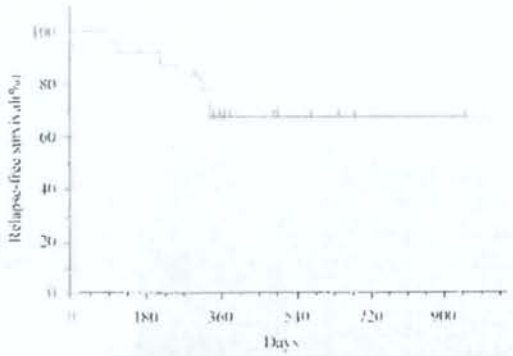

Figure 1. Relapse-free survival.

of the appearance of an adverse event, neutropenia, immediately after the start of the trial. In the ANITA trial, CDDP at 100 mg/m^2 was administered on Day 1 and VNR at 30 mg/m^2 was administered on Days 1, 8, 15 and 22 every 4 weeks, and each 4-week treatment schedule was designated as 1 cycle.

With regard to the patient characteristics, the gender distribution in this study was approximately the same as that in the JBR 10 trial, and the proportion of female patients was higher in this study than in the ANITA trial. The age of the patients was mostly the same in all the trials. In the JBR 10 trial, Stage IIIA NSCLC was not included in the inclusion criteria. The proportion of patients with Stage IB was lower in this study than in the ANITA trial. The reason for the lower proportion of the patients with Stage IB NSCLC in this study seems to be that an alternative choice of UFT for Stage-IB adenocarcinoma is available in Japan, and the results of the meta-analysis by lung adjuvant CDDP evaluation (LACE) (15) did not indicate the usefulness of CDDP-based postoperative chemotherapy for Stage IB patients. With regard to the histological type, the proportion of adenocarcinoma patients was higher in this study than in both the JBR 10 trial and the ANITA trial. In the ANITA trial in particular, the proportion of patients with squamous cell carcinoma was high. The proportions of the patients treated by lobectomy and that of patients with a favorable PS were higher in this study (Table 3). Taking into consideration the treatment schedule and the patient characteristics, this study was comparable to the JBR 10 trial and the ANITA trial in terms of the incidence of adverse events and the compliance (Table 4). There were no significant differences among the trials in terms of the incidence of Grade 3 and 4 adverse events, i.e. of hematological toxicities such as neutropenia and anemia and non-hematological toxicities such as nausea, vomiting, anorexia and febrile neutropenia, although the frequency of fatigue tended to be markedly lower in this study.

High compliance was observed in this study as compared with that in the JBR 10 trial and ANITA trial, and there were no treatment-related deaths. Of the reasons for

Table 3. Comparison among JBR 10, ANITA and this study: patient characteristics

	JBR 10 (n = 242)	ANITA (n = 407)	This study (n = 25)
Gender [No. (%)]			
Male	160 (66%)	346 (85%)	18 (72%)
Female	82 (34%)	59 (14%)	7 (28%)
Age (years)			
Median	61	59	62
Range	35–82	32–75	39–74
Stage [No. (%)]			
IB	111 (46%)	146 (36%)	1 (4%)
IIA	39 (16%)	89 (22%)	8 (32%)
IIB	92 (38%)		6 (24%)
IIIA	0 (0)	166 (41%)	10 (40%)
Hisology [No. (%)]			
Ad.	128 (53%)		16 (64%)
Sq.	90 (37%)	240 (59%)	4 (16%)
Large	24 (10%)		3 (12%)
Others			2 (8%)
Operation [No. (%)]			
Lobectomy	182 (75%)	253 (62%)	22 (88%)
PN	60 (25%)	155 (38%)	3 (12%)
PS			
0	141 (58%)	195 (48%)	19 (76%)
1	141 (58%)	191 (47%)	6 (24%)

ANITA, Adjuvant Navelbine International Trialist Association; PN, pneumonectomy

treatment discontinuation in the JBR 10 trial, treatment

Table 4. Comparison among JBR 10, ANITA and this study: Grade 3/4 adverse events, compliance and treatment-related death

	JBR 10 (%)	ANITA (%)	This study (%)
Neutropenia	73	85	76
Anemia	7	14	12
Thrombocytopenia	1	3	0
Fatigue	15	28	4
Anorexia	10	15	12
Nausea	10	27	12
Vomiting	7	27	4
Constipation	3	5	0
Febrile neutropenia	7	9	4
Treatment-related death	0.8	2.0	0
Treatment complete	48	CDDP 49 VNR 50	92

CDDP, cisplatin; VNR, vinorelbine

rejection by the patient and toxicity accounted for 29% and 13%, respectively (16). These observations suggest that the low frequency of treatment rejection by the patients in this study might have been the reason for the high compliance, and in turn, the reason for the low frequency of rejection of treatment by the patients could have been that the fatigue was mild. However, there is the possibility that non-hematological toxicity was evaluated milder in retrospective setting and that more patients rejected treatment continuation in the JBR 10 trial and ANITA trial because the benefit of adjuvant chemotherapy had not been demonstrated at that time. With regard to the relationships of the PS and surgical stress to compliance, there was no correlation between the surgical stress and compliance in the ANITA trial. In the JBR 10 trial, on the other hand, a correlation was noted between surgical stress and compliance, but not between surgical stress and the PS. In this study, however, there were many patients who showed favorable PS and only slight surgical stress, and the proportion of the patients rejecting treatment was also low, presumably because of the mild toxicity. These factors were estimated to account for the high compliance. The difference in the socio-cultural characteristics between the Japanese and Western populations may also have had an influence on the high compliance.

There was a better trend of overall survival in favor of higher total CDDP dose in LACE (15). On the other hand, there was no significant interaction between total CDDP dose and overall survival in IALT (17). The total of CDDP and VNR dose in this study was comparable to those in JBR 10 trial as the mean cumulative dose of CDDP was 275 mg·m^{-2} and the mean cumulative dose of VNR was 199 mg·m^{-2} in JBR 10 trial (15).

CDDP at 80 mg/m^2 administered on Day 1 plus VNR at 25 mg/m^2 administered on Days 1 and 8 every 3 weeks is effective and commonly used for Stage IIIB/IV NSCLC in Japanese patients (14). Major grade 3 or 4 adverse events of this chemotherapeutic regimen for Stage IIIB/IV NSCLC in Japanese patients were neutropenia in 88%, anemia in 30%, anorexia in 21%, febrile neutropenia in 18% and nausea in 14% of the patients (14). Adverse events in this study were milder because PS and main organ function would be better in adjuvant setting than in advanced and metastatic settings.

All the above-described study observations indicate that postoperative combined adjuvant chemotherapy with CDDP administered on Day 1 and VNR administered on Days 1 and 8 every 3 weeks is safe and that the rate of completion of treatment is also satisfactory in Japanese patients.

Conflict of interest statement

None declared.

