# Clinical implication of aminoacyl-tRNA synthetase interacting multi-functional protein 2–exon 2 deletion variant in lung cancer

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# Clinical implication of aminoacyl-tRNA synthetase interacting multi-functional protein 2–exon 2 deletion variant in lung cancer

Directed by Professor Joon Chang

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**ABSTRACT**

# Clinical implication of aminoacyl-tRNA synthetase interacting multi-functional protein 2–exon 2 deletion variant in lung cancer

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**Backgrounds:** Aminoacyl-tRNA synthetase interacting multi-functional protein 2 (AIMP2) promotes cell death through activation of p53 and TNF-α signaling. Its alternatively spliced form lacking exon 2 (AIMP2-DX2) competes with AIMP2 for binding to target proteins, compromising the tumor suppressive activity of AIMP2. In this study, the clinical implication and mechanism of AIMP2-DX2 overexpression was investigated in lung cancer.

**Methods:** AIMP2-DX2 expression was evaluated by immunohistochemistry (IHC) and immunoblotting in lung tissues from lung cancer mouse models and surgical specimens from lung cancer patients. Autoantibodies against AIMP2-DX2 and AIMP2 were measured in the blood from lung cancer patients and controls by enzyme-linked immunosorbent assay.

**Results:** AIMP2-DX2 was overexpressed in lung cancer tissues more often than in non-neoplastic lung tissues from mice and humans. AIMP2-DX2 overexpression in lung cancer tissues was not related with mTORC1 activity. Among various types of aminoacyl-tRNA synthetases and anti-apoptotic molecules, AIMP2-DX2 was positively correlated with Bcl-xL expression. Transfecting A549 and H460 cells with a dominant-negative mutant of signal transducer and activator of transcription 3 (STAT3) decreased the expression of serine/arginine-rich splicing factor 1 (SRSF1), AIMP2-DX2, and Bcl-xL. Autoantibodies against AIMP2-DX2 and AIMP2 were detectable in the serum of lung cancer patients and controls. A high AIMP2-DX2/AIMP2 autoantibody ratio was an independent significant prognostic factor for poor clinical outcome in lung cancer patients (hazard ratio = 1.83, 95% confidence intervals 1.11 – 3.01).

**Conclusions:** These findings suggest that AIMP2-DX2 is a useful biomarker for lung cancer diagnosis and prediction of prognosis. AIMP2-DX2 warrants further study for development as a target molecule in lung cancer.

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**Key words:** aminoacyl-tRNA synthetase, aminoacyl-tRNA synthetase interacting multi-functional protein 2, aminoacyl-tRNA synthetase interacting multi-functional protein 2-exon 2 deletion, lung cancer

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## I. INTRODUCTION

Lung cancer is the second most common malignancy, with approximately 242,550 new cases diagnosed in the US in 2014.[1] Moreover, lung cancer accounts for more than one quarter of all cancer deaths, making it the most common cause of cancer-related death.[1] In Korea, lung cancer was the fourth most common cancer overall with 22,118 new cases in 2011, and was most common cancer in male aged 65 years or older.[2] Lung cancer also showed the highest mortality rate in Korea among all cancers.[2]

Despite efforts toward targeted drug development and biomarker discovery, lung cancer has poor prognosis. Understanding the biology and developing new

therapeutic strategies based on specific subtypes of lung cancer is needed to improve clinical outcomes.

Aminoacyl-tRNA synthetase interacting multi-functional proteins (AIMPs) are scaffolding proteins for the assembly of the macromolecular tRNA synthetase complex. Among the 3 types of AIMPs, AIMP2 (also known as p38 and JTV1) possesses anti-proliferative and cell death-promoting activity through multiple pathways such as tumor-derived growth factor (TGF)-β, p53, tumor necrosis factor (TNF)-α, and Wnt signaling.[3-9] Mice lacking AIMP2 die neonatally due to respiratory failure resulting from uncontrolled proliferation of lung epithelial cells. AIMP2 heterozygous mouse (with reduced expression of AIMP2) show a higher susceptibility to tumorigenesis.[6] These findings indicate that AIMP2 is a haploinsufficient tumor suppressor with a unique working mechanism.[3]

A variant of AIMP2 in which exon 2 is deleted by alternative splicing, AIMP2-DX2, has been detected in cancer cell lines and tissues.[10] AIMP2-DX2 competes with AIMP2 for binding to the target proteins (namely far upstream element binding protein [FBP], TNF receptor-associated factor 2 [TRAF2], and p53), consequently inhibiting the tumor suppressive activity of AIMP2. Increased ratio of AIMP2-DX2 versus AIMP2 showed positive correlation to poor clinical outcome of lung cancer, as well as chemoresistance of ovarian cancer.[3,10] The synthetic compound, BC-DXI01 suppressed the AIMP2-DX2 mRNA transcript, leading to the inhibition of the AIMP2-DX2 activity and tumor suppression.[11] Taken together, these data suggest that AIMP2-DX2 is an attractive candidate for cancer diagnosis

and therapy.[11]

In this study, AIMP2-DX2 expression was examined in lung cancer using K-ras and K-ras:$p53^{fl/fl}$ mouse lung cancer models and human lung cancer tissues to evaluate its clinical implications. To explore signaling pathways and mechanisms related to AIMP2-DX2 expression, we evaluated the relationship between AIMP2-DX2 expression and components of mTOR pathway and Bcl-xL, which play a critical role in cancer growth and proliferation. Moreover, the presence of autoantibodies against AIMP2-DX2 and AIMP2 in the blood was investigated by enzyme-linked immunosorbent assay (ELISA). We sought to find the diagnostic and prognostic usefulness of these autoantibodies in lung cancer patients.

## II. MATERIALS AND METHODS

### *1. Tissue samples*

Mouse tissues were obtained from wild type C57BL/6, LSL K-ras G12D, and LSL K-ras G12D:$p53^{fl/fl}$ mice. For mouse lung cancer tissues, LSL K-ras G12D and LSL K-ras G12D:$p53^{fl/fl}$ mice inhaled $5 \times 10^7$ PFU AdCre particles at 8 weeks of age and were sacrificed 8 and 24 weeks after inhalation, respectively (http://mouse.ncifcrf.gov/). This animal study was approved by our Institutional Animal Care and Use Committee (2014-0229-1), following the guidelines of the American Association for the Assessment and Accreditation of Laboratory Animal Care. A total 275 human lung cancer tissues (formalin fixation and paraffin embedding (FFPE)), and 12 pairs of lung cancer tissue and adjacent normal lung tissue lysates were obtained from tissue archives of affiliated hospitals of Yonsei University College of Medicine. The use of clinical samples was approved by the Institutional Review Boards of Severance Hospital (#4-2013-0556) and Gangnam Severance Hospital (#3-2014-0838).

### *2. Serum samples*

The serum samples from lung cancer patients and control subjects were obtained from those who had presented to Severance Hospital and Gangnam Severance Hospital between September 2011 and January 2015. Lung cancer cases were confirmed pathologically and did not present with any other types of cancer. Control cases were 1:1 age-, gender- and smoking status-matched and did not present with

any other types of cancer. To exploit their clinical implication, a total 165 samples from lung cancer patients were recruited. Informed consent was obtained from all study subjects, and all protocols were approved by the above-mentioned review boards. The study was carried out in accordance with the Declaration of Helsinki and Korean GCP guidelines.

***3. Cell lines, chemicals, and antibodies***

A549 and H1703 cells were purchased from the ATCC (Manassas, VA, USA). H460, H358, H596, H1299, H1650, H2009, and HCC2279 cells were obtained from the Korean Cell Line Bank (Seoul, Korea). Anti-AIMP2-DX2, anti-methionyl-tRNA synthetase (MRS), and anti-leucyl-tRNA synthetase (LRS) antibodies were purchased from Neomix Inc (Suwon, Gyeonggi-do, Korea) and other antibodies were obtained from Cell Signaling Technology (Danvers, MA, USA).

***4. Immunohistochemistry (IHC)***

Expression of AIMP2-DX2 in mouse lung tissues and human lung cancer tissues was analyzed by IHC using the LABS®2 System (Dako, Carpinteria, CA, USA) according to the manufacturer's protocol. Briefly, sections were deparaffinized, rehydrated, immersed in $H_2O_2$ methanol solution, and then incubated overnight with primary antibodies against AIMP2-DX2 obtained by rabbit immunization (Neomix Inc, Korea) at a 1:2,000 dilution. Sections were incubated for 10 min with biotinylated linker and processed using avidin/biotin IHC techniques. 3,3′-diaminobenzidine (DAB) was used as a chromogen in conjunction with the Liquid

DAB Substrate kit (Novocastra, UK). Expression of AIMP2-DX2 was evaluated using a scoring system that takes into account the product of staining intensity and percentage of positive cells. Staining intensity was classified as 0, 1, 2, or 3 and frequency was classified as 0 (<10%), 1 (10–50%), 2 (51–80%), or 3 (>80%). The cases were classified into 2 groups according to the AIMP2-DX2 expression in the cytoplasm and nucleus. Cases with cytoplasmic AIMP2-DX2 expression equal to or greater than 4 were defined as having high expression; those with 3 or less were defined as having low expression. Cases with nuclear AIMP2-DX2 expression equal to or greater than 1 were defined as having high expression; those with 0 were defined as having low expression.

