# Epithelial-mesenchymal Transition (EMT) is Correlated with Patient's Prognosis of Lung Squamous Cell Carcinoma

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**Epithelial-mesenchymal transition (EMT) is an important step leading to invasion and migration of various cancer cells, and are characterized by decreased E-cadherin as an epithelial marker, and increased vimentin as a mesenchymal marker. The present study focused on the clinicopathological significance of E-cadherin and vimentin expression in lung squamous cell carcinoma (SqCC). Immunohistochemically, E-cadherin expression patterns were classified into two types: preserved or reduced; and vimentin expression patterns were also divided into two types: positive or negative. The univariate analyses showed six factors associated with increased mortality: tumor size (P = 0.031), lymph node metastasis (P < 0.001), lymphatic invasion (P < 0.001), histological differentiation (P = 0.036), E-cadherin reduced expression (P < 0.001), and vimentin positive expression (P = 0.004). Multivariate analysis demonstrated that E-cadherin reduced expression (P < 0.001), vimentin positive expression (P = 0.028), lymph node metastasis (P < 0.001), and age (P = 0.020) were independent predictors of patient mortality. There may be some correlation between E-cadherin and vimentin expression (P = 0.017), but the correlation coefficient was 0.235. The complete EMT and the incomplete EMT type were associated with a poor prognosis (p < 0.001 and p=0.036, respectively). The overall survival rate after curative resection was significantly lower in patients with the complete EMT type (reduced E-cadherin / positive vimentin). In conclusion, both E-cadherin and vimentin are independent predictors of mortality, and the EMT phenotype is a significant indicator of poor prognosis in lung SqCC.**

**Key words: Lang cancer, Epithelial-mesenchymal transition, vimentin, E-cadherin, Squamous cell carcinoma**

## INTRODUCTION

Lung cancer is the most common malignancy of the respiratory tract and the leading cause of cancer death globally [1]. The number of surgically resected cases of lung cancer has increased because of the recent advances in imaging diagnosis. Although complete surgical resection is performed, the prognosis of lung cancer is generally poor [2]. It was reported that recurrence rates of lung cancer were approximately 15–30% and 5-year survival rates were 60–70% [3]. Lung cancer is commonly classified into four histological types: squamous cell carcinoma (SqCC), adenocarcinoma, large cell carcinoma, and small cell carcinoma [2, 4, 5], and adenocarcinoma and SqCC are considered as the two major types. Customized chemotherapy for unresectable or recurrent lung cancers is frequently used for adenocarcinoma than for SqCC [6, 7]. Recently, anti-angiogenic therapy and molecular-targeting have been developed for adenocarcinoma [8–12, 23, 24, 26, 28, 39]. However, there are limited therapeutic options for unresectable or recurrent squamous cell carcinoma of the lung, and it is therefore important to examine the histopathological features in order to clarify a poor prognosis group of SqCC.

Cadherins are comprised of a large family of transmembrane or membrane-associated glycoproteins that mediate specific cell-cell adhesion, functioning as key molecules in the morphogenesis of a variety of organs [13–15]. E-cadherin included in the family is expressed by most normal epithelial tissues and many epithelium-derived cancer cells have lost E-cadherin [16]. E-cadherin down-regulation and vimentin up-regulation are widely known as important phenomena in the Epithelial-mesenchymal transition (EMT) [17]. EMT has been considered to be a critical event for invasion and metastasis of carcinoma cells [18]. Many studies reported that reduced expression of E-cadherin promoted EMT [19, 20]. Vimentin is one of the cellular intermediate filaments in normal and tumor mesenchymal cells and is thought to be one of the primary indicators of the development of EMT in carcinomas [21]. Several stadies have reported that expression of E-cadherin or vimentin in non-small cell lung cancer has been analyzed separately [22–28]. The present study focused on immnohistochemical expression of both E-cadherin / vimentin, and its clinicopathological significance in the progression of lung SqCC.

## MATERIAL AND METHODS

### Patient materials

One hundred and three lung squamous cell carcinoma cases were surgically resected at Tokai University Hospital from January 2001 to December 2006. All

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patients provided informed consent, in accordance with the regulations of the Institutional Review Board of Tokai University Hospital (#14R-076). These 103 patients (97 males and 6 females; age range, 43–85 years; mean age, 67.2 ± 9.1 years) with lung squamous cell carcinoma underwent surgery with a radical approach (lobectomy and mediastinum lymphadenectomy) at Tokai University Hospital (Kanagawa, Japan).

