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Chemotherapy of Human Colon Cancer Transplanted in Nude Mouse†

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= **Abstract** =Human colon carcinomas, transplantable in BALB/c nude mice, designated as SCN-1, SCN-2 and SCN-4, were serially passed for 8, 12 and 8 generations respectively. A piece of each tumor excised to 2 mm in diameter was inoculated subcutaneously on the back of nude mice. Experimental chemotherapies with a single drug, adriamycin, mitomycin C or 5-fluorouracil were started when the tumors reached 5-6 mm in diameter. The results showed that mitomycin C and 5-fluorouracil significantly suppressed the growth of colon cancer. The histologic features of transplanted tumor were similar to those of the original tumors.

Key Words: ***Chemotherapy, Human colon cancer transplanted, Nude mouse***

INTRODUCTION

Experimental animal tumors and cultured cells have generally been used as the screening system for the development of antitumor agents or as the model for experimental chemotherapy. In view of the selectivity of the drug action on malignant tissues, experimental systems with human tumors have been awaited for a long time. Heterotransplantation of human tumors is one of these systems, and well-known hosts are hamsters' cheek pouches (Lemon *et al.* 1952; Chute *et al.* 1952) and immune-deprived animals (Gallagher and Korson 1959; Kutner and Southam 1960; Levin *et al.* 1969).

With the introduction of a congenitally athymic mouse mutant, "nude", many human tumors have been transplanted successfully (Rygaard and Povlsen 1969; Giovanella *et al.* 1972).

Since April 1984, we have transplanted 10 human colon adenocarcinomas to BALB/c nude mice, and established 3 serially transplantable lines (Park *et al.* 1985). This paper deals with results of the single agent chemotherapy with 5-fluorouracil (5-FU), mitomycin-C(MMC), and adriamycin (ADR) on transplantable colon carcinomas (SCN-1, SCN-2, and SCN-4).

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MATERIALS AND METHODS

Three tumor lines selected for this study were obtained originally from surgical materials of colon cancer patients. Tumor line SCN-1 (well-differentiated adenocarcinoma, in 57-year-old male), line SCN-2 (moderately differentiated, in 43-year-old female) and line SCN-4 (poorly differentiated, in 35-year-old male) were obtained from primary tumor of resected colon adenocarcinoma patients. These tumors maintained by serial passage in nude mice were used for experiments between the 8th and 12th passage (Table 1).

Male or female nude mice of BALB/c background, seven to ten weeks old, were obtained from CLEA, Tokyo, Japan, and maintained under specific pathogen free condition. A fragment of tumor, approximately 2 mm in diameter, was inoculated subcutaneously into the back of each mouse. Test mice were randomized into groups of four mice: in group I as control, 0.2 ml of normal saline was given once a week; in group II, adriamycin(ADR) 5 mg/kg once a week; in group III, mitomycin C (MMC) 3 mg/kg once a week; in group IV, 5-fluorouracil (5-FU) 80 mg/kg six times a week. Drug administrations were started intraperitoneally when the tumors reached 5-6 mm in dia-

Table 1. Clinical and pathological characteristics of human colon cancers transplanted in nude mice.

Tumor line	Sex	Age	Site(colon)	Dukes stage	Differentiation	Transfer No.
SCN-1	M	57	Ascending	B	Well	8
SCN-2	F	43	Cecum	C	Moderate	12
SCN-3	M	49	Descending	A	Well	—
SCN-4	M	35	Splenic flexure	C	Poor	8
SCN-5	F	77	Cecum	C	Poor	3*
SCN-6	F	69	Sigmoid	B	Moderate	—
SCN-7	M	69	Rectosigmoid	C	Moderate	—
SCN-8	F	63	Ascending	B	Well	—
SCN-9	M	40	Descending	B	Signet ring cell	2*
SCN-10	M	64	Hepatic flexure	B	Well	—

