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Original contribution

Chaperone protein L-isoaspartate (D-aspartyl) *O*-methyltransferase as a novel predictor of poor prognosis in lung adenocarcinoma☆,☆☆,★,★★

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Summary Endoplasmic reticulum stress and chaperone dysfunction have recently been associated with poor prognoses in various cancers. The newly discovered chaperone protein L-isoaspartyl (D-aspartyl) *O*-methyltransferase (PIMT) regulates the viability of cancer cells in various cancers, although no clinical information regarding the relationship between lung cancer and PIMT expression has been reported. In this study, we aimed to elucidate the relationship between PIMT expression and the prognosis of lung adenocarcinoma. Paraffin-embedded lung tissues obtained from 208 patients with surgically resected lung adenocarcinoma were subjected to immunohistochemical analyses using primary antibodies against PIMT. Kaplan-Meier curves, log-rank tests, and the Cox proportional hazards model were used to analyze the association between PIMT expression and patient survival. Strong PIMT expression was detected in 106 (50.9%) patients, being particularly observed in patients with advanced stages of lung adenocarcinoma. Strong PIMT expression was associated with that of 78-kDa glucose-regulated protein, a marker of endoplasmic reticulum stress. Patients with strong PIMT expression had a shorter survival time (Kaplan-Meier analysis, $P < .001$). Multivariate Cox hazard regression analysis demonstrated that strong PIMT expression was an independent predictor of poor prognosis of lung adenocarcinoma, including those with stage I disease (hazard ratios, 6.45 and 6.81, respectively; 95% confidence intervals, 2.46-16.9 and 1.79-25.8, respectively; $P < .001$ and $P = .005$, respectively). Collectively, strong PIMT expression was a predictive marker of poor prognosis for surgically resected lung adenocarcinoma, and this finding might help clinicians determine the need for postoperative adjuvant chemotherapy in patients with stage I lung adenocarcinoma.

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1. Introduction

Lung cancer is a major global health concern, and non–small cell lung cancer is the most common type of lung cancer, accounting for 80% to 85% of all cases [1]. Despite advances in chemotherapy, radiation, and surgery, the prognosis of lung cancer remains unsatisfactory [2]. Multiple processes are involved in the development of lung cancers, including non–small cell lung cancer, such as carcinogenesis, proliferation, invasion, and distant metastasis of cancer cells [3,4]. Various types of stresses are exerted on cancer cells through these processes, resulting in the accumulation of unfolded proteins in the endoplasmic reticulum (ER), as demonstrated by reports that ER stress markers are up-regulated in cancer [5–7]. Molecular chaperones function by folding other cellular proteins into conformations that permit their structural, enzymatic, and signaling functions. An increasing body of clinical information indicates that increased expression of chaperone proteins is typically associated with poor prognoses for cancers, and the experimental up-regulation of chaperone proteins allows cancer cells to survive apoptosis induced by ER stresses [8–11].

In proteins, L-asparatic acid (L-Asp) and L-asparagine are residues are nonenzymatically modified via dehydration (L-Asp) or deamidation (L-asparagine) during stress-related conditions such as age-related cataracts, sunburn, and hypoxic stress.; These changes are followed by the formation of L-isoaspartateyl, D-isoaspartateyl, and D-aspartic acid, which results in structurally nonfunctional proteins [12,13]. In this context, protein L-isoaspartyl (D-aspartyl) *O*-methyltransferase (PIMT) functions as a chaperone for the conversion of isomerized L-isoaspartateyl and D-aspartic acid residues into L-Asp. PIMT is ubiquitously expressed in all living organisms (from bacteria to humans), and its expression has been detected in mammalian brain, liver, and heart tissues [14–17]. Recently, it was reported that PIMT expression is associated with poor prognoses for several types of cancers. In that report, PIMT expression was closely linked to an antiapoptosis and carcinogenic effect in cancer via inhibition of the p53 pathway, rather than its standard chaperone function [18]. Although the mechanistic understanding of PIMT's role in cancer has increased, the clinical association between PIMT and cancer has yet to be fully elucidated.

Similarly, limited information is available regarding the clinical and pathogenic significance of ER stress and chaperone proteins in non–small cell lung cancer [7,19–21]. In the present study, we aimed to immunohistochemically characterize the expression of PIMT in surgically resected lung adenocarcinoma, which is the most common type of non–small cell lung cancer. We additionally compared these results to the expression of p53 and 78-kDa glucose-regulated protein (GRP78), a marker of ER stress, which was previously determined to be involved in lung cancer, and attempted to clarify whether PIMT functions in lung adenocarcinoma as an inhibitor of the p53 pathway or as a standard chaperone [22].

