# Prognostic significance of ABCB1 in stage I lung adenocarcinoma

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**Abstract.** Cancer stem cell (CSC) properties have been recently proposed to explain tumor carcinogenesis and multidrug resistance in several human cancers, including non-small cell lung cancer (NSCLC). The present study examined the protein expression of three CSC-associated markers, namely ATP binding cassette subfamily B member 1 (ABCB1), aldehyde dehydrogenase 1 family member A1 (ALDH1A1) and cluster of differentiation (CD) 44, by immunohistochemistry in 194 NSCLC patients who underwent complete resection of NSCLC tumors. The association between the expression of these proteins and patient prognosis was evaluated to clarify the prognostic significance of CSC-associated markers in NSCLC patients. Positive staining for ABCB1 demonstrated a trend toward worse survival compared with negative staining in stage I-III NSCLC. Negative staining for ALDH1 or CD44 exhibited a trend toward worse survival compared with positive staining in stage I-III NSCLC. It was observed that patients with stage I lung adenocarcinoma (ADC) showing positivity for ABCB1 expression had significantly poorer survival than those with negative ABCB1 staining (P=0.03). Furthermore, stage I ADC patients with wild-type epidermal growth factor receptor (EGFR) who exhibited positive staining for ABCB1 had significantly shorter disease-free survival (DFS) compared with patients with negative staining for ABCB1 (P<0.01). Analyses by univariate and multivariate Cox proportional hazards models revealed that ABCB1-positive staining was significantly associated with DFS and was an independent prognostic factor (hazard ratio, 3.49; P<0.05) in these patients. These results suggest that ABCB1 protein expression is useful for predicting prognosis and selecting patients for post-operative therapy in stage I lung ADC patients, particularly those harboring wild-type EGFR.

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*Key words:* ABCB1, lung adenocarcinoma, prognosis, EGFR

## Introduction

Lung cancer was the leading cause of cancer-associated mortality in Japan and worldwide in 2013 (1). Recently, oncogenic driver mutations in non-small cell lung cancer (NSCLC) patients, including gene alterations in epidermal growth factor receptor (EGFR) and fusion of the anaplastic lymphoma kinase (ALK) gene, have been identified (2-4). Several tyrosine kinase inhibitors (TKIs) are currently approved or under clinical development for the treatment of NSCLC, particularly lung adenocarcinoma (ADC) (2-4). However, although the recent beneficial use of molecular targeted therapy for advanced ADC prolonged progression-free survival, patient prognosis is still poor due to drug resistance (5-7). Furthermore, the prognosis of NSCLC patients without specific driver mutations is even more unfavorable (8). Therefore, the identification of sensitive and specific biomarkers for prognosis and drug resistance will be of great clinical benefit to ADC patients.

The ability of lung cancer to recur despite systemic therapy is correlated with the presence of a small number of residual cancer cells termed cancer stem cells (CSCs), which consist of a population with the capacity for self-renewal and differentiation, biological functions that are generally limited to normal somatic stem cells (5). The CSC theory is based on a myriad of experimental and clinical observations suggesting that the malignant phenotype is sustained by a subset of cells characterized by their capacity for self-renewal, differentiation, and innate resistance to chemotherapy and radiation (5). CSCs may be responsible for resistance to anticancer agents and disease recurrence following definitive therapy such as chemotherapy and molecular-targeted therapy in solid tumors (5-7). Several putative CSC-associated markers for NSCLC, including ATP binding cassette subfamily B member 1 [ABCB1, also known as multidrug resistance protein 1 (MDR1)], aldehyde dehydrogenase 1 family member A1 (ALDH1A1) and cluster of differentiation (CD) 44, have been identified (6). A previous study demonstrated that CSCs were involved in the acquired resistance to EGFR TKIs in mutant EGFR NSCLC (6). Our group recently reported that overexpression of ABCB1 was associated with CSCs as a mechanism of resistance to mesenchymal-epithelial transition factor (MET) inhibition in NSCLC cells (7). However, the potential correlation between

these CSC-associated marker proteins and patient survival remains to be clarified.

In the present study, the prognostic significance of the above three CSC-associated markers was evaluated in ADC patients by immunohistochemical (IHC) analysis. It was noticed that ABCB1 could be useful for the prognosis of stage I ADC patients. Furthermore, ABCB1 overexpression was associated with recurrence, and could be used as a post-operative recurrence prediction factor in ADC patients harboring wild-type EGFR. The present findings may be useful for the selection of stage I ADC patients who would benefit from adjuvant chemotherapy, particularly those with wild-type EGFR.

## Materials and methods

*Clinical samples*. A total of 194 stage I-III NSCLC patients who received surgical treatment from February 2001 to December 2009 at Nippon Medical School Hospital (Tokyo, Japan) were enrolled in the present study. In total, 128 specimens were collected from ADC patients, while 66 were collected from lung squamous cell carcinoma (SCC) patients. All tissues were freshly collected during surgery, snap-frozen and stored at -80˚C. Tumor-node-metastasis (TNM) stage and grade were classified according to the World Health Organization TNM staging system, 7th edition (9,10). Information on patient survival and recurrence during 5 years of follow-up was available for all the 194 cases. EGFR mutation status was examined using the peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp method, which was conducted by LSI Medience Corporation (Tokyo, Japan). IHC staining of the NSCLC samples was carried out in accordance with the principles embodied in the 2008 Declaration of Helsinki (11). All included patients provided written informed consent for the use of their tissue specimens for medical research. The study protocol was approved by an ethics committee review board at Nippon Medical School Hospital.

