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Research paper

Single treatment with cisplatin or UFT, but not their combination treatment enhances the metastatic capacity of mouse fibrosarcoma cells

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To elucidate the roles of chemotherapeutic agents in tumor progression, we examined whether *cis*-diamminedichloroplatinum (II) (cisplatin) and UFT would promote malignant progression of a weakly tumorigenic and poorly metastatic fibrosarcoma cell line QR-32SK *in vivo*. C57BL/6 mice, transplanted with QR32SK, were treated with either cisplatin or UFT alone and in combination. After treatment with or without cisplatin and/or UFT, we established *in vitro* culture cell lines from the tumors arising in the mice on day 21 and then i.v. injected the established cell lines into syngeneic mice. As a result, the cell lines established from cisplatin-treated mice and UFT-treated mice had significantly higher metastatic capacity in lung compared to the ones from control untreated mice (64 and 65%, respectively, versus 26.7%). The cell lines established from the mice with the combination therapy showed lower lung metastasis (11%) than the ones from control untreated mice. Furthermore, we found the incidences of these experimental metastases were closely related with *in vitro* chemotactic activities and the production of MMP-9 of the cultured cell lines. These results indicate that cisplatin and UFT as a single agent promote the chemotactic activities and the production of MMP-9 in non-metastatic fibrosarcoma cells, resulting in the conversion to highly metastatic ones, and that cisplatin and UFT in combination failed to promote the chemotactic activities and the conversion. These results suggest that the combination therapy with cisplatin and UFT is useful in preventing the emergence of more malignant tumor cells after chemotherapy. [© 1999 Lippincott Williams & Wilkins.]

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Introduction

Clinically repeated sessions of chemotherapies can intervene in recuperation, and often result in an eventual emergence of even highly resistant and increasingly malignant tumor cells.[1,2] However, it has not been reported how chemotherapies cause the emergence of highly resistant and malignant tumor cells *in vivo*, since no adequate *in vivo* experimental model has been established.

We have previously reported that a small number of QR-32 cells (2×10^5), derived from a mouse fibrosarcoma, spontaneously regressed in normal syngeneic c57BL/6 mice, when s.c. injected. QR-32 cells could also grow in mice which had been immunosuppressed by irradiation or depletion of helper/inducer T cells.[3] We have also observed that the cell lines established from the tumors arising in mice after the co-implantation of QR-32 cells with a foreign body such as gelatin sponge or plastic plates exhibited biologically more malignant characteristics than those of parent QR-32 cells such as enhanced s.c. tumorigenicity, lung metastatic capacity and production of prostaglandin E_2. It was found that this malignant progression was induced by inflammatory host cells responding to the foreign body.[4,5]

To further elucidate whether chemotherapeutic agents play a role in tumor progression, we examined in this study the effects of chemotherapeutic agents, *cis*-diamminedichloroplatinum(II) (cisplatin) and/or UFT on the malignant progression of a weakly tumorigenic and poorly metastatic cloned cell line, QR-32SK.

Cisplatin is thought to be one of the most effective chemotherapeutic drugs for a variety of human malignancies including ovarian, testicular, bladder, head and neck, esophageal, and small cell lung cancers.[6,7] UFT is also reported to have a greater antitumor activity because the degradation of 5-fluorouracil (5-FU) by hepatic dihydropyrimidine dehydrogenase is inhibited by uracil mixed with tegafur.[8,9] 5-FU and cisplatin in combination have shown synergistic cytotoxicity and low side effects in many experimental and clinical studies.[10-12]

Most cancer chemotherapeutic agents are known to be mutagens.[13-15] Thus, in addition to inducing or increasing the number of resistant cells, mutagenic effects of the therapy could well stimulate the appearance of more malignant variants. Although there have been many reports discussing the details of pharmacological mechanisms of their actions, experimental evidence is not adequate to explain how chemotherapeutic agents promote the malignancy of tumor cells. In this study, we focussed on the effects of *in vivo* treatment of tumor-bearing mice with chemotherapeutic agents, cisplatin and/or UFT, on the subsequent metastatic capacity of these tumor cells in normal syngeneic mice.

