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Histopathologic analysis of brain metastasis in pulmonary adenocarcinoma: Necrosis is a new risk factor

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ABSTRACT

Background: Studies have shown that 30–50% of non-small cell lung cancer (NSCLC) patients develop brain metastasis (BM). Since BM shortens overall survival and decreases the quality of life, early detection and treatment of BM are vital. While data are available for clinical risk factors of NSCLC with BM, histopathological factors are not well understood. Therefore, we evaluated the histopathological related factors which will help early detection and selection of effective treatment options.

Materials and methods: A total of 117 surgical lung specimens diagnosed as NSCLC with BM were included as a study group. We included 237 cases without BM as a control group. One pathologist reviewed H&E slides and analyzed the histopathologic factors of all cases.

Results: In pulmonary adenocarcinoma, vascular invasion, N stage, micropapillary pattern and necrosis were significantly associated with BM in multivariate analysis (vascular invasion, $p = 0.009$; micropapillary pattern, $p = 0.024$; others, $p < 0.001$). Tumor with extensive necrosis had higher hazard ratio and shorter time to BM ($p < 0.001$).

Conclusion: Our findings suggest that necrosis is a new predictive factor of BM in pulmonary adenocarcinoma. Short term follow-up is needed especially when extensive necrosis is present.

1. Introduction

Lung cancer is well known for its high mortality rate. According to the 2015 WHO statistics, it is the most common cause of cancer-related death. In non-small cell lung cancer (NSCLC), which accounts for approximately 85% of lung cancer [1], 30–50% of patients will develop brain metastasis (BM) [2] which is a higher percentage than other cancers [3]. Untreated NSCLC patients with BM have 1–2 months median overall survival (OS) [4,5], but aggressive management can increase OS to 12.1 months [6]. Although many recent publications have suggested that chemotherapy or targeted therapy may be an effective treatment option for BM, the treatment of BM differs from that of metastases to other organs [7]. Since there is a limited delivery of chemotherapeutic agents due to the blood-brain barrier, most clinicians choose surgery or radiotherapy instead of chemotherapy for the treatment. Furthermore, BM is associated with many neurologic symptoms which can significantly affect the quality of life. When it comes to edema, emergent situations like seizure or brain herniation may occur. Since BM shortens overall survival and decreases the quality of life, it is important to detect and treat BM early.

To date, risk factors for BM in NSCLC have been reported, including young age, adenocarcinoma, large primary tumor size, and high N stage [8–12]. While data are available for clinical risk factors of NSCLC with BM, histopathological risk factors are not well understood. We reviewed H&E slides and evaluated the histopathological related factors which will help early detection and selection of effective treatment options.

Abbreviations: NSCLC, non-small cell lung cancer; BM, brain metastasis; OS, overall survival; STAS, spread through air spaces; TAF, tumor associated fibrosis; VEGF, vascular endothelial growth factor

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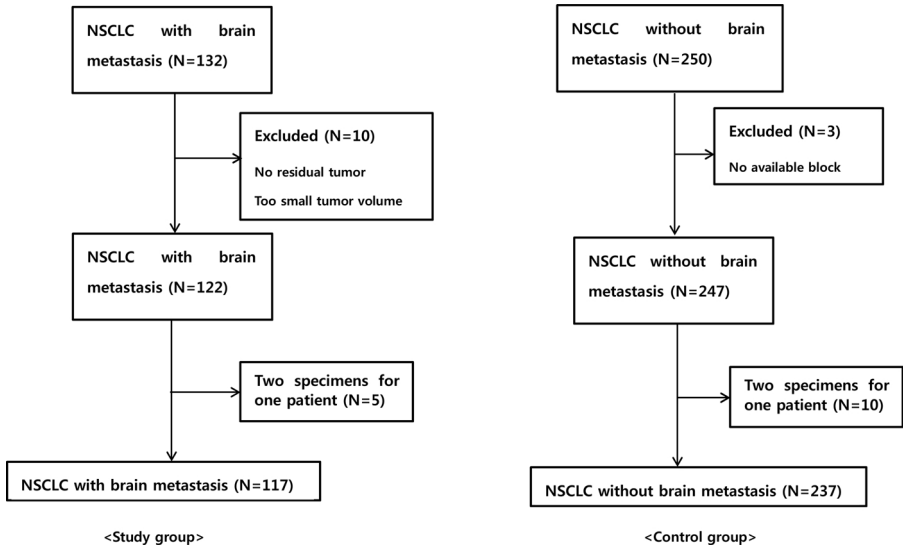

Fig. 1. Flow charts of study and control groups of non-small cell lung cancer (NSCLC) patients.