*IHC*. IHC staining was performed on snap-frozen surgical samples, which were fixed in 10% neutral-buffered formalin and paraffin-embedded. Following deparaffinization, antigen retrieval was carried out with 10 mmol/l citrate buffer (pH 6.0) (LSI Medience Corporation) using an autoclave at 120˚C for 15 min. Upon blocking with swine serum albumin (Vector Laboratories, Inc., Burlingame, CA, USA) at room temperature for 20 min, the sections were washed with PBS and incubated with mouse anti-human CD44 monoclonal antibody (cat. no. 156-3c11; dilution, 1:100; Cell Signaling Technology, Inc., Danvers, MA, USA), mouse anti-human MDR1 monoclonal antibody (cat. no. D-11; 1:100 dilution; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) or rabbit anti-human ALDH1A1 antibody (cat. no. EP1933Y; 1:100 dilution; Abcam, Cambridge, UK) at 4˚C overnight. Upon washing with PBS, the sections were incubated with biotinylated goat anti-mouse immunoglobulin (Ig) G (cat. no. BA-9200; dilution, 1:200; Vector Laboratories, Inc.) or biotinylated goat anti-rabbit IgG (cat. no. BA-1000; dilution, 1:200; Vector Laboratories, Inc.) for 30 min at room temperature. Visualization was then conducted with the ABC Peroxidase Staining kit (Funakoshi Co., Ltd, Tokyo, Japan). Negative controls were prepared by omitting the primary antibody under the same experimental conditions.

*Evaluation of ABCB1, CD44 and ALDH1A1 protein expression*. IHC scoring was performed using the Histoscore (H-score) (12,13). CD44 expression level was scored on a scale according to a previous study as follows: No expression, 0; low expression, 1+; and high expression, 2+ and 3+ (14). ABCB1 and ALDH1A1 expression were scored on a scale according to a previous study (15) as follows: A semi-quantitative H-score for each tissue sample was calculated by multiplying the staining intensity of tumor cells (0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining) by a proportion score based on the percentage of positive tumor cells (0, ≤10%; 1, 10-39%; 2, 40-69%; and 3, ≥70%). For ABCB1 expression, the score was graded as follows: Low expression, H-score ≤3; and high expression, H-score >3. ALDH1A1 expression was graded as follows: Low expression, H-score ≤2; and high expression, H-score >2. IHC status was determined independently by two investigators (F.Z. and R.N.), who were blinded to the clinical data, and consensus was reached for any discordant cases.

*Statistical analyses*. Correlations between protein expression and patients' characteristics were assessed by $\chi^2$ tests. Overall survival (OS) and disease-free survival (DFS) were calculated from the date of surgery. Kaplan-Meier survival curves were represented for OS and DFS, and the results were compared by log-rank test. Univariate and multivariate analyses were performed using the Cox regression model as previously described (16). For each analysis, $P<0.05$ was considered to indicate a statistically significant difference. All statistical analyses were carried using SPSS version 21 (IBM SPSS, Inc., Armonk, NY, USA).

## Results

*Expression and prognostic significance of ABCB1, ALDH1A1 and CD44 in NSCLC*. Samples from 194 NSCLC patients were available for IHC analysis of ABCB1, ALDH1A1 and CD44. The expression levels of ABCB1, ALDH1A1 and CD44 were high in 25 (13%), 61 (31%) and 117 (60%) of the 194 NSCLC specimens, respectively (Fig. 1). The correlations between ABCB1, ALDH1A1 and CD44 protein expression and patients' characteristics were evaluated. Significant positive correlations of CD44 expression with sex ($P=0.02$), tobacco smoking ($P=0.02$) and histology ($P<0.01$) were observed (data not shown). No significant correlations were noticed between ABCB1 or ALDH1 and patients' characteristics. Next, the prognostic significance of the expression of the aforementioned three CSC-associated proteins was evaluated. Positive staining for ABCB1 demonstrated a trend toward worse survival compared with negative staining in stage I-III and stage I NSCLC. Negative staining for ALDH1 or CD44 exhibited a trend toward worse survival compared with positive staining in stage I-III and stage I NSCLC. However, these differences were not statistically significant (data not shown).