***5. Immunoblotting***

Tissues were harvested using 2×LSB lysis buffer containing protease and phosphatase inhibitors (GenDepot, Korea) on ice. After homogenization and sonication, 30–50 mg of lysates was separated by gel electrophoresis on 7.5–12% polyacrylamide gels and transferred onto nitrocellulose membranes (Bio-Rad Laboratories, Inc., Richmond, CA, USA). The expression level of each protein was measured using Image J (http://rsbweb.nih.gov/ij/) and quantified relative to that of β-actin.

***6. Measurement of serum autoantibodies against AIMP2 and AIMP2-DX2***

The 96–well plates were coated with either His-tagged AIMP2-DX2 or His-tagged

AIMP2 protein (Neomix Inc, Korea) overnight at 4°C, and then were blocked with 1% bovine serum albumin for 1 hour at room temperature. After adding 1:500 diluted serum, 1:10,000 diluted anti-human immunoglobulin G was added and incubated for another 1 hour at room temperature. The enzyme activity was visualized by adding 3,3′,5,5′-tetramethylbenzidine substrate (TMB; Thermo Fisher Scientific) for 10 minutes and the reaction was stopped by 2N $H_2SO_4$. The absorbance was measured at 450 nm. The intra-assay variation of AIMP2-DX2 and AIMP2 were 8.7 % (6.8–11.5 %) and 6.3 % (1.6–10.4 %), respectively. The inter-assay (plate-to-plate) variation was 15.9% (13.7%–17.9%) and 12.2% (8.9%–16.1%), respectively. Lung cancer patients were classified into 2 groups, high and low, on the basis of the median value of AIMP2-DX2, AIMP2, and AIMP2-DX2/AIMP2 ratio.

***7. Statistics.***

Significant differences in clinical characteristics according to AIMP2-DX2 expression determined by IHC in lung tissues, and AIMP2-DX2, AIMP2, and AIMP2-DX2/AIMP2 autoantibodies ratio were analyzed using the $\chi^2$-test, Fisher's exact test, and independent 2-sample *t*-test. Predictive factors for overall survival (OS) and progression/recurrence-free survival (PFS) were calculated using the Kaplan-Meier method and Cox proportional hazards model. Diagnostic usefulness was evaluated by receiver operative characteristic (ROC) curve generation. All tests for significance were 2-tailed, and *P*-values less

than 0.05 were interpreted as statistically significant. All analyses were performed using the SPSS version 23 (SPSS Inc., Chicago, IL, USA).

## III. RESULTS

### 1. AIMP2-DX2 in lung cancer

#### *가. AIMP2-DX2 is frequently and specifically overexpressed in lung cancer compared to non-neoplastic lung tissue in mouse and human.*

To explore AIMP2-DX2 as a biomarker of lung cancer, its expression was evaluated in non-neoplastic lung tissue from 8-week-old wild type C57BL/6 mice (Fig. 1A). The majority of the cells in alveolar structure did not express AIMP2-DX2 while bronchial epithelial cells showed weak expression.

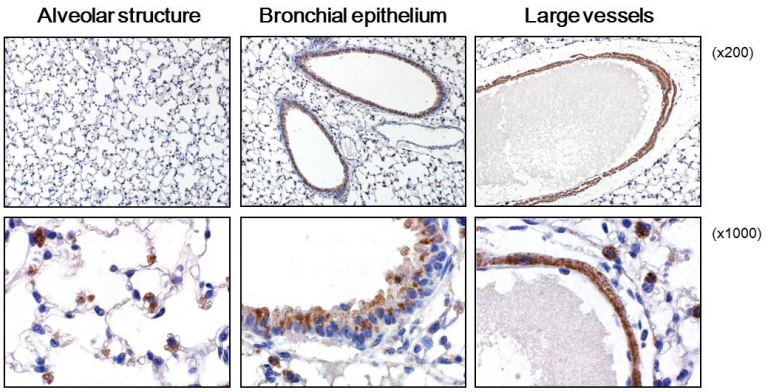



Figure 1A. Weak AIMP2-DX2 expression in non-neoplastic mouse lung tissue as determined by immunohistochemistry.

AIMP2-DX2 expression was also evaluated in normal tissues adjacent to lung cancer (Fig. 1B). AIMP2-DX2 was not expressed in type I or type II pneumocytes

that comprise alveolar structures, and it was weakly expressed in the epithelial cells of bronchioles and bronchi.

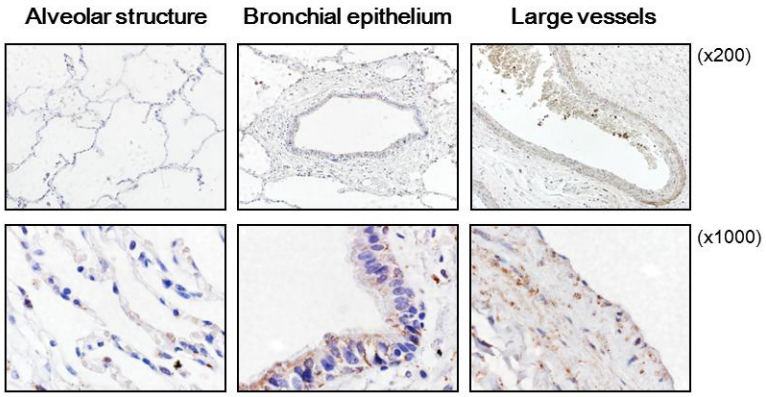



Figure 1B. Weak AIMP2-DX2 expression in normal adjacent human lung tissue as determined by immunohistochemistry.

To evaluate if AIMP2-DX2 expression was specific to lung cancer, its expression was evaluated in lung cancer tissues. Lung cancer-specific AIMP2-DX2 overexpression was observed in LSL K-ras G12D and LSL K-ras G12D:$p53^{fl/fl}$ murine lung cancer models induced by AdCre inhalation (Fig. 1C). Lung cancer specific AIMP2-DX2 overexpression was further evaluated by immunoblotting of lung tissue lysates from wild type mouse and lung cancer model mouse (Fig. 1D). Stronger AIMP2-DX2 expression was observed in mouse lung cancer tissues than in wild type normal lung tissue.

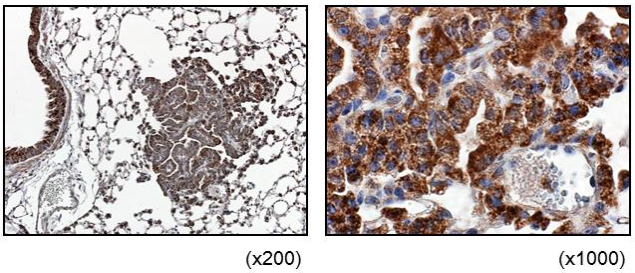



Figure 1C. AIMP2-DX2 expression in LSL K-ras G12D:p53$^{fl/fl}$ murine lung cancer tissue.

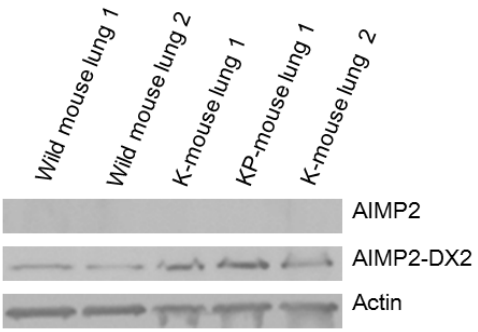



Figure 1D. AIMP2-DX2 expression in mouse lung cancer tissues and wild type normal lung tissues by immunoblotting.

Lung cancer-specific AIMP2-DX2 overexpression was further evaluated by immunoblotting of lysates from human lung cancer tissues and adjacent normal lung tissues. The baseline characteristics of 12 patients are shown in Table 1. Among the 12 pairs of cancer and normal tissue lysates, 7 pairs showed overexpression of AIMP2-DX2 in lung cancer tissue compared to adjacent normal lung tissue (Fig. 1E).