2. Materials and methods

2.1. Data collection

We collected a total of 132 surgical lung specimens diagnosed as NSCLC with BM as a study group at a single institution from January 2012 to June 2017. We collected BM cases diagnosed via: surgery, CSF tapping, and brain MRI. The cases of which the results of CSF tapping were 'suspicious for malignancy' or 'malignancy', and which brain MRI exhibited definitive evidence of BM, were included. Patients with extrathoracic metastases not involving the brain were excluded to analyze factors only related to BM. Ten cases that had no residual tumors or small tumor volume after pre-operative therapy were also excluded from this study. Five patients underwent lobectomy twice. Two separate nodules were found in four out of five patients over time, and on another patient upon the first diagnosis. More details are described on 'histopathologic analysis' of Materials and methods. Finally, 117 surgical lung specimens were included in the study group (Fig. 1).

We selected 250 cases without BM as a control group. Patients with extrathoracic metastases not involving the brain were excluded to analyze factors only related to BM. Three cases without blocks were also excluded from this study. Ten patients underwent lobectomy twice. Multiple nodules were found in eight out of ten people over time and others at the first diagnosis. Finally, 237 surgical lung specimens were included in the control group (Fig. 1). All patients of the control group underwent sufficient follow-up considering median time to BM [13,14]. All patients of both groups had no previously diagnosed or suspected cancers other than lung cancer.

Clinical data included sex, age at diagnosis, follow up period, and time to BM. Histopathologic data included a type of tumor cells, tumor size, presence of vascular invasion, lymphatic invasion, perineural invasion, pleural invasion, resection margin status, and the pathologic stage according to the AJCC 8th edition. Clinical and histopathological data were extracted from the electronic medical records. This study was approved as a retrospective study without informed consent by the institutional review board of the hospital (2018-03-140).

2.2. Histopathologic analysis

One pathologist reviewed H&E slides of both the study and control groups. When the patients underwent two operations, the slides from the two operations were compared to determine whether the tumor was metastatic or synchronous. In the case of metastasis, only the primary tumor was included, while in the case of a synchronous tumor, only the larger tumor was included in the analysis. Such decision was based on a previous article, which stated that tumor size was a risk factor for BM [11]. If the tumor was an adenocarcinoma, the following additional factors were evaluated: dominant growth pattern, presence of solid, cribriform, and micropapillary patterns, and the degree of spread through air spaces (STAS), tumor associated fibrosis (TAF), and necrosis (Fig. 2).

2.2.1. Examination of STAS

We examined all tumor edges to select some fields with abundant STAS, then classified them as predominantly micropapillary/solid or single cell type. Micropapillary/solid type cases were classified as having no STAS, low STAS (1–4 clusters), or high STAS ($\geq$5 clusters). Single cell type cases were classified as having no STAS, low STAS (1–4 cells), or high STAS ($\geq$5 cells). Above criteria were based on the previous publications [15,16].

2.2.2. Examination of TAF

We defined tumor associated fibrosis (TAF) as stromal fibroblasts running side by side with the tumor (Fig. 2E). We evaluated the degree of TAF from all H&E slides and classified them as having no TAF, low TAF ($<$ 5%), intermediate TAF ($<$ 10%), or high TAF ($\geq$10%). TAF was not evaluated in case of pre-operative therapy, because it was difficult to distinguish them from therapy-related fibrosis.

2.2.3. Examination of necrosis

We reviewed all H&E slides and evaluated the degree of necrosis. They were classified as having no necrosis, focal necrosis, or extensive necrosis. We defined small punctuated necrosis which can be observed at high magnification (10×, 20×, or 40×) as focal necrosis and large necrosis which can be observed at low magnification (4×) as extensive necrosis. Necrosis was not evaluated in cases of pre-operative therapy, because it was difficult to distinguish them from therapy-related necrosis.

2.3. Statistical analysis

Continuous variables were expressed as medians within the range. Follow up period was analyzed using student T-test. All categorical variables were first analyzed by univariate analysis then entered into multivariate analysis with $p < 0.05$ using Cox proportional hazards model. Since T stage reflects tumor size and pleural invasion, it was