* Terminated at transfer number indicated

Table 2. Summary of experiment

Tumor line	Sex of mice	No. of mice	No. of tumors taken	Drug	Route administration	Dosage	Times	Schedule
SCN-1 (8)	M	4	8	Control (Normal Saline)	ip	0.2 ml	4	1/wk
		4	8	ADR	ip	5 mg/kg	4	1/wk
		4	7	MMC	ip	3 mg/kg	4	1/wk
		4	8	5-FU	ip	80 mg/kg	24	6/wk
SCN-2(12)	F	4	8	Control (Normal Saline)	ip	0.2 ml	3	1/wk
		4	8	ADR	ip	5 mg/kg	3	1/wk
		4	8	MMC	ip	3 mg/kg	3	1/wk
		4	8	5-FU	ip	80 mg/kg	18	6/wk
SCN-4 (8)	M	4	8	Control (Normal Saline)	ip	0.2 ml	3	1/wk
		4	8	ADR	ip	5 mg/kg	3	1/wk
		4	8	MMC	ip	3 mg/kg	3	1/wk
		4	8	5-FU	ip	80 mg/kg	18	6/wk

meter. As the tumor became ulcerative at 4-6 weeks after inoculation, experiments were designed to be completed within this period (Table 2).

Tumor size in each animal was measured by three dimension twice a week during treatment. Responses to experimental therapies were analysed by comparing tumor growth curve of treated and untreated control groups. Tumor volume was computed according to the formula (AXBXC)/2, where A,B, and C were the lengths of the long and short axes and heights respectively. Inhibition rate in each treatment was expressed by the formula:100 - mean volume of treated tumors/mean volume of control tumors x 100. Histological changes by chemotherapy were also observed.

RESULTS

In body weight changes of SCN-1, SCN-2 or SCN-4 tumor transplanted nude mice, a statistical significance between control and 5-FU treated group was found ($p<0.01$), however, no significant difference was observed between control and MMC or ADR treated group ($p>0.05$) (Fig. 1,2,3).

Growth curves of SCN-1 are shown in Fig.4. In

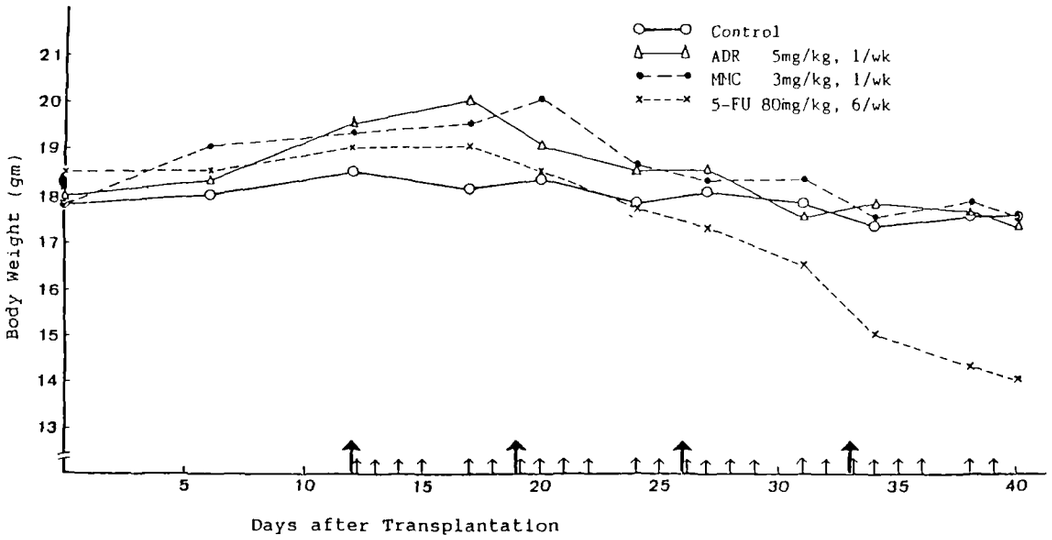

Fig. 1. Effect of chemotherapy on body weight of human colon cancer (SCN-1) bearing nude mice. Arrows indicate administration of drugs.