Table 1. Baseline characteristics of 12 lung cancer patients

| No. | Gender | Age | cell type | Differentiation | smoking | pack-year | TNM | Stage | EGFR mutation | KRAS mutation | CEA (ng/mL) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 76 | Adeno | - | never | 0 | T3N0M0 | IIB | 0 | 0 | 51.72 |
| 2 | F | 48 | Adeno | acinar type | never | 0 | T1aN2M0 | IIIA | 1 | 0 | 2.19 |
| 3 | M | 75 | Adeno | solid predominant | former | 30 | T2aN0M0 | IB | 0 | 0 | 5.49 |
| 4 | F | 75 | Adeno | papillary predominant | never | 0 | T1bN0M0 | IA | 0 | 0 | 1.95 |
| 5 | M | 67 | Squamous | - | never | 0 | T2aN0M0 | IB | - | - | 3.41 |
| 6 | M | 68 | Squamous | moderate | current | 55 | T2aN1M0 | IIA | - | - | 9.24 |
| 7 | M | 65 | Squamous | poor | former | 40 | T2aN0M0 | IB | - | - | 4.09 |
| 8 | M | 62 | Adeno | micropapillary predominant pattern | former | 48 | T2aN1M0 | IIA | 1 | 0 | - |
| 9 | M | 59 | Adeno | micropapillary predominant with extracellular mucin formation | current | 39 | T2aN2M0 | IIIA | 1 | 0 | 4.33 |
| 10 | M | 71 | Adeno | acinar predominant | current | 92 | T1bN2M0 | IIIA | 0 | 0 | 3.18 |
| 11 | M | 66 | Squamous | moderate | former | 25 | T2bN2M0 | IIIA | - | - | 2.38 |
| 12 | F | 74 | Adeno | acinar predominant | never | 0 | T2aN0M0 | IB | 1 | 0 | 3.08 |

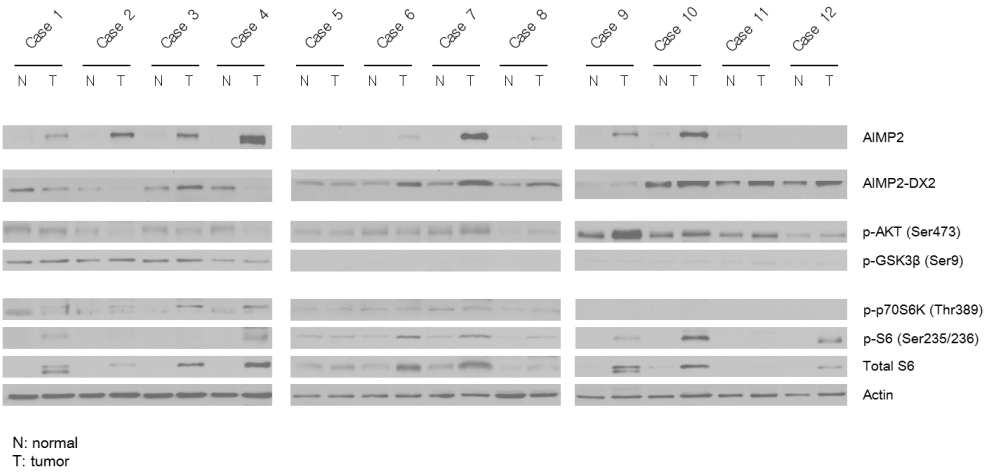



Figure 1E. AIMP2-DX2 overexpression was observed in lung cancer tissue of patients 3, 6, 7, 8, 10, 11, and 12, and was related with mTORC1 activity (p-p70S6K, p-S6, and total S6)(N: normal, T: tumor).

To determine whether other proteins were associated with AIMP2-DX2 overexpression, signaling molecules related to the mammalian target of rapamycin (mTOR) pathway were evaluated by immunoblotting human lung cancer and adjacent normal lung tissue lysates from the patients described in Table 1. mTOR serves as a central regulator of cell metabolism, growth, proliferation and survival, which is also a pivotal role of AIMP2.[6,12] AIMP2-DX2 level was related to mTORC1 activity (p-p70S6K, p-S6, and total S6) more than it was related to mTORC2 activity (Fig. 1E). Taken together, AIMP2-DX2 expression was frequently detected and highly specific to lung cancer, suggesting it can be a candidate for lung cancer diagnosis or therapy.

### 나. *Overexpression of AIMP2-DX2 in lung cancer is related with Bcl-xL.*

To investigate the relationship of AIMP2-DX2 with different types of aminoacyl-tRNA synthetases and anti-apoptotic molecules, the correlation of AIMP2-DX2 expression with MRS, LRS, p-S6, Bcl-xL, KRAS, and HSP70 was determined by immunohistochemistry (Fig. 2A). A significant positive correlation was observed between AIMP2-DX2 and Bcl-xL (r=0.196, $P = 0.011$)(Table 2).

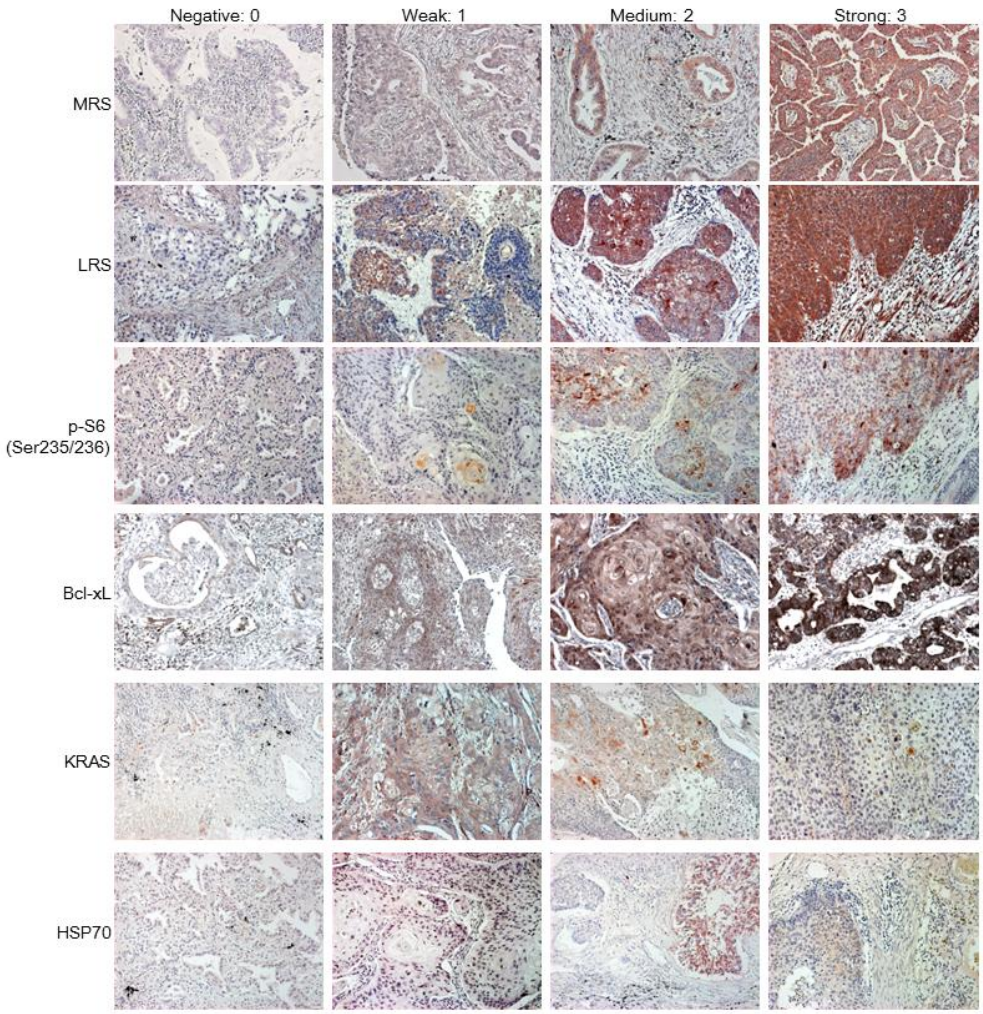



Figure 2A. Expression of MRS, LRS, p-S6, Bcl-xL, KRAS, and HSP70 in human lung cancer tissue as determined by immunohistochemistry.

Table 2. Correlation between AIMP2-DX2 expression and expression of related molecules

| Related molecules | No. of cases | Pearson correlation coefficient | *P*-value |
|---|---|---|---|
| MRS | 172 | 0.028 | 0.661 |
| LRS | 117 | 0.053 | 0.503 |
| p-S6 (Ser235/236) | 114 | -0.016 | 0.839 |
| Bcl-xL | 114 | 0.196 | 0.011 |
| KRAS | 120 | 0.033 | 0.673 |
| HSP70 | 118 | -0.143 | 0.065 |

### *다. DN-STAT3 inhibits AIMP2-DX2 expression by down-regulating SRSF1.*

A previous report conducted serine/arginine-rich (SR) protein-binding motif analysis in exon 2 with the exonic splicing enhancer (ESE) finder program ver. 3.0 to identify splicing factors involved in the deletion of AIMP2 exon 2.[3] Analysis found that ESEs for serine/arginine-rich splicing factor 1 (SRSF1) were located in mutated sites of exon 2.[3] SRSF1 is a member of the SR protein family, which is involved in constitutive and alternative splicing.[13] SRSF1 expression is up-regulated in several human neoplasms, including lung cancer, and participates in the establishment and maintenance of a transformed phenotype.[14] SRSF1 expression was evaluated in relation to AIMP2-DX2 by immunoblotting of human lung cancer and adjacent normal lung tissue lysates from the 12 lung cancer patients described in Table 1. Among the 7 patients exhibiting SRSF1 overexpression, 5 patients also showed AIMP2-DX2 overexpression (Fig. 3A).