The results indicate that single treatment with either cisplatin or UFT frequently converted non-metastatic cells into highly metastatic cells, whereas combination treatment with cisplatin and UFT did not convert tumor cells into more malignant ones.

Materials and methods

Animals and chemicals

Female C57BL/6 mice between 2 and 4 months of age were obtained from Japan SLC (Hamamatsu, Japan), and maintained in specific pathogen-free conditions. Cisplatin was purchased from Kyowa Hakko (Tokyo, Japan). UFT was provided by Taiho (Tokyo, Japan).

Tumor cells and culture conditions

The tumor used in the experiment was QR-32SK, which is a subclone of a fibrosarcoma cell clone, QR-32, and gives tumorigenicity in syngeneic mice implanted with a large number of the cells (1×10^7). The origin and characteristics of the fibrosarcoma cells used in this study have been described previously.[4] Briefly, a mouse fibrosarcoma cell line, BMT-11, was established from a tumor induced by 3-methylcholanthrene in a C57BL/6 mouse. Its transplantable clone, BMT-11cl-9, was exposed *in vitro* to quercetin and further cloned by limiting dilution. One of the established clones, QR-32, is weakly tumorigenic and poorly metastatic. The tumor cells were maintained in a monolayer culture in Eagle's minimum essential medium (MEM) containing 10% fetal bovine serum (FBS) (BioWhittaker, Walkersville, MD), sodium pyruvate, non-essential amino acids and L-glutamine, at 37°C in a humidified 5% CO_2/95% air mixture.

Treatment with drugs

The experimental scheme is shown in Figure 1. The mice were randomized into treatment or control groups (five mice per group) according to body weight of mice. QR-32SK tumor cells (1×10^7) were transplanted s.c. into normal syngeneic mice. After 5 days of tumor implantation, UFT (20 mg/kg/day) was administered p.o. for 17 consecutive days. Eleven days after the tumor implantation, cisplatin (4 mg/kg/day) was injected i.p. into QR-32SK tumor-bearing mice every other day for 3 times.

Establishment of culture cell lines from *in vivo* growing tumors

Twenty-one days after the tumor implantation, culture cell lines were established from each of arising tumors in the mice untreated (QRL) and treated with either cisplatin (QRL/cisplatin) or UFT (QRL/UFT) and cisplatin combined with UFT (QRL/cisplatin/UFT).

Evaluation of metastatic capacity

Tumor cells (1×10^6) of the established culture lines were injected i.v. into five normal mice, which were killed 21 days after inoculation. Lungs were fixed with Bouin's solution and superficial metastatic nodules were counted macroscopically.

Zymography

Samples of conditioned medium (CM) were obtained by culturing the cells for 24 h at confluence in serum-free MEM, and SDS-gelatin-containing gels (0.75 mm thickness) were prepared according to the method reported by Houssen and Dowdle[16] with some modification. The serum-free samples were mixed at 2:1 with sample buffer. Electrophoresis was carried out by Laemmli's method[17] under cooling conditions. After the electro-

