Table 4 Treatment results and outcomes of all patients

Patients	Age	Stage	Cell type	Regimen	Cycles	Responses	Surgery	Adjuvant	Follow-up period (months)	PFS (months)	OS (months)	Outcome
1	52	IIb	ASC	DC	2	CR	Incomplete	CCRT	21	12	21	DOD
2	50	Ib2	MAC	TC	2	CR	Complete	NT	90	90	90	NED
3	55	IIb	ASC	DC	2	CR	Complete	CT	62	62	62	NED
4	39	Ib2	MAC	DC	3	CR	Complete	CT	51	11	51	AWD
5	36	Ib2	MAC	DC	2	CR	Complete	CT	22	22	22	NED
6	32	IIb	ASC	DC	3	PR	Incomplete	NT	19	11	19	DOD
7	49	Ib2	MAC	DC	2	PR	Complete	CCRT	78	78	78	NED
8	60	IIb	ASC	DC	2	PR	Complete	NT	68	30	68	AWD
9	54	IIb	EDC	TC	1	PR	Complete	CT	68	68	68	NED
10	40	Ib2	MAC	TC	2	PR	Complete	CT	67	67	67	NED
11	38	IIb	MAC	DC	2	PR	Complete	CT	9	6	9	DOD
12	63	IIb	CCC	DC	2	PR	Complete	RT	48	48	48	NED
13	50	IIb	EDC	DC	2	PR	Complete	CT	35	35	35	NED
14	53	Ib2	EDC	DC	2	PR	Complete	CT	38	38	38	NED
15	54	IIb	MAC	DC	2	PR	Complete	CT	29	29	29	NED
16	52	IIb	MAC	TC	3	PR	Incomplete	CT	27	7	27	DOD
17	45	IIb	EDC	DC	2	PR	Complete	CT	10	10	10	NED
18	51	IIb	EDC	DC	3	PR	Complete	CT	13	13	13	NED
19	45	IIb	MAC	DC	2	SD	Incomplete	CT	10	3	10	DOD
20	52	IIb	ASC	DC	2	SD	Complete	CT	13	6	13	DOD
21	56	IIb	ASC	DC	2	SD	Complete	CCRT	56	56	56	NED
22	61	IIb	ASC	DC	3	SD	Incomplete	RT	35	8	35	DOD
23	45	IIb	MAC	DC	2	SD	Complete	CT	26	26	26	NED

ASC adenosquamous cell carcinoma, *MAC* mucinous adenocarcinoma, *EDC* endometrioid adenocarcinoma, *CCC* clear cell adenocarcinoma, DC docetaxel + carboplatin, *TC* paclitaxel + carboplatin, *CR* complete response, *PR* partial response, *SD* stable disease, *NT* no treatment, *CT* chemotherapy, *RT* radiotherapy, *CCRT* concurrent chemoradiation therapy, *PFS* progression-free survival, *OS* overall survival, *NED* no evidence of disease, *AWD* alive with disease, *DOD* died of disease

In the analysis of adverse events, severe neutropenia developed in 91.3 % of patients, but subsided in response to short-term treatment with a G-CSF preparation. During the first course of DC therapy, grade 3 febrile neutropenia developed in 2 cases; the dose of both agents was reduced for the next course of treatment. All signs, specific to taxanes, of peripheral neuropathy were grade 1 or less, allowing for continuation of treatment while preserving the quality of life of the individual patients. No serious adverse events occurred, and the response rate was 78.3 %. This study demonstrated a high response rate of bulky non-squamous cell carcinoma of the cervix to NAC using taxanes (paclitaxel or docetaxel) and carboplatin. It also demonstrated the safety of the medications in this regimen. The completion rate of radical hysterectomy, however, was only 78.3 %; thus, the treatment outcomes in this study were not satisfactory. Possible reasons for the low surgery completion rate include the rapid progression of non-squamous cell carcinoma, frequent invasion of tissues and organs surrounding the uterus, and frequent lymph node metastasis.

The treatment results and outcomes of all patients were shown in Table 4. Unfortunately, all patients with incomplete surgery ultimately experienced disease recurrence and died of their primary disease. Thus, the significance of NAC at present may not be to prolong survival time. Instead, in our view, NAC should be performed to fully optimize patients' conditions with its antitumor effect in order to improve the chances of complete surgery. Further study is needed regarding the long-term outcomes of NAC.

Conflict of interest The authors have no conflict of interest to declare.

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Original Articles

Efficacy of neoadjuvant chemotherapy followed by radical hysterectomy in locally advanced non-squamous carcinoma of the uterine cervix: a retrospective multicenter study of Tohoku Gynecologic Cancer Unit

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Summary

Objective: Radical hysterectomy (RH) is a standard treatment for locally advanced non-squamous cell carcinoma (N-SCC) of the uterine cervix, but there have been no reports on whether neoadjuvant chemotherapy (NAC) followed by radical hysterectomy could improve the outcome of patients with this disease. *Materials and Methods:* This multicenter retrospective study enrolled 77 patients with Stage IB2 to IIB N-SCC of the uterine cervix. Of these, 27 patients were treated with NAC prior to radical hysterectomy (NAC group) and 50 with RH alone (RH group). The two-year recurrence-free survival (RFS) rate, progression-free survival (PFS), and overall survival (OS) were compared between the two groups. Clinical parameters such as clinical stage, histological type, and post-operative treatment were also examined between the groups. *Results:* While the two-year RFS rates were 81.5% and 70.0% in NAC and RH groups, respectively ($p = 0.27$) and the median PFS was 51 months and 35 months in NAC and RH groups, respectively ($p = 0.35$), the median OS was 58 months and 48 months in NAC and RH groups, respectively, which was significant ($p = 0.0014$). The median OS of patients with mucinous adenocarcinoma in NAC group was significantly higher than that in RH group: 58 months versus 37 months ($p = 0.03$). *Conclusion:* NAC prior to RH may offer the prognostic advantage of patients with locally advanced N-SCC of the uterine cervix, especially mucinous adenocarcinoma.

Key words: Uterine cervical carcinoma; Non-squamous cell carcinoma; Neoadjuvant chemotherapy; Radical hysterectomy; Outcome.

Introduction

Radical hysterectomy and radiotherapy are a traditional therapeutic modality for invasive carcinoma of the uterine cervix in Japan. Since some observations showed that chemo-radiotherapy with cisplatin offered the advantage of clinical outcome in locally advanced carcinoma of the uterine cervix, chemotherapy has become the treatment of preference of uterine cervical carcinoma [1-7]. The Italian multicenter randomized study, which enrolled patients with locally advanced Stage IB2 to IIB squamous cell carcinoma of the uterine cervix, showed that NAC prior to RH improved the patient outcome as compared to conventional radiation therapy alone [8]. Combination of docetaxel and carboplatin in the neoadjuvant setting for patients with advanced or recurrent uterine cervical malignancy showed complete or partial response in all of patients with uterine cervical adenocarcinoma, suggesting that the combination may be quite promising for treatment of uterine cervical adenocarcinoma [9]. However, there is no evidence that NAC improves the outcome of patients with uterine cervical adenocarcinoma. The aim of this multicenter study was to retrospectively evaluate whether NAC can improve the outcome of patients with locally advanced N-SCC of the uterine cervix.

Materials and Methods

This study enrolled 77 patients with Stage IB2 to IIB N-SCC of the uterine cervix who underwent RH at the institutions belonging to the Tohoku Gynecologic Cancer Unit (TGCU) between January 1996 and December 2008. Of these, 27 patients were treated with NAC prior to RH (NAC group), and 50 patients were treated with RH alone (RH group). The two-year recurrence-free survival (RFS) rate, progression-free survival (PFS), and overall survival (OS) were compared between the two groups. Clinical parameters, such as: clinical stage, histological type, and postoperative treatment were also examined between the groups.

The PFS and OS in the two groups were calculated by the Kaplan-Meier method, and the statistical significance of differences in the cumulative curves between the two groups was evaluated by log-rank test. Categorical variables comparisons were conduced by two-tailed Chi square or Mann-Whitney U test where appropriate. A result was deemed significant at $p < 0.05$.

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Results

Patient characteristics

The median age was 49 and 45 years in NAC and RH groups, respectively. Eleven (40.7%) and 29 (58.0%) patients had Stage IB2 disease in NAC and RH groups, respectively, and 16 (59.3%) and 21 (42.0%) patients had Stage II disease NAC and RH groups, respectively. In regard to the histological type, 13 patients had mucinous adenocarcinoma, four had endometrioid adenocarcinoma, three had clear cell carcinoma, and seven had adenosquamous carcinoma in the NAC group, while 27 patients had mucinous adenocarcinoma, nine had endometrioid adenocarcinoma, two had clear cell carcinoma, nine had adenosquamous carcinoma, and three had other types in RH group. Of the 27 patients in NAC group and 50 in RH group, 19 (70.4%) and 40 (80.0%) underwent any postoperative treatments, respectively (Table 1).