Table 1 Patient characteristics

Clinical parameters	No. of patients (n = 208)
Age (y)	
Median (range)	70 (36-87)
<65	67
>65	141
Sex	
Male	96
Female	112
Smoking history	
No [a]	154
Yes [b]	54
Pathological staging	
I	154
II	14
III	35
IV	5
Invasion [c]	
Preinvasive	16
Minimally invasive	48
Invasive	144

NOTE. Preinvasive refers to preinvasive lesions; Minimally invasive, minimally invasive adenocarcinoma; Invasive, invasive adenocarcinoma and variants of invasive adenocarcinoma.

[a] Data are never smokers.

[b] Data are current smokers and former smokers.

[c] Histologic diagnosis was performed using the new World Health Organization adenocarcinoma classification [23].

We found that PIMT expression was closely associated with GRP78 expression and confirmed that strong PIMT expression was a predictor of poor prognosis for lung adenocarcinoma, including stage I disease, using Cox hazard regression analyses. Our results might help clinicians to determine the need for chemotherapy after surgery in these cases.

2. Materials and methods

2.1. Subjects

A series of 263 patients diagnosed as having lung adenocarcinoma who underwent surgical resection in Iwate Medical University Hospital (Morioka, Japan) from 2004 to 2010 were selected for study. In total, 55 patients who underwent wedge resection and segmentectomy were excluded from the present study because it was difficult to determine the exact pathological stage. The remaining 208 patients were included in the present study, and all patients were treated by lobectomy. Histologic diagnosis was performed using the latest World Health Organization classification system [23]. Pathological staging of the tumors was performed according to the International System for