References

1. Goya T, Asamura H, Yoshimura H, Kato H, Shimokata K, Tsuchiya R, et al. Prognosis of 6644 resected non-small cell lung cancers in Japan: a Japanese lung cancer registry study. *Lung Cancer* 2005;50:227–34.
2. Nitanda A, Nitanda-Kouhnen S, Kouri M, Assendelft A, Mattson K, Pyrhönen S. Adjuvant chemotherapy after radical surgery for non-small-cell lung cancer: a randomized study. *J Clin Oncol* 1992;10:1927–32.
3. Tada Y, Tsuchiya R, Ichinose Y, Koike T, Nishizawa N, Nagai K, et al. A randomized trial comparing adjuvant chemotherapy versus surgery alone for completely resected pN2 non-small cell lung cancer (JCOG9304). *Lung Cancer* 2004;43:167–73.
4. Waller D, Peake RJ, Stephens RJ, Gower NH, Milroy R, Parmar MK, et al. Chemotherapy for patients with non-small cell lung cancer: the surgical setting of the Big Lung Trial. *Eur J Cardiothorac Surg* 2004;26:173–82.
5. Scagliotti GV, Fossati R, Torri V, Crinò L, Giaccone G, Silvano G, et al. Randomized study of adjuvant chemotherapy for completely resected stage I, II or IIIA non-small-cell lung cancer. *J Natl Cancer Inst* 2003;95:1453–61.
6. Non-Small Cell Lung Cancer Group. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. *BMJ* 1995;311:899–909.
7. Arriagada R, Bergman B, Dunant A, Le Chevalier T, Pignon JP, Vansteenkiste J, et al. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. *N Engl J Med* 2004;350:351–60.
8. Winton TL, Livingston R, Johnson D, Rigas J, Johnston M, Butts C, et al. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. *N Engl J Med* 2005;352:2589–97.
9. Douillard J, Rosell R, Delena M, Carpagnano F, Ramlau R, Gonzáles-Larriba JL, et al. Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB–IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): a randomised controlled trial. *Lancet Oncol* 2006;7:719–27.
10. Strauss GM, Herndon JE, Maddaus MA, Johnstone DW, Johnson EA, Watson DM, et al. Randomized clinical trial of adjuvant chemotherapy with paclitaxel and carboplatin following resection in stage IB non-small cell lung cancer (NSCLC): report of Cancer and Leukemia Group B (CALGB) Protocol 9633. *J Clin Oncol* 2004;22:62ls. (Abstract #7019).
11. Strauss GM, Herndon JE II, Maddaus MA, Johnstone DW, Johnson EA, Watson DM, et al. Adjuvant chemotherapy in Stage IB non-small cell lung cancer (NSCLC): update of Cancer and Leukimia Group B (CALGB) protocol 9633. *J Clin Oncol* 2006;24:365s. (Abstract #7007).
12. Pisters KM, Evans WK, Azzoli CG, Kris MG, Smith CA, Desch CE, et al. Cancer care Ontario and American society of clinical oncology adjuvant chemotherapy and adjuvant radiation therapy for stages I-IIIA resectable non-small-cell lung cancer guideline. *J Clin Oncol* 2007;25:5506–18.
13. Gebbia V, Galetta D, Lorusso V, Caruso M, Verderame F, Pezzella G, et al. Cisplatin plus weekly vinorelbine versus cisplatin plus vinorelbine on days 1 and 8 in advanced non-small cell lung cancer: a prospective randomized phase III trial of the G.O.I.M. (Gruppo Oncologico Italia Meridionale). *Lung Cancer* 2008; available online use.
14. Ohe Y, Ohashi Y, Kubota K, Tamura T, Nakagawa K, Negoro S, et al. Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. *Ann Oncol* 2007;18:317–23.
15. Pignon JP, Tribodet H, Scagliotti GV, Douillard JY, Shepherd FA, Stephens RJ, et al. Lung Adjuvant Cisplatin Evaluation: A Pooled Analysis by the LACE Collaborative Group. *J Clin Oncol* 2008;26:3552–9.
16. Alam N, Shepherd FA, Winton T, Graham B, Johnson D, Livingston R, et al. Compliance with post-operative adjuvant chemotherapy in non-small cell lung cancer an analysis of National Cancer Institute of Canada and intergroup trial JBR.10 and a review of the literature. *Lung Cancer* 2005;47:385–94.
17. Chevalier TL, Dunant A, Arriagada R, Bergman B, Chabowski M, LePechoux C, et al. Long-term results of the International Adjuvant Lung Cancer Trial (IALT) evaluating adjuvant cisplatin-based chemotherapy in resected non-small cell lung cancer (NSCLC). *J Clin Oncol* 2008;26:15s. (abstr. #7507).

Circ J 2008; **72:** 2069–2074

Gender Differences in Chronic Thromboembolic Pulmonary Hypertension in Japan

Ayako Shigeta, MD; Nobuhiro Tanabe, MD; Hidefumi Shimizu, MD; Susumu Hoshino, MD; Miki Maruoka, MD; Seiichiro Sakao, MD; Yuji Tada, MD; Yasunori Kasahara, MD; Yuichi Takiguchi, MD; Koichiro Tatsumi, MD; Masahisa Masuda, MD*; Takayuki Kuriyama, MD

Background The predominance of chronic thromboembolic pulmonary hypertension (CTEPH) in females and association of HLA-B*5201 with CTEPH have been reported in Japan. However, the clinical characteristics of female CTEPH remain uncertain. The purpose of the present study is to clarify the clinical phenotype of female CTEPH in Japan.

Methods and Results The 150 consecutive patients (female 103, male 47; age 52.8±12.4 years SD) were admitted to Chiba University Hospital, and diagnosis was confirmed using right cardiac catheterization and pulmonary angiography. Among these patients, 78 underwent pulmonary endarterectomy. Clinical characteristics, pulmonary hemodynamics, extent of central disease and surgical outcome in females were compared with those in males. The female patients were elderly and had less deep vein thrombosis, less acute embolic episodes, better cardiac function, lower arterial oxygen tension and more peripheral thrombi, and showed less improvement through surgery than males. When the patients were identified using HLA-B*5201, HLA-B*5201-positive female patients had less embolic episodes and better cardiac function with lower operative mortality. In contrast, HLA-B*5201-negative female patients had less embolic episodes, and more peripheral thrombi, resulting in less improvement by surgery.

Conclusion The clinical phenotype of female CTEPH differed from that of male CTEPH. Additionally, gender differences of HLA-B*5201-positive type were dissimilar to those of HLA-B*5201-negative type. (*Circ J* 2008; **72:** 2069–2074)

Key Words: Chronic thromboembolic pulmonary hypertension; Gender difference; HLA; Pulmonary embolism

Chronic thromboembolic pulmonary hypertension (CTEPH) has been considered to be caused by single or recurrent pulmonary emboli arising from deep vein thrombosis (DVT)[1,2] However, the incidence of DVT in this disease is only 35 to 45% in the USA and 12 to 38% in Japan[2–6] It was reported that the risk of recurrent venous thromboembolism was higher in men than women[7] The female-to-male ratio in CTEPH was 2.1 in Japan, which is much higher than that of 0.7 in the USA[3,8] However, the incidence of DVT in females was similar to that in males, even in Japan[9] Jamieson reported a female predominance in type 3 disease (distal segmental arteries only type)[10] In addition, we previously reported that female predominant CTEPH without DVT exists in Japan, and that the disease was associated with HLA-B*5201 and HLA-DPB1*0202. HLA-B*5201-positive patients were predominantly female, and this was unrelated to DVT[11]

It remains uncertain whether the clinical phenotype in female CTEPH differed from male CTEPH, especially in the Japanese series.

The purpose of the present study is to clarify the clinical phenotype in female CTEPH in Japan. We also examined the clinical phenotype of female CTEPH when patients were analysed according to HLA-B*5201 status because the HLA-B*5201-positive type could indicate a female-predominant Japanese-specific type, while the HLA-B*5201-negative type could indicate a DVT-related type similar to Western countries. Because of the female predominance in HLA-B*5201 patients, gender differences in the clinical parameters might be more related to the HLA-B*5201-positive type than the female gender itself. We added multivariate analysis to clarify whether female gender or HLA-B*5201 had the main effects on clinical parameters.

Methods

Study Subjects

We studied 150 patients, 103 females and 47 males, with CTEPH, diagnosed at Chiba University Hospital, Chiba, Japan. CTEPH was defined as mean pulmonary arterial pressure (Ppa) >25 mmHg with normal wedge pressure in patients with symptoms for >6 months. Chronic thromboembolic findings were confirmed using pulmonary angiography. All patients were examined using blood gas examination, right-heart catheterization, pulmonary angiography and computed tomographic angiography. Seventy-eight of the patients underwent pulmonary endarterectomy.

