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Drug Sensitivity of Small Cell Gastrointestinal Carcinoma

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Summary. **Small cell gastrointestinal carcinoma frequently denotes a poor prognosis. In this study, we carried out drug sensitivity tests *in vitro*, using four small cell gastrointestinal carcinoma cell lines and seven different anti-cancer agents, and the results were compared with those obtained in small cell lung carcinoma (SCLC) and common gastric carcinoma cell lines.**

In addition to the SCLC lines, Lu-130 and Lu-139, the ECC18 cell line was sensitive to mitomycin C (MMC) and vincristine (VCR), while the other three small cell gastrointestinal carcinoma cell lines, ECC10, ECC12 and ECC4, were not sensitive to these agents. Only ECC4 cells were sensitive to 5-fluorouracil (5-FU). Our previous study showed that the ECC18 cell line had similarities to the biochemical variant of SCLC cells, and the three other small cell gastrointestinal carcinoma cell lines were similar to the morphological variant of SCLC cells. The present findings suggest that a regimen including MMC and VCR should be selected as the first choice for treatment of the biological variant of small cell gastrointestinal carcinoma, while most anti-cancer drugs in use at the present time are not effective in the treatment of the morphological variant of small cell gastrointestinal carcinoma. Thus, the development of new anti-cancer agents seems necessary for the morphological variant of small cell gastrointestinal carcinoma.

INTRODUCTION

Small cell gastrointestinal carcinoma is a rare tumor whose nature is not well known. It readily metastasizes to distant organs, frequently indicating a poor prognosis.[1-3] This tumor has been thought to be identical histologically and biologically to small cell lung carcinoma (SCLC).[4] Chemotherapy has been shown to have a substantial impact on the management of patients with SCLC. A number of chemosensitivity tests for SCLC *in vitro* have contributed to the planning of clinical management.[5-9] SCLCs have been studied in detail by using several different cultured cell lines. It is known that some cell lines derived from human colorectal carcinomas have endocrine properties.[10,11] There is, however, no laboratory which possesses plural small cell gastrointestinal cell lines. Furthermore, there are only a few case reports describing clinical trials using chemotherapeutic agents in the treatment of small cell gastrointestinal carcinoma.[12-14] The drug sensitivity of small cell gastrointestinal carcinoma remains poorly understood.

In this study, we examined the *in vitro* drug sensitivity of four small cell gastrointestinal carcinoma cell lines, comparing these with that of SCLC and common gastric carcinoma cell lines.

MATERIALS AND METHODS

Cell lines

Four cell lines derived from small cell gastrointestinal carcinoma, designated ECC18, ECC10, ECC12, and ECC4, were used for this study.[15] For a comparative study, we used the human SCLC cell lines, Lu-130 (small cell carcinoma, classic type, lung) and Lu-139 (small cell carcinoma, classic type, lung),[16] and common gastric adenocarcinoma cell lines, MKN28 (moderately differentiated tubular adenocarcinoma, stomach) and MKN45 (poorly differentiated adenocarcinoma, stomach).[17] The Lu-130 and Lu-139 cell lines were obtained from the Riken Cell Bank (Tsukuba, Japan) (Table 1).

Drug sensitivity test

Weisenthal's dye exclusion assay was used.[18] This assay is closely correlated with MTT assay[19-21] and easily performed without any expensive equipment.

Table 1. Cell lines used in this study.

Cell line	Parent tumor		Type of	Doubling	Previous
	Location	Histology[a]	cultured cell[b]	time (h)	chemotherapy[c]
ECC18	Esophagus	SC	SC-BV	92	(−)
ECC10	Stomach	SC+Ad (well)+Sq	SC-MV	71	(−)
ECC12	Stomach	SC+Ad (well)	SC-MV	69	(−)
ECC4	Rectum	SC+Ad (poor)	SC-MV	56	(−)
Lu-130	Lung	SC	SC-C	78	N.D.
Lu-139	Lung	SC	SC-C	47	N.D.
MKN28	Stomach	Ad (well)	Ad	28	(−)
MKN45	Stomach	Ad (poor)	Ad	30	(−)

[a] SC: Small cell carcinoma, Ad: Adenocarrcinoma, Sq: Squamous cell carcinoma, well: well differentiated type, poor: poorly differentiated type,
[b] BV: Biochemical variant, MV: Morphological variant, C: Classic type, Ad: Adenocarcinoma,
[c] N.D.: Not described.