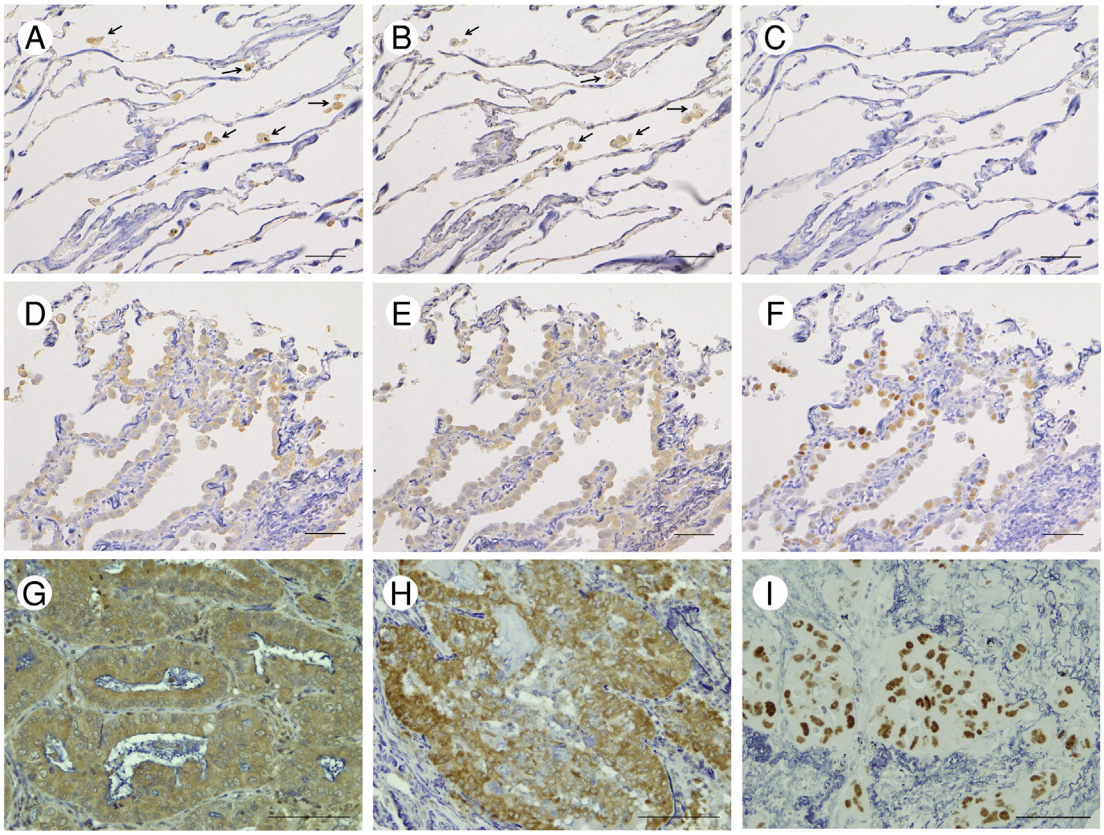

Fig. 1 Immunohistochemical staining for PIMT, GRP78, and p53 expression in normal lung tissue and in lung adenocarcinoma. In normal lung tissue, (A) PIMT and (B) GRP78 expression is detectable in the alveolar macrophages within the adjacent noncancerous tissues (arrows), whereas (C) p53 is barely expressed in normal regions. The expression of the 3 molecules is significantly lower in preinvasive lesions (D-F) than in invasive adenocarcinoma (G-I). PIMT (A, D, and G), GRP78 (B, E, and H), and p53 (C, F, and I). Immunostaining was counterstained using resorcin-fuchsin and hematoxylin. Scale bar, 100 μm.

Staging Lung Cancer [24]. Adjuvant chemotherapy was administered according to the guidelines in effect at the time of resection. The median follow-up period of patients alive at the end point of analysis was 72.0 months. The median follow-up period of patients alive without recurrence was 84.0 months. The clinicopathological characteristics of the test cohort are provided in Table 1. The use of all samples in the present study was approved by the Ethical Committees of Iwate Medical University. The requirement for informed consent was waived because of the retrospective nature of this study.

2.2. Immunohistochemistry

All lung specimens were fixed using 20% formalin and embedded in paraffin wax. The slides were subsequently deparaffinized and rehydrated prior to analysis. Antigen retrieval was performed using citrate buffer (pH 6.0), as previously described, and this step was followed by treatment with 3% H_2O_2 for 15 minutes to quench the endogenous peroxidase activity [25]. For the primary antibodies, we used rabbit antihuman PIMT (Abcam, Cambridge, UK; dilution 1:400), goat antihuman GRP78 (Santa Cruz Biotechnology, Santa Cruz, CA; dilution 1:50), and mouse antihuman p53 antibodies (Santa Cruz Biotechnology; dilution 1:50). As the negative controls for each antibody, normal rabbit and normal goat sera (Vector Labs, Burlingame, CA) were used. After blocking with the normal serum and incubation with the primary antibodies, the antigen-antibody reaction was visualized using 3,3′-diaminobenzidine tetrahydrochloride. These processes were performed using an autostainer (Autostainer Link 48; Dako Japan, Tokyo, Japan). In addition, the sections were counterstained using resorcin-fuchsin and hematoxylin.

2.3. Evaluation of immunostaining

The slides were evaluated individually under a light microscope by 2 separate pathologists (N. Y. and T. S.) who were blinded to the patients' background information. Staining for PIMT and GRP78 was scored using the Allred 8-unit system, which uses the sum of the proportion score (ranging from 0 to 5) and intensity score (ranging from 0 to 3). The proportion score was defined as the fraction of

Table 2 Correlation between PIMT, GRP78, and p53 expression and the clinicopathological characteristics of adenocarcinoma in all patients

Clinical parameters	PIMT			GRP78			p53		
	Weak (n = 102)	Strong (n = 106)	P*	Weak (n = 106)	Strong (n = 102)	P*	Weak (n = 118)	Strong (n = 90)	P*
Age (y)			.376			0.103			.653
<65	36	31		40	27		40	27	
>65	66	75		66	75		78	63	
Sex			.581			0.211			.051
Male	45	51		44	52		47	49	
Female	57	55		62	50		71	41	
Smoking history			.343			0.085			.007
No [a]	79	75		84	70		96	58	
Yes [b]	23	31		22	32		22	32	
Pathological staging			<.001			<0.001			<.001
I	88	66		91	63		100	54	
II, III, IV	14	40		15	39		18	36	
Invasion [c]			.002			0.003			<.001
Preinvasive + minimally invasive	42	22		43	21		50	14	
Invasive	60	84		63	81		68	76	

NOTE. Preinvasive + minimally invasive refers to preinvasive lesions and minimally invasive adenocarcinoma; Invasive, invasive adenocarcinoma and variants of invasive adenocarcinoma.

[a] Data are never smokers.

[b] Data are current smokers and former smokers.

[c] Histologic diagnosis was performed using the new World Health Organization adenocarcinoma classification [23].

