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Pitfall in Chemotherapy for Ovarian Cancer

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Concerning the biological properties of recurrent ovarian cancer, other than drug resistance, we revealed that the expressions of mutant p53 and CD44v6 genes were significantly greater in recurrent ovarian cancer than in those of its primary counterpart. These findings suggest that chemotherapeutic agents may modify some biological characteristics of cancer by altering gene expressions. The biological behavior concerning the metastatic potential of a recurrent disease must be elucidated in order to develop an optional treatment regimen against recurrent tumors. Therefore, we established *in-vivo* cisplatin-resistant cell lines by repeated administration, in order to find a more suitable model for reflecting the biological aggressiveness of clinically recurrent ovarian cancer following chemotherapy. Chemotherapeutic agents have given a substantial advantage to cancer patients. It must be borne in mind that the cancer cells surviving following chemotherapy possibly present different biological properties from primary cancer cells, and that these properties might be developed by the chemotherapeutic agents.

Key Words: Chemotherapy, biological behavior

INTRODUCTION

About half of all ovarian cancer patients present with advanced disease. Therefore chemotherapy is essential for residual disease after cytoreductive surgery. Before cisplatin was available at the beginning of 1980's, most of patients with advanced ovarian cancer died within 3 years. After that time, approximately 20% of patients have been able to live longer than 5 years following the initial treatment. Conversely, 80% of advanced ovarian cancers recur despite cytoreductive surgery and cisplatin based chemotherapy. The biological character of recurrent ovarian cancer has, until now, only been analyzed in terms of drug resistance. In fact, both *in vitro* and *in vivo* studies have revealed chemotherapeutic agents could induce an increased expression of drug resistance-related proteins. Moreover, our clinical observation has revealed that recurrent ovarian cancer tends to metastasize to distant organs, although ovarian cancer is known to be an intraperitoneal disease. In patients with a first recurrence, 47% presented distant metastasis. On the other hand, in the same patients only 16% had presented distant metastasis (stage IV) at the diagnosis of primary disease.[1]

Concerning the biological properties of recurrent ovarian cancer, other than drug resistance, we revealed that the expressions of mutant p53 and CD44v6 genes were significantly greater in recurrent ovarian cancer than in those of its primary counterpart (Table 1). These findings suggest that chemotherapeutic agents may modify some biological characteristics of cancer by altering gene expressions.[2]

Table 1. Alteration in the p53 and CD44v6 Expressions of Paired Primary and Recurrent Ovarian Cancers[2]

Expression	p53	CD44v6
Primary > recurrence	3	4
Primary=recurrence	27*	26†
Primary < recurrence	13	13

Wilcoxon signed-rank test.
*p=0.0055.
†p=0.0071.

The biological behavior concerning the metastatic potential of a recurrent disease must be elucidated in order to develop an optional treat-

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ment regimen against recurrent tumors. Therefore we established *in-vivo* -cisplatin-resistant cell lines by repeated administration, in order to find a more suitable model for reflecting the biological aggressiveness of clinically recurrent ovarian cancer following chemotherapy.[3]

Metastatic potential evaluated by *in vivo* model

As a model for recurrent ovarian cancer, two *in vivo* cisplatin resistant human ovarian cancer cell lines (M1/DDPTx and M2/DDPTx) were established by repeated cisplatin administration to parental tumor-bearing nude mice (Fig. 1). Whereas, as a model for untreated cancer, which is impossible to be observed clinically, the *in vivo* passaged control cell lines were established by repeated administration of phosphate-buffered saline (M1/P6 and M2/P6). M1/DDPTx and M2/DDPTx became 1.5 and 2.6 fold more resistant to cisplatin than their parental cell lines (MET-1 and MET-2), respectively.

Metastatic properties

To evaluate the spontaneous metastatic properties, the visible lung nodules were counted after intrafoot pad inoculation, which was followed by amputation of the tumor-bearing legs. M1 and M2/DDPTx showed a significantly higher tendency to metastasize than their parental cell lines and the M1 and M2/P6 also (Table 2).

