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Original Study

Clinical and Prognostic Significance of the Epithelial–Mesenchymal Transition in Stage IA Lung Adenocarcinoma: A Propensity Score–Matched Analysis

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Abstract

We aimed to analyze the clinical significance of the epithelial–mesenchymal transition (EMT) in stage IA lung adenocarcinoma. Tumors with the EMT phenotype were identified in 43.1% of patients with a high ratio of consolidation to tumor diameter. Propensity score matching indicated a significant association of the EMT with shorter survival.

Background: The epithelial–mesenchymal transition (EMT) describes the process through which cells lose epithelial characteristics and gain a mesenchymal phenotype. The EMT contributes to tumor invasion and cancer progression, and is associated with metastasis and poor survival of patients with non–small-cell lung cancer. However, little is known about the relationships between the EMT and the clinicopathologic characteristics of patients with stage IA lung adenocarcinoma. **Patients and Methods:** We conducted immunohistochemical analysis of the expression of the EMT markers E-cadherin and vimentin of specimens acquired from 183 consecutive patients with stage IA lung adenocarcinoma. The clinicopathologic significance of the association of the EMT status with E-cadherin and vimentin expression was analyzed after propensity score matching. **Results:** E-cadherin and vimentin were detected in 68.3% and 18.6% of stage IA lung adenocarcinomas, respectively. The presence of cells with EMT conversion was associated with older patient age. A propensity score–matched cohort (128 patients) was used for further analyses. Computed tomography revealed that tumors with EMT conversion showed solid-dominant nodules compared to those without conversion. Survival analysis after propensity score matching showed that the EMT correlated with poor disease-free survival (hazard ratio = 2.57, $P = .0451$) and overall survival (hazard ratio = 4.23, $P = .0471$). Multivariate analysis revealed that the EMT was an independent predictor of shorter disease-free survival. **Conclusion:** The EMT was a significant predictor of poor prognosis of patients with stage IA lung adenocarcinoma. The EMT status may serve as an indicator for administering adjuvant therapy.

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Keywords: Early stage, E-cadherin, Lung cancer, Prognosis, Vimentin

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Introduction

Lung cancer is the leading cause of cancer-related death worldwide, and the most common histologic lung cancer subtype is adenocarcinoma. The recent development of a multidisciplinary approach for treating lung cancer that includes surgery, radiotherapy, and chemotherapy has improved outcomes of patients with non–small-cell lung cancer (NSCLC). Surgical resection is the standard treatment for early-stage adenocarcinomas, particularly

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pathologic stage I lung cancer.[1] Patients with pathologic stage IA NSCLC experience longer survival after complete resection[2,3]; however, the survival rate after recurrence is poor.[4,5] Therefore, it is important to identify survival-associated molecular markers for stage IA NSCLC patients.

The epithelial–mesenchymal transition (EMT) describes the process through which cells down-regulate their epithelial characteristics and acquire a mesenchymal phenotype. The EMT is integral to development, wound healing, and stem-cell behaviors and contributes to fibrosis, tumor invasion, and metastatic spread, which drive cancer progression.[6,7] During the EMT, epithelial cells lose junctions, reorganize their cytoskeletons, and reprogram their gene expression profiles to acquire motility an invasive phenotype.[8] E-cadherin is an epithelial marker that maintains cell–cell adhesion and inhibits cell mobility and invasiveness.[9] Vimentin is a type III intermediate filament that is a marker of the mesenchymal phenotype of the EMT. The down-regulation of E-cadherin expression and concomitant up-regulation of vimentin expression provide the best indicators of the EMT in carcinomas.[10] Although evidence indicates that the EMT is associated with metastasis and poor prognosis of certain malignant tumors, including lung cancer,[11-13] the clinical significance of EMT phenotypes of stage IA lung adenocarcinoma is unknown.

Here we investigated the expression of EMT markers of stage IA lung adenocarcinoma and applied propensity score–matched analysis to evaluate the clinical and prognostic significance of EMT status.

Patients and Methods

Patients

We retrospectively analyzed data of consecutive patients who underwent surgical resection for primary lung adenocarcinomas at the Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, between January 2003 and December 2012. We ultimately enrolled 183 patients who were pathologically diagnosed with stage IA lung adenocarcinomas and had available paraffin-embedded specimens. Pathologic tumor stage was defined according to the criteria of the 7th edition of the International Association for the Study of Lung Cancer staging system. We examined the clinicopathologic features as follows: age at surgical resection, sex, smoking history, histologic growth type of adenocarcinoma, lymphovascular invasion, and epidermal growth factor receptor (*EGFR*) mutation status. Furthermore, we reviewed radiologic findings acquired using computed tomography (CT) and positron-emission tomography/computed tomography (PET/CT) upon surgical resection. After surgical resection, clinical examinations (physical examination, blood tests including serum tumor markers, and chest radiograph) were performed at 3-month intervals for the first 3 years and twice a year thereafter. Chest and upper abdominal CT was performed twice a year for the first 3 years and annually thereafter.