* *P* values were determined using the χ^2 analysis.

positively stained tumor cells as follows: 0, none; 1, 1/100; 2, 1/100 to 1/10; 3, 1/10 to 1/3; 4, 1/3 to 2/3; and 5, ≥2/3. The staining intensity score was classified as follows: 0, none; 1, weak; 2, medium; and 3, strong [26]. Tumors with an Allred total score higher than 5 were assumed to have strong expression because it showed median values [27]. Evaluation of p53 staining was decided on the basis of the known article [28]. Tumors with more than 10% staining were assumed to have strong expression [28]. In addition, the Allred 8-unit system was used to evaluate the correlation of p53 with PIMT.

2.4. Statistical analysis

The statistical significance of differences between 2 groups was evaluated using the Mann-Whitney *U* test or Fisher exact test, as appropriate. The strength of the association between the PIMT and GRP78 scores and between the PIMT and p53 scores was assessed using the Spearman rank correlation test. The Kaplan-Meier method was used to estimate patient survival, and differences were analyzed using the log-rank test. The Cox proportional hazards model was used for multivariate analysis of the prognostic factors. Statistical analyses were performed using SPSS Statistics software (IBM Japan, Tokyo, Japan), and differences with a *P* value less than .05 were considered statistically significant.

3. Results

3.1. Patient characteristics and the relationships of PIMT, GRP78, and p53 expression with the patients' clinicopathological findings

The clinicopathological characteristics of the 208 patients are shown in Table 1. Among them, 16, 48, and 154 patients had preinvasive, minimally invasive, and stage I lung adenocarcinoma, respectively. The results of the immunohistochemical analysis using anti-PIMT, anti-GRP78, and p53 antibodies are shown in Fig. 1. PIMT and GRP78 expression was detectable in the alveolar macrophages within the adjacent noncancerous tissues, whereas p53 was barely expressed in normal regions (Figs. 1A-C). Strong expression of PIMT, GRP78, and p53 was observed in tumor samples from 106 (50.9%), 102 (49.0%), and 90 (43.3%) patients with lung adenocarcinoma, respectively (Table 2). The expression of the 3 molecules was significantly lower in patients with stage I or preinvasive lesions than in those with stage II-IV or invasive adenocarcinoma (Fig. 1D-I; Table 2). In addition, we attempted to quantify apoptotic cells in cancer cells based on the presence of apoptotic bodies. However, apoptotic bodies were barely observed in any stage of lung adenocarcinoma in which a glandular structure was maintained, although they were detectable outside adenocarcinoma, in which the structure was lost (Supplementary Fig.).