Table 1. — *Patient characteristics.*

Variable	NAC (n = 27)	RH (n = 50)	p value
Median age in years [range]	49 [30-63]	45 [25-76]	$p = 0.85$*
Stage			
IB2	11 (40.7)	29 (58.0)	$p = 0.15$**
II			
IIA	0	6 (12.0)	
IIB	16 (59.3)	15 (30.0)	
Histological type			
Adenocarcinoma			
mucinous	13 (48.1)	27 (54.0)	$p = 0.98$**
endometrioid	4 (14.9)	9 (18.0)	
clear cell	3 (11.1)	2 (4.0)	
Adenosquamous carcinoma	7 (25.9)	9 (18.0)	
Others	0	3 (6.0)	
Adjuvant therapy			
administered	8 (29.7)	10 (20.0)	$p = 0.34$**
not administered			
Chemotherapy	9 (33.3)	16 (32.0)	
Chemoradiation therapy	5 (18.5)	14 (28.0)	
Radiotherapy	5 (18.5)	10 (20.0)	

*Mann-Whitney U test, **Chi-square test, numbers of parenthesis represent %.

NAC regimens and number of cycles

Because this was a retrospective and multicenter study, the combination of anti-cancer agents utilized was heterogeneous as shown in Table 2. Of the 27 patients in NAC group, eight received DC: seven patients received two cycles and one patient received three cycles. Five patients received cisplatin alone. Four patients received MEP: one patient received one cycle, two patients received two cycles, and one patient received three cycles. Three patients received TC of two cycles. Three patients received FCAP: one patient received one cycle and two patients received three cycles. Other four patients received cisplatin/CPT-11 of two cycles, cisplatin/Adriamycin of two cycles, cisplatin/mitomycin C of three cycles, and carboplatin/actinomycin D of three cycles, respectively.

Table 2. — *List of NAC regimens.*

Regimen	No. of patients
DC (Docetaxel 70 mg/m², carboplatin AUC6 day 1 q21 days)	8
Cisplatin alone (total 200 mg/body for 3 days)	5
MEP (MMC 10 mg/m² day 1, etoposide100 mg/m² days 1,3,5, cisplatin 50 mg/m² day 1, q28 days)	4
TC (Paclitacel 175 mg/m², carboplatin AUC6 day 1 q21 days)	3
FCAP (5-FU 200 mg/body, CPM100 mg/body, cisplatin 20 mg/m² days 1-7, ADM 35 mg/m² day 7)	3
Cisplatin/CPT-11 (cisplatin 70 mg/m² day 1, CPT-11 70 mg/m² days 1,8 q21 days)	1
Cisplatin/ADM (cisplatin 100 mg/body, ADR 40 mg/body days 1,2 q21 days)	1
Cisplatin/MMC (cisplatin 50 mg/body, MMC 4 mg/body day 1 q21 days)	1
Carboplatin/Actinomycin D (Carboplatin 300 mg/body, Actinomycin D 1.5 mg/body day 1 q21 days)	1

MMC: mitomycin C; CPM: cyclophosphamide; ADM: adriamycin.

Comparison of clinical outcome between NAC and RH groups

The two-year RFS rate was 81.5% in NAC group and 70.0% in RH group ($p = 0.27$, Table 3). The median PFS was 51 months (range, 14-157 months) in NAC group and 35 months (range, 4-157 months) in RH group ($p = 0.35$, Table 3). On the other hand, the median OS was 58 months (range, 15-157 months) in NAC group and 48 months (range, 9-157 months) in RH group, which was significant ($p = 0.0014$, Table 3 and Figure 1A).

Table 3. — *Comparison of the clinical outcome between the two groups.*

	NAC (n = 27)	RH (n = 50)	p value
Two-year RFS rate	81.5% (22/27)	70.0% (35/50)	$p = 0.27$
Median PFS (range)	51 months (14-157)	35 months (4-157)	$p = 0.35$
Median OS (range)	58 months (15-157)	48 months (9-157)	$p = 0.0014$

RFS: recurrence free survival; PFS: progression-free survival; OS: overall survival.

Comparison of clinical outcome according to clinical parameters

There were no significant differences in the median PFS and OS between NAC and RH groups according to stage, histological type and adjuvant therapy, except mucinous adenocarcinoma (Table 4). While the median PFS of patients with mucinous adenocarcinoma was 58 months (range, 8-124 months) in NAC group and 33 months (range, 4-125 months) in RH group ($p = 0.34$), the median OS of those with mucinous adenocarcinoma was 58 months (range, 24-124 months) in NAC group and 37 months (range, 9-125 months) in RH group, which was significant ($p = 0.03$) (Table 4 and Figure 1B).

Clinical outcome according to therapeutic modality after NAC and radical surgery

The outcome of patients who underwent chemotherapy or chemoradiotherapy or radiotherapy after NAC and RH were compared. As shown in Table 5, chemotherapy after NAC and surgery prolonged PFS and OS, and increased

Table 4. — *Comparison of the clinical outcome according to clinical parameters.*

Clinical parameters	Median PFS			Median OS		
	NAC	RH	*p* value	NAC	RH	*p* value
Stage						
IB2	64 (11-157)	37 (9-157)	0.26	64 (15-157)	54 (12-157)	0.26
II	33 (4-124)	45 (4-92)	0.45	39 (16-124)	45 (9-92)	0.40
Histological type						
Adenocarcinoma						
mucinous	58 (8-124)	33 (4-125)	0.34	58 (24-124)	37 (9-125)	0.03
endometrioid	31 (10-97)	70 (14-97)	0.29	31 (10-97)	70 (20-97)	0.49
clear cell	22 (4-108)	64 (12-106)	0.89	22 (16-108)	83 (60-106)	0.41
Adenosquamous	36 (12-157)	45 (12-157)	0.11	43 (21-157)	46 (12-157)	0.31
Adjuvant therapy						
administered	77 (25-157)	31 (5-157)	0.18	77 (35-157)	32 (12-157)	0.24
not administered	33 (4-124)	47 (4-106)	0.66	39 (16-124)	51 (9-106)	0.61

Numbers show months: Parenthesis means range. PFS: progression-free survival; OS: overall survival.

Table 5. — *Clinical outcome according to therapeutic modality after NAC and radical surgery.*

	Chemotherapy (n = 9)	Chemoradiotherapy (n = 5)	Radiotherapy (n = 5)
PFS (months)	42 (10-108)	30 (4-76)	22 (8-97)
OS (months)	42 (19-108)	30 (16-76)	31 (22-97)
Two-year RFS rate	88.9%	60.0%	60.0%

PFS: progression-free survival; OS: overall survival; RFS: recurrence free survival.

the two-year RFS rate compared to chemoradiotherapy or radiotherapy after NAC and surgery, although they did not reach significance.

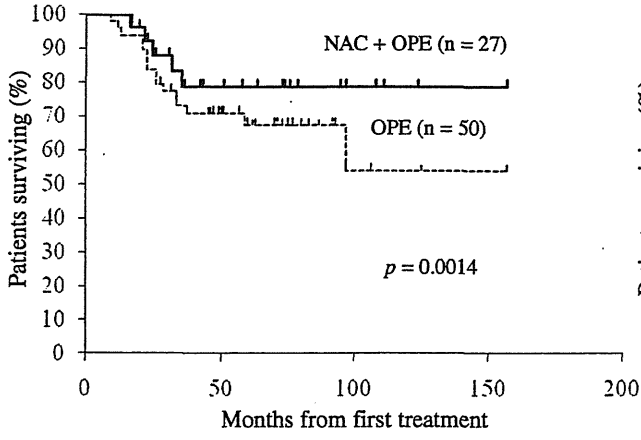
Discussion

Numerous phase II studies have reported the favorable effects of NAC in the treatment of locally advanced carcinoma of the uterine cervix. The authors have previously reported the efficacy and safety of NAC with cisplatin plus irinotecan in this disease [10]. However, few randomized clinical trials (RCT) have evaluated the effect of NAC in the clinical outcome of patients with this disease. Sardi *et al.* reported a significant improvement of the seven-year survival rate in patients treated by NAC and radical surgery and radiotherapy (65%), as compared with that in those treated by radical surgery and radiotherapy (41%) in a four-arm randomized controlled trial (RCT) (NAC and radical surgery and radiotherapy, radical surgery and radiotherapy, radiotherapy alone, and NAC and radiotherapy) [11]. However, the retrospective study did not show obvious improvement of the five-year survival rate in patients with Stage IB2 carcinoma of the uterine cervix treated by NAC prior to surgery, as compared with that in those treated by surgery alone (80% versus 69%) [12]. These two reports were conducted in patients with SCC of the uterine cervix. Since N-SCC of the uterine cervix has recently increased in Japan, it is an important issue to evaluate the effectiveness of NAC in the outcome of patients with N-SCC of the uterine cervix. Some evidence showed that the outcome of patients with N-SCC of the uterine cervix was poorer than that of patients with SCC of the uterine cervix [13, 14], because of the higher incidence of lymph node metastases at a relatively early stage of the disease, and a lower sensitivity to radiotherapy in N-SCC of the uterine cervix [15, 16]. Chemotherapy is therefore expected to have a greater beneficial effect on the outcome of patients with N-SCC than radiotherapy or chemoradiation therapy. Because the present study was conducted retrospectively in multicenters, the combination of anti-cancer agents used was heterogeneous. The NAC regimens used in this study invariably included one of platimun derivatives, such as cisplatin and carboplatin, so platimun agents seem favorable for chemotherapy prior to surgery in N-SCC of the uterine cervix.

