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p53-regulated *GML* gene expression in non-small cell lung cancer: a promising relationship to cisplatin chemosensitivity

M. Higashiyama[a,*], Y. Miyoshi[b], K. Kodama[a], H. Yokouchi[a], K. Takami[a], M. Nishijima[b], T. Nakayama[b], H. Kobayashi[c], K. Minamigawa[c], Y. Nakamura[b,d]

[a]*Department of Thoracic Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Nakamichi 1-3-3, Higashirariku, Osaka 537-8511, Japan*

[b]*Division of Clinical Genetics, Department of Medical Genetics, Biomedical Research Center, Osaka University Medical School, Yamadaoka 2-2, Suita, Osaka 565-0871, Japan*

[c]*Research Laboratory Division, Nitta Gelatin Inc., Futamata 2-22, Yao, Osaka 581-0024, Japan*

[d]*Laboratory of Molecular Medicine, The Institute of Medical Science, The University of Tokyo, Shirokanedai 4-6-1, Minatoku, Tokyo 108-0071, Japan*

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Abstract

The *GML* gene (glycosylphosphatidylinositol-anchored molecule-like protein gene) is a novel gene specifically induced by wild-type p53, which may participate in cell cycle control or the cell apoptotic pathway. Recent experiments suggest that the expression of this novel gene in cancer cells is closely associated with sensitivity to certain anticancer drugs. To elucidate the role of the gene expression in cisplatin (CDDP) chemosensitivity of non-small cell lung cancer (NSCLC), 30 surgically resected materials were examined by reverse transcriptase-polymerase chain reaction (RT-PCR). *GML* gene expression was detected in 9 (30%) samples. Its incidence was significantly higher in immunohistochemically p53-negative ($P=0.040$) or wild-type p53 tissues ($P=0.041$). On *in vitro* chemosensitivity testing using 29 primary tissues, six samples with *GML* gene expression showed good sensitivity to CDDP. In particular, in tissues with immunohistochemically p53-negative accumulation, those with *GML* gene expression showed significantly better *in vitro* sensitivity to CDDP ($P=0.012$). Clinically a good response to CDDP-based chemo(thermo)therapy for NSCLC patients with tumour residue or recurrence, was observed only in those with p53-negative accumulation and *GML* gene expression, in agreement with *in vitro* results. Thus, although the number of tested samples was small, *GML* gene expression is commonly detected in immunohistochemically p53-negative NSCLCs in close association with good sensitivity to CDDP. *GML* gene expression analysis may serve as a predictor of CDDP-based chemotherapy for patients with NSCLC.

Keywords: *GML*; NSCLC; Cisplatin; Chemosensitivity; p53; CD-DST

1. Introduction

The *TP53* tumour-suppressor gene is thought to be involved in cell-cycle regulation [1,2]. The wild-type p53 protein may possess antiproliferative and anti-transforming activities, and in some cases, may promote apoptotic cell death through the activation or suppression of transcription of such representative genes as the *WAF/CIP 1* gene [3], the *MDM2* gene [4], the *GADD45* gene [5], and the *BAX* or *BCL-2* genes [6].

Recently, the *TP53* gene has been demonstrated to play an important role in cell death following damage to DNA by anticancer drugs or irradiation. In contrast, cells lacking wild-type p53 function, for example, by mutation, fail to initiate apoptosis or suffer delays in this process. Thus, the *TP53* gene is closely associated with the response to anticancer drugs or irradiation [7,8].

The *GML* gene (glycosylphosphatidylinositol-anchored molecule-like protein gene) was firstly isolated by Furuhata and colleagues [9], as a novel gene specifically induced by wild-type p53. This gene product shows structural homology to the family of GPI (glycosylphosphatidylinositol)-anchored membrane proteins such as E48 [10], an antigen involved in keratinocyte cell-to-cell adhesion [10], CD59, an inhibitor of complement lysis [11] and murine Ly-6 glycoproteins [12]. These GPI-anchored membrane proteins are thought to play important roles in cell adhesions or attachment to the cell matrix and signal transduction through the activation of tyrosine kinases and phosphatases [13].