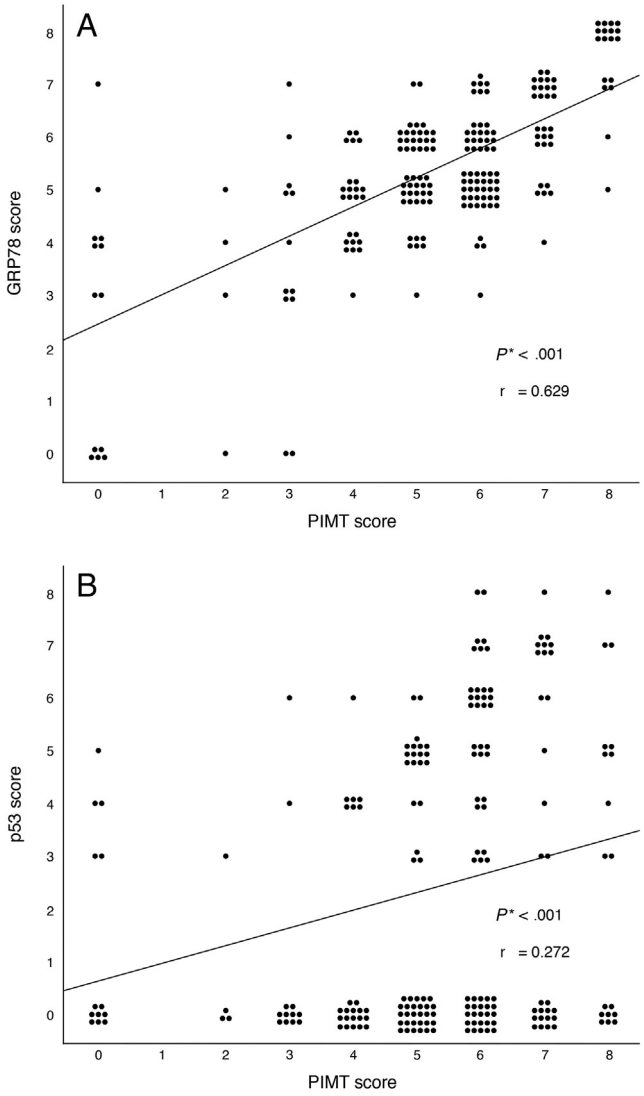

Fig. 2 Correlation of PIMT expression with GRP78 and p53 expression in patients with lung adenocarcinoma. A statistically significant correlation is observed between PIMT and GRP78 expression scores in patients with lung adenocarcinoma (A; $r = 0.629$, $P < .001$), whereas the correlation between PIMT and p53 expression scores is not meaningful (B; $r = 0.272$, $P < .001$). *P value was determined using Spearman analysis.

3.2. Correlation of PIMT expression with GRP78 and p53 expression

When the expression of PIMT and GRP78 was semiquantitatively estimated using the Allred total score (0-8), a statistically significant correlation was observed on the basis of the results of Spearman correlation test ($r = 0.629$, $P < .001$; Fig. 2A). Conversely, a mild correlation was detected between PIMT and p53 expression ($r = 0.272$, $P < .001$).

3.3. Prognostic significance of PIMT and GRP78 expression

The prognostic variables were analyzed using the log-rank test (Fig. 3). Strong expression of PIMT, GRP78, and p53 was significantly correlated with poor outcomes for all stages of lung adenocarcinoma ($P < .001$; Fig. 3A-C). The hazard ratios for mortality among patients with strong PIMT, GRP78, and p53 expression (relative to cases with weak expression) were 10.3, 7.24, and 3.04, respectively (95% confidence intervals [CIs], 4.07-26.1, 3.23-16.2, and 1.66-5.59, respectively; $P < .001$, via univariate Cox regression analysis; Table 3). The hazard ratio for mortality in patients with an advanced pathological stage (relative to patients with stage I adenocarcinoma) was 7.96 (95% CI = 4.34-14.6; $P < .001$, via univariate Cox regression analysis). Multivariate analysis revealed that strong PIMT expression was the most significant independent risk factor for mortality in all cancer stages (hazard ratio, 6.45; 95% CI, 2.46-16.9; $P < .001$), followed by advanced pathological stage and strong GRP78 expression (hazard ratios, 3.93 and 3.15, respectively; 95% CIs, 1.99-7.76 and 1.38-7.18, respectively; $P < .001$ and $P = .006$, respectively) (Table 3).

In the subgroup analyses, strong PIMT expression was significantly correlated with poor prognoses for subjects with stage I or advanced-stage lung adenocarcinoma ($P < .001$, via the log-rank test) (Fig. 3D and G). Similarly, strong GRP expression was also significantly correlated with poor prognoses for subjects with stage I or advanced-stage lung adenocarcinoma ($P < .001$ and $P = .005$, respectively, via the log-rank test) (Fig. 3E and H). However, strong p53 expression was weakly correlated with poor prognoses for subjects with advanced-stage lung adenocarcinoma but not among those with not stage I disease ($P = .019$ and $P = .499$, respectively, via the log-rank test) (Fig. 3I and F). Multivariate analysis revealed that strong PIMT expression was a significant independent risk factor for mortality for patients with stage I and stage II-IV adenocarcinoma (hazard ratios, 6.81 and 7.39, respectively; 95% CIs, 1.79-25.8 and 1.70-32.0, respectively; $P = .005$ and $P = .008$, respectively; Tables 4 and 5). Strong GRP78 expression was not significantly correlated with poor prognoses for patients with stage I or stage II-IV adenocarcinoma based on the results of multivariate analysis (Tables 4 and 5).

4. Discussion

In the present study, we attempted to assess PIMT expression in surgically resected specimens obtained from patients with lung adenocarcinoma. Strong PIMT expression was observed in approximately half of the patients with lung adenocarcinoma, and this expression was associated with a poor prognosis. In addition, strong PIMT expression was identified as an independent predictor of poor prognosis in all patients, even among those with early and advanced pathological stage lung adenocarcinoma, based on multivariate Cox hazard regression analysis. Furthermore, strong

