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Plasminogen Activator Inhibitor Type 1 (*PAI-1*) A15T Gene Polymorphism Is Associated with Prognosis in Patients with *EGFR* Mutation Positive Pulmonary Adenocarcinoma

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Background: Plasminogen activator inhibitor type 1 (PAI-1), an important regulator of plasminogen activator system which controls degradation of extracellular membrane and progression of tumor cells, and *PAI-1* gene polymorphic variants have been known as the prognostic biomarkers of non-small cell lung cancer patients. Recently, experimental in vitro study revealed that transforming growth factor-β1 initiated *PAI-1* transcription through epithelial growth factor receptor (EGFR) signaling pathway. However, there is little clinical evidence on the association between *PAI-1* A15T gene polymorphism and prognosis of Korean population with pulmonary adenocarcinoma and the influence of activating mutation of EGFR kinase domain.
Methods: We retrospectively reviewed the medical records of 171 patients who were diagnosed with pulmonary adenocarcinoma and undergone *EGFR* mutation analysis from 1995 through 2009.
Results: In all patients with pulmonary adenocarcinoma, there was no significant association between *PAI-1* A15T polymorphic variants and prognosis for overall survival. However, further subgroup analysis showed that the group with AG/AA genotype had a shorter 3-year survival time than the group with GG genotype in patients with *EGFR* mutant-type pulmonary adenocarcinoma (mean survival time, 24.9 months vs. 32.5 months, respectively; p=0.015). In multivariate analysis of 3-year survival for patients with pulmonary adenocarcinoma harboring mutant-type *EGFR*, the AG/AA genotype carriers had poorer prognosis than the GG genotype carriers (hazard ratio, 7.729; 95% confidence interval, 1.414–42.250; p=0.018).
Conclusion: According to our study of Korean population with pulmonary adenocarcinoma, AG/AA genotype of *PAI-1* A15T would be a significant predictor of poor short-term survival in patients with pulmonary adenocarcinoma harboring mutant-type *EGFR*.

Keywords: Plasminogen Activator Inhibitor 1; Polymorphism, Single Nucleotide; Carcinoma, Non-Small-Cell Lung; Prognosis; Receptor, Epidermal Growth Factor

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Introduction

Lung cancer is ranked second of the newly developed cancer and is a major cause of cancer-related mortality in the United States in 2010[1]. TNM staging system which has been used to anticipate the clinical outcomes of patients who diagnosed with non-small cell lung cancer (NSCLC) keeps many limitations[2,3]. Overcoming these drawbacks of TNM staging system, genetic polymorphisms of candidate genes for molecules involved in NSCLC invasion and metastasis have been suggested as the more precise predictors of clinical outcomes in NSCLC patients[4-8].

Plasminogen activator (PA) system which is known as one of the accelerator of tumor invasion has been researched to define their prognostic role in patients with NSCLC[9]. PA system is comprised of plasmin converted from plasminogen by PA, PA receptor (PAR) and plasminogen activator inhibitor type 1 and 2 (PAI-1 and -2). Among them, plasmin catalyzes degradation of extracellular membrane, thereby inducing further progression of tumor cells. Both PAI-1 and -2 are important regulators of plasmin. Previous study had been reported paradoxical result that higher PAI-1 level in tumor extracts is a predictor of poor prognosis in patients with NSCLCs[10]. But, in that study, the prognostic effect of PAI-1 depends on the histologic subtypes. The higher level of PAI-1 was significantly associated poor prognosis in patients with squamous cell carcinoma, but not in those with pulmonary adenocarcinoma. Contrary to this, higher level of PAI-1 was related to the poor prognosis of patients with pulmonary adenocarinoma in other study[11]. Like this, there is still controversy in the prognostic role of PAI-1 in different histologic type, especially in pulmonary adenocarcinoma. Moreover, recent study in the United Kingdom reported that polymorphic variants of *PAI-1* A15T (rs6092) and *PAI-2* S413C (rs6104) influence on the prognosis of NSCLC patients[12]. But, the prognostic role of *PAI-1* A15T in patients with pulmonary adenocarcinoma was not studied in that study. Consequently, further investigation is necessary to confirm the prognostic role of PAI-1 in pulmonary adenocarcinoma. Adding to this, lately, experimental in vitro study revealed that PAI-1 transcription is initiated by transforming growth factor-β1 (TGF-β1)[13]. But, more interesting result in that study was that this process requires epithelial growth factor receptor (EGFR) signaling pathway. It has been widely known that the presence of activating mutation in EGFR kinase domain in patients with pulmonary adenocarcinoma is strongly associated with the response to the EGFR tyrosine kinase inhibitor[14]. All things taken together, we hypothesized that the prognostic impact of *PAI-1* A15T on patients with pulmonary adenocarcinoma would be influenced by the presence of activating mutation in EGFR kinase domain. So, we designed this study to test whether *PAI-1* A15T gene polymorphism is related to the prognosis and the clinical characteristics of Korean populations with pulmonary adenocarcinoma and to test whether the presence of activating mutation in EGFR kinase domain can influence on the prognostic impact of *PAI-1* A15T.