*Correlation between CSC-associated marker expression and patient survival in ADC*. The prognostic significance of CSC-associated marker expression was next examined based on the histological types. The 194 NSCLC samples evaluated included 128 ADC and 66 SCC specimens. The characteristics of the 128 ADC patients are shown in Table I. There were

significant positive correlations of ALDH1A1 expression with age (P=0.03) and disease grade (P=0.01). CD44 expression was associated with T stage (P=0.02) and pathological stage (P=0.02; Table I). Next, the prognostic significance of the expression of the aforementioned three CSC-associated markers was evaluated in ADC patients (Fig. 2). ALDH1A1 expression did not show any significant association with ADC patient survival (Fig. 2A). Stage I-III patients with a CD44-positive status displayed significantly better survival than those who were negative for CD44 expression (P=0.04; Fig. 2B). However, CD44 status did not show any significant correlation with survival in stage I cases. By contrast, positivity for ABCB1 expression in stage I ADC patients was significantly associated with poorer survival than in ABCB1-negative cases (P=0.03; Fig. 2C).

*Prognostic significance of ABCB1 expression in stage I ADC*. The present study further evaluated the prognostic significance of ABCB1 expression in stage I ADC cases. Among 75 ADC patients with stage I disease, 56 cases harbored wild-type EGFR. Positivity for ABCB1 expression in stage I ADC patients with wild-type EGFR was correlated with significantly poorer prognosis than in ABCB1-negative cases (P<0.05) (Fig. 3A). By contrast, no significant correlation between ABCB1 staining and patient prognosis was observed in 19 stage I ADC patients with mutant EGFR (data not shown). The present study also evaluated the correlation between ABCB1 expression and DFS in ADC patients with stage I disease, and it was observed that ABCB1-positive cases with stage I disease exhibited a trend toward worse DFS compared with patients who are ABCB1-negative, although the difference was not statistically significant (P<0.10; Fig. 3B). However, among stage I ADC patients with wild-type EGFR, ABCB1-positive cases exhibited significantly worse DFS than ABCB1-negative cases (P<0.01; Fig. 3C).

*Univariate analysis and multivariate Cox proportional hazards models for factors associated with mortality and DFS in ADC patients with stage I disease*. The present study further investigated whether the prognostic ability of ABCB1 was affected by underlying clinical variables by performing univariate and multivariate Cox proportional hazards survival analyses in stage I cases (Table II). Univariate analysis revealed that EGFR and ABCB1 statuses were significantly associated with mortality [hazard ratio (HR), 2.98 and 3.11; P=0.02 and P=0.04, respectively]. However, multivariate analysis revealed that EGFR and ABCB1 statuses were not significantly associated with mortality (HR, 2.64 and 2.53; P=0.08 and P=0.13, respectively; Table II). Univariate and multivariable analyses were also performed in stage I ADC patients with wild-type EGFR, but no significant associations were detected (Table II).

Furthermore, univariate and multivariate Cox proportional hazards models for factors associated with DFS in stage I ADC patients were also performed. No significant associations between DFS and clinical variables were observed in stage I cases by multivariate analysis (Table III). In stage I patients with wild-type EGFR, age and ABCB1 status were significantly correlated with DFS by univariate analysis (HR, 4.71 and 4.29; P=0.04 and P=0.01, respectively; Table III). Multivariate Cox proportional hazards model analysis demonstrated that only ABCB1 status was an independent prognostic indicator of DFS in stage I cases with wild-type EGFR (HR, 3.49; P<0.05; Table III).