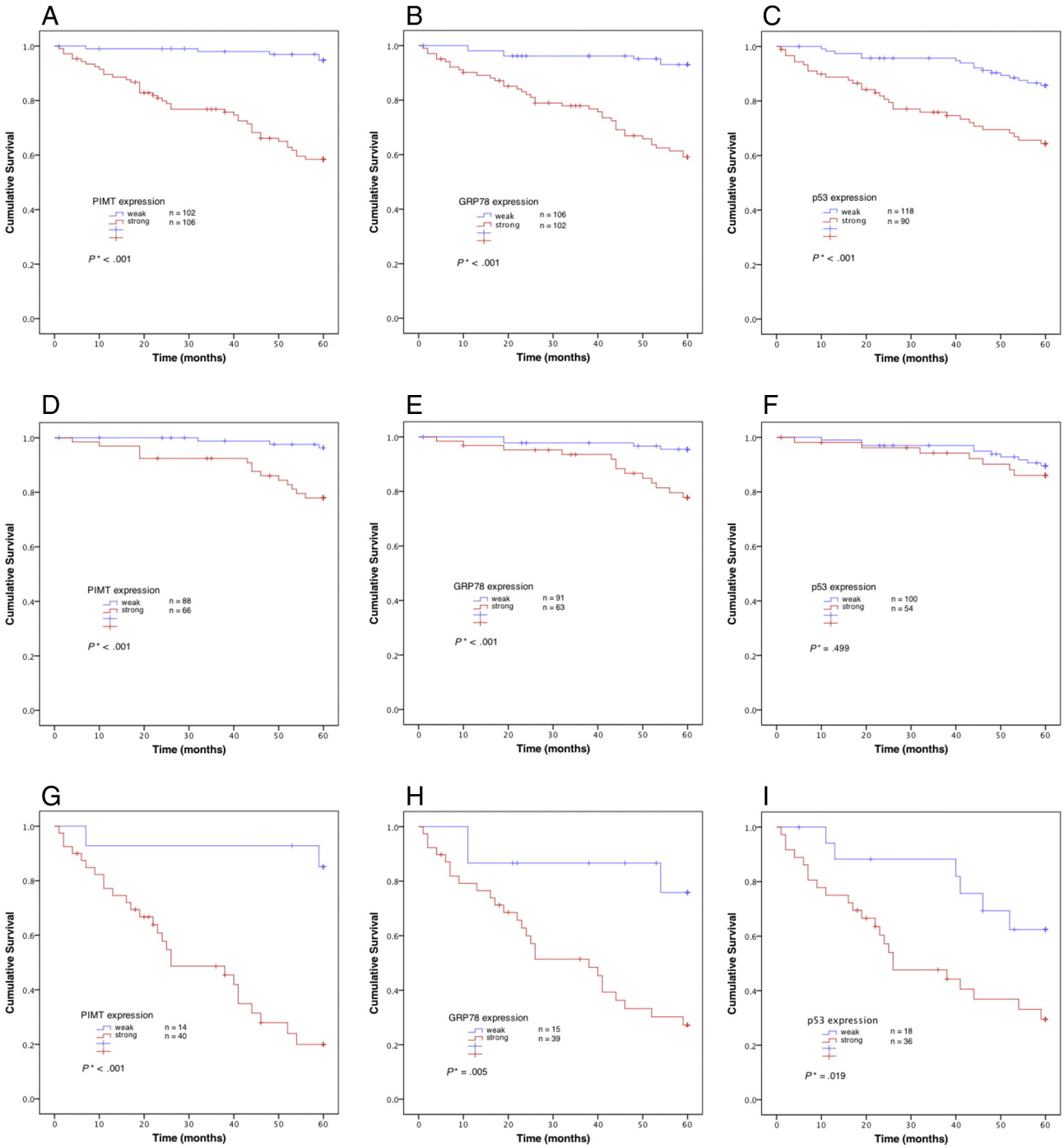

Fig. 3 Prognostic significance of PIMT, GRP78, and p53 expression in lung adenocarcinoma. A-C, Kaplan-Meier estimates for overall survival in patients with stage I-IV lung adenocarcinoma and strong (red) or weak (blue) PIMT (A), GRP78 (B), and p53 (C) expression. D-F, Kaplan-Meier estimates for overall survival in patients with stage I lung adenocarcinoma and strong (red) or weak (blue) PIMT (D), GRP78 (E), and p53 (F) expression. G-I, Kaplan-Meier estimates for overall survival in patients with stage II-IV lung adenocarcinoma and strong (red) or weak (blue) PIMT (G), GRP78 (H), and p53 (I) expression. **P* value is determined using the log-rank test.

PIMT expression was strongly correlated with strong GPR78 expression in patients with lung adenocarcinoma.

