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. 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. 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.

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. 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. 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 adenocarcinoma in other study. 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. 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.

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’-TCCCTGTTGTCCCGTGTC-3’. PAI-1 A15T genetic polymorphisms were genotyped by using minisequencing assay. The sequence of minisequencing primer was 5’-CCTGCCACTGCCCCGGGATAA-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 \( \chi^2 \) 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 IIA 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 IIIB 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

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Table 1. Baseline characteristics

| Clinical characteristic                  | PAI-1 A15T genotype | p-value |
|------------------------------------------|---------------------|---------|
|                                          | All patients (n=171) | GG (n=144) | AG/AA (n=27) |
| 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.
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) | 62 (81.6) | 12 (80.0) |
| Adjuvant chemotherapy*  |  |  | 0.636 |  |  | 0.886 |
| Recurrence†             |  |  | 47 (79.7) | 8 (72.7) | 60 (79.4) | 8 (66.7) |
| EGFR TKI use‡           |  |  | 0.743 |  |  | 0.427 |
| Death                   |  |  | 50 (50.8) | 5 (45.5) | 47 (74.6) | 5 (41.7) |
| Death                   |  |  | 14 (46.7) | 1 (20.0) | 28 (59.6) | 1 (20.0) |

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).
Additionally, 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).

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       | 1.059−24.104 | 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 | 4.053       | 1.913−8.397 | <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).
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=0.046, 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. In the previous study conducted in the UK, 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.

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.
Contrary, 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. But, because of 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. 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. On the basis of these findings, it is thought that PAI-1 may be multifunctional protease inhibitor, thereby promoting cancer invasion and progression. 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. identified 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. 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. They did not confirm the association between the expression levels of PA system and prognosis of those patients. Recently, Offersen et al. 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. 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. 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). 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. 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. 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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