45 in the fibronectin-coated group. There was no significant difference in invasion or migration between MKN-45 and MKN-45P. COX-1 and COX-2 proteins were observed in both cell lines. These results suggested that secretion of IL-6, IL-8, VEGF and MMP-2 from cancer cells, and adhesion of cancer cells to fibronectin, were related to the establishment of peritoneal dissemination.

Key words gastric cancer, peritoneal dissemination, cytokine, adhesion assay

INTRODUCTION

The detection rate of early gastric cancer, and the overall survival rate of gastric cancer have been increasing because of improved diagnostic techniques and the promotion of routine mass screenings for gastric cancer. However, we still experience cases of far advanced gastric cancer with poor prognosis. Peritoneal dissemination is the most common form of recurrence in gastric cancer, and it is associated with a poor prognosis. Treatment of peritoneal dissemination is one of the most important challenges in the treatment of gastric cancer. However, no standard treatment for peritoneal dissemination has yet been proposed. Surgery alone or chemotherapy alone has no beneficial effect on survival. New anti-cancer drugs are being used for advanced and recurrent gastric cancer in many institutions [1]. A recent paper has suggested that neoadjuvant chemotherapy, such as preoperative intraperitoneal chemotherapy (IPC)

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Abbreviations: AMFR, autocrine motility factor; bFGF, basic fibroblast growth factor; BSA, bovine serum albumin; COX, cyclooxygenase; ELISA, enzyme-linked immunosorbent assay; ECL, enhanced chemiluminescence FBS, fetal bovine serum; HGF, hepatocyte growth factor; IL, interleukin; IPC, intraperitoneal chemotherapy; MMP, matrix metalloproteinase; PBS, phosphate-buffered saline; TGF-β1, transforming growth factor-β1; TIMP, tissue inhibitor of metalloproteinases; VEGF, vascular endothelial growth factor.

combined with systemic chemotherapy using S-1 and docetaxel may have a strong survival benefit for patients with scirrhous gastric cancer [2].

However, many questions remain unanswered, such as whether neoadjuvant chemotherapy is better than adjuvant chemotherapy, and whether IPC is better than systemic chemotherapy [2]. Moreover, there is as yet no effective treatment against peritoneal dissemination. The development of peritoneal metastasis is a multistep process, beginning with the detachment of cancer cells from the primary tumors, their attachment to peritoneal mesothelial cells, retraction of the mesothelial cells, and exposure of the basement membrane. After attachment to the basement membrane, the cancer cells degrade the extracellular matrix and proliferate [3]. Finally, the cancer cells induce angiogenesis and lymphangiogenesis. Many cytokines, adhesive factors, growth factors, matrix metalloproteinases (MMPs), and angiogenic factors play important roles in these steps. Briefly, cadherin and MMPs are related to the detachment of cancer cells from the gastric wall [4,5]. CD44 and integrin are important factors for the attachment of free cancer cells to the peritoneum, directly [6,7]. After attachment, migration factors such as c-met and autocrine motility factor (AMFR), and MMPs are needed for cancer cells to infiltrate the submesothelial matrix [5,8]. After infiltration, growth factors such as epidermal growth factor stimulate the growth of cancer cells, while simultaneously angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), interleukin-6 (IL-6), and IL-8 induce angiogenesis, and finally peritoneal dissemination is established [9-11]. Cyclooxygenase (COX) is a key enzyme in the conversion of arachidonic acid to prostaglandin, and COX-2 is significantly correlated with lymph node metastasis, stage, and prognosis in gastric carcinoma, suggesting that COX-2 might be correlated with invasion and metastasis of gastric carcinoma [12].

The second cascade of peritoneal metastasis is called trans-lymphatic metastasis. In this cascade, cancer cells migrate into the submesothelial lymphatic vessels through milky spots, stomata or initial lymphatics. There are four entrance gates of peritoneal free cancer cells into the submesothelial lymphatic vessels [13].

Recently, molecular targeting therapies combined with anti-cancer drugs are being utilized to treat various cancers and have shown a survival benefit for patients with cancer. The study of factors relevant to the development of peritoneal metastasis may have a significant impact on the treatment of gastric cancer. In the present study we compared cytokines, MMPs, angiogenic factors, invasion, adhesive ability, migration, and the expression of COX in the gastric cancer cell line MKN-45 and the high-potential peritoneal dissemination gastric cancer cell line MKN-45P which Miyagi et al. [14] established in our department.

MATERIALS AND METHODS

Cell lines

We used the high-potential peritoneal dissemination cell line MKN-45P, which was established from the human gastric cancer cell line MKN-45 (derived from a poorly differentiated adenocarcinoma in a 62-year-old woman; Health Science Research Resources Bank, Tokyo, Japan), in our institution as described previously [14]. Briefly, nude mice (BALB/c nu/nu) were subcutaneously inoculated with MKN-45 cells and the subcutaneous nodules were removed and injected into other mice intraperitoneally. The cancer cells from the peritoneal nodules were injected into the abdominal cavity of other mice. The process was continued through to a seventh generation. The resulting high potential peritoneal dissemination cell line was named MKN-45P. MKN-45 and MKN-45P cells were maintained in RPMI-1640 medium (Nihon Seiyaku Co., Komaki, Aichi, Japan), supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco Uxbridge, Middlesex, UK), 2mM-glutamine, and penicillin-streptomycin (50 IU/mL and 50 μg/mL, respectively) at 37.0°C in humidified air with 5% CO_2.

Measurement of cytokines in conditioned medium

For measurement of cytokines in conditioned medium, MKN-45 cells (1×10^6 cells/10mL) or MKN-45P cells (1×10^6 cells/10mL) were placed in 100 mm tissue culture dishes (IWAKI Co., Funabashi, Chiba, Japan) and cultured for 72 h in medium containing 10% FBS at 37.0°C in humidified air with 5% CO_2. The number of cells in each cell line was evaluated visually at 12, 24, 48, and at 72 h (values: mean of three fields). The supernatant was then collected, and the concentrations of IL-1β, IL-6, IL-8, IL-10, hepatocyte growth factor (HGF), transforming growth factor-β1 (TGF-β1), VEGF, MMP-2, MMP-9, and tissue inhibitor of metalloproteinases-1 (TIMP-1) proteins were measured using an enzyme-linked immunosorbent assay (ELISA) method (IL-1β, IL-8 and IL-10: Bio Source Europe S. A., Nivelles, Belgium; IL-6: Fujirebio Inc., Tokyo, Japan; HGF: Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan; TGF-β1 and VEGF: R & D Systems Inc., Minneapolis, MN, USA;

MMP-2, MMP-9 and TIMP-1: Daiichi Fine Chemical Co. Ltd., Takaoka, Toyama, Japan). Each cytokine was measured in 5 samples, and the means of these cytokines were compared between the MKN-45 cells and the MKN-45P cells.

Invasion assay

An invasion assay was performed in vitro to confirm interstitial invasion in the gastric cancer cell lines. Experiments were conducted using 24-well chemotaxicell chambers (Kurabo Industries Ltd., Osaka, Japan). Matrigel (BD Bioscience San Jose, CA, USA) was diluted in phosphate-buffered saline (PBS) to a concentration of 0.75 mg/mL, and 100 μL of this solution was placed into the chambers and air-dried overnight (Matrigel coating). Then, 200 μL of RPMI-1640 medium, containing 0.1% bovine serum albumin (BSA) was added to the Matrigel-coated chambers, and after allowing the film to swell (for 1h), the MKN-45 or MKN-45P cell suspensions (2.5×10^4 cells) were placed in the chambers. Then, 700 μL of chemoattractant solution (adjusted to a fibronectin level of 10 μg/L with serum-free RPMI-1640) was added to the bottom chamber and the cells were cultured for 48 h in a 5% CO_2 incubator. At 48 h, the cells on the upper surface of the filter were removed using a swab, and the cells that had infiltrated to the bottom surface of the filter were fixed in 70% ethanol for 30 min, subjected to Giemsa staining, and observed visually under a microscope (at 200 × magnification). The number of invasive cells was the average of ten random views. The non-Matrigel-coated chambers were used as controls. Percent invasion was calculated as the mean of cells invading through the Matrigel insert membrane/mean of cells migrating through control insert membrane × 100.

Adhesion assay

Adhesion assay was performed to confirm cell-matrix adhesion in the gastric cancer cell lines. MKN-45 or MKN-45P cells (1×10^4 cells) were seeded on fibronectin, laminin or type IV collagen-coated 96-well plates, and were cultured for 6 h in a 5% CO_2 incubator. At 6 h, after unattached cells were removed, Tetra Color ONE (Cell Proliferation Assay System) (Seikagaku Co., Tokyo, Japan) was added to each 96-well plate and the cells were incubated for 2 h at 37°C. Then the percent adhesion was calculated as absorbance at 450 nm using a 96-well microplate reader.

Scratch wound assay

Cells were grown to confluence in a 100 mm Tissue Culture Dish (Iwaki Co., Funabashi, Chiba, Japan). After 24 h, the monolayers were scratched using a 200 μL, sterile plastic pipette tip and washed twice with complete medium. The cells were allowed to migrate onto a plastic surface and photographed. Two random pictures under a microscope (at 4 × magnification) were taken for each wound immediately after the wound was inflicted to the cell monolayer and 12, 24 h.