(Received April 17, 2008; revised manuscript received July 7, 2008; accepted July 15, 2008; released online October 17, 2008)

*Department of Respirology, Graduate School of Medicine, Chiba University, *National Hospital Organization, Chiba Medical Center, Chiba, Japan*

Mailing address: Ayako Shigeta, MD, Department of Respirology, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuou-ku, Chiba 260-8670, Japan. E-mail: ayakosh@nifty.com

All rights are reserved to the Japanese Circulation Society. For permissions, please e-mail: cj@j-circ.or.jp

Table 1 Clinical Characteristics of All Patients With CTEPH (n=150)

Age (years)	*52.8±12.4*
F/M (n)	*103/47*
Acute embolic episodes (%)	*45.3*
Underlying disease	
DVT (%)	*38.7*
Pelvic surgery (%)	*13.3*
Coagulopathy (%)	*31.3*
(Anti-cardiolipin antibody) (%)	*24.7*
Malignancy (%)	*4.0*
Heart disease (%)	*7.3*
*HLA-B*5201 (%)*	*31.3*
Cardiorespiratory variables	
Mean Pra (mmHg)	*5.2±4.4*
Mean Ppa (mmHg)	*44.2±11.1*
Cardiac index ($L \cdot min^{-1} \cdot m^{-2}$)	*2.54±0.63*
PVR ($dynes \cdot s \cdot cm^{-5}$)	*827±382*
PaO_2 (Torr)	*58.6±9.8*
WHO functional classification I/II/III/IV	*2/37/97/14*

Values are mean±SD or n (%).
CTEPH, chronic thromboembolic pulmonary hypertension; DVT, deep vein thrombosis; Pra, right atrium pressure; Ppa, pulmonary arterial pressure; PVR, pulmonary vascular resistance; PaO_2, arterial oxygen tension.

Measurements

At least 3 months after an acute episode, pulmonary hemodynamics, cardiac output (using thermodilution technique) and blood gases were measured in the supine position while breathing normally. The cardiac index was calculated as cardiac output divided by body surface area. Pulmonary vascular resistance (PVR) was calculated conventionally as the ratio of the difference between mean Ppa and pulmonary wedge pressure to cardiac output. Cardiorespiratory variables were also measured after surgery. To evaluate the effectiveness of surgery, the % decrease in PVR was calculated using [preoperative PVR−postoperative PVR]×100 (%)/preoperative PVR.

HLA Typing

HLA typing was analyzed in 126 patients. Serological HLA typing of A and B antigens was done using a standard microcytotoxicity test[12] Genomic DNA was obtained from peripheral blood leukocytes using a QIAamp DNA blood minikit (Qiagen). DNA typing of HLA-B and –DPB1 genes was performed using a RELI-typing kit (Dynal) and/or using SSO probes as previously reported[13,14]

Assessment of Central Extent of Thrombi

Using the Bergin's method, central arteries were defined as vessels proximal to the segmental branches and were divided into 4 portions. These portions included the right and left main pulmonary arteries proximal to the upper lobe branches and the right and left descending portions of the central arteries between the upper lobes and the segmental branches. The central disease score was quantified by adding up the number of abnormal central portions in each patient up to a maximum score of 4[15] Two investigators retrospectively calculated the scores independently by workstation, and if the score differed, it was changed to either one score up or down by consensual agreement of the 2 investigators.

During the operation, thromboembolic disease was visualized and each patient was classified into one of 4 groups as reported by Thistlethwaite (intraoperative classification): type 1, fresh thrombus in the main-lobar pulmonary arteries; type 2, intimal thickening and fibrosis proximal to the segmental arteries; type 3, disease within distal segmental arteries only; and type 4, distal arteriolar vasculopathy without visible thromboembolic disease[16]

Pulmonary Endarterectomy

The selection criteria for pulmonary thromboendarterectomy were slightly modified from those defined by Moser[17] Our criteria were: (1) mean Ppa >30 mmHg, resulting in calculated PVR >300 $dynes \cdot s \cdot cm^{-5}$ even after oral anticoagulant therapy for >6 months; (2) WHO functional class ≥3; (3) thrombi defined as accessible to current surgical techniques (presence at main, lobar, segmental arteries); and (4) absence of severe associated disease[4,18] Although we have used a lateral thoracotomy in 16 previous cases, since 1990 we used median sternotomy with the application of deep hypothermia and circulatory arrest in 62 cases[4,19]

The Human Subject Committee of Chiba University approved the study, written informed consent was obtained from all patients and the study protocol for HLA typing was approved by the Research Ethics Committee of Chiba University School of Medicine.

Clinical characteristics were compared between males and females in all patients, and in patients with or without HLA-B*5201, respectively.

Statistical Analysis

Comparison of males and females was performed using unpaired Student's t-test when data were continuous variables, and by chi-square test or Mann-Whitney test when data were categorical, where appropriate. We performed a 2-way factorial analysis of variance (ANOVA) for parametric data and multiple regression analysis for categorized data, using gender and HLA-B*5201 as independent variables, and other clinical parameters as dependent variables. A p-value<0.05 was considered significant.

Results

Patient Characteristics

Characteristics of the patients are shown in Table 1. There were more female (n=103) than male patients (n=47). Age at catheterization varied from 18 to 78 years, with a mean±SD of 52.8±12.4. Sixty-eight patients (45.3%) had a history of acute embolic episodes. Fifty-eight patients (38.7%) had a history of DVT. Forty-seven patients (31.3%) revealed abnormalities in the screening for coagulopathy. Thirty-seven patients (24.7%) were diagnosed with antiphospholipid syndrome. Mean Ppa, cardiac index, PVR and PaO_2 were 44.2±11.1 mmHg, 2.54±0.63 $L \cdot min^{-1} \cdot m^{-2}$, 827±382 $dynes \cdot s \cdot cm^{-5}$ and 58.6±9.8 Torr, respectively. The patients were classified as WHO functional class I (n=2), class II (n=37), class III (n=97) and class IV (n=14).

Comparison of Clinical Characteristics Between Males and Females

As shown in Table 2, female patients were significantly older than males (54.3±11.3 vs 49.6±14.1 years, p=0.03). Female patients showed significantly less acute embolic episodes (34.0 vs 70.2%, p<0.001) and less history of DVT (31.1 vs 55.3%, p=0.005) compared with males. Female patients had a significantly greater history of pelvic surgery compared with males (19.4 vs 0.0%, p=0.012), while females had significantly less heart disease than males (3.9 vs 14.9%, p=0.016).

The cardiac index was significantly higher in females than in males (2.65±0.62 vs 2.36±0.66 $L \cdot min^{-1} \cdot m^{-2}$, p=0.01), and

Table 2 Gender Differences of Clinical Characteristics in CTEPH Patients

	Female (n=103)	Male (n=47)	p value
Age (years)	54.3±11.3	49.6±14.1	0.03
Acute embolic episodes (%)	34.0	70.2	<0.001
Underlying disease			
DVT (%)	31.1	55.3	0.005
Pelvic surgery (%)	19.4	0.0	0.012
Coagulopathy (%)	27.1	40.4	0.105
(Anti-cardiolipin antibody) (%)	23.2	30.4	0.35
Malignancy (%)	1.9	8.5	0.057
Heart disease (%)	3.9	14.9	0.016
HLA-B*5201 (%)	41.6	27.0	0.124
Haemodynamics			
Pra (mmHg)	4.3±3.5	7.1±5.5	0.0002
Mean Ppa (mmHg)	44.8±11.1	43.0±11.3	0.36
Cardiac index ($L \cdot min^{-1} \cdot m^{-2}$)	2.65±0.62	2.36±0.66	0.01
PVR ($dynes \cdot s \cdot cm^{-5}$)	850±393	777±357	0.28
PaO_2 (Torr)	56.4±9.8	61.7±9.8	0.005
Location of thrombi			
Central disease score	1.09±1.01	1.83±1.27	0.0002
Intra-operative classification			
Type 1/2/3/4	29/10/8/0	28/0/2/1	
Non-type 1 (%)	38.3	9.7	0.005
WHO functional classification I/II/III/IV	1/23/69/10	1/14/28/4	0.71

Values are mean±SD or n (%).
Abbreviations see in Table 1.