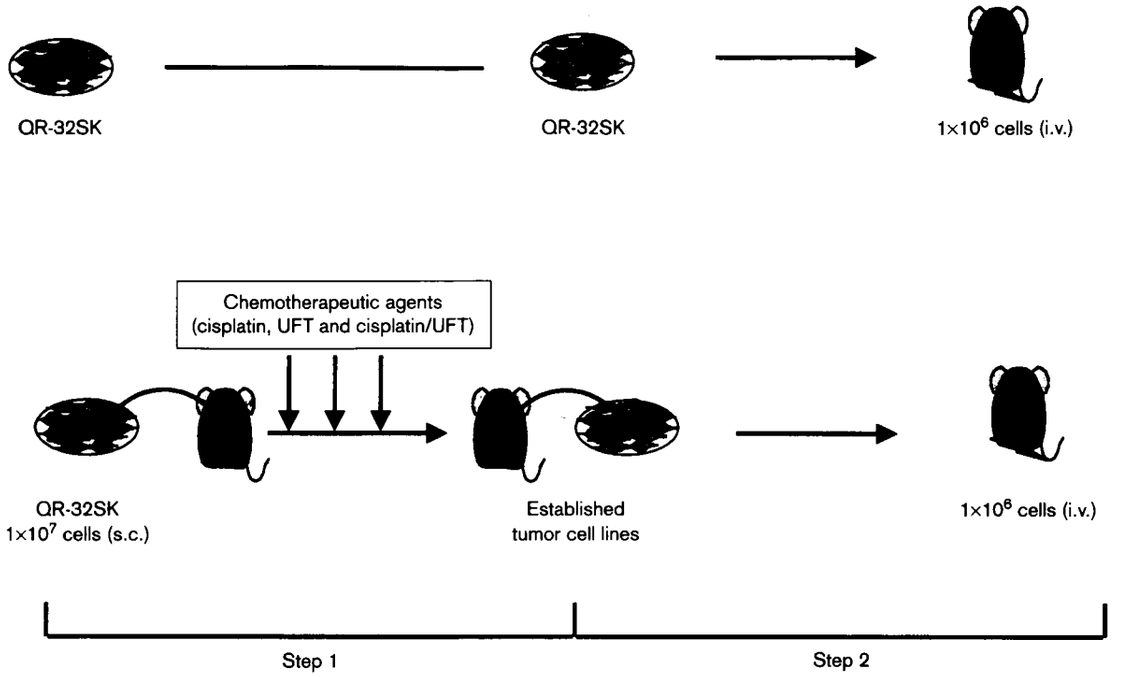

Figure 1. Experimental scheme of the tumor progression model. *In vivo* experiment was in two steps. Step 1: 1×10^7 QR-32 cells were implanted s.c. on day 0 and chemotherapeutic agents (cisplatin, UFT and cisplatin plus UFT) were administered as described in Materials and methods. On day 21, culture cell lines were established from each of the arising tumors in the mice and cultured in Eagle's MEM containing 10% FBS serum. Step 2: these cultured cell lines were injected into five normal syngeneic mice i.v.

phoresis was completed, the gels were soaked at room temperature for 2 h in the presence of 2.5% Triton X-100 to remove the SDS and then incubated at 37°C for 36 h in buffer containing 10 mM $CaCl_2$, 150 mM NaCl and 50 mM Tris-HCl buffer (pH 7.5) containing sodium azide. After incubation, the gels were stained with 0.05% Coomassie brilliant blue R-250 (BioRad, Richmond, CA) in isopropanol, acetic acid and water at 1:1:8, and destained with isopropanol, acetic acid and water at 1:1:8.

Reverse transcription-polymerase chain reaction (RT-PCR)

The RNA (5 mg) underwent cDNA synthesis in 50 μl of a reaction buffer [75 mM KCl, 50 mM Tris-HCl (pH 8.3), 3 mM $MgCl_2$, 10 mM dithiothereitol, 0.5 mM each dNTP, 2 mg random primer and 1000 U M-MLV reverse transcriptase (Gibco/BRL, Grand Island, NY)] by incubation at 37°C for 1 h. PCR amplification of cDNA (5 ml) was performed in 50 μl reaction buffer [50 mM KCl, 10 mM Tris-HCl (pH 9.0), 2.5 mM $MgCl_2$, 0.1% Triton X-100, 0.2 mM each dNTP, 1 mM each specific primer and 1 U Taq polymerase (Gibco/BRL)]. The primer pairs were chosen from separate DNA exons of genes. The primer sequences and expected sizes of amplified cDNA fragments were as follows: TIMP-1 5′ primer, 5′-TCC CCA GAA ATC AAC GAG AC-3′; 3′ primer, 5′-CTC AGA GTA CGC CAG GGA AC-3′, 251 bp, β-actin 5′ primer, 5′-TTC GAG CAA GAG ATG GCC ACG GCT-3′; 3′ primer, 5′-ATA CTC CTG CTT GCT GAT CCA CAT-3′, 478 bp, respectively. The reactions were run for 25 cycles on a thermal cycler (Barnstead/Thermolyne, Dubuque, IA) as follows: 1 min at 94°C, 1 min at 60°C and 2 min at 72°C. Aliquots of 9 μl of each PCR sample were mixed with 1 μl of 10× gel loading buffer, electrophoresed through 1% agarose gel and stained with ethidium bromide. For evaluation of the semiquantitativeness of the presently used RT-PCR, β-actin and TIMP-1 mRNA expression were analyzed by the use of serial 2-fold dilution of each cDNA. This analysis showed that semiquantitative RT-PCR could be utilized to determine the amount of 2-fold difference in mRNA expression.