Materials and Methods

1. Patients

We conducted retrospective observational study and patients who were diagnosed with pulmonary adenocarcinoma and underwent *EGFR* mutation analysis from 1995 through 2009 at the Severance hospital (tertiary referral hospital in Seoul, Korea) were reviewed. When tissue samples were not available, patients were excluded. One hundred seventy-one patients were finally selected. We retrospectively reviewed the medical records which included age, sex, smoking history, histology type, tumor grade, surgery type when patient underwent a surgical resection, and treatment modalities other than surgery such as chemotherapy or concurrent chemoradiation therapy. Then, we classified study population into different subgroups according to *PAI-1* A15T genotypes (GG genotype or AG/AA genotype) and the type of *EGFR* mutation (wild-type or mutant-type *EGFR*). Finally, study population was classified into four subgroups as follows: group 1, patients with the GG genotype and mutant-type *EGFR*; group 2, patients with the AG/AA genotype and mutant *EGFR*; group 3, patients with the GG genotype and wild-type *EGFR*; group 4, patients with the AG/AA genotype and wild-type *EGFR*.

Tumor staging at presentation was determined by using the International TNM classification system[15]. Histology type and tumor grade were reviewed by pathologist according to the World Health Organization classification of lung tumors[16]. Informed consents were obtained from all participants and this study was approved by Ethical Review Committee of Severance Hospital.

2. *PAI-1* A15T gene polymorphism and genotyping

Genomic DNA was extracted from microdissected tissue blocks of 10-μm thickness. *PAI-1* A15T was amplified with the forward primer, 5'-AGGGCAAGATGGGCGAAGACTCC-3' and the reverse primer, 5'-TCCCCTGGTGTCCCGTGGCTC-3'. *PAI-1* A15T genetic polymorphisms were genotyped by using minisequencing assay. The sequence of minisequencing primer was 5'-CCTGCCACTGCCCGGGGATAA-3'. Minisequencing assay was processed by ABI BigDye Terminator version 3.1 Ready Reaction Cycle Sequencing Kit (Applied biosystems, Foster City, CA, USA).

3. *EGFR* mutation analysis

Genomic DNAs which were extracted from tissue blocks

containing tumor regions were prepared. Then, nucleotide sequencing of EGFR kinase domain (exons 18–21) was performed using nested polymerase chain reaction amplification of individual exons. The detail process was explained elsewhere[17].

4. Statistical methods

The primary end point was to compare overall outcomes and clinical characteristics of study population between groups with different genotypes. Survival time was defined as from the date of surgery when patient underwent an operation or from the date of diagnosis when patient did not undergo an operation to the date of death from any cause. Disease-free survival (DFS) was defined as from the date of surgery to the date of recurrence or death from any cause. When patients were censored, the status of survival and recurrence were determined according to the information at last contact. The second end point was to compare overall survival and short-term survival between different genotypic groups (GG genotype or AG/AA genotype) which were classified into subgroups depending on the type of *EGFR* mutation state (wild-type or mutant-type *EGFR*). For statistical analysis, common allele homozygotes, the GG genotype was defined as reference group and the AA genotype was combined with the AG genotype because the frequency of the AA homozygote was too low. To compare survival times between groups with different genotypes, log-rank test were used. The Cox proportional hazards model was used for a multivariate analysis of survival. The χ^2 test and Fisher's exact test were used to examine the association between single nucleotide polymorphism (SNP) genotypes and known prognostic factors. Two-sided p-values less than 0.05 were considered to be statistically significant. The computer software SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