Table 3 Gender Differences for Surgical Outcome by Pulmonary Endarterectomy

	Female (n=47)	Male (n=31)	p value
Operative mortality (%)	11	23	0.15
Postoperative PVR ($dynes \cdot s \cdot cm^{-5}$)	406±282	257±119	0.02
% decrease in PVR (%)	51.9±25.4	65.2±21.4	0.04

Values are mean±SD or n (%).
% decrease in PVR, [preoperative PVR-postoperative PVR]×100(%)/preoperative PVR. Other abbreviation see in Table 1.

mean right atrial pressure (Pra) was significantly lower in females than in males (4.3±3.5 vs 7.1±5.5 mmHg, p=0.0002). However, PaO_2 in females was significantly lower than in males (56.4±9.8 vs 61.7±9.8 Torr, p=0.005).

With regard to the WHO functional classification, there was no significant difference between females and males.

Central disease score in females was significantly lower than in males, indicating a peripheral type (1.09±1.01 vs 1.83±1.27, p=0.0002). With regard to intra-operative classification, female patients showed significantly more non-type 1 disease compared with male patients (38.3 vs 9.7%, p=0.005).

Surgical Outcome and Gender

Although there was no significant difference in mortality between males and females, postoperative PVR in females was significantly higher than in males (406±282 vs 257±119 $dynes \cdot s \cdot cm^{-5}$, p=0.02), and the percentage decrease in PVR in females was significantly less than in males (51.9±25.4 vs 65.2±21.4%, p=0.04) (Table 3).

*Association With Gender Differences in Clinical Characteristics and HLA-B*5201*

As shown in Table 4, in HLA-B*5201-positive patients, females showed less embolic episodes (27.0 vs 80.0%, p=0.002). The cardiac index in females was significantly higher than in males (2.77±0.61 vs 2.23±0.38 $L \cdot min^{-1} \cdot m^{-2}$, p=0.001), and mean Pra in females was significantly lower than in males (3.9±3.7 vs 8.2±5.9 mmHg, p=0.0006). The surgical mortality was significantly lower in females than in males (0 vs 40%, p=0.0098).

In contrast, as shown in Table 5, in HLA-B*5201-negative patients, female CTEPH patients had less embolic episodes (40.4 vs 66.7%, p=0.03), lower central disease score (0.93±0.98 vs 2.04±1.32, p<0.0001) and more non-type 1 disease (48.0 vs 0.0%, p=0.0005), indicating the peripheral type of emboli. As a result, female patients showed higher postoperative PVR (405±303 vs 234±115 $dynes \cdot s \cdot cm^{-5}$, p=0.05) and a modest percentage decrease in PVR compared with males (55.6±21.3 vs 69.9±18.3%, p=0.04).

Two-way factorial ANOVA and multiple regression analysis revealed that the HLA-B*5201-positive type was significantly correlated with the absence of DVT (p=0.005), but female gender was not correlated with the absence of DVT (p=0.06). HLA-B*5201 did not show any correlation with any other clinical parameters (p>0.10). In contrast, female gender correlated with the absence of acute embolic episodes (p=0.0007), higher cardiac index (p=0.01), lower mean Pra (p<0.0001), lower central disease score (p=0.03) and non-type 1 disease (p=0.01).

Differences of Clinical Phenotype in Female CTEPH Based on HLA Types

To identify the differences of clinical phenotype of female

Table 4 Characteristics of Gender Difference in HLA-B*5201-Positive Type

	Female (n=37)	Male (n=10)	p value
Age (years)	53.9±10.5	50.4±18.9	0.45
DVT (%)	13.5	40.0	0.06
Embolic episode (%)	27.0	80.0	0.002
Pra (mmHg)	3.9±3.7	8.2±5.9	0.0006
Mean Ppa (mmHg)	43.7±11.3	44.6±12.2	0.79
Cardiac index ($L \cdot min^{-1} \cdot m^{-2}$)	2.77±0.61	2.23±0.38	0.001
PVR ($dynes \cdot s \cdot cm^{-5}$)	837±460	857±396	0.86
PaO_2 (Torr)	57.1±10.5	63.2±9.8	0.04
Central disease score	1.24±0.98	1.50±1.08	0.48
Intraoperative classification			
Type 1/2/3/4	13/1/1/0	4/0/0/1	
Non-type 1	13.3	20.0	0.72
Operative mortality (%)	0.0	40.0	0.0098
Postoperative PVR ($dynes \cdot s \cdot cm^{-5}$)	365±223	232±112	0.34
% decrease in PVR (%)	53.7±25.5	65.6±23.2	0.46

Values are mean±SD or n (%).
Abbreviations see in Tables 1,3.

Table 5 Characteristics of Gender Difference in HLA-B*5201-Negative Type

	Female (n=52)	Male (n=27)	p value
Age (years)	54.2±11.6	53.1±11.4	0.71
DVT (%)	42.3	55.6	0.26
Embolic episode (%)	40.4	66.7	0.03
Pra (mmHg)	4.6±3.3	6.3±5.1	0.07
Mean Ppa (mmHg)	45.8±10.9	41.9±10.6	0.12
Cardiac index ($L \cdot min^{-1} \cdot m^{-2}$)	2.59±0.56	2.46±0.73	0.38
PVR ($dynes \cdot s \cdot cm^{-5}$)	862±316	717±319	0.06
PaO_2 (Torr)	57.1±9.8	61.7±9.8	0.06
Central disease score	0.93±0.98	2.04±1.32	<0.0001
Intraoperative classification			
Type 1/2/3/4	13/8/4/0	18/0/0/0	
Non-type 1	48.0	0.0	0.0005
Operative mortality (%)	4.0	5.6	0.81
Postoperative PVR ($dynes \cdot s \cdot cm^{-5}$)	405±303	234±115	0.05
% decrease in PVR (%)	55.6±21.3	69.9±18.3	0.04

Values are mean±SD or n (%).
Abbreviations see in Tables 1,3.

Table 6 Characteristics of Female in HLA-B*5201-Positive or -Negative Type

	HLA-B*5201 positive (n=37)	HLA-B*5201 negative (n=52)	p value
Age (years)	53.9±10.5	54.2±11.6	0.90
DVT (%)	13.5	42.3	0.0036
Embolic episode (%)	27.0	40.4	0.19
Pra (mmHg)	3.9±3.7	4.6±3.3	0.25
Mean Ppa (mmHg)	43.7±11.3	45.8±10.9	0.08
Cardiac index ($L \cdot min^{-1} \cdot m^{-2}$)	2.77±0.61	2.59±0.56	0.15
PVR ($dynes \cdot s \cdot cm^{-5}$)	837±460	862±316	0.14
PaO_2 (Torr)	57.1±10.5	57.1±9.8	0.35
Central disease score	1.24±0.98	0.93±0.98	0.11
Intraoperative classification			
Type 1/2/3/4	13/1/1/0	13/8/4/0	
Non-type 1	13.3	48.0	0.02
Operative mortality (%)	0.0	4.0	0.43
Postoperative PVR ($dynes \cdot s \cdot cm^{-5}$)	365±223	405±303	0.68
% decrease in PVR (%)	53.7±25.5	55.6±21.3	0.81

Values are mean±SD or n (%).
Abbreviations see in Tables 1,3.

CTEPH based on HLA types, we compared the clinical characteristics of HLA-B*5201-positive and -negative females. As shown in Table 6, HLA-B*5201-positive females had less history of DVT (13.5 vs 42.3%, p=0.0036) and less non-type I disease (13.3 vs 48%, p=0.02) compared with HLA-B*5201-negative females.

