The Alternatively Spliced Actinin-4 Variant as a Prognostic Marker for Metastasis in Small-cell Lung Cancer

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Abstract. *Background: The alternatively spliced actinin-4 variant (ACTN4va) is expressed in small-cell lung cancer (SCLC) and is thought to be a potential diagnostic marker. However, ACTN4va expression has not been examined in transbronchial biopsy specimens. Materials and Methods: We retrospectively examined the relationship between ACTN4va expression, clinical factors and survival in 104 consecutive newly-diagnosed SCLC patients. Results: Of the 104 screened cases, 83 (median age=69 years; transbronchial biopsy, 71) were included in our study. Survival was significantly different in the group with no distant metastasis (1996 vs. 422 days, respectively; p=0.000115) but was not significantly different with regard to ACTN4va expression in the group with distant metastasis (293 vs. 254 days, respectively; p=0.678). Conclusion: ACTN4va expression was identifiable in small biopsy samples. ACTN4va expression was also significantly related to distant metastasis and could stratify SCLC patients according to prognosis.*

Small-cell lung cancer (SCLC) accounts for 15% of all lung cancers and is characterized by rapid progression and poor overall survival (1). Although the ability to predict prognosis at the time of diagnosis is difficult, patients with limited-stage disease (LD) may be cured by chemoradiotherapy, while those with extensive-stage disease (ED) have a poor prognosis.

Cell motility has a close relationship with cancer invasion and distant metastasis (2). Key factors, such as actin and the actin binding protein alpha-actinin-4 (ACTN4), regulate formation of the actin cytoskeleton and cell motility (3). In SCLC and normal testes, the alternatively spliced actinin-4 variant (ACTN4va) is expressed in place of full-length ACTN4 and is considered a potential diagnostic marker or new molecular target (4). Although ACTN4 expression has been examined in surgical biopsy specimens, it has not been examined by immunohistochemistry of transbronchial biopsy (TBB) specimens (5). As the majority of SCLC cases are diagnosed by TBB, it is necessary to reliably examine the immunohistochemical status of this small specimen.

In the present study, we investigated the relationship between ACTN4va expression in TBB and clinical factors of prognosis for SCLC.

Materials and Methods

Patients. We retrospectively examined 104 consecutive SCLC patients newly diagnosed between January 2004 and December 2007 at the Osaka Prefectural Medical Center for Respiratory and Allergic Diseases. This study was approved by the appropriate institutional review boards.

Our analysis included patients receiving first-line chemotherapy. Data concerning age, sex, biopsy method, stage, survival time, chemotherapy regimen, radiotherapy and response to treatment were collected. LD was defined as carcinoma confined to one hemithorax, including the bilateral, mediastinal lymph nodes and supraclavicular lymph nodes. Invasion beyond the hemithorax and the previously listed lymph nodes was defined as ED. The tumor/node/metastasis classification was identified by the new Union for International Cancer Control staging system for lung cancer (7th edition) (6). Survival information was calculated from the date of diagnosis. Cases of unknown final condition or survival at the end of the study were treated as censored cases. The treatment effect was based on the Response Evaluation Criteria in Solid Tumors (7). Curative (concurrently or sequentially) radiation was defined as thoracic radiation.

ACTN4va immunohistochemistry. Formalin-fixed, paraffin-embedded SCLC tissues were cut into 4-mm sections. Immunohistochemistry was performed with anti-ACTN4va primary monoclonal antibody

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Key Words: Small-cell lung cancer, actinin-4, transbronchial biopsy, biomarker, prognosis.

Table I. *Patients' characteristics*.

		ACTN4va (−)	ACTN4va (+)	*p*-Value
Age (range in years)		69 (49-85)		-
Overall survival (days)		370 (303-498)		-
Gender	Male	27	47	0.30
	Female	5	4	
PS	0-1	27	39	0.42
	2-4	5	12	
Stage	LD	23	23	0.023*
	ED	9	28	
Tumor size	T1–T2	16	18	0.25
	T3-T4	16	33	
Lymph node status	N0–N1	11	9	0.114
	N2-N3	21	42	
Distant metastasis	M0	21	22	0.070
	M1a-M1b	11	29	
Response to TRT	CR, PR	26	42	0.80
	PD	4	9	
Thoracic radiation	Yes	9	13	0.80
	No	23	28	
PCI	Yes	4	6	1.0
	No	28	45	

ACTN4va, Alternatively spliced actinin-4 variant expression; PS, performance status; TRT, thoracic radiation therapy; CR, complete response; PR partial response; PD, progressive disease; PCI, prophylactic cranial irradiation; LD, limited-stage disease; ED, extensive-stage disease.