* Corresponding author. Fax: +81-6-6981-8055.

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GML, like the genes described above, contains p53-binding sites in its promoter region or introns and, therefore may play a significant role in cell growth suppression by wild-type p53 [9,14]. Interestingly, this gene was also shown to be linked to *in vitro* sensitivity to anticancer drugs in some cancer cell lines, suggesting the *GML* gene product may participate in cell cycle control or apoptosis induced by wild-type p53 after DNA damage [9]. However, to date, this gene has been studied only *in vitro*, and so the gene expression status in human malignant tissues remains unknown.

In Japan, lung cancer is the leading cause of cancer mortality especially in men, and most lung cancers are of histologically non-small cell cancer type [15]. Whilst the best therapeutic modality for this disease is, if possible, surgical resection, most patients are compelled to undergo chemotherapy because of systemic dissemination. At present, the standard medical treatment for this cancer is a cisplatin (CDDP)-based chemotherapy, which has poor clinical and prognostic effects. Nevertheless, the possibility that CDDP-based chemotherapy might improve survival has encouraged efforts for intensive treatment in a chemosensitive subgroup of this patient population [16,17].

Thus, to determine the clinicopathological and therapeutic value of this novel p53-regulated *GML* gene expression in patients with non-small cell lung cancer (NSCLC), we examined the gene expression of *GML*, correlated it with CDDP chemosensitivity, and attempted to compare the data with clinical findings.

2. Patients and methods

2.1. Clinical samples

A total of 30 samples of NSCLC, surgically resected in our institute between August and December 1996, were used in this study. Of these, eight samples were squamous cell carcinoma, 16 were adenocarcinoma, one was adenosquamous cell carcinoma and five were large cell carcinoma. At the time of the operation, 18 were in stage I (IA: 3, IB: 15), two were in stage IIB, nine were in stage III (IIIA: 2, IIIB: 7), and one was in stage IV [18]. These samples were not treated with chemo- or radiation therapy before surgery.

2.2. GML *gene expression analysis*

GML gene expression was examined using the reverse transcriptase-polymerase chain reaction (RT-PCR) method described by Furuhata and colleagues [9] with minor modification. Briefly, total RNAs were isolated with TRIZOL Reagent (Gibco BRL, Grand Island, NY, USA) according to the manufacturer's protocol. Three micrograms of total RNA was reverse transcribed into single-strand cDNA using oligo$(dT)_{15}$ as a primer and superscript II reverse transcriptase (Gibco BRL). Each single-strand cDNA was diluted for subsequent PCR reactions by monitoring glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a quantitative control. The PCR reaction was carried out in a 20 μl reaction mixture with an initiation step at 94°C, 5 min, followed by 18 cycles (GAPDH) or 35 cycles (*GML*) at 94°C for 30 sec, 58°C (GAPDH) or 62°C (*GML*) for 30 sec, and 72°C for 30 sec, on the GeneAmp PCR system 9600 (Perkin-Elmer, Foster City, CA, USA). The primer sequences used for the reactions were; GAPDH F, 5′-CAACTACATGGTTTACATGTT-3′; GAPDH R, 5′-GCCAGTGGACTCCACGAC-3′; *GML* F, 5′-GGCTCCTGCGTGAAGTGATGC-3′; *GML* R, 5′-ATGGAGATTGTCATACAGCGCC-3′. The PCR products were resolved on a 2% agarose gel and visualised by ethidium bromide staining using ultraviolet (UV) transillumination.