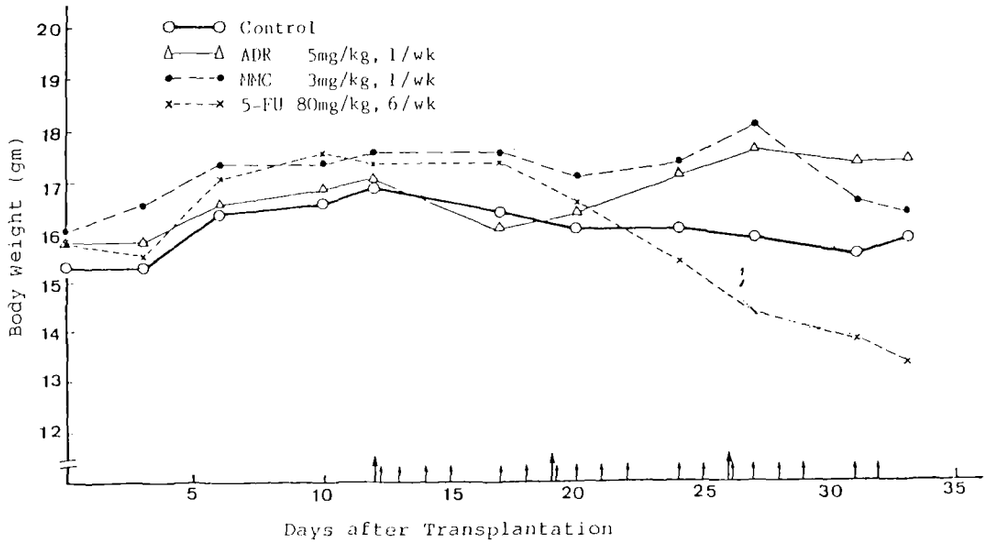

Fig. 2. Effect of chemotherapy on body weight of human colon cancer (SCN-2) bearing nude mice. Arrows indicate administration of drugs.

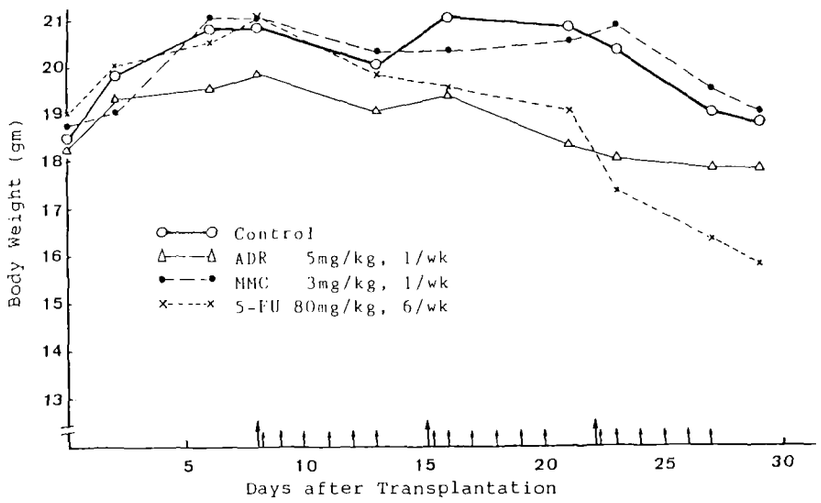

Fig. 3. Effect of chemotherapy on body weight of human colon cancer (SCN-4) bearing nude mice. Arrows indicate administration of drugs.