The two-year RFS rate and the median PFS were better in NAC group than in RH group, which were not significant, whereas the median OS in NAC group was significantly longer than in RH group ($p = 0.0014$). Furthermore, prognostic analysis in clinical parameters showed that the median OS of patients with mucinous adenocarcinoma in NAC group was significantly longer than in RH group ($p = 0.03$), although other histological types and postoperative treatment did not significantly affect the prognosis of patients between NAC and RH groups. These results suggest that NAC may offer the prognostic advantage of patients with locally advanced N-SCC of the uterine cervix, especially mucinous adenocarcinoma. Because mucinous adenocarcinoma accounts for approximately 70% out of adenocarcinomas of the uterine cervix, NAC may improve prognosis of patients with N-SCC of the uterine cervix, although NAC should be used individually at the present time.

The present study showed that chemotherapy after NAC and surgery prolonged PFS and OS, compared to chemoradiotherapy or radiotherapy after NAC and surgery, which did not reach significance. Tattersall *et al.* reported that primary chemotherapy followed by radiotherapy significantly decreased the survival rate of patients with uterine cervical carcinoma compared to those who were treated by radiotherapy alone [17]; furthermore, meta-analysis showed that chemotherapy followed by radiotherapy did not improve the survival time

ig. 1A



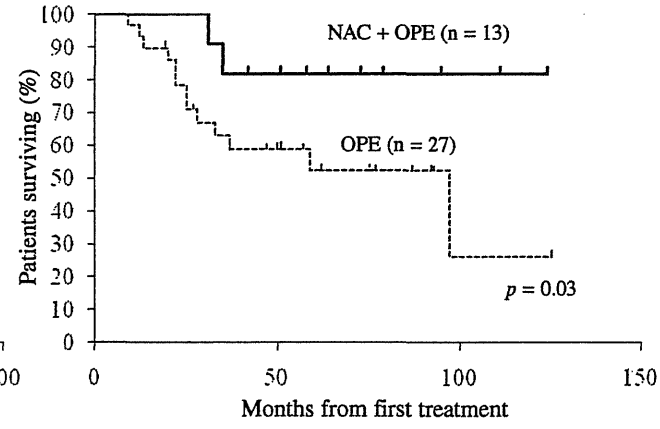


Fig. 1B

Figure 1. — 1A: overall survival in all patients who underwent neoadjuvant chemotherapy followed by radical hysterectomoy (NAC) or radical hysterectomy alone (RH). 1B: overall survival in patients with mucinous adenocarcinoma who underwent neoadjuvant chemotherapy followed by radical hysterectomoy (NAC) or radical hysterectomy alone (RH).

in uterine cervical carcinoma [18]. Considering these reports together with the present results, chemoradiotherapy or radiotherapy after NAC and surgery may contribute to unfavorable outcome of the patients with uterine cervical adenocarcinoma compared to chemotherapy after NAC and surgery, although further investigation is necessary to confirm the appropriate therapeutic modality follwing NAC and surgery.

The recent reports demonstrated that taxanes were used effectively in NAC for uterine cervical adenocarcinoma [19, 20]. Most of the institutions joining TGCU had adopted cisplatin-based regimens in the 1990s, and switched to the regimens combining taxanes and platinum derivatives after 2000. Despite the diverse NAC regimens and the small sample size, the authors believe that the present results have provided constructive ideas for the development of new therapeutic strategy for N-SCC of the uterine cervix. An effective chemotherapeutic regimen for N-SCC of the uterine cervix should be urgently integrated in a phase II study and then a RCT that compares a new single NAC and radical surgery with radical surgery alone, is warranted to confirm the present results.

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Are platinum agents, paclitaxel and irinotecan effective for clear cell carcinoma of the ovary? DNA damage detected with γH2AX induced by anticancer agents

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Abstract

Objectives: Differences in the incidences and types of DNA damage induced by antitumor agents for clear cell carcinoma (CCC) were determined in 2 ovarian CCC cell lines using γH2AX.

Material and methods: The antitumor activity of anticancer agents, CDDP, CBDCA, PTX and SN-38, was examined using ovarian clear cell carcinoma cultured cell lines (OVISE and RMG-I). After culture, each cell line was treated with each anticancer agent, the cells were collected, fixed, and then reacted with the anti-γH2AX antibody. γH2AX and nuclear DNA were then simultaneously detected by flow cytometry using FITC and propidium iodide, respectively, to determine γH2AX in each cell cycle phase.

Results: After administration of CDDP, DNA damage was frequent in S-phase cells, while cell-cycle arrest occurred in the G1 and G2/M phases and γH2AX did not increase in CDDP-resistant cells. Sensitivities to CDDP and CBDCA differed between the two cell lines. The antitumor effect of PTX is induced by G2/M arrest, and combination treatment with CBDCA, inducing DNA damage in G2/M-phase cells, might be effective.

Conclusions: This is the first study in Japan to evaluate the antitumor activity of anticancer agents by focusing on the relationship between the cell cycle and DNA damage using γH2AX as an indicator. The immunocytochemical method used in this study detects γH2AX, which indicates DNA damage even at very low concentrations and with high sensitivity. Therefore, a promising method of easily and rapidly identifying agents potentially effective against CCC.

Keywords: γH2AX, Clear cell carcinoma, Ovarian cancer, DNA damage, Apoptosis, Chemotherapy

Introduction

Clear cell adenocarcinoma (CCC), a subtype of epithelial ovarian cancer, is less sensitive to chemotherapy and is thus classified as a refractory ovarian cancer [1]. It has been shown that a combination of carboplatin (CBDCA) and paclitaxel (PTX), a standard therapy for ovarian cancer [2,3], is effective against serous adenocarcinoma and endometrioid adenocarcinoma, with a response rate of approximately 75%, while CCC has lower response rates ranging from 18% to 50% [4]. The incidence of CCC has been increasing and is now 25% in Japan, while that in Europe is 5-6%. As yet, no treatment for this histological subtype of ovarian cancer has been established. Histopathology remains the gold standard for classifying epithelial ovarian cancer subgroups; however, there is emerging evidence indicating different genetic and molecular profiles. Consequently, there is no international consensus regarding the necessity of establishing treatment strategies based on histological subtypes. In fact, global clinical trials of CCC and mucinous adenocarcinoma have already begun. Although which cytotoxic agents have true efficacy against CCC remains unknown, small trials in Japan and basic studies have suggested the efficacy of irinotecan

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(CPT-11) [5-7]. The Japanese Gynecologic Oncology Group (JGOG) started an international randomized controlled trial (RCT) of cisplatin (CDDP)/CPT-11 therapy with a control arm of CBDCA/TXL (TC) therapy (JGOG3017/GCIG); patient accrual is ongoing and approximately 560 patients had been enrolled in the trial as of July 2010. In addition, an ongoing translational study, as part of the JGOG3017/GCIG trial, also aims to establish an updated treatment strategy.

Nucleosomes, units of chromatin, consist of core histones wrapped in 146 bp of DNA and linker DNA. Core histones are octamers designated H2A, H2B, H3 and H4. Histone H2AX is a variant of histone H2A and accounts for 10-15% of all variants. When DNA damage occurs, serine 139 of histone H2AX in chromatins on both sides of a damaged site is phosphorylated by two enzymes: ataxia telangiectasia mutated (ATM) protein kinase and by ATM and Rad3 related (ATR) protein kinase [8,9]. Phosphorylated histone H2AX is called γH2AX. Dot γH2AX, which is detectable using γH2AX-specific antibody, is considered to correspond to specific DNA damage. Therefore, DNA damage can be immunocytochemically detected [10]. DNA damage in individual cells has been detected employing a single-cell DNA gel electrophoresis technique (comet assay), in which the extent and length of the comet's tail indicate the severity of DNA damage. Recently, however, it has become apparent that phosphorylation of histone H2AX, one of the variants of the nucleosome core histone H2A, can provide a sensitive and reliable marker of DNA damage. More specifically, DNA damage, particularly that involving the formation of DNA double-strand breaks (DSBs), induces phosphorylation of histone H2AX on Ser-139; phosphorylated H2AX is defined as γH2AX. The phosphorylation takes place on H2AX molecules on both sides of DSBs along a megabase length of DNA. Although DSBs generated during DNA fragmentation in the course of apoptosis also induce γH2AX, the degree of γH2AX induction in apoptotic cells is much greater than that in primary DSBs induced by antitumor drugs or radiation. The presence of γH2AX in cells can be detected immunocytochemically in the form of distinct nuclear γH2AX immunofluorescent foci and each focus is considered to correspond to a single DSB. This immunocytochemical approach has made it possible to assay DNA damage and *in situ* repair of the chromatin of individual cells. The immunocytochemical approach is significantly more sensitive than the comet assay. The use of multi-parameter flow cytometry in measurements of γH2AX immunofluorescence allows DNA damage to be correlated with cellular DNA content and, therefore, the cell-cycle phase. Determination of the cell-cycle phase targeted by the drug is of importance in elucidating the mechanism of antitumor drug activity.

