Cancer Chemother Pharmacol (1999) 43(Suppl): S26–S31

Saburo Sone · Seiji Yano · Masaki Hanibuchi
Hiroshi Nokihara · Naoki Nishimura
Toyokazu Miki · Yasuhiko Nishioka
Tsutomu Shinohara

Heterogeneity of multiorgan metastases of human lung cancer cells genetically engineered to produce cytokines and reversal using chimeric monoclonal antibodies in natural killer cell-depleted severe combined immunodeficient mice

Abstract Lung cancer is a major cause of cancer deaths, most of which can be attributed to distant multiorgan metastases. To examine the cellular and molecular mechanisms of lung cancer metastasis to distant organs, we have established novel models of human lung cancer (small cell and non-small cell lung cancer) metastasis in natural killer cell-depleted severe combined immunodeficient (SCID) mice. We investigated whether local production of the cytokines responsible for regulation of macrophage function at tumor growth sites affects the pattern of lung cancer metastasis in distant organs. Several lung cancer cell lines were genetically engineered to produce human macrophage colony-stimulating factor (M-CSF) and monocyte chemoattractant protein-1 (MCP-1), and their metastatic potentials were assessed. Interestingly, M-CSF gene transduction had an antimetastatic effect for the liver and lymph nodes, but not the kidneys. In contrast, MCP-1 gene-modified lung cancer cells and their parent cells had identical metastatic potentials. These findings indicate a possible role for cyotokines and suggest that lung cancer has metastatic heterogeneity. Examining ways of controlling human lung cancer metastases, we investigated the antimetastatic effect of chimeric monoclonal antibodies (MAbs) against P-glycoprotein and ganglioside GM2 (MH162 and KM966, respectively). Both MAbs, when given on days 2 and 7, inhibited the development of distant metastases of lung cancer in a dose-dependent fashion. Combined use of anti-P-glycoprotein MAb with M-CSF or MCP-1 gene transduction caused complete inhibition of metastasis of H69/VP cells. The antimetastatic effect of these MAbs in vivo was mainly due to an antibody-dependent cell-mediated cytotoxicity reaction mediated by mouse macrophages. These findings suggest that the mouse-human chimeric MAb in combination with cytokine gene transduction may be useful for the eradication of lung cancer metastases in humans.

Key words Lung cancer · Multiorgan metastasis · Macrophage colony-stimulating factor · Chimeric antibody

Work presented at the 14th Bristol-Myers Squibb Nagoya International Cancer Treatment Symposium, "Challenges in Cancer Metastasis," 11–12 September 1998, Nagoya, Japan

S. Sone · S. Yano · M. Hanibuchi · H. Nokihara · N. Nishimura · T. Miki · Y. Nishioka · T. Shinohara
Third Department of Internal Medicine,
University of Tokushima School of Medicine,
Tokushima, Japan

S. Sone (✉)
Third Department of Internal Medicine,
University of Tokushima School of Medicine,
3 Kuramoto-cho, Tokushima 770-8503, Japan

Introduction

Lung cancer is a leading cause of malignancy-related deaths worldwide, and >90% of deaths from lung cancer can be attributed to metastases [21]. The disease is histologically subdivided into two groups, small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC), based on clinical behavior. Lung cancer has often metastasized to lymph nodes and multiple distant organs by the time a diagnosis is made, and therefore metastasis is a critical problem in the therapy of human lung cancer.

Several models in immunodeficient animals, such as nude and severe combined immunodeficient (SCID) mice, have been used to try to elucidate the mechanisms of human lung cancer metastasis [14]. We recently established reproducible models of human small cell and squamous cell lung carcinoma metastasis in natural killer (NK) cell-depleted SCID mice [10, 30]. Moreover, we developed a model of malignant pleural effusion of human adenocarcinoma cells in SCID mice [31].

It has been demonstrated that interactions between tumor and host cells are important in the formation of metastases in specific organs [4]. Organ-specific host cells in different organ microenvironments can influence tumor cells through the production of various cytokines

or components of the extracellular matrix [1, 2]. For example, monocytes/macrophages are thought to have dual roles in the growth of primary and metastatic tumor cells [15]. Unstimulated infiltrating macrophages promote tumor growth whereas after activation they have tumor cytocidal effects. Since the migration and/or activation of macrophages are important in tumor progression and metastasis formation, we believed it interesting to examine whether monocyte chemoattractant protein-1 (MCP-1), a chemoattractant for monocytes [5], influences metastatic patterns. Moreover, we examined whether local production of M-CSF, which is responsible for differentiation, migration, and activation of monocytes/macrophages [24] at tumor growth sites, affects the metastatic pattern of lung cancer in distant organs.