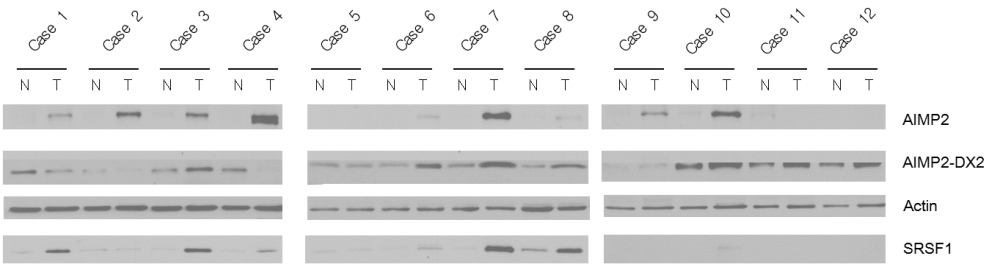



Figure 3A. Among 7 patients with SRSF1 overexpression, 5 patients (patient 3, 6, 7, 8, and 10) also showed AIMP2-DX2 overexpression as determined by immunoblotting.

To further explore the relationship between SRSF1 and AIMP2-DX2, the same immunoblotting strategy was applied to 9 lung cancer cell lines. However, these *in vitro* experiments showed that SRSF1 expression was not closely related with AIMP2-DX2 expression, because only 4 lung cancer cell lines (A549, H460, H1299, and H2009) showed simultaneous overexpression of SRSF1 and AIMP2-DX2 (Fig. 3B).

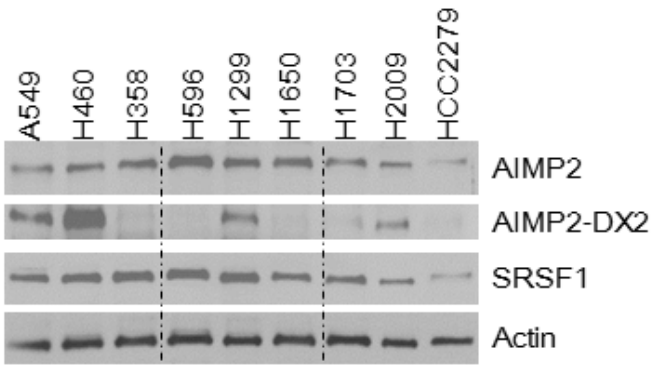



Figure 3B. Expression of SRSF1 and AIMP2-DX2 in various lung cancer cell lines as determined by immunoblotting.

To evaluate the functional significance of SRSF1 on AIMP2-DX2 expression, SRSF1 was overexpressed in A540 and H460 cells or was down-regulated with a specific siRNA. Forced expression of SRSF1 using an SRSF1 expression vector did not increase AIMP2-DX2 expression (Fig. 3C). However, SRSF1 knockdown partially decreased AIMP2-DX2 level (Fig. 3D).

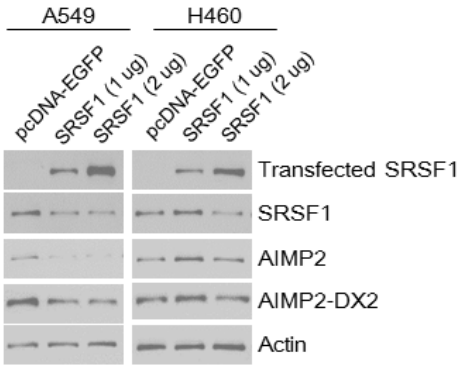



Figure 3C. SRSF1 overexpression in A549 and H460 lung cancer cell lines did not increase AIMP2-DX2 expression.

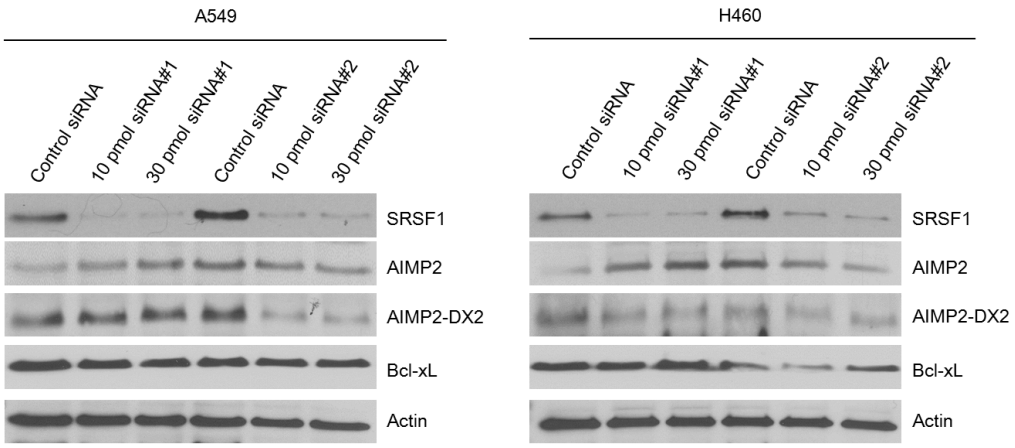



Figure 3D. SRSF1 knockdown partially down-regulated AIMP2-DX2 expression in A549 and H460 lung cancer cell lines.

Since partial down-regulation of AIMP2-DX2 expression was observed upon SRSF1 knockdown, we evaluated whether signal transducer and activator of transcription 3 (STAT3) was an upstream regulator of AIMP2-DX2. According to our analysis of the relationship between AIMP2-DX2 and anti-apoptotic molecules by IHC (Table 2), Bcl-xL expression was significantly correlated with AIMP2-DX2. STAT3, a transcription factor, promotes Bcl-xL expression, which is associated with cell survival and proliferation.[15,16] To further explore the relationship between AIMP2-DX2, SRSF1, and Bcl-xL, STAT3 was inhibited in cells by expressing a dominant negative construct. STAT3 knockdown decreased SRSF1, AIMP2-DX2, and Bcl-xL expression (Fig. 3E). Therefore, STAT3 and SRSF1 are possible regulators of AIMP2-DX2.

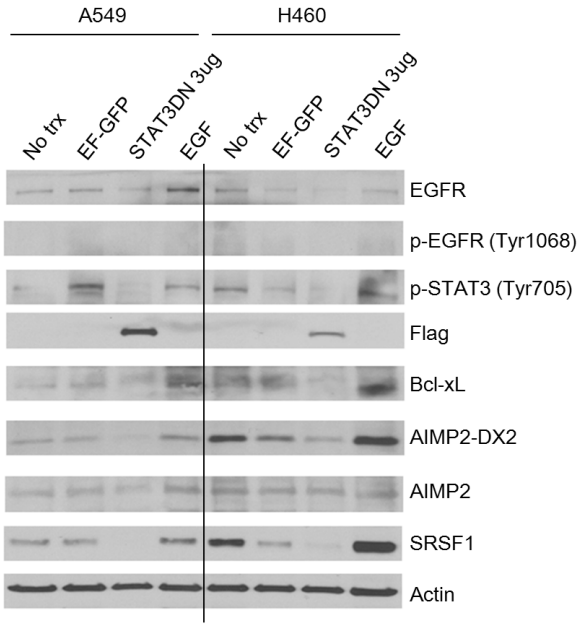



Figure 3E. STAT3 inhibition using a dominant negative construct decreased the expression of SRSF1, AIMP2-DX2, and Bcl-xL.

***라. AIMP2-DX2 expression by immunohistochemistry is not prognostically useful for lung cancer.***

To further determine the clinical implications of AIMP2-DX2 in lung cancer, its expression was assessed by IHC in 275 paraffin-embedded human non-small cell lung cancer (NSCLC) tissues. The baseline characteristics of these 275 patients are shown in Table 3.

Table 3. Baseline characteristics of 275 cancer patients[a]

| Characteristics | n= 275 |
|---|---|
| Age, yr | 61.3 ± 10.3 |
| Male | 206 (74.9) |
| Smoking status (n=256) | |
| Current | 113 (44.1) |
| Former | 69 (27.0) |
| Never | 74 (28.9) |
| Stage | |
| I | 82 (29.8) |
| II | 98 (35.6) |
| III | 91 (33.1) |
| IV | 4 (1.5) |
| Lung cancer cell type | |
| Adenocarcinoma | 134 (48.7) |
| Squamous cell carcinoma | 134 (48.7) |
| Other | 7 (2.5) |

[a] Data are presented as numbers (percentages) for categorical variables. Continuous variables are presented as means ± standard deviations.