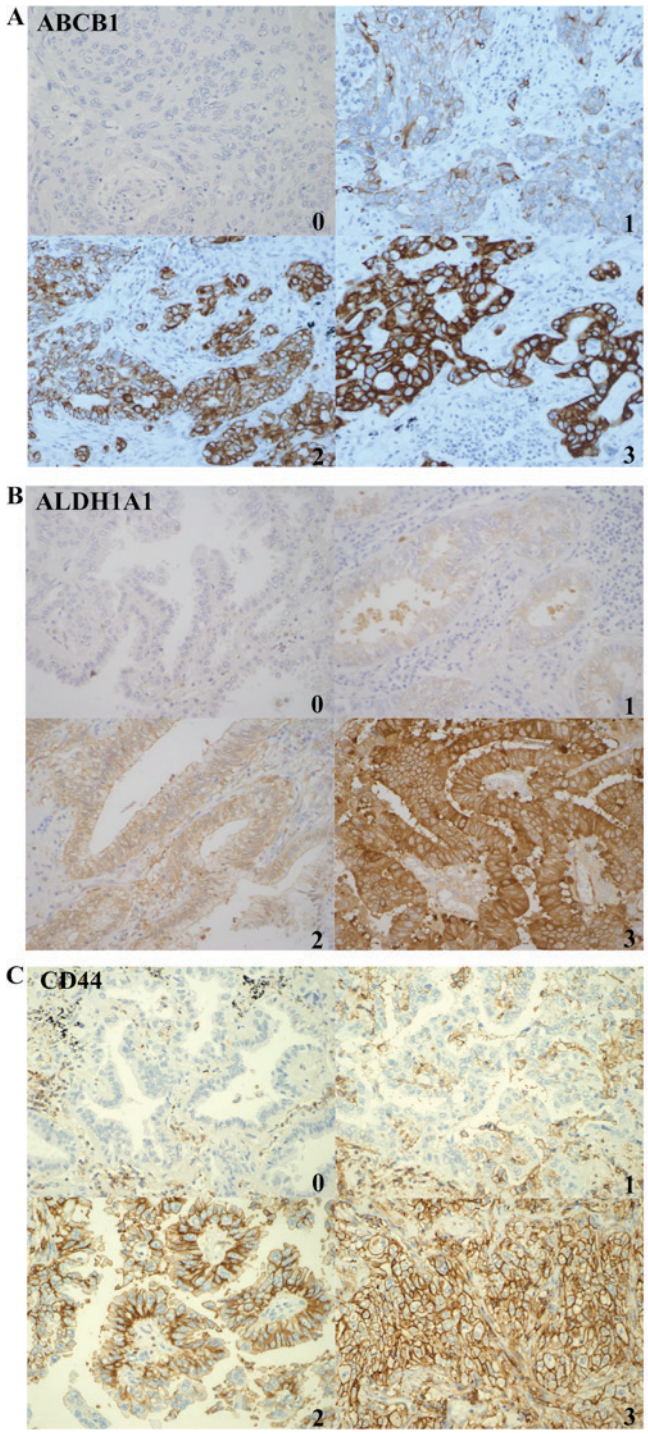


Figure 1. IHC staining for ALDH1A1, CD44 and ABCB1 from different patients. IHC staining for (A) ABCB1, (B) ALDH1A1 and (C) CD44 protein expression in tumor cells. Scores 0, 1, 2 and 3 correspond to negative, weak, moderate and strong staining, respectively (magnification, x20). IHC, immunohistochemical; ABCB1, ATP binding cassette subfamily B member 1; ALDH1A1, aldehyde dehydrogenase 1 family member A1; CD, cluster of differentiation.

*Expression and clinical significance of ABCB1, ALDH1A1 and CD44 in lung SCC*. The present study also evaluated the prognostic significance of ABCB1 in lung SCC. The

Table I. Associations between ABCB1, ALDH1A1 and CD44 expression levels and characteristics of patients with lung adenocarcinoma.

| | | ABCB1 expression | | | ALDH1A1 expression | | | CD44 expression | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variables | Total no. (%) | Positive [no. (%)] | Negative [no. (%)] | P-value | Positive [no. (%)] | Negative [no. (%)] | P-value | Positive [no. (%)] | Negative [no. (%)] | P-value |
| No. of cases | 128 (100) | 20 (16) | 108 (84) | | 43 (34) | 85 (66) | | 67 (52) | 61 (48) | |
| Age, years | | | | 0.40 | | | 0.03 | | | 0.38 |
| <65 | 47 (37) | 9 (19) | 38 (81) | | 10 (21) | 37 (79) | | 27 (57) | 20 (43) | |
| ≥65 | 81 (63) | 11 (14) | 70 (86) | | 33 (41) | 48 (59) | | 40 (49) | 41 (51) | |
| Sex | | | | 0.22 | | | 0.09 | | | 0.16 |
| Male | 67 (52) | 13 (19) | 54 (81) | | 27 (40) | 40 (60) | | 39 (58) | 28 (42) | |
| Female | 61 (48) | 7 (11) | 54 (89) | | 16 (26) | 45 (74) | | 28 (46) | 33 (54) | |
| Tobacco smoking | | | | 0.84 | | | 0.97 | | | 0.08 |
| Yes | 80 (63) | 13 (16) | 67 (84) | | 27 (34) | 53 (66) | | 47 (59) | 33 (41) | |
| No | 47 (37) | 7 (15) | 40 (85) | | 16 (34) | 31 (66) | | 20 (43) | 27 (57) | |
| Unknown | 1 (1) | 0 (0) | 1 (1) | | 0 (0) | 1 (1) | | 0 (0) | 1 (1) | |
| Grade | | | | 0.18 | | | 0.01 | | | 0.13 |
| 1 | 42 (17) | 4 (10) | 38 (90) | | 7 (17) | 35 (83) | | 26 (62) | 16 (38) | |
| 2+3 | 86 (83) | 16 (19) | 70 (81) | | 36 (42) | 50 (58) | | 41 (48) | 45 (52) | |
| T stage | | | | 0.36 | | | 0.56 | | | 0.02 |
| T1 | 40 (31) | 8 (20) | 32 (80) | | 12 (30) | 28 (70) | | 27 (68) | 13 (33) | |
| T2+T3 | 88 (69) | 12 (14) | 76 (86) | | 31 (35) | 57 (65) | | 40 (45) | 48 (55) | |
| N stage | | | | 0.51 | | | 0.81 | | | 0.36 |
| N0 | 91 (71) | 13 (14) | 78 (86) | | 30 (33) | 61 (67) | | 50 (55) | 41 (45) | |
| N1+N2 | 37 (29) | 7 (19) | 30 (81) | | 13 (35) | 24 (65) | | 17 (46) | 20 (54) | |
| Pathological stage | | | | 0.89 | | | 0.76 | | | 0.02 |
| I | 75 (59) | 12 (16) | 63 (84) | | 26 (35) | 49 (65) | | 46 (61) | 29 (39) | |
| II+III | 53 (41) | 8 (15) | 45 (85) | | 17 (32) | 36 (68) | | 21 (40 | 32 (60) | |
| EGFR mutation status | | | | 0.55 | | | 0.47 | | | 0.32 |
| Positive | 28 (22) | 4 (14) | 24 (86) | | 11 (39) | 17 (61) | | 17 (61) | 11 (39) | |
| Negative | 100 (78) | 16 (16) | 84 (84) | | 32 (32) | 68 (68) | | 50 (50) | 50 (50) | |

ABCB1, ATP binding cassette subfamily B member 1; ALDH1A1, aldehyde dehydrogenase 1 family member A1; CD, cluster of differentiation; EGFR, epidermal growth factor receptor.