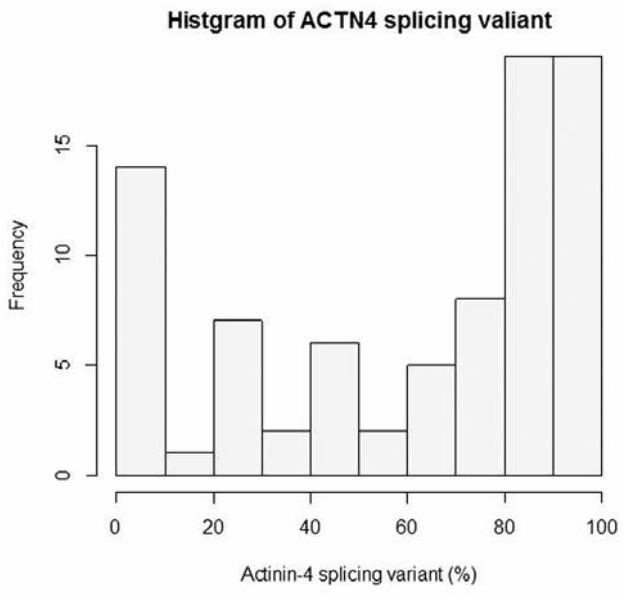

Figure 1. *Distribution of the alternatively spliced actinin-4 variant (ACTN4va) expression. Mean and median expressions were 63.5% and 80%, respectively.*

Table II. *Biopsy method and first-line chemotherapy.*

Category		N
Biopsy Method	TBB	71
	EBUS-TBNA	6
	Surgery	3
	Pleural biopsy	3
Chemotherapy	CBDCA+ETP	42
	CDDP+CPT-11	19
	CDDP+ETP	12
	CDDP+TOP	2
	AMR	2
	Others	6

TBB, Transbronchial biopsy; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; CBDCA, carboplatin; ETP, etoposide; CDDP, cisplatin; CPT-11, irinotecan; TOP, topotecan; AMR, amrubicin.

(clone 15H2; TransGenic Inc., Kobe, Japan) and a Ventana BenchMark GX (Roche Diagnostics K.K, Tokyo, Japan). ACTN4va expression was scored (positive cancer cells/all cancer cells) by 2 independent pathologists blinded to the clinical information. When opinions differed between the 2 pathologists, a final decision was made by consensus.

Statistical analysis. Patients' background data were compared using the Fisher's exact test for categorical data. A p-value <0.05 was considered statistically significant. Variables were divided into binary categories as follows: sex, an Eastern Cooperative Oncology Group performance status (PS) of 0-1 or 2-4, LD or ED, a tumor size of T1–T2 or T3–T4, lymph node status of N0–N1 or N2–N3, distant metastasis of M0 or M1a–M1b, response to treatment (complete response, partial response or progressive disease), thoracic radiation and prophylactic cranial irradiation. Survival time was measured from the date of diagnosis until death or censoring. Kaplan–Meier curves were used to estimate the survival time and log-rank tests were used to analyze differences in survival. Univariate Cox proportional hazard models were used to calculate hazard ratios (HR) and 95% confidential intervals (CI) between groups. Statistical analysis was performed using the R software (version 2.15.2; http://www.r-project.org).

Results

Patients and biopsy samples. One-hundred four cases of SCLC were diagnosed at our Institution from January 2004 to December 2007. Thirty-one samples were excluded: 10 could not be cut for additional slices, 8 were duplicates and 3 were obtained from patients treated at another Institution; therefore, a total of 83 cases (median age=69 years) were included in our study. Table I summarizes the clinical characteristics of the patients. Samples were obtained by surgical biopsy (3), TBB (71), endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) (6) and percutaneous pleural biopsy (3). Treatment included operation plus chemotherapy (2), chemoradiotherapy (22) and chemotherapy (59) (Table II).