There is limited information available regarding the clinical relationship between PIMT and lung cancer. Lee et al [18] conducted a preclinical study of PIMT expression in several types of cancer, including lung cancer. In their study, semiquantified PIMT expression was estimated using immunoblotting of the whole lung tissue, and they reported that strong PIMT expression was associated with a poor prognosis for lung cancer. However, the authors did not provide detailed

Table 3 Hazard ratios for mortality among the all patients with lung adenocarcinoma

Clinical parameters	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P**	HR	95% CI	*P**
Age (y)						
<65/> 65	1.94	0.96-3.91	.063			
Sex						
Women/Men	1.91	1.05-3.46	.032	0.98	0.49-1.95	.956
Smoking history						
No [a]/Yes [b]	4.19	2.34-7.50	<.001	2.54	1.24-5.20	.011
Pathological staging						
I/II, III, IV	7.96	4.34-14.6	<.001	3.93	1.99-7.76	<.001
PIMT						
Weak/Strong	10.3	4.07-26.1	<.001	6.45	2.46-16.9	<.001
GRP78						
Weak/Strong	7.24	3.23-16.2	<.001	3.15	1.38-7.18	.006
p53						
Weak/Strong	3.04	1.66-5.59	<.001	1.27	0.65-2.49	.473

Abbreviations: HR, hazard ratio; CI, confidence interval.
[a] Data are never smokers.
[b] Data are current smokers and former smokers.
* *P* values were determined using the Cox regression analysis.

patient characteristics. In addition, as shown in the present study, PIMT expression was also detectable in the adjacent noncancerous tissues, indicating that semiquantifiable immunoblotting of the whole lung may not necessarily represent the actual PIMT expression in lung cancer. Therefore, our results confirm that PIMT expression can be immunohistochemically detected in in situ lung adenocarcinoma.

Our Kaplan-Meier survival analysis indicated that patients with lung adenocarcinoma and strong PIMT expression had a significantly shorter survival time. Strong PIMT expression in lung adenocarcinoma was identified as an independent predictor of poor prognosis based on the results of our multivariate Cox hazard regression analysis. Our results indicate that PIMT might be a useful marker for predicting survival in patients with surgically resected lung adenocarcinoma. Notably, even among patients with stage I lung adenocarcinoma, our results indicated that strong PIMT expression was a predictor of poor prognosis. It is controversial whether chemotherapy after surgery improves the prognoses of patients with stage I lung adenocarcinoma [29]. Our results suggest that patients with stage I lung adenocarcinoma and strong PIMT expression should receive adjuvant chemotherapy postoperatively.

The pathogenic role of PIMT expression in cancer cells has yet to be elucidated. Lee and coworkers [18]. Demonstrated a mutually interfering relationship between

Table 4 Hazard ratios for mortality among the stage I patients with lung adenocarcinoma

Clinical parameters	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P**	HR	95% CI	*P**
Age (y)						
<65/> 65	3.89	0.89-17.0	.071			
Sex						
Women/Men	2.51	0.93-6.80	.069			
Smoking history						
No [a]/Yes [b]	4.49	1.73-11.6	.002	5.88	2.19-15.7	<.001
PIMT						
Weak/Strong	6.74	1.93-23.4	.003	6.81	1.79-25.8	.005
GRP78						
Weak/Strong	5.26	1.71-16.1	.004	2.37	0.72-7.76	.154
p53						
Weak/Strong	1.39	0.53-3.66	.501			

Abbreviations: HR, hazard ratio; CI, confidence interval.
[a] Data are never smokers.
[b] Data are current smokers and former smokers.
* *P* values were determined using the Cox regression analysis.

Table 5 Hazard ratios for mortality among the stage II-IV patients with lung adenocarcinoma

Clinical parameters	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P* *	HR	95% CI	*P* *
Age (y)						
<65/> 65	1.82	0.80-4.14	.153			
Sex						
Women/Men	1.16	0.55-2.44	.686			
Smoking history						
No [a]/Yes [b]	1.641	0.78-3.44	.191			
PIMT						
Weak/Strong	10.2	2.38-43.9	.002	7.39	1.70-32.0	.008
GRP78						
Weak/Strong	4.72	1.42-15.6	.011	2.96	0.88-9.89	.077
p53						
Weak/Strong	2.81	1.13-6.95	.025	1.78	0.71-4.44	.213

Abbreviations: HR, hazard ratio; CI, confidence interval.

[a] Data are never smokers.

[b] Data are current smokers and former smokers.