In vitro motility and invasion assays

In vitro motility assay was done using a Transwell

chamber (Costar, Cambridge, MA) with 6.5 mm diameter tissue-treated membranes of 8 μm pore size according to Repesh with some modification.[18,19] Tumor cells (1×10^5/ml) were suspended in 0.2 ml serum-free MEM supplemented with 0.1% BSA. The cell suspension (200 μl) was placed into the upper compartment of a Transwell chamber. Then 600 μl of medium containing 25 μg/ml fibronectin was placed in the lower compartment of the Transwell chamber as a chemoattractant. The Transwell chambers were incubated for 6 h at 37°C in a CO_2 incubator. At the end of the incubation, the cells that had migrated to the under surface of the membranes were fixed with 5% glutareldehyde/PBS solution for 30 min and stained with Giemsa solution. The cells on the upper surface of the filter were completely removed by wiping with a cotton swab. Numbers of the migrated cells were counted at 10 different fields under a microscope at × 200 magnification. Each assay was done in triplicate. The migration capacity was expressed as the number of migrated cells per field.

Tumor cell invasion to Matrigel (Collaborative Research, Bedford, MA) was assayed according to the method reported by Albini *et al.* with some modifications.[20,21] The 8 μm pore membranes of Transwell chambers were coated with 100 μl of a 1:20 dilution of Matrigel in cold serum-free MEM. The Matrigel-coated membranes of Transwell chambers were dried under a hood overnight. The Matrigel was washed twice with 100 μl of serum-free MEM and incubated with MEM for 1 h at room temperature. Then 600 μl of medium containing 25 μg/ml fibronectin was placed in the lower compartment of the Transwell chamber as a chemoattractant. After the medium was removed, 2×10^4 tumor cells suspended in 0.2 ml MEM containing 10% FCS were placed into the upper compartments. After 48 h incubation, the membranes were treated in the same manner as in the *in vitro* migration assay. The invasion capacity was expressed by the number of migrated cells per membrane.

Evaluation of sensitivity to cisplatin and UFT

The established tumor line cells were plated at 2×10^4 onto 1% agarose-coated 96-well plates. After 12 h incubation, cell aggregates were exposed to various concentrations of cisplatin or 5-FU, a substitute for UFT. After 5-day incubation at 37°C in a humidified 5% CO_2/95% air mixture, the growth of the cells was monitored by a colormetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazorium bromide (MTT) assay (Promega, Madison, WI). Briefly, the cells in the 96-well plates were incubated with tetrazolium dye solution for 4 h at 37°C in a humidified 5% CO_2 atmosphere. The plates were then protected from light and kept overnight in a humidified atmosphere for complete solubilization of the formazan crystals. The absorbance at 570 nm wavelength with a reference of 630 nm was measured with an ELISA plate reader (BioRad). The absorbance showed a linear relationship with number of cells. Each assay was done in triplicate.

Statistical analysis

Significant differences in the frequency of metastasis in lung were determined by the χ^2-test and those in chemotactic activities were determined by Student's *t*-test.

Results

Growth of QR-32SK tumor cells in syngeneic mice treated with cisplatin and/or UFT

We examined the antitumor effects of cisplatin and UFT on the growth of QR-32SK tumor cells in syngeneic mice. As shown in Figure 2, QR-32SK tumor cells grew lethally in nine out of 10 mice (90%) when 1×10^7 cells were s.c. injected. QR-32SK tumor cells in the mice treated with cisplatin or UFT grew lethally in 10 out of 10 (100%) or eight out of 10 mice (80%), respectively; and those in the mice treated with cisplatin and UFT grew lethally in nine out of 10 mice (90%). Namely, we did not observe any therapeutic effect of the drugs in any of the groups. Figure 2 shows the mean individual tumor growth curves of each experimental group. No significant difference was observed among the groups.