**Histopathological examination**

Lung cancer specimens were routinely fixed with 10% formalin for 24–48 hr, embedded in paraffin, cut into 4-μm sections, and stained with hematoxylin and eosin. One representative histological specimen, including both intraepithelial (in situ) spread and subepithelial invasion of SqCC lesion, was selected for immunohistochemistry. Tumor stages were defined according to the TNM classification of the International Union Against Cancer (UICC) [29] and the histological types according to the World Health Organization classification [30]. The median postoperative follow-up duration was 1,572 days (range 41–3,837 days). The degree of lymph node metastasis was classified as follows: n0, no lymph node metastasis; n1, ipsilateral peribronchial and/or ipsilateral hilar lymph node metastasis; or n2, ipsilateral mediastinal and/or subcarinal lymph node metastasis, based on the TNM classification [29]. The degree of lymphatic invasion was classified as follows: ly0, no lymphatic invasion; ly1, mild lymphatic invasion; ly2, preserved lymphatic invasion; or ly3, severe lymphatic invasion. Then, these categories were divided into two groups: ly0/1 and ly2/3. Vascular and pleural invasion was evaluated using the elastica van Gieson method. The degree of venous invasion was classified as follows: v0, no venous invasion; v1, minimal venous invasion, that is, one or two foci of venous invasion in one histological section; v2, preserved venous invasion, that is, three or four foci; or v3, severe venous invasion with five or more foci. These were divided into two groups: v(-) and v(+). The stromal infiltration (INF) types, i.e., cancer-stroma relationship patterns of lung squamous cell carcinoma, were also classified into three groups: a medullary type with scanty stroma (INFa), a scirrhous type with abundant stroma (INFc), and an intermediate type (INFb), according to the general rules for gastric cancer [31].

**Immunohistochemical analysis**

Five-micrometer-thick sections were mounted on a silane-coated glass slide Immunohistochemical examination was performed on deparaffinized sections using the strent avidin-biotin-peroxidase complex method with automated immunostainer (Benchmark XT; Ventana medical system, Tucson, AZ, USA). The primary antibodies for immunohistochemistry used in immunohistchemical analyses were anti-human E-cadherin (clone 36B5, mouse monoclonal, 1: 100 dilution; cat. no. PA0387; LeicaBiosystems. com, Newcastle UK) and mouse monoclonal anti-vimentin (clone v9; 1: 100 dilution; cat. no. MO725; Dako Denmark A/S, Glostrup, Denmark).

**Evaluation of immunohistochemistry**

Immunohistochemically, E-cadherin expression was evaluated in the invasive region of tumors, and was classified into the following two groups: reduced, ≤ 20% of staining in tumor cells; or preserved, > 20% of staining in tumor cells. Vimentin expression was determined by the presence of cytoplasmic staining, particularly in the tumor/stoma interface, and divided based on the following two criteria: negative, < 10% vimentin expression at the invasive front of the cancer cells; positive, ≥ 10% vimentin expression.

**Definition for expression of EMT phenotype**

According to the immunohistochemical expression, the cases were divided into three groups as follows: (i) non-EMT type, defined as preserved E-cadherin expression with negative vimentin expression (Fig. 1A–1C); (ii) incomplete EMT type, defined as reduced E-cadherin expression with negative vimentin expression and preserved E-cadherin expression with positive vimentin expression (Fig. 1D–1F); and (iii) complete EMT type, defined as reduced E-cadherin expression with positive vimentin expression (Fig. 1G–1I).

**Statistical analysis**

Univariate analysis was performed by chi-square test. Cox proportional hazards regression analysis was used to determine the net effects of each predictor while controlling for the effects of the other factors by uni- and multivariate analyses. Independent prognostic factors were analyzed by Cox's proportional hazard regression model. Hazard ratios (HR) and their 95% confidence intervals (CI) were used to assess the independent contributions of significant factors. $P < 0.05$ was considered to indicate a significant difference. The patients' overall survival was measured from the date of operation; death from all causes (without discrimination between deaths resulting from lung squamous cell carcinoma or other causes) was taken as the outcome. Survival curves were calculated by the Kaplan-Meier method and analyzed by the log-rank test. All analyses were performed using the SPSS statistical software, version 21 (SPSS, International Business Machines C. P., Armonk, NY, USA).