We measured the greatest consolidation diameter and the entire tumor diameter, including ground glass opacity, using 2-dimensional CT scanning of 1 mm or 2 mm sections, and calculated the ratio of consolidation diameter to tumor diameter (C/T). We acquired the standardized maximum uptake value (SUV_{max}) of tumors from PET/CT images to the extent possible.

Written informed consent was obtained from each patient before study inclusion. The institutional review board of Kyushu University approved our study (No. 30-41).

Immunohistochemistry

Formalin-fixed, paraffin-embedded specimens were sectioned into 4 μm thick sections, deparaffinized in xylene, and rehydrated through a graded ethanol series. Different immunohistochemical (IHC) techniques were used to detect the expression of E-cadherin and vimentin. The E-cadherin IHC protocol was as follows: (1) Target Retrieval Solution (Dako, Glostrup, Denmark) was used for antigen retrieval at 115° C for 15 minutes after inhibiting endogenous peroxidase activity with 3% hydrogen peroxidase in methanol. (2) Sections were incubated with an anti–E-cadherin monoclonal antibody (HECD-1, 1:1000; Takara, Shiga, Japan) at 4° C overnight. (3) Immunostaining was performed using the Envision Detection System (Dako). (4) Hematoxylin was used as a counterstain.

The vimentin IHC protocol was as follows: (1) Sections were incubated for 30 minutes in 3% hydrogen peroxidase in methanol without antigen retrieval. (2) Sections were incubated with an anti-vimentin monoclonal antibody (V-9, 1:25; Dako) at room temperature for 60 minutes. (3) Immunostaining was performed using the Envision Detection System (Dako). (4) Hematoxylin was as a counterstain.

Evaluation of IHC Staining

The levels of E-cadherin and vimentin were independently evaluated by at least two investigators. Vimentin expression was evaluated according the cytoplasmic staining of tumor cells (TCs) because the intensity of vimentin expression appeared to be evenly distributed throughout. In this study, the positive proportion score of vimentin expression was defined as $\geq$ 3% of stained TCs because nonspecific staining might be also considered as positive if positivity was defined as $\geq$ 1% of TCs. E-cadherin expression was evaluated using staining intensity and the proportion of TCs as previously reported.[14-16] E-cadherin staining intensities were classified as follows: 1 = weak staining, 2 = moderate staining, 3 = strong staining. The staining of positive TCs was expressed as percentages, and scores were assigned follows: 0 = 0, +1 = 1%-20%, +2 = 21%-40%, +3 = 41%-60%, and +4 = 61%-100%. The scores were then multiplied to calculate a final E-cadherin staining score. Final scores of $\geq$ 8 were judged positive for E-cadherin expression according to the receiver operating characteristic curve. If the independent assessments did not agree, then the stained slides were reviewed by another investigator to achieve consensus.

Patients were then divided into subgroups according to E-cadherin and vimentin expression as follows: (1) negative for vimentin and positive for E-cadherin, (2) positive for both vimentin and E-cadherin, (3) negative for both for vimentin and E-cadherin, and (4) positive for vimentin and negative for E-cadherin. We classified the staining patterns into groups as

Table 1 Analysis of Expression of E-Cadherin and Vimentin

	Vimentin Expression Undetected (N = 149, 81.4%)	Vimentin Expression Detected (N = 34, 18.6%)
E-cadherin Expression Negative (N = 58, 31.7%)	I: 44 (24.0%)	M: 14 (7.7%)
E-cadherin Expression Positive (N = 125, 68.3%)	E: 105 (57.4%)	I: 20 (10.9%)

Abbreviations: E = epithelial; I = intermediate; M = mesenchymal.