1. Baseline characteristics

The baseline characteristics of all subjects are summarized in Table 1. The median follow-up time was 25.9 months (range, 0.3–143.0 months). The mean age was 59.4±10.1 years old. One hundred two (59.6%) were female and 119 (69.6%) had no smoking history. The genotypes of *PAI-1* A15T could be summarized as follows: 144 (84.2%) had the GG genotype and 27 (15.8%) had the AG/AA genotypes. The mutation state of *EGFR* also could be summarized as follows: 80 (46.8%) had mutant-type *EGFR* and 91 (53.2%) had wild-type *EGFR*. Finally, 68 were classified as group with the GG genotype and mutant-type *EGFR*, 12 as group with the AG/AA genotype and mutant-type *EGFR*, 76 as group with the GG genotype and wild-type *EGFR* and 15 as group with the AG/AA genotype and wild-type *EGFR*. Among 144 subjects had operations, 33 patients (23%) did not receive adjuvant chemotherapy. Among those, 10 patients with completely resected stage IA did not need further chemotherapy and 9 patients could not receive chemotherapy owing to decreased performance status or refusal. The remainder, 14 patients with completely resected stage IB did not receive chemotherapy according to the clinician's decision.

Recurrence rate of patients who did not receive adjuvant chemotherapy was slightly higher than that of patients who received adjuvant chemotherapy, but there was no statistical significance (15/33, 45.5% vs. 71/111, 64%; p=0.06). Only 1 among patients with stage IIIA was treated with concurrent chemo-radiation therapy. Recurrence occurred in 87 (60.0%) among 145 subjects who underwent surgical resection or concurrent chemo-radiation therapy. After recurrence, 44 patients (50.6%) were treated with EGFR tyrosine kinase inhibitor (TKI). The frequency of EGFR TKI use was higher in group with GG genotype and mutant-type *EGFR* than in group with AG/AA type and mutant-type *EGFR*, though there was no statistically significant difference (14/30, 46.7% vs. 1/5, 20%; p=0.265). In patients with stage IIIB and IV were treated with platinum based doublet chemotherapy. The median DFS time was 33.3 months. Eighty two (48%) were died during follow-up period. The estimated median survival time was 54.1 months.

2. Clinical features according to different *PAI-1* A15T genotypes

PAI-1 A15T gene polymorphism analysis showed that 144 (84.2%) had the GG genotype, 26 (15.2%) had the AG genotype and only 1 subject had the AA genotype. There was no significant correlation between *PAI-1* A15T genotypes and clinical characteristics such as age, gender, smoking history, the degree of bronchioloalveolar carcinoma (BAC) component, grade of histologic differentiation, TNM stage, and *EGFR* mutation state (Table 1). After classifying subjects into four subgroups by *EGFR* mutation state and *PAI-1* A15T genotypes, we also could not find a significant correlation between clinical characteristics and *PAI-1* A15T genotypes and *EGFR* mutation state except recurrence. The recurrence rate was higher in group with wild-type EGFR and the GG genotype than group with wild-type EGFR and the AG/AA genotype (74.6% vs. 41.7%, respectively; p=0.023) (Table 2).

3. Interaction between *PAI-1* A15T variants and prognosis

Seventy-two patients (50%) of 144 patients with the GG genotype died and 10 patients (37%) of 27 patients with the AG or AA genotype died in follow-up period. But, *PAI-1* A15T