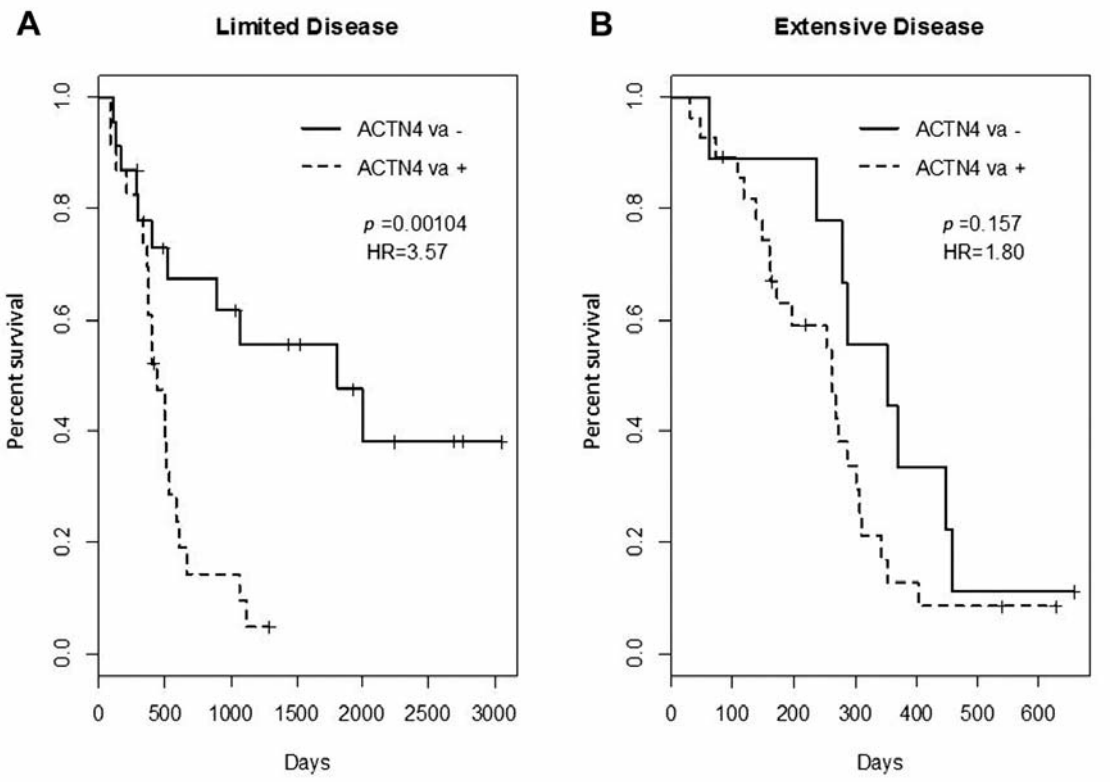

Figure 2. *A: Survival curves of limited disease patients divided into 2 groups according to the alternatively spliced actinin-4 variant (ACTN4va) status. The solid line shows patients with ACTN4va expression <65% and the broken line shows patients with ACTNva expression ≥65%. B: Survival curves of extensive-disease patients, divided into 2 groups according to ACTN4va status. The solid line shows patients with ACTN4va expression <65% and the broken line shows patients with ACTNva expression ≥65%. HR, hazard ratio.*

Relationship between ACTN4va expression and clinical factors. We show a histogram of ACTN4va in Figure 1. The mean and median ACTN4va expression values were 63.5% and 80%, respectively. Using the mean as a cut-off value, patients were divided into 2 groups: ACTNva-negative (expression <65%) and ACTN4va-positive (expression ≥65%). The patients' background ACTN4va statuses are summarized in Table I. The patient population (n=83) was predominantly male and the ACTN4va-positive group had significantly more cases of ED (p=0.015). ACTN4va positivity increased (p=0.07) when metastases were found. The most frequent chemotherapy regimen was carboplatin-plus-etoposide and there was no difference between treatment responses with regard to ACTN4va expression (p=0.80).