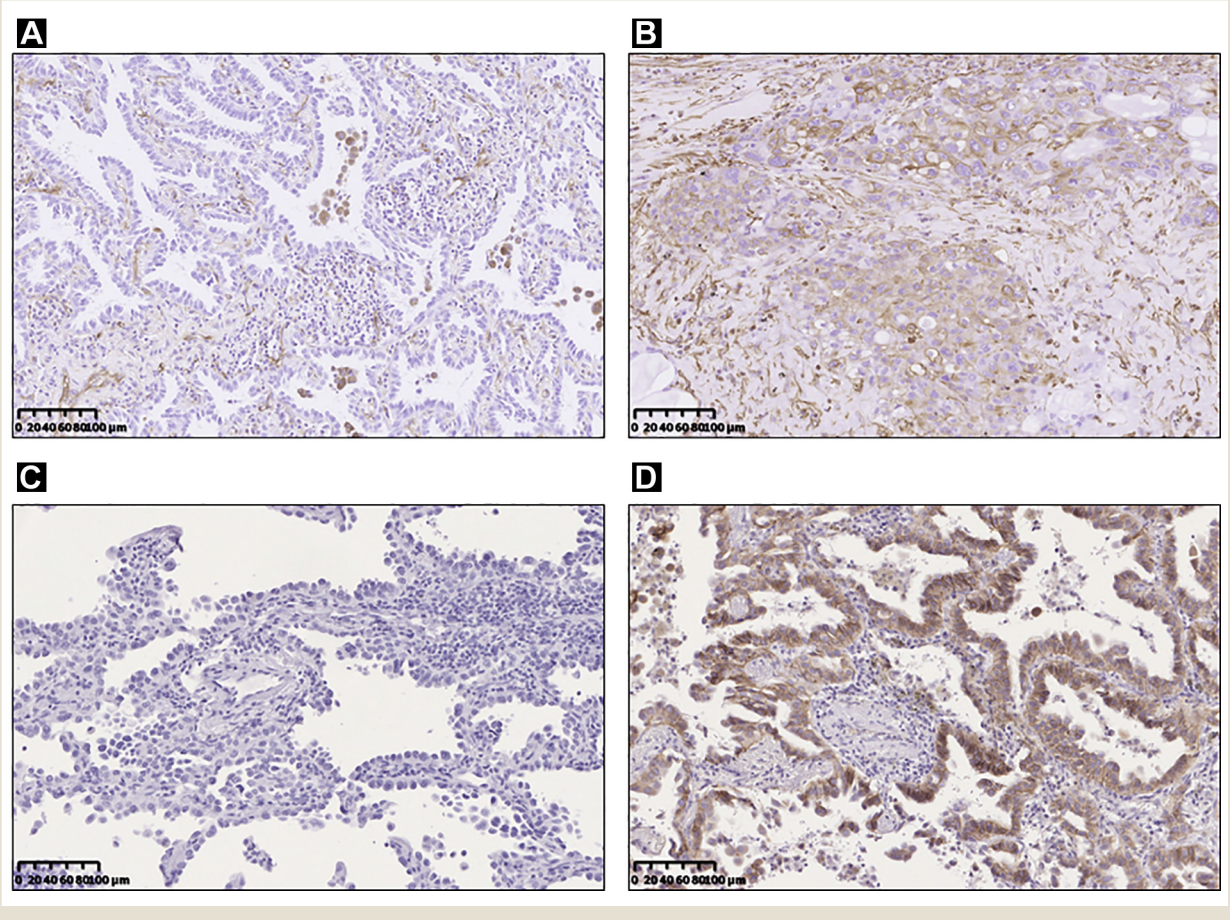
follows: E, epithelial = 1; I, intermediate = 2 and 3; and M, mesenchymal = 4 (Table 1).

Statistical Analysis

Continuous variables are expressed as the mean ± standard deviation, and categorical variables are expressed as numbers. We performed statistical evaluations using JMP 13 software (SAS Institute, Cary, NC). For continuous variables, differences were evaluated using a 2-sided Student *t* test. For categorical variables, statistical differences between groups were evaluated by the chi-square test or the Fisher exact test. Univariate analysis of the associations between the EMT and clinical factors was performed using logistic regression analysis. Disease-free survival (DFS) was defined as the interval between the date of surgery and the date of recurrence or death, and was censored at last follow-up. Overall survival (OS) was defined as the interval between the date of surgery and the date of death, and was censored at last follow-up. DFS and OS rates were estimated by the Kaplan-Meier method with the log-rank test. Univariate and multivariate analyses were used to estimate the hazard ratios (HRs) for independent prognostic values via Cox proportional hazards regression models with the backward elimination method. All factors assessed in the univariate analysis were included in the multivariate analysis. $P < .05$ was considered statistically significant.

Propensity score matching was performed to reduce the bias inherent in a retrospective study. A logistic regression model was

Figure 1 Vimentin and E-Cadherin Expression in Lung Adenocarcinoma Specimens. (A) Results of IHC that did not Detect Vimentin Expression. (B) IHC Showing Increased Vimentin Expression, Mainly in Cytoplasm of Tumor Cells. (C) IHC Showing Reduced E-Cadherin Expression. (D) IHC Showing That E-Cadherin Expression was Mainly Detected on Surface and in Cytoplasm of Tumor Cells



Abbreviation: IHC = immunohistochemistry.

used to calculate the propensity score using the following variables: age, sex, predominant histologic growth type, and lymphovascular invasion. A difference in propensity score of 0.20 was adopted as the maximum caliper width.

Results

Patient Characteristics

The clinicopathologic characteristics of the 183 enrolled patients with pathologic stage IA lung adenocarcinoma who underwent surgical resection are shown in Supplemental Table 1 in the online version. The median follow-up time was 60.3 months (range, 1.5-139.2 months). The median age of patients was 68 years (range, 34-85 years), 83 patients (45.3%) were men, and 88 patients (48.1%) had a history of smoking. One hundred twenty patients (65.6%) underwent lobectomy with standard dissection of the hilar and mediastinal lymph nodes. *EGFR* mutation status was available for 125 patients; of these, 57 (45.6%) were wild type and 68 (54.4%) harbored *EGFR* mutations, mainly exon 19 deletions or the L858R point mutation.