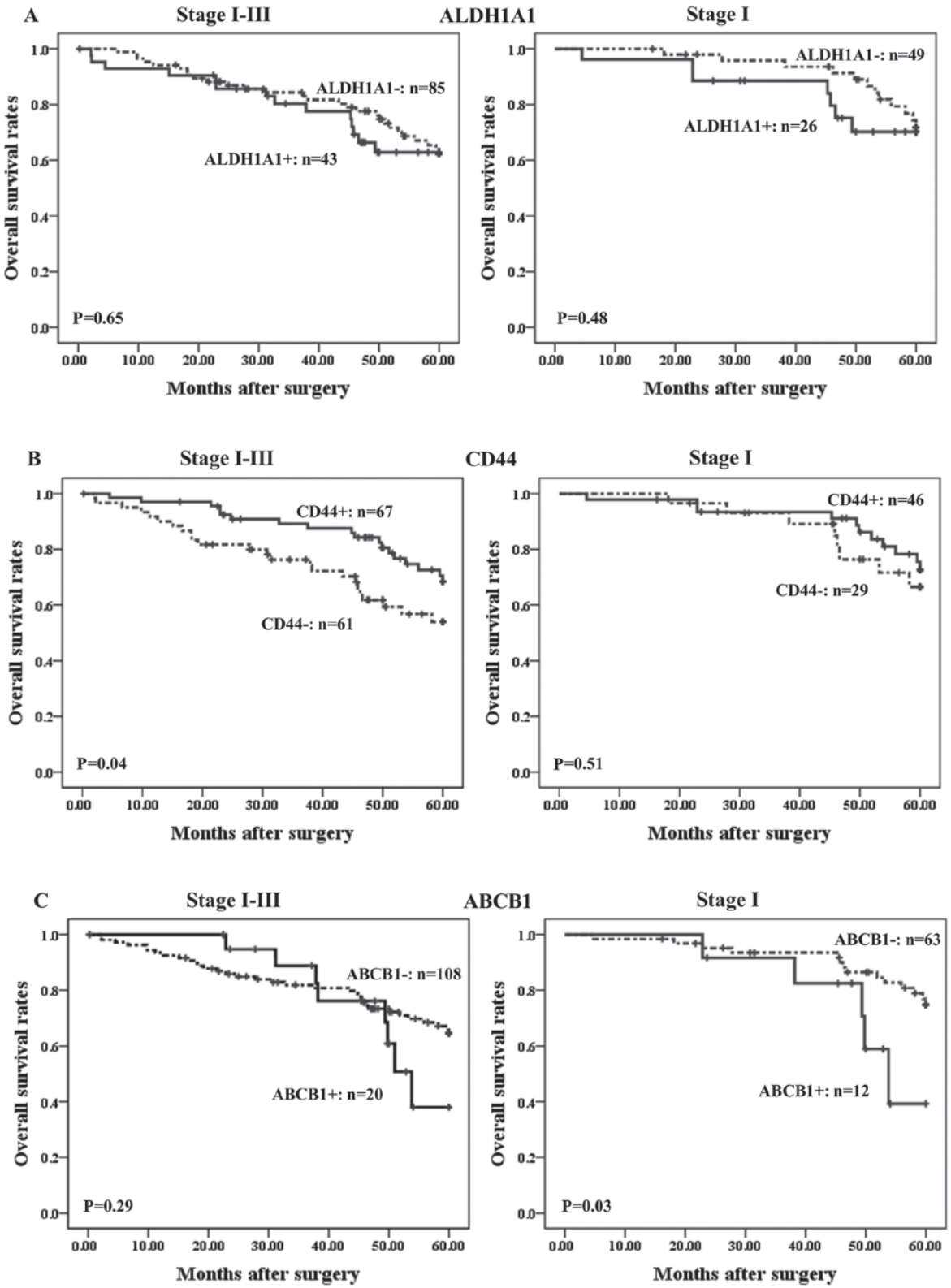


Figure 2. Kaplan-Meier analyses of OS in adenocarcinoma patients. OS curves of patients with positive or negative expression of (A) ALDH1A1, (B) CD44 and (C) ABCB1. The significance of the differences in OS between subgroups was analyzed by the log-rank test. OS, overall survival; ABCB1, ATP binding cassette subfamily B member 1; ALDH1A1, aldehyde dehydrogenase 1 family member A1; CD, cluster of differentiation.