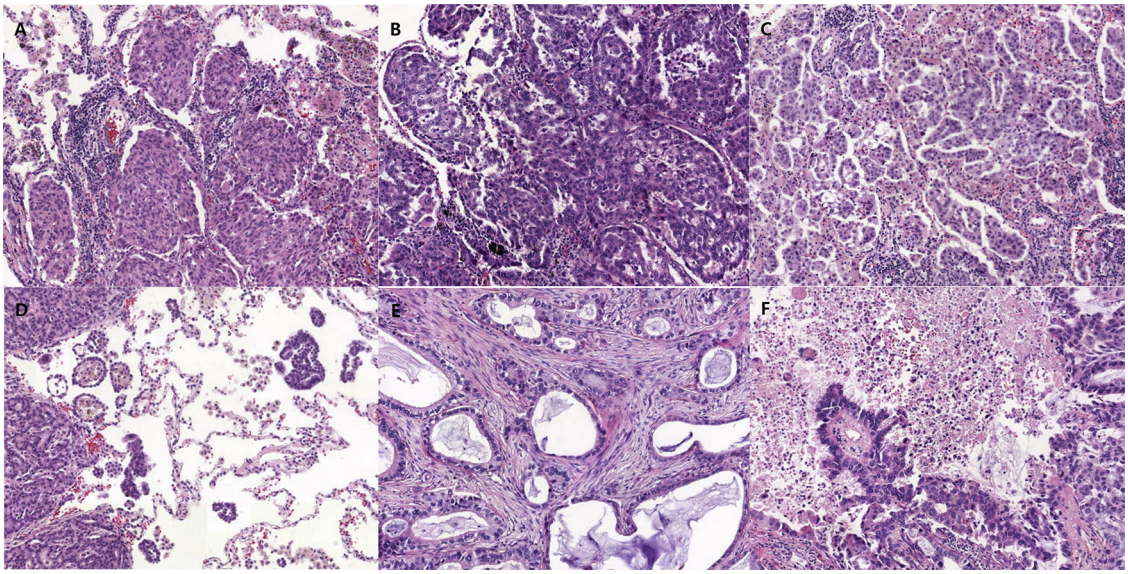

Fig. 2. Representative histopathologic findings of adenocarcinoma. (A) Solid pattern forming sheets of tumor cells (B) Cribriform pattern formed by back-to-back fused glands with lacking intervening stroma (C) Micropapillary pattern growing in papillae that lack fibrovascular cores (D) Spreading through air spaces (STAS) which spreads of tumor cells within air spaces beyond the edge of the main tumor (E) Tumor associated fibrosis (TAF) which is a proliferation of fibroblasts running side by side with the tumor cells (F) Necrosis.

applied as a representative factor in multivariable analysis. N stage reflecting the lymphatic invasion was applied representatively. Time to BM curves were generated by the Kaplan-Meier method and were compared using the log-rank test. P-value < 0.05 was interpreted as significant, and was indicated in bold in following tables. All statistical analyses were performed using SPSS software version 25.

3. Results

3.1. Clinicopathologic features of non-small cell carcinoma

A total of 354 patients with NSCLC with BM (n = 117) and NSCLC without BM (n = 237) were enrolled in the study. The clinical characteristics of the two groups of the patients are presented in Table 1. There was no significant difference in sex and age between the two groups (p = 0.207, 0.139). Follow up period showed significant difference between the two groups (p = 0.031).

Tumor size, which was subdivided by T stage, vascular invasion, lymphatic invasion, perineural invasion, pleural invasion, T stage and N stage showed a significant association with BM (perineural invasion, p = 0.012; others, p < 0.001). However, there was no significant difference in tumor type, and margin status (< 1 cm) between the two groups (p = 0.822, 0.571).

On multivariate analysis, vascular invasion, T stage and N stage were significantly associated with BM (p = 0.002, 0.006, and < 0.001). Vascular invasion increased the risk of BM by 2.342 times. High T stage increased the risk of BM by 1.856 (T2 vs T1), 2.347 (T3 vs T1), and 3.464 (T4 vs T1) times. Lymph node metastasis also increased the risk of BM by 3.332 (N0 vs N1), and 4.935 (N0 vs N2) times.

The details of the clinical and histopathological factors in the two groups are presented in Table 1. Tumor cell types of the two groups are presented in Supplementary Tables 1 and 2.

3.2. Clinicopathologic features of adenocarcinoma

We selected adenocarcinoma cases from each group and reviewed the representative H&E slides. A total of 287 cases consisting of 97 BM cases and 190 control cases were included. The most frequent dominant pattern was papillary (51.5%) in the study group and acinar (47.4%) in the control group. Solid, cribriform, and micropapillary patterns were significantly associated with BM (p < 0.001). STAS, TAF, and necrosis also exhibited a significant association (p < 0.001).

On multivariable analysis, vascular invasion (p = 0.009), N stage (p < 0.001), micropapillary pattern (p = 0.024), and necrosis (p < 0.001) were significant risk factors for BM in lung adenocarcinoma. Vascular invasion and micropapillary pattern increased the risk of BM by 2.501 and 2.128 times. Lymph node metastasis increased the risk of BM by 5.046 (N0 vs N1), and 4.460 (N0 vs N2) times. Necrosis increased the risk of BM by 5.227 (No vs Focal), and 8.713 (No vs Extensive) times.

The details of the clinical and histopathological factors in the two groups can be found in Table 2. The results of other factors were similar to those seen with NSCLC in Table 1, but follow up period did not show significant difference between the two groups (p = 0.318).

3.2.1. Time to brain metastasis

There were statistically significant differences in time to BM depending on vascular invasion, N stage, micropapillary pattern, and necrosis (p < 0.001, Fig. 3). The higher degree of necrosis, the shorter the time to BM.