Metastatic capacities of QRL, QRL/cisplatin, QRL/UFT and QRL/cisplatin/UFT cells

As shown in Table 1, QRL/cisplatin (64%) and QRL/UFT cell lines (65%) showed significantly higher metastatic capacities in lung compared to those of QRL cell lines (26.7%), but QRL/cisplatin/UFT cell lines (11%) did not. It was found that QR-32SK cells acquired metastatic properties even after their growth in syngeneic mice for 21 days (QRL). The single treatment with cisplatin or UFT definitely enhanced the acquisition of metastatic capacity. We also found that the

grade of metastatic capacity of the individual cell lines tended to have inclination (Table 2). Cell lines with high-grade metastatic capacity were more frequent in QRL/cisplatin and QRL/UFT groups.

Gelatinolytic activities and TIMP-1 mRNA expression of the tumor cell lines

Figure 3 shows the metalloproteinase activities and TIMP-1 mRNA expression of the parent and established cell lines. Production of MMP-9 in these cell lines correlated well with their metastatic capacities, except for QRL-2 and QRL/cisplatin/UFT cell lines. To elucidate this discrepancy, we examined the mRNA expression of TIMP-1, a natural inhibitor of MMP-9; however, the TIMP-1 expression was not enhanced in either QRL-2 or QRL/cisplatin/UFT cell lines (Figure 3B).

Chemotactic activities of established cell lines

We examined the chemotactic migration of QR-32SK cells, QRL, QRL/cisplatin, QRL/UFT and QRL/cisplatin/UFT cell lines to fibronectin, using Transwell chambers with non-coated filters. As shown in Figure 4, the

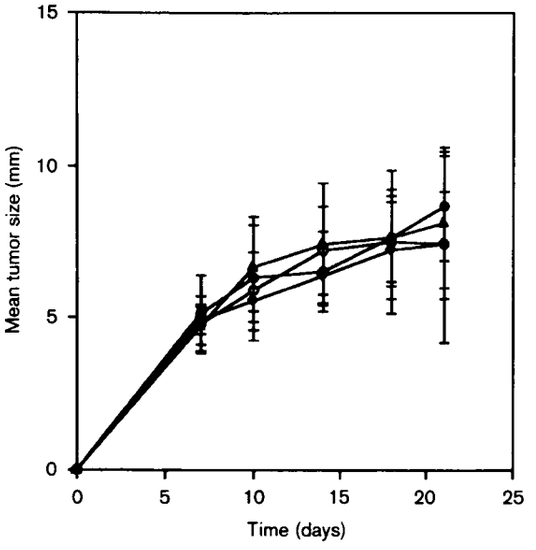

Figure 2. Mean tumor growth curves of QR32SK tumors in the C57BL/6 mice (First step). QR-32SK tumor cells 1×10^7 were implanted s.c. on day 0. UFT (20 mg/kg/day) was administered p.o. from day 5 to day 20, consecutively. On the 11th day after the tumor implantation, cisplatin (4 mg/kg/day) was injected i.p. into QR-32 tumor-bearing mice every other day for 3 times. Each point presents the mean ± SD of average tumor diameter (Experiment 1 plus Experiment 2). ○, Control untreated group; ◆, Cisplatin administration group; ●, UFT administration group; ▲, Cisplatin plus UFT administration group.

Table 1. Altered metastatic capacities of the cells promoted by cisplatin and/or UFT administration

Tumor cell lines[a]	No. of mice with metastasis in lung/no. of mice tested[b]	
QR32SK	0/15 (0%)	
QRL	12/45 (26.7%)	*
QRL/cisplatin	32/50 (64%)	
QRL/UFt	26/40 (65%)	*
QRL/Cisplatin/UFT	5/45 (11%)	

[a]QRL: control group (no treatment). QRL/cisplatin: cisplatin administration group. QRL/UFT: UFT administration group. QRL/cisplatin/UFT: cisplatin plus UFT administration group.
[b]Tumor cells (1×10^6) of the established culture lines were injected i.v. into five normal mice, which were killed 21 days after inoculation. Lungs were fixed with Bouin's solution and superficial metastatic nodules were counted macroscopically.