Western blotting

Whole-cell lysates were prepared for Western blot analyses using a lysis buffer containing 0.75 mM Tris (pH7.5), 10% glycerol, and 2% SDS. After centrifugation the supernatant was collected as total proteins. Protein concentrations were determined using the Bio-Rad detergent-compatible protein assay (Bio-Rad Laboratories, Richmond, VA, USA). Sixμg of proteins were denatured in a SDS sample buffer [0.5 M Tris-HCl (pH 6.8), 10% glycerol, 10% SDS, 10% mercaptoethanol, and 0.01% Bromophenol blue] at 100°C for 5 min. Samples were separated by a denaturing 10% SDS-polyacrilamide gel and transferred to polyvinylidene difluoride membranes (Immobilon, Bedford, MA, USA). Membranes were blocked with 1% skimmed milk in 0.5% Tween20 adding TBS (20 mM Tris-HCl (pH7.6), 137 mM NaCl) at 4°C overnight and incubated with primary polyclonal antibodies that recognize COX-1 (1 : 1,000, Santa Cruz, Inc. Santa Cruz, CA, USA), or COX-2 (1 : 1000, Santa Cruz). Secondary anti-rabbit IgG antibody conjugated to horseradish peroxidase (Vector Laboratories, Inc. Burlingame, CA, USA) was used at 1 : 500 to detect primary antibodies, and enzymatic signals were visualized by enhanced chemiluminescence (ECL).

Statistical analysis

Statistical tests were performed by Student's *t* -test and χ^2 test. P values <0.05 were considered significant.

RESULTS

Measurement of cytokines in condition medium

The number of MKN-45 or MKN-45P cells was counted at 24, 48 and 72 h. There was no difference in the number of cancer cells between the two cell lines.

The concentration of cytokines in conditioned media from MKN-45 or MKN-45P were shown in Table 1. The concentrations of IL-6, IL-8, VEGF and MMP-2 protein in the culture supernatants of MKN-45P were significantly higher than those of MKN-45 (M= 0.045, P= 0.011, P= 0.013, and P= 0.021, respectively) (Table 1).

TABLE 1.
Comparison of cytokines between MKN-45 and MKN-45P

	IL-1β (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	VEGF (pg/mL)	MMP-2 (ng/mL)	TIMP-1 (ng/mL)
MKN-45	0.9±0.7	1.2±0.7	381.9±147.1	1335.0±624.3	0.3±0.1	2.7±1.8
MKN-45P	0.4±0.2	2.9±0.6	891.4±210.2	3806.0±229.8	0.7±0.5	6.0±4.0
P value	0.109	0.045	0.011	0.013	0.021	0.126

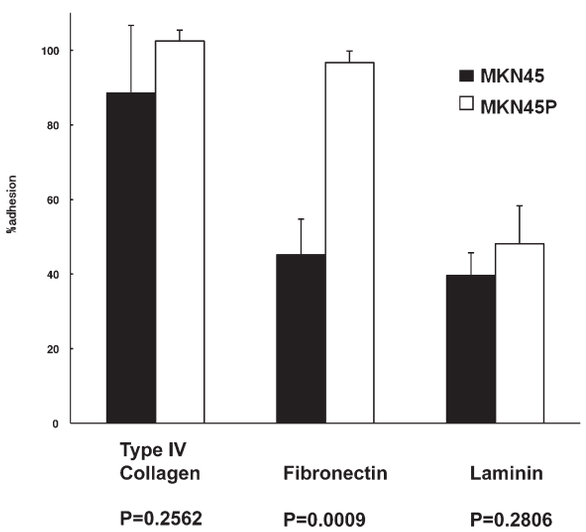

Fig. 1. The result of adhesion assay. In the type IV collagen or laminin coating group, there was no significant difference in the %adhesion between the MKN-45 cells and the MKN-45P cells (P=0.0616 and P=0.6236). However, in the fibronectin group, the %adhesion in the MKN-45P cells was significantly higher than that in the MKN-45 cells (P=0.0239).

Invasion assay

The mean number of invasive MKN-45 and MKN-45P cells was counted by invasion assay. There was no significant difference in percent invasion between the MKN-45 cells and the MKN-45P cells (P= 0.7579).

Adhesion assay

Adhesion assay was performed to compare the adhesive ability of MKN-45 cells and MKN-45P cells to matrix. In the type IV collagen or laminin-coated groups there was no significant difference in percent adhesion between the MKN-45 cells and the MKN-45P cells (P= 0.2562 and P= 0.2806). However, in the fibronectin-coated group, percent adhesion of MKN-45P cells was significantly higher than that of MKN-45 cells (P= 0.0009) (Fig. 1).

Scratch wound assay

Using the scrape wound-healing migration assay to compare migration and motility, there were no differences in migration between the MKN-45 cells and the MKN-45P cells at 12 h and 24 h.

Western blotting

Western blotting was performed to compare the

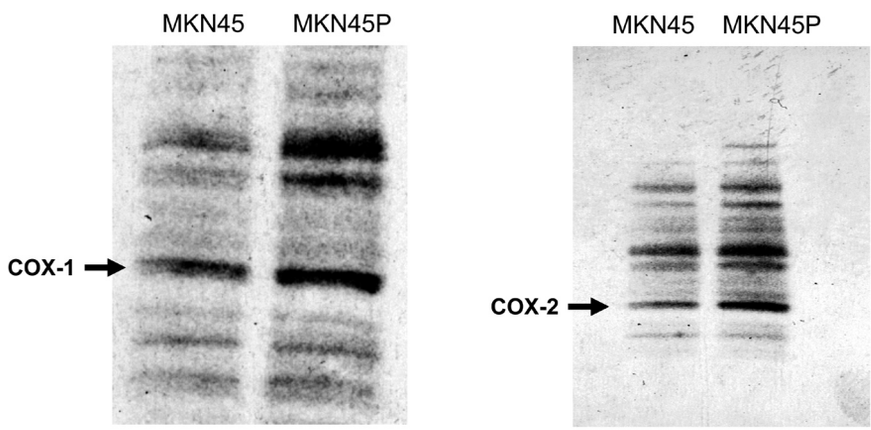

Fig. 2. COX-1 and COX-2 protein expression on Western blotting.
The expressions of COX-1 and COX-2 were detected in both cell lines.

expressions of COX-1 and COX-2 in MKN-45 cells and MKN-45P cells. We observed the expression of COX-1 and COX-2 in both cell lines (Fig. 2).

DISCUSSION

We compared the concentration of cytokines in culture supernatant of the gastric cancer cell line MKN-45 with that in the high-potential peritoneal dissemination gastric cancer cell line MKN-45P which had been established from MKN-45. The concentrations of IL-6, IL-8, VEGF and MMP-2 protein were significantly higher in the supernatant of culture medium of MKN-45P than in MKN-45.

IL-6 is a 26 kDa molecular weight protein that is produced by monocytes, macrophages, T lymphocytes, and endothelial cells. It is related to hemopoiesis, immunity, and inflammation, and is a multifunctional regulator in the nervous and endocrine systems [15]. Moreover, IL-6 has been reported as a prognostic factor in gastric carcinoma, and was significantly correlated with the incidence of lymph node metastasis and of liver metastasis [15]. IL-8 is an 8kDa molecular weight polypeptide and is one of the α-chemokines which belong to the C-X-C family, which are produced mainly by monocytes, macrophages, and endothelial cells, and is related to the migration of white corpuscles and inflammation [16]. Moreover, IL-8 has been reported as a prognostic factor in gastric carcinoma, and was significantly correlated with the depth of invasion and vessel infiltration [16]. IL-6 and IL-8 are related to the accomplishment of peritoneal dissemination by inducement of angiogenesis [10,11].

VEGF is a 34-45 kDa molecular weight typical angiogenic factor which is secreted mainly from monocytes, macrophages, pituitary cells, cancer cells, and smooth muscle cells, and is significantly related to angiogenesis in gastric cancer [17]. VEGF, as well as functioning as a growth factor, is able to function as a vascular permeability factor. Increased permeability of blood vessels facilitates the extravasation of proteins and the formation of ascites [18]. In previous reports, the expression level of VEGF has been found to be directly associated with the production of ascites and carcinomatosis [18,19]. Aoyagi et al. [20] reported that immunohistochemical expression of VEGF in gastric cancer tissue was correlated with peritoneal metastasis from gastric cancer, and that the expression of VEGF in cancer cells was a useful indicator of peritoneal recurrence. Moreover, Imaizumi et al. [21] reported that bevacizumab, which is a humanized monoclonal antibody against VEGF, suppressed peritoneal dissemination from gastric cancer in a peritoneal metastasis model. These studies provide clear evidence that VEGF is an essential element in the development of peritoneal metastasis and support the result of our in vitro study.

Degradation of the extracellular matrix is considered to be a prerequisite for peritoneal metastasis, and MMPs are thought to play an important role in this process [22,23]. There are many reports that highly invasive cancer cells with a high potential for metastasis stimulate the production of MMPs [22], and MMP-2 is significantly correlated with depth of invasion, lymph node metastasis, and distant metastasis of gastric cancer [24].

A loss in balance or a disequilibrium between MMPs and TIMPs, which inhibit the activity of MMPs, is thought to be a major factor leading to invasion and metastasis [25]. Some studies have reported that increased TIMP-1 or administration of TIMP-1 inhibited peritoneal invasion [25,26]. Miyagi et al. [14] reported that the TIMP-1 gene was transferred to MKN-45P by an adenoviral vector, and that peritoneal dissemination was significantly inhibited in a TIMP-1 transfected group compared with a non-virus group and a Lac-Z transfected group, using a peritoneal metastasis model.