AIMP2-DX2 was mainly expressed in the cytoplasm in lung cancer tissues. AIMP2-DX2 expression was graded with regards to intensity and frequency (Fig. 4A). The expression score was obtained by the product of intensity and frequency scores. Distribution of cytoplasmic and nuclear expression scores of AIMP2-DX2

are shown in Table 4. Among the 275 patients, 270 (98.2%) lung cancer patients showed positive AIMP2-DX2 cytoplasmic expression while 91 (33.1%) patients showed positive nuclear expression.

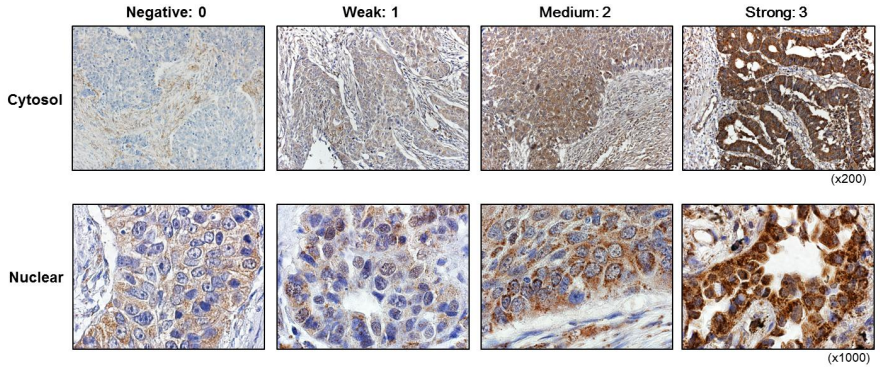



Figure 4A. Representative immunohistochemical images of cytoplasmic and nuclear AIMP2-DX2 expression in lung cancer.

Table 4. Distribution of AIMP2-DX2 expression in cytoplasm and nucleus by immunohistochemistry

| AIMP2-DX2 Expression | Cytoplasm, n (%) | Nucleus, n (%) |
|---|---|---|
| 0 | 5 (1.8) | 184 (66.9) |
| 1 | 0 (0) | 23 (8.5) |
| 2 | 17 (6.2) | 25 (9.1) |
| 3 | 104 (37.8) | 29 (10.5) |
| 4 | 15 (5.5) | 7 (2.5) |
| 6 | 92 (33.5) | 5 (1.8) |
| 9 | 42 (15.2) | 2 (0.7) |

To determine the clinical implications of AIMP2-DX2 expression in lung cancer, the patients were classified into 2 groups, high and low expression, according to the AIMP2-DX2 expression in the cytoplasm and nucleus. Clinical and pathologic

characteristics are compared between the groups in Table 5. Age, sex, smoking status, and tumor size were not different between the groups. However, high cytoplasmic expression (39.6% vs. 28.6%, $P = 0.055$) tended to be more frequently observed and high nuclear expression (42.9% vs. 30.4%, $P = 0.042$) was significantly more frequent in advanced stages (III-IV) than in earlier stages (I-II).

Table 5. Baseline characteristics of high and low AIMP2-DX2 expression groups in the cytoplasm and nucleus[a]

| Characteristics | Cytoplasmic expression | | | Nuclear expression | | |
|---|---|---|---|---|---|---|
| | Low (0-3) n=126 | High (4-9) n=149 | *P*-value | Low (0) n=184 | High (≥1) n=91 | *P*-value |
| Age, yr | 61.9 ± 9.8 | 61.4 ± 10.0 | 0.687 | 61.7 ± 9.7 | 61.6 ± 10.3 | 0.970 |
| Male | 94 (74.6) | 112 (75.2) | 0.914 | 138 (75.0) | 68 (74.7) | 0.961 |
| Ever smokers | 88 (75.2) | 94 (67.6) | 0.213 | 124 (72.5) | 58 (68.2) | 0.477 |
| Size > 3cm | 88 (47.6) | 97 (52.4) | 0.351 | 124 (67.0) | 61 (33.0) | 0.904 |
| Stage | | | | | | |
| I-II | 90 (71.4) | 90 (60.4) | 0.055 | 128 (69.6) | 52 (57.1) | 0.042 |
| III-IV | 36 (28.6) | 59 (39.6) | | 56 (30.4) | 39 (42.9) | |
| Pathology (n=268) | | | | | | |
| Adenocarcinoma | 49 (40.2) | 85 (58.2) | 0.003 | 82 (45.8) | 52 (58.4) | 0.052 |
| Squamous cell ca | 73 (59.8) | 61 (41.8) | | 97 (54.2) | 37 (41.6) | |

[a] Data are presented as numbers (percentages) for categorical variables. Continuous variables are presented as means ± standard deviations.

AIMP2-DX2 expression level was not related with clinical outcome, showing no significant differences in OS or PFS by Kaplan-Meier curves (Fig. 4B).

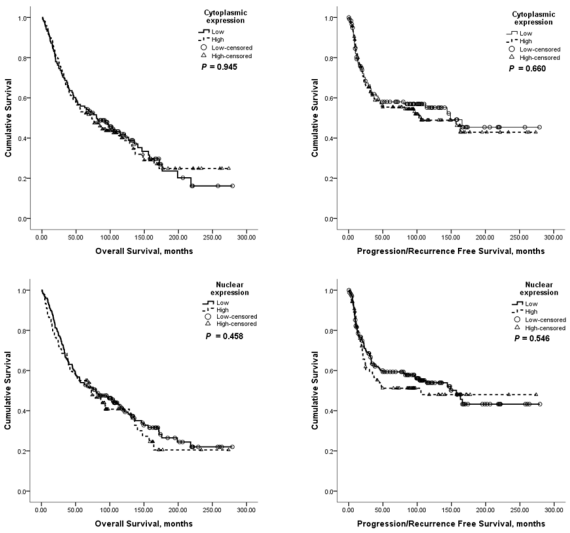



Figure 4B. Kaplan-Meier curves for overall survival and progression/recurrence free-survival in high and low AIMP2-DX2 expression groups in the cytoplasm and nucleus. Expression of AIMP2-DX2 was not related with clinical outcome. *P*-value was obtained by log-rank test.

## 2. Autoantibodies against AIMP2-DX2 and AIMP2

### 가. *Autoantibodies against AIMP2-DX2 and AIMP2 are detected in serum of lung cancer patients.*

To further evaluate the diagnostic validity of AIMP2-DX2, the level of autoantibodies against AIMP2-DX2 and AIMP2 were measured in the serum of controls and lung cancer patients. The baseline characteristics of age, gender, and smoking status were not different between control (n=80) and lung cancer (n=80) groups (Table 6). The distribution of autoantibody levels against AIMP2-DX2 and AIMP2, and AIMP2-DX2/AIMP2 ratio are shown with histograms (Fig. 5A).

Table 6. Baseline characteristics of controls and lung cancer patients [a]

| Baseline characteristics | Control (n=80) | Lung cancer (n=80) | *P*-value |
|---|---|---|---|
| Age | 68.2 ± 12.5 | 68.5 ± 9.3 | 0.869 |
| Gender, male | 63 (78.8) | 61 (76.3) | 0.705 |
| Smoking status, ever-smoker | 59 (73.8) | 59 (73.8) | 1.000 |
| Pack years (including never smokers) | 32.6 ± 28.8 | 35.5 ± 30.5 | 0.549 |

[a] Data are presented as numbers (percentages) for categorical variables. Continuous variables are presented as means ± standard deviations.

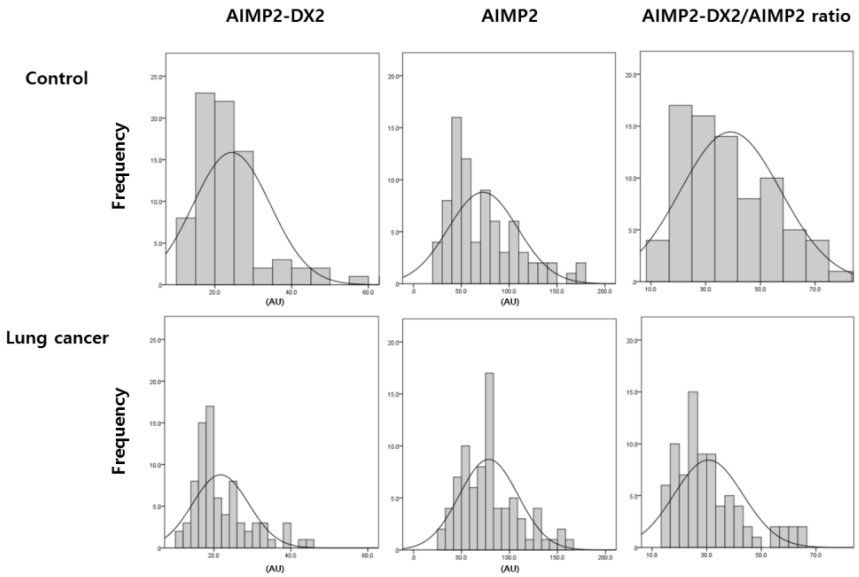
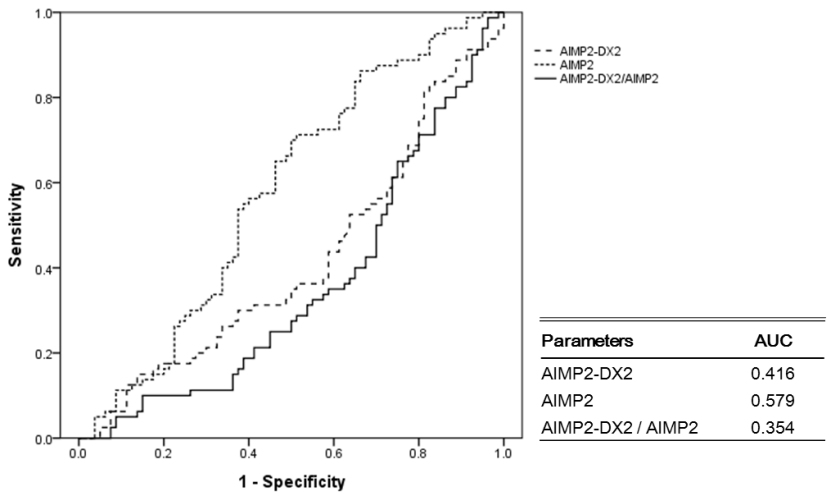