3.3. Clinicopathologic features of squamous cell carcinoma

We selected squamous cell carcinoma cases from each group. A total of 53 cases consisting of 14 BM cases and 39 control cases were included. Only T stage and N stage were significantly associated with BM (p = 0.038, 0.002). On multivariate analysis, N stage was a significant risk factor of BM in squamous cell carcinoma. Lymph node metastasis increased the risk of BM by 4.893 (N0 vs N1), and 8.721 (N0 vs N2) times. The details of the clinical and histopathological factors in the two groups can be found in Table 3.

3.4. Clinicopathologic features of non-adenocarcinoma/non-squamous cell carcinoma

We selected non-adenocarcinoma/non-squamous cell carcinoma cases including combined two types of carcinoma, pleomorphic carcinoma, or salivary gland-type tumor from each group. A total of 14 cases consisting of 6 BM cases and 8 control cases were included. There was no significantly related factor identified. The details of the clinical and

Table 1
Cox univariate and multivariate analysis of clinicopathologic features of non-small cell lung carcinoma.

a. univariate analysis

	BM (N = 117)	Control (N = 237)	HR (95% CI)	p value
Clinical factor				
Sex (M:F)	65:52	117:120	1.265(0.878-1.822)	0.207
Age at diagnosis (median)	59 (36-79)	63 (37-90)	1.315(0.915-1.889)	0.139
≤60 yrs	59	96		
> 60 yrs	58	141		
Follow up period (median, months)	38 (5-86)	43 (17-104)		**0.031**
Time to brain metastasis (median, months)	13 (0-60)			
Histopathologic factor				
Tumor type				
Adenocarcinoma	97 (82.9%)	190 (80.2%)	0.946(0.585-1.531)	0.822
Squamous cell carcinoma	14 (12.0%)	39 (16.4%)		
others	6 (5.1%)	8 (3.4%)		
Tumor size*				< **0.001**
≤3cm	36 (39.6%)	158 (68.7%)		
≤5cm	32 (35.1%)	50 (21.7%)	2.370(1.471-3.816)	< **0.001**
≤7cm	15 (16.5%)	14 (6.1%)	3.688(2.015-6.750)	< **0.001**
> 7cm	8 (8.8%)	8 (3.5%)	4.909(2.255-10.686)	< **0.001**
Vascular invasion				< **0.001**
Absent	88 (75.2%)	229 (96.6%)		
Present	29 (24.8%)	8 (3.4%)	4.424(2.888-6.778)	
Lymphatic invasion				< **0.001**
Absent	53 (45.3%)	183 (77.2%)		
Present	64 (54.7%)	54 (22.8%)	2.993(2.077-4.312)	
Perineural invasion				**0.012**
Absent	106 (90.6%)	230 (97.0%)		
Present	11 (9.4%)	7 (3.0%)	2.227(1.196-4.149)	
Pleural invasion				< **0.001**
Absent	86 (73.5%)	216 (91.1%)		
Present	31 (26.5%)	21 (8.9%)	2.580(1.708-3.895)	
Margin (< 1 cm)				0.571
≥1 cm	97 (85.1%)	204 (87.9%)		
< 1 cm	17 (14.9%)	28 (12.1%)	1.161(0.693-1.943)	
T stage*				< **0.001**
T1	27 (29.7%)	151 (65.7%)		
T2	38 (41.7%)	55 (23.9%)	3.045(1.858-4.990)	< **0.001**
T3	15 (16.5%)	16 (6.9%)	4.168(2.214-7.848)	< **0.001**
T4	11 (12.1%)	8 (3.5%)	6.962(3.404-14.237)	< **0.001**
N stage**				< **0.001**
N0	46 (40.4%)	207 (90.4%)		
N1	20 (17.5%)	12 (5.2%)	4.491(2.651-7.609)	< **0.001**
N2	47 (41.2%)	10 (4.4%)	6.941(4.594-10.487)	< **0.001**
N3	1 (0.9%)	0 (0%)	6.437(0.884-46.849)	0.066

b. multivariate analysis

Risk factor	HR (95% CI)	p value
Vascular invasion	2.342 (1.360-4.032)	**0.002**
T stage		**0.006**
T2 (vs T1)	1.856 (1.097-3.138)	**0.021**
T3 (vs T1)	2.347 (1.196-4.609)	**0.013**
T4 (vs T1)	3.464 (1.621-7.402)	**0.001**
N stage		< **0.001**
N1 (vs N0)	3.332 (1.796-6.181)	< **0.001**
N2 (vs N0)	4.935 (2.925-8.327)	< **0.001**

Bold values are $P < 0.05$.
* Tumor size and T stage are evaluated after excluding pre-operative therapy cases.
** N stage is evaluated after excluding Nx cases.

histopathological factors in the two groups can be found in Table 4.

4. Discussion

In this study, we retrospectively investigated the histopathologic factors related to BM by reviewing H&E slides of 354 cases from a single institution. In pulmonary adenocarcinoma, we found that 1) necrosis is a new independent risk factor of BM; 2) the higher degree of necrosis, the shorter the time to BM. Taken together, our findings suggest that necrosis is a new predictive factor of BM and short term follow-up is needed when extensive necrosis is present in pulmonary adenocarcinoma.