The development of peritoneal metastasis is a multistep process, beginning with the detachment of cancer cells from the primary tumor, attachment to peritoneal mesothelial cells, retraction of the mesothelial cells and exposure of the basement membrane, degradation of the extracellular matrix, proliferation of the cancer cells, and angiogenesis, and it is clear that many agents are involved at the various stages of this process [3]. Therefore, the invasion, adhesion, and migration abilities of cancer cells are very important to the accomplishment of peritoneal dissemination. The present study found no significant difference in invasion or migration between MKN-45 and MKN-45P cells. However, the adhesion of MKN-45P to fibronectin was significantly higher than that of MKN-45. Integrins play an important role in the attachment of gastric cancer cells to the submesothelial basement membrane of the peritoneum. Over-expression of integrin-α2β1 and α3β1 are recognized in MKN-45P and OCUM-2MD3, which are high frequency peritoneal metastasis gastric cancer cell lines, and anti-α2β1- and α3β1-integrin antibody significantly reduced the number of cancer cells on the peritoneum in nude mice [7,27]. Moreover, α3β1-integrin mediates cell-binding to fibronectin, which is a component of basement membrane, and these are thought to be important for attachment of free gastric cancer cells to

the peritoneum [7].

COX is a key enzyme in the conversion of arachidonic acid to prostaglandin, and two isoforms of COX, namely COX-1 and COX-2, have been identified [28]. COX-1 is constitutively expressed in many tissues and is considered to be involved in various physiological functions, whereas COX-2 is induced by pathological stimuli, such as inflammation, various growth factors and cytokines produced by tumor cells [28]. Increased COX-2 expression has been reported in colorectal, pancreatic, hepatocellular and other cancers [29]. Li et al. [29] reported that COX-2 expression in gastric adenocarcinoma was higher than that in the paracancerous tissues, and was related to lymph node metastasis and the depth of invasion, suggesting COX-2 might be correlated to the occurrence and advancement of gastric carcinoma. Leung et al. [30] reported that overexpression of COX-2 in gastric cancer is associated with up-regulation in VEGF and angiogenesis. However, in this study, we observed the expression of COX-1 and COX-2 in both cell lines on Western blotting.

These results suggested that the secretion of IL-6, IL-8, VEGF and MMP-2 from cancer cells was related to the establishment of peritoneal dissemination by promoting angiogenesis and degradation of the extracellular matrix, and by increasing the ability of cancer cells to adhere to the peritoneum.

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Case Report

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Recurrence of Invasive Micropapillary Carcinoma of the Breast with Different Ultrasound Features according to Lesion Site: Case Report

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Summary: Invasive micropapillary carcinoma (IMPC) of the breast is a distinct variant of breast cancer. Extensive lymphatic penetration, lymph node metastasis, and local recurrence are seen at a relatively high frequency. On ultrasound (US) findings, IMPC has been reported to be an irregular or lobulated mass with hypoechoic internal areas, but as yet there is no consensus regarding typical findings. A 52-year-old female noticed a mass less than 3 cm in diameter in her left upper breast. US findings indicated an irregularly shaped, hypoechoic tumor with indistinct margins. The diagnosis according to fine-needle aspiration cytology was invasive ductal carcinoma. She underwent lymph node dissection with mastectomy of the left breast. Histological examination revealed mixed-type IMPC. Three years and three months after surgery, IMPC recurred under the skin of the surgical scar. US findings indicated a hyperechoic tumor in this region. Eight months after further surgery, a tumor in the anterior chest wall was observed. US findings indicated an oval hypoechoic tumor with posterior acoustic enhancement. US findings differed between primary and recurrent IMPC because of differences in the occupancy and distribution of IMPC. We describe here a comparison between US and histological findings, as well as differences in IMPC between primary, secondary and tertiary sites.

Key words invasive micropapillary carcinoma, ultrasound, breast cancer, recurrence

INTRODUCTION

Invasive micropapillary carcinoma (IMPC) of the breast is a relatively rare, distinct variant of breast cancer [1]. This carcinoma has been reported to show lymphovascular invasion, lymph node metastasis, local recurrence, and distant metastasis at a relatively high frequency. IMPC has been reported to be associated with a poor prognosis [2-4].

We previously reported that the Ki-67 labeling index was higher in IMPC components than in invasive ductal carcinoma (IDC) components, and that IMPC might occur by squamous differentiation, suggesting a more aggressive behavior than that seen with IDC [5]. Ultrasound (US) has shown IMPC to be an irregular or lobulated mass showing hypoechoic areas with internal echoes [6,7]. However, no consensus on typical findings has been reached.

We report a case of IMPC with repeated recurrence in whom US findings were different at each site. We compared US and histological findings.

CASE REPORT

Primary lesion

A 52-year-old female noticed a nodule −3 cm in

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Abbreviations: CEF, cyclophoshamide, epirubicin and fluorouracil; FNAC, fine-needle aspiration cytology; IDC, invasive ductal carcinoma; IMPC, invasive micropapillary carcinoma; MRI, magnetic resonance imaging PET, positron emission tomography; TVDT, tumor volume doubling time; us, ultrasound.

diameter on her left upper breast and complained of pain. Mammography was not undertaken because of the pain. US findings indicated an irregularly shaped hypoechoic tumor with indistinct margins, accompanied by several high-echo spots within the tumor. The lesion corresponded to category 4b (suspicious abnormality) according to the BI-RADS® Ultrasound Lexicon Classification [8] (Fig. 1). Left axillary lymph node metastasis was suspected. Dynamic contrast-enhanced magnetic resonance imaging (MRI) showed rapid initial enhancement. Fine-needle aspiration cytology (FNAC) showed papillary clustering of hyperchromatic cells with irregular and crowded nuclei, suggesting malignancy. Histologically, cancer cells showed a micropapillary structure and were observed floating in stromal spaces with a mixed scirrhous component. Based on these findings, the tumor was diagnosed as IMPC with scirrhous carcinoma. We therefore undertook a mastectomy and lymph node dissection. Extensive intraductal components were also seen (Fig. 2). Immunohistochemically, estrogen and progesterone receptors were positive. HER2/neu was 2+ (10% cutoff at that time). It was retested by fluorescence *in-situ* hybridization and judged to be positive. Adjuvant chemotherapy comprised six cycles of the cyclophosphamide, epirubicin and fluorouracil (CEF) regimen. Each dose consisted of 500 mg/m^2 cyclophosphamide, 100 mg/m^2 epirubicin, and 500 mg/m^2 5-fluorouracil injected intravenously. Hormone therapy (25 mg exemestane, p.o.) was continued after completion of chemotherapy.

Secondary lesion

Three years and three months after surgery, a lump (diameter, −4 cm) was palpable under the skin of the

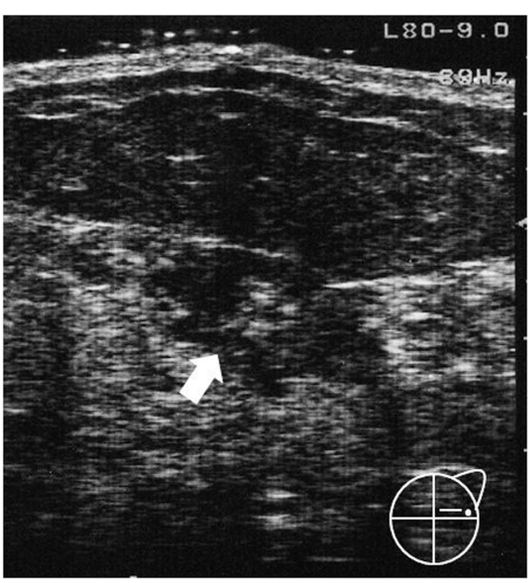

Fig. 1. Ultrasound findings in the primary lesion.

Ultrasound findings showed a solid hypoechoic mass with irregular shape and indistinct margins measuring 3.5 cm in the outer upper left breast. Several high-echo spots within the tumor were observed (arrow).

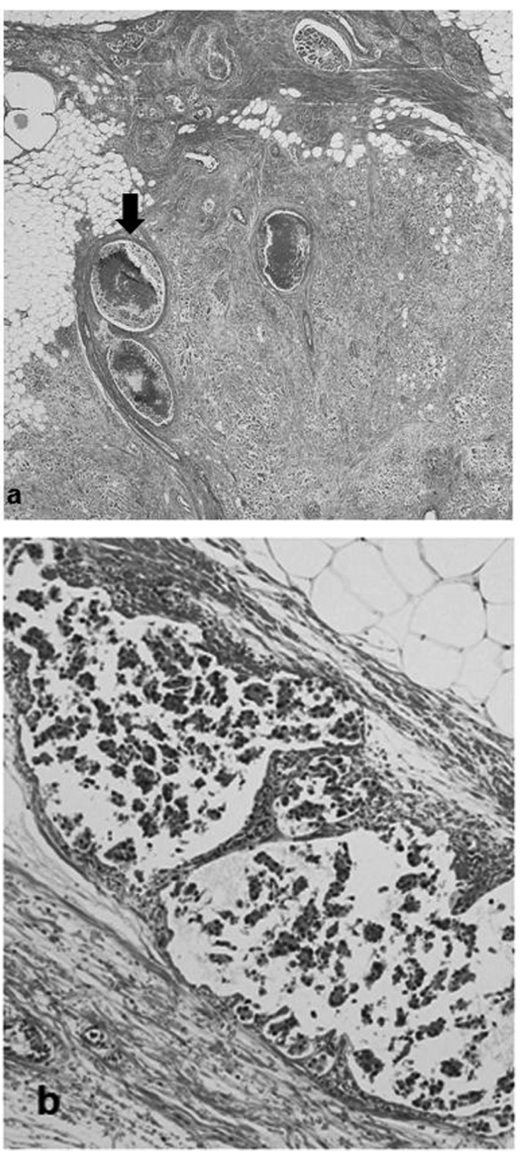

Fig. 2. Histological findings in the primary lesion.

a. Invasive micropapillary carcinoma has grown extensively in the stroma. Intraductal carcinoma with comedo necrosis is also seen (arrow).
Low magnification, H&E staining.

b. Invasive micropapillary carcinoma has infiltrated into lymphatic vessels. High magnification, H&E staining.