In the present study, we conducted flow cytometric bivariate analyses of γH2AX and DNA contents in two different cell lines of CCC treated with CDDP, CBDCA, PTX or CPT-11 (SN-38), which have been used in the aforementioned international clinical randomized trial targeting CCC, and examined effects of these drugs with regard to the induction of DNA damage, apoptosis and cell-cycle progression vis-à-vis the cell-cycle phase.

Materials and methods

Cell culture

We used two CCC cell lines (OVISE and RMG-I) were obtained from the Health Science Research Resources Bank (Osaka, Japan). OVISE was established from a patient with metastatic disease after completion of six cycles of platinum combination therapy, and was grown in dishes (Becton Drive, Franklin Lakes, NJ, USA) in RPMI1640 medium (Sigma Chemical Co., St Louis, MO, USA) with 10% fetal bovine serum. RMG-I was established from a chemotherapy-naïve patient with ascites, and was reported to be primary platinum resistant (Table 1) [11]. RMG-I was grown in dishes (Becton Drive) in Ham F-12 medium supplemented with 10% fetal bovine serum. The media for the two cell lines were supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin (Meiji Seika Co., Ltd., Tokyo, Japan). All cell lines were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air.

Drugs

CDDP, PTX and SN-38 (CPT-11) were dissolved in dimethyl sulfoxide (DMSO, Sigma); the final concentration of DMSO in the culture medium never exceeded 0.1% (w/v). CBDCA was dissolved in phosphate-buffered saline (PBS). The concentration of each agent was set to correspond to the blood concentration at a standard clinical dose (Table 2).

Immunohistochemistry

Both cells floating in the medium and the cells that remained attached after trypsinization were collected and fixed with 1% methanol-free formaldehyde (Polysciences Inc., Warrington, PA, USA) in PBS at 0 °C for 15 minutes and post-fixed with 80% ethanol for at least 2 hours at -20 °C. The fixed cells were washed twice in PBS and

Table 1 Clinical biological characteristics of the cell line

Cell Line	Source	Histopathology	Pretreatment	Median doubling time
OVISE	Solid metastatic	CCC	CAP×6 courses	60 hours
RMG- 1	Ascites	CCC	No	60 hours

CCC; clear cell carcinoma
CAP; cyclophosphamide, doxorubicin, cisplatin

Table 2 Minimum effective concentration (MEC)

	Cmax	MEC
PTX	10 μg/ml	50 ng/ml
CDDP	7 μg/ml	1 μg/ml
CBDCA	55 μg/ml	10 μg/ml
SN-38	30 μg/ml	1 ng/ml

Cmax; clinical maximum drug concentration.

suspended in a 1% (w/v) solution of bovine serum albumin (BSA) (Sigma) in PBS to suppress non-specific antibody binding. The cells were then incubated in 100 μl of 1% BSA containing 1:100 diluted anti-phosphohistone H2AX (Ser-139) monoclonal antibody (Upstate, Lake Placid, NY, USA) for 2 hours at room temperature, washed twice with PBS and resuspended in 100 μl of 1:20 diluted fluorescein isothiocyanate (FITC)-conjugated $F(ab')^2$ fragment of goat anti-mouse immunoglobulin (Dako, Glostrup, Denmark) for 30 minutes at room temperature in the dark. The cells were then counterstained with 5 μg/ml propidium iodide (PI) (Sigma) in the presence of 100 μl of RNaseA (Sigma) for 30 minutes.

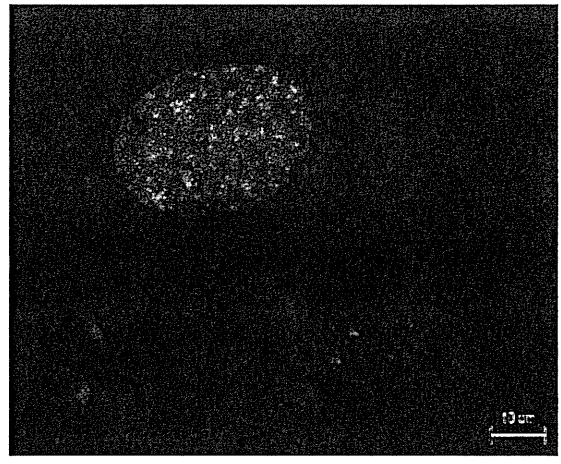

Figure 1 Immunohistochemistry. Representative microscopic images of OVISE cell line after exposure to 10 μg/ml CDDP for 24 h. γH2AX foci in a nuclear are stained green and red, respectively. An increase in green dots, indicating elevation of γH2AX, can be seen after exposure to CDDP. Thus, DNA damage is visually recognizable in each nucleus. Apoptotic bodies (insert) are distinguishable by the entire cell being intensely positive for γH2AX.

Fluorescence measurements by flow cytometry

The FITC (green) and PI (red) fluorescence of individual cells in suspension induced by excitation with a 488-nm argon ion laser was measured using a FACScan flow cytometer (Becton-Dickinson, San Jose, CA, USA). The green and red fluorescence from each cell was separated and quantified using standard optics and Cell Quest software (Becton-Dickinson). Ten thousand cells were measured per sample. All experiments were repeated at least three times.

After γH2AX and DNA staining, the DNA content was represented on the x axis and the γH2AX content on the y axis using flow cytometry. The γH2AX in each cell cycle was determined, thereby allowing the relationships between cell kinetics and DNA damage induced by antitumor agents to be examined.

Results

Platinum Agents

CDDP

OVISE cells showed an increase in the number of distinct green dots (γH2AX foci) after exposure to 10 μg/ml CDDP for 24 h, which indicates that CDDP caused DNA damage (Figure 1). Using folw cytometry, DNA damage was evident from the increase in γH2AX. After 24-hour treatment with CDDP, DNA damage in OVISE and RMG-I was seen gradually in the S phase at concentrations of 100 ng/ml and 1 ng/ml (Figure 2A). In both cell lines, treatment with 100 ng/ml or more CDDP for 24 hours caused DNA damage throughout the cell cycle. The cells with DNA damage gradually underwent apoptosis, as was evident by the presence of cells with markedly elevated γH2AX and fractional (sub-G1) DNA contents. In OVISE, DNA damage in the S and G2/M phases after treatment with 100 ng/ml CDDP was seen for 24 and 72 hours, respectively (Figure 2B). Although in RMG-I, 100 ng/ml CDDP caused DNA damage in the S phase, in other phases of the cell cycle it was not apparent even with longer treatment. In both cell lines, the cells with damaged DNA underwent apoptosis. The number of cells in the G2/M phase in OVISE decreased gradually indicating S-phase arrest. . On the other hand, in RMG-I showed G1 and G2/M-phases arrest. RMG-I was found to be less susceptible to DNA damage and subsequent apoptosis than OVISE.

CBDCA

DNA damage in the S phase was seen gradually after exposure to CBDCA for 24 hours in OVISE and RMG-I lines at 1 μg/ml and 10 μg/ml, respectively (Figure 3A). Subsequently, the cells with damaged DNA underwent apoptosis. Gradually both cell lines showed DNA damage in the G2/M phase and underwent apoptosis. OVISE showed S and G2/M- phases arrest, while RMG-I G2/M-phase arrest (Figure 3B).

PTX

Exposure to 10 ng/ml or more PTX for 24 hours caused apoptosis without primary DNA damage in both cell lines. (Figure 4A). Although, further apoptotic effects were not seen at doses exceeding 50 ng/ml. PTX induced both cell lines G2/M-phase arrest, but some cells remained 120 hours after exposure without primary DNA damage (Figure 4B).