2.3. p53 analysis

p53 abnormalities in these samples were analysed by immunohistochemical staining (IHS) [19]. Thin sections (4 μm) of formalin-fixed and paraffin-embedded blocks including the maximum cut surface of the tumour tissue were used. IHS using DO7 (Novocastra Laboratories, Newcastle, UK) as the primary antibody against the p53 protein, including both wild and mutant types, was routinely performed with microwave irradiation pretreatment. Samples showing immunohistochemical staining in less than 10% of cancer cells within the tissue were considered p53-negative, and those showing immunohistochemical staining in 10% or more, p53-positive. Of these p53-positives, the samples with a diffuse staining pattern (80% or more of cancer cells showing positive staining) were subclassified as strongly p53-positive (++), and others as weakly p53-positive (+).

Of these samples, 17 deeply-frozen materials were also used to examine *TP53* gene mutation status by the fluorescence-based polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) method, as described previously by Katsuragi and coworkers [20]. This technique was used for the detection of point mutations in the *TP53* gene in exons 5, 6, 7 and 8 by an automated DNA sequencer and software. In addition, in order to ascertain base changes and exclude the cases with non-missense mutations from mutant cases classified with PCR-SSCP, direct sequencing was performed using chemiluminescent DNA sequencing kit (Toyobo, Osaka, Japan).

2.4. In vitro *chemosensitivity to cisplatin (CDDP)*

The CDDP *in vitro* chemosensitivity of these samples was analysed by collagen gel droplet embedded culture

drug sensitivity test (CD-DST), as previously described but with minor modifications [19,21–23]. Briefly, surgically resected specimens were digested in dispersion collagenase enzyme, and the dispersed cancer cells were incubated in a collagen gel-coated flask. Then, the viable cells alone adhering to the collagen gel layer were collected and added into the reconstructed Type I collagen solution (cell matrix® Type CD™ Nitta Gelatin, Yao, Japan). Three drops of these mixtures were placed in each well of a 6-well multiplate, and then CDDP (0.2 μg/ml) was added to each well for a 24 h incubation. After removal of the medium containing cisplatin, each well was incubated with PCM-2 medium (Nitta Gelatin) for 7 days. Then, neural red was added to stain the colonies in the collagen gel droplets, which were finally fixed with formalin. The *in vitro* chemosensitivity effect of CDDP was expressed as a ratio of the total colony volume (T) of treated cells to that of untreated cells (C). Samples with ratios T to C of 50% or less, >60%, and between 50 and 60%, were regarded as sensitive, resistant and borderline, respectively.

2.5. Clinical treatment using cisplatin CDDP for recurrent or residual tumours

7 patients had undergone chemotherapy using CDDP for recurrent or residual tumours by the time of this study. Intravenous CDDP-based combined chemotherapy was performed in 2 cases due to bone metastasis, in 1 due to lung metastasis, and in 1 case due to brain metastasis (combination with 5-fluorouracil; 1 patient, with vindesine sulphate; 2 patients, with vindesine sulphate plus 30 Gy radiation therapy; 1 patient, respectively), and intrapleural CDDP-based chemothermotherapy was performed in 3 cases due to residual pleural dissemination [24].

In principle the experiments were performed in duplicate except for the *GML* expression analyses which were carried out in triplicate.

Table 1
Summary of *GML* gene expression and *TP53* abnormality in non-small cell lung cancer

	GML gene expression	
	–	+
p53 status (IHS, $n=30$)[a]		
Negative (–)	10	8
Positive (+, + +)	11	1
TP53 status (PCR-SSCP, $n=17$)[b]		
Wild	5	7
Mutant	5	0

IHS, immunohistochemical staining; PCR-SSCP, polymerase chain reaction single-stranded conformation polymorphism.

[a] $P=0.040$ (Fisher's exact probability test).

[b] $P=0.041$ (Fisher's exact probability test).

3. Results

3.1. GML gene expression, TP53 abnormality status and clinicopathological factors

GML gene expression was observed in nine samples (30%) in the series. A representative example is shown in Fig. 1. p53 abnormality was detected in 12 samples (40%) by IHS, of which 10 were strongly p53-positive, and 2 were weakly p53-positive. Of 17 samples examined by PCR-SSCP, *TP53* mutations were found in 5 (29%, one in exon 5, one in exon 6, two in exon 7 and one in exon 8, respectively), four of which were from the strongly p53-positive group and the other from the weakly p53-positive group.