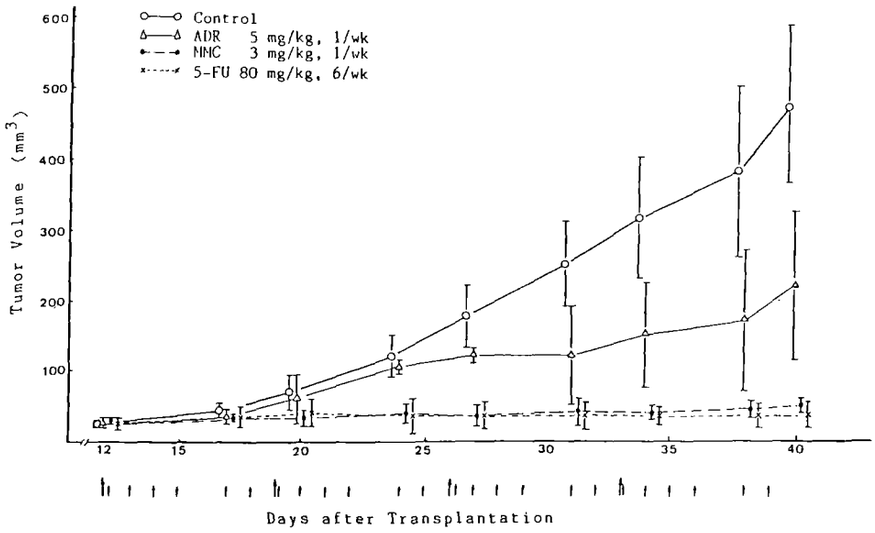

Fig. 4. Effect of chemotherapy on the growth of human colon cancer (SCN-1) transplanted in nude mice. Arrows indicate administration of drugs.

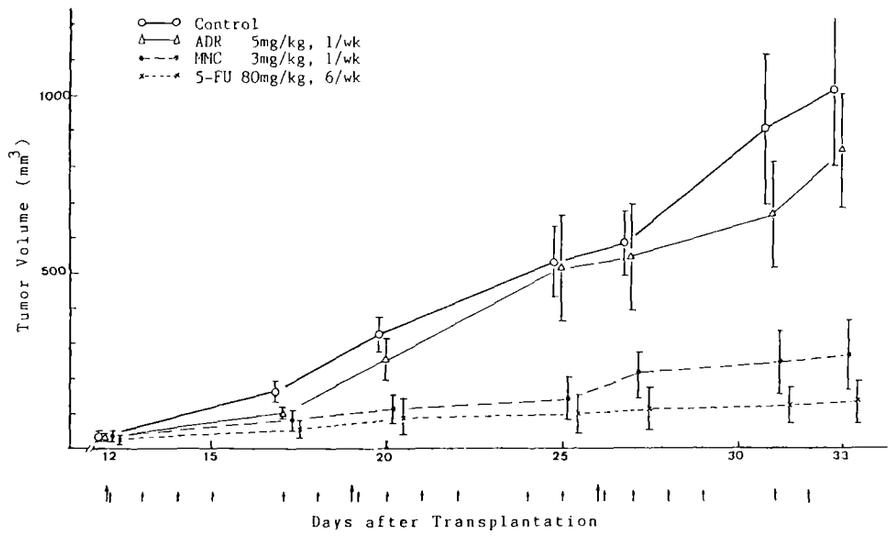

Fig. 5. Effect of chemotherapy on the growth of human colon cancer (SCN-2) transplanted in nude mice. Arrows indicate administration of drugs.