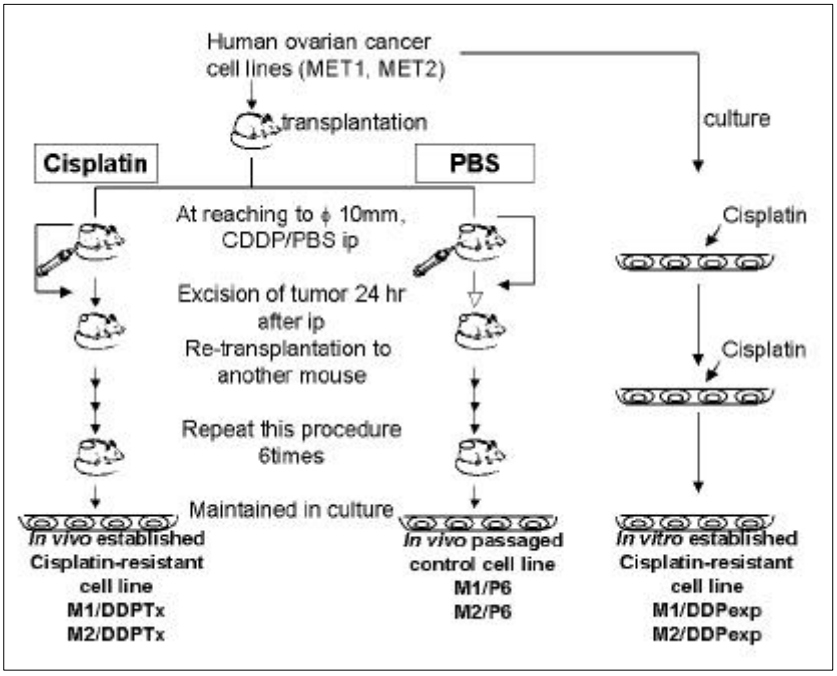

Fig. 1. Establishment of the cisplatin-resistant cell lines, *in vivo*. (PBS: phosphate-buffered saline) As a model for recurrent ovarian cancer, two *in vivo* cisplatin resistant human ovarian cancer cell lines (M1/DDPTx and M2/DDPTx) were established by repeated cisplatin administration to parental tumor bearing nude mice. Whereas, as a model for untreated cancer, the *in vivo* passaged control cell lines (M1/P6 and M2/P6) were established by repeated administration of phosphate-buffered saline.

Table 2. Evaluation of Spontaneous Lung Metastatic Abilities

Cell lines	No. of mice	Median No. of lung colonies(range)
MET-1	4	2 (1-3)
M1/DDPexp	4	0 (0-5)
M1/P6	4	9 (4-14)
M1/DDPTx	4	31 (15-54)
MET-2	5	0 (0)
M2/DDPexp	5	0 (0)
M2/P6	6	7 (2-10)
M2/DDPTx	6	16 (14-29)

Growth and invasiveness

To elucidate the main factors responsible for the increased metastatic properties of the *in-vivo* cisplatin-resistant cell lines established, we observed their abilities to grow and become invasive. Their growth potential was evaluated by both *in vitro* and *in vivo* methods. M1 and M2/DDPTx showed almost the same growth as the other cell lines. The tumor invasiveness was assessed by the tumor invasion into the upper thigh from the inoculated footpad. The *in vivo* established cisplatin resistant cell line indicated a highly invasive tendency compared with the other cell lines (Table 3). These results suggest that the increased metastatic ability of the *in vivo* cisplatin resistant cell lines established was caused by an enhanced invasiveness, rather than by growth. Liotta et al. reported a "three-step theory" of tumor invasion, where the invasion is thought to consist of three biological factors: cell attachment to the extracellular matrix, the secretion of proteolytic enzymes and cell motility. We analyzed these three factors in order to elucidate the mechanism of the enhanced invasiveness in the *in vivo* cisplatin resistant cell lines established.

Cell attachment to the extracellular matrix

M1/DDPTx showed a higher adhesion to Matrigel, fibronectin, type IV collagen and laminin, which are the main components of the extracellular matrix, than the MET-1, M1/P6 (Fig. 2). On the other hand, the attachment to the extracellular matrix was almost the same for all MET-2 sublines.