Table 1. Baseline characteristics

Clinical characteristic	*PAI-1* A15T genotype			
	All patients (n=171)	GG (n=144)	AG/AA (n=27)	p-value
Mean±SD (age at diagnosis), yr	59.4±10.1	58.6±10.2	62.7±8.8	0.055
Age, yr				0.414
<65	113 (66.1)	97 (67.4)	16 (59.3)	
≥65	58 (33.9)	47 (32.6)	11 (40.7)	
Gender				0.184
Female	102 (59.6)	89 (61.8)	13 (48.1)	
Male	69 (40.4)	55 (38.2)	14 (51.9)	
Smoking state				0.203
Never	119 (69.6)	103 (71.5)	16 (59.3)	
Ever	52 (30.4)	41 (28.5)	11 (40.7)	
BAC component				0.632
Pure AC	107 (62.6)	91 (6.2)	16 (59.3)	
Mixed type	57 (33.3)	48 (33.3)	9 (33.3)	
Pure BAC	7 (4.1)	5 (3.5)	2 (7.4)	
Histologic differentiation				0.870
Well	20 (11.7)	17 (11.8)	3 (11.2)	
Moderate	53 (31.0)	45 (31.2)	8 (29.6)	
Poor	33 (19.3)	29 (20.2)	4 (14.8)	
Unknown	65 (38.0)	53 (36.8)	12 (44.4)	
TNM stage				0.522
Stage I	63 (36.8)	55 (38.2)	8 (29.6)	
Stage II	19 (11.1)	15 (10.4)	4 (14.8)	
Stage III	67 (39.2)	54 (37.5)	13 (48.1)	
Stage IV	22 (12.9)	20 (13.9)	2 (7.4)	
Surgery	144 (84.2)	121 (84.0)	23 (85.2)	0.880
Adjuvant chemotherapy*	111 (77.1)	95 (78.5)	16 (69.6)	0.349
Recurrence†	87 (60.0)	77 (63.1)	10 (43.5)	0.078
Local recurrence	14 (16.1)	11 (14.5)	3 (27.3)	
Distant recurrence	74 (83.9)	65 (85.5)	8 (72.7)	
EGFR mutation state				0.791
Mutant-type	80 (46.8)	68 (47.2)	12 (44.4)	
Wild-type	91 (53.2)	76 (52.8)	15 (55.6)	
EGFR TKI use after recurrence‡	44 (50.6)	42 (54.5)	2 (20.0)	0.040
Death	82 (48.0)	72 (50.0)	10 (37.0)	0.216

Values are presented as number (%).
*An analysis of frequency within 144 patients who underwent surgical resection with curative aim. †An analysis of frequency within 145 patients who had surgical resection and concurrent chemoradiation therapy with curative aim. ‡An analysis of frequency within 87 patients who experienced recurrence.
PAI-1: plasminogen activator inhibitor type 1; BAC: bronchioloalveolar carcinoma; AC: adenocarcinoma; *EGFR*: epidermal growth factor receptor; TKI: tyrosine kinase inhibitor.

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Table 2. Comparison of clinicopathological characteristics between groups classified by *EGFR* mutation state and *PAI-1* A15T genotypes

Clinical characteristic	*PAI-1* A15T genotype					
	EGFR mutant-type (n=80)			*EGFR* wild-type (n=91)		
	GG (n=68)	AG/AA (n=12)	p-value	GG (n=76)	AG/AA (n=15)	p-value
Age, yr			1.000			0.178
<65	43 (63.2)	8 (66.7)		54 (71.1)	8 (53.3)	
≥65	25 (36.8)	4 (33.3)		22 (28.9)	7 (46.7)	
Gender			0.237			0.481
Female	46 (67.6)	6 (50.0)		43 (56.6)	7 (46.7)	
Male	22 (32.4)	6 (50.0)		33 (43.4)	8 (53.3)	
Smoking state			0.330			0.415
Never	54 (79.4)	8 (66.7)		49 (64.5)	8 (53.3)	
Ever	14 (20.6)	4 (33.3)		27 (35.5)	7 (46.7)	
BAC component			0.638			0.896
Pure AC	39 (57.4)	6 (50.0)		52 (68.4)	10 (66.7)	
Mixed type	27 (39.7)	5 (41.7)		21 (27.6)	4 (26.7)	
Pure BAC	2 (2.9)	1 (8.3)		3 (3.9)	1 (6.7)	
Histologic differentiation			0.643			0.263
Well	10 (14.7)	1 (8.3)		7 (9.2)	2 (13.3)	
Moderate	18 (26.5)	3 (25.0)		27 (35.5)	5 (33.3)	
Poor	8 (11.8)	3 (25.0)		21 (27.6)	1 (6.7)	
Unknown	32 (47.1)	5 (41.7)		21 (27.6)	7 (46.7)	
TNM stage			0.730			0.813
Stage I+II	32 (47.1)	5 (41.7)		38 (50.0)	7 (46.7)	
Stage III+IV	36 (52.9)	7 (58.3)		38 (50.0)	8 (53.3)	
Surgery	59 (86.8)	11 (91.7)	0.636	62 (81.6)	12 (80.0)	0.886
Adjuvant chemotherapy*	47 (79.7)	8 (72.7)	0.607	48 (77.4)	8 (66.7)	0.427
Recurrence†	30 (50.8)	5 (45.5)	0.743	47 (74.6)	5 (41.7)	0.023
EGFR TKI use‡	14 (46.7)	1 (20.0)	0.265	28 (59.6)	1 (20.0)	0.090
Death	30 (44.1)	5 (41.7)	0.875	42 (55.3)	5 (33.3)	0.120