Table 1 shows an association between *GML* gene expression and *TP53* abnormality. Eight (89%) of the nine samples positive for *GML* gene expression were included in the p53-negative group by IHS. *GML* gene expression was more commonly observed in the p53-negative group from the immunohistochemical analysis ($P=0.040$, Fisher's exact probability test). Also, by PCR-SSCP analysis, its incidence was higher in the tissues with wild-type *TP53* status ($P=0.041$, Fisher's exact probability test).

No association was demonstrated between *GML* gene expression and histological type, but seven samples with positive *GML* gene expression were early stage cancers (one in stage IA and six in stage IB, respectively).

3.2. GML gene expression and in vitro chemosensitivity to CDDP

An *in vitro* chemosensitivity test to CDDP was performed in 29 samples. The samples from 6 (67%) of the 9 patients with positive *GML* gene expression were sensitive to CDDP *in vitro*, one was borderline, and two were resistant. Tissues from 6 of the 20 patients (30%)

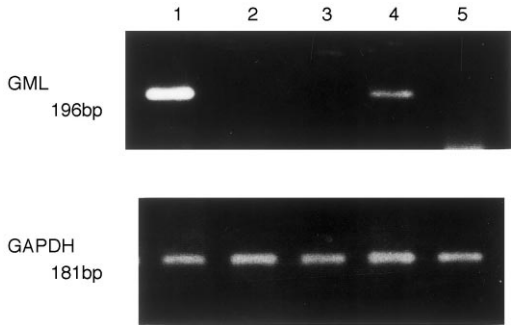

Fig. 1. *GML* gene expression in non-small cell lung cancer tissues. *GML* gene expression was detected in lanes 1 (p 13) and 4 (p 19), but not in lanes 2 (p 5), 3 (p 16) and 5 (p 14). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts were observed in all lanes as control.

without *GML* gene expression were sensitive to CDDP *in vitro*, one was borderline, and the remaining 13 were resistant.

Table 2
Summary of *GML* gene expression and *in vitro* chemosensitivity to cisplatin (CDDP) according to immunohistochemical p53 status

	p53 (−, $n=17$)[a] *GML* gene expression		p53 (+, ++, $n=12$) *GML* gene expression	
	−	+	−	+
CDDP *in vitro* sensitivity				
Sensitive	2	6	4	0
Borderline	0	1	1	0
Resistant	7	1	6	1

[a] Sensitive + borderline versus resistant $P=0.012$ (Fisher's exact probability test).

Table 2 shows an association between *GML* gene expression and *in vitro* chemosensitivity to CDDP according to immunohistochemical p53 status. In tissues with immunohistochemically p53-negative accumulation ($n=17$), *GML* gene expression was significantly correlated with *in vitro* chemosensitivity to CDDP ($P=0.012$, Fisher's exact probability test): tissues with *GML* gene expression showed better sensitivity to CDDP, whilst those without *GML* gene expression were mainly resistant to CDDP. In contrast, there was no association between *GML* gene expression and *in vitro* chemosensitivity to CDDP in tissues with immunohistochemically p53-positive accumulation.

Table 3 shows a summary of tumour histology, stage, *GML* gene expression, p53 status, and *in vitro* chemosensitivity to CDDP in each patient. One exceptional case with *GML* gene expression, despite immunohistochemically p53-positive staining and wild-type p53 on PCR-SSCP analysis, was resistant *in vitro* to CDDP (patient 9).