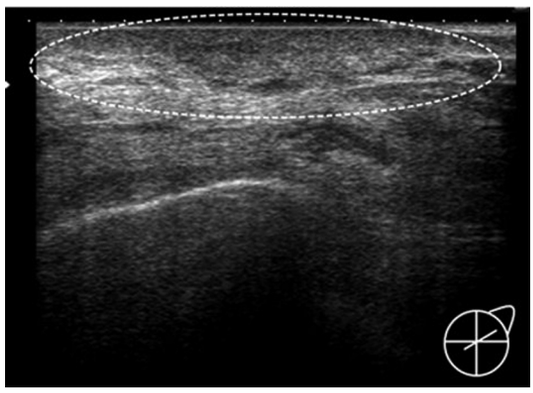

Fig. 3. Ultrasound findings in the secondary lesion.

Internal echoes, heterogeneous (hyperechoic or isoechoic), and no posterior acoustic features are recognized.

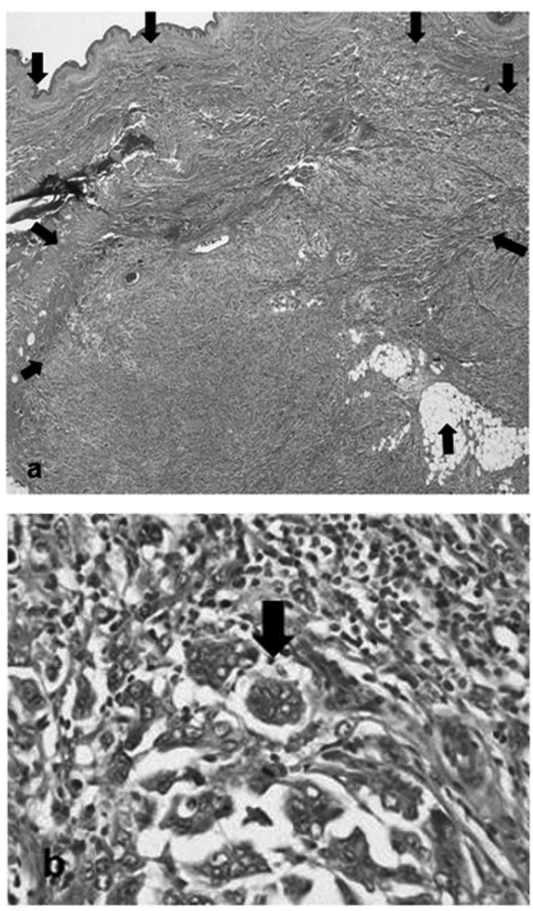

Fig. 4. Histological findings in the secondary lesion.

a. Invasive micropapillary carcinoma is seen in the collagen fibers under the epidermis and dermis.

b. Papillae lack true fibrovascular cores. Cell clusters and arranged so that the polarity of the cells is reversed. Cancer cells are floating in the empty spaces of the stroma (arrow).

surgical scar of the left breast. US findings indicated a hyperechoic tumor without posterior acoustic features along the skin of the surgical scar. The shape of the tumor was not obvious (Fig. 3). CT revealed early enhancement of the skin in the left breast lesion, suggesting local recurrence. Histologically, IMPC was observed in the collagen fibers under the epidermis and dermis (Fig. 4).

Third lesion

Eight months after further surgery, a new nodular lesion was observed in the left anterior chest wall on US. US findings indicated an irregular-shaped hypoechoic mass with indistinct margins (1.5×1.3 cm) without posterior acoustic features.

Two months later, a new circular tumor (diameter, 0.9 cm) was observed projecting from the existing tumor. Three months later the tumor had grown rapidly (2.2×1.8×1.3 cm). US findings demonstrated homogeneous oval hypoechoic areas with posterior acoustic enhancement (Fig. 5). Tumor volume doubling time (TVDT) [9] was −19 days, which was fairly short. MRI findings suggested invasion into soft tissue. Positron emission tomography (PET) findings revealed tumor spread to the left parasternal lymph nodes and left pectoral muscle lymph nodes. Chemotherapy was started (40 mg Navelbine, 360 mg Herceptin), but the tumor continued to increase in size. A new treatment plan was formulated involving radiation therapy {left chest wall: 50 Gy in 25 fractions compared with local areas compared with 70 Gy in five fractions}, chemotherapy (docetaxel 20 mg/body

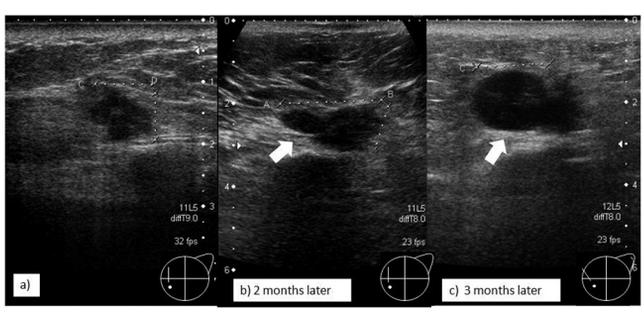

Fig. 5. Ultrasound findings in the third lesion (mass in the left anterior chest wall).

a. Solid hypoechoic mass with an irregular shape and indistinct margin (1.5×1.3 cm); no posterior acoustic features.

b. A new tumor (0.9×0.5×0.4 cm) and showed an oval shape protruding outwards (arrow)

c. The protruding tumor grew rapidly (2.2×1.8×1.3 cm). The tumor was a hypoechoic mass, round in shape, with posterior acoustic enhancement (arrow).

weekly for 6 weeks) and hyperthermia once a week on five occasions. However, 2 months later, PET revealed that the tumor had spread to the left supraclavicular, parasternal, left cervical and right axillary lymph nodes. Radiation therapy was being continued against these lesions.

DISCUSSION

We detailed differences in IMPC between primary and recurrent sites, comparing US and histological findings.

At the primary site, histological analyses showed primary IMPC mixed with scirrhous carcinoma. US findings revealed an irregular-shaped hypoechoic tumor with indistinct features. Plural duct dilatation with comedo features was observed as high-echo spots. Tumor margin corresponded to the histologic features of carcinoma cells invading fat tissue admixed with adipocytes and elastic fiber [10,11]. The primary site included scirrhous components, which also appeared on the image findings.

At the secondary site, histological analyses demonstrated IMPC under the skin of the surgical scar with extensive proliferation of cancer cells intermingled with collagen. Cancer cells were scattered in complicated scar tissue. Reflection and scattering were detected and were considered to be caused by heterogenous structures smaller than sound waves, such as cellular tissue [11,12]. Therefore, the reflection source and backscatter were increased, and internal echoic regions showed high-echo spots on US. We speculated that the carcinoma cells had entered lymphatic vessels under the skin and then spread widely. Therefore, the shape of the tumor was ambiguous on US.

In the skin of surgical scars, it is difficult to distinguish recurrent skin lesions from hypertrophic scars. However, relapse of the carcinoma to the skin has been reported to occur in 12% of patients [13], so we should be careful when observing changes in the skin after surgery. In normal skin, the outermost surface layer is hyperechoic (boundary of the stratum corneum and petroleum jelly), the second layer is slightly hypoechoic (epidermis), and the third layer is very hyperechoic (dermis) [14]. If these three layers of the skin are irregular, careful observation is necessary.

In the third lesion (recurrence in the anterior chest wall), the tumor showed rapid growth and was a homogeneous oval hypoechoic mass with posterior acoustic enhancement. Histological correlation of internal and posterior echoes demonstrated that internal hypoechoic masses were composed of fibroblastic cells with marked collagenization in the stroma, analogous to cases in which carcinoma cells proliferated in a monotonous, solid and/or expanding manner. Attenuation of posterior echo was detected in the cases associated with hyperplasia of collagenized fibroblastic stroma. An increased cellularity in the mass with prominent large tumor nests and little fibrous stroma demonstrated the accentuation or no alterations of the posterior echo [11]. We considered that the cancer had increased rapidly in size because it was solid and high-grade. Kuroishi et al. [9] reported that the growth rate of breast tumors was associated with histological type. The geometric mean of the doubling time for papillotubular carcinoma was the longest, that for the solid-tubular carcinoma was the shortest, and that for scirrhous carcinoma was intermediate. More rapidly growing tumors were more malignant, suggesting that cells of rapidly growing tumors may be more active in cell proliferation, and may have a higher potential to metastasize [9,15].

Common findings for IMPC on US include a homogeneously hypoechoic, irregular or microlobulated mass with posterior acoustic shadowing or normal sound transmission. Axillary lymph nodes are frequently involved, especially if the mass is >2 cm [6]. In the present case, primary IMPC showed common US findings such as irregularly shaped and hypoechoic tumors with indistinct features. However, recurrent IMPC was different because the secondary lesion had hyperechoic internal areas, and the third lesion had homogeneous hypoechoic areas with posterior acoustic enhancement. US findings were different at each site because of differences in the manner of spread and the density of cancer cells.

According to one report of IMPC, the prevalence of lymph node metastasis was 71.4%, 5-year recurrence was 62.6%, 5-year survival was 50.5%, and the prognosis was poor compared with IDC [16]. A higher proportion of IMPC components within the tumor also suggested a poor prognosis [16]. Luna-More et al. [17] reported that IMPC showed similar lymph-node metastasizing capacity, with massive spread to axillary nodes. Also, the invasive capacity of IMPC to the lymphatic vessels was high.

We reported a case of recurrent IMPC in which the US findings differed at each site. Although we were not able to clarify the typical US findings of IMPC, our results suggest that US findings might change depending on the proportion and distribution of IMPC cells in the tumor, and/or the manner of spread of IMPC cells.