Figure 5A. Histograms for the autoantibodies against AIMP2-DX2 and AIMP2, and AIMP2-DX2/AIMP2 ratio in controls and lung cancer patients.

According to the ROC curves, the AUCs of autoantibodies against AIMP2-DX2 and AIMP2, and AIMP2-DX2/AIMP2 ratio were 0.416, 0.579, and 0.354, respectively, suggesting that these parameters have limited diagnostic value in lung cancer (Fig. 5B).




| Parameters | AUC |
|---|---|
| AIMP2-DX2 | 0.416 |
| AIMP2 | 0.579 |
| AIMP2-DX2 / AIMP2 | 0.354 |

Figure 5B. ROC curve analysis indicating the diagnostic value of autoantibodies against AIMP2-DX2 and AIMP2, and AIMP2-DX2/AIMP2 ratio in lung cancer.

### *나. Higher level of autoantibody against AIMP2-DX2 in patients with lung adenocarcinoma*

Levels of autoantibodies against AIMP2-DX2 and AIMP2, and AIMP2-DX2/AIMP2 ratio were measured and analyzed in 165 lung cancer patients. Their baseline characteristics are demonstrated in Table 7. The mean age was 65 years old and 64.8% were male. About one third of patients were never smokers. According to histologic classification, adenocarcinoma was the most common type (62.4%) and squamous cell carcinoma was second most common (25.5%). The levels of autoantibodies toward AIMP2-DX2 and AIMP2, and AIMP2-DX2/AIMP2 ratio were 21.6 AU, 76.7 AU, and 31.7, respectively. The distributions of these levels are shown using histograms (Fig. 6).

Table 7. Baseline characteristics of 165 lung cancer patients[a]

| Characteristics | n=165 (%) |
|---|---|
| Age, yr | 65.3 ± 9.4 |
| Sex, male | 107 (64.8) |
| Smoking status | |
| Current | 58 (35.2) |
| Former | 50 (30.3) |
| Never | 57 (34.5) |
| Histology | |
| Adenocarcinoma | 103 (62.4) |
| Squamous cell carcinoma | 42 (25.5) |
| Small cell carcinoma | 16 (9.7) |
| Large cell carcinoma | 4 (2.4) |
| Size > 3cm[b] | 23 (18.5) |
| Stage (n=149)[c] | |
| I | 47 (31.5) |
| II | 8 (5.4) |
| III | 36 (24.2) |
| IV | 58 (38.9) |
| Tumor markers | |
| Elevated CEA[d] | 74 (49.3) |
| Elevated CYFRA 21-1[e] | 68 (47.6) |
| Parameters | |
| AIMP2-DX2, AU | 21.6 ± 9.2 |
| AIMP2, AU | 76.7 ± 33.8 |
| AIMP2-DX2/AIMP2 | 31.7 ± 13.8 |
| Mortality (n=163)[f] | 82 (50.3) |

[a] Data are presented as numbers (percentages) for categorical variables. Continuous variables are presented as means ± standard deviations.
[b] n=124
[c] Small cell carcinoma was excluded
[d] n=150, > 5.0 ng/mL
[e] n=143, > 3.3 ng/mL
[f] Survival of two patients are unknown

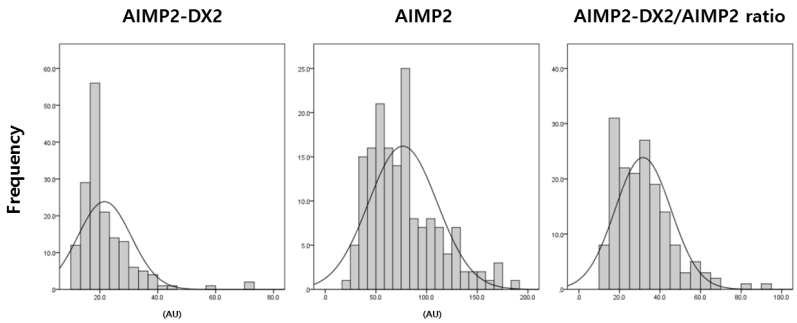



Figure 6. Histograms for levels of autoantibodies against AIMP2-DX2 and AIMP2, and AIMP2-DX2/AIMP2 ratio in lung cancer patients.

To explore the clinical relevance of autoantibodies against AIMP2-DX2, AIMP2, and AIMP2-DX2/AIMP2 ratio in lung cancer, patients were classified into two groups, high and low, based on the median value of AIMP2-DX2 and AIMP2, and AIMP2-DX2/AIMP2 ratio (Table 8). There was no statistical difference between the gender, smoking status, histologic diagnosis, or tumor stages between high and low groups of autoantibodies toward AIMP2-DX2 and AIMP2, and AIMP2-DX2/AIMP2 ratio. However, age (66.8 yr vs. 63.8 yr, $P = 0.040$) and proportion of tumor size larger than 3 cm (26.9% vs. 12.7%, $P = 0.045$) was higher in the high AIMP2-DX2 ratio group compared to the low group. An elevated CYFRA-21 level was more frequently observed in the high AIMP2-DX2/AIMP2 ratio group than in the low ratio group (58.8% vs. 41.2%, $P = 0.037$) while the proportion of patients with elevated CEA level was similar between the groups. Moreover, the mortality rate was higher in the high AIMP2-DX2/AIMP2 ratio group than in the low ratio group (58.5% vs. 42.0%, $P = 0.034$).

Table 8. Comparison between high and low AIMP2-DX2, AIMP2, and AIMP2-DX2/AIMP2 autoantibodies ratio[a]

| Characteristics | AIMP2-DX2 | | | AIMP2 | | | AIMP2-DX2/AIMP2 ratio | | |
|---|---|---|---|---|---|---|---|---|---|
| | Low (n=82) | High (n=83) | *P*-value | Low (n=82) | High (n=83) | *P*-value | Low (n=82) | High (n=83) | *P*-value |
| Age, yr | 63.8 ± 10.1 | 66.8 ± 8.4 | 0.040 | 64.1 ± 9.4 | 66.5 ± 9.3 | 0.113 | 64.7 ± 9.5 | 65.9 ± 9.2 | 0.443 |
| Sex, male | 52 (63.4) | 55 (67.1) | 0.623 | 51 (62.2) | 56 (67.5) | 0.478 | 54 (65.9) | 53 (63.9) | 0.788 |
| Smoking | | | | | | | | | |
| Current | 32 (39.0) | 26 (31.7) | | 27 (32.9) | 31 (37.3) | | 29 (35.4) | 29 (34.9) | |
| Former | 23 (28.0) | 27 (32.9) | 0.603 | 23 (28.0) | 27 (32.5) | 0.484 | 27 (32.9) | 23 (27.7) | 0.686 |
| Never | 27 (32.9) | 29 (35.4) | | 32 (39.0) | 25 (30.1) | | 26 (31.7) | 31 (37.3) | |
| Histology | | | | | | | | | |
| Adenocarcinoma | 55 (67.1) | 48 (57.3) | | 53 (64.6) | 50 (60.2) | | 53 (64.6) | 50 (60.2) | |
| Squamous cell carcinoma | 18 (22.0) | 24 (29.2) | 0.478 | 16 (19.5) | 26 (31.3) | 0.216 | 20 (24.4) | 22 (26.5) | 0.580 |
| Small cell carcinoma | 8 (9.7) | 8 (9.8) | | 10 (12.2) | 6 (7.2) | | 7 (8.5) | 9 (10.8) | |
| Large cell carcinoma | 1 (1.2) | 3 (3.7) | | 3 (3.7) | 1 (1.2) | | 2 (2.4) | 2 (2.4) | |
| Size > 3cm | 9 (12.7) | 14 (26.9) | 0.045 | 12 (18.2) | 11 (19.0) | 0.911 | 13 (20.0) | 10 (16.9) | 0.662 |
| Stage (n=149) | | | | | | | | | |
| I-II | 34 (41.5) | 27 (33.3) | 0.284 | 28 (34.1) | 34 (41.5) | 0.334 | 36 (44.4) | 45 (55.6) | 0.083 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| III-IV | 48 (58.5) | 54 (66.7) | | 54 (65.9) | 48 (58.5) | | 26 (31.3) | 57 (68.7) | |
| Tumor markers | | | | | | | | | |
| Elevated CEA[b] | 42 (56.8) | 32 (43.2) | 0.119 | 40 (54.1) | 34 (45.9) | 0.193 | 35 (47.3) | 39 (52.7) | 0.514 |
| Elevated CYFRA-21[c] | 38 (55.9) | 30 (44.1) | 0.307 | 37 (54.4) | 31 (45.6) | 0.278 | 28 (41.2) | 40 (58.8) | 0.037 |
| Mortality | 37 (45.1) | 45 (56.3) | 0.157 | 42 (51.9) | 40 (48.8) | 0.695 | 34 (42.0) | 48 (58.5) | 0.034 |