## RESULTS

**E-cadhelin / vimentin expression and Clinicopathological findings in lung SqCC**

Relationships between the E-cadherin and vimentin expression and clinicopathological factors are summarized in (Table 1). E-cadherin expression patterns were classified into two types: preserved (36, 35.0%), and reduced (67, 65.0%). Preserved E-cadherin expression was diffusely found at the membrane of tumor cells, and reduced expression was detected at the basolateral membrane of several tumors. Vimentin expression patterns were classified into two types: positive (42, 40.8%) and negative (61, 59.2%). E-cadherin expression was significantly correlated with tumor size ($P = 0.007$), but there were no significant correlation better vimentin expression and any clinicopathological findings of lung SqCC. The aforementioned univariate analyses identified six factors associated with increased mortality in patients with lung SqCC (Table 2): tumor size (HR, 1.897; 95% CI, 1.059–3.396, $P = 0.031$), lymph node metastasis (HR, 3.028; 95% CI, 1.785–5.136, $P < 0.001$), lymphatic invasion (HR, 3.416; 95% CI, 1.909–6.113, $P < 0.001$), histological differentiation (HR,

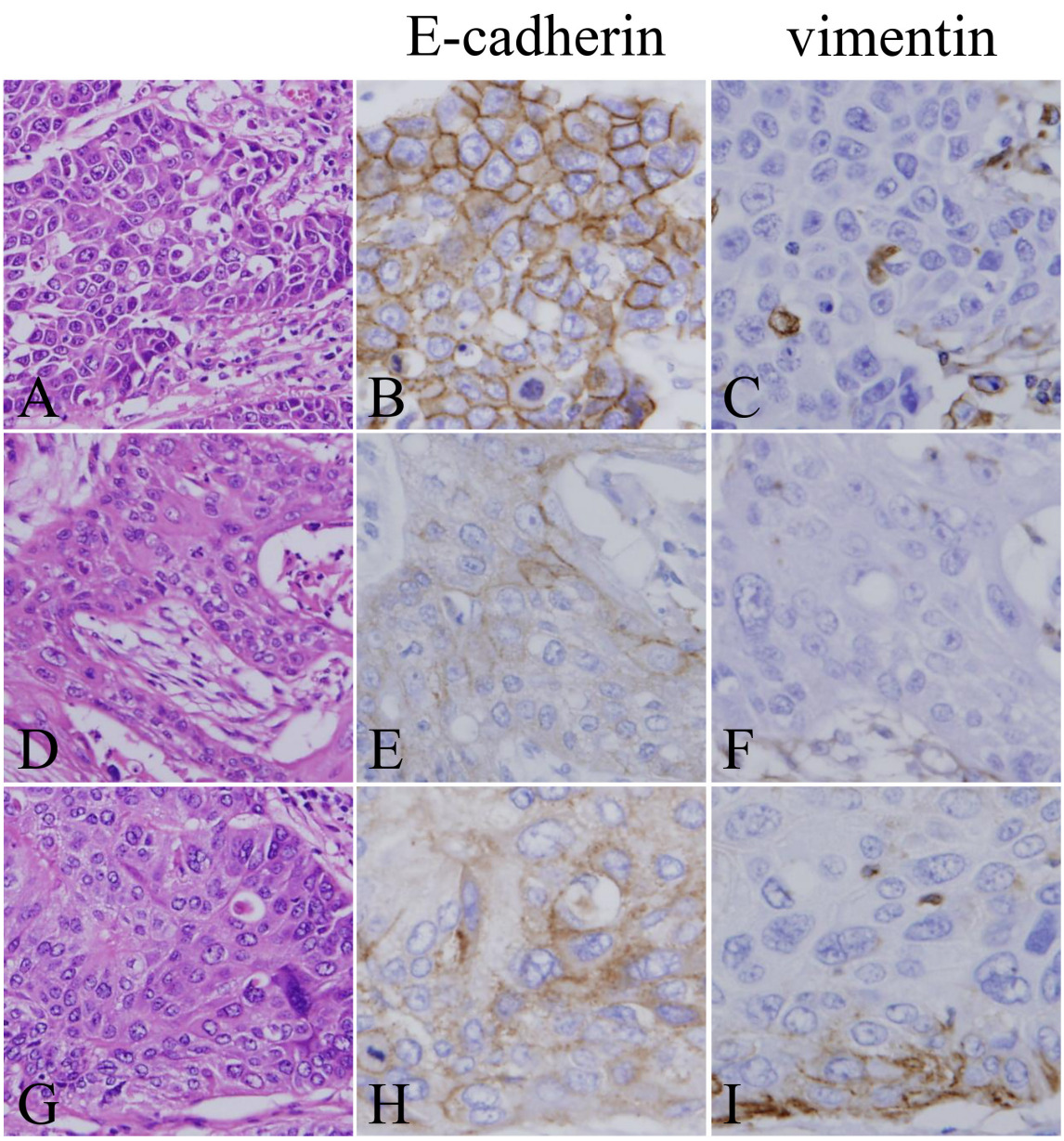


**Fig. 1** Hematoxylin-eosin and immunohistochemical findings of lung squamous cell carcinoma invasion and E-cadherin / vimentin expression patterns. Non-EMT type, hematoxylin and eosin staning (A), preserved E-cadhelin expression (B) with negative vimentin expression (C); incomplete EMT type, hematoxylin and eosin staning (D), reduced E-cadhelin expression (E) with negative vimentin expression (F); complete EMT type, hematoxylin and eosin staning (G), reduced E-cadhelin expression (H) with positive vimentin expression (I).