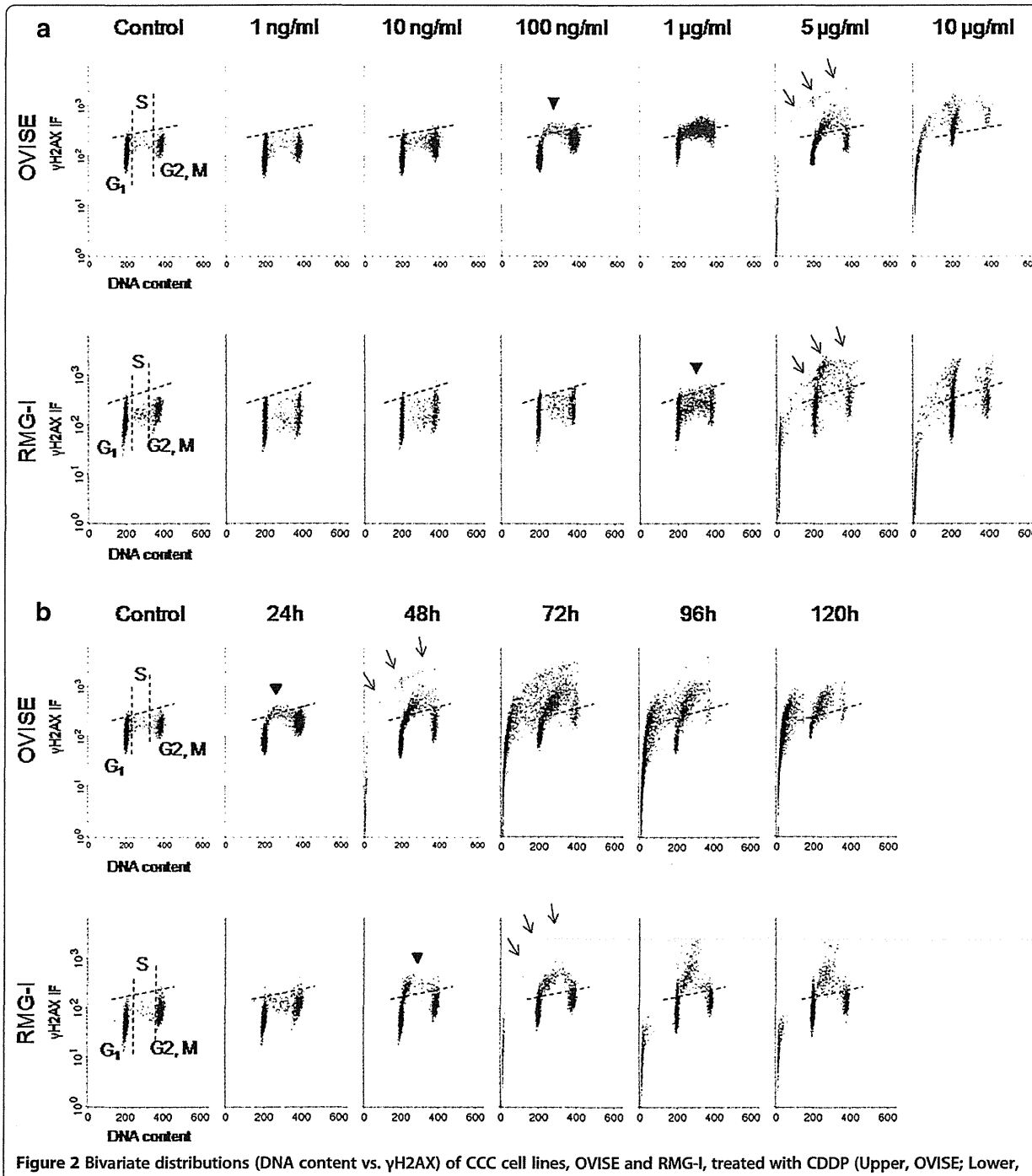

Figure 2 Bivariate distributions (DNA content vs. γH2AX) of CCC cell lines, OVISE and RMG-I, treated with CDDP (Upper, OVISE; Lower, RMG-I). The dotted lines indicate the upper level of γI I2AX immunofluorescence for 95% of cells in the untreated (control) culture. Arrow heads indicate elevation of γH2AX that means DNA damage. Arrows indicate apoptotic cell populations with marked increase in γH2AX and gradual decrease in DNA. (A) Both cell lines treated with various concentrations of CDDP for 24 h. OVISE and RMG-1 cell lines exhibited DNA damage in S-phase cells at minimum concentrations of 100 ng/ml and 1 μg/ml, respectively. Both cell lines were subjected to DNA damage concentration-dependently at every cell cycle, and apoptosis was induced at concentrations of 5 μg/ml or higher. More cells in RMG-1 remained free of DNA damage as compared to OVISE. (B) Both cell lines were treated with 100 ng/ml, the minimum concentration inducing DNA damage in either cell line, for various reaction times. S-phase cells of OVISE showing DNA damage progressed to apoptosis after 48 h. In addition, S-phase arrest was observed. DNA damage was induced in S-phase cells of RMG-I after 48 h. Cells with DNA damage progressed to apoptosis after 72 h. Furthermore, cell-cycle arrest occurred in all cells.

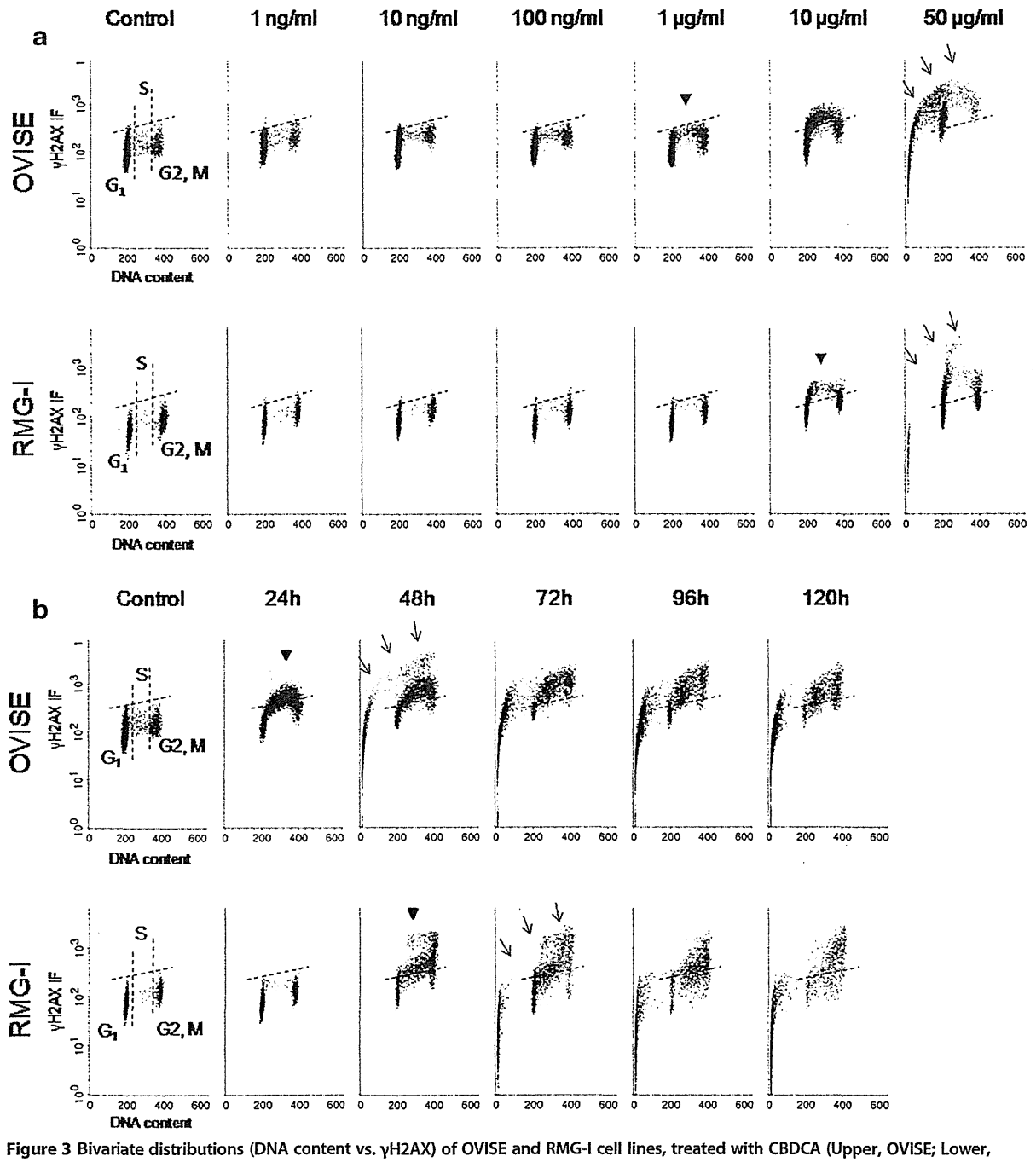

Figure 3 Bivariate distributions (DNA content vs. γH2AX) of OVISE and RMG-I cell lines, treated with CBDCA (Upper, OVISE; Lower, RMG-I). The dotted lines indicate control. Arrow heads and arrows indicate DNA damage and apoptosis, respectively. (A) Both cell lines treated with various concentrations of CBDCA for 24 h. DNA damage was observed in the S-phase cells at 1 μg/ml and 10 μg/ml concentrations in OVISE and RMG-1, respectively. DNA damage was found in both cell lines at every cell cycle as the concentration increased, and apoptosis occurred at a concentration of 50 μg/ml. More cells remained free of DNA damage in RMG-1 than in OVISE. (B) Both cell lines were treated with 1 μg/ml, the minimum concentration inducing DNA damage in either cell line, for various reaction times. In OVISE, S-phase cells with DNA damage progressed to apoptosis after 48 h. DNA damage was also found in G2/M-phase cells after 48 h, but most did not progress to apoptosis. S and G/2 M-phase arrests were observed. DNA damage was found in S and G2/M-phase cells after 48 h in RMG-I. The S-phase cells with DNA damage progressed to apoptosis 78 h later, but G2/M-phase cells showing DNA damage remained. S and G/2 M-phase arrests were observed.