Table 2. Individual metastatic capacities by cisplatin and/or UFT administration

Tumor cell[a]	Pulmonary metastasis after i.v. injection in mice (no. of metastatic nodules in lung)[b]	
	Experiment 1	Experiment 2
QR-32SK	0/10	0/5
QRL	1, 0/5 2, 2/5 (2, 1) 3, 0/5 4, 4/5 (23, 11, 10, 3) 5, 0/5	6, 4/5 (18, 12, 8, 5) 7, 0/5 8, 0/5 9, 2/5 (8, 1)
QRL/cisplatin	1, 2/5 (2, 1) 2, 5/5 (7, 4, 2, 2, 1) 3, 5/5 (15, 8, 4, 2, 2) 4, 3/5 (2, 1, 1) 5, 0/5	6, 3/5 (4, 3, 1) 7, 5/5 (25, 25, 20, 4, 2) 8, 3/5 (5, 1, 1) 9, 5/5 (15, 10, 9, 4, 3) 10, 1/5 (2)
QRL/UFT	1, 3/5 (2, 2, 2) 2, 4/5 (25, 9, 5, 4) 3, 5/5 (35, 25, 25, 8, 6) 4, 4/5 (13, 12, 11, 2) 5, 3/5 (3, 2, 2)	6, 2/5 (8, 2) 7, 3/5 (4, 4, 3) 8, 2/5 (4, 1)
QRL/cisplatin/UFT	1, 0/5 2, 1/5 (5) 3, 0/5 4, 1/5 (1)	5, 0/5 6, 1/5 (2) 7, 1/5 (1) 8, 1/5 (1) 9, 0/5

[a]QRL: control group (no treatment). QRL/cisplatin: cisplatin administration group. QRL/UFT: UFT administration group. QRL/cisplatin/UFT: cisplatin plus UFT administration group.
[b]Tumor cells (1×10^6) of the established culture lines were injected i.v. into five nromal mice, which were killed 21 days after inoculation. Lungs were fixed with Bouin's solution and superficial metastatic nodules were counted macroscopically.

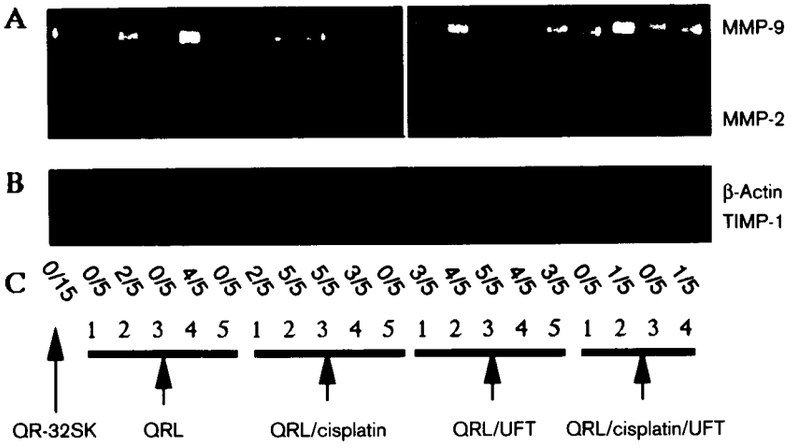

Figure 3. Gelatinolytic activities and TIMP-1 mRNA expression of the tumor cell lines. (A) Gelatinase activities in serum-free conditioned media (CM) of the cell lines. CM was separated by electrophoresis through a non-reducing SDS–polyacrylamide gel containing 0.1% gelatin and processed as described in Materials and methods. (B) RT-PCR analysis of TIMP-1 mRNA expression in each culture cell lines. Total RNAs were extracted and amplified by RT-PCR for 25 cycles. The PCR samples were electrophoresed and stained with ethidium bromide. β-Actin is an internal marker for the applied RNA amounts. (C) *In vivo* metastasis in lung of each tumor cell line (no. of mice with metastasis in lung/no. of mice tested). Tumor cells (1×10^6) of the established culture lines were injected i.v. into five normal syngeneic mice that were killed 21 days later. The lungs were fixed with Bouin's solution and superficial metastatic nodules in each lung were counted macroscopically.