Discussion

The incidence of pulmonary thromboembolism in Japan in 2004 (4,106 patients) was much lower than in the USA (630,000)[20] The absence of factor V leiden and prothrombin mutation, and low lipid levels in the Japanese might be involved in the difference between Japanese and Caucasian populations with this disease[21,22] However, female predominance and a higher incidence ratio of chronic to acute pulmonary thromboembolism in Japan as compared to those in the USA were recently reported[1–3] From an annual report in Japan, the total number of CTEPH patients in Japan was 800 in 2006[23] It was reported that the risk of recurrent venous thromboembolism was higher in men than women, but the female-to-male ratio in CTEPH is 2.1 in Japan, much higher than that of 0.7 in the USA[3,7,8] In addition, we previously reported that HLA-B*5201-positive patients with Japanese CTEPH were predominantly females and were unrelated to DVT[11] The frequency of HLA-B*5201 among the normal population in Japan was reported to be 20%, much higher than 2% in western countries[24–26] Then we considered that the HLA-B*5201-positive type indicates Japanese-specific CTEPH, and that HLA-B*5201-negative type could indicate CTEPH related to DVT, similar to Western countries.

We investigated whether the clinical phenotype in female CTEPH differs from male CTEPH, especially in the Japanese series.

Female patients were elderly and had less DVT, less acute embolic episodes, a higher cardiac index, lower mean Pra, lower PaO_2, more peripheral thrombi and less improvement through surgery than males. When the patients were divided according to HLA-B*5201 status, in HLA-B*5201-positive patients, females showed less embolic episodes, higher cardiac index and lower mean Pra with lower operative mortality. In contrast, in HLA-B*5201-negative patients, females showed less embolic episodes and more peripheral thrombi, resulting in less improvement through surgery.

This is the first report to reveal gender differences in the clinical characteristics of CTEPH, and that gender differences in the HLA-B*5201-positive type were dissimilar to those in HLA-B*5201-negative type.

Several issues need to be considered in the interpretation of these results. First, female CTEPH showed peripheral thrombi according to the central disease score and intra-operative classification. These findings were similar to those of Jamieson et al, who reported that females predominate in type 3 disease (distal segmental arteries only type)[10] When the patients were divided according to HLA-B*5201 status, only in HLA-B*5201-negative patients did females show more peripheral type than males, but in HLA-B*5201-positive patients such difference could not be observed. It remains uncertain why female CTEPH showed more of the peripheral type that in the USA as well as in HLA-B*5201-negative type in Japan. Although distal small DVT could induce peripheral type CTEPH, it is possible that peripheral pulmonary arteriopathy, similar to pulmonary arterial hypertension, in situ thrombosis might cause peripheral type CTEPH? As shown in Table 6, in females, the frequencies of non-type I in intra-operative classifications were significantly higher in HLA-B*5201-negative than in -positive type patients. We have already shown that the frequencies of HLA-B*5201 were higher in CTEPH with central predominant type[10] It seems that the existence of HLA-B*5201 might be related to a more proximal location of thrombi only in females.

Second, female patients showed less history of DVT. However, there was no significant difference in DVT between males and females when the patients were divided by HLA-B*5201 status. Additionally, multiple regression analysis revealed that the HLA-B*5201-positive type significantly correlated with the absence of DVT, but female gender did not show significant correlation with DVT. It is likely that a significant correlation with female gender might be related to female predominance in the HLA-B*5201-positive type. Takayasu arteritis is epidemiologically known for its female predominance, and the association of HLA-B*5201 with this disease has been well documented in Japan[13,14,27] Takayasu arteritis is a chronic vasculitis, mainly involving the aorta and its major branches, as well as the coronary and pulmonary arteries[28] The frequency of HLA-B*5201 in CTEPH was similar to that reported in Takayasu arteritis[27] We previously reported that the HLA-B*5201-positive type showed female predominance and was unrelated to DVT, and that this Japanese-specific type might include underdiagnosed pulmonary arteritis secondary to thrombi, although Takayasu arteritis was clinically excluded by CT angiographies. In our series, the frequency of HLA-B*5201-positive type (27.0%) in male CTEPH was not dissimilar to that in the normal Japanese population (20.0%), while that in female CTEPH (41.6%) was much higher. Only female CTEPH in HLA-B*5201-positive type could be a specific Japanese type unrelated to DVT, caused by underdiagnosed arteritis secondary to thrombi.

Third, univariate analysis showed that female patients had less history of acute embolic episode regardless of the existence of HLA-B*5201. The results obtained by multiple logistic regression also showed that acute embolic episode was influenced by gender more strongly than the existence of HLA-B*5201. The peripheral type of CTEPH and lower frequency of DVT might be related to less embolic episodes.

Fourth, female patients showed higher cardiac index and lower mean Pra, indicating preserved right ventricular function. Although preserved right ventricular function in females was significant only in HLA-B*5201-positive type, 2-way factorial ANOVA revealed that female gender had the main effect on higher cardiac index and lower mean Pra independent of the existence of HLA-B*5201. Several studies of the left ventricle have suggested that female gender is associated with more favorable myocardial adaptations to hemodynamic overload, including a better preserved contractile response and a greater adaptive hypertrophic reserve[29–31] Our data for the right ventricle were consistent with these data. The use of diuretics did not differ between males and females (62.5 vs 62.7%, p=0.99). Only fifteen patients had systemic hypertension, with 3 of them taking an angiotensin converting enzyme inhibitor. Therefore, it is unlikely that such medications might have strong effects on gender difference in terms of right ventricular function.

Female patients had less heart disease compared with males. After excluding patients with heart disease, females still showed higher cardiac index and lower mean Pra. In

addition, there was no significant differences in pulmonary capillary wedge pressure (7.3 vs 6.1 mmHg, p=0.83) between male and female CTEPH. Further studies on gender differences regarding right ventricular function will be needed.

Fifth, female patients showed higher postoperative PVR and a modest percentage decrease in PVR in all patients as well as in the HLA-B*5201-negative type. In contrast, the mortality of female patients was lower than that of males in only the HLA-B*5201-positive type despite similar postoperative PVR and percentage decrease in PVR. The peripheral type of emboli in females could be related to less improvement using surgery in all patients as well as in the HLA-B*5201-negative type. In contrast, it is likely that better right ventricular function in females contributes to lower mortality in HLA-B*5201-positive type. We previously reported that the female HLA-B*5201-positive type had a tendency to be the central predominant type![1] In the present study, HLA-B*5201-positive females showed more type I disease compared with HLA-B*5201-negative females, although there was no significant difference in central disease score. More type I disease in HLA-B*5201-positive females could be related to lower mortality.

Finally, the present study is based on the results from a single institution, and the number of patients in each group subcategorized according to gender and HLA type was small. Nonetheless, it will be important to manage patients while taking into account gender differences and HLA type. Larger studies are needed to confirm the relationship of gender difference and clinical phenotype.

In conclusion, to our knowledge, this is the first study to report that clinical phenotype in female CTEPH differed from that in males, and that gender differences in HLA-B*5201-positive type were dissimilar to those in HLA-B*5201-negative type.

Acknowledgement

The present study was supported in part by a grant to the Respiratory Failure Research Group from the Japanese Ministry of Health, Labor and Welfare, and a research grant for Cardiovascular Diseases (19-9) from the Ministry of Health, Labor and Welfare of Japan.

Disclosure

The authors have no conflicts of interest to disclose.