characteristics of 66 SCC patients were evaluated. There were significant positive correlations of ABCB1 expression with N stage and p-stage in lung SCC samples (P=0.04 and P=0.02, respectively; data not shown). ABCB1-positive cases with stage I-III disease exhibited a trend toward worse survival compared with patients who are ABCB1-negative; however, the difference was not statistically significant (data not shown). The prognostic significance of ABCB1 in stage I SCC could

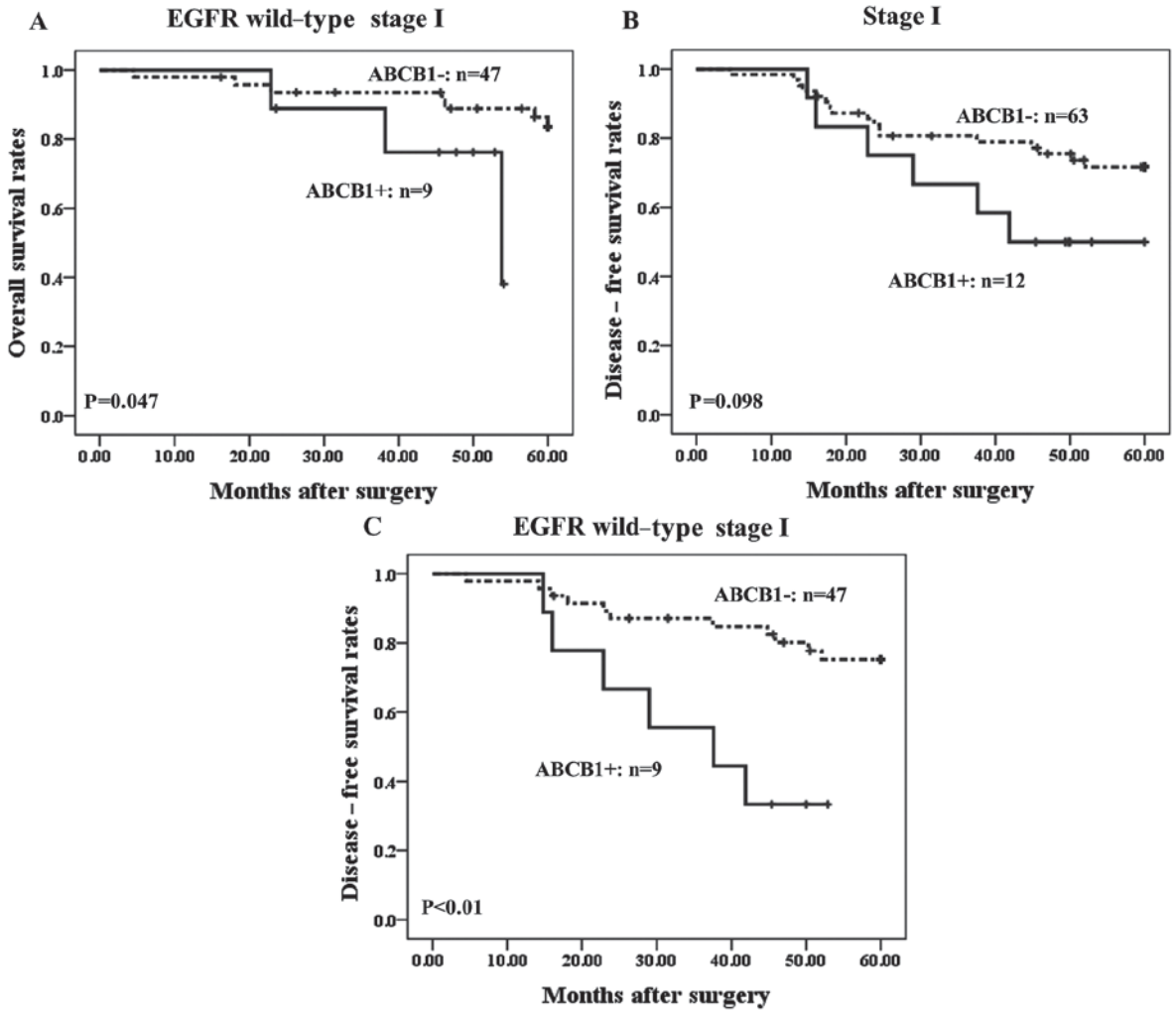


Figure 3. Kaplan-Meier analyses of OS and DFS in stage I adenocarcinoma patients based on ABCB1 expression. (A) Wild-type EGFR patients with positivity for ABCB1 expression exhibited significantly poorer OS than those with ABCB1-negative staining (P=0.047). (B) Cases with positive ABCB1 expression exhibited poorer DFS than those that were negative for ABCB1 (P=0.098). (C) Wild-type EGFR patients with positive ABCB1 expression exhibited significantly poorer DFS than those with negativity for ABCB1 (P<0.01). The significance of differences in OS or DFS was analyzed by the log-rank test. OS, overall survival; DFS, disease-free survival; ABCB1, ATP binding cassette subfamily B member 1; EGFR, epidermal growth factor receptor.

not be evaluated, since none of these cases exhibited positivity for ABCB1. Thus, these data indicated that ABCB1 expression was associated with metastatic ability in lung SCC patients.