Proteolytic enzyme activity

The conditioned medium prepared from each subline was assayed to determine the proteolytic activity by gelatin zymography. The *in vivo* cisplatin resistant cell lines established secreted 62-kDa gelatinase. Whereas the control cell line, M1/P6, secreted no detectable gelatinase. Although the M2/P6 also secreted 62-kDa gelati-

Table 3. Assessment of Tumor Invasiveness

Invasive tumor / No. of all mice Cell lines	No. of mice with	
	MET-1	MET-2
Parent	1/12	0/11
In vitro CDDP treated	0/12	0/11
In vivo Control	4/13	3/12
In vivo CDDP treated	10/13	9/12

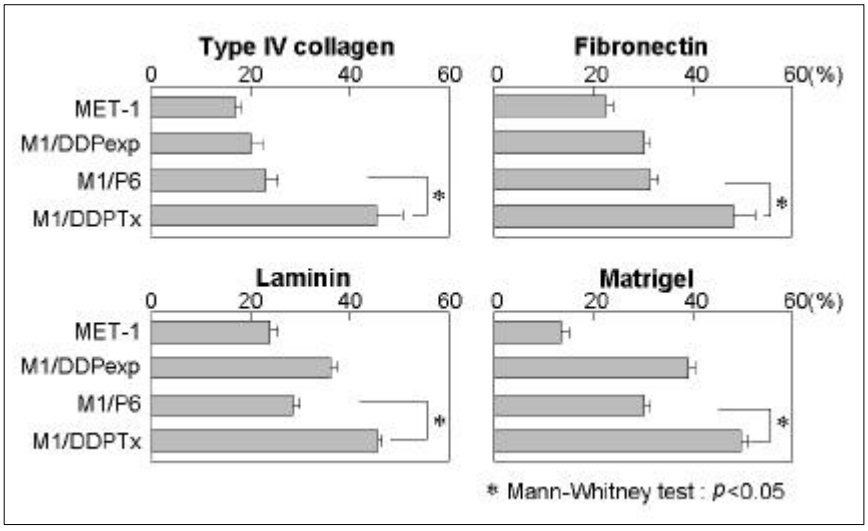

Fig. 2. Cell attachment to the extracellular matrix. M1/DDPTx showed a higher adhesion to matrigel, fibronectin, type IV collagen and laminin, than did MET-1, M1/P6.

nase, the amount was much smaller than that by M2/DDPTx.

Cell motility

Cell motility was evaluated by a phagokinetic track assay. The *in vivo* established cisplatin resistant cell lines migrated more vigorously than their parental cell lines or their *in vivo* passaged control cell lines. The cell motility of the M1/DDPTx especially, was highly enhanced, being almost double that of the M1/P6 (Fig. 3).

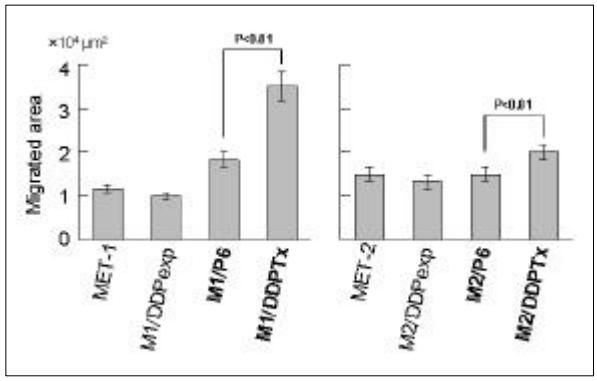

Fig. 3. Cell motility as evaluated by phagokinetic track assays.

CONCLUSION

These studies have shown that the *in vivo* cisplatin resistant cell lines established had significantly enhanced metastatic properties compared with the control cell lines. These enhanced metastatic properties were caused by the tumors' invasiveness in combination with various levels of cell attachment enhancement, proteolytic enzyme activity and cell motility. All these data, obtained from the *in vivo* study, could support the phenomenon observed in the clinical recurrent ovarian cancer in terms of the metastatic potential.

Chemotherapeutic agents have given a substantial advantage to cancer patients. It must be borne in mind that the cancer cells surviving following chemotherapy possibly present different biological properties from primary cancer cells, and that these properties might be developed by the chemotherapeutic agents.

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