* *P* values were determined using the Cox regression analysis.

PIMT and wild-type p53 in their preclinical experiments, which used several cancer cell lines. Based on this finding, they hypothesized that the association between strong PIMT expression and poor prognosis for cancer may arise from the antiapoptotic effects that PIMT exerts in cancer cells via the suppression of p53 expression. In general, the expression of wild-type p53 was undetectable via immunohistochemistry because p53 turnover is usually rapid [30]. By contrast, the expression of mutant p53 is competitive to that of wild-type p53, but mutant p53 is nevertheless immunohistochemically detectable in human specimens [31]. In the present study, the results of immunohistochemical analyses using anti-p53 antibody illustrated that strong expression of mutant p53, but not wild-type p53, was associated with a poor prognosis among patients with lung adenocarcinoma and that mutant p53 expression was correlated with PIMT expression, which was consistent with previous reports [32]. Therefore, PIMT expression was apparently opposite to that of wild-type p53 expression. However, apoptotic cells were barely observed in any stage of lung adenocarcinoma that maintained a glandular structure when estimated by the presence of apoptotic bodies, whereas apoptotic bodies were often observed outside advanced stages of lung adenocarcinoma. In the present study, although we could not determine whether strong PIMT expression is involved in the carcinogenesis of lung adenocarcinoma via the suppression of wild-type p53 expression, which was hypothesized by Lee and coworkers [18], strong PIMT expression is likely related to cancer progression in concert with strong mutant p53 expression.

GRP78 expression was moderately correlated with PIMT expression. These findings suggest that the standard chaperone function of PIMT may exert antiapoptotic effects in lung adenocarcinoma cells via the compensative clearance of the unfolded proteins that is induced by the increased ER stress, although the exact client proteins in lung adenocarcinoma corresponding to PIMT have not been identified. As mentioned previously, strong PIMT expression was associated with poor prognosis, even in patients with stage I or stage II-IV adenocarcinoma. In addition, the proportion of patients with strong PIMT expression significantly increased in advanced-stage adenocarcinoma compared with the findings in patients with stage I disease. These results suggested that PIMT is deeply linked to the pathogenesis of cancer progression. Lapointe et al [33] reported that the expression and activity of PIMT decrease with the continued progression of human astrocytic tumors, which is seemingly inconsistent with our finding. However, astrocytes express PIMT in normal conditions, differently than alveolar epithelial cells [15]. Therefore, we could not exclude the possibility that PIMT expression plays heterogeneous roles among the different types of cancer.

Several studies reported the clinical and pathogenic significance of GRP78 expression in non–small cell lung cancer [7,20]. In these studies, the results of immunohistochemical analyses indicated that strong GRP78 expression was inversely correlated with low TNM stage in invasive non–small cell lung cancer. In Kaplan-Meier survival analyses, patients with strong GRP78 expression had a longer survival time than did those with weak expression. In the present study, we found that patients with stage I or stage II-IV adenocarcinoma and strong GRP78 expression had a shorter survival time than did those with weak expression. These findings were inconsistent with previously published results. This discrepancy may have 2 possible explanations. First, we evaluated a different population of patients with lung cancer (exclusively lung adenocarcinoma), whereas the previous studies included patients with various lung cancers, including adenocarcinoma and squamous cell carcinoma.

Based on this difference, it is possible that GRP78 plays different roles in squamous carcinoma and adenocarcinoma. Second, we evaluated GRP78 expression based on the Allred 8-unit system involving the sum of the proportion and intensity scores, whereas the previous studies assumed that GRP78 expression was positive when the individual intensity and extent scores were higher than 2. Although we reestimated the relationship between GRP78 expression and the prognosis of patients with invasive lung adenocarcinoma using the latter method, no relevant differences were observed between the 2 methods (data not shown).

The main limitation of this study is its retrospective nature. Consequently, there are some missing data such as survival time. In particular, we could not determine the relationship between PIMT expression and progression-free survival. Although we excluded patients who underwent lung reduction surgery from the present study, the involvement of any selection bias is unlikely.

5. Conclusion

We firstly determined PIMT expression in lung adenocarcinoma tissues using in situ analysis and identified the predictive value of PIMT expression for the prognosis of patients with lung adenocarcinoma, which greatly exceeded those of GRP78 expression and pathological staging. Our results highlight the potential importance of PIMT expression in the pathogenesis of lung cancer, and our findings might facilitate appropriate decision making in the clinical treatment of lung adenocarcinoma.

Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.humpath.2015.11.006.

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