Values are presented as number (%).
*An analysis of frequency within 144 patients who underwent surgical resection with curative aim. †An analysis of frequency within 145 patients who had operations and concurrent chemoradiation therapy with curative aim. ‡An analysis of frequency within 87 patients who experienced recurrence.
EGFR: epidermal growth factor receptor; *PAI-1*: plasminogen activator inhibitor type 1; BAC: bronchioloalveolar carcinoma; AC: adenocarcinoma; TKI: tyrosine kinase inhibitor.

genotypes were not significantly associated with overall survival time in Kaplan-Meier curve and log-rank test (p=0.88). Univariate analysis showed that the histologic subtype with pure BAC, TNM stage (I–II) and the absence of recurrence were statistically significantly associated with favorable overall survival (Table 3). To the multivariate analysis, the following variables were entered: age, smoking history, BAC component, TNM stage, recurrence, EGFR TKI use after recurrence, *EGFR* mutation state and *PAI-1* A15T genotype. The result of the multivariate analysis was same as that of univariate analysis (Table 3). After then, we conducted subgroup analysis to check out whether the *EGFR* mutation state would be concerned in the prognostic role of *PAI-1* A15T. However, there was no significant association between *PAI-1* A15T genotypes and overall survival in both groups with wild-type and mutant-type *EGFR* (log-rank test, p=0.43 and p=0.43, respectively). Ad-

Table 3. Univariate and multivariate analysis of overall survival in patients with pulmonary adenocarcinoma

Clinical characteristic		Univariate			Multivariate		
		HR	95% CI	p-value	HR	95% CI	p-value
Age, yr	<65 vs. ≥65	0.996	0.624–1.592	0.988	1.100	0.625–1.935	0.742
Smoking history	Never vs. Ever	1.339	0.830–2.158	0.232	1.026	0.597–1.818	0.930
BAC component	Pure BAC vs. Others	5.386	1.208–22.669	0.022	5.084	1.059–24.404	0.042
TNM stage	I+II vs. III+IV	2.486	1.560–3.964	<0.0001	1.756	1.014–3.043	0.045
Recurrence	No vs. Yes	3.185	1.649–6.153	0.001	4.055	1.913–8.597	<0.001
EGFR TKI use after recurrence	Yes vs. No	0.773	0.498–1.199	0.251	1.774	0.970–3.246	0.063
EGFR mutation state	Mutant vs. Wild-type	1.305	0.837–2.034	0.239	1.096	0.634–1.895	0.743
PAI-1 A15T genotype	GG vs. AG/AA	0.949	0.487–1.849	0.879	1.676	0.746–3.764	0.211

HR: hazard ratio; CI: confidence interval; BAC: bronchioloalveolar carcinoma; *EGFR*: epithelial growth factor receptor; TKI: tyrosine kinase inhibitor; *PAI-1*: plasminogen activator inhibitor type 1.