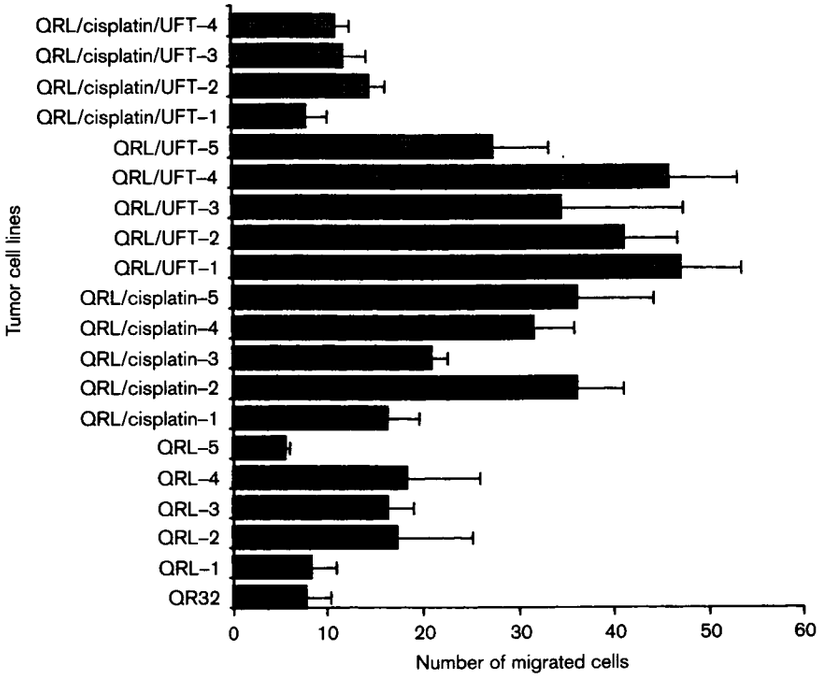

Figure 4. Chemotactic capacities of tumor cell lines to fibronectin. The cell line of each group originates from *in vivo* Experiment 1. The QRL group consists of five cell lines, QRL/cisplatin(n=5), QRL/UFT (n=5) and QRL/cisplatin/UFT ($n=4$), respectively. *In vitro* motility assays were performed using Transwell chambers as described in Materials and methods.

Table 3. Drug sensitivity of the established cell lines to cisplatin or 5-FU

Tumor cell lines	Drug sensitivity (IC_{50}) [mean ± SD (μg/ml)]	
	Cisplatin	5-FU
QR32SK	12.633 ± 0.214	0.066 ± 0.045
QRL	10.921 ± 2.391	0.168 ± 0.111
QRL/cisplatin	19.205 ± 12.424	0.448 ± 0.401
QRL/UFT	14.382 ± 1.887	0.250 ± 0.162
QRL/cisplatin/UFT	16.452 ± 3.03	0.359 ± 0.204

Sensitivity assays were performed using cell spheroid-forming plates as described in Materials and methods. Each cell line raised from *in vivo* Experiment 1 (QRL $n=5$, QRL/cisplatin $n=5$, QRL/UFT $n=5$ and QRL/cisplatin/UFT $n=4$).

chemotactic activities of the relatively high metastatic cell lines were significantly higher than that of QR32 cells, except QRL-4. On the contrary, the chemotactic activities of the relatively low metastatic cell lines were low, except QRL/cisplatin-5.

Drug resistance of established tumor cells

We also investigated drug sensitivities (IC_{50}) of each tumor cell line to cisplatin and to 5-FU in order to examine whether the metastatic capacity of established cell lines was correlated with acquisition of drug resistance. As shown in Table 3, we did not observe significant differences among them in IC_{50} to both drugs. Thus there was no correlation between the resistance to cisplatin or UFT and their metastatic capacities of the tumor cells.

Discussion

In the present study, we have demonstrated that single treatment with either cisplatin or UFT of mice bearing poorly metastatic fibrosarcoma, QR-32SK, caused the enhanced acquisition of metastatic properties of the tumor cells and that combined treatment with cisplatin plus UFT did not.