Our first major finding is that necrosis is a new independent risk factor of BM. Angiolymphatic invasion is a well-known risk factor [17] and micropapillary pattern had been reported several times in previous reports [17,18]. The relationship between necrosis and BM has yet to be reported, although their positive association can be sensibly postulated. Due to the high rate of proliferation, tumors are well-associated to hypoxic environment, leading to necrosis where many genes related to hypoxia and angiogenesis of tumor cells are upregulated [19]. Many

Table 2
Cox univariate and multivariate analysis of clinicopathologic features of adenocarcinoma.

a. univariate analysis

	BM (N = 97)	Control (N = 190)	HR (95% CI)	p value
Clinical factor				
Sex (M:F)	49:48	77:113	1.403(0.942-2.090)	0.095
Age at diagnosis (median)	59 (36-79)	61.5 (37-90)	1.423(0.952-2.127)	0.085
≤60 yrs	55	86		
> 60 yrs	42	104		
Follow up period (median, months)	42 (5-86)	43 (18-104)		0.318
Time to brain metastasis (median, months)	15 (0-60)			
Histopathologic factor				
Tumor size*				< **0.001**
≤3cm	33 (44.6%)	143 (77.3%)		
≤5cm	28 (37.8%)	32 (17.3%)	2.975(1.796-4.927)	< **0.001**
≤7cm	9 (12.2%)	7 (3.8%)	3.700(1.768-7.745)	**0.001**
> 7cm	4 (5.4%)	3 (1.6%)	5.738(2.006-16.410)	**0.001**
Vascular invasion				< **0.001**
Absent	75 (77.3%)	188 (99.0%)		
Present	22 (22.7%)	2 (1.0%)	5.120(3.154-8.314)	
Lymphatic invasion				< **0.001**
Absent	39 (40.2%)	155 (81.6%)		
Present	58 (59.8%)	35 (18.4%)	4.361(2.898-6.564)	
Perineural invasion				**0.001**
Absent	89 (91.8%)	189 (99.5%)		
Present	8 (8.2%)	1 (0.5%)	3.663(1.771-7.576)	
Pleural invasion				< **0.001**
Absent	72 (74.2%)	177 (93.2%)		
Present	25 (25.8%)	13 (6.8%)	2.707(1.715-4.272)	
Margin				0.075
≥1 cm	83 (88.3%)	175 (95.1%)		
< 1 cm	11 (11.7%)	9 (4.9%)	1.768(0.944-3.312)	
T stage*				< **0.001**
T1	24 (32.4%)	136 (73.5%)		
T2	35 (47.3%)	37 (20.0%)	3.892(2.312-6.552)	< **0.001**
T3	9 (12.2%)	9 (4.9%)	4.006(1.861-8.625)	< **0.001**
T4	6 (8.1%)	3 (1.6%)	8.244(3.314-20.504)	< **0.001**
N stage**				< **0.001**
N0	36 (38.3%)	170 (93.4%)		
N1	15 (16.0%)	4 (2.2%)	6.434(3.509-11.796)	< **0.001**
N2	42 (44.7%)	8 (4.4%)	7.741(4.919-12.180)	< **0.001**
N3	1 (1.0%)	0 (0%)	7.358(1.003-53.993)	0.050
Dominant pattern				
Lepidic	0 (0%)	4 (2.1%)		
Acinar	9 (9.3%)	90 (47.4%)		
Papillary	50 (51.5%)	77 (40.5%)		
Solid	22 (22.7%)	12 (6.3%)		
Micropapillary	1 (1.0%)	2 (1.1%)		
Cribriform	12 (12.4%)	5 (2.6%)		
Unclassifiable	3 (3.1%)	0 (0%)		
Solid pattern				< **0.001**
Absent	60 (61.9%)	168 (88.4%)		
Present	37 (38.1%)	22 (11.6%)	3.335(2.206-5.042)	
Cribriform pattern				< **0.001**
Absent	38 (39.2%)	150 (78.9%)		
Present	59 (60.8%)	40 (21.1%)	3.733(2.479-5.622)	
Micropapillary pattern				< **0.001**
Absent	23 (23.7%)	101 (53.2%)		
Present	74 (76.3%)	89 (46.8%)	2.821(1.766-4.507)	
STAS***				< **0.001**
No	36 (37.1%)	153 (80.5%)		
Low	7 (7.2%)	6 (3.2%)	3.181(1.414-7.157)	**0.005**
High	54 (55.7%)	31 (16.3%)	4.640(3.029-7.108)	< **0.001**
TAF****				< **0.001**
No	15 (20.3%)	133 (71.9%)		
Low	24 (32.4%)	26 (14.1%)	5.612(2.941-10.706)	< **0.001**
Intermediate	9 (12.2%)	8 (4.3%)	7.685(3.338-17.689)	< **0.001**
High	26 (35.1%)	18 (9.7%)	9.669(5.080-18.403)	< **0.001**
Necrosis				< **0.001**
No	20 (27.0%)	154 (83.3%)		
Focal	26 (35.1%)	20 (10.8%)	6.918(3.847-12.438)	< **0.001**
Extensive	28 (37.9%)	11 (5.9%)	10.950(6.099-19.658)	< **0.001**

b. multivariate analysis

(*continued on next page*)