2.092; 95% CI, 1.050−4.168, P = 0.036), E-cadherin expression (HR, 3.926; 95% CI, 1.920−8.031, P < 0.001), and vimentin expression (HR, 2.190; 95% CI, 1284−3.736, P = 0.004). Multivariate analysis demonstrated that reduced E-cadherin expression (HR, 4.017; 95% CI, 1.932−8.351, P < 0.001), vimentin expression (HR, 1.868; 95% CI, 1.071−3.260, P = 0.028), lymph node metastasis (HR, 3.453; 95% CI, 1.984−6.008, P < 0.001), and age (HR, 1.899; 95% CI, 1.105−3.265, P = 0.020) were independent predictors of patient mortality in lung SqCC (Table 3). Cases with preserved E-cadherin expression showed significantly better overall survival than reduced cases (P < 0.001, log rank test, Fig. 2A), and those with positive vimentin expression demonstrated significantly poorer overall survival than negative cases (P = 0.003, log rank test, Fig. 2B).

**Relationship between E-cadherin and vimentin expression**

The relationship between E-cadherin and vimentin expression is shown in (Table 4). There was a mild correlation between E-cadherin and vimentin expression (P = 0.017), but the correlation coefficient was 0.235. It can be said that E-cadherin and vimentin expression were confirmed as independent predictors of patient mortality in lung SqCC. According to the combination of E-cadherin and tumor vimentin expression, the cases were divided into three groups as follows. Twenty-seven (26.2%) cases were the non-EMT type; forty-three cases (41.8%) were the incomplete EMT type; and thirty-three cases (32.0%) were the complete EMT type. The aforementioned multivariate analyses also demonstrated that the EMT phenotype is associated with increased mortality in patients with lung SqCC: complete EMT type (HR, 6.237; 95% CI, 2.549−15.265,

