Modification of p53 Immunoexpression Associated with Chemotherapy Regimens in Advanced and Refractory Ovarian Cancers

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For 34 advanced and refractory cases of epithelial ovarian cancer, we immunohistochemically analyzed modification of p53 expression in tissue from both primary and secondary surgeries, focusing on the relationship with chemotherapeutic effects caused by two different regimens: high-dose chemotherapy (HDC) and conventional chemotherapy (CONV). Following the primary surgery, 18 cases were treated with HDC and 16 cases with CONV. The p53 expression was more markedly decreased in the HDC-treated cases (78% for the primary; 39% for the secondary), compared to that in the CONV-treated cases (50% for the primary;

43% for the secondary). Sixty-seven percent (12/18) of the HDC-treated cases showed an obvious decrease in p53 expression, whereas 81% (13/16) of the CONV-treated cases showed no change in expression. In the comparison of these two groups, the survival ratio was higher in the 12 HDC-treated cases than that in the 13 CONV-treated cases ($p < 0.05$). In conclusion, as p53 expression in epithelial ovarian cancers is considerably influenced by chemotherapy regimen, it was suggested that the regimen yielding a distinct decrease in expression could yield a more favorable outcome.

Key words: p53, immunohistochemistry, high-dose chemotherapy, ovarian cancer

I. Introduction

Regarding epithelial ovarian cancers, no significant difference in the treatment outcome is recognized between CAP (cyclophosphamide+adriamycin+cisplatin) and cyclophosphamide+cisplatin [5, 15]. The latter has been widely favored because of the lighter side effects for patients. Later, paclitaxel as an effective anti-cancer drug for ovarian cancer appeared, and the combination of paclitaxel+cisplatin+cyclophosphamide has been proven to give a relatively good clinical outcome [13]. Recently, the mixture of paclitaxel+carboplatin has become the golden standard of chemotherapy for ovarian cancers due to its fewer side effects [3, 16]. However, it is not fully satisfactory when the long-term prognosis results are analyzed.

Our department started high-dose chemotherapy

(HDC) as a specific regimen for epithelial ovarian cancers 16 years ago. Compared to conventional chemotherapy (CONV), the regimen of HDC is particularly characterized by the dose-dependent effect of platinum. We considered that an increased dose of platinum over the secondary threshold would be enforced by autologous stem cell support [1, 24]. As a result, the clinical outcome has been considerably improved as well as the safety for the patients [19, 20]. In ovarian cancers, oncogenes such as *HER-2* [22], *c-myc* [29], and *k-ras* [4] were reported to be amplified, although the frequencies were not very high. Compared to these oncogenes, the abnormality of p53 expression is more frequently manifested in association with the morphological feature, clinicopathological state and prognosis not only in ovarian cancers, but also neoplasms of various organs [7, 11, 23, 25–27]. Namely, the p53 expression appears at a high rate according to the advance of the stage [10]. Overexpression of p53 is believed to be one of the most reliable pathologic factors that influence the prognosis of ovarian cancers [6, 9, 10].

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The present study focused on the retrospective analysis of the relationship between the modification of p53 expression and treatment effect or prognosis, associated with the different chemotherapy regimens, HDC and CONV. Our results suggested that p53 expression tended to be influenced more strongly by HDC than CONV.

II. Patients and Methods

Case selection

Of 226 ovarian cancers which were surgically treated at Tokai University Hospital during 1983 to 1995, 34 advanced and refractory epithelial cancers were chosen on the following conditions. a) The primary surgery was optimal or sub-optimal. b) The secondary surgery after chemotherapy was performed to reduce or resect the recurrent tumors. The patients were aged from 32 to 74 years old (mean 54). The stage according to classification of International Federation of Gynecology and Obstetrics (FIGO), histology and residual tumor size are shown in Table 1. The residual tumor size at the primary surgery was divided into two groups: minimal and >1 cm. There was no significant difference in the survival ratio between the HDC-treated cases and CONV-treated cases (Fig. 1).

Following the primary surgery, 18 patients were treated with HDC (cisplatin 300 mg/m² and cyclophosphamide 3000 mg/m²) and 16 with CONV (cisplatin 50–100 mg/m², adriamycin 50 mg/m², cyclophosphamide 500 mg/m² or carboplatin 1500 mg/m²). As for the neoadjuvant chemotherapy, only platinum was administered intraperitoneally or intravenously to 19 patients, mainly because of the massive ascites storage with cancer cell dissemination.

Table 1. Ovarian epithelial cancers studied

	No. of case
FIGO stage	
Ic/II	4
III	19
IV	11
Histological type	
Serous	24
Non-serous	10
Residual tumor	
minimal	16
<1 cm	18

FIGO: International Federation of Gynecology and Obstetrics.

Two tissue samples for each case were obtained from the primary and secondary surgery specimens, respectively. The secondary surgery specimens included lymph nodes, intestinal tract, liver, spleen, and peritoneum.