IHC analysis of TCs localized E-cadherin to the cell membrane or the cytoplasm (Figure 1A and B), and vimentin was detected in the cytoplasm (Figure 1C and D). E-cadherin was not detectably expressed in 58 samples (31.7%), and vimentin expression was detected in 34 samples (18.6%; Table 1). We classified 105 (57.4%), 64 (34.9%), and 14 (7.7%) patients into the E, I, and M groups, respectively. The I and M groups were regarded as the EMT conversion group (I/M).

Association Between EMT Conversion and Clinicopathologic Factors of Stage IA Lung Adenocarcinoma

A comparison of clinicopathologic features according to EMT status is shown in Table 2. The EMT conversion occurred more often in patients ≥ 68 years of age (64.1 vs. 35.9%, $P = .0048$). There were no significant differences between the EMT and other clinicopathologic factors.

Propensity score matching was performed to create a balanced cohort. The 128 matched patients were included in the propensity score–matched analysis (64 and 64 patients from groups E and I/M, respectively). After matching, the distribution of patients' characteristics among the groups was well balanced (Table 2). There were no significant differences in sex, smoking history, histologic type, lymphovascular invasion, and *EGFR* mutation status. The most variable clinical factor was the surgical procedure ($P = .3522$). We used this propensity score–matched population for further analyses.

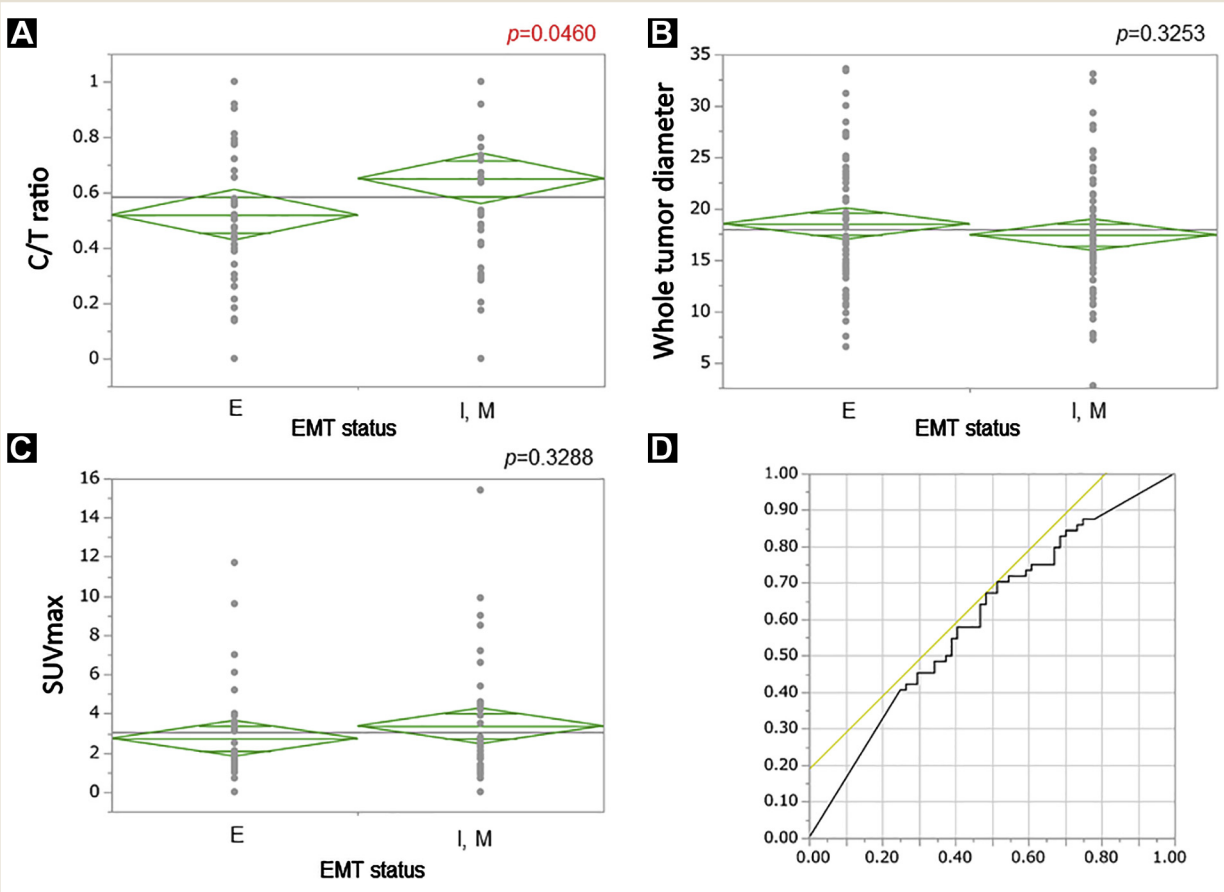
We investigated the radiologic features of tumors associated with the EMT conversion in the propensity score–matched cohort (Figure 2). A significantly greater C/T ratio was observed in tumors