Table 2. Reference concentrations of drugs used *in vitro* tests.

Anti-cancer agent common name (abbreviation)	Concentration (μg/ml)	Exposure time (h)
Etoposide (VP-16)	125	1
Cis-platin (CDDP)	1	Continuous
Adriamycin (ADM)	1.2	1
Mitomycin C (MMC)	0.5	Continuous
Methotrexate (MTX)	20	Continuous
5-Fluorouracil (5FU)	10	Continuous
Vincristine (VCR)	0.8	1

Table 3. Cell survival rate (%).

Drug	ECC18	ECC4	ECC10	ECC12
ADR	43.7±22.1	90.5±3.9	82.8±11.5	72.0±6.7
CDDP	45.6±12.3	73.9±6.6	76.7±21.4	79.2±12.4
VCR	17.7±3.0	99.3±5.8	69.9±22.4	84.4±1.1
MMC	19.6±14.6	75.6±2.4	70.2±11.8	62.9±8.9
MTX	58.7±30.4	78.0±3.6	64.8±13.2	67.5±15.5
5-FU	49.6±15.9	18.9±6.0	84.0±6.4	37.8±5.9
VP-16	57.3±13.6	84.8±11.1	56.2±33.5	56.9±15.4

Tumor cells were exposed to seven different individual drugs known to be clinically effective against SCLCs or gastrointestinal tract carcinomas. These effectivities were evaluated 4 days later. These drugs included adriamycin (ADM), vincristine (VCR), methotrexate (MTX), cisplatin (CDDP), mitomycin C (MMC), 5-fluorouracil (5-FU), and etoposide (VP-16). The concentration and exposure time were determined in accordance with reports by Weisenthal et al.[18] and Gazdar et al.[7] (Table 2).

Each drug concentration was tested in triplicate or more, and in each experiment, at least 500 cells were counted. The cell survival rate was determined using the following formula:

$$\frac{\dfrac{\text{No. of treated living tumor cells}}{\text{No. of treated living tumor cells + No. of treated dead cells}}}{\dfrac{\text{No. of untreated living tumor cells}}{\text{No. of untreated living tumor cells + No. of untreated dead cells}}} \times 100(\%)$$

In accordance with Weisenthal et al.[18] the drug was considered to be effective against the cell line when the cell survival rate was less than 30% at the reference concentration. To confirm these results, a drug sensitivity test was performed at 10 and 1/10 times concentrations.

RESULTS

Small cell gastrointestinal carcinoma cell lines

The drug sensitivities of small cell gastrointestinal carcinoma are summarized in Table 3 and Fig. 1. The ECC18 cell line was sensitive to VCR and MMC, showing greater sensitivitiy when compared with other small cell gastrointestinal carcinoma cell lines in each concentration. The cell survival rates with VCR and MMC were 17.3% and 26.4%, respectively. However, ECC18 cells were resistant to the other five drugs. The ECC4 cell line was sensitive to 5-FU, and the cell survival rate was 18.9%. However, the ECC4