Immunohistochemical staining of p53

The surgical specimens of ovarian epithelial cancer used were routinely formalin-fixed and paraffin-embedded. The representative 68 sections in 4 µm thick were deparaffinized and processed with heat antigen retrieval at 90°C for 5 min in 0.01 M phosphate buffered saline (PBS), pH 7.2, using a microwave. The labeled streptavidin-biotin method was applied. The primary antibody (clone DO7, Novocastra Laboratories Tyne, UK) was incubated at a dilution of 1:50. Sections were counterstained with 5% methyl green. As a

Fig. 1. The survival curve of CONV and HDC in the 34 cases (Kaplan–Meier Method). HDC, high-dose chemotherapy; CONV, conventional chemotherapy.

positive control, known colon carcinomas were used. For the negative control study, sections were incubated with a non-immunized serum instead of the primary antibody. A labeling index, which was defined as positive cells/1,000 tumor cells, was categorized into five groups: less than 5%, negative; +, 5–25%; 2+, 25–50%; 3+, 50–75%; 4+, 75%<.

Statistical analysis

The data were analyzed by means of the χ^2 or Fisher's exact test. $p < 0.05$ was regarded as significant. The Kaplan–Meier curve was utilized to evaluate the survival ratio.

III. Results

The alterations in the positive ratios of p53 expression from the primary to the secondary surgery are shown in Table 1. The overall positive ratios in the primary and secondary were 65% (22/34) and 41% (14/34), respectively. For the 18 HDC-treated cases, the positive ratio was decreased from the primary to the secondary surgery: 78% (14/18) to 39% (7/18) ($p < 0.02$), as shown in Table 2. In contrary, no remarkable decrease was noted in the 16 CONV-treated cases: 50% (8/16) to 43% (7/16), as shown in Table 2. According to the follow-up period between the primary to the secondary surgery, the 34 cases were divided into 2 groups: 16 cases, <1 year, and 18 cases, >1 year. The decrease of positive ratios was more evident in the latter: from 72% (13/18) to 33% (6/18) ($p < 0.02$) than in the former: from 56% (9/16) to 50% (8/16), as shown in Table 3.

The modification of p53 expression after HDC and CONV was semi-quantitatively analyzed, as shown in Table

4. Sixty-seven percent (12/18) of the HDC-treated cases exhibited a decrease in the positive ratio, however, in 81% (13/16) of the CONV-treated cases no obvious change was noted. Among 7 of 12 HDC-treated cases with varying decrease of p53 expression, no positive reaction was observed in the secondary surgery. One case in each group showed a p53 expression increase. A difference in modification between the two groups of $p < 0.02$ was regarded as statistically significant.

In the comparison of the survival ratio for the 12 HDC-treated cases showing a decrease in p53 expression and that for the 13 CONV-treated cases showing no change, as demonstrated in Fig. 2, a more favorable outcome was shown in the HDC-treated cases ($p < 0.05$).

IV. Discussion

The main reasons for focusing on p53 expression are that this protein can be easily applied for evaluating the biological behavior of various kinds of neoplasms [7, 11, 23, 25–27], including ovarian cancers [6, 9, 10], and that many reports have mentioned that the overexpression of p53 is closely associated with an unfavorable outcome. For ovarian cancers with a high grade and/or in an advanced stage, mutant p53 protein accumulation is not a significant prognosis-related factor that reflects an increased risk for relapse and death for tumors [10]. Kiyokawa *et al.* [8] reported that no significant correlation was found between the aberrant expression of p53 and stage or histologic type, and that there was a concordant expression level of p53 in primary and matched metastatic lesions. Silvestrini *et al.* [21] also men-

Table 2. p53 expression: Comparison of positive ratios in primary and secondary surgery

	No. of cases	Primary surgery	Secondary surgery	p value
		p53 expression	p53 expression	
		Positive ratio % (n)	Positive ratio % (n)	
Total cases	34	65 (22)	41 (14)	$p < 0.05$
HDC	18	78 (14)	39 (7)	$p < 0.02$
CONV	16	50 (8)	43 (7)	NS

HDC, high-dose chemotherapy; CONV, conventional chemotherapy.

Table 3. p53 expression: Comparison of positive ratios in primary and secondary surgery

Interval	No. of cases	Primary surgery	Secondary surgery	p value
		p53 expression	p53 expression	
		Positive ratio % (n)	Positive ratio % (n)	
<1 year	16	56 (9)	50 (8)	NS
≥1 year	18	72 (13)	33 (6)	$p < 0.02$

Interval: period during primary to secondary surgery.