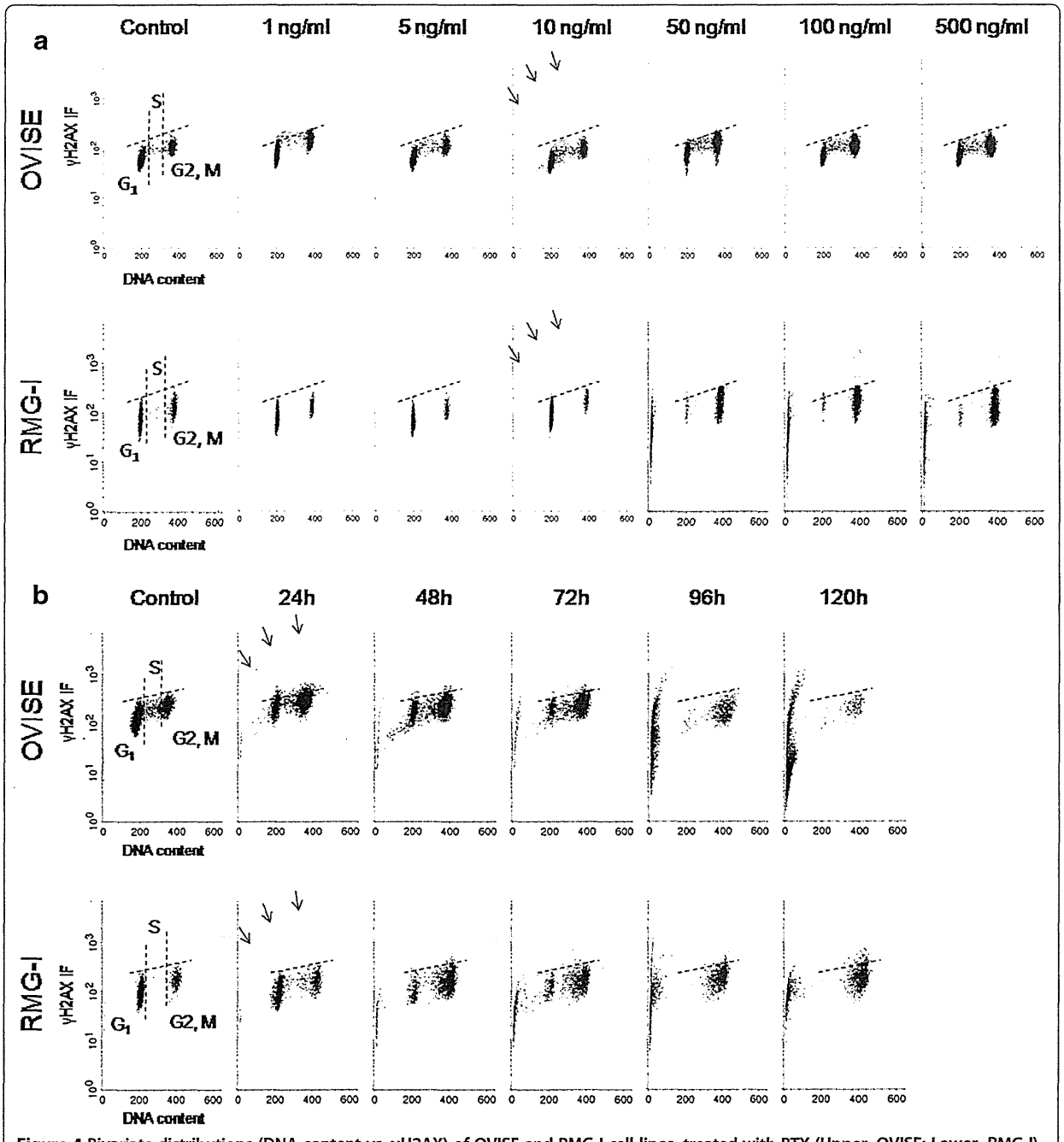

Figure 4 Bivariate distributions (DNA content vs. γH2AX) of OVISE and RMG-I cell lines, treated with PTX (Upper, OVISE; Lower, RMG-I). The dotted lines and arrows indicate control and apoptosis, respectively. (**A**) Both cell lines treated with various concentrations of PTX for 24 h. Both cell lines included cells that had progressed to apoptosis without direct DNA damage induced by a minimum concentration of 10 ng/ml. No concentration-dependent differences were observed at concentrations of 50 ng/ml or higher. (**B**) Both cell lines were treated with 10 ng/ml, the minimum concentration inducing DNA damage in either cell line, for various reaction times. G2/M-phase arrest occurred in both cell lines.

SN-38

After treatment with 0.5 ng/ml or more of SN-38 for 24 hours, both cell lines distinguished DNA damage in the S phase. Nevertheless even 10 ng/ml which is nearly the clinical maximum blood concentration did not cause apoptosis (Figure 5A). Apoptosis was seen barely 72 hours after exposure to 0.5 ng/ml SN-38 in a portion of OVISE while in RMG-I after 96 hours (Figure 5B). However, the cells with significant DNA damage remained in the S phase over 120 hours after exposure.

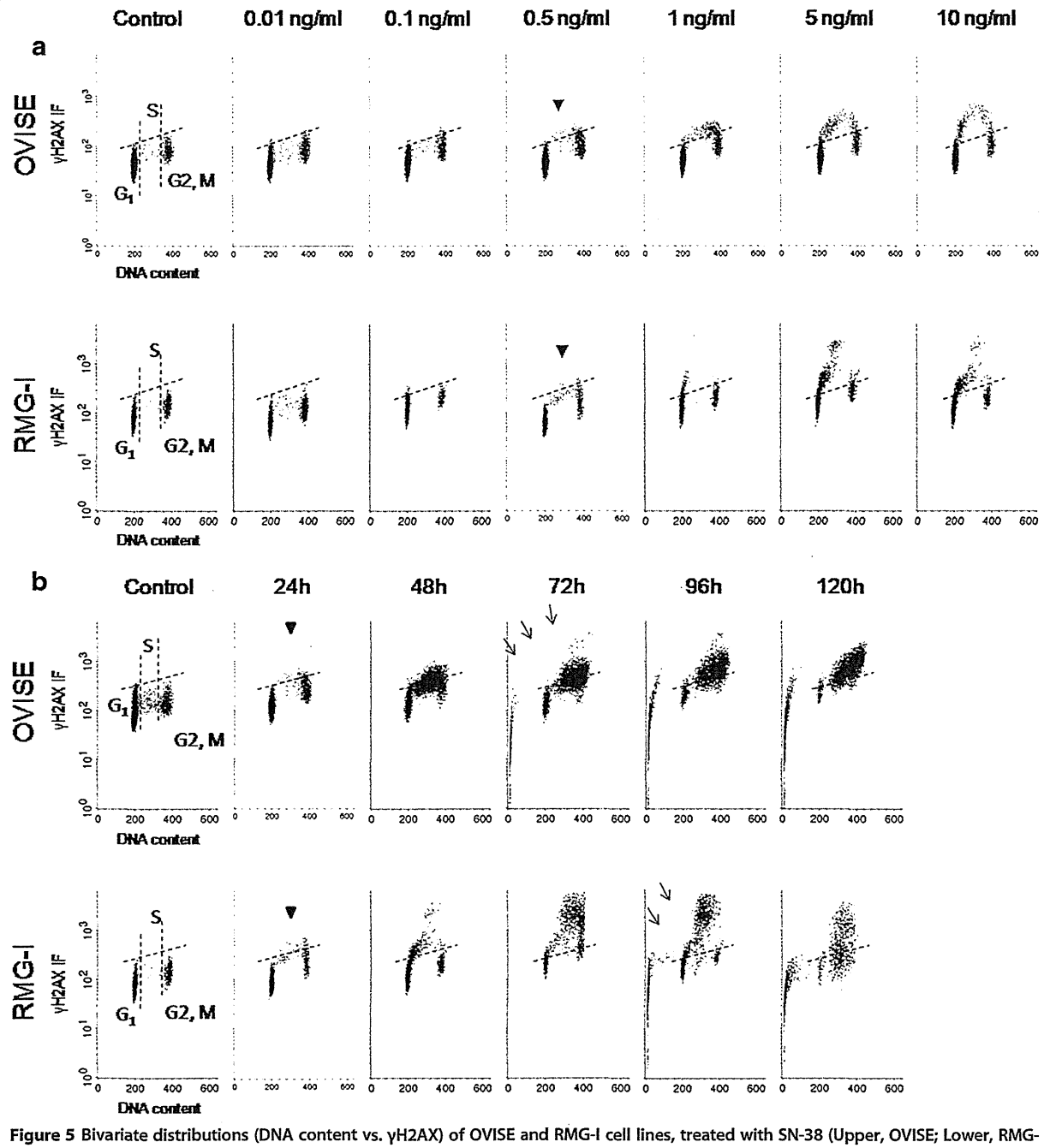

Figure 5 Bivariate distributions (DNA content vs. γH2AX) of OVISE and RMG-I cell lines, treated with SN-38 (Upper, OVISE; Lower, RMG-I). The dotted lines indicate control. Arrow heads and arrows indicate DNA damage and apoptosis, respectively. (**A**) Both cell lines treated with various concentrations of SN-38 for 24 h. Both cell lines exhibited DNA damage in S-phase cells at a minimum concentration of 0.5 ng/ml. (**B**) Both cell lines were treated with 0.5 ng/ml, the minimum concentration inducing DNA damage in either cell line, for various reaction times. S-phase cells with DNA damage started progressing to apoptosis after 72 and 96 h in OVISE and RMG-1, respectively. Both cell lines showed S-phase arrest, and large numbers of cells with DNA damage were still present even after 120 h.