Table 3
Association between *TP53* abnormality, *GML* gene expression and *in vitro* chemosensitivity to cisplatin (CDDP) in patients with non-small cell lung cancer

Patient No.	Histology	Stage	p53/*TP53* status		*GML* gene expression	*In vitro* chemosensitivity to CDDP (T/C rate, %)	
			IHS	PCR-SSCP			
1	Sq	IIB	−	NT	+	41%	Sensitive
2	Sq	IB	−	Wild-type	−	13%	Sensitive
3	Sq	IB	−	Wild-type	−	84%	Resistant
4	Sq	IA	−	NT	−	73%	Resistant
5	Sq	IB	++	Mutant (Ex 5, Co 137, CTG→ATG)	−	75%	Resistant
6	Sq	IIIB	++	NT	−	47%	Sensitive
7	Sq	IB	−	NT	+	59%	Borderline
8	Sq	IB	++	Mutant (Ex 6, Co 220, TAT→TCT)	−	37%	Sensitive
9	Ad	IB	++	Wild-type	+	75%	Resistant
10	Ad	IIIB	++	Mutant (Ex 7, Co 248, CGG→CAG)	−	61%	Resistant
11	Ad	IB	−	NT	−	80%	Resistant
12	Ad	IA	+	Mutant (Ex 8, Co 273, CGT→CAT)	−	99%	Resistant
13	Ad	IA	−	Wild-type	+	45%	Sensitive
14	Ad	IB	−	Wild-type	−	76%	Resistant
15	Ad	IIIA	−	Wild-type	−	80%	Resistant
16	Ad	IIIB	++	NT	−	65%	Resistant
17	Ad	IB	−	Wild-type	−	93%	Resistant
18	Ad	IIIB	−	NT	−	83%	Resistant
19	Ad	IIIB	−	Wild-type	+	49%	Sensitive
20	Ad	IB	−	Wild-type	+	45%	Sensitive
21	Ad	IB	−	Wild-type	+	22%	Sensitive
22	Ad	IB	−	Wild-type	+	83%	Resistant
23	Ad	IV	+	NT	−	51%	Borderline
24	Ad	IB	−	NT	−	NT	NT
25	As	IIIB	++	NT	−	98%	Resistant
26	La	IB	++	NT	−	47%	Sensitive
27	La	IIB	−	NT	−	37%	Sensitive
28	La	IIIB	++	Mutant (Ex 7, Co 245, GGC→CGC)	−	37%	Sensitive
29	La	IB	−	Wild-type	+	43%	Sensitive
30	La	IIIA	++	NT	−	80%	Resistant

Sq, squamous cell carcinoma; Ad, adenocarcinoma; As, adenosquamous cell carcinoma; La, large cell carcinoma; IHS, immunohistochemical staining; PCR-SSCP, polymerase chain reaction single-strand conformation polymorphism; Ex, exon; Co, codon; NT, not tested.

In vitro chemosensitivity to cisplatin (CDDP), CD-DST by Kobayashi and colleagues [23].

3.3. Clinical effect of CDDP-based chemotherapy according to GML gene expression status

Table 4 shows a summary of the clinical effect of undergoing CDDP-based chemotherapy in relation to p53 status, *GML* gene expression and *in vitro* chemosensitivity to CDDP in the primary tumour tissue. 2 patients with p53-negative and *GML* gene expression showed good local response to CDDP-based chemotherapy (patients 19 and 21), and in agreement with *in vitro* chemosensitivity results. In particular, patient 19, despite having malignant effusion at the time of surgical treatment, is both locally and systemically relapse-free 33 months after CDDP-based intrathoracic chemothermotherapy [24]. In contrast, other patients, in whom *GML* gene expression was not detected in the primary tumour tissue, showed no response to CDDP-based chemotherapy.