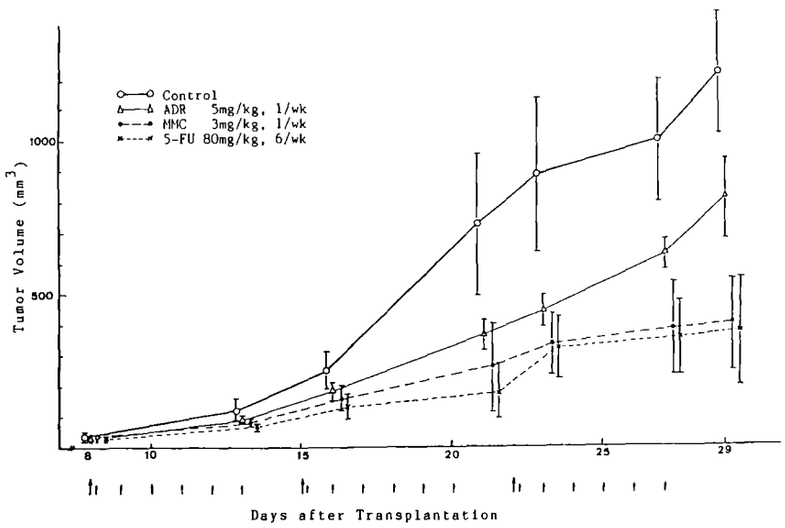

Fig. 6. Effect of chemotherapy on the growth of human colon cancer (SCN-4) transplanted in nude mice. Arrows indicate administration of drugs.

Table 3. Chemosensitivity according to inhibition rate of 3 human colon cancers transplanted in nude mice

Drug \ Tumor line	SCN-1	SCN-2	SCN-4
ADR	53.6%	22.7%	34.7%
MMC	89.2%	76.6%	67.6%
5-FU	92.1%	88.2%	69.4%

Inhibition Rate =

$$100 - \frac{\text{Mean tumor volume of treated group}}{\text{Mean tumor volume of control group}} \times 100$$

contrast to the control group, growth was suppressed in drug treated groups with statistical significance (ADR:$p<0.01$; MMC:$p<0.005$; FU:$p<0.005$). Growth curves of SCN-2 are shown in Fig.5. In contrast to the control group, growth was suppressed in MMC or 5-FU treated group with significance (MMC:$p<0.005$), but in ADR treated group without significance ($p>0.005$). Growth curves of SCN-4 are shown in Fig.6. In contrast to the control group, growth was suppressed in drug treated groups with statistical significance (ADR:$p<0.01$; MMC:$p<0.005$; 5-FU: $p<0.05$).

Table 3 shows the chemosensitivity according to inhibition rate of tumors transplanted in nude mice.

The histologic feature of serially transplanted tumors was similar to those of the original tumors, although the tumor became necrotic and showed lower degree of differentiation than the original tumors. Also, the amount of stroma decreased (Table 4,5,6). In drug treated groups, nuclear pyknosis and cytoplasmic swelling were observed.

DISCUSSION

In 1969, Rygaard and Povlsen successfully heterotransplanted the first human tumor -a colon carcinoma- directly from the patient into nude mice. In 1972, Giovanella *et al.* successfully transplanted a cultured human melanoma cell line in a nude mice.

Sharkey *et al.* (1978) heterotransplanted a wide variety of human tumors into nude mice (342 cases). Progressive growth was noted in 35.7% and takes were higher for recurrent tumors (54.2%) and metastases (49.1%), whereas only 26.9% from primary sites grew. Differences in take rates were also noted among the various tumor groups, as follows: malignant melanoma, 86%; adenocarcinoma of colon, 72%; kidney, 50%; gastrointestinal tract, 20%; breast, 16%.

In this study, we have transplanted ten cases of colon carcinomas and growth was obtained with 5 tumors, but two tumors were lost. So SCN-1, SCN-2 and SCN-4 lines were used for experiment.