Relationship between ACTN4va expression and survival. Figure 2 displays the patients' survival curves by clinical stage. Survival was significantly different with regard to ACTN4va expression in the LD group (negative *vs.* positive expression: 1807 *vs.* 440 days, respectively; p=0.00104; HR=3.57, 95%CI=1.60-7.99) (Figure 2A). However, survival was not significantly different with regard to ACTN4va expression in the ED group (353 *vs.* 262 days, respectively; p=0.157; HR=1.80, 95%CI 0.79-4.08) (Figure 2B). Survival was significantly different in the group with no distant metastasis (1996 *vs.* 422 days, respectively; HR=5.41, 95%CI=2.10-13.9, p=0.000115) (Figure 3A) but was not significantly different with regard to ACTN4va expression in the group with distant metastasis (293 *vs.* 254 days, respectively; p=0.678; HR=1.17, 95%CI=0.57-2.41) (Figure 3B). Survival was significantly different with regard to ACTN4va expression in patients who received thoracic radiation (negative *vs.* positive expression: 1,996 *vs.* 498 days, p=0.00148; HR=8.31, 95%CI=1.80-38.4), 3-year survival rate: 8.97% *vs.* 72.8%, respectively) (Figure 4).

Discussion

Splice variants of oncogenic genes play an important role in tumor biology and may provide a tool for molecular-targeted therapy (8). Up-regulation of ACTN4 may increase the bundling of actin fibers and stimulate cytoskeletal rearrangement, thus facilitating cell motility and the formation of distant metastases. Interestingly, ACTN4va had a higher affinity for actin fibers (4).

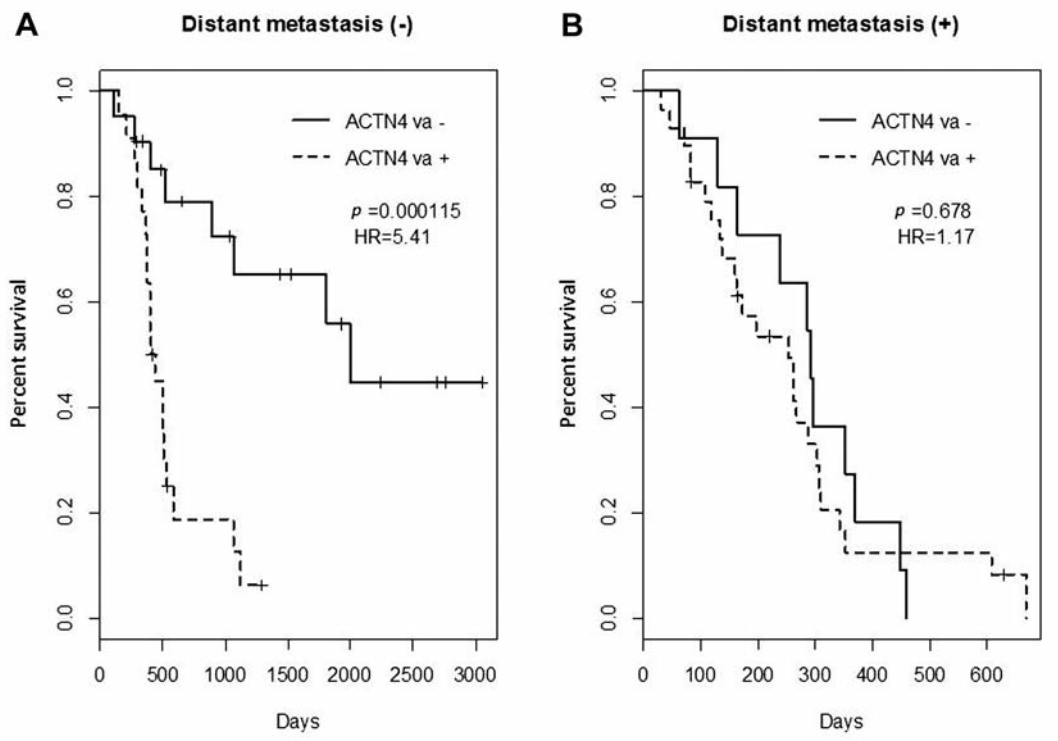

Figure 3. *A: Survival curves of patients without distant metastasis divided into 2 groups according to the alternatively spliced actinin-4 variant (ACTN4va) status. The solid line shows patients with ACTN4va expression <65% and the broken line shows patients with ACTN4va expression ≥65%. B: Survival curves of patients with distant metastasis divided into 2 groups according to ACTN4va status. The solid line shows patients with ACTN4va expression <65% and the broken line shows patients with ACTN4va expression ≥65%. HR, hazard ratio.*