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Case Report

Kurume Medical Journal, 58, 87-90, 2011

Concurrent Lung Cancer in Non-Tuberculous Mycobacteriosis: Case Report

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Summary: An 82-year-old woman was admitted to our hospital after multiple round opacities were detected in chest X-rays performed during a routine health screening. *Mycobacterium avium* complex (MAC) was found in sputum cultures, and compatible pathological findings on biopsy confirmed pulmonary MAC infection, whereas biopsies from another opacity revealed adenocarcinoma of the lung.
Curative surgery for the lung cancer confirmed a concurrence of lung cancer and pulmonary MAC infection. Since the prevalence of both of these lung diseases is increasing, suspicion of concurrence is critical to provide appropriate care.

Key words lung cancer, mycobacteriosis, *Mycobacterium avium* complex

INTRODUCTION

Non-tuberculous mycobacteria (NTM) are widely distributed worldwide, and have gained attention as important pathogens among patients with compromised immunity such as AIDS. However, recent investigations have shown that lung diseases caused by NTM are common even in immunocompetent individuals [1]. *Mycobacterium avium* complex (MAC), in particular, is known to affect elderly women [2]. MAC lung infection in immunocompetent individuals presents with distinctive radiological patterns including fibrocavitary lesions and bronchiectasis with nodular opacities. However, it is becoming clear that NTM lung infection may also present radiological manifestations mimicking lung cancer [3,4]. Therefore, it is possible that these two conditions may coexist, and this raises challenges in terms of diagnosis and management.

CASE REPORT

An 82-year-old woman was referred to Asakura Medical Association Hospital for the evaluation of incidental findings on a chest radiograph, which was taken as a part of a routine health screening. She had no remarkable subjective symptoms. The patient was a life-long non-smoker, and had worked as a secretary until she retired. She denied occupational exposure to dust or soil. Her medical history included breast cancer at the age of 70, for which she underwent a curative left mastectomy. The patient was 146 cm tall and weighed 32.2 kg , and a physical examination was not remarkable except for bilateral minimum inspiratory crackles on auscultation of the thorax. Routine blood tests were not significant (Table). There were multiple round opacities in chest radiographs (Fig. 1). Computed tomography (CT) of the thorax revealed multiple nodular opacities and bronchiectasis in both lungs (Fig. 2A). Sputum culture gave positive results for myco-

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Abbreviations: CT, computed tomography; MAC, *Mycobacterium avium* complex, NTM, non-tuberculous mycobacteria; PCR, polymerase chain reaction.

TABLE
Laboratory tests

Biochemical		Complete Blood Count	
ALB [g/dl]	4.1	WBC [×100/μl]	53
AST(GOT) [U/l]	18	RBC [×10^4/μl]	449
ALT(GPT) [U/l]	8	Hb [g/dl]	13.9
LD(LDH) [U/l]	144	Hct [%]	44
ALP [U/l]	211	Plt [×10^4/μl]	30.7
γ-GTP [U/l]	13	Arterial Blood Gas (room air)	
BUN [mg/dl]	16	pH	7.434
CRE [mg/dl]	0.74	Pco2 [mmHg]	39.1
CRP [mg/dl]	0.09	Po2 [mmHg]	74.8
CA15-3 [U/ml]	3.2	HCO_3^- [mmol/l]	25.6
CEA [ng/dl]	3.3	B. E. [mmol/l]	1.4

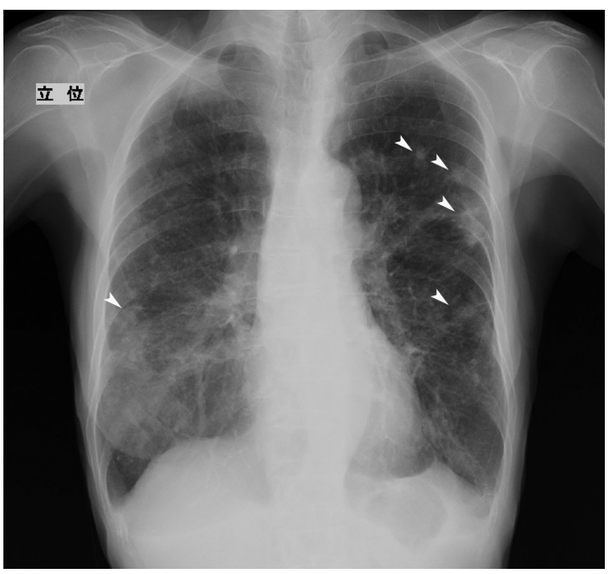

Fig. 1. Chest radiograph shows multiple round opacities in both lungs.

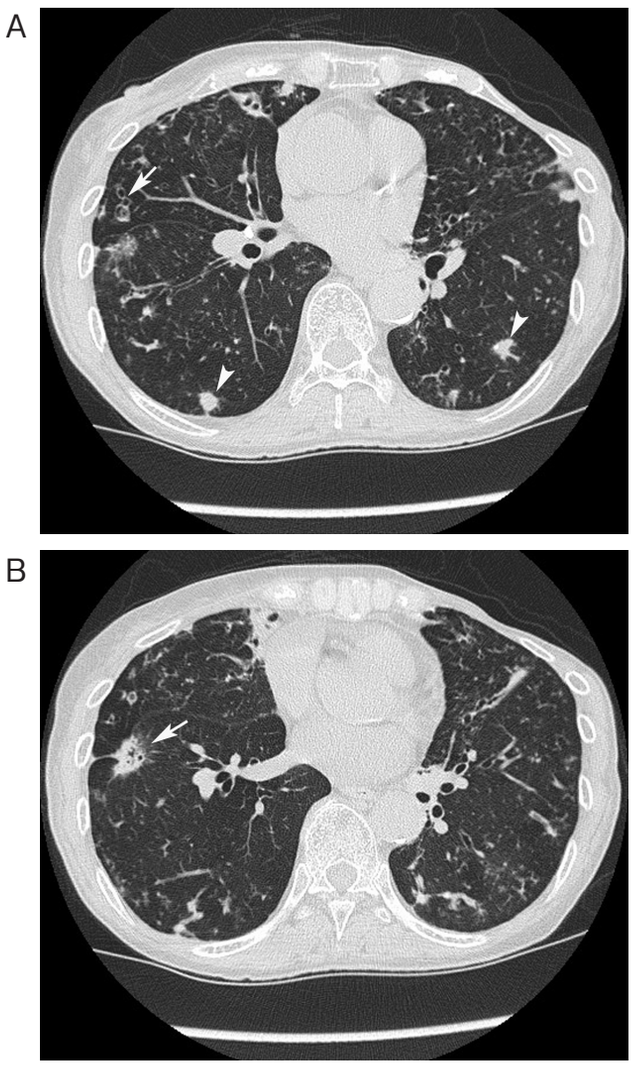

Fig. 2. Thoracic CT shows multiple nodular opacities (arrowheads) and bronchiectasis (arrow) (A). Another slice (B) shows spiculated opacity (arrow) in the right S8.

bacteria, which was identified as *M. intracellulare* by polymerase-chain reaction (PCR). Transbronchial lung biopsy specimens obtained from the left S^{1+2} demonstrated granulomatous lesions accompanied by multinucleated giant cells, suggesting mycobacterial infection. Since *M. intracellulare* was identified by PCR in the bronchial washings recovered in the same examination, she was diagnosed as having pulmonary MAC infection [1,5]. Cytology was negative in the specimen. However, another opacity in the right S^8 (Fig. 2B), showing radiological spiculation, was marked as possible lung cancer in the review of the thoracic CT, and another transbronchial lung biopsy was performed. As a result, the lesion was pathologically diagnosed as adenocarcinoma of the lung. Since there were no signs of mediastinal lymphadenopathy or distant metastasis, the patient underwent right lower lobectomy as a curative treatment for the lung cancer. Pathological studies of the excised right lower lobe confirmed a diagnosis of lung mycobacteriosis (Fig. 3A) coexisting with adenocarcinoma (Fig. 3B) staged pT1N0M0. Antimicrobial treatment with clarithromycin, rifampicin, and ethanbutol were initiated against pulmonary MAC infection, after which the relevant radiological findings improved (Fig. 4). There has been no evidence of recurrence or metastasis of lung cancer thus far.

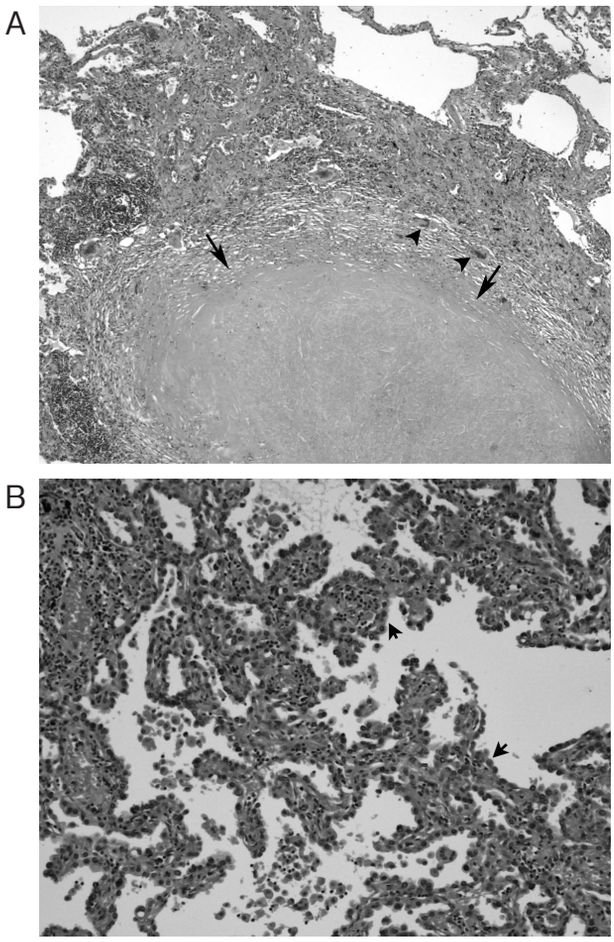

Fig. 3. Granulomatous inflammation characterized by central caseous necrosis (arrows) and multi-nucleated giant cells (arrwoheads) was noted, suggesting mycobacterial infection of the lung (A). Ziehl-Neelsen stain of the specimen identified mycobacteria in the granuloma (not shown). Another section demonstrating adenocarcinoma (arrows) replacing alveolar epithelium (B), suggestive of primary lung cancer.