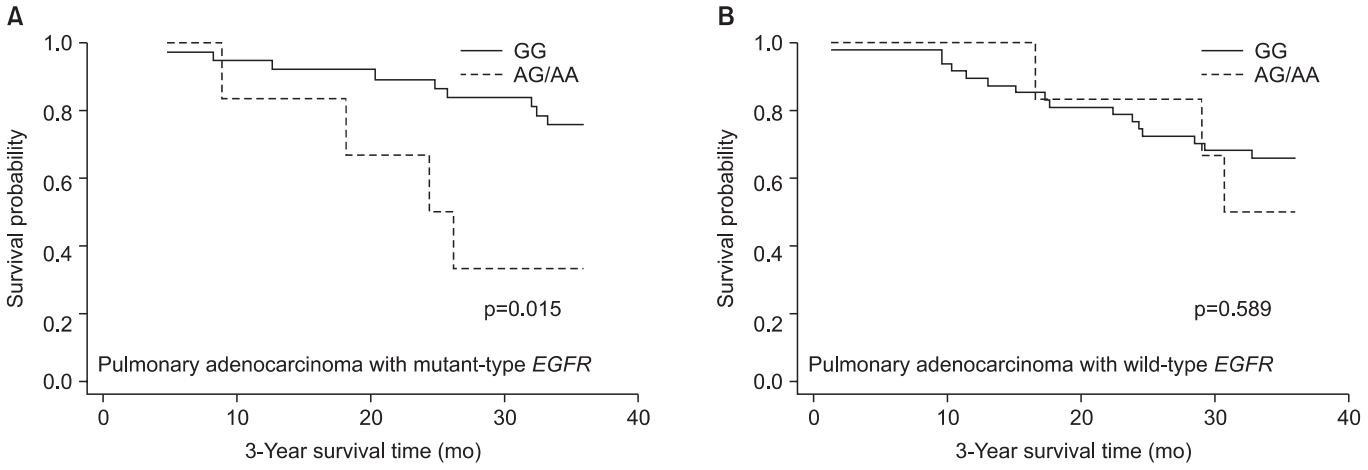

Figure 1. (A) Kaplan-Meier curves for the relationship between plasminogen activator inhibitor type 1 (*PAI-1*) A15T genotype and 3-year survival in patients with pulmonary adenocarcinoma harboring mutant-type. Subgroup analysis of 3-year survival for the patients with pulmonary adenocarcinoma harboring wild-type epithelial growth factor receptor (*EGFR*) and mutant-type *EGFR* revealed that group with AG/AA genotype and mutant-type *EGFR* had a shorter survival time than group with GG genotype and mutant-type *EGFR* (mean survival time, 24.9 months vs. 32.5 months, respectively; p=0.015). (B) Kaplan-Meier curves for the relationship between PAI-1 A15T genotype and 3-year survival in patients with pulmonary adenocarcinoma harboring wild-type *EGFR*. No correlation existed between the genotypes and 3-year survival among patients with pulmonary adenocarcinoma harboring wild-type *EGFR* (log-rank test, p=0.589).

ditionally, there was also no significant association between *PAI-1* A15T genotypes and DFS in both groups with wild-type and mutant-type *EGFR* (data not shown).

4. The effects of *PAI-1* A15T gene polymorphic variants and activating mutation in *EGFR* kinase domain on 3-year survival

According to an analysis for 3-year survival, though it was not statistically significant, we found that patients with the AG/AA genotypes had shorter survival time than patients with the GG genotype (mean survival time, 27.8 months vs. 31.1 months, respectively; p=0.05). Although it was not statistically significant, patients with the AG/AA genotype showed a trend of poor prognosis of 3-year survival in Cox regression analysis (hazard ratio [HR], 2.266; 95% confidence interval [CI], 0.978–5.251; p=0.056). Further subgroup analysis of 3-year survival for those patients with pulmonary adenocarcinoma harboring wild-type *EGFR* and mutant-type *EGFR*, respectively revealed that group with the AG/AA genotype and mutant-type *EGFR* had shorter survival time than group with the GG genotype and mutant-type *EGFR* (mean survival time, 24.9 months vs. 32.5 months, respectively; p=0.015) (Fig. 1A). No correlation existed between the genotypes and 3-year survival among patients with pulmonary adenocarcinoma harboring wild-type *EGFR* (log-rank test, p=0.589) (Fig. 1B).

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Table 4. Univariate and multivariate analysis of 3-year survival in patients with pulmonary adenocarcinoma harboring mutant-type *EGFR*

Clinical characteristics		Univariate			Multivariate		
		HR	95% CI	p-value	HR	95% CI	p-value
Age, yr	<65 vs. ≥65	0.697	0.214–2.263	0.547	0.866	0.223–3.368	0.836
Smoking history	Never vs. Ever	2.002	0.616–6.508	0.249	1.050	0.271–4.068	0.944
BAC component	Pure BAC vs. Others	1.391	0.004–1.431	0.483	2.946	0.001–3.105	0.988
TNM stage	I+II vs. III+IV	4.663	1.031–21.084	0.046	6.284	1.149–34.354	0.034
Recurrence	No vs. Yes	4.662	1.032–21.060	0.045	6.388	1.334–30.592	0.020
EGFR TKI use after recurrence	Yes vs. No	1.899	0.421–8.569	0.404	3.433	0.686–17.184	0.133
PAI-1 A15T genotype	GG vs. AG/AA	3.954	1.201–13.022	0.024	7.729	1.414–42.250	0.018

EGFR: epithelial growth factor receptor; HR: hazard ratio; CI: confidence interval; BAC: bronchioloalveolar carcinoma; TKI: tyrosine kinase inhibitor; *PAI-1*: plasminogen activator inhibitor type 1.