References

1. Moser KM, Auger WR, Fedullo PE. Chronic major-vessel thromboembolic pulmonary hypertension. *Circulation* 1990; **81:** 1735–1743.
2. Fedullo PF, Auger WR, Kerr KM, Rubin LJ. Chronic thromboembolic pulmonary hypertension. *N Engl J Med* 2001; **345:** 1465–1472.
3. Nakamura M, Okada O, Sakuma M, Nakanishi N, Miyahara Y, Yamada N, et al. Incidence and clinical characteristics of chronic pulmonary thromboembolism in Japan compared to acute pulmonary embolism results of multicenter registry of the Japanese society of pulmonary embolism research. *Circ J* 2002; **66:** 257–260.
4. Tanabe N, Okada O, Nakagawa Y, Masuda M, Kato K, Nakajima N, et al. The efficacy of pulmonary thromboendarterectomy on long-term gas exchange. *Eur Respir J* 1997; **10:** 2066–2072.
5. Kamieda T, Naito M, Ookubo S, Yoshioka K, Nakanishi N, Oobayashi Y. Clinical aspects and diagnosis of pulmonary thromboembolism. *Nihon Kyobu Shikkan Gakkai Zasshi* 1988; **26:** 463–472.
6. Tanabe N, Amano S, Tatsumi K, Kominami S, Igarashi N, Shimura R, et al. Angiotensin-coverting enzyme gene polymorphisms and prognosis in chronic thromboembolic pulmonary hypertension. *Circ J* 2006; **70:** 1174–1179.
7. Kyrle PA, Minar E, Bialonczyk C, Hirschl M, Weltermann A, Eichinger S. The risk of recurrent venous thromboembolism in men and women. *N Engl J Med* 2004; **350:** 2558–2563.
8. Archibald CJ, Auger WR, Fedullo PF, Channick RN, Kerr KM, Jamieson SW, et al. Long-term outcome after pulmonary thromboendarterectomy. *Am J Respir Crit Care Med* 1999; **160:** 523–528.
9. Hoshino S, Sadogawa H. Deep venous thrombolism. *Phlebology* 1997; **8:** 97–100.
10. Jamieson SW, Kapelanski DP. Pulmonary endarterectomy. *Curr Probl Surg* 2000; **36:** 165–252.
11. Tanabe N, Kimura A, Amano S, Okada O, Kasahara Y, Tatsumi K, et al. Association of clinical features with HLA in chronic pulmonary thromboembolism. *Eur Respir J* 2005; **25:** 131–138.
12. Terasaki PI. Microdroplet lymphocytotoxicity test. *In*: Ray JG, Hare DB, Pedersen PD, editors. Manual of tissue typing techniques. DHEW publications NO. (NIH) 76-545. Bethesda: 1976; 69–80.
13. Yoshida M, Kimura A, Katsuragi K, Numano F, Sasazuki T. DNA typing of HLA-B gene in Takayasu's arteriitis. *Tissue Antigens* 1993; **42:** 87–90.
14. Dong RP, Kimura A, Numano F, Nishimura Y, Sasazuki T. HLA linked susceptibility and resistance to Takayasu arteritis. *Heart Vessels* 1992; **7:** 73–80.
15. Bergin CJ, Sirlin C, Deutsch R, Fedullo P, Hauschildt J, Huynh T, et al. Predictors of patient response to pulmonary thromboendarterectomy. *Am J Review* 2000; **174:** 509–515.
16. Thistlethwaite PA, Mo M, Madani MM, Deutsch R, Jamieson SW. Operative classification of thromboembolic disease determines outcome after pulmonary endarterectomy. *J Thorac Cardiovasc Surg* 2002; **124:** 1203–1211.
17. Moser KM, Auger WR, Fedullo PF, Jamieson SW. Chronic thromboembolic hypertension: Clinical picture and surgical treatment. *Eur Respir J* 1992; **5:** 334–342.
18. Yoshimi S, Tanabe N, Masuda M, Sakao S, Uruma T, Shimizu H, et al. Survival and quality of life for patients with peripheral type chronic thromboembolic pulmonary hypertension. *Circ J* 2008; **72:** 958–965.
19. Nakajima N, Masuda M, Mogi K. The surgical treatments for chronic pulmonary thromboembolism: Our experience and current review of the literature. *Ann Thorac Cardiovasc Surg* 1997; **3:** 15–21.
20. Sugimura K, Sakuma M, Shirato K. Potential risk factor and incidence of pulmonary thromoboembolism in Japan: Results from an overview of mailed questionnaires and a matched case-control study. *Circ J* 2006; **70:** 542–547.
21. Kumasaka N, Sakuma M, Shirato K. Incidence of pulmonary thromboembolism in Japan. *Jpn Circ J* 1999; **63:** 439–441.
22. Murata M. Genetic polymorphisms associated with thrombotic disorders in the Japanese population. *Fibrinolysis and Proteolysis* 2000; **14:** 155–164.
23. http://www.nanbyou.or.jp/what/nan_kouhu1.htm Japan intractable disease information center. Data last updated: December 3, 2007.
24. Saito S, Ota S, Yamada E, Inoko H, Ota M. Allelic frequencies and haplotypic associations defined by allelic DNA typing at HLA class I and II loci in the Japanese population. *Tissue Antigens* 2000; **56:** 522–529.
25. Nakajima F, Nakamura J, Yokota T. Analysis of HLA haplotypes in Japanese, using high resolution allele typing. *MHC* 2001; **8:** 1–3.
26. Imanishi T, Akazawa T, Kimura A, Tokunaga K, Gojobori T. Allele and haplotype frequencies for HLA and complement loci in various ethnic groups. *In*: Tsuji K, Aizawa M, Sasazuki T, editors. HLA 1991, vol 1. New York: Oxford University Press; 1992; 1065–1220.
27. Kimura A, Kitamura H, Date Y, Numano F. Comprehensive analysis of HLA genes in Takayasu arteritis in Japan. *Int J Cardiol* 1996; **54(Suppl):** 61–69.
28. Lupi HF, Sanchez TG, Horwitz S, Gutierrez FF. Pulmonary artery involvement in Takayasu's arteritis. *Int J Cardiol* 2000; **75(Suppl):** 105–110.
29. Weinberg EO, Thienelt CD, Katz SE, Bartunek J, Tajima M, Rohrbach S, et al. Gender differences in molecular remodeling in pressure overload hypertrophy. *J Am Coll Cardiol* 1999; **34:** 264–273.
30. Olsson MC, Palmer BM, Leinwand LA, Moore RL. Gender and aging in a transgenic mouse model of hypertrophic cardiomyopathy. *Am J Physiol* 2001; **280:** 136–144.
31. Tamura T, Said S, Gerdes AM. Gender-related differences in myocyte remodeling in progression to heart failure. *Hypertension* 1999; **33:** 676–680.

Gene therapy for malignant pleural mesothelioma: presence and future

Yuji Tada[1], Yuichi Takiguchi[1], Kenzo Hiroshima[2], Hideaki Shimada[3,5], Taro Ueyama[1,6], Makoto Nakamura[1,6], Koichiro Tatsumi[1], Takayuki Kuriyama[1] and Masatoshi Tagawa[4,6]

Department of [1]Respirology, [2]Diagnostic Pathology, [3]Molecular Diagnosis and [4]Molecular Oncology, Graduate School of Medicine, Chiba University, Chiba, Japan

[5]Division of Gastroenterological Surgery, Chiba Cancer Center, [6]Division of Pathology, Chiba Cancer Center Research Institute, Chiba Japan

Running title: gene therapy for mesothelioma

Correspondence: Masatoshi Tagawa, Division of Pathology, Chiba Cancer Center Research Institute. 666-2 Nitona, Chuo-ku, Chiba 260-8717, Japan

Phone: +81-43-264-5431 ext 5101

Fax: +81-43-265-4459

E-mail: mtagawa@chiba-cc.jp

Abstract

Malignant pleural mesothelioma is relatively rare in frequency but one of the intractable diseases linked with asbestos exposure. Clinical outcomes with the present treatment modalities are unsatisfactory and no effective prevention method has been reported. Growing numbers of the patients in the Western countries with a long latent period need development of a novel therapeutic strategy. Gene therapy is a candidate for mesothelioma treatment because of its easy accessibility of a vector-mediated gene medicine into the intrapleural cavity. Several preclinical studies demonstrated that the gene medicine produced anti-tumor effects, suggesting the feasibility in clinical settings. In this article, we review the current status of gene therapy and clinical trials targeting mesothelioma and address possible directions to improve the efficacy.