4. Discussion

The *GML* gene product is considered to be induced by wild-type p53, not mutant-type p53 in some human colon and oesophageal cancer cell lines and, therefore, there is a clear correlation between *GML* gene expression and p53 status *in vitro* [9]. The present data using human NSCLC tissues also supported this correlation (Table 1): 8/9 tissues (89%) with *GML* gene expression were immunohistochemically p53-negative ($P = 0.040$), and the majority of these had wild-type *TP53* status in the PCR-SSCP analysis ($P = 0.041$). Even an exceptional case (patient 9) with *GML* gene expression despite immunohistochemically p53-positive accumulation, showed no mutation of the *TP53* gene as far as we could discern and, therefore, in this case, the protein might be overexpressed by some other mechanism. Conversely, in our series, no case with mutant-type p53 on PCR-SSCP showed *GML* gene expression. Thus, to our knowledge, this was the first study as regards *GML* gene expression status in surgically resected NSCLCs, although the number of samples was small. Furthermore, in a preliminary experiment, *GML* gene expression was also observed in some wild-type *TP53* NSCLC cell lines established in our institute, and from sequence analysis, the 196 bp region of the *GML* gene, in *GML*-positive NSCLC tissues as well as NSCLC cell lines was identical in structure to that previously reported in oesophageal cancer cell lines (data not shown).

According to previous experimental reports [9,25], *GML* gene expression in cancer cells partially suppresses cell growth, suggesting that the product of this gene is also associated with the malignant potential of the cell. In this study, although the number of samples examined was small, 7 *GML* gene-positive cases were early stage cancers. *TP53* abnormality in NSCLCs, especially in adenocarcinomas, is now considered to be strongly associated with tumour progression and

Table 4
Clinical effect of cisplatin (CDDP)-based chemotherapy on recurrent or residual tumours of surgically resected NSCLC

Patient's No.	Histology	p53/*TP53* status		*GML* gene expression	*In vitro* sensitivity to CDDP (T/C rate, %)		Tumour	Chemotherapy	Effect
		IHS	PCR-SSCP						
10	Ad	++	Mutant (Exon 7, Codon 248, CGG→CAG)	–	61%	Resistant	Pleuritis	CDDP 80 mg ipl×2[a] CBDCA 300 mg ipl×1[a]	NC
15	Ad	–	Wild-type	–	80%	Resistant	Bone meta.	CDDP 80 mg i.v. VDS 3 mg i.v. ×1 course plus RT (30 Gy)	PD
16	Ad	++	NT	–	65%	Resistant	Pleuritis	CDDP 100 mg ipl×1[a] CBDCA 150 mg ipl×1[a]	PD
19	Ad	–	Wild-type	+	49%	Sensitive	Pleuritis	CDDP 50 mg ipl×1[a] CBDCA 450 mg ipl×1[a]	CR
21	Ad	–	Wild-type	+	22%	Sensitive	Bone meta.	CDDP 150 mg i.v. VDS 5 mg i.v. ×3 courses	PR
23	Ad	+	NT	–	51%	Borderline	Brain meta.	CDDP 100 mg i.v. 5-FU 750 mg i.v.×5 days ×2 courses	PD
24	Ad	–	NT	–	NT	NT	Lung meta.	CDDP 100 mg i.v. VDS 5 mg i.v. ×1 course	NC

NSCLC, non-small cell lung cancer; Ad, adenocarcinoma; IHS, immunohistochemical staining; PCR-SSCP, polymerase chain reaction single-strand conformation polymorphism; NT, not tested; VDS, vindesine sulphate; CBDCA, carboplatin; 5-FU, 5-fluorouracil; meta., metastasis; ipl, intrapleural injection; i.v., intravenous injection; RT, radiation therapy; NC, no change; CR, complete response; PD, progressive disease; PR, partial response.

[a] Intrathoracic chemothermotherapy by Kodama and associates [24].

prognosis [26,27] and, therefore, considering the inverse association between *GML* gene expression and *TP53* abnormality, *GML* gene expression in NSCLCs may occur in relatively slow growing or early stage tumours. Thus, it seems that the *GML* gene product in NSCLCs may also play an important role in cell cycle control via wild-type p53. In contrast, expression of the *GML* gene product in some cancer cells was experimentally induced by mutant-type p53, although cell growth was not inhibited [9,25].