Table 2 Associations Between EMT Phenotype and Patient Clinicopathologic Characteristics

	Before Propensity Score Matching			After Propensity Score Matching		
Characteristic	**E (N = 105)**	**I, M (N = 78)**	***P***	**E (N = 64)**	**I, M (N = 64)**	***P***
Age						
<68 years	60 (57.1%)	28 (35.9%)		28 (43.8%)	25 (39.1%)	
≥68 years	45 (42.9%)	50 (64.1%)	.0048	36 (56.2%)	39 (60.9%)	.7199
Sex						
Male	52 (49.5%)	31 (39.7%)		35 (54.7%)	35 (54.7%)	
Female	53 (50.5%)	47 (60.3%)	.2300	29 (45.3%)	29 (45.3%)	1.0000
Smoking History						
Never smoker	53 (50.5%)	42 (53.9%)		33 (51.6%)	33 (51.6%)	
Smoker	52 (49.5%)	36 (46.1%)	.6572	31 (48.4%)	31 (48.4%)	1.0000
Histologic Type						
Solid/micropapillary	31 (29.5%)	14 (18.0%)		15 (23.4%)	14 (21.9%)	
Other	74 (70.5%)	64 (82.0%)	.0838	49 (76.6%)	50 (78.1%)	1.0000
Lymphatic Invasion						
Absent	101 (96.2%)	72 (92.3%)		62 (96.9%)	62 (96.9%)	
Present	4 (3.8%)	6 (7.7%)	.3288	2 (3.1%)	2 (3.1%)	1.0000
Vascular Invasion						
Absent	91 (86.7%)	70 (89.7%)		56 (87.5%)	57 (89.1%)	
Present	14 (13.3%)	8 (10.2%)	.6476	8 (12.5%)	7 (10.9%)	1.0000
Surgical Procedure						
Sublobar resection	34 (32.4%)	29 (37.2%)		19 (29.7%)	25 (39.1%)	
Lobectomy	71 (67.6%)	49 (62.8%)	.5316	45 (70.3%)	39 (60.9%)	.3522
***EGFR* Status**						
Wild type	30 (41.7%)	27 (50.9%)		21 (51.2%)	22 (50.0%)	
Mutant	42 (58.3%)	26 (49.1%)	.3646	20 (48.8%)	22 (50.0%)	1.0000

Abbreviations: E = epithelial; EGFR = epidermal growth factor receptor; EMT = epithelial–mesenchymal transition; I = intermediate; M = mesenchymal.

Figure 2 Analyses of Association Between EMT Status and Radiologic Characteristics. (A) Tumors With EMT Conversion had Higher Preoperative C/T Compared to Those Without Conversion. CT Imaging did not Reveal a Significant Association Between EMT Status and Entire Tumor diameter (B) or SUV_{max} Determined Using PET/CT (C). (D) ROC Curve Analysis of C/T Ratio, Using EMT Status as State Variable. Optimal C/T Ratio Cutoff Value = 0.5



Abbreviations: C/T = consolidation diameter to tumor diameter; CT = computed tomography; EMT = epithelial–mesenchymal transition; ROC = receiver operating characteristic; SUV_{max} = standardized maximum uptake value.

with EMT conversion compared to those with the epithelial phenotype ($P = .046$; Figure 2A). In contrast, there was no significant difference in the radiologically determined entire tumor size associated with EMT status ($P = .3253$; Figure 2B. The mean value of SUV_{max} was 2.73 (range, 0.0-11.7) in the E group and 3.18 (range, 0.0-15.4) in the I/M group; however, the difference was not significant ($P = .3288$; Figure 2C).