**Table 1** E-cadherin/vimentin expression and clinicopathological features of lung squamous cell carcinoma

| Variable | No. of patients (%) | E-cadherin | | P-value | Vimentin | | P-value |
|---|---|---|---|---|---|---|---|
| | | preserved | reduced | | negative | positive | |
| Age at surgery (years) | | | | | | | |
| < 68 | 53 (51.5) | 15 (28.3) | 38 (71.7) | 0.145 | 36 (67.9) | 17 (32.1) | 0.064 |
| ≥ 68 | 50 (48.5) | 21 (42.0) | 29 (58.0) | | 25 (50.0) | 25 (50.0) | |
| Gender | | | | | | | |
| Male | 97 (94.2) | 35 (36.1) | 62 (63.9) | 0.333 | 57 (58.8) | 40 (41.2) | 0.702 |
| Female | 6 (5.8) | 1 (16.7) | 5 (83.3) | | 4 (66.7) | 2 (33.3) | |
| Tumor size (mm) | | | | | | | |
| ≤ 30 | 39 (37.9) | 20 (51.3) | 19 (48.7) | 0.007 | 26 (66.7) | 13 (33.3) | 0.230 |
| > 30 | 64 (62.1) | 16 (25.0) | 48 (75.0) | | 35 (54.7) | 29 (45.3) | |
| Lymph node metastasis | | | | | | | |
| n(-) | 70 (68.0) | 26 (37.1) | 44 (62.9) | 0.497 | 43 (61.4) | 27 (38.6) | 0.507 |
| n(+) | 33 (32.0) | 10 (30.3) | 23 (69.7) | | 18 (54.5) | 15 (45.5) | |
| Lymphatic invasion | | | | | | | |
| ly(0, 1) | 83 (80.6) | 30 (36.1) | 53 (63.9) | 0.605 | 47 (56.6) | 36 (43.4) | 0.275 |
| ly(2, 3) | 20 (19.4) | 6 (30.0) | 14 (70.0) | | 14 (70.0) | 6 (30.0) | |
| Venous invasion | | | | | | | |
| v(-) | 53 (51.5) | 19 (35.8) | 34 (64.2) | 0.844 | 32 (60.4) | 21 (39.6) | 0.806 |
| v(+) | 50 (48.5) | 17 (34.0) | 33 (66.0) | | 29 (58.0) | 21 (42.0) | |
| Histological differentiation | | | | | | | |
| well | 28 (27.2) | 12 (42.9) | 16 (57.1) | | 16 (57.1) | 12 (42.9) | |
| mod | 62 (60.2) | 22 (35.5) | 40 (64.5) | | 39 (62.9) | 23 (37.1) | |
| poorly | 13 (12.6) | 2 (15.4) | 11 (84.6) | 0.227 | 6 (46.2) | 7 (53.8) | 0.518 |
| Stromal type | | | | | | | |
| medullary, intermediate | 70 (68.0) | 25 (35.7) | 45 (64.3) | 0.813 | 43 (61.4) | 27 (38.6) | 0.507 |
| scirrhous | 33 (32.0) | 11 (33.3) | 22 (66.7) | | 18 (54.5) | 15 (45.5) | |

n(-)/n(+): lymph node metastasis-negative/positive
v(-)/v(+): venous invasion-negative/positive

P < 0.001), and incomplete EMT type (HR, 2.812; 95% CI, 1.144−6.911, P < 0.001). Multivariate analysis showed the phenotype and lymph node metastasis both predict patient mortality (p < 0.001, both) (Table 5), and also identified the incomplete EMT type as an independent predictor of mortality (HR, 2.695; 95% CI, 1.064−6.82, P = 0.036). The post operative overall survival rate was lower in patients with the complete EMT type than in those with the incomplete EMT type or the non-EMT type (p = 0004 and p < 0.001, respectively, log-rank test), and lower with the incomplete EMT type than with the non-EMT type. Cases of complete EMT type showed lower the postoperative overall survival rate than non-EMT type and incomplete EMT type. (P < 0.001, log-rank test, Fig. 3A). In regards to the incomplete EMT type such as, the survival rate was lower in patients with the complete EMT type than in those with the E-cadherin reduced/ vimentin negative type or the E-cadherin preserved/ vimentin positive type (p < 0.001 and p = 0028, respectively, log-rank test). And then, Cases of the E-cadherin reduced/ vimentin negative type showed lower the postoperative overall survival rate than the E-cadherin preserved/ vimentin positive type and incomplete EMT type. (P = 0.009, log-rank test, Fig. 3B).

## DISCUSSION

In this study, we focused on immunohistochemical expression-related EMT and the other clinicopathological factors of lung SqCC, and clarified that E-cadherin and vimentin expression is important in regards to the malignant potential of lung SqCC. By using the combination of E-cadherin and vimentin expression, we found that the complete EMT type showed a lower overall survival rate. To our knowledge, this is the first report that describes the relationship between the EMT phenotype and prognosis in lung SqCC.