Ovejera *et al.* (1977) reported that the average tumor weights of human colon cancer transplanted mice treated with 5-FU, MMC, cyclophophamide or methyl CCNU were 57-74 percent smaller than

Table 4. Pathologic characteristics of SCN-1 tumors during passages and chemotherapy

Passage	Type	Nuclear variation	Necrotic area(%)	Stromal reaction		
				Fibrosis	Inflammation	Edema
Original	W/D, tubular P + mucinous	mild	−	++	++	+
1	M/D, tubular + mucinous	mild	−	++	++	+
2	M/D, mucinous + tubular	mild	−	++	+	+
3	M/D, tubular + mucinous	moderate	10	++	++	+
4	M/D, tubular + mucinous	moderate	10	+	+	+
5	M/D, mucinous + tubular	moderate	20	±	±	±
6	M/D, mucinous + tubular	moderate	20	+	−	−
7	M/D, tubular + mucinous	moderate	30	+	±	+
8:Control	M/D, tubular	moderate	30	++	+	+
ADR	M/D, tubular	moderate	20	+	+	+
MMC	M/D, tubular + mucinous	moderate	10	+	+	±
5-FU	M/D, tubular + mucinous	moderate	20	+	+	+

W/D :well differentiated

W/D :moderately differentiated

Table 5. Pathologic characteristics of SCN-2 tumors during passages and chemotherapy

Passage	Type	Nuclear variation	Necrotic area (%)	Stromal reaction		
				Fibrosis	Inflammation	Edema
Original	M/D, tubular	moderate	10	+++	++	++
1	M/D, cribriform	moderate	−	+	+	+
2	M/D, cribriform	moderate	10	+	+	+
3	M/D, solid	moderate	10	+	+	+
4	M/D, solid and cribriform	moderate	10	±	+	±
5	M/D, solid	moderate	20	±	+	±
7	P/D, solid	moderate	10	+	+	+
8	M/D, cribriform	moderate	20	+	+	+
9	P/D, solid	moderate	30	+	+	+
11	P/D, solid	moderate	40	+	+	+
12:Control	P/D, solid	moderate	70	±	+	±
ADR	M/D, solid	moderate	70	±	+	+
MMC	P/D, solid	severe	50	+	+	+
5-FU	M/D, solid	severe	50	+	+	++

M/D: moderately differentiated
P/D: poorly differentiated

Table 6. Pathologic characteristics of SCN-4 tumors during passages and chemotherapy

Passage	Type	Nuclear variation	Necrotic area (%)	Stromal reaction		
				Fibrosis	Inflammation	Edema
Original	P/D, sheet & nest	moderate	20	+++	++	−
1	P/D, sheet	moderate	20	+++	++	−
2	P/D, solid & lacy	moderate	30	+++	++	+
3	P/D, solid	moderate	20	++	+	−
4	P/D, solid	moderate	20	+	+	±
5	P/D, solid	moderate	20	+	+	−
6	P/D, solid	moderate	50	+	++	−
7	P/D, solid	moderate	50	+	++	±
8:Control	P/D, solid	moderate	70	+	+	±
ADR	P/D, solid	severe	50	+	+	+
MMC	P/D, solid	severe	50	+	+	±
5-FU	P/D, solid	severe	70	+	++	+

P/D: poorly differentiated

those of the controls and the methotrexate was inactive against that tumor model.

In this study, the effectiveness of chemotherapy on colon cancer transplanted in nude mice was investigated by change of body weight, inhibition rate on tumor growth. MMC and 5-FU suppressed the growth of colon cancer significantly but 5-FU administration showed severe body weight loss and ADR was not effective on this tumor model. Many investigators have reported that nude mouse-grown human tumors showed close similarity in histopathology and cytology to the human tumor of origin, even after long periods of transplantation (Povlsen and Rygaard 1971, 1972; Povlsen 1976). Tumors produced by cultural human cell lines also presented histology similar to the original human tumor (Giovanella *et al.* 1972, 1974; Carrel *et al.* 1976).

The study of Fogh *et al.* (1978) has shown that the cells of a cultured line, established from a nude

mouse tumor produced by the human melanoma line MeWo, were identical to MeWo cells in cellular morphology, ultrastructure, growth pattern and rate in vitro, susceptibility to poliovirus, as well as in the isozyme phenotypes of a number of polymorphic enzymes.