Table 2 (*continued*)

Risk factor	HR (95% CI)	p value
Vascular invasion	2.501 (1.252-4.993)	**0.009**
N stage		< **0.001**
N1 (vs N0)	5.046 (2.375-10.720)	< **0.001**
N2 (vs N0)	4.460 (2.354-8.451)	< **0.001**
Solid	1.741 (0.946-3.204)	0.075
Micropapillary	2.128 (1.105-4.098)	**0.024**
Necrosis		< **0.001**
Focal (vs No)	5.227 (2.425-11.269)	< **0.001**
Extensive (vs No)	8.713 (3.953-19.205)	< **0.001**

Bold values are P < 0.05.

* Tumor size and T stage are evaluated after excluding pre-operative therapy cases.

** N stage is evaluated after excluding Nx cases.

*** STAS (spreading through air space).

**** TAF (tumor associated fibrosis).

steps are involved in metastasis including detachment from a primary tumor, invasion of the stroma, and penetration of lymphatic or vascular channels. Activated angiogenesis indicates that tumor cells have a higher chance of reaching the vessels and increasing the probability of BM [20]. According to a previous report, lung adenocarcinoma cells produced large, fast-growing BMs with high expression of vascular endothelial growth factor (VEGF) mRNA and protein; the lesion of BMs regressed when initiation of angiogenesis was failed [20]. Also, the addition of bevacizumab to chemotherapy increased progression-free survival and overall survival in advanced NSCLC patients [20]. While the exact mechanism is still unclear, we suggest that angiogenesis is a critical step to BM.

Micropapillary predominant adenocarcinoma is significantly associated with BM and a prognostic factor for decreased brain metastasis-free survival [17]. In this study, micropapillary pattern was an important risk factor of BM (p = 0.024) regardless of proportion. Although such finding was expected regarding the previous papers on a close association between micropapillary pattern and lymph node metastasis [17,21–23], it is meaningful to suggest that the presence of micropapillary pattern might be important regardless of proportion. Then, is micropapillary pattern associated only with BM, and not with metastasis to other organs? Considering the association between the pattern and lymph node metastasis, the answer is no. However, according to the previous article, micropapillary predominant adenocarcinomas is more significantly associated with BM than metastases to other organs [17]. When we excluded cases involving extrathoracic metastasis other than brain to find the risk factors associated only with BM, the result was similar to the previous study. The reason why a micropapillary pattern shows brain-specific metastasis is unclear, and can be investigated by further studies.

Our second major finding is that the time to BM is significantly shorter in cases with necrosis, especially extensive necrosis. This can also be explained by the mechanism stated above. If the tumor is active in angiogenesis, the time to BM will be reduced. Vascular invasion, lymph node metastasis, and micropapillary pattern of tumors imply that the tumor cells have either reached the angiolymphatics or detached from the primary tumor. Hence, it can be explained that tumors with such characteristics take shorter time to BM.

In contrast to the previous reports, T stage showed no association with BM in multivariate analysis. Tumor size and T stage were not