Cadherins are cell-cell adhesion proteins. E-cadherin generally inhibits invasion via epithelial cell-cell adhesion. During EMT, E-cadherin is often downregulated [32], and downregulated E-cadherin expression is correlated with tumor progression and prognosis of non-small cell lung cancer patients [33]. Zhang *et al.* reported that downregulation of E-cadherin was correlated with poor prognosis of lung SqCC [34]. In this study, E-cadherin expression was also correlated with poorer prognosis, and this result is not inconsistent with the previous reports [41].

**Table 2** Univariate analysis of clinicopathological features and patients' survival in lung squamous cell carcinoma

| Variable | No. of patients | P-value | Hazard ratio | 95% Confidence interval |
|---|---|---|---|---|
| Age at surgery (years) | | | | |
| < 68 | 53 (51.5) | 0.131 | 1.502 | 0.885−2.548 |
| ≥ 68 | 50 (48.5) | | | |
| Gender | | | | |
| Male | 97 (94.2) | 0.904 | 0.939 | 0.339−2.602 |
| Female | 6 (5.8) | | | |
| Tumor size (mm) | | | | |
| ≤ 30 | 39 (37.9) | 0.031 | 1.897 | 1.059−3.396 |
| > 30 | 64 (62.1) | | | |
| Lymph nodal metastasis | | | | |
| n(-) | 70 (68.0) | < 0.001 | 3.028 | 1.785−5.136 |
| n(+) | 33 (32.0) | | | |
| Lymphatic invasion | | | | |
| ly(0, 1) | 84 (81.6) | < 0.001 | 3.416 | 1.909−6.113 |
| ly(2, 3) | 19 (18.4) | | | |
| Venous invasion | | | | |
| v(-) | 53 (51.5) | 0.145 | 1.486 | 0.873−2.530 |
| v(+) | 50 (48.5) | | | |
| Histological differentiation | | | | |
| well, mod | 90 (87.4) | 0.036 | 2.092 | 1.050−4.168 |
| poorly | 13 (12.6) | | | |
| Stromal type | | | | |
| medullary, intermediate | 70 (68.0) | 0.465 | 1.229 | 0.706−2.139 |
| scirrhous | 33 (32.0) | | | |
| E-cadherin | | | | |
| preserved | 36 (35.0) | < 0.001 | 3.926 | 1.920−8.031 |
| reduced | 67 (65.0) | | | |
| Vimentin | | | | |
| negative | 61 (59.2) | 0.004 | 2.190 | 1.284−3.736 |
| positive | 42 (40.8) | | | |

n(-)/n(+): lymph node metastasis-negative/positive. v(-)/v(+): venous invasion-negative/positive.

Vimentin is an intermediate filament protein which is characteristically upregulated in cells undergoing EMT. Several studies have reported that vimentin expression is associated with poor prognosis in several squamous cell carcinoma [35, 36] and in non-small lung cancer [23]. The aforementioned article reported that vimentin expression was a prognostic factor in univariate analysis [34], and a meta-analysis revealed that an upregulation of vimentin may predict an unfavorable survival of non-small cell lung cancer. In this study, vimentin expression was considered to indicate poor prognosis in the multivariate analysis. As this may be due to the difference in the number of evaluation items used for analysis, further studies using a large patient population are needed.

In this study, we investigated the correlation between E-cadherin and vimentin expression in lung SqCC. We demonstrated they were related with each other (P = 0.017), but the correlation coefficient was 0.235. The complete EMT and the non-EMT type were only 55.2% of the SqCC cases, whereas the incomplete EMT type was 41.8%. Previous studies have reported that the incomplete EMT type ranged from 24.8% to 52.3% of SqCC [37, 38]. The large proportion of incomplete EMT type was considered to be one of the causes for the low correlation in this study. Some studies have reported results suggesting that EMT is not a necessary prerequisite for metastasis [39, 40]. Therefore, we plan to perform studies to clarify the relationship between the EMT phenotype and invasiveness using tumor budding [41] and other immunochemical staining for lung SqCC.