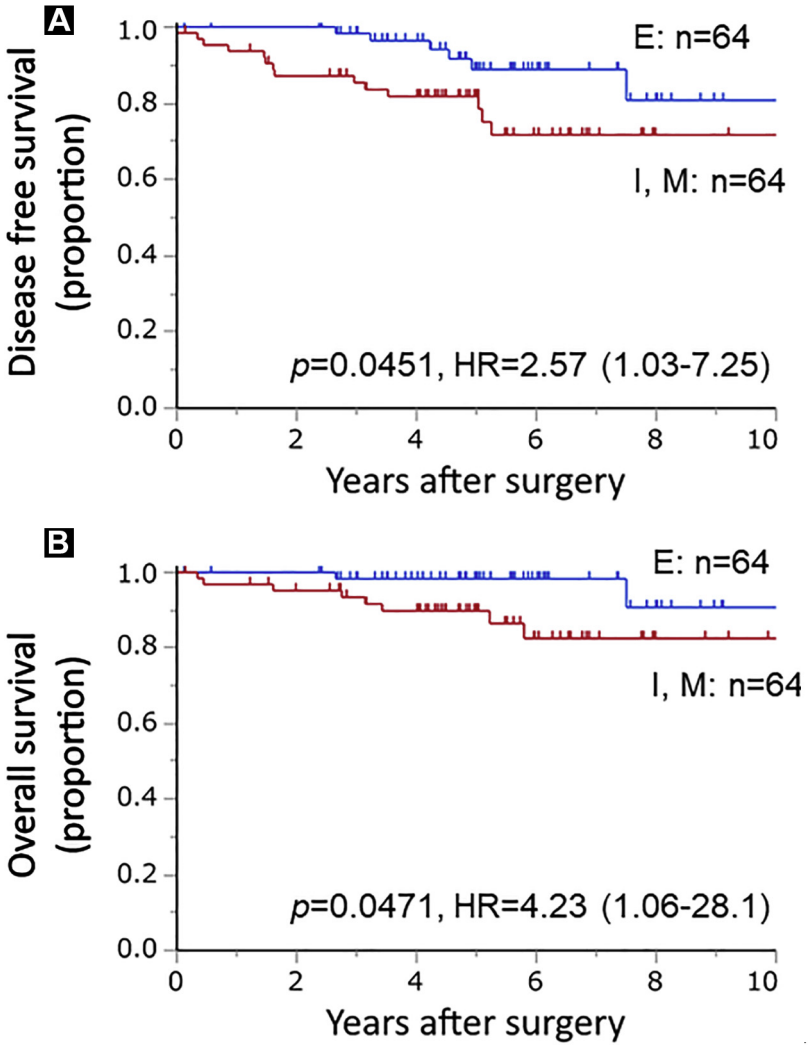
The cutoff value of the C/T ratio was determined using the receiver operating characteristic curve. EMT status was used as the state variable. The optimal C/T ratio cutoff value was 0.5, with an area under the curve, sensitivity, and specificity of 0.6018, 0.6719, and 0.5156, respectively (Figure 2D). Logistic regression analysis revealed that the group with high C/T ratios tended to be associated with EMT conversion (odds ratio = 1.92, $P = .0733$).

Prognostic Significance of EMT Conversion for Survival of Patients With Stage IA Lung Adenocarcinoma

We assessed the significance of the associations between an EMT phenotype and patient survival after surgical resection in the propensity score–matched population. The EMT conversion group was significantly associated with shorter DFS and OS compared to the E group (DFS: $P = .0451$, HR = 2.57; OS: $P = .0471$, HR = 4.23; Figure 3A and B). The 5-year survival rate of the EMT conversion group was significantly shorter compared to that of the E group (89.8% vs. 98.3%, respectively).

We then analyzed the independent predictive prognostic value of variables using Cox proportional hazards models in the propensity score–matched population (Table 3). Univariate and multivariate analysis of OS using this method may be uninformative because the number of events was very small. Therefore, we analyzed only DFS. Univariate analysis revealed that age $\geq$ 68 years (HR = 3.01, $P = .0305$), male sex (HR = 3.55, $P = .0062$), smoking history (HR = 2.50, $P = .0454$), sublobar resection (HR = 2.59, $P = .0349$), and EMT conversion (HR = 2.57, $P = .0430$) were associated with shorter DFS. Multivariate analysis revealed that male sex and EMT conversion were independent predictors of poor DFS (HR = 3.62, $P = .0056$ and HR = 2.63, $P = .0380$, respectively).

Figure 3 Kaplan-Meier Analysis of Survival Associated With EMT Status and Log-Rank Test Analysis of Propensity Score–matched Cohort EMT Conversion was Associated With (A) Poor DFS (HR = 2.57; 95% CI, 1.03-7.25; $P = .0451$) and (B) OS (HR = 4.23; 95% CI, 1.06-28.1; $P = .0471$) in Patients With Stage IA Lung Adenocarcinoma



Abbreviations: CI = confidence interval; DFS = disease-free survival; EMT = epithelial–mesenchymal transition; HR = hazard ratio; OS = overall survival.

Discussion

The phenotype of the EMT facilitates tumor invasion and progression, and is associated with poor survival of patients with NSCLC.[13,14,17] However, the associations between the EMT and clinicopathologic factors, including survival of patients with stage IA adenocarcinomas, is unclear. The present study found that stage IA adenocarcinoma with EMT conversion, which was defined as high levels of vimentin, low levels of E-cadherin, or both, was associated with poor survival.

The classic characteristics of the EMT include loss of epithelial polarity; loss of epithelial markers such as E-cadherin, cytokeratin, and occludin; and acquisition of mesenchymal markers such as vimentin and N-cadherin.[18] The EMT initiates with the dissociation of epithelial cell–cell contacts, which include tight junctions, adherens junctions, desmosomes, and gap junctions.[7] E-cadherin is cleaved and subsequently degraded during the destabilization of adherens junctions.[19] The dissociated epithelial cells subsequently undergo biochemical changes that generate the mesenchymal phenotype.