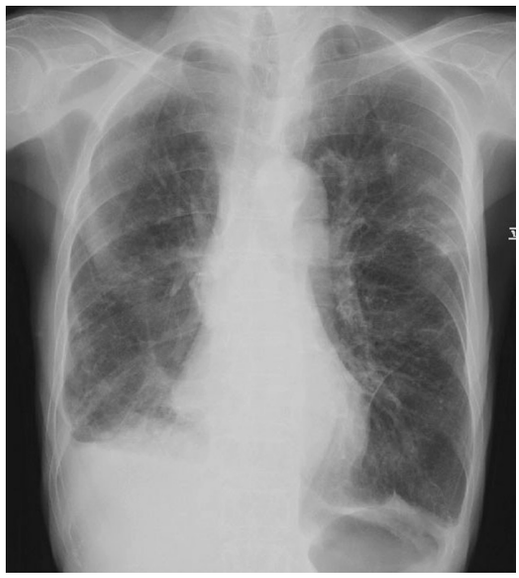

Fig. 4. Chest radiograph obtained two years after the initiation of the anti-mycobacterial treatment shows resolution of the multiple round opacities in both lungs.

DISCUSSION

The present patient illustrates the challenge of diagnosing lung diseases when cancer and MAC infection coexist. A study at a single institution in Japan reported that lung cancer coexisted in 1.8% of the patients with pulmonary NTM (including 660 MAC, 126 *M. kansasii*, and 19 *M. fortuitum*) infection [6]. The figure for a series of 302 patients with *M. kansasii* infection was 6%, and most of these were male smokers [7]. Since the prevalence of pulmonary NTM infections, especially MAC, is on the rise, the likelihood of encountering patients with these infections and concurrent lung cancer may increase. Although a causal association between lung cancer and pulmonary NTM infection is not clear, the high prevalence of lung cancer lesions at old *M. kansasii* sites suggested the possibility of scar tumor, similar to those after pulmonary tuberculosis [7].

Radiological examinations, (i.e. chest radiograph and CT), will probably provide the first clues in investigating these diseases, however, it is often difficult to distinguish them based on those tests alone. MAC infections typically manifest either cavitary lesions or fibronodular bronchiectasis in radiological examinations, with the latter being predominant among elderly women without immunosuppression [5]. The radiological manifestations of the present patient fall into this category, although nodules and masses were prominent. Importantly, pulmonary NTM infections can present radiological findings which resemble lung malignancies [4,8]. Even more advanced radiological modalities, such as fluorodeoxy-glucose positron emission tomography, which was not applied to the present patient, have a limited role in discriminating those conditions [9]. Therefore, at present, invasive procedures such as biopsy or surgical interventions in these patients are required to obtain an accurate diagnosis of the lung lesions [4].

Identification of the microorganisms in respiratory tract specimens such as sputum is essential in diagnosing mycobacteriosis [1]. Conversely, it is possible that positive results for mycobacteria may leave concur-

rent lung cancer unattended. The present patient presented positive results for *M. intracellulare* in sputum and bronchial washing cultures by PCR. These results in addition to the radiological and pathological findings established a diagnosis of pulmonary *M. intracellulare* infection. However, prominent spiculations of a lesion in the right S8 were suspicious for concomitant lung cancer and prompted us to perform another biopsy. Several radiological features may be useful in discriminating between benign and malignant lung lesions [10]. These features may be applicable when concurrent lung cancer is suspected in patients with mycobacteriosis. Interestingly, lung cancer was located in the ipsilateral lung in most of the cases of NTM infection, in contrast to patients with pulmonary tuberculosis in whom there is no predilection as regards cancer location [6]. According to a few reported descriptions of the radiological features of cases where lung cancer and pulmonary MAC infection coexisted, those lesions can be round [11], as in the present patient, or infiltrative [12], suggesting that there are no specific radiological presentations in these cases. Therefore, it would be advisable to pay close attention to the ipsilateral lung for possible coexisting lung cancer if the patient has NTM infection, although it is not rare for NTM infection to affect both lungs, as in the present patient. Other non-surgical modalities to detect concurrent lung malignancies may be needle biopsy and careful observation of chronological changes with/without appropriate antimicrobial agents. Nonetheless, a suspicion of concurrent malignancy is essential.

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Case Report

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Ectopic Pregnancy Occurred after Oocyte Intrauterine Transfer (OUT) - A Case Report -

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Summary: Although still an experimental procedure, it is hoped that oocyte intrauterine transfer (OUT) could become a convenient technique for initiating pregnancy. A 33-year old woman received OUT treatment after a period of infertility lasting for 3 years. Two weeks later the result of pregnancy test was positive, but shortly thereafter she complained of vaginal bleeding. Ultrasonography revealed a cystic lesion in her right adnexal area without any ascites. At laparotomy, a right side tubal pregnancy was confirmed. This is the first case report of ectopic pregnancy occurring after OUT. It was speculated that the OUT may have caused the tubal pregnancy. However, since the precise mechanism for embryonic implantation to the tubal epithelium is unknown, the causal relationship between OUT and tubal pregnancy remains unclear.

Key words oocyte transfer, ectopic pregnancy, invitrofertilization and embryotransfer, transvaginal hydrolaparoscopy

INTRODUCTION

Recent advances in artificial reproductive techniques (ART) have been encouraging. However, patients are usually required to make repeated visits to the hospital for follicular monitoring, hormone therapy and finally oocyte retrieval. The clinical application of ART is, therefore, expensive, time consuming, and highly stressful for the patient.

Against this backdrop, it has been suggested that oocyte intrauterine transfer (OUT) could become a convenient new procedure in the ART repertoire [1]. The results of previous studies describing same-day transfer of oocytes and spermatozoa to the uterus are promising [2,3]. Since actual experience with OUT is limited, however, its clinical value remains to be determined.

The fallopian tube plays an essential role in gamete transport, fertilization and the early development of the embryo [4], and damage to the fallopian tube and pelvic inflammatory diseases are usually regarded as major risks for ectopic pregnancy [5-7]. Hence, most infertile women requiring ART should have an increased risk for ectopic pregnancy. We present here the first ever report of a case of tubal pregnancy occurring after OUT, and briefly discuss the causal relationship between ART and tubal pregnancy.

CASE REPORT

A 33-year old null-gravid woman suffered from infertility lasting for 3 years. Her husband already had 3 children with a former wife, suggesting his normal reproductive capacity. Her hormonal profiles of the pituitary-gonadal axis were not remarkable. Hysterosalpigography revealed that both tubes were blocked, indicating tubal atresia. Transvaginal hydrolaparoscopy also showed perifallopian tube adhesion, presumably due to chlamydia trachomatis infection (Fig. 1 and Fig. 2). OUT was performed after obtain-

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Abbreviations: OUT, oocyte transfer; ART, artificial reproductive technique; IVF-ET, invitrofertilization and embryotransfer; THL, transvaginal hydrolaparoscopy.

ing informed consent from the patient and approval for the procedure by the institutional review board. Short-term administration of gonadotropin releasing hormone (GnRH) agonist was used to achieve controlled ovarian stimulation. When the diameter of dormant follicles reached about 2 cm, 5,000 IU human chorionic gonadotropin (HCG) was administered. Transvaginal oocyte retrieval was performed 35 h after the HCG administration. Eight oocytes were retrieved and incubated for 5 h in a fertilization medium (Quinn TM, Australia) in 5% CO_2 and 80% Nitrogen gases at 37°C. According to the recommendations of the Japan Society of Fertility and Sterility [8], three oocytes were transferred to the uterus using an echo marked tip (ET) catheter after sexual intercourse. Fourteen days after OUT, she complained of a spotting bloody discharge, and showed serum HCG of 333 mIU. At day 21 following OUT, although the level of serum HCG elevated to 1,800 mIU, no intrauterine gestational sac could be identified. Instead, a cystic lesion was observed in her right adnexal area without ascites, and the genital bleeding continued. She finally underwent a laparotomy to confirm the right tubal pregnancy (Fig. 3 and 4), and chorionic villi were observed in the resected tissue (Fig. 5). The serum level of HCG then decreased to 2.0 mIU within two weeks.

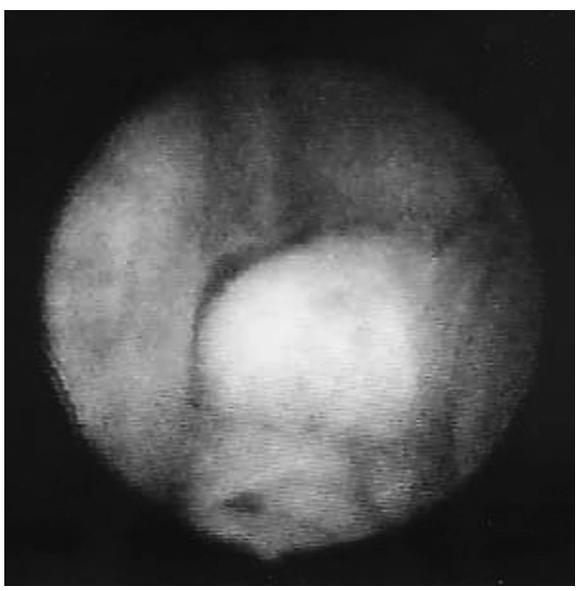

Fig. 1. Transvaginal hydrolaparoscopy, showing peritubal adhesion with sigmoid colon of the left Fallopian tube.