In conclusion, both reduced E-cadherin and positive vimentin expression are significantly correlated with prognosis, and the utility of this expression pattern may be enhanced by combining the reduced-type with

**Table 3** Multivariate analyasis of clinicopathological features and patients' survival in lung squamous cell carcinoma

| Variable | No. of patients | P-value | Hazard ratio | 95% Confidence interval |
|---|---|---|---|---|
| E-cadherin | | | | |
| preserved | 36 (35.0) | < 0.001 | 4.017 | 1.932–8.351 |
| reduced | 67 (65.0) | | | |
| Vimentin | | | | |
| negative | 61 (59.2) | 0.028 | 1.868 | 1.071–3.260 |
| positive | 42 (40.8) | | | |
| Age at surgery (years) | | | | |
| < 68 | 53 (51.5) | 0.020 | 1.899 | 1.105–3.265 |
| ≥ 68 | 50 (48.5) | | | |
| Tumor size (mm) | | | | |
| ≤ 30 | 39 (37.9) | 0.452 | 1.263 | 0.687–2.321 |
| > 30 | 64 (62.1) | | | |
| Lymph node metastasis | | | | |
| n(-) | 70 (68.0) | < 0.001 | 3.453 | 1.984–6.008 |
| n(+) | 33 (32.0) | | | |

n(-)/n(+): lymph node metastasis-negative/positive

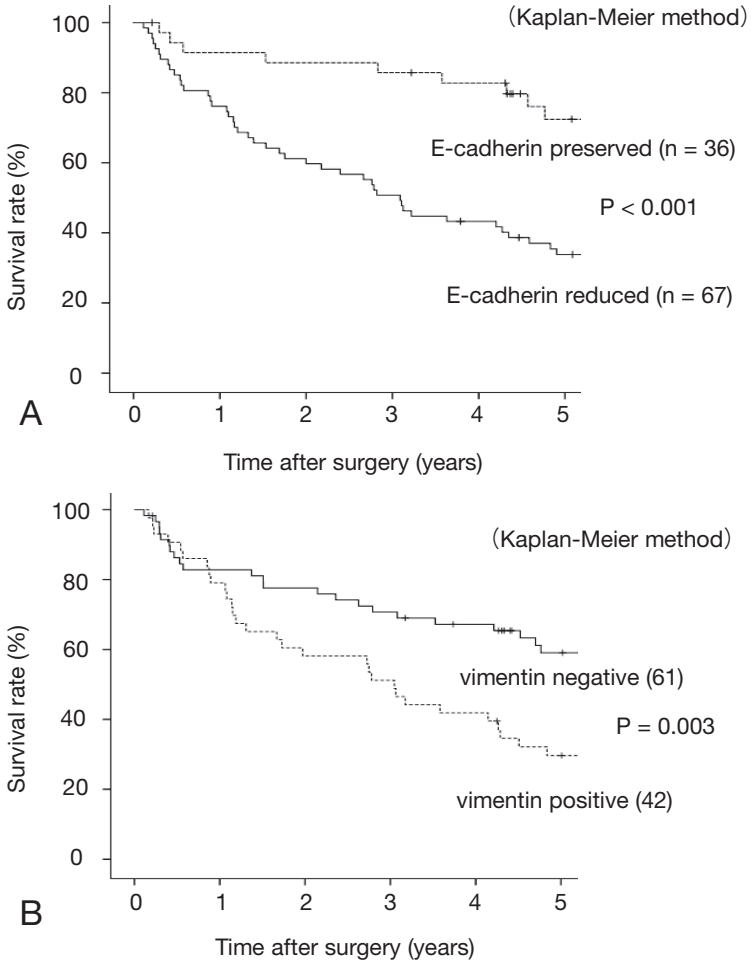


**Fig. 2** E-cadherin (A) and vimentin (B) expression patterns, and cumulative survival of patients with lung squamous cell carcinoma.