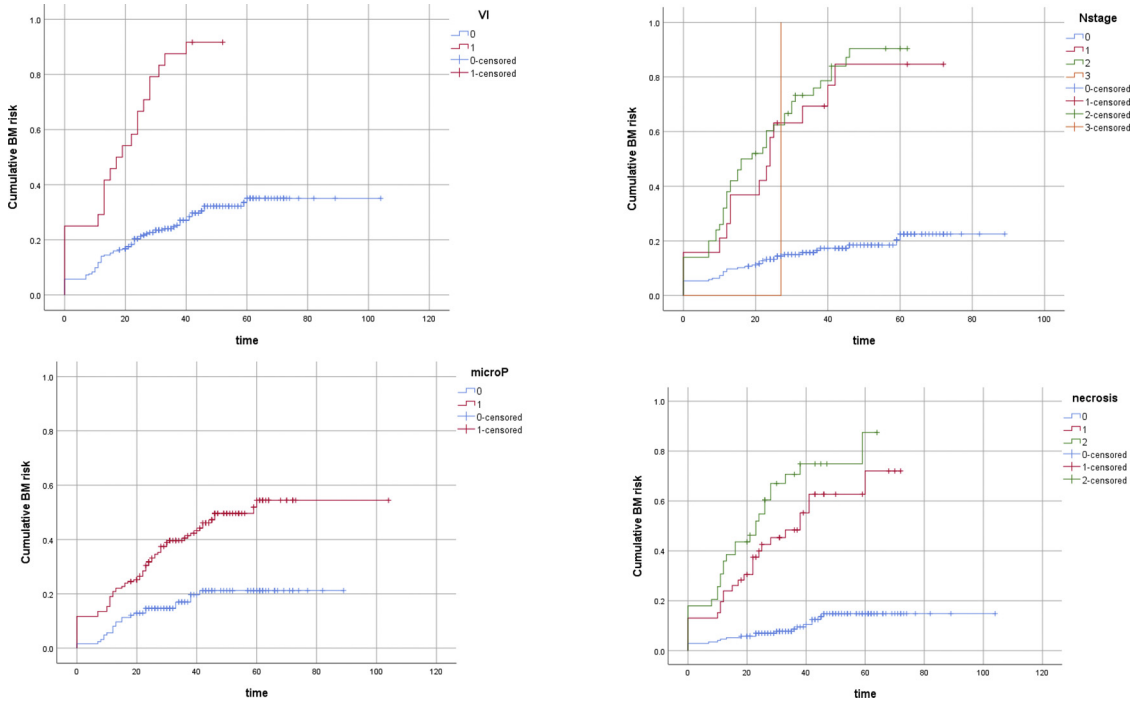

Fig. 3. Kaplan-Meier curves of time to brain metastasis (BM) in pulmonary adenocarcinoma. Vascular invasion (left upper), N stage (right upper), micropapillary pattern (left lower), and necrosis (right lower).

Table 3
Cox univariate and multivariate analysis of clinicopathologic features of squamous cell carcinoma.

a. univariate analysis

	BM (N = 14)	Control (N = 39)	HR (95% CI)	p value
Clinical factor				
Sex (M:F)	14:0	35:4	***	0.449
Age at diagnosis (median)	64.5 (54-79)	70 (54-82)	1.246 (0.279-5.570)	0.773
≤60 yrs	2	4		
> 60 yrs	12	35		
Follow up period (median, months)	19 (11-72)	40 (17-94)		**0.018**
Time to brain metastasis (median, months)	11 (0-26)			
Histopathologic factor				
Tumor size*				0.188
≤3cm	3 (25.0%)	11 (29.7%)		
≤5cm	2 (16.7%)	16 (43.3%)	0.506 (0.085-3.029)	0.456
≤7cm	4 (33.3%)	6 (16.2%)	2.417 (0.540-10.825)	0.249
> 7cm	3 (25.0%)	4 (10.8%)	2.739 (0.547-13.723)	0.220
Vascular invasion				0.115
Absent	10 (71.4%)	34 (87.2%)		
Present	4 (28.6%)	5 (12.8%)	2.548 (0.797-8.146)	
Lymphatic invasion				0.674
Absent	9 (64.3%)	23 (59.0%)		
Present	5 (35.7%)	16 (41.0%)	0.791 (0.265-2.360)	
Perineural invasion				0.803
Absent	12 (85.7%)	35 (89.7%)		
Present	2 (14.3%)	4 (10.3%)	1.210 (0.271-5.408)	
Pleural invasion				0.276
Absent	10 (71.4%)	32 (82.1%)		
Present	4 (28.6%)	7 (17.9%)	1.911 (0.597-6.116)	
Margin				0.741
≥1 cm	9 (64.3%)	24 (61.5%)		
< 1 cm	5 (35.7%)	15 (38.5%)	0.831 (0.278-2.484)	
T stage*				**0.038**
T1	3 (25.0%)	11 (29.7%)		
T2	1 (8.4%)	16 (43.3%)	0.265 (0.028-2.547)	0.250
T3	4 (33.3%)	6 (16.2%)	2.424 (0.541-10.853)	0.247
T4	4 (33.3%)	4 (10.8%)	3.176 (0.701-14.400)	0.134
N stage**				**0.002**
N0	5 (35.7%)	31 (79.5%)		
N1	5 (35.7%)	8 (20.5%)	3.241 (0.937-11.205)	0.063
N2	4 (28.6%)	0 (0.0%)	13.239 (3.377-51.909)	< **0.001**
N3	0 (0.0%)	0 (0.0%)		

b. multivariate analysis

Risk factor	HR (95% CI)	p value
T stage		0.070
T2 (vs T1)	0.233 (0.024-2.250)	0.208
T3 (vs T1)	2.718 (0.592-12.483)	0.198
T4 (vs T1)	1.944 (0.319-11.868)	0.471
N stage		**0.021**
N1 (vs N0)	4.893 (1.371-17.471)	**0.014**
N2 (vs N0)	8.721 (1.053-72.262)	**0.045**

Bold values are P < 0.05.
* Tumor size and T stage are evaluated after excluding pre-operative therapy cases.
** N stage is evaluated after excluding Nx cases.
*** Since coefficients did not converge, no further models will be fitted.

evaluated in cases of pre-operative therapy, due to difficulty in measuring the amount of residual tumor. T stage is determined by taking many factors into account, including tumor size, pleural invasion, distance from carina, structures invaded by tumor, and the presence and location of other tumor nodules. Thus, the association with BM may not be evident. To clarify this relationship, further studies are needed. Adenocarcinoma was not associated with BM as opposed to the previous papers [8–11,17]. In this article, the proportion of adenocarcinomas was 80.1% in the study group and 78.3% in the control group, which was higher than other articles. It is theorized that these higher proportions are affected by the selection bias. There was no relationship between age and BM, although previous articles suggested that young age was a risk factor of BM [8,10]. Similarly, we believe that our results are due to the selection bias.