Univariate analysis showed that TNM stage (III–IV), recurrence and *PAI-1* A15T AG/AA genotype were significant predictors of poor short-term survival in patients with pulmonary adenocarcinoma harboring mutant-type *EGFR* (p=0.046, p=0.045 and p=0.024, respectively) (Table 4). To the multivariate analysis, the following variables were entered: age, smoking history, BAC component, TNM stage, recurrence, EGFR TKI use after recurrence and *PAI-1* A15T genotype. Multivariate analysis also showed same results that TNM stage (III–IV), recurrence and *PAI-1* A15T AG/AA genotype were significant predictors of poor short-term survival (HR, 6.284; 95% CI, 1.149–34.354; p=0.034; HR, 6.388; 95% CI, 1.334–30.592; p=0.020; and HR, 7.729; 95% CI, 1.414–42.250; p=0.018, respectively) (Table 4).

Discussion

To the best of our knowledge, this study is the first trial about the prognostic role of *PAI-1* A15T gene polymorphic variants in patients with pulmonary adenocarcinoma harboring *EGFR* mutation. Although there have been many researches about the relationship between PAI-1 level in tumor extracts and prognosis of NSCLC patients, the prognostic role of polymorphic variants within *PAI-1* gene in NSCLC has been rarely studied. It is also meaningful in the way that we found out the association between the prognostic impact of *PAI-1* A15T gene polymorphic variants and activating mutation in EGFR kinase domain.

In this study, although *PAI-1* A15T gene polymorphic variants were not significantly correlated with overall survival and the clinical characteristics of patients with pulmonary adenocarcinomas, the AG/AA genotype carriers showed a poor 3-year survival in patients with pulmonary adenocarcinoma harboring mutant-type *EGFR*.

Our results were partially inconsistent with findings of previous study indicating that *PAI-1* A15T gene polymorphic variants were independent predictors of overall survival in patients with NSCLC[12]. In the previous study conducted in the UK[12], median survival time in the NSCLC patients with the AG/AA genotype was significantly shorter than the NSCLC patients with the GG genotype (median survival time, 8.4 months vs. 10.9 months, respectively; p=0.005). Many reasons can account for this discrepancy. First, all of the subjects enrolled in this study were Koreans. Racial difference between two studies could explain the discordant result. Second, the number of patients enrolled in our study was comparatively too small to reveal the differences of overall survival between different genotype groups. The previous study in the UK included 522 patients with NSCLC, but in our study, only 171 patients diagnosed with pulmonary adenocarcinoma and underwent *EGFR* mutation analysis. Third, patients with pulmonary adenocarcinoma with early stage accounted for approximately one-half of all (stage I–II: 82/144, 48%). Therefore, the influence of *PAI-1* gene polymorphic variants on patients with advanced NSCLC would be weekly reflected. Fourth, because only patients with pulmonary adenocarcinoma were selected, the association between *PAI-1* A15T gene polymorphic variants and overall survival of patients with various histologic types were not fully investigated in our study. These are the explanations why our study did not show a significant association between *PAI-1* A15T gene polymorphism and overall survival in our patients.

Nevertheless, the AG/AA genotype carriers showed a poor 3-year survival in patients with pulmonary adenocarcinoma mutant-type *EGFR*. This suggests that *PAI-1* A15T genetic polymorphisms would be able to discriminate the subgroups with poor prognosis from those with better prognosis among patients with pulmonary adenocarcinoma harboring mutant-type *EGFR*. These can be plausible in that way that genetic polymorphisms influence on individual differences and the diverse outcomes of patients under the same clinical conditions.

Contrarily, *PAI-1* A15T polymorphism was not significantly associated with the 3-year survival in patients with pulmonary adenocarcinoma harboring wild-type *EGFR*. Previous reports identified that the PAI-1 expression is induced by TGF-β1 via EGFR signaling pathway in human cancers[18]. But, because the impact of activating mutation in *EGFR* kinase domain on the PAI-1 expression has not been explored, there is any room for further research.