## Discussion

The present study examined by IHC the prognostic significance of the protein expression of the CSC-associated markers ABCB1, ALDH1A1 and CD44 in NSCLC patients. It was demonstrated that ABCB1 protein expression could be used as a prognostic marker in stage I ADC patients. In particular, ABCB1 expression was associated with recurrence in stage I ADC patients with wild-type EGFR. In SCC patients, ABCB1 expression was associated with lymph node metastasis (data not shown).

ABCB1 encodes the transport protein P-glycoprotein and is localized in the plasma membrane to protect sensitive tissues from potentially toxic xenobiotics (17,18). However, ABCB1 is considered a 'double-edged sword', since the ABCB1 protein can also remove therapeutic agents out of cancer cells and diminish the efficacy of anticancer drugs (19-21). Thus, ABCB1 can affect the pharmacokinetics of administered drugs and the efficacy of chemotherapy agents for cancer. In fact, previous studies reported that increased ABCB1 expression conferred resistance to chemotherapeutic agents in several cancers (21,22). ABCB1 is closely associated with CSC-like properties and is considered one of the CSC-associated markers (6). Cancer cells with CSC-like properties, which are characterized by their capacity for pluripotency and self-renewal, have been attracting interest as a source of cancer cells (23). The therapeutic significance of CSC-like properties has been reported in NSCLC patients (6,7,24,25); however, the prognostic significance of CSC-associated markers remains to be clarified. Embryonic signaling pathways, including Hedgehog, Notch, WNT and B lymphoma MLV insertion region 1, are associated with the renewal of normal stem cells and the maintenance of tissue homeostasis (26). CSCs exhibit similar properties to those of normal stem cells, suggesting that these signaling pathways are important in maintaining CSCs in a variety of cancers, including lung (5,27). Previous studies reported that ABCB1 expression was affected by the above signaling pathways (28-30). However, the association between ABCB1 and these signaling pathways should be further confirmed in NSCLC patients.

Table II. Univariate and multivariate Cox proportional hazards models for factors associated with mortality in stage I lung adenocarcinoma patients.

A, Stage I (n=75)

| | | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|---|
| Characteristics | Comparison | HR | 95% CI | P-value | HR | 95% CI | P-value |
| Age, years | <65 vs. ≥65 | 0.93 | 0.37-2.30 | 0.87 | 0.96 | 0.33-2.79 | 0.94 |
| Sex | Female vs. male | 0.95 | 0.39-2.34 | 0.91 | 1.94 | 0.60-6.22 | 0.27 |
| Tobacco smoking | No vs. yes | 0.57 | 0.23-1.42 | 0.23 | 0.49 | 0.15-1.56 | 0.23 |
| Grade | G1 vs. G2+G3 | 1.59 | 0.57-4.43 | 0.37 | 1.41 | 0.41-4.83 | 0.59 |
| T stage | T1 vs. T2-T4 | 1.29 | 0.51-3.28 | 0.59 | 1.44 | 0.46-4.53 | 0.53 |
| UFT | No vs. yes | 1.19 | 0.46-3.08 | 0.72 | 1.00 | 0.36-2.81 | 1.00 |
| EGFR expression | $EGFR^-$ vs. $EGFR^+$ | 2.98 | 1.21-7.36 | 0.02 | 2.64 | 0.88-7.92 | 0.08 |
| ABCB1 status | $ABCB1^-$ vs. $ABCB1^+$ | 3.11 | 1.08-8.97 | 0.04 | 2.53 | 0.76-8.47 | 0.13 |

B, EGFR wild-type (n=56)

| | | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|---|
| Characteristics | Comparison | HR | 95% CI | P-value | HR | 95% CI | P-value |
| Age, years | <65 vs. ≥65 | 5.50 | 0.69-43.14 | 0.11 | 4.73 | 0.55-40.71 | 0.16 |
| Sex | Female vs. male | 1.27 | 0.33-4.91 | 0.73 | 1.91 | 0.36-10.16 | 0.45 |
| Tobacco smoking | No vs. yes | 1.56 | 0.33-7.33 | 0.58 | 1.30 | 0.18-9.29 | 0.80 |
| Grade | G1 vs. G2+G3 | 1.27 | 0.33-4.92 | 0.73 | 0.54 | 0.12-2.47 | 0.42 |
| T stage | T1 vs. T2-T4 | 5.86 | 0.74-46.42 | 0.09 | 6.82 | 0.67-69.08 | 0.10 |
| UFT | No vs. yes | 0.57 | 0.15-2.20 | 0.42 | 0.26 | 0.06-1.09 | 0.07 |
| ABCB1 status | $ABCB1^-$ vs. $ABCB1^+$ | 3.92 | 0.92-16.78 | 0.07 | 3.58 | 0.70-18.41 | 0.13 |

HR, hazard ratio; CI, confidence interval; EGFR, epidermal growth factor receptor; ABCB1, ATP binding cassette subfamily B member 1; UFT, uracil-tegafur.