Recently much attention has been paid to several target molecules, such as P-glycoprotein [27] and ganglioside GM2 [22] which are overexpressed in particular types of lung cancer [13, 17], because the selective killing of tumor cells expressing these molecules seems to be important for successful cancer therapy. Since chimeric antibodies against these two molecules were found to induce macrophage/monocyte-mediated tumor cell killing [9, 25], we examined whether chimeric monoclonal antibodies (MAbs) could therapeutically overcome the heterogeneity in the multiorgan metastases of human lung cancer.

Materials and methods

Fetal bovine serum was purchased from M.A. Bioproducts (Walkerville, MD, USA). The mouse–human chimeric anti-human P-glycoprotein MAb, MH162, and mouse anti-human P-glycoprotein MAb, MRK-16 (IgG2a), were obtained as described previously [7, 8]. The anti-mouse IL-2 receptor β chain MAb TM-β1 (IgG2b) was kindly provided by Drs M. Miyasaka and T. Tanaka, Osaka University, Osaka, Japan [26]. The mouse–human chimeric anti-ganglioside GM2 MAb KM966 was a gift from Kyowa Hakko Kogyo Co. (Tokyo, Japan) [17].

The human SCLC cell lines used in the present study were SBC-3, SBC-5, H69, and its etoposide-resistant variant H69/VP [16]. The human NSCLC cell lines used were PC-14 and A549 (adenocarcinoma), PC-13 (large cell carcinoma), and RERF-LC-AI (squamous cell carcinoma). Cell cultures were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum and gentamicin at 37°C in a humidified atmosphere of 5% CO_2 in air. H69/VP cells expressing P-glycoprotein reacted to MH162, but H69 cells did not. In contrast, chimeric anti-ganglioside GM2 MAb KM966 bound to SBC-3 (SCLC) cells.

The pRc/CMV-MCSF plasmid [12] containing a 1.8-kb *Eco*RI cDNA fragment of human M-CSF cDNA was kindly supplied by Dr T. Tsuruo, University of Tokyo, Tokyo, Japan. RERF-LC-AI and H69/VP cells were transduced with pRc/CMV-MCSF using LipofectinTM Reagents (Life Technologies, Gaithersburg, MD, USA). After 18 h, the medium was changed to fresh CRPMI 1640 medium containing G418 800 μg/mL in 24-well plates. Cell subclones resistant to G418 were assayed for expression of human M-CSF mRNA by Northern blotting. For establishment of cell lines that expressed M-CSF stably, the subclones positive for M-CSF mRNA were cloned by limiting dilution. Stable clones transduced with pRc/CMV and expressing Neo mRNA were established by a similar method. Similarly, H69/VP cells were transduced with BCMGSNeoMCAF [18] containing a 400-bp *Pst*I fragment of human MCP-1 cDNA using Lipofectin Reagents as described in detail elsewhere [11].

Six- to 8-week-old male SCID mice were obtained from Charles River (Yokohama, Japan). All mice were maintained under specific pathogen-free conditions and experiments were performed under the guidelines of our university.

In this study, we equated experimental metastasis with the blood-borne spread of tumor cells, as described previously [30]. TM-β1 (1 mg ip) was injected into SCID mice 2 days before tumor inoculation to deplete NK cells. Tumor cells were washed with and resuspended in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS) to the desired cell concentration. Cell viability as determined by trypan blue dye exclusion was >90%. We injected 0.2 mL of tumor cells (5×10^6) into the lateral tail veins of unanesthetized SCID mice pretreated with TM-β1. These mice were treated with PBS, KM966, or anti-P-glycoprotein MAbs (MRK16 or MH162) at the indicated times. Six or 8 weeks after tumor inoculation, the mice were killed and the number of metastatic lymph nodes was counted. Nodules >1 mm in diameter in the liver and kidneys were counted with the aid of a dissecting microscope. In some experiments, the survival of mice was also assessed.

The statistical significance of differences between groups was analyzed using the Student 2-tailed *t* test or Cox-Mantel test.