ACTN4 expression is related to lymph node and distant metastases and has been reported as a prognositic marker in non-small cell lung (9), bladder (10), colon (11), ovarian (12, 13) and pancreatic cancers (14, 15). ACTN4va has been reported as a prognostic factor in SCLC using SCLC surgical samples and large cell neuroendocrine carcinomas (5). In clinical practice, the majority of SCLCs are diagnosed by bronchoscopy specimens, rather than surgical biopsy, which are small and have a tendency to be crushed because SCLC cells have a small amount of cytoplasm (16). Therefore, expression discrimination would be difficult if the cytoplasm was the main source of staining. ACTN4va can be found in both the membrane and cytoplasm; therefore, it can be regarded as a useful marker. Although EBUS-TBNA results in a specimen size smaller than that of TBB, all 6 specimens obtained by EBUS-TBNA in this study had successful ACTN4va staining. One report suggested that EBUS-TBNA could recover a sufficient specimen for genetic analysis, including fluorescence *in situ* hybridization (17).

ACTN4va expression reflected the prognosis of SCLC without distant metastasis. A previous multidisciplinary approach for the treatment of LD-SCLC had a 3-year survival rate of 30% (18). In this study, the 3-year survival rates with chemoradiotherapy were 72.9% and 8.97% in the ACTN4va-negative and -positive groups, respectively. ACTN4va expression strongly reflected distant metastasis and invasion and could stratify patients according to their risk of metastasis. We believe that ACTN4va-negative cases could be alleviated of SCLC, while ACTN4va-positive cases have the possibility of distant metastasis and should be considered as candidates for prophylactic cranial irradiation and more powerful anti-cancer therapies.

This study had several limitations. First, this was a retrospective study at a single Institute with a small sample size. Second, the cut-off ACTN4va value was the average value in this case and it may vary with increasing sample size.

Conclusion

ACTN4va expression was pathologically identifiable by TBB, significantly related to distant metastasis and could stratify SCLC patients according to prognosis. ACTN4va expression may be a strong prognositic marker of metastasis in cases of SCLC. ACTN4va expression may also be useful for detecting cases of complete cure in LD-SCLC patients and making correct evaluations for the risk of recurrence. Future studies

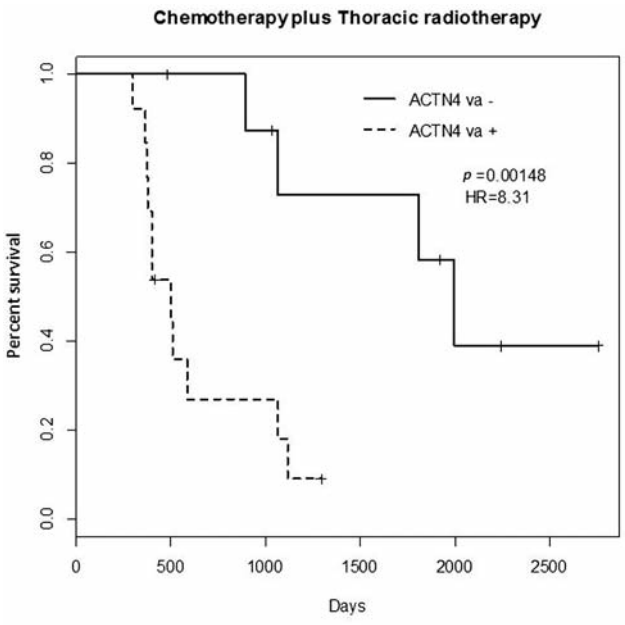

Figure 4. *Survival curves of patients receiving chemotherapy and curative thoracic radiation divided into 2 groups according to the alternatively spliced actinin-4 variant (ACTN4va) status. The solid line shows patients with ACTN4va expression <65% and the broken line shows patients with ACTNva expression ≥65%. HR, hazard ratio.*

involving the prospective analysis of a multidisciplinary approach regarding ACTN4va status are warranted.

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