Discussion

After CDDP administration, DNA damage was observed mainly in the S phase. It is reasonable to assume that the DNA was structurally altered by CDDP, leading to DNA replication fork arrest and ultimately resulting in apoptosis. This result was consistent with a known pharmacological effect of CDDP [12]. In RMG-I, apoptotic cells were minimally increased in the S phase, moreover the cells showing arrest in the G1 and G2/M phases without DNA damage were increased as

compared with OVISE. Therefore, the results of the present study support the clinical experience that RMG-I is CDDP-resistant [11,13]. After CBDCA administration, DNA damage was seen in the S and G2/M phases in both cell lines. OVISE contained a remarkable cell population rescued from apoptosis and surviving with DNA damage. On the other hand, most RMG-I cells with DNA damage underwent apoptosis. These results suggest that cell lines respond differently to platinum agents, i.e., RMG-I was CDDP-resistant but responded to CBDCA. PTX directly induced apoptosis in M-phase cells but not via DNA damage, an observation consistent with a known pharmacological effect of PTX, i.e. microtubule inhibition [14]. PTX was confirmed to induce apoptosis through a p53-independent pathway; it was, therefore, expected to have an effect on CCC, in which the p53 mutation is rare [15,16]. The mechanism underlying the antitumor effect of PTX is G2/M arrest. Therefore, the combination with CBDCA, an agent inducing DNA damage, in G2/M-phase arrested cells might be effective, at least theoretically. As shown in this study, it is noteworthy that sensitivities to CDDP and CBDCA differed between the CCC cell lines. In practice, CCC is less sensitive to CBDCA/PTX treatment [4,6,17], which is the standard regimen for ovarian cancer. Since the effect of PTX was independent of both the concentration and the response time, these results raise the possibility that repeated administration of PTX at a low dose increases the antitumor effect more than a single administration. These findings support the results of the JGOG3016, i.e. that weekly CBDCA (AUC6)/PTX (80 mg/m^2, weekly × 3) is more effective than tri-weekly CBDCA (AUC 6)/PTX (175 mg/m^2) treatment [18].

On the other hand, after administration of SN-38, DNA damage occurred in S-phase cells, followed by apoptosis. This confirmed that SN-38 acts as a type I topoisomerase inhibitor [19]. Furthermore, it appears that SN-38 had an effect on the cell cycle because S-phase arrest continued for more than 120 hours. It is, therefore, possible that the improved administration method for SN-38 increases its antitumor effect. Cells rescued from apoptosis remained in S phase with DNA damage; consequently, the efficacy of combining SN-38 with CDDP, which induces DNA damage mainly in S-phase cells, was supported.

In conclusion, the present results suggest that an effective treatment for CCC with a slow growth rate and a low ratio of S-phase cells would be a combination of agents arresting the cell cycle, thereby causing accumulation of cells in the S phase or the G2/M phase, and agents specifically inducing DNA damage in S-phase cells. The method used in this study allows immunocytochemical detection of γH2AX, which indicates DNA damage even at very low concentrations and has high sensitivity in comparison with the comet assay. Employing this method, we were able to analyze relationships between anti-tumor effects and cell cycle perturbations. Therefore, γH2AX detection is a promising method of simply and rapidly identifying agents potentially effective against CCC.

Competing interest
The authors have no conflicts of interest to report.

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Authors' contributions
ET participated in all aspects of the study, from design to clinical and laboratory performance, and manuscript writing. TS participated in design, data analysis and drafting of the manuscript. AK participated in the design of the study and technical assistance. SK, TS and TS participated in design and analysis of clinical data. All authors have read and approved the manuscript.

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Case of peptide-YY-producing strumal carcinoid of the ovary: A case report and review

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Abstract

Ovarian carcinoid is a rare tumor accounting for approximately 0.1% of all ovarian malignancies. We describe a case of peptide-YY-producing strumal carcinoid of the ovary associated with severe constipation. A 48-year-old woman was found to have a pelvic mass on ultrasonography when she visited her primary doctor for a health check-up. She was thus referred to our department. Magnetic resonance imaging revealed a solid right ovarian tumor 60 × 50 mm in size. The patient underwent a right adnexectomy and was histopathologically diagnosed as having strumal carcinoid of the ovary. On immunohistochemical examination, the tumor cells were positive for peptide YY. The patient's constipation resolved rapidly after surgery. Based on her clinical course, her constipation was considered to have been caused by the strumal carcinoid of the ovary. The clinical course of this case supports the previously recognized correlation between peptide-YY-producing ovarian carcinoid and constipation.

Key words: constipation, new carcinoid syndrome, peptide YY, strumal carcinoid.

Introduction

Carcinoid is a tumor originating from argyrophil cells (peripheral endocrine cells) located in organs throughout the body including the mucosa of the gastrointestinal tract. Ovarian carcinoid is relatively rare, accounting for approximately 1.3% of all carcinoids. Only about 150 cases have been reported worldwide, and 30 of these cases in Japan. Strumal carcinoid is histologically characterized by an intimate mixture of follicles containing thyroid tissue and carcinoid components, and only about 60 such cases have been reported worldwide. Ovarian carcinoid is a rare tumor accounting for approximately 0.1% of ovarian tumors. It is generally considered to be a borderline malignancy. This tumor is often accompanied by other types of tumors, such as teratoma and mucinous adenoma, and tends to be identified incidentally when specimens from these tumors are examined. The clinical characteristics and histogenesis of ovarian carcinoids remain unclear. We describe a case of strumal carcinoid of the ovary with severe constipation (a newly recognized carcinoid syndrome), with a review of the relevant literature.

Case Report

The patient was a 48-year-old woman who had a history of 2 gravitas, 2 para, an inguinal hernia at age 17 and appendectomy at age 34. Her family history was unremarkable. The patient visited her primary doctor for a health check-up. She was found to have a pelvic mass on ultrasonography and was thus referred to our department. On pelvic examination, the uterus was egg-sized and a movable eagle egg-sized mass was palpated at the right side of the uterus. Transvaginal

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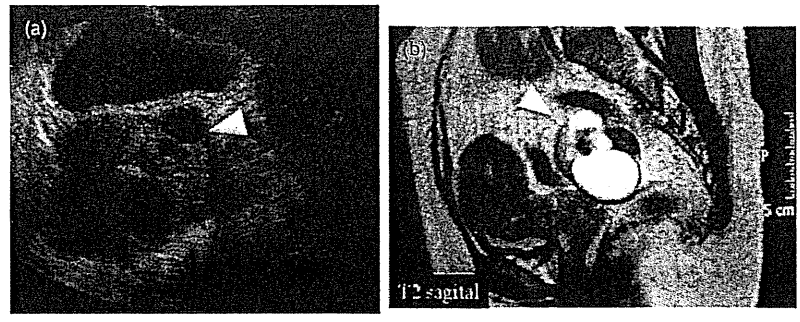

Figure 1 (a) Transvaginal ultrasonography and (b) magnetic resonance imaging of the pelvic cavity showed a 5-cm diameter mass in the right ovary comprised of solid and cystic parts.

Figure 2 Macroscopic finding of right ovary. (a) Macroscopic appearance of a smooth-surfaced, firm, mobile, unruptured right ovarian tumor measuring approximately 6 cm at its largest diameter. (b) On excision, the yellow, gray, and white part was found to contain solid components, whereas the cystic part contained brown fluid.

ultrasonography revealed a mass shadow 51 × 39 mm in size on the right side of the uterus (Fig. 1a). There was no ascites in the Pouch of Douglas. Levels of the tumor markers were 1.5 ng/mL for carcinoembryonic antigen (CEA), 14.1 U/mL for carbohydrate antigen (CA)19-9, and 14.6 IU/mL for CA125. Magnetic resonance imaging revealed a mass, 60 × 50 mm in size, consisting of a solid component with a wide range of signal intensities (Fig. 1b). The patient underwent laparotomy based on a suspicion of ovarian malignancy. Intraoperatively, a fist-sized multilocular ovarian mass with a smooth surface was identified (Fig. 2a). The cut surface of the tumor was solid, showing a mixture of yellow, gray, and white (Fig. 2b). Since ascites was minimal and no abnormalities were found in the left adnexa, the uterus, or the abdominal cavity, the patient underwent right uterine adnexectomy only.

On histopathological examination of the isolated tumor, most of the tissue was arranged in a trabecular formation and the tumor cells had medium-sized round nuclei, showing a pattern consistent with trabecular carcinoid (Fig. 3). On immunohistological examination, the tumor cells were positive for synaptophysin and chromogranin A (Fig. 4a,b). The tumor was diagnosed as a strumal carcinoid because there was a mixture of thyroid tissue and carcinoid components. The carcinoid tumor cells were positive for thyroglobulin (Fig. 4c). Furthermore, the tumor cells were strongly positive for peptide YY (Fig. 4d). The postoperative pathological diagnosis was strumal carcinoid of the ovary stage Ia (pT1A, NX, M0), and ascites cytology was negative. As no other lesions were identified on gastrointestinal endoscopy or computed tomography, the patient was diagnosed as having primary strumal carcinoid of the ovary. After this diagnosis had been made, a more detailed medical history was obtained from the patient, and it was found that she had suffered severe constipation (a newly recognized carcinoid syndrome). After adnexectomy, the patient recovered from this constipation. She has remained alive and well, without any evidence of recurrence, for 18 months, to date, since surgery.