We also investigated the clinical and histopathologic factors related to BM in squamous cell carcinoma and non-adenocarcinoma/non-squamous cell carcinoma. There were limitations to these analyses due to small number of cases. In squamous cell carcinoma, only N stage was a significant risk factor of BM, and non-adenocarcinoma/squamous cell carcinoma had no related factor. The number of cases was too small and the group of non-adenocarcinoma/squamous cell carcinoma was heterogeneous; thus, it was difficult to obtain reliable results.

BM is a common and lethal complication of NSCLC patients and is treated in a different way from other distant metastases. Therefore, this study is meaningful as it confirmed that necrosis is an associated factor to BM. It was also revealed that the time to BM is significantly shorter in

Table 4
Cox univariate analysis of clinicopathologic features of non-adenocarcinoma/non-squamous cell carcinoma.

a. univariate analysis

	BM (N = 6)	Control (N = 8)	HR (95% CI)	p value
Clinical factor				
Sex (M:F)	2:4	5:3	0.432 (0.079-2.374)	0.334
Age at diagnosis (median)	64 (54-76)	56 (53-66)	0.306 (0.056-1.677)	0.172
≤60 yrs	2	6		
> 60 yrs	4	2		
Follow up period (median, months)	28.5 (13-35)	45 (23-65)		**0.015**
Time to brain metastasis (median, months)	9 (0-27)			
Histopathologic factor				
Tumor size*				0.878
≤3cm	0 (0.0%)	4 (50.0%)		
≤5cm	2 (40.0%)	2 (25.0%)	***	
≤7cm	2 (40.0%)	1 (12.5%)	***	
> 7cm	1 (20.0%)	1 (12.5%)	***	
Vascular invasion				0.095
Absent	3 (50.0%)	7 (87.5%)		
Present	3 (50.0%)	1 (12.5%)	4.090 (0.783-21.365)	
Lymphatic invasion				0.330
Absent	5 (83.3%)	5 (62.5%)		
Present	1 (16.7%)	3 (37.5%)	0.341 (0.039-2.965)	
Perineural invasion				0.690
Absent	5 (83.3%)	6 (75.0%)		
Present	1 (16.7%)	2 (25.0%)	0.646 (0.075-5.541)	
Pleural invasion				0.363
Absent	4 (66.7%)	6 (85.7%)		
Present	2 (33.3%)	1 (14.3%)	2.221 (0.399-12.369)	
Margin				0.274
≥1 cm	5 (83.3%)	4 (50.0%)		
< 1 cm	1 (16.7%)	4 (50.0%)	0.301 (0.035-2.586)	
T stage*				0.878
T1	0 (0.0%)	4 (50.0%)		
T2	2 (40.0%)	2 (25.0%)	***	
T3	2 (40.0%)	1 (12.5%)	***	
T4	1 (20.0%)	1 (12.5%)	***	
N stage**				0.690
N0	0 (0.0%)	6 (75.0%)		
N1	0 (0.0%)	0 (0.0%)		
N2	1 (100.0%)	2 (25.0%)	0.646 (0.075-5.541)	
N3	0 (0.0%)	0 (0.0%)		

Bold values are P < 0.05.
* Tumor size and T stage are evaluated after excluding pre-operative therapy cases.
** N stage is evaluated after excluding Nx cases.
*** Since coefficients did not converge, no further models will be fitted.

cases with extensive necrosis. This finding will be helpful for follow-up of NSCLC patients and establishment of treatment plans. Angiolymphatic invasion and micropapillary pattern were also associated with BM, which was supported by previous articles [11,14,17].

The present study had some limitations. First, there was selection bias and time trend bias, because it is a retrospective study in a single institution. A number of young adenocarcinoma patients was included in the study due to the characteristics of this institution. In addition, there was a possibility that BM in the control group may appear after the end of the study. Second, the tumors were not entirely embedded and the all H&E slides were not reviewed due to missing blocks. As a result, any histologic finding could have been omitted. Third, conventional imaging may not have detected all metastatic lesions. If the metastatic lesion was subclinical, imaging would not have been performed. Finally, the risk factors of BM were evaluated without considering intrathoracic metastases. This was due to difficulty in distinguishing intrathoracic metastases from a recurrent tumor and from a synchronous tumor. Additional studies are needed to explore these aspects.

Competing interests

All the authors report no conflicts of interest

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.prp.2019.01.023.

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