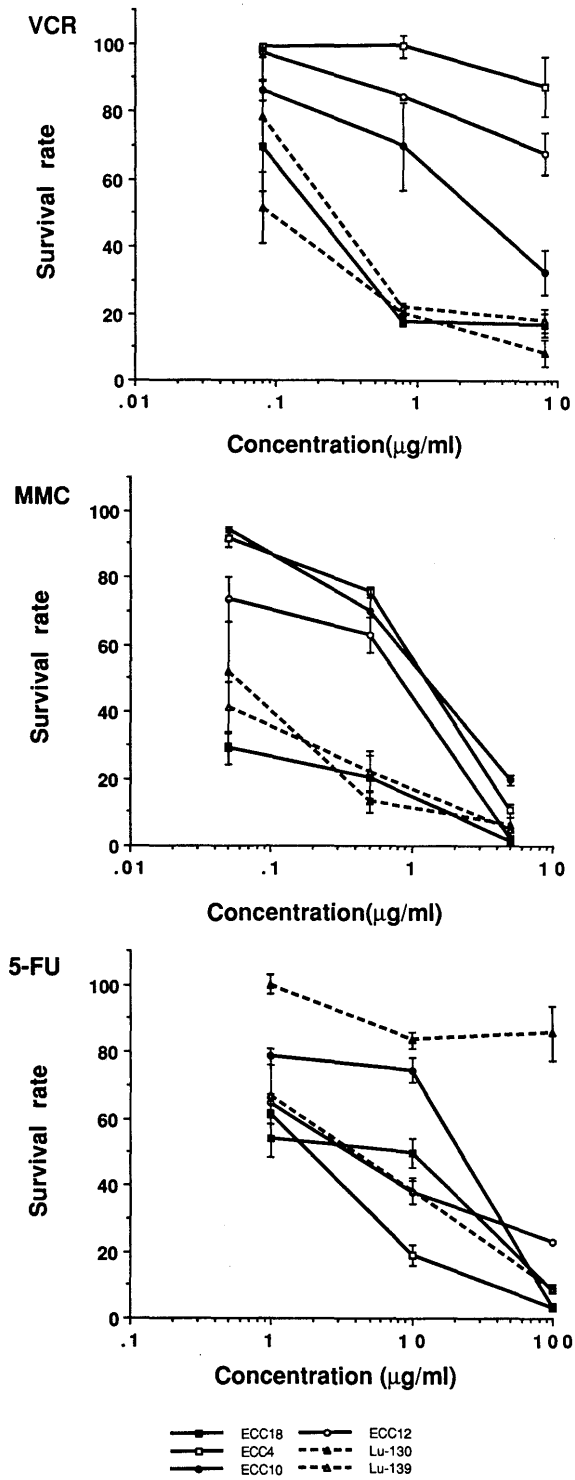

Fig. 1. Dose-response curves. In VCR and MMC, the ECC18 cell line is most sensitive to VCR and MMC among small cell gastrointestinal carcinoma cell lines. 5-FU is effective for only the ECC4 cell line. Each bar represents the mean±SE.

Table 4. Cell survival rate (%)

Drug	Lu-130	LU-139	MKN28	MKN45
ADR	31.5±7.1	32.9±14.3	84.4±5.2	82.9±1.4
CDDP	31.3±12.4	41.4±4.0	75.2±6.9	69.4±11.2
VCR	20.3±5.0	21.8±5.2	100.9±2.7	76.5±11.4
MMC	13.1±5.7	22.1±10.8	61.9±8.6	33.0±22.2
MTX	39.6±13.9	34.3±17.5	85.9±2.3	51.5±4.2
5-FU	86.2±7.0	34.7±13.0	53.7±16.9	53.6±10.0
VP-16	40.2±13.2	58.3±14.8	44.7±5.9	40.5±2.7

cell line was resistant to the other six drugs. The ECC10 cell line was resistant to all seven drugs. The ECC12 cell line had an intermediate degree of resistance to 5-FU (cell survival rate: 37.8%), but was resistant to the other six drugs.

SCLC and common gastric cancer cell lines

Drug sensitivities of SCLC and common gastric cancer are summarized in Table 4 and Fig. 1. Both the Lu-130 and Lu-139 cell lines were sensitive to VCR and MMC. Although the MKN28 cell line showed an intermediate degree of sensitivity to VP-16 (cell survival rate: 44.7%), it was resistant to the other five drugs. The MKN45 cell line showed an intermediate degree of resistance to MMC (cell survival rate: 33.0%) and VP-16 (cell survival rate: 40.5%), but was resistant to the other four drugs.