The mesenchymal marker vimentin is a type III intermediate filament that is expressed by mesenchymal cells of various tissues.[20] Here we used E-cadherin and vimentin as epithelial and mesenchymal markers, respectively. Table 4 presents a summary of IHC studies that used E-cadherin and vimentin as markers of the EMT to investigate the effects of survival associated with the EMT.[21-25] Some of these studies performed survival analysis associated with EMT as a function of the expression of E-cadherin and vimentin.[23,24] For example, studies of the associations between the

Table 3 Univariate and Multivariate Analyses of Disease-Free Survival in 128 Patients

Characteristic	N (%)	Univariate Analysis HR (95% CI)	*P*	Multivariate Analysis HR (95% CI)	*P*
Age, ≥ 68/< 68 years	75/53 (58.6%/41.4%)	3.01 (1.10-10.5)	.0305		
Sex, male/female	70/58 (54.7%/45.3%)	3.55 (1.42-10.0)	.0062	3.62 (1.45-10.2)	.0056
Smoking history, smoker/never smoker	62/66 (48.4%/51.6%)	2.50 (1.02-6.67)	.0454		
Histologic type, solid, micropapillary/other	29/99 (22.7%/77.3%)	3.04 (0.87-19.2)	.0865		
Lymphatic invasion, present/absent	4/124 (3.1%/96.9%)	1.51 (0.08-7.30)	.7067		
Vascular invasion, present/absent	15/115 (11.7%/88.3%)	1.97 (0.56-5.39)	.2585		
Surgical procedure, sublobar/lobectomy	44/84 (34.4%/65.6%)	2.59 (1.07-6.43)	.0349		
EGFR status, wild type/mutant	42/43 (49.4%/50.6%)	1.91 (0.60-7.15)	.2781		
EMT phenotype I, M/E	64/64 (50.0%/50.0%)	2.57 (1.03-7.25)	.0430	2.63 (1.05-7.43)	.0380

Abbreviations: E = epithelial; EGFR = epidermal growth factor receptor; EMT = epithelial–mesenchymal transition; HR = hazard ratio; I = intermediate; M = mesenchymal.

survival of patients with NSCLC and EMT status found that the null EMT conversion group (positive E-cadherin and negative vimentin) had the best prognosis.[23,24] The study population of the former[23] included approximately 80% of patients with pathologic stage I disease, and that of latter[24] included approximately 20% at the same disease stage. Despite the differences in patients' backgrounds, the results of their survival analyses are consistent with our present findings.

An important consideration for interpreting these results is that these previous studies included patients with an advanced stage of disease.[21-25] The EMT plays a crucial role in tumor invasion, metastatic spread, and progression.[6,7] Therefore, it is not unexpected that the EMT was associated with shorter survival in survival analyses that included patients with disease at an advanced stage. However, few studies address the prognostic significance of the EMT in patients with early-stage lung cancer.[26] The study cited found that the expression of EFHD2, which is a calcium-binding protein involved in immune cell activation, promoted the EMT and significantly correlated with postsurgical recurrence of stage I lung adenocarcinoma. However, this study did not accurately assess the influence of the EMT on the prognosis of stage I lung cancer.

Here we used propensity score matching to better evaluate the effects of the EMT on the prognosis of patients with stage IA lung adenocarcinoma. Furthermore, our multivariate analysis revealed that the EMT was an independent predictor of shorter DFS (HR = 2.63, $P = .0380$). Although numerous, the associations of diverse sets of prognostic clinical variables are available for stage I disease.[27-30] EMT status can predict DFS and OS during the early stages of disease, such as stage IA lung adenocarcinoma. The EMT has received attention as a mechanism that confers TCs with resistance to anticancer agents such as cytotoxic chemotherapy and molecularly targeted therapy.[31-33] The present study suggests that EMT status may serve as a novel criterion for administering adjuvant therapy to patients with stage IA lung adenocarcinoma.

Is it possible to predict tumors with EMT conversion before surgery? To answer this important question, we analyzed the preoperative radiologic characteristics according to EMT status. We found that tumors with EMT conversion had a high C/T ratio in preoperative CT images, but no significant association was obtained using the findings of PET/CT studies (Figure 2). Moreover, logistic regression analysis revealed that a relatively higher C/T ratio (> 0.5) can serve as a predictive factor of EMT conversion of stage IA lung

Table 4 Summary of IHC Studies That Aimed to Determine Association Between Survival of Patients With Lung Cancer and EMT, as Indicated by Expression of E-Cadherin and Vimentin