In this study the histologic feature of serially transplanted tumors was similar to those of the original tumors, although the tumor became necrotic and showed lower degree of differentiation than the original tumors. Also, the amount of stroma and mitotic count decreased.

Effectiveness of experimental chemotherapy on cancer xenografts in nude mice was parallel to the corresponding clinical responses in their donor patients in others studies (Kubota *et al.* 1978; Fujita *et al.* 1980; Fujita and Taguchi 1982). So, prospective selection of anticancer agents active against individual tumor xenografts will be useful for performing effective chemotherapy to the donor patient with recurrent disease.

REFERENCES

Carrel S, Sordat B, Merenda C. Establishment of a cell line (CO-115) from a human colon carcinoma transplanted into nude mice. Cancer Res. 1976, 36:3978-3984

Chute RN, Sommer SC, Warren S. Heterotransplantation of human cancer. II. Hamster cheek pouch. Cancer Res. 1952, 12:912-914

Fogh J, Bean MA, Brüggen J, Fogh H, Fogh JM, Hammer SP, Kodera Y, Loveless JD, Sorg C, Wright WC. Comparison of a human tumor cell line before and after growth in the nude mouse. In Fogh J and Giovanella BC(Ed), The nude mouse in experimental and clinical research. Academic Press, New York, San Francisco, London, 1978:pp.215-234

Fujita M, Hayata S, Taguchi T. Relationship of chemotherapy on human cancer xenografts in nude mice to clinical response in donor patient. J. Surg. Oncol. 1980, 15:211-219

Fujita M, Taguchi T. Comparison between the chemotherapy of human cancer xenografts in nude mice and the clinical responses observed in the donor patients. In Reed ND(Ed), Proceedings of the third international workshop on nude mice. Gustav Fisher, New York, Stuttgart, 1982:pp.621-629

Gallagher FW, Korson R. Growth of human cancer(HEp 3) in normal rats. Proc. Soc. Exp. Biol. Med. 1959, 100:805-807

Giovanella BC, Stehlin JS, Williams LJ Jr. Heterotransplantation of human malignant tumors in "nude" thymusless mice. II. Malignant tumors induced by injection of cell cultures derived from human solid tumors. J. Natl. Cancer Inst. 1974, 52:921-930

Giovanella BC, Yim SO, Stehlin JS, Williams LJ Jr. Brief communication: development of invasive tumors in the "nude" mouse after injection of cultured human melanoma cells. J. Natl. Cancer Inst. 1972, 48:1531-1533

Kubota T, Shimosato Y, Nagai K. Experimental chemotherapy of carcinoma of the human stomach and colon serially transplanted in nude mice. Gann 1978, 69:299-309

Kutner LJ, Southam CM. Growth of human cancer cells (HEP 2) in newborn rats. Proc. Soc. Exp. Biol. Med. 1960, 104:785-787

Lemon HM, Lutz BR, Pope R, Parsons L, Handler AH, Patt DI. Survival and growth of human tissues transplanted to hamster cheek pouch. Science 1952, 115:461-465

Levin AG, Friberg S Jr, Klein E. Xenotransplantation of a Burkitt lymphoma culture line with surface immunoglobulin specificity. Nature 1969, 222:997-998

Ovejera AA, Houchens DP, Baker AD. Sensitivity of a human tumor xenograft in nude (athymic) mice to various clinically-active drugs. In Nomura T, Ohsawa N, Tamaoki N, Fujiwara K (Ed), Proceedings of the second international workshop on nude mice. University of Tokyo Press 1977

Park JG, Yang HK, Kim JP, Kim WH, Kim YI, Chang WH, Lee MH. Human colon carcinoma transplanted into nude mouse. J. Korean Cancer Res. Assoc. 1985, 17:1-9

Povlsen CO. Heterotransplantation of human malignant melanomas to the mouse mutant nude. Acta Pathol. Microbiol. Scand. (A) 1976, 84:9-16