The fact that chemotherapy can increase metastatic spreads in experimental animals has been widely documented.[1,22,23] The phenomenon has been investigated in two types of experimental models. Namely, in one model, we have known that the influence of chemotherapy on the host may facilitate tumor metastasis.[24-26] For this phenomenon, immunosuppression and vascular damage are thought to be drug-induced modifiers of the host.[27-30] On the other hand, a few experimental reports support the other model that chemotherapeutic agents have a direct effect on the malignancy of tumor cells.[31,32] Frost *et al.* reported that *in vitro* exposure of a non-metastatic murine tumor to 2'-deoxy-5-azacytidine or hydroxyurea converted the ability of this tumor to metastasize spontaneously.[33] Although we treated tumor-bearing mice with chemotherapeutic agents, we observed influence of the agents only on tumor cells since metastatic properties of the established tumor cells were examined in normal syngeneic mice (Figure 1).

For elucidation of the direct effects of chemotherapeutic agents on the tumor cell lines, considerable attention has been given to characterization of mechanisms of drug resistance, with few exceptions,[34,35] but the metastatic behavior of tumor cell lines has not been fully evaluated. Drug sensitivity (IC_{50}) data in this study also showed that drug-promoted metastatic capacity had little relation to drug resistance (Table 3). Although genetic mutation, amplified genes and altered gene expression are caused by the direct effects of chemotherapeutic agents on the tumor cells themselves,[36-39] the exact molecular mechanism for the acquisition of metastases is obscure. In this present study, we speculated that augmentation in metastatic activities might arise as a consequence of alterations in levels of expression of genes in the metastatic variants.

From these points of view, we investigated *in vitro* metastasis-related cell biological properties, and chemotactic, invasive and gelatinolytic activities of the tumor cell lines. The chemotactic activity of the relatively high metastatic cell lines was significantly higher than that of QR-32SK cells, except QRL-4. However, the chemotactic activity of the relatively low metastatic cell lines was not active, except QRL/cisplatin-5 (Figure 4). We further examined the invasive activities of these cells, using a reconstituted Matrigel membrane. However, we could not find any correlation between the invasive activities and metastasis because their invasive activities were very low (unpublished observation). In the zymographic analysis using gelatin-containing SDS-PAGE, production of MMP-9 in these cell lines correlated well with their metastatic capacities, except those of QRL-2 and QRL/cisplatin/UFT cell lines. These exceptions might be because of their low chemotactic activity as shown in Figure 4.

As stated above, neither of these *in vitro* characteristics of tumor cells by themselves could completely explain the mechanisms responsible for *in vivo* metastatic capacities. However, we think our present results are reasonable since metastasis is a complex multistep process.[40,41] Namely, these variations in

metastatic capacities by cisplatin and/or UFT closely correlated with MMP-9 production and chemotactic activities of each cell line.

Although we did not investigate the genetic mechanisms for altered metastatic capacity by the chemotherapeutic agents, these *in vitro* metastatic properties might explain the alteration of metastasis-related gene expression. Interestingly, the use of cisplatin and UFT together does not enhance such metastatic behavior as regularly seen with treatment with a single application of each drug (Table 1). It is important to investigate molecular mechanisms responsible for the combined effects of cisplatin and UFT in further experiments. The effects of combined use of cisplatin and UFT on the emergence of metastatic tumor cells may be relevant in the clinic as reported by Kemeny *et al.*[11]

In conclusion, our model indicates that under certain conditions combination chemotherapy of cisplatin and UFT may be a desirable treatment regime.

Conclusion

We observed that a single treatment with cisplatin or UFT enhanced metastatic capacity of a poorly metastatic fibrosarcoma cell line, QR-32SK, *in vivo*. However, combination therapy of these chemotherapeutic agents failed to promote metastatic capacity. The *in vivo* metastatic capacities were closely correlated with *in vitro* chemotactic activities and the production of MMP-9 of the cultured cell lines. These results suggest that the combination therapy with cisplatin and UFT is useful to prevent the emergence of more malignant tumor cells after chemotherapy.

Acknowledgments

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