PAI-1, the serine protease inhibitors, inhibits the activity of uPA by catalyzing convert of plasminogen into plasmin which promotes tumor invasion and metastasis by degradation of extracellular matrix[19]. Considering this inhibitory role of PAI-1 in tumor invasion and metastasis, it is expected that higher PAI-1 level would be correlated with favorable survival and prognosis of patients with malignancy. But, contrary to the expectations, many studies about the various cancer types such as breast, ovarian, gastric, colorectal, head and neck cancer revealed that higher PAI-1 level in tumor extracts was correlated with poor survival and prognosis[20-24]. On the basis of these findings, it is thought that PAI-1 may be multifunctional protease inhibitor, thereby promoting cancer invasion and progression[25-27]. Until recently, many studies about the prognostic role of PAI-1 in NSCLC have been published. But, the paradoxical role of PAI-1 is not consistently observed in the study of NSCLC patients. Pedersen et al.[11] indentified that higher PAI-1 levels in tumor extracts were significantly associated with shorter survival in patients with pulmonary adenocarcinoma. On the contrary to this, the authors did not find the association in patients with squamous cell carcinoma and large cell carcinoma[28]. Whether the expression levels of PA, PAR, PAI-1 and PAI-2 in tumors could influence on the prognosis of patients with resected NSCLCs were investigated by Salden et al[29]. They did not confirm the association between the expression levels of PA system and prognosis of those patients. Recently, Offersen et al.[30] investigated the association between uPA and PAI-1 in tumor extracts and the prognosis of patients with NSCLC and angiogenic parameters. They suggested the possibility that uPA and PAI-1 could influence on enhancing angiogenesis of NSCLC. Though monoclonal anti-PAI-1 antibodies were developed to neutralize PAI-1, there is a lack of sufficient evidence about the role of PAI-1 in NSCLC progression[31]. More studies are necessary to define the prognostic impact of PAI-1 especially on pulmonary adenocarcinoma or NSCLC and to use the PAI-1 level for decision making for NSCLC patients.

In our study *PAI-1* A15T genotype frequencies were similar with those reported in the other studies[12,32-34]. One study embracing the subjects with different races reported that the combined frequency of AG and AA genotypes was significantly higher in Whites than in Blacks, but not significantly higher than in Hispanics (30.9%, 12.5% and, 20% respectively)[33]. To compare our study with the others, the combined frequency of AG and AA genotypes of our study stands between that in Hispanics and that in Blacks.

SNP of *PAI-1* A15T results in a G to A transition within coding sequences, thereby changing the encoded protein[32,35]. This subtle change is expected to alter the function of PAI-1, though it is not confirmed yet. Moreover, we cannot exclude the possibility that the function of *PAI-1* A15T polymorphism is attributed to linkage disequilibrium with 4G/5G insertion/deletion functional polymorphism which is associated with the higher PAI-1 level[35]. Thus, further study is necessary to demonstrate the direct effect of *PAI-1* A15T polymorphism on the PAI-1 level and function.

Our study has many limitations. As mentioned to earlier, first is the relatively small number of enrolled patients. Second, this study was a single center study conducted in South Korea. Multicenter based more large scale study is needed to confirm the prognostic role of *PAI-1* A15T polymorphism in patients with pulmonary adenocarcinoma. Third, treatment modalities after tumor recurrence or disease progression were not unified. Largely, patients in this study were treated with doublet chemotherapy based on platinum. But, this point might function as compounding factor of our study. Fourth, it is thought that follow-up duration time was relatively short. For the analysis of overall survival, there is the need for long-term follow-up system. Fifth, we explored the role of only one SNP in the *PAI-1* gene. Given the complexity of phenotype which is not determined by single SNP, multiple genetic variations in *PAI-1* gene should be examined in the future study. Sixth, we could not exclude the possibility that the more frequent use of EGFR TKI after recurrence in group with GG type and mutant-type *EGFR*, though there was no statistically significant difference, might result in more favorable 3-year survival in patients with GG type and mutant-type *EGFR* than AG/GG type and mutant-type *EGFR*.

In summary, we did not find out the significant association between *PAI-1* A15T gene polymorphic variants and overall survival in patients with pulmonary adenocarcinoma. However, the subgroup analyses of 3-year survival for patients with pulmonary adenocarcinoma harboring mutant-type *EGFR* showed that the AG/AA genotype of *PAI-1* A15T polymorphism would be a significant predictor of poor short-term survival. But, judging from the fact that our study contained many limitations, we should be more cautious to accept the prognostic role of *PAI-1* A15T polymorphism in patients with pulmonary adenocarcinoma.

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