Povlsen CO, Rygaard J. Heterotransplantation of human adenocarcinomas of the colon and rectum to the mouse mutant nude. A study of nine consecutive transplantations. Acta Pathol. Microbiol. Scand. (A) 1971, 79:159-169

Povlsen CO, Rygaard J. Heterotransplantation of human epidermoid carcinomas to the mouse mutant nude. Acta Pathol. Microbiol. Scand. (A) 1972, 80:713-717

Rygaard J, Povlsen CO. Heterotransplantation of a human maligant tumor to nude mice. Acta Pathol. Microbiol. Scand.(D) 1969, 77:758-760

Sharkey FE, Fogh JM, Hajdu SI, Fitzgerald PJ, Fogh J. Experience in surgical pathology with human tumor growth in the nude mouse. In Fogh J and Giovanella BC(Ed), The nude mouse in experimental and clinical research. Academic Press, New York, San Francisco, London, 1978:pp.187-214

＝국문초록＝

누드 마우스에 이식된 인체대장암에 대한 화학요법

서울대학교 의과대학 외과학교실 및 암연구소

정성은 · 박재갑 · 김진복

大腸癌 患者들인 57세 男子(SCN-1), 43세 女子(SCN-2), 35세 男子(SCN-4)의 癌組織을 BALB/c 누드 마우스의 肩胛下皮下에 移植後 자란 腫瘍을 각기 8代, 12代, 8代씩 계대이식하였다. 각 腫瘍群마다 16마리씩 32개의 腫瘍을 만들어 네 群으로 나눈 後 항암제 投與를 실시하여 각 항암제에 의한 마우스의 體重變化, 腫瘍의 增殖抑制率, 病理所見 등을 觀察하였다.

腫瘍의 直徑이 5～6 mm로 자란 후 약물투여를 시작하였으며, 對照群에는 0.2 ml의 生理食鹽水를 1주일에 1회씩, adriamycin(ADR)은 5 mg/㎏로 1주일에 1회씩, mitomycin-C(MMC)는 3 mg/kg로 1주일에 1회씩, 5-fluorouracil(5-FU)는 80 mg/kg로 1주일에 6회씩, 腹腔內로 注入하였다.

SCN-1에서 腫瘍의 增殖抑制率은 ADR 53.6%, MMC 89.2%, 5-FU 92.1%였으며 腫瘍은 對照群 5.4%, ADR群 11.2%, MMC群 9.3%, 5-FU群 26.3%씩 減少하였다.

SCN-2에서 腫瘍의 增殖抑制率은 ADR 22.7%, MMC 76.6%, 5-FU 88.2%였으며 體重은 對照群 5.9% 減少, ADR群 1.8% 增加, MMC群 5.8% 減少, 5-FU 群 25.3% 減少하였다.

SCN-4에서 腫瘍의 增殖抑制率은 ADR 34.7%, MMC 67.6%, 5-FU 69.4%였으며, 體重은 對照群 9.6%, ADR群 10.0%, MMC群 9.5%, 5-FU群 24.8%씩 減少하였다.

病理的 所見上 移植되어 자란 腫瘍들을 原組織에 가까운 形態를 유지하였으나 腫瘍의 壞死는 심해지고 分化度는 低下되는 傾向이 있었으며, 間質의 量과 核分烈數는 減少하였다. 한편 MMC投與群과 5-FU 投與群에서는 腫瘍 細胞核의 濃縮과 細胞質의 膨化 등이 觀察되었다.

以上의 成績을 綜合하여 보면, 大腸癌은 MMC와 5-FU에 뚜렷한 腫瘍의 增殖抑制效果를 나타냈으나 5-FU는 심한 體重 減少를 동반하였으며, 移植된 腫瘍은 原腫瘍과 類似한 病理組織學的 所見을 보였다.