Key words: Mesothelioma; Gene therapy; Clinical trial; Adenovirus

Introduction

Malignant pleural mesothelioma (MPM) is a rare solid tumor of the mesenchymal origin developed in the thoracic cavity. MPM extends into the vicinity organs to disturb the functions of vital organs such as lungs, large vessels and heart, which results in respiratory failure, massive fluid accumulation in the pleural space, cardiac tamponade and spinal cord compression. These symptoms damage the patient's quality of life. Development of MPM has been closely associated with asbestos exposure in most of the cases and recent studies suggested the involvement of SV40 large T antigen (1). The latent period of MPM is extremely long which could be beyond 30 years (2) and no effective prevention remains undeveloped. The majority of the patients is often found in the advanced stage and only a few patients can survive long even when they are diagnosed in an early stage. Statistical analyses based on asbestos consumption and the average latent periods imply that the incidence possibly has reached its peak in USA, whereas in Australia, Japan and European countries, anticipated numbers of the patients are increasing in the next a few decades and could be summed up to 30,000, 103,000 and 250,000 patients in this 40 years, respectively. The MPM incidence in other industrialized countries will also mark a peak in the next 2 to 3 decades (3). Despite inhibition of asbestos usage in the Western countries, other industrialized countries such as China continue to use asbestos. Continued increase in the incidence needs accelerated research to develop therapeutic options to the potential patients in future.

The prognosis of MPM is poor with 6 to 9 months of the median survival time after the diagnosis. Conventional therapies for MPM consist of surgery, radiation and chemotherapy. In an early stage of the diseases, extrapleural pneumonectomy can be curative although the numbers of the eligible patients are quite limited. Majority of the

patients who undergo such radical debulking surgery frequently results in local recurrence with no further effective treatment modalities; thereby, the mortality rates remain high even with surgical resection. MPM cells are resistant to radiation and the clinical responses to radiotherapy proved to be disappointing. Radical radiation therapy against widespread tumors can result in radiation-induced pneumonitis, carditis and spinal cord toxicity. The indication for radiation therapy is therefore restricted and the therapy is currently applicable only in combination with surgery and/or for palliative procedures. MPM cells are also resistant to chemotherapeutic agents and many clinical trials using cisplatin, doxorubicin, gemcitabine and docetaxel, even in a combinatory use have failed to prolong the overall survival. Recently, pemetrexed, a novel multi-targeted anti-folate agent, has extended the survival period from 9.3 months with cisplatin treatment alone to 12.1 months in combination with cisplatin (4). The clinical outcome even with the updated combination chemotherapy is not satisfactory and an innovative therapeutic approach is required to improve the prognosis.

Gene therapy as a monotherapy or in combination with the conventional therapies can be an option for MPM treatment. Gene therapy has an advantage for MPM over other types of cancer because it developed in a closed cavity. MPM is thereby relatively easy for viral vectors to access through the chest wall. Administration of the vectors directly into tumors and/or the intrapleural cavity is technically feasible and the vectors localized to tumor sites without being washed out or diluted by blood stream. For example, adenoviruses (Ad) which are frequently used for current cancer gene therapy often cause liver damages due to their nonspecific integration into the organ; however, administration of Ad into the thoracic cavity usually does not cause severe liver injury since a large amount of Ad administered is not transferred into liver (5). Preclinical

studies of gene therapy for MPM demonstrated possible clinical applications. To our knowledge, however, there have been only four clinical studies at a phase I level reported and the patient numbers were limited (6-11). It is therefore inappropriate to compare the clinical efficacy of gene therapy between MPM and non-MPM malignancy even with the same vector system. In the present article, we review clinical data of gene therapy for MPM and updated preclinical studies, and discuss possible future directions.

Clinical trials for MPM

There have been conducted 4 clinical trials for MPM in the Western world including 3 phase I and I pilot study (Table 1), which included a suicide gene therapy and an immune gene therapy using interferon (IFN)-β.

(1) Suicide gene therapy

A suicide gene therapy that has been frequently investigated is a combination of expression of the herpes simplex virus thymidine kinase (HSVtk) gene and administration of anti-viral prodrug ganciclovir (GCV). Gene transfer of HSVtk gene renders the cells sensitive to the prodrug. GCV is phosphorylated by HSVtk but not by the mammalian tk gene, and phosphorylated and diphosphorylated GCV are incorporated into DNA to terminate DNA synthesis. Conversion of GCV into the cytotoxic molecules is mediated solely by the HSVtk gene and thus the combination of the gene expression and the prodrug administrateded leads to cytotoxic effects to the mammalian cells expressing the gene. Administration of Ad or adeno-associated viruses bearing the HSVtk gene into MPM cells or into the thoracic space of tumor-bearing host animals, efficiently eradicated established tumors with systemic administration of GCV, depending on the promoter activity to activate the HSVtk gene and experimental models

(12-14). These preclinical studies used constitutive active promoter rather than tissue-specific promoter to activate the target gene such as the Rous sarcoma virus (RSV) promoter (12, 13).

A phase I clinical trial with Ad bearing the HSVtk gene (Ad-HSVtk) powered by the RSV promoter and subsequent GCV administration was conducted at the University of Pennsylvania, which enrolled 34 patients without prior chemotherapy (Table 1) (11). Patients received single intrapleural injection of Ad-HSVtk at $5x10^9$-$5x10^{13}$ virus particle (vp) in the dose escalation study and the patients tolerated well up to $5x10^{13}$ vp. The protocol used 2 types of Ad-HSV-tk, the E1/E3 region-deleted and the E1/E4 region-deleted, both of which were essentially non-replicative and were not different in the clinical results. The study demonstrated that the gene therapy with Ad-HSVtk was conducted safe with minimal adverse reactions and successful gene transfer into the tumors was observed in 17 of 25 evaluable patients. The clinical stages of the MPM patients varied including from stage I to IV cases and moreover some of the patients also received conventional therapies as well. It could be difficult to evaluate the clinical outcomes and the efficacy was not a purpose of the phase I study; however, it is interesting to note that the study showed 3 cases survived more than 5 years. All the long lived cases were in stage I or II but 2 of them did not receive any other treatments besides the gene therapy. Objective responses of the tumor regression in general were not observed with an imaging technology but metabolic imagings clearly showed the anti-tumor responses. The authors also suggest that immune responses were generated in the patients since immunoblot analyses with patient sera detected several antibody-reactive molecules after the treatment. Consequently, they implied that immune responses played a crucial or certain role in the anti-tumor responses in

particularly in long survived patients. Repeated administration of Ad-HSVtk induced humoral and cell-mediated immunity against the Ad (15). Some of the patients had already developed anti-Ad antibody before the Ad administration and interestingly, antibody for HSVtk protein was also generated with the gene therapy. The humoral immunity however did not deteriorate the efficacy of gene transfer nor induced serious adverse effects. Raised antibody can be rather a surrogate marker for successful gene transfer. Although the antibody titers developed in pleural effusion was as high as that in serum, successful transduction despite the presence of such neutralizing antibody was encouraging. Several studies demonstrated that presence of anti-Ad antibody prevented systemic distribution of Ad but did not influence the gene transduction as long as administered locally such as intratumoral injection.

Harrison et al. administered gene-modified ovarian cancer cells expressing HSVtk into the intrapleural cavity of MPM patients in a phase I trial (6, 7). The clinical trial showed that the cell-based gene therapy was safe and the patients tolerated to receive up to 10^{10} allogenic gene modified cells. The study also showed that the modified cells that were administered into the cavity successfully homed to and adhered to the MPM cells lining the thoracic cavity. The precise clinical results as well as the outcomes however were not reported. This type of gene therapy contained several points to be considered. A small cell fraction of a tumor mass, when they become HSVtk-positive, can damage to HSVtk-negative tumor cells in the vicinity of the positive cells (bystander effects, see below). It allows tumor cell killing more than those expressing the suicide gene, achieving better anti-tumor effects. Moreover, it is relatively with easy to prepare large amounts of transduced cells at a standard quality. On the contrary, allogenic tumor cells are instantly rejected and the delivery of phosphorylated GCV to targeted MPM cells

would be hampered. Repeated injection of the allogenic tumor cells cannot be successful due to strong allogenic immune responses. It is consequently a better strategy to use autologous cells such as fibroblasts and bone-marrow-derived mesenchymal cells of the patient origin. Simultaneous administration of corticosteroid to alleviate acute inflammatory reactions caused by Ad administration did not disturb gene transfer (16) although the effects of such agents remain unclear.