Interestingly, a causal linkage between *GML* gene expression and some anticancer drug sensitivities has been proposed [9,25]. In oesophageal cancer cell lines, those with *GML* gene expression were sensitive *in vitro* to bleomycin and CDDP in cell lines with wild-type *TP53* status, whereas those with no *GML* gene expression and mutant *TP53* were resistant *in vitro* to such DNA damaging drugs. In particular, an oesophageal cancer cell line, TE-10, which has no *GML* gene expression, was originally resistant to bleomycin but showed higher sensitivity upon transfection of the *GML* gene. In the present study using human NSCLC tissues, the majority of the samples with *GML* gene expression showed higher *in vitro* sensitivity to CDDP. This observation supports previous results. Furthermore, it was recently described that *GML* gene expression in the TE-10 oesophageal cancer cell line correlated with *in vitro* sensitivity to paclitaxel, which is thought not to damage DNA, but to stabilise the polymerisation of tubulin [25]. It has also been reported that *GML* gene expression sensitises cancer cells to ionising radiation [28]. This mechanism may be due to increasing G2/M arrest and apoptosis on treatment [9,25,28]. Thus, the *GML* gene in cancer cells would confer sensitisation to various types of anticancer therapeutic modalities.

We previously reported no significant association between immunohistochemical p53 status and CDDP sensitivity in NSCLCs *in vitro* [19]. However, especially in our series of immunohistochemically p53-negative samples, *GML* gene expression significantly correlated with CDDP sensitivity *in vitro*. Therefore, considering not only p53 status but also *GML* gene expression, CDDP sensitivity in this disease may potentially be more accurately predicted. Furthermore, we are currently investigating the effects of *GML* gene transfection on CDDP sensitivity in tumour cell lines with p53+ and *GML*− status.

Moreover, in the present study, 2 patients with p53-negative and *GML* gene expression in the primary tumour tissue of NSCLC showed a good local response to CDDP-based chemotherapy even for metastatic lesions. These responses also coincided with the *in vitro* chemosensitivity results. Although the number of patients undergoing CDDP-based chemotherapy for NSCLC with recurrent and residual tumours was extremely small, the clinical effect we observed was also in agreement with not only *in vitro* chemosensitivity test results but also *GML* gene expression status. Therefore, *GML* gene expression status in NSCLCs may be, although preliminarily, potentially applied as a predictor of chemotherapeutic sensitivity for recurrent and residual tumours. Further larger studies will be needed to confirm this predictive potential.

Various *in vitro* chemosensitivity tests for malignant tumours, especially lung cancer, have been described [29,30]. In this study, CD-DST was used as an *in vitro* chemosensitivity assay [19,21–23], which was a technically different method from that used by Furuhata and coworkers [9]. CD-DST is now considered to also provide information on the chemosensitivity of anticancer drugs. However, although *in vitro* chemosensitivity testing using clinical tissues is becoming increasingly popular, the process has one practical disadvantage: samples must be taken in the culture system without contamination. To ensure this, such chemosensitivity-associated indicators in lung cancer as p53 [31,32], P-glycoprotein [33], multidrug resistance-associated protein (MRP) [34], Bcl-2 [35] and glutathione S-transferase-π (GST-π) [36] have been proposed. From this point of view, *GML* gene expression status in NSCLCs may be a novel candidate for this marker in combination with p53 status. Although *GML* gene expression in tumour tissue was examined using RT-PCR in this study, it may be practically more useful and easier to use an immunohistochemical technique with anti-*GML* gene product antibody [28], as used for the analysis of p53 status.

Currently, CDDP-based chemotherapy is considered a standard regimen for the treatment of NSCLCs [16,17]. We propose, based on our study, that to select better responders for this chemotherapeutic modality, *GML* gene expression status in NSCLCs should be applied in addition to p53 status [31,32]. Moreover, chemotherapy using a combination of paclitaxel for NSCLC patients is a promising modality [17,37,38] and, therefore, the use of this novel gene product could also be considered in these cases, although a correlation between p53 status and sensitivity to paclitaxel has yet to be demonstrated [39–41]. Further study using clinical materials is warranted in assessing the association between the *GML* gene product and therapeutic efficacy using both various types of anticancer drugs and irradiation.

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