Results

Models of metastasis of different human lung cancer cell lines were established in NK cell-depleted SCID mice [10, 30, 31]. The results are summarized in Table 1. Using this metastasis model, we examined the metastastic potential of the M-CSF gene transduced into human lung cancer cells in SCID mice. RERF-LC-AI (2×10^6 cells/mouse) or H69/VP (1×10^7 cells/mouse) with or without the M-CSF gene was injected subcutaneously into groups of SCID mice. Injection of the parent cells or mock-transfected cells resulted in palpable tumors in all recipient mice. M-CSF high-producing cells formed palpable tumors in 50–75% of the SCID mice; however, their in vivo growth rates were less than those of parent or mock-transduced cells.

Under these experimental conditions, we examined the metastatic potential of M-CSF gene-transduced human lung cancer cells. For this, RERF-LC-AI (1×10^6) and H69/VP (5×10^6) cells, with or without the M-CSF gene, were inoculated intravenously into SCID mice depleted of NK cells and the mice were killed on days 42 and 56, respectively. Human lung squamous cell carci-

Table 1 Multiorgan metastasis patterns of human lung cancer cell lines in NK cell-depeleted SCID mice

Histologic type	Cell line	Frequency of metastasis*			
		Lymph node	Liver	Kidney	Lung
Small cell	H69	++	++	+	–
	H69/VP	+++	++	+	–
	SBC-3	++	++	++	–
	SBC-5	++	+++	++	++
Squamous	RERF-LC-AI	+	+++	++	–
Adenocarcinoma	PC-14	–	+	+	+++
	A549	–	+	+	+++
Large cell	PC-13	+	+++	+	–

* – none; + few; ++ intermediate; +++ many

noma RERF-LC-AI and mock-transduced cells (Neo-AI-3) formed numerous metastases in the liver and kidneys and a few in the lymph nodes. M-CSF gene transfectants (MCSF-AI-9-18 and MCSF-AI-9-24) produced fewer liver metastases than those of parent and control cells (Table 2). Liver metastases from MCSF-AI-9-1 cells producing low levels of M-CSF tended to be smaller. However, no significant differences were observed in the formation of kidney tumors by M-CSF gene transfectants and their parent or control cells.

In a parallel experiment, SCLC H69/VP and mock-transduced cells (Neo-VP-51) formed metastases in the liver, kidneys, and systemic lymph nodes, as shown in Table 2. M-CSF-gene transfectants (MCSF-VP-5) produced fewer lymph node and liver metastases than parent and mock-transduced cells. Interestingly, no significant differences were observed in the incidence of kidney metastases produced by M-CSF gene transfectants and parent or mock-transduced cells.

We previously demonstrated that mouse anti-P-glycoprotein MAb MRK-16 caused regression of the growth of MDR1-positive tumor cells inoculated subcutaneously into nude mice [23, 25]. We investigated the effect of mouse anti-P-glycoprotein MAb MRK-16 (IgG2a) and its mouse–human chimeric MAb, MH162, on metastasis of SCLC (H69/VP) cells in NK cell-depleted SCID mice. Treatment with MRK-16 and with MH162 on days 2 and 7 markedly reduced multiorgan metastases in the liver, kidneys, and lymph nodes (Table 3).

Table 2 Metastatic potential of human lung cancer cells genetically engineered to produce M-CSF in NK cell-depleted SCID mice

Cell line	M-CSF production ($ng/10^6$/48 h)	Incidence of metastasis (positive mice/total mice)		
		Lymph node	Liver	Kidney
RERF-LC-AI	<0.20	11/16	16/16	16/16
Neo-AI-3	<0.20	7/12	12/12	12/12
MCSF-AI-9-1	1.7	2/4	4/4	4/4
MCSF-AI-9-18	55.0	1/12	5/12	11/12
H69/VP	<0.20	9/9	9/9	9/9
Neo-VP-51	<0.20	9/9	9/9	9/9
MCSF-VP-5	37.6	2/9	2/9	9/9

Similarly, we examined the therapeutic effect of anti-GM2 MAb (KM966) on distant metastasis formation by GM2-positive SCLC (SBC-3) cells in SCID mice. Systemic treatment with KM966 on days 2 and 7 significantly reduced the numbers of metastatic colonies in the liver, kidneys, and lymph nodes in a dose-dependent manner (Table 3).