**Table 4** E-cadherin/vimentin expression and clinicopathological features of lung squamous cell carcinoma

| Variable | No. of patients (%) | E-cadherin | | P value |
|---|---|---|---|---|
| | | preserved | reduced | |
| Vimentin | | | | |
| negative | 61 (59.2) | 27 (44.3) | 34 (55.7) | 0.017 |
| positive | 42 (40.8) | 9 (21.4) | 33 (78.6) | |

**Table 5** Clinicopathological features and patients' survival in lung squamous cell carcinoma

| Variable | No. of patients | P-value | Hazard ratio | 95% Confidence interval |
|---|---|---|---|---|
| EMT | | | | |
| non-EMT/incomplete EMT | 70 (68.0) | < 0.001 | 3.336 | 1.916–5.810 |
| complete EMT | 33 (32.0) | | | |
| Age at surgery (years) | | | | |
| < 68 | 53 (51.5) | 0.036 | 1.782 | 1.039–3.053 |
| ≥ 68 | 50 (48.5) | | | |
| Tumor size (mm) | | | | |
| ≤ 30 | 39 (37.9) | 0.112 | 1.626 | 0.892–2.963 |
| > 30 | 64 (62.1) | | | |
| Lymph node metastasis | | | | |
| n(-) | 70 (68.0) | < 0.001 | 3.333 | 1.909–5.820 |
| n(+) | 33 (32.0) | | | |

n(-)/n(+): lymph node metastasis-negative/positive

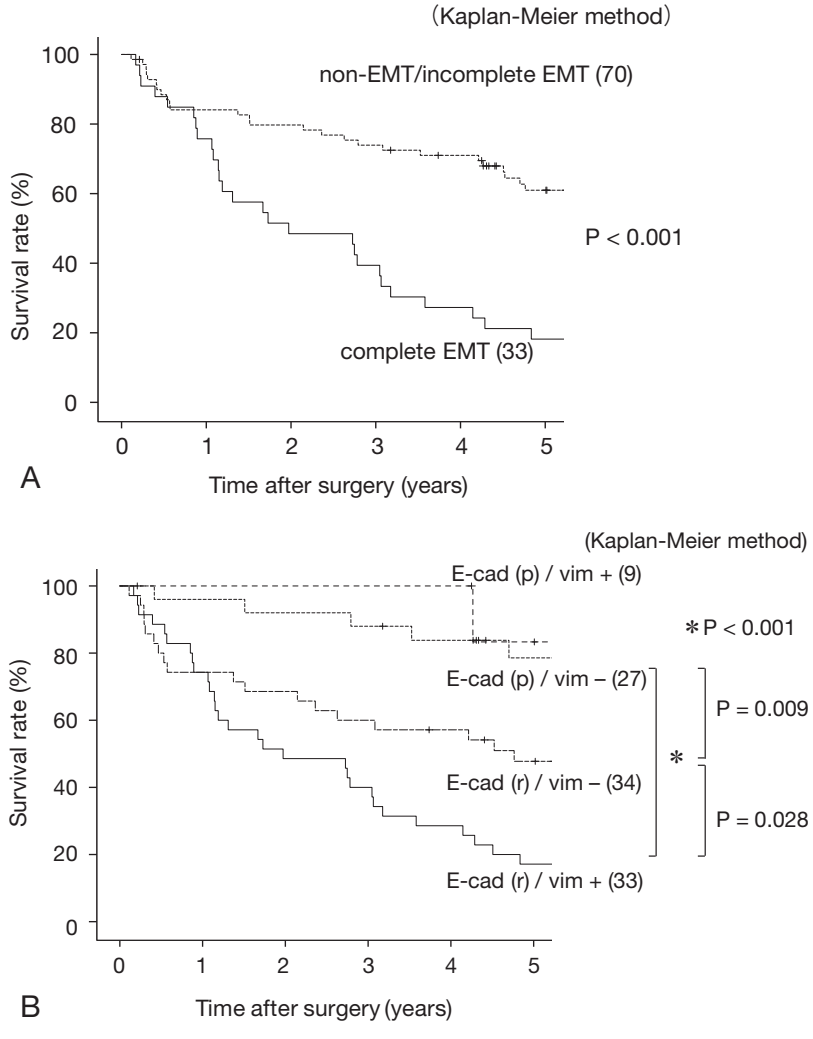


**Fig. 3** Epithelial-mesenchymal transition phenotype and cumulative survival of patients with lung squamous cell carcinoma (A). Combining E-cadherin expression with vimentin and cumulative survival of patients with lung squamous cell carcinoma (B).

the positive-type assay. In other words, the complete EMT type may become a useful indicator of prognosis for lung SqCC.

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