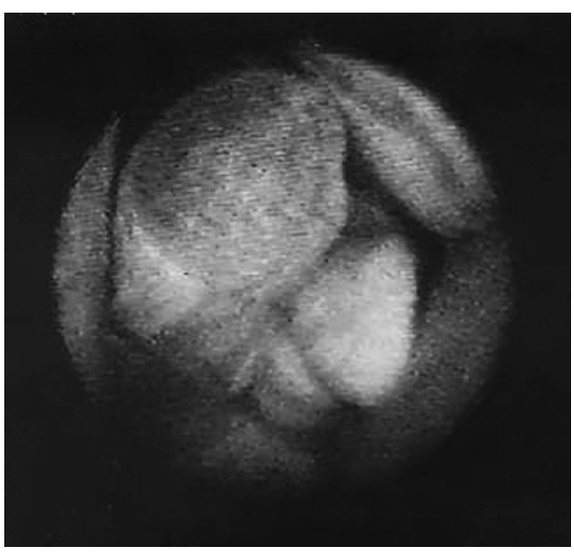

Fig. 2. Transvaginal hydrolaparoscopy. The balloon-like swelling was observed in the right side Fallopian tube.

Fig. 3. At laparotomy, the Fallopian tube swelling was revealed.

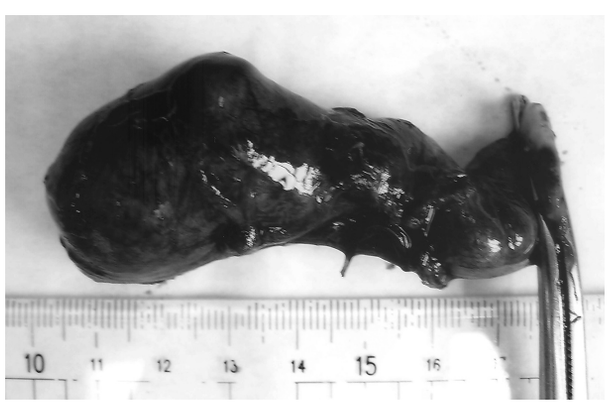

Fig. 4. In the resected tissue, the Fallopian tube was filled with coagulated blood.

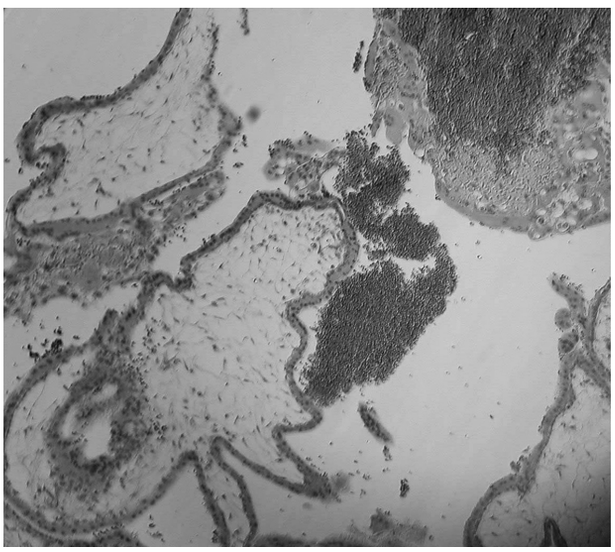

Fig. 5. In histological examination, chorionic villi were observed in the resected tissue. Original magnification 100×(HE)

DISCUSSION

Unfortunately, our clinical trial of OUT for an infertile patient resulted in the occurrence of tubal pregnancy, and the OUT itself seemed to be a likely cause of the ectopic pregnancy. However, it remains uncertain whether the tubal pregnancy resulted from the OUT or from other factors.

A number of risk factors for ectopic pregnancy are known in humans, and ART also may increase the risk of tubal pregnancy. However, in approximately one-third [9] to one half [10] of ectopic pregnancies, no risk factors can be identified.

The era of ART began in 1978 with the first report of in vitro fertilization [11]. The earliest record of ectopic pregnancy was described by Abu al-Qasim al-Zahrawi, the father of modern surgery, in 963 [12], indicating that ART was not essential for the development of tubal pregnancy.

Current knowledge of the aetiology of tubal ectopic pregnancy has focused on embryo-tubal transport. Effective tubal transport of ova, sperm and embryo is a prerequisite for successful spontaneous pregnancy, and transport of the embryo through the Fallopian tube is controlled by smooth muscle contraction and cilliary beating [13,14].

The interaction between the genital tract and developing embryo is not fully understood, and tubal pregnancy is currently thought to be caused by a combination of retention of the embryo within the Fallopian tube due to impaired embryo-tubal transport and alterations in the tubal environment allowing early implantation to occur.

Embryonic implantation consists of three related and consecutive phases: apposition, adhesion between trophoectoderm and endometrial epithelium, and invasion. In order to establish pregnancy, the initial apposition and adhesion of the blastocyst to maternal endometrium must occur in a coordinated manner.

Among animal species, tubal ectopic pregnancy would appear to be restricted to primates. Given the striking absence of tubal ectopic pregnancy in other mammals, attention has been focused on the fact that the compositions of tubal fluid and uterine fluid are similar in primates [15]. However, the roles played by tubal fluid and uterine fluid in human embryonic implantation are not clear [16].

The mechanisms responsible for ectopic pregnancy, thus, are still largely unknown. Patients considering ART should be informed of the increased risk for ectopic tubal pregnancy.

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Case Report

Kurume Medical Journal, 58, 95-98, 2011

Successful Treatment of T4 Renal Cell Carcinoma After a Neoadjuvant Targeted Therapy Using Sunitinib: Report of a Case

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Summary: Since the introduction of targeted therapy, treatment of metastatic renal cell carcinoma (RCC) has undergone dramatic changes. Responses to targeted therapy within the primary tumor and metastatic lesions are novel findings not seen with immunotherapeutic-based strategies. We report here a case of T4 RCC in which cytoreductive nephrectomy became possible after a neoadjuvant targeted therapy using sunitinib. Our experience with the present case suggests that targeted therapy in the neoadjuvant setting may have a variety of potential applications. Further investigations should be encouraged.

Key words renal cell carcinoma, sunitinib, tyrosine kinase inhibitors, neoadjuvant therapy

INTRODUCTION

Sunitinib (multitargeted tyrosine kinase inhibitor) treatment has shown significant improvement in the overall survival of patients with metastatic renal cell carcinoma (RCC) as well as significant shrinkage of both metastases and the primary tumor [1]. Some recent publications have suggested promising results with neoadjuvant therapy, including partial remission of the primary tumor, vena cava thrombus, and lymph node metastases [2-7]. We present the case of a patient who underwent successful resection of T4 RCC after a neoadjuvant targeted therapy using sunitinib, and review the pertinent literature.

CASE REPORT

A 70-year-old man developed lumbago and pain in both lower extremities and consulted the orthopedics department at Kurume University Hospital. Supine magnetic resonance imaging (MRI) detected a metastatic lesion of the fourth lumbar vertebra and bone biopsy of the site confirmed metastasis from RCC. The patient was referred to our department in September 2008. Computer tomography (CT) scan and MRI of the abdomen found a tumor measuring 108 mm in diameter at the middle pole of the right kidney with direct invasion of the liver (Fig. 1A, B). Thoracic CT scan showed several nodules with a maximum diameter of 22 mm in the lung (Fig. 1C). The RCC was classified as clinical stage T4N0M1 according to TNM classification. Due to pain, his performance status was considered as 3 on admission. Laboratory studies showed slight anemia (Hb:11.5 g/dl) and mild liver dysfunction (AST/ALT:56/48 U/ml). The patient was classified as intermediate risk according to the Memorial Sloan-Kettering Cancer Center (MSKCC) criteria. The patient first received palliative radiotherapy (2 Gy×10 times) for bone metastasis, and then was administered sunitinib 50 mg daily for 4 wks, repeated at 6 wk intervals. After four cycles of treatment, a 28.5% regression of the tumor was observed on CT based on the response evaluation criteria in solid tumors (RECIST), and invasion of the liver and lung metastasis had disappeared (Fig. 2A, B). Although grade 2 hypothyroidism, thrombocyto-

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Abbreviations: CT, computer tomography; MRI, magnetic resonance imaging; RCC, renal cell carcinoma; TKI, tyrosine kinase inhibitor.