Discussion

Carcinoids usually arise in the gastrointestinal tract, lungs, bronchial tubes, thymus gland, and mediastinum. Ovarian carcinoid is relatively rare, accounting for approximately 1.3% of all carcinoids.[1] Furthermore, ovarian carcinoid accounts for approximately 0.1% of

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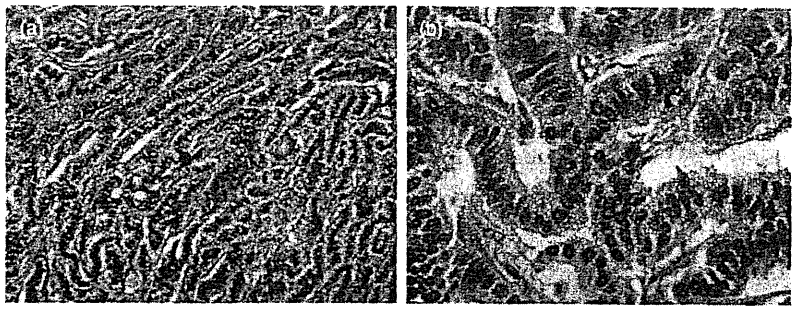

Figure 3 Hematoxylin–eosin staining of the strumal carcinoid tumor. (a) Carcinoid tumor composed of solid parts and showing a trabecular growth pattern (original magnification ×10). (b) Carcinoid cells with oblong nuclei and columnar cytoplasm presented with long, wavy, parallel ribbon-like arrangement (original magnification ×40).

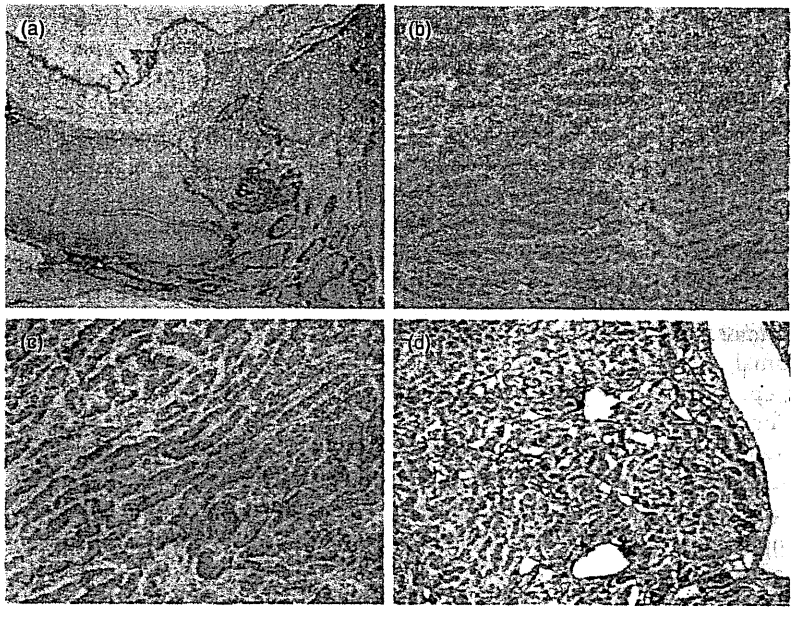

Figure 4 Immunohistochemical staining of strumal carcinoid showing positive staining for (a) thyroglobulin, ×4 (b) synaptophysin, ×10 (c) chromogranin A, ×10 and (d) anti-peptide YY antibody, ×10. Most of the cytoplasm of the carcinoid cells was stained with the anti-PYY antibody.

all ovarian malignancies.[2] According to the General Rules for Clinical and Pathological Management of Ovarian Tumors, carcinoid, strumal carcinoid, and mucinous carcinoid fall under the heading of 'monodermal and highly specialized teratomas,' a category which includes germ cell tumors. Carcinoids are classified into insular and trabecular types, based on their histopathological patterns.[3] Strumal carcinoid is histologically characterized by an intimate mixture of follicles containing thyroid tissue and carcinoid components. According to the report by Robboy *et al.* on 50 cases ranging in age from 21 to 77 years with strumal carcinoids of the ovary, half the cases showed trabecular carcinoids while two had insular carcinoids, and the others showed a mixture in which either the trabecular or the insular pattern predominated.[4] The thyroid component was either normal thyroid tissue or follicular adenoma.

Patients with insular carcinoids present with carcinoid syndrome (flushing, diarrhea, and so on), whereas those with a trabecular or strumal carcinoid rarely develop carcinoid syndrome, although some do reportedly have severe constipation.[5–10]

Peptide YY is a 36-amino acid gastrointestinal hormone localized in endocrine cells of the distal small bowel. This peptide exerts an inhibitory effect on the peristaltic actions of the jejunum and colon. The reported correlation between peptide-YY-producing ovarian carcinoids and constipation is assumed to reflect this inhibitory effect on peristalsis.[5–10] Matsuda

et al. examined eight patients with severe constipation, and found that five had strumal carcinoids and three had trabecular carcinoids. As for peptide YY, at least 80% of the tumor cells were positive in seven of their cases and at least 50% were positive in the one remaining case. Matsuda *et al.* concluded that peptide YY was strongly related to the severe constipation observed in their cases.[7] Utsumi *et al.* reported peptide YY to be associated with severe constipation and hirsutism.[8] Our patient did not have hirsutism. Carcinoids occurring in Japanese patients are rarely accompanied by carcinoid syndrome. We consider this to be attributable to the tumor cells in Japanese patients tending to show a trabecular rather than an insular arrangement, which is generally seen in Western patients. It has been reported that ovarian carcinoid with a trabecular arrangement produces a large amount of peptide YY, leading to persistent constipation. This clinical entity is now recognized as a new carcinoid syndrome.[6] The clinical course of our case was compatible with this syndrome, because a detailed postoperative interview regarding her medical history revealed that this patient had suffered severe constipation. In addition, her constipation resolved after surgical removal of the ovarian carcinoid. In our present case, the carcinoid tumor had a trabecular structure, as is seen in most strumal carcinoids, and the tumor cells were positive for peptide YY by immunostaining. Given the rapid resolution of constipation postoperatively, peptide YY production by the tumor was considered to be the cause of constipation in this case. To date, to our knowledge, only four cases with peptide-YY-producing strumal carcinoid have been reported.[7,9–11] Thus, this is the fourth case report worldwide (Table 1).

As ovarian carcinoid does not have characteristic imaging findings, preoperative diagnosis of this disease is difficult. The present case was no exception. The patient was diagnosed with strumal carcinoid after undergoing a right adnexectomy. The clinical course described herein suggests that when a patient with an ovarian tumor presents with severe constipation, we should consider the possibility of ovarian carcinoid. Regardless of tumor size, surgical treatment is indicated in such cases.

Although strumal carcinoid is categorized as a borderline malignancy, the prognosis is relatively favorable. Davis *et al.* reported the cure rate for stage I of this disease, at 5 or 10 years, to be nearly 100%.[12] However, a case with bone and breast metastases, despite having clinical stage Ia disease, was reported.[13] In addition, two fatalities due to strumal carcinoid have been reported.[4,14] Furthermore, Talerman reported metastasis rates of primary carcinoid of the ovary to be 7.1% for the insular type and 4.8% for the trabecular type.[15] Patients must be closely followed after surgery, in the same manner as those with other ovarian cancers.

We experienced a case of peptide-YY-producing strumal carcinoid of the ovary constituting a newly recognized carcinoid syndrome. This is the fifth such case reported worldwide. The rapid amelioration of constipation after surgery in this case suggested peptide YY production by the tumor to possibly have caused the constipation.

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Disclosure

The authors have no conflicts of interest.

Table 1 Previously reported cases with ovarian strumal carcinoid producing peptide YY

Case	Author/ reference	Age (years)	Stage	Tumor size	Surgery	Major complaint	Status	Follow up (months)
1	Shigeta *et al.*[10]	53	Ic	16 cm	BSO, Hys	Abdominal tumor	NED	31
2	Matsuda *et al.*[7]	50	Ia	20 cm	LSO	Abdominal tumor	AWD	27
3	Kawano *et al.*[9]	47	Ic(b)	12 cm	BSO, Hys	Severe constipation	NED	18
4	Matsunami *et al.*[11]	45	NA	8 cm	BC	No Symptom	NED	12
5	Present case	48	Ia	6 cm	RSO	No Symptom	NED	18

The tumor cells were strongly positive for peptide YY in all cases. AWD, alive with disease; BC, bilateral cystectomy; BSO/LSO/RSO, bilateral/left/right salpingo-oophorectomy; Hys, hysterectomy; NA, not available; NED, no evidence of disease.