An advantage of the HSVtk-mediated suicide gene therapy is to produce cytotoxic activities not only to the transduced cells but also to surrounding non-infected tumor cells through bystander effects, which resulted in better anti-tumor effects by damaging non-infected tumors. The mechanisms of the bystander effects are not completely understood but transfer of cytotoxic phosphorylated GCV to the neighbors through cell-cell junctions is one of the suggested mechanisms. Transfer of the cytotoxic GCV between irrelevant cells as is the case of allogenic ovarian tumors and MPM cells (6, 7) remain controversial since the junction structure is in general formed among the same cell type. It could be much beneficial to modify Ad to express the HSVtk gene selectively in tumor cells, for instance by placing a tumor-specific regulatory region in the upstream of the HSVtk gene, which can avoid the adverse effects to normal tissues. The HSVtk with GCV system has also been examined for the clinical benefits to treat other tumor types and none of them has been proved to be clinically effective despite numerous encouraging preclinical data. The typical case is intratumoral injection of packaging cells producing retroviruses bearing HSVtk gene into glioblastoma (17). The phase III study did not prove the therapeutic efficacy.

(2) Immune gene therapy

MPM is a typical non-immunogenic tumor and often produces a latent form of transforming growth factor (TGF)-β, a cytokine that induces fibrous stroma formation and tolerance of host immunity. In fact few cytotoxic T cells (CTLs) infiltrations were observed in thick stromal tissues in MPM patient's specimens (18). Tumors also escape from attacks of the host immune system under the tolerance state; nonetheless, several types of immune gene therapy have shown the efficacy and achieved sufficient anti-tumor effects, demonstrating its feasibility as a therapeutic modality. The goal of immune gene therapy is to activate a host immune system and to break the tolerance. Forced expression of cytokine genes in tumor cells is a technique to enhance host immunity against the tumors. The cytokine actions influence multiple steps of immune responses, depending on properties of the cytokine used. The actions are to assist the activation steps for enhanced cell-mediated immunity, which include (i) acquisition of putative tumor-specific antigens by professional antigen presenting cells, such as dendritic cells (DCs), and presentation of them to CTLs, (ii) effective differentiation of naïve T cells into type I helper T cells and effective CTLs, (iii) proliferation of CTLs and maintenance of immunological memories.

Experimental animal models demonstrated that expression of IFN-β, IFN-γ and interleukin (IL)-12 in MPM cells enhanced the anti-tumor immunity against the tumors (19-21). Ad-mediated gene transfer of IFN-γ and IL-12 in tumors induced regression of the tumors and subsequently generated systemic anti-tumor immunity, activating $CD4^+$, $CD8^+$ T cells, antigen presenting cells and NK cells. The immunity induced lasted long and protected hosts from subsequent challenges of the tumor implantation. Enhanced co-stimulatory signaling pathways, such as stimulation of CD40 with CD40 ligand

(CD154), can generate anti-tumor immunity in the inoculated host (22). Friedlander et al. reported that over-expression of CD40 ligand in MPM cells induced recruitment of $CD8^+$ T cells and established long-lasting immunity against the tumors (23).

There have been two clinical trials of the immunology-based gene therapy: vaccinia viruses bearing IL-2 (8) and Ad harboring IFN-β (Ad-IFN-β) (9). The former was a pilot study to examine the safety of vaccinia viruses that were injected intratumorally. The study showed no major toxicities accompanied by the viruses administration and increased antibody formation against the viruses in the patients. The report also mentioned that no clinical responses were observed although precise data were not presented. It seems that no further subsequent study was reported. A number preclinical studies demonstrated that forced expression of IL-2 in tumors generated anti-tumor immune responses and several clinical studies suggested the potential usage in clinical settings; however, to our knowledge there has been no preclinical study on MPM with IL-2. The latter was a phase I study with a single intrapleural injection into 10 cancer patients including 7 MPM patients. The study demonstrated IFN-β protein in MPM cells but not IFN-α in any pleural fluid of the patients despite activation of cellular and humoral immunity. IFN-β has a number of biological functions including up-regulated class I expression of the major histocompatibility complexes and induction of anti-viral molecules. Preclinical data showed that intraperitoneal injection of Ad-IFN-β into MPM-bearing tumors produced anti-tumor effects in immunocompetent but not in immunodeficient mice and both $CD4^+$ and $CD8^+$ T cells were involved in the anti-tumor responses (24). CTLs were also induced and the anti-tumor effects were enhanced in the combination of surgical procedures (19). The study used from $9x10^{11}$ to $3x10^{12}$ vp for intrapleural injection and the investigators concluded that maximal tolerance dose was

$9x10^{11}$ vp since a patients showed elevated serum transaminase (9). Later the dose was scaled up since the adverse reaction was turned out to be not related with Ad administered. The clinical trial showed the evidence of the regressed MPM in some cases based on images with positron emission and computed tomography (CT) despite relative low gene transduction efficacy. In some patients, Ad-IFN-β administration activated natural killer cells but CTLs induction was minimal. Although the gene therapy was combined with conventional chemotherapy, some of the MPM patients showed prolonged survival (more than 26 months) after the treatment. It could be due to immune responses induced by IFN-β secretion. In fact the investigators detected humoral responses against mesothelin (see below) in the patients; moreover, immunoblot with patient sera evidenced antibody-reactive molecules that were not detected with the pretreatment sera. These analyses suggest that the immune system was activated against mesothelin, a possible target for CTLs, and putative tumor antigens which came to be recognized followed by the tumor destruction. The study concluded that single intrapleural injection of Ad-INF-β was feasible and well-tolerated, and could be applicable to MPM patients. Enhanced anti-tumor responses however are required for better therapeutic effects. Multiple injections and a high dose administration of Ad-IFN-β are possible treatment modalities. In particular, multiple administration with short intervals would be a choice since generation of anti-Ad antibody takes about a week and the induced antibody will inhibit subsequent Ad-mediated gene transfer. Combinatory use of other treatment strategies including low dose chemotherapy that deplete regulatory T cells could contribute to increased anti-tumor effects.

Experimental studies and possible directions

(1) Re-constitution of tumor suppressor genes

MPM has a unique molecular pathological feature about a loss of tumor suppressor genes, which could be good target for the re-construction gene therapy. Mutations in the two principal tumor suppressor genes, p53 and Rb, were not found in the majority of MPM cells. In contrast, a homozygous deletion of the INK4a/ARF locus on human chromosome 9p21 is predominant with the frequency of more than 70% in MPM cell lines and the clinical specimens. The two major products encoded by the INK4a/ARF gene are $p16^{INK4a}$ which inhibits cyclin-dependent kinase and $p14^{ARF}$ which blocks MDM2-mediated p53 inhibition. Deletion of the INK4a/ARF gene thereby leads to function losses of P53 and Rb; consequently, the genetic deletion results in uncontrolled cell proliferation and resistant to apoptotic stimuli despite the wild-type p53 and Rb genes in MPM cells. Ad-mediated $p14^{ARF}$ gene transfer in MPM cells in fact restored p53 and $p14^{ARF}$ expression levels, which subsequently induced G0 arrest and apoptotic cell death (25, 26). Functional P53-mediated pathways are essential to induce the cytotoxicity mediated by $P14^{ARF}$. Similarly, over-expression of $P16^{INK4a}$ in MPM cells by Ad-$p16^{INK4a}$ inhibited the cell proliferation and induced the apoptosis (27, 28). In addition, instillation of Ad-$p16^{INK4a}$ into the peritoneal cavity successfully produced anti-tumor effects against the xenografts in a peritoneal MPM model. These reconstitution data indicate that the signal pathways downstream of p53- and Rb-mediated pathways seem to be intact in MPM cells.

ONYX-015, conditional replication-competent Ad lacking E1B 55kDa molecules, was initially reported to kill selectively tumor cells that lack the wild-type p53 gene. Subsequent studies showed that replication of ONYX-015 was not directly linked with the p53 status in target tumors; however, ONYX-015 can replicate preferentially in