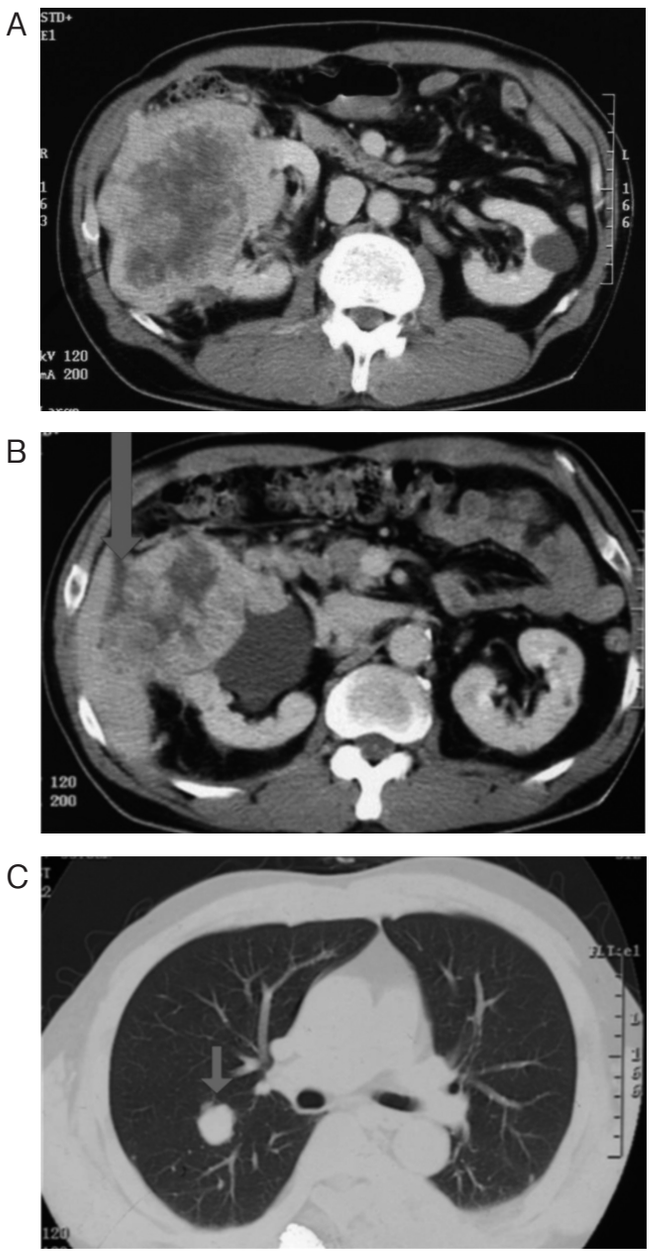

Fig. 1 (A, B, C). CT scan at diagnosis. Abdominal CT revealing a 108-mm diameter heterogeneously enhanced mass at the middle pole of the right kidney (A) with direct invasion of the liver (B). Thoracic CT scan showing several nodules with a maximum diameter of 22 mm in the lung (C).

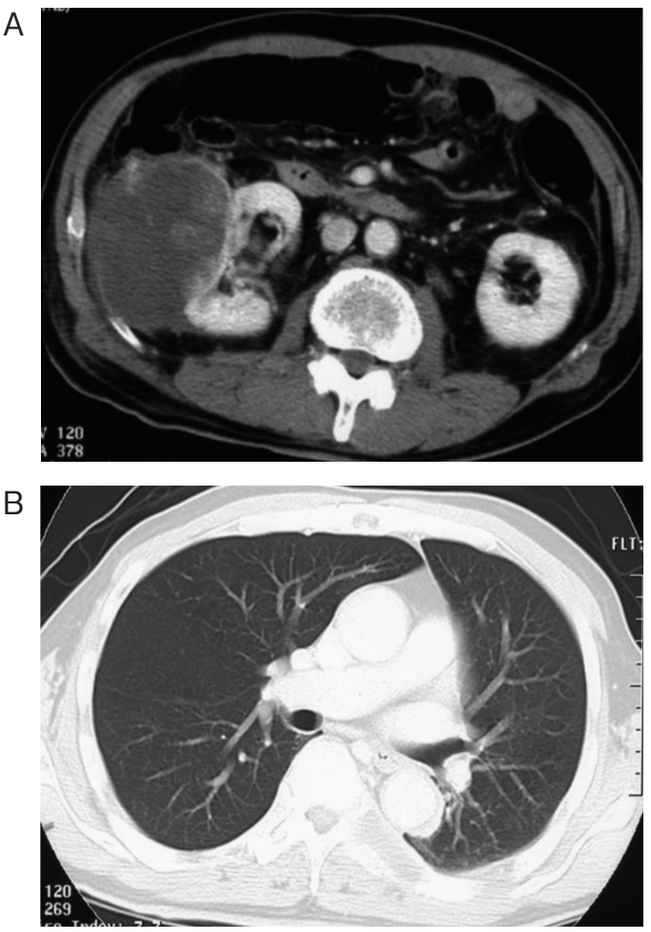

Fig. 2 (A, B). Abdominal CT after 4 cycles of sunitinib showing 28.5% tumor reduction according to RECIST criteria (A) and lung metastasis had disappeared (B).

penia and hand-foot syndrome were observed during the treatment, sunitinib administration was well tolerated with no grade 3/4 adverse treatment-related events. Radical nephrectomy was performed by an abdominal approach. During the nephrectomy, tight perirenal tissues, and adherence to the abdominal walls and surrounding liver were observed, but there was no direct invasion of the liver. Operation time was 4 h 40 min and estimated blood loss was 1485 ml. There were no complications during the perioperative period. Macroscopic examination of the right kidney showed a middle-pole tumor with significant necrosis (Fig. 3). Pathological examination showed a 90 mm clear cell type of RCC. The tumor had a membrane structure, and capsular invasion of the tumor was not observed although a few remaining viable cells were detected around the edge of the tumor (Fig. 4). Finally, pathological examination indicated complete resection of the primary renal tumor. The surgical wound healed well without complications. A bone scan at 3 months after surgery indicated a slight increase in uptake at the metastatic bone lesion, so zoledronic acid was administered postoperatively. The patient has been doing well for 2 years postoperatively without evidence of progression.

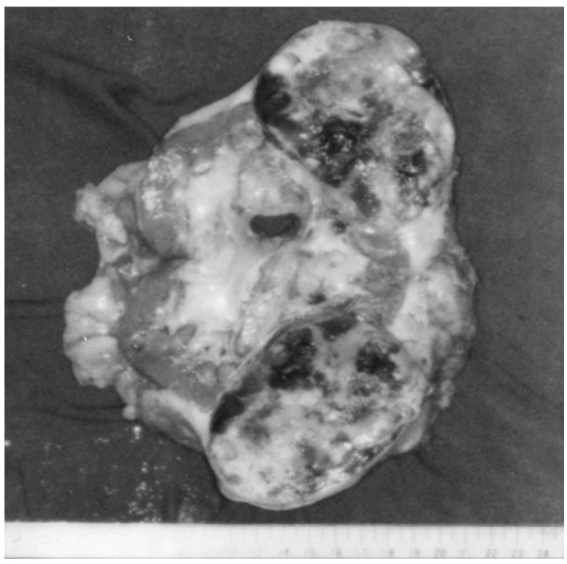

Fig. 3. Macroscopic appearance of the resected specimen showing the right renal tumor with significant tumor necrosis.

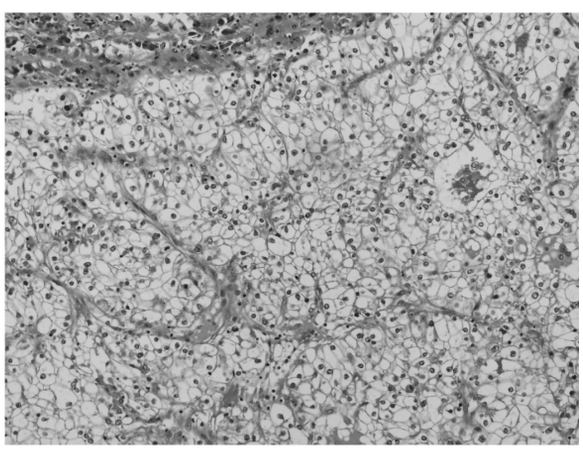

Fig. 4. Microscopic appearance of tissue slides from the renal tumor. A few viable cells remained around the edge of the tumor, but there were no cancer cells in the surgical margin (HE ×100).

DISCUSSION

Neoadjuvant therapy is commonly used to downstage locally advanced tumors and to improve survival. In patients with RCC, neoadjuvant studies have been limited during the cytokine era because of poor responses in the primary tumor and the significant toxicity associated with treatment [8]. Compared with immunotherapeutic-based therapy, targeted therapy has higher response rates in both primary and metastatic sites, and a favourable safety profile. These findings suggest that the concept of neoadjuvant therapy in RCC should be revived, particularly in patients deemed to have unresectable disease. Furthermore, neoadjuvant therapy might extend beyond these uses to patients with localized or advanced disease.

The present case illustrates the potential of neoadjuvant targeted therapy in the surgical management of RCC. The patient had T4 RCC with a direct invasion of liver and metastasis to lung and bone. The administration of sunitinib was successful in decreasing the primary tumor and lung metastasis, and making it possible to achieve a complete resection. One of the primary concerns of neoadjuvant targeted therapy is the potential to make conditions worse for surgical resection because of bleeding complications or poor wound healing. Recent publications have reported interesting effects of sunitinib as neoadjuvant therapy [6,7]. Lauren reported that pre-surgical tyrosine kinase inhrbitor (TKI) use was associated with increased incidence and grade of intra-operative adhesions but was not associated with a significant increase in peri-operative complications or peri-operative bleeding [9]. In the present case, surgery was well tolerated with low peri-operative morbidity, despite the fact that surgery was performed within 1 week after the cessation of sunitinib. Moreover, compared with our institutional standards, the operative time and surgical approach were not affected by the use of sunitinib. Additional studies are needed to clarify the optimal timing of the discontinuation of the drug, because sunitinib, sorafenib and other targeted drugs have different half-lives. The half-life for sorfenib is 25-48 h [10], while sunitinib and its active metabolite have a half-life of 40-60 h and 80-100 h, respectively [11].

Currently, several phase II trials are ongoing to determine appropriate patient selection and timing of cytoreductive nephrectomy in patients with metastatic RCC treated with sunitinib or other targeted drugs [12]. Our observations support the suggestion that sunitinib could prove useful in the neoadjuvant setting by facilitating surgical procedures. Future clinical trials are needed to assess the use of neoadjuvant therapy in both localized and advanced RCC.

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