The 5-year survival rate of stage I NSCLC patients who undergo complete resection is ~70% (31). However, 20-30% of early stage NSCLC patients undergo a relapse even upon complete surgical resection of their tumor (32,33). Platinum-based adjuvant chemotherapy for NSCLC patients with stage IB-IIIA disease has been investigated in several clinical trials (34-37). In Japan, NSCLC patients with stage IB lung ADC can benefit from uracil-tegafur (UFT) treatment following complete resection of the tumor (38). However, only 5-15% of NSCLC patients could benefit from UFT or platinum-based adjuvant chemotherapy (38,39). Therefore, the identification of prognostic biomarkers to select NSCLC patients with poor prognosis and to develop tailored treatment strategies may have a clinical benefit. A previous study indicated that evaluation of excision repair cross-complementation group 1 (ERCC1) and ribonucleotide reductase 1 proteins has prognostic value in NSCLC patients with stage I disease (40). Although several studies have evaluated the expression levels of ERCC1 by IHC, no consensus has been reached due to the difficulty in detecting the unique functional ERCC1 isoform (41). Actin 4 has been identified as a potential prognostic biomarker in stage I lung adenocarcinoma (42). The present study revealed that ABCB1 could be used as a prognostic marker in lung ADC patients with stage I disease, and is associated with recurrence in stage I ADC patients with wild-type EGFR. EGFR mutations or ALK rearrangement contributes to the therapeutic effects of certain cancer treatments, resulting in significant improvement of patient prognosis (2-4). By contrast, the therapeutic strategy for NSCLC patients without specific driver mutations is still undeveloped. ABCB1 status may be useful for selecting ADC patients with poor prognosis and identifying ADC patients harboring wild-type EGFR who may benefit from post-surgical adjuvant therapy.

In conclusion, the present study demonstrated that ABCB1 protein expression may have prognostic value in stage I ADC patients. In particular, overexpression of ABCB1 was observed to be associated with recurrence and was recognized as a post-operative recurrence prediction factor in ADC patients with wild-type EGFR. The present findings may be useful for the selection of stage I NSCLC patients who would benefit from adjuvant chemotherapy.

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Table III. Univariate and multivariate Cox proportional hazards models for factors associated with disease-free survival in stage I lung adenocarcinoma patients.

A, Stage I (n=75)

| | | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|---|
| Characteristics | Comparison | HR | 95% CI | P-value | HR | 95% CI | P-value |
| Age, years | <65 vs. ≥65 | 1.87 | 0.74-4.74 | 0.19 | 1.94 | 0.70-5.38 | 0.20 |
| Sex | Female vs. male | 1.97 | 0.81-4.80 | 0.13 | 2.47 | 0.78-7.88 | 0.13 |
| Tobacco smoking | No vs. yes | 1.62 | 0.60-4.38 | 0.34 | 1.49 | 0.44-5.06 | 0.52 |
| Grade | G1 vs. G2+G3 | 1.36 | 0.54-3.47 | 0.51 | 0.75 | 0.27-2.07 | 0.57 |
| T stage | T1 vs. T2-T4 | 2.70 | 1.00-7.28 | 0.05 | 2.08 | 0.62-7.01 | 0.24 |
| UFT | No vs. yes | 2.54 | 1.06-6.08 | 0.04 | 2.22 | 0.85-5.77 | 0.10 |
| EGFR expression | $EGFR^-$ vs. $EGFR^+$ | 1.17 | 0.46-2.96 | 0.75 | 2.03 | 0.63-6.46 | 0.23 |
| ABCB1 status | $ABCB1^-$ vs. $ABCB1^+$ | 2.16 | 0.85-5.53 | 0.11 | 1.47 | 0.51-4.25 | 0.47 |

B, EGFR wild-type (n=56)

| | | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|---|
| Characteristics | Comparison | HR | 95% CI | P-value | HR | 95% CI | P-value |
| Age, years | <65 vs. ≥65 | 4.71 | 1.07-20.64 | 0.04 | 4.31 | 0.96-19.40 | 0.06 |
| Sex | Female vs. male | 2.66 | 0.76-9.27 | 0.12 | 1.91 | 0.44-8.31 | 0.39 |
| Tobacco smoking | No vs. yes | 3.30 | 0.75-14.45 | 0.11 | 2.30 | 0.39-13.61 | 0.36 |
| Grade | G1 vs. G2+G3 | 1.23 | 0.43-3.52 | 0.69 | 0.51 | 0.15-1.77 | 0.29 |
| T stage | T1 vs. T2-T4 | 3.08 | 0.88-10.73 | 0.08 | 2.36 | 0.49-11.35 | 0.29 |
| UFT | No vs. yes | 1.58 | 0.61-4.10 | 0.35 | 0.84 | 0.29-2.47 | 0.75 |
| ABCB1 status | $ABCB1^-$ vs. $ABCB1^+$ | 4.29 | 1.54-11.92 | 0.01 | 3.49 | 1.03-11.89 | 0.05 |

HR, hazard ratio; CI, confidence interval; EGFR, epidermal growth factor receptor; ABCB1, ATP binding cassette subfamily B member 1; UFT, uracil-tegafur.

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