Cytokines, which induce activation and accumulation of effector cells around the tumor, have been shown to be effective in augmenting the therapeutic potential of anti-P-glycoprotein MAbs for solid tumors growing subcutaneously [19, 23, 25]. Therefore we examined the effects of the combined use of anti-P-glycoprotein MAbs and M-CSF gene or MCP-1 gene transduction in this model.

MCSF-VP-5 cells transfected with pRc/CMV-MCSF produced M-CSF (6.43 $ng/10^6$ cells/48 h), but H69/VP cells and Neo-VP-51 cells transduced with pRc/CMV alone did not. Moreover, transfection of the M-CSF gene into human lung cancer cells did not cause significant changes in P-glycoprotein expression. Under these experimental conditions, Neo-VP-51 cells formed metastases in the liver, kidneys, and systemic lymph nodes, as shown in Table 4. M-CSF gene transfectants (MCSF-VP-5) produced fewer lymph node and liver metastases than parent and mock-transduced cells, but no significant differences were observed in the incidence of kidney metastases. Treatment with MRK-16 0.1 μg significantly reduced Neo-VP-51 cell metastasis, but did not cure all mice. Treatment with the same dose of MRK-16 completely inhibited MCSF-VP-5 cell metastasis.

We also tested whether treatment with MRK16 was effective against multiorgan metastases of MCP-1 gene-modified SCLC cells using a late-treatment model. For this, control H69/VPneo3 cells and the MCP-1 gene-modified cells (H69/VP-MCP17) (5×10^6) genetically engineered to produce MCP-1 (33.5 ng/mL) were inoculated intravenously into NK cell-depleted mice, which were killed on day 56 thereafter. Control cells and the gene-modified cells (H69/VPMCP17) formed numerous metastases, mainly in the systemic lymph nodes and the liver, with a few metastases in the kidneys (Table 5). No significant differences were observed in the incidence of metastases with MCP-1 gene transfectants and mock-transduced cells. Under these experimental conditions,

Table 3 Effect of anti-P-glycoprotein MAbs MRK-16 and MH162 and anti-ganglioside GM2 MAb KM966 on multiorgan metastases of SCLC cells in NK cell-depeleted SCID mice

Cell type	Treatment	MAb dose (μg)	No. of mice with metastases	No. of metastatic colonies		
				Lymph node	Liver	Kidney
Set 1: H69/VP	PBS		15/15	29.2 ± 5.0	15.6 ± 2.7	4.6 ± 4.5
	MRK-16	1	6/9	7.8 ± 1.9*	0.2 ± 0.1*	0.1 ± 0.1*
		10	6/9	2.2 ± 1.0*	0	0
	MH162	1	10/11	16.2 ± 4.1	1.0 ± 0.5*	1.8 ± 1.0
		10	8/11	5.6 ± 1.6*	1.2 ± 0.8*	0.3 ± 0.1*
Set 2: SBC-3	PBS		8/8	14.5 ± 4.1	14.1 ± 2.2	13.0 ± 2.0
	KM966	1	6/8	2.1 ± 2.2*	3.3 ± 3.6*	2.6 ± 2.9*
		10	2/8	0.3 ± 0.5*	0.1 ± 0.4*	0.1 ± 0.3*

* $P < 0.05$

Table 4 Combined use of MRK-16 and M-CSF gene transduction on multiorgan metastasis of H69/VP cells

Cell line	MRK-16 dose (μg)	Mice with tumors/total	Metastatic colonies (mean ± SD)		
			Lymph node	Liver	Kidney
Experiment 1					
H69/VP	0	4/4	27.5 ± 5.5	22.0 ± 6.3	5.0 ± 2.2
Neo-VP-51	0	3/3	21.7 ± 7.2	34.0 ± 21.4	8.0 ± 4.6
	0.1	3/3	8.8 ± 2.3*	5.7 ± 2.1*	1.0 ± 1.0*
MCSF-VP-5	0	3/3	1.0 ± 1.0	0.7 ± 1.2	4.3 ± 1.2
	0.1	1/3	0.3 ± 0.6	0	0
Experiment 2					
Neo-VP-51	0	4/4	57.8 ± 4.0	38.0 ± 11.3	5.8 ± 1.0
	0.1	4/4	25.3 ± 5.9*	3.5 ± 1.9*	0
MCSF-VP-5	0	3/3	0.7 ± 0.6	1.7 ± 1.5	3.3 ± 2.1
	0.1	0/3	0	0	0
Experiment 3					
Neo-VP-51	0	4/4	28.8 ± 7.2	39.3 ± 11.8	6.0 ± 2.5
	0.1	4/4	12.8 ± 8.5*	4.0 ± 3.2*	0.8 ± 1.0*
MCSF-VP-5	0	4/4	1.8 ± 1.3	0.8 ± 1.0	4.8 ± 1.5
	0.1	0/4	0	0	0