[a] Data are presented as numbers (percentages) for categorical variables. Continuous variables are presented as means ± standard deviations.
[b] n=75 for low group, n=75 for high group
[c] n=72 for low group, n=71 for high group

***다. Elevated AIMP2-DX2/AIMP2 autoantibody ratio was related to poor lung cancer outcome.***

To further evaluate the relationship of autoantibodies against AIMP2-DX2 and AIMP2, and AIMP2-DX2/AIMP2 ratio with clinical outcomes, OS and PFS of the two groups were compared using Kaplan-Meier estimators (Fig. 7). There were no significant differences in OS and PFS between high and low AIMP2-DX2 and AIMP2 autoantibody groups. However, high AIMP2-DX2/AIMP2 ratio group showed significantly shorter OS compared to the low ratio group (18.4 months vs. 48.3 months, $P = 0.021$)(Table 9).

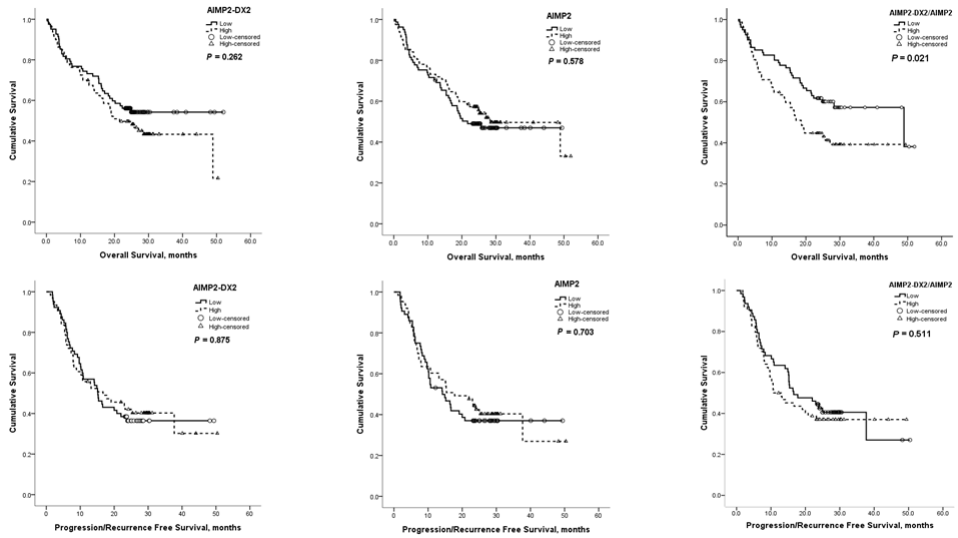



Figure 7. Kaplan-Meier curves for overall survival and progression/recurrence free survival in high and low groups for autoantibodies against AIMP2-DX2 and AIMP2, and AIMP2-DX2/AIMP2 autoantibody ratio. High AIMP2-DX2/AIMP2 ratio was significantly correlated with improved overall survival. *P*-value was obtained by log-rank test.

Table 9. Median overall survival and progression/recurrence free survival in high and low AIMP2-DX2, AIMP2, and AIMP2-DX2/AIMP2 autoantibodies ratio

| Median | AIMP2-DX2 | | AIMP2 | | AIMP2-DX2/AIMP2 ratio | |
|---|---|---|---|---|---|---|
| | Low | High | Low | High | Low | High |
| OS months (95% CI) | - | 21.1 (13.04-29.37) | 21.4 (-) | 27.9 (15.64--40.17) | 48.3 (14.62-81.98) | 18.4 (15.09-21.70) |
| PFS months (95% CI) | 14.9 (10.12-19.68) | 15.0 (6.20-23.79) | 13.9 (8.67-19.13) | 17.5 (8.14-26.86) | 16.2 (9.71-22.69) | 10.7 (6.61-14.79) |

To evaluate the hypothesis that high AIMP2-DX2/AIMP2 autoantibodies ratio is an independent prognostic factor in lung cancer, univariate and multivariate analyses were performed using a Cox regression hazard model (Table 10). Univariate analysis revealed that age, female sex, ever-smoker, advanced stage (III-IV), elevated CEA level, elevated CYFRA-21 level, and high AIMP2-DX2/AIMP2 ratio were significant predictors of poor OS while adenocarcinoma histology was a significant predictor of better OS. Multivariate analysis showed that advanced stages (III–IV)(HR = 6.66, 95% CI 3.20–13.89) and high AIMP2-DX2/AIMP2 ratio (HR = 1.83, 95% CI 1.11–3.01) were significant independent factors for poor OS while adenocarcinoma histology (HR = 0.42, 95% CI 0.23–0.76) was a predictor for better OS.

Table 10. Univariate and multivariate Cox-regression analysis of overall survival

| Characteristics | Univariate Analysis | | | Multivariate Analysis | | |
|---|---|---|---|---|---|---|
| | HR | *P*-value | 95% CI | HR | *P*-value | 95% CI |
| Age, yr | 1.03 | 0.022 | 1.01-1.06 | 1.02 | 0.201 | 0.99-1.05 |
| Sex, female | 0.53 | 0.011 | 0.32-0.86 | 0.76 | 0.655 | 0.22-2.58 |
| Smoking, ever-smoker | 1.71 | 0.029 | 1.06-2.77 | 1.12 | 0.858 | 0.33-3.77 |
| Histology | | | | | | |
| Adenocarcinoma | 0.54 | 0.006 | 0.35-0.84 | 0.42 | 0.004 | 0.23-0.76 |
| Squamous cell carcinoma | 1.51 | 0.084 | 0.95-2.42 | | | |
| Size > 3 cm | 0.56 | 0.180 | 0.24-1.31 | | | |
| Stage, stage III-IV vs. I-II | 6.71 | < 0.001 | 3.45-13.05 | 6.66 | < 0.001 | 3.20-13.89 |
| Tumor markers | | | | | | |
| Elevated CEA | 1.92 | 0.007 | 1.20-3.07 | 1.44 | 0.176 | 0.85-2.42 |
| Elevated CYFRA-21 | 2.98 | < 0.001 | 1.82-4.86 | 1.40 | 0.231 | 0.81-2.41 |
| High level of | | | | | | |
| AIMP2-DX2 | 1.28 | 0.263 | 0.83-1.98 | | | |
| AIMP2 | 0.88 | 0.579 | 0.57-1.37 | | | |
| AIMP2-DX2/AIMP2 | 1.67 | 0.023 | 1.07-2.60 | 1.83 | 0.018 | 1.11-3.01 |

HR: hazard ratio, CI: confidence interval

## IV. DISCUSSION

This study demonstrated a mechanism related with AIMP2-DX2 expression and elucidated the clinical implication of AIMP2-DX2 overexpression in lung cancer. AIMP2-DX2 was specifically overexpressed in human and mouse lung cancer tissues compared to adjacent normal tissues. Expression of AIMP2-DX2 in lung cancer was related with mTORC1 signaling activity and was positively correlated with expression of the anti-apoptotic protein, Bcl-xL. Down-regulation of SRSF1 partially decreased AIMP2-DX2 expression. STAT3 inhibition decreased expression of SRSF1, AIMP2-DX2, and Bcl-xL. IHC analysis showed that AIMP2-DX2 expression in lung cancer tissue was associated with advanced stage and histology subtypes although its validity in relation to clinical outcome was limited. Autoantibodies against AIMP2-DX2 and AIMP2 existed at detectable levels in human blood. Increased level of the ratio between autoantibodies for AIMP2-DX2 and AIMP2 was an independent prognostic factor for poor OS in patients with lung cancer.