DISCUSSION

Patients with small cell gastrointestinal carcinoma frequently require chemotherapy. Despite this, we have little knowledge on the drug sensitivity of small cell gastrointestinal carcinoma. Jass et al.[4] described small cell gastrointestinal carcinoma as being biologically identical to SCLC. However, only one small cell gastrointestinal carcinoma cell line, ECC18, showed similarities in drug sensitivity to the classic SCLC cell lines. The ECC18 cell line, as well as the Lu-130 and Lu-139 cell lines, was sensitive to MMC and VCR (Tables 3 and 4). Although it has been shown that MKN45 cells are more sencitive to MMC than MKN28 cells,[22] the present study indicates that ECC18 cells are more sensitive to MMC than MKN45 cells. While MMC is usually used for patients with common gastric carcinoma, it has been seldom used for patients with small cell gastrointestinal carcinoma in Japan. There has been only one case report

indicating that MMC is effective in patients with SCLC.[23]

Incidentally, there are a few reports indicating that a chemotherapeutic regimen including VCR is effective in cases of small cell gastrointestinal carcinoma.[12,13]

Our previous study showed that the ECC18 cell line corresponds to the biochemical variant of SCLC cells, while the ECC10, ECC12, and ECC4 cell lines belong to the morphological variant, according to the classification of SCLC.[15,24] The biochemical variant, as well as the classic type, probably arises directly from true endocrine cells, while the morphological variant may occur *via* "neoplastic neometaplasia" from an adenocarcinoma cell to endocrine cells.[15] Our findings indicate that a regimen including MMC and VCR should be selected for the treatment of small cell gastrointestinal carcinoma, which is a biochemical variant.

Although the ECC4 cell line showed considerable sensitivity to 5-FU, the drug was not effective against the ECC10 and ECC12 cell lines. Small cell gastrointestinal carcinoma cell lines of the morphological variant type were resistant to all drugs examined, except for 5-FU. These findings suggest that most drugs now in use are not effective for the treatment of the morphological variant of small cell gastrointestinal carcinoma. Thus, the development of new anti-cancer agents is necessary for effective treatment of the morphological variants of small cell gastrointestinal carcinoma.