* $P < 0.05$ vs mice not treated with MRK-16 by the Student *t*-test

Table 5 Effect of MCP-1 gene transduction into H69/VP cells and MRK-16 treatment on multiorgan metastasis in NK cell-depleted SCID mice

Day of MAb treatment	No. of metastatic nodules (mean ± SD)					
	Liver		Kidney		Lymph node	
	H69/VP neo3	H69/VP MCP17	H69/VP neo3	H69/VP MCP17	H69/VP neo3	H69/VP MCP17
PBS	54 ± 11	62 ± 16	7 ± 1	6 ± 2	44 ± 13	47 ± 7
9 and 14	15 ± 7	2 ± 2*	2 ± 1	0 ± 0*	26 ± 13	6 ± 3*
23 and 28	59 ± 15	12 ± 10*	6 ± 2	1 ± 1*	52 ± 7	27 ± 9*

* $P < 0.05$ vs value for H69/VPneo3

treatment with MRK16 on days 9 and 14 or days 23 and 28 was more effective in inhibiting multiorgan metastasis by MCP-1-producing H69/VP than that of mock-transduced cells.

Discussion

There is accumulating evidence of marked differences in the biological properties of tumor cells in the microenvironment of the subcutaneous space and that of organs [3, 6]. For example, organ-specific host factors can enhance or suppress the growth, vascularization, invasion, and metastasis of human tumors implanted into immunodeficient mice [1, 2], indicating heterogeneity in organ microenvironments. Therefore studies on new therapeutic modalities to inhibit distant multiorgan metastases of tumors should be performed in appropriate animal models of cancer metastasis to various organs. Recently, we established novel and reproducible metastasis models of human lung cancers of different histological origin (SCLC and NSCLC) in NK cell-depleted SCID mice, which formed distant metastases with patterns similar to those observed clinically. These experimental models appear to be more efficient and quantitative than the previously reported models of human lung cancer implanted orthotopically in nude mice [28, 29] due to their simplicity and reproducibility for large-scale assays. Moreover, this model may be suitable for testing the antimetastatic effects of agents that modify the interaction between host factors and tumor cells in vivo.

Transduction of cytokine genes into tumor cells is a useful modality for analysis of cytokine-mediated biological effects on tumor growth. In this study, we examined whether local production of the cytokines responsible for the differentiation, migration, and activation of monocytes/macrophages at the sites of tumor growth affected the metastatic pattern of lung cancer. Although M-CSF gene transduction into human SCLC cells was previously found to result in significant inhibition of subcutaneous growth [25], we found that M-CSF gene transduction resulted in inhibition of metastasis formation by SCLC and NSCLC cells only in the liver and lymph nodes. In contrast, transduction of the M-CSF gene did not affect metastasis formation in the kidneys. Thus the antimetastatic effect of M-CSF gene transduction is organ specific. In a parallel experiment, we found that MCP-1 gene transduction into multidrug-resistant human SCLC (H69/VP) cells did not affect the development of multiorgan metastases. These findings, together with observations for B16 melanoma metastases [1] and considering the heterogeneity of organ microenvironments, indicate that it may be impor-

tant to examine the effect of antimetastatic modalities in different organs using various metastasis models.