AIMP2 plays an important role in controlling cell fate. It demonstrates anti-proliferative activity by enhancing growth-arresting TGF-β signaling.[4] AIMP2 also promotes cell death through activating p53 and the apoptotic signaling of TNF-α.[5,6] AIMP2-DX2 competes with AIMP2 for binding to FBP, TRAF2, and p53, consequently interrupting the tumor-suppressive roles of AIMP2.[3,10]

To explore the molecules related with the AIMP2-DX2 overexpression, signaling molecules involved in the mTOR pathway were evaluated. AIMP2-DX2 expression was related to the level of mTORC1, p-p70S6K, and p-S6. Elevated p70S6K has been found in many tumor cells and is related with increased tumor size, whereas inhibition of p70S6K prevents cell proliferation.[17] shDX2 suppressed mTOR phosphorylation at p70S6K, regulating cancer growth and proliferation.[8]

STAT3 is a transcription factor activated by Janus kinase 1 (JAK1) and JAK2. After translocation from the cytoplasm to the nucleus, nuclear STAT3 transactivates numerous genes that sustain cellular survival and proliferation by promoting the expression of anti-apoptotic molecules including Bcl-xL.[15,16] Down-regulation of STAT3 decreased SRSF1, AIMP2-DX2, and Bcl-xL expression. AIMP2-DX2 expression may be induced by STAT3 directly or by SRSF1 through STAT3, because SRSF1 knockdown partially decreased AIMP2-DX2 expression.

Cytoplasmic-nuclear shuttling of AIMP2-DX2 is expected to scarcely occur because the AIMP2-DX2 sequence does not contain a nuclear localization sequence. In human lung cancer tissues, AIMP2-DX2 was expressed strongly in the cytoplasm of 98% of the patients but weakly expressed in the nucleus of a few patients.

Although autoantibodies against AIMP2-DX2 were detectable in the serum, whether AIMP2-DX2 or AIMP2 possesses stronger properties as a secretary protein has not been defined yet. Investigating the structure of AIMP2-DX2 and AIMP2 would be helpful to explore the structural differences between AIMP2-DX2 and AIMP2. Then, hydrophilicity and hydrophobicity of those could be predicted.

Previous studies demonstrated the essential role of AIMP2-DX2 in tumorigenesis, but only few studies have evaluated the possible role of AIMP2-DX2 as a biomarker for diagnosis, treatment, and prognosis in lung cancer. Besides lung cancer, AIMP2-DX2 was investigated whether it can be an effective target for overcoming chemoresistance in ovarian cancer.[10] It was found that the direct delivery of siRNA against AIMP2-DX2 into abdominal ovarian cancer metastases significantly suppressed the growth rate of tumors in a mouse model of ovarian cancer suggesting that AIMP2-DX2 down-regulation can be a potent adjuvant therapeutic approach for chemoresistant epithelial ovarian cancer through aberrant NF-kB activity.[10]

High AIMP2-DX2 expression in tissues as determined by IHC was related with advanced disease stage and was more frequent in adenocarcinoma than squamous cell carcinoma. Unlike the previous study, this study could not demonstrate the prognostic value of AIMP2-DX2 to predict mortality and disease progression. This discrepancy may be related with different methods of detecting AIMP2-DX2 and AIMP2, and a different definition of high expression.[3]

This study showed that the ratio of autoantibodies against AIMP2-DX2/AIMP2 is a potential prognostic biomarker for lung cancer. The prognostic validity of autoantibody level was investigated in lung cancer patients with evenly distributed cancer stages. Most current lung cancer biomarkers identify gene mutations. KRAS, Epidermal Growth Factor Receptor (EGFR), and anaplastic lymphoma kinase (ALK) are common biomarkers seen in NSCLC.[18] The KRAS gene is mutated in about 25%

of NSCLC patients and EGFR is defective in about 10% of NSCLC patients (nearly 50% of lung cancer patients never smoked) while ALK gene rearrangements occur in up to 5 % of NSCLC patients.[18] The relationship between AIMP2-DX2 and KRAS was evaluated, but AIMP2-DX2 was not correlated with KRAS. Moreover, the group with low AIMP2-DX2 and KRAS expression and the group with high AIMP2-DX2 and KRAS expression showed similar OS and PFS (data not shown). However, these comparisons were only determined by IHC, so further investigation is required with more quantitative and qualitative measurement of biomarkers. For this purpose, methods of directly measuring AIMP2-DX2 and AIMP2 in the blood need to be developed.

## V. CONCLUSION

In conclusion, AIMP2-DX2 expression was specifically overexpressed in lung cancer tissues. Expression of AIMP2-DX2 was related with mTORC1 activity and was correlated with Bcl-xL expression. SRSF1 knockdown partially decreased AIMP2-DX2 level, and STAT3 inhibition down-regulated the expression of SRSF1, AIMP2-DX2 and Bcl-xL. This suggests that STAT3 and SRSF1 regulate AIMP2-DX2 expression. Autoantibodies against AIMP2-DX2 and AIMP2 were detectable in the serum and the ratio of autoantibodies against AIMP2-DX2/AIMP2 was an independent significant prognostic factor for poor clinical outcome in lung cancer patients. Although this study suggests that AIMP2-DX2 is a new possible prognostic biomarker in lung cancer, further studies are necessary to explore AIMP2-DX2 as a biomarker for diagnosis, therapeutic guidance, and prediction of disease recurrence/progression.

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## ABSTRACT (IN KOREAN)

# 폐암에서 aminoacyl-tRNA synthetase complex interacting multi-functional protein 2-exon 2 deletion 이형의 임상적 의의

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**배경:** Aminoacyl-tRNA synthetase interacting multi-functional protein 2 (AIMP2) 는 p53과 TNF-α 신호 전달 경로를 통해 세포사를 유도한다. AIMP2의 RNA 대체 접합 (alternative splicing)으로 exon 2가 결실이 된 AIMP2-exon 2 deletion (AIMP2-DX2)은 표적 단백에 AIMP2와 경쟁적으로 결합하여, AIMP2의 종양 억제 기능을 방해한다. 본 연구에는 폐암에서의 AIMP2-DX2 과발현의 임상적 의미와 기전을 탐구하고자 하였다.

**방법:** AIMP2-DX2 발현을 폐암 쥐 모델과 수술적 치료 후 얻어진 폐암 환자의 조직에서 면역조직화학법과 면역학적 블로팅으로 분석하였다. 폐암 환자와 대조군의 혈청에서 효소결합 면역흡수 분석법을 이용하여, AIMP2-DX2와 AIMP2에 대한 자가 항체 농도를 측정하였다. 분석 및 측정된 결과는 임상적 요소들과 비교 분석하여, 의의를 조사하였다.

**결과:** 폐암 생쥐 모델과 폐암 환자의 폐암과 정상 조직을 이용한 실험에서 AIMP2-DX2는 폐암조직에서의 특이적 과발현이 58%에서 관찰되었다.

폐암 조직에서 AIMP2-DX2의 과발현은 mTORC1 신호 전달 경로의 활성과 뚜렷한 관련이 없었고, 다양한 aminoacyl-tRNA synthetase와 항고사 단백 발현과의 상관관계분석 결과 Bcl-xL 발현과 양의 상관관계를 보였다. 폐암 세포주인 A549와 H460에 signal transducer and activator of transcription 3 (STAT3)의 우성음성돌연변이체 플라스미드 형질주입으로 억제하였을 때, serine/arginine-rich splicing factor 1 (SRSF1), AIMP2-DX2, 그리고 Bcl-xL 발현이 감소하였다. SRSF1 억제는 AIMP2-DX2 발현을 일부 감소시켜, AIMP2-DX2는 STAT3에 의해 발현되며, 일부는 SRSF1에 의해 발현되는 것임을 확인하였다. 폐암 환자와 대조군 혈액에서 AIMP2-DX2와 AIMP2 자가 항체 측정을 통해 검출이 가능함을 확인하였다. 폐암 환자의 혈액에서 AIMP2-DX2/AIMP2 비율이 높은 군은 낮은 군에 비해서 생존율이 낮으며 (18.4 개월 vs. 48.3개월; $P$ = 0.021), AIMP2-DX2/AIMP2의 높은 비율은 폐암환자의 독립적인 예후 인자로 의의가 있었다 (위험비 = 1.83, 95% 신뢰구간 1.11 – 3.01).

**결론:** 본 연구결과는 AIMP2-DX2 면역조직화학적 과발현이 폐암 진단에 유용하고, AIMP2-DX2/AIMP2의 자가 항체 비율 증가가 폐암 예후 예측에 유효한 바이오마커임을 시사한다. 따라서, AIMP2-DX2는 폐암의 표적 물질로서의 개발에 지속적인 연구가 필요할 것으로 사료된다.

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**핵심되는 말:** aminoacyl-tRNA synthetase, aminoacyl-tRNA synthetase interacting multi-functional protein 2, aminoacyl-tRNA synthetase interacting multi-functional protein 2-exon 2 deletion, 폐암