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REFERENCES

1) Sarsfield P, Anthony PP: Small cell undifferentiated ('neuroendocrine') carcinoma of the colon. *Histopathology* **16**: 353-358, 1978.
2) Gaffey MJ, Mills SE, Lack EE: Neuroendocrine carcinoma of the colon and rectum. A clinicopathologic, ultrastructural, and immunohistochemical study of 24 cases. *Am J Surg Pathol* **14**: 1010-1023, 1990.
3) Burke AB, Shekitka KM, Sobin LH: Small cell carcinomas of the large intestine. *Am J Clin Pathol* **95**: 315-321, 1991.
4) Jass JR, Sobin LH, Watanabe H: The world health organization's histologic classification of gastrointestinal tumors. A commentary on the second edition. *Cancer* **66**: 2162-2167, 1990.
5) Ruckdeschel JC, Carney DN, Oie HK, Russel EK, Gazdar AF: *In vitro* chemosensitivity of human lung cancer cell lines. *Cancer Treat Rep* **71**: 697-704, 1987.
6) Carmichael J, Michell JB, Degraff WG, Gamson J, Gazdar AF, Johnson BE, Glastein E, Minna JD: Chemosensitivity testing of human lung cancer cell lines using the MTT assay. *Br J Cancer* **57**: 540-547, 1988.
7) Gazdar AF, Steinberg SM, Russel EK, Linnoila RI, Oie HK, Ghosh BC, Cotelingam JD, Johnson BE, Minna JD, Ihde DC: Correlation of *in vitro* drug-sensitivity testing results with responce to chemotherapy and survival in extensive-stage small cell lung cancer: A prospective clinical trial. *J Natl Cancer Inst* **82**: 117-124, 1990.
8) Campling BG, Pym J, Baker HM, Cole SPC, Lam YM: Chemosensitivity testing of small cell lung cancer using the MTT assay. *Br J Cancer* **63**: 75-83, 1991.
9) Smit EF, Vries EGE, Timmer-Bosscha H, Leij LFHM, Oosterhuis JW, Scheper RJ, Weening JJ, Postmus PE, Mulder NH: *In vitro* response of human small-cell lung-cancer cell lines to chemotherapeutic drugs; No correlation with clinical data. *Int J Cancer* **51**: 72-78, 1992.
10) Park JG, Oie HK, Sugarbaker PH, Henslee JG, Chen TR, Johnson BE, Gazdar AF: Characteristics of cell lines established from human colorectal carcinoma. *Cancer Res* **47**: 6710-6718, 1987.
11) Quinn LA, Moore GE, Morgan RT, Woods LK: Cell lines from human colon carcinoma with unusual cell products, double minutes, and homogeneously staining regions. *Cancer Res* **39**: 4914-4924, 1979.
12) Rosenthal SW, Lemkin JA: Multiple small cell carcinoma of the esophagus. *Cancer* **51**: 1944-1946, 1983.
13) Hussen AM, Feun LG, Sridhar KS, Benedetto P, Waldman S, Otrakji CL: Combination chemotherapy and radiotherapy for small cell carcinoma of the esophagus. *Am J Clin Oncol* **13**: 369-373, 1990.
14) Moertel CG, Kvols LK, O'Connell MJ, Rubin J: Treatment of neuroendocrine carcinomas with combined etoposide and cisplatin. *Cancer* **68**: 227-232, 1991.
15) Fujiwara T, Motoyama T, Ishihara N, Watanabe H, Kumanishi T, Kato K, Ichinose H, Nagatsu T: Characterization of four new cell lines derived from small cell gastrointestinal carcinoma. *Int J Cancer* **54**: 1-7, 1993.
16) Terasaki T, Simosato Y, Nakajima T, Tsumura M, Morinaga S, Hirohashi S, Yamaguchi K, Kato K, Ichinose H, Nagatsu T: Changes in cell characteristics due to culture conditions in cell lines from human small cell lung cancer. *Jap J Clin Oncol* **16**: 203-212, 1986.
17) Motoyama T, Hojo H, Watanabe H: Comparison of seven cell lines derived from human gastric carcinomas. *Acta Pathol Jap* **36**: 65-83, 1986.

18) Weisenthal LM, Marsden JA, Dill PL, Macaluso CK: A novel dye exclusion method for testing *in vitro* chemosensitivity of human tumors. *Cancer Res* **43**: 749-757, 1983.
19) Mosmann T: Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assyas. *J Immunol Methods* **65**: 55-63, 1983.
20) Carmichael J, Ðegraff WG, Gazdar AF Minna JD, Mitchell JB: Evaluation of a Tetrazolium-based semiautomated colorimetric assay: Assessment of chemosensitivity testing. *Cancer Res* **47**: 936-942, 1987.
21) Pieters R, Huismans DR, Leyva A, Veerman AJP: Comparison of the rapid automated MTT-assay with dye exclusion assay for chemosensitivity testing in childhood leukemia. *Br J Cancer* **59**: 217-220, 1989.
22) Motoyama T, Hojo H, Suzuki T, Oboshi S: Evaluation of the regrowth assay method as an *in vitro* drug sensitivity test and its application to cultured human gastric cancer cell lines. *Acta Med Biol* **27**: 49-63, 1979.
23) Ota K, Nishimura M: Present status of lung cancer chemotherapy in Japan. In: Carter SK, Sakurai Y, (eds) Recent results in cancer research. Springer-Verlag, Berlin, Vol 70, 1980, p 167-177.
24) Carney DN, Gazdar AF, Bepler G, Guccion JG, Marangos PJ, Moody TW, Zweig MH, Minna JD: Establishment and identification of small cell lung cancer cell lines having classic and variant features. *Cancer Res* **45**: 2913-2923, 1985.