To overcome such heterogeneity in multiorgan metastasis, we tried to evaluate the therapeutic effect of mouse–human chimeric MAbs MH162 and KM966, against P-glycoprotein and ganglioside GM2, respectively, on distant metastases. In this study, we demonstrated that treatment with MH162 or KM966 dose-dependently reduces multiorgan metastasis formation by H69/VP and SBC-3 cells, respectively. The number of metastases in the kidneys also appeared to be reduced by treatment with these MAbs. Interestingly, it should be noted that the therapeutic efficacy of both antibodies (MH162 and KM966) was not affected by the heterogeneity of the organ microenvironment. Moreover, combined use of anti-P-glycoprotein MAb with M-CSF gene transduction resulted in complete inhibition of multiorgan metastasis by H69/VP cells. We also found that MCP-1 gene transduction significantly enhanced the therapeutic efficacy of the anti-P-glycoprotein MAb, with prolongation of survival [20]. These findings indicate that these chimeric antibodies have the potential to kill P-glycoprotein- or ganglioside GM2-positive metastatic tumor cells in various organs. However, since treatment with MAbs alone was not enough to cure human lung cancer multiorgan metastasis, combined modality treatment, such as that incorporating MAb plus cytokine gene transduction to augment the therapeutic efficacy of chimeric MAbs, might be required to eradicate metastatic lung cancer in humans.

References

1. Anasagasti MJ, Olaso E, Calvo F, Mendoza L, Martin JJ, Bidaurrazaga J, Vidal-Vanaclocha F (1997) Interleukin 1-dependent and -independent mouse melanoma metastases. J Natl Cancer Inst 89: 645
2. Fidler IJ (1985) Macrophages and metastasis—a biological approach to cancer therapy: presidential adress. Cancer Res 45: 4714
3. Fidler IJ (1990) Critical factors in the biology of human cancer metastasis: twenty-eighth G.H.A. Clowes Memorial Award Lecture. Cancer Res 50: 6130
4. Fidler IJ (1990) Orthotopic implantation of human colon carcinomas into nude mice provides a valuable model for biology and therapy of cancer metastasis. Cancer Metastasis Rev 10: 229
5. Furutani Y, Nomura H, Notake M, Oyamada Y, Fukui T, Yamada M, Larsen CG, Oppenheim JJ, Matsushima K (1989) Cloning and sequencing of the cDNA for human monocyte chemotactic and activating factor (MCAF). Biochem Biophys Res Commun 159: 249
6. Gutman M, Singh RK, Xie K, Bucana CD, Fidler IJ (1995) Regulation of interleukin-8 expression in human melanoma cells by the organ environment. Cancer Res 55: 2470
7. Hamada H, Tsuruo T (1986) Functional role for the 170- to 180-kDa glycoprotein specific to drug-resistant tumor cells as revealed by monoclonal antibodies. Proc Natl Acad Sci USA 83: 7785
8. Hamada H, Miura K, Ariyoshi K, Heike Y, Sato S, Kameyama K, Kurosawa Y, Tsuruo T (1990) Mouse-human chimeric antibody against the multidrug transporter P-glycoprotein. Cancer Res 50: 3167
9. Hanibuchi M, Yano S, Nishioka Y, Yanagawa H, Sone S (1996) Anti-ganglioside GM2 monoclonal antibody-dependent killing of human lung cancer cells by lymphocytes and monocytes. Jpn J Cancer Res 87: 497
10. Hanibuchi M, Yano S, Nishioka Y, Yanagawa H, Tetsuya K, Sone S (1998) Therapeutic efficacy of mouse-human chimeric anti-ganglioside GM2 monoclonal antibody against multiple organ micrometastases of human lung cancer in NK cell-depleted SCID mouse. Int J Cancer 78: 480
11. Hayashi H, Kamohara S, Nishioka Y, Kanai F, Miyake N, Fukui Y, Shibasaki F, Takenawa T, Ebina Y (1992) Insulin treatment stimulates the tyrosine phosphorylation of the a-type 85-kDa subunit of phosphatidylinositol 3-kinase in vivo. J Biol Chem 267: 22575
12. Heike Y, Sone S, Yano S, Seimiya H, Tsuruo T, Ogura T (1993) M-CSF-gene transduction in multidrug-resistant human cancer cells to enhance anti-P-glycoprotein antibody-dependent macrophage-mediated cytotoxicity. Int J Cancer 54: 851
13. Holzmayer TA, Hilsenbeck S, Von Hoff DD, Roninson IB (1992) Clinical correlates of MDR1 (P-glycoprotein) gene expression in ovarian and small-cell lung carcinomas. J Natl Cancer Inst 84: 1486
14. Kuo T, Kubota T, Watanabe M, Furukawa T, Kase S, Tanino H, Nishibori H, Saikawa Y, Teramoto T, Ishibiki K, Kitajima M, Hoffman RM (1992) Orthotopic reconstitution of human small-cell lung carcinoma after intravenous transplantation in SCID mice. Anticancer Res 12: 1407
15. Mantovani A, Bottazzi B, Colotta F, Sozzani S, Ruco L (1992) The origin and function of tumor-associated macrophages. Immunol Today 13: 265
16. Minato K, Kanzawa F, Nishio K, Nakagawa K, Fujiwara Y, Saijo N (1990) Characterization of an etoposide-resistant human small-cell lung cancer cell line. Cancer Chemother Pharmacol 26: 313
17. Nakamura K, Koike M, Shitara K, Kuwana Y, Kiuragi K, Igarashi S, Hasegawa M, Hanai N (1994) Chimeric anti-ganglioside GM2 antibody with antitumor activity. Cancer Res 54: 1511
18. Nakano Y, Kasahara T, Mukaida N, Ko YC, Nakano M, Matsushima K (1994) Protection against lethal bacterial infection in mice by monocyte-chemotactic and -activating factor. Infect Immun 62: 377
19. Nishioka Y, Yano S, Fujiki F, Mukaida N, Matsushima K, Tsuruo T, Sone S (1997) Combined therapy of multidrug-resisstant lung cancer with anti-P-glycoprotein antibody and monocyte chemoattractant protein-1 gene transduction: The possibility of immunological overcoming of multidrug resistance. Int J Cancer 71: 170
20. Nokihara H, Nishioka Y, Yano S, Mukaida N, Matsushima K, Tsuruo T, Sone S (1999) Monocyte chemoattractant protein-1 gene modification of multidrug-resistant human lung cancer enhances antimetastatic effect of therapy with anti-P-glycoprotein antibody in SCID mice. Int J Cancer (in press)
21. Paesmans M, Sculier JP, Libert P, Bureau G, Dabouis G, Thiriaux J, Michel J, Van Cutsem O, Sergysels R, Mommem P, Klastersky J (1995) Prognostic factors for survival in advanced non-small-cell lung cancer: univariate and multivariate analyses including recursive partitioning and amalgamation algorythms in 1,052 patients. J Clin Oncol 13: 1221
22. Ruan S, Lloyd KO (1992) Glycosylation pathways in the biosynthesis of gangliosides in melanoma and neuroblastoma cells: relative glycosyltransferase levels determine ganglioside patterns. Cancer Res 52: 5725
23. Shinohara T, Sugimoto Y, Sato S, Sone S, Tsuruo T (1997) Combination therapy with antibody and interleukin-2 gene transfer against multidrug-resistant cancer cells. Jpn J Cancer Res 88: 1100
24. Sieff CA (1988) Hematopoietic growth factors. J Clin Invest 79: 1549
25. Sone S, Tsuruo T, Sato S, Yano S, Nishioka Y, Shinohara T (1996) Transduction of the macrophage colony-stimulating factor gene into human multidrug resistant cancer cells: en-