First Author, Journal (Year)	Histologic Type	No. of Patients	Stage	Results, HR (*P*)
Chikaishi, *Anticancer Res* (2011)[21]	Adenocarcinoma	183	I-IIIB	• NS (E-cadherin, vimentin)
				• E-cadherin: 1.73 (.183), vimentin: 1.326 (.452)
Kong, *Oncol Rep* (2014)[22]	NSCLC	NM	I-IV	• Poor OS (E-cadherin, vimentin)
				• NM (.008, .002, respectively)
Sowa, *Cancer Med* (2015)[23]	Adenocarcinoma	239	I-IIIB	• Poor OS (E-cadherin, vimentin, and EMT status)
				• NM (.003, .005, NM, respectively)
Zhou, *World J Surg Oncol* (2015)[24]	NSCLC	312	I-IIIA	• Poor Disease-specific survival (EMT status)
				• NM (< .001)
Tsoukalas, *Anticancer Res* (2017)[25]	NSCLC	112	I-IV	• Poor OS (vimentin)
				• 1.13 (.026)
Present study (2018)	Adenocarcinoma	183	IA	• Poor DFS and OS (EMT status)
				• DFS: 2.57 (.0451), OS: 2.51 (.0471)

Abbreviations: DFS = disease-free survival; EMT = epithelial–mesenchymal transition; HR = hazard ratio; IHC = immunohistochemistry; NM = not mentioned; NS = not significant; NSCLC = non–small-cell lung cancer; OS = overall survival.

adenocarcinoma (odds ratio = 1.92, $P = .0733$). Briefly, it is possible to predict a tumor with EMT conversion to some extent by measuring the C/T ratio in preoperative CT imaging.

In lung adenocarcinomas, radiologic ground glass opacity is associated with histologically noninvasive or minimally invasive TC phenotypes,[34] and several reports demonstrate that a higher C/T ratio is associated with tumor invasiveness and poor prognosis.[35-37] For example, the risk of recurrence of clinical stage IA adenocarcinoma is indicated by a high C/T ratio (> 0.5), and multivariate analysis indicates that sublobar pulmonary resection is an independent risk factor of DFS.[34] Moreover, even in stage IA lung adenocarcinoma, a high C/T ratio (particularly C/T ratios > 0.5) is likely associated with the EMT conversion. Thus, as a surgical approach for such tumors, lobectomy with standard lymph node dissection may be more desirable than limited resection. Phase 3 trials comparing lung lobectomy versus segmentectomy for lung cancers ≤ 2 cm with C/T ratios > 0.5 are in progress.[38,39] There is an association between ^{18}F-fludeoxyglucose uptake on PET and the EMT in NSCLC,[40,41] which was not observed here. The reason may be that both studies analyzed patients with advanced stages of disease and that we were unable to accumulate PET data from all cases in our cohort.

There are some limitations to our study. First, we retrospectively analyzed consecutive patients who underwent surgical resection, so we did not attempt a power analysis and could not perform any sample size justification. Second, we classified the EMT stage using IHC for E-cadherin and vimentin. Other transcription factors induce the EMT, such as SNAIL1/2, ZEB1/2, and TWIST.[42,43] It may therefore be possible to analyze the effects more precisely of the EMT on stage IA lung adenocarcinomas by evaluating these EMT-associated molecules.

Conclusion

The EMT conversion exerted a significantly unfavorable effect on the prognosis of stage IA lung adenocarcinoma patients. EMT status is important as a mechanism of resistance to anticancer drugs as well as a criterion for indicating the administration of adjuvant therapy to patients with stage IA lung adenocarcinoma.

Clinical Practice Points

- The EMT is associated with tumor invasion, cancer progression, and poor survival of malignant tumors, including NSCLC. However, the clinical and prognostic significance of the EMT in stage IA lung adenocarcinoma is unknown.
- We used IHC to analyze the expression of E-cadherin and vimentin in 183 patients with stage IA lung adenocarcinoma. Propensity score matching was used to evaluate the significance of the association of the EMT with survival.
- Tumors with EMT conversion had high preoperative C/T ratios that were significantly associated with poor DFS and OS. The EMT was an independent risk factor of DFS.
- These findings suggest that EMT status may serve as a novel criterion for administering adjuvant therapy.

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Disclosure

The authors have stated that they have no conflict of interest.

Supplemental Data

Supplemental tables and figures accompanying this article can be found in the online version at https://doi.org/10.1016/j.cllc.2019.04.006.

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Supplemental Table 1 Characteristics of 183 Patients With Pathologic Stage IA Lung Adenocarcinoma

Characteristic	Value
Age (years)	68 ± 9.0
Sex	
Male	83 (45.4%)
Female	100 (54.6%)
Smoking History	
Never smoker	95 (51.9%)
Smoker	88 (48.1%)
Histologic Type	
Solid/micropapillary	45 (24.6%)
Other	138 (75.4%)
Lymphatic Invasion	
Absent	173 (94.5%)
Present	10 (5.5%)
Vascular Invasion	
Absent	161 (88.0%)
Present	22 (12.0%)
Surgical Procedure	
Sublobar resection	63 (34.4%)
Lobectomy	120 (65.6%)
***EGFR* Status**	
Wild type	57 (31.1%)
Mutant	68 (37.2%)
Unknown	58 (31.7%)

Abbreviation: EGFR = epidermal growth factor receptor.