Table 4. Modification of p53 expression from primary to secondary surgery

	HDC-treated cases (18) % (n)	CONV-treated cases (16) % (n)
Decrease	^a 67 (12)	^b 13 (2)
No change	^c 28 (5)	^d 81 (13)
Increase	5 (1)	6 (1)

decrease: + to – [6 cases (CONV: 1 cases, HDC: 5 cases)], 2+ to – (HDC: 1 case), 3+ to – (HDC: 1 case), 3+ to 2+ (CONV: 1 case), 4+ to 3+ (HDC: 5 cases)

no change: – to – [12 cases (CONV: 8 cases, HDC: 4 cases)], + to + (CONV: 2 cases), 4+ to 4+ [4 cases (CONV: 3 cases, HDC: 1 case)]

increase: 2+ to 3+ (HDC: 1 case), 3+ to 4+ (CONV: 1 case)

p=0.02: Fisher's exact test [between (a, d) and (b, c)]

tioned that alterations in the accumulated p53 protein are not correlated with clinical response or survival after cisplatin treatment. In our experience so far, regarding refractory ovarian epithelial cancers, immunohistochemical p53 expression profiles seem to be less changeable, however, a distinct decrease of p53 expression has been occasionally recognized after chemotherapy.

In the present study, we attempted to clarify the modification of p53 expression from the viewpoint of the association with therapeutic effects caused by two different chemotherapy regimens. In the 18 HDC-treated cases, the positive ratios were drastically decreased from 78% for the primary surgery to 39% for the secondary surgery ($p<0.02$) (Table 2). On the contrary, no significant decrease was noted in the CONV-treated cases. Sixty-seven percent (12/18) of the HDC-treated cases and 37% (6/16) of the CONV-treated

cases had a follow-up period of more than 1 year, respectively. Thus, the chemotherapeutic effects seem to be reflected in the elongation of the follow-up period between the primary and secondary surgeries (Table 3), and are considered to be closely associated with a decrease in p53 expression.

The semi-quantitative analysis with the modification of the p53 expression from the primary to the secondary surgery provides a reasonable explanation for the above interpretation (Table 4). In fact, a decrease in the grade of the positive extent was noted in 67% of the HDC-treated cases, but in only 13% of the CONV-treated cases. In the majority (81%) of the CONV-treated cases, no significant difference in p53 expression was noted between the primary and secondary surgeries. The difference in the survival ratios was found considerably favorable with the 12 HDC-treated cases showing a decrease in p53 expression, compared to the 13 CONV-treated cases showing no change ($p<0.05$) (Fig. 2). In the analysis of the relationship between p53 expression and chemosensitivity [17, 18, 28], Righetti *et al.* [17] mentioned that a significant correlation was found between p53 accumulation, type of mutation, and pathological response to cisplatin-based therapy. Buttitta *et al.* [2] also reported that ovarian cancers showing p53 aberrations are significantly less sensitive to chemotherapy and more aggressive than those with functional p53. In addition, they observed that patients with p53 mutations had a significantly shorter progression-free survival than those with p53-negative tumors [2]. From our results, it is speculated that immunohistochemical p53 expression could be more susceptible to decrease by HDC than CONV, and that this phenomenon may be related to the difference in prognosis between HDC-treated cases and CONV-treated cases. Using the cell lines

Fig. 2. Comparison of survival ratios of ^aHDC-treated cases and ^bCONV-treated cases (Kaplan–Meier Method). HDC, high-dose chemotherapy; CONV, conventional chemotherapy.

Fig. 3. HDC-treated case with endometrioid adenocarcinoma. Intense p53 expression before HDC (a) completely disappeared after HDC (b). Original magnification $\times 50$

Fig. 4. CONV-treated case with serous adenocarcinoma. No significant change in p53 expression was noted between before (a) and after CONV (b). Original magnification $\times 50$

of ovarian cancer in a variable p53 status, it has been demonstrated that occurrence of apoptosis depends on the drug and the drug dose used [14]. The effect of high cisplatin concentration for a cell line exhibiting mutant p53 could be the same as that of the low cisplatin concentration for a cell line exhibiting wild p53. Therefore, irrespective of the abnormal p53 expression in the primary surgical specimen, the expression is expected to be downregulated by appropriate chemotherapy for the recurrent or residual tumors. It seems that, on this point, p53 overexpression may not be necessarily an indicator of the poor clinical outcome. Although, there is a statement that a significant dose-response effect of cisplatin is not seen in patients with p53 positive ovarian tumors, unlike those with p53 negative ovarian tumors [12].

In the present study, the number of cases used was not sufficient to perform multivariate analyses for the relationship between p53 expression and histologic type, stage, effect of neo-adjuvant therapy, and residual tumor size. Nevertheless, we stress that the HDC-treated cases tended to exhibit a decreased immunohistochemical expression of p53 in comparison to the CONV-treated cases. Focusing

on the appropriate selection of chemotherapy regimens for ovarian cancer patients, we are trying to investigate the optimal conditions for inducing apoptosis, using human ovarian cancer cell lines with or without p53 mutations. In parallel to this project, a prospective randomized trial for epithelial ovarian cancers applying HDC and CONV is being attempted [19].

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VI. References

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