hanced therapeutic efficacy of monoclonal anti-P-glycoprotein antibody in nude mice. Jpn J Cancer Res 87: 757
26. Tanaka T, Tsudo M, Karasuyama H, Kitamura F, Kono T, Hatakeyama M, Tanigushi T, Miyasaka M (1991) A novel monoclonal antibody against murine IL-2 receptor β-chain: characterization of receptor expression in normal lymphoid cells and EL-4 cells. J Immunol 147: 2222
27. Tsuruo T (1988) Mechanisms of multidrug resistance and implications for therapy. Jpn J Cancer Res 79: 285
28. Wang X, Fu X, Kubota T, Hoffman RM (1992) A new patient-like metastatic model of human small-cell lung cancer constructed orthotopically with intact tissue via thoracotomy in nude mice. Anticancer Res 29: 1403
29. Wang X, Fu X, Hoffman RM (1992) A new patient-like metastatic model of human lung cancer constructed orthotopically with intact tissue via thoracotomy in immunodeficient mice. Int J Cancer 51: 992
30. Yano S, Nishioka Y, Izumi K, Tsuruo T, Tanaka T, Miyasaka M, Sone S (1996) Novel metastasis model of human lung cancer in SCID mice depleted of NK cells. Int J Cancer 67: 211
31. Yano S, Nokihara H, Hanibuchi M, Parajuli P, Shinohara T, Kawano T, Sone S (1997) Model of malignant pleural effusion of human lung adenocarcinoma in SCID mice. Oncol Res 9: 573