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学位論文

Tumor-associated macrophage infiltration is associated with a higher rate of tumor spread through air spaces in resected lung adenocarcinomas

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Tumor-associated macrophage infiltration is associated with a higher rate of tumor spread through air spaces in resected lung adenocarcinomas

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ABSTRACT

Objective: Lung cancer can spread in numerous ways, one of which has been suggested to be spread through air spaces (STAS). The tumor immune microenvironment appears to play a significant role in this spread. Particularly, tumor-associated macrophages (TAMs) can create a favorable microenvironment for tumor progression. In this study, we analyzed data from 709 patients with stage 0–IIIA lung adenocarcinoma, resected between 1999 and 2016, and investigated whether immune cell infiltration was associated with the occurrence of STAS and clinical outcome of the disease.

Materials and methods: Tissue microarrays were constructed, and immunohistochemical analysis was performed for CD3, CD4, CD8, CD45RO, CD25, CD20, and CD68. The three tumor areas with the highest density of immune cells were photographed, and the immune cells were quantified. Associations between variables were analyzed using chi-square tests and Mann–Whitney *U* tests. Recurrence-free probability and overall survival were analyzed using log-rank tests and Cox proportional hazards models.

Results: After analyzing the associations between STAS and each type of immune cell infiltration, high density of CD68 + TAMs was identified as an independent predictor of a high STAS rate ($p = 0.014$) and was found to be associated with a high risk of recurrence, using univariate analysis ($p = 0.008$). After adjusting for CD68+ TAMs, pathological stage, and lymphovascular invasion, STAS remained significantly associated with a high risk of recurrence (HR = 3.50, $p < 0.001$).

Conclusion: We demonstrated that a high density of CD68 + TAMs is an independent predictor of an increased STAS rate. Additionally, STAS is correlated with aggressive tumor behavior characteristics.

1. Introduction

Spread through air spaces (STAS) is a spreading phenomenon of lung cancer, which is defined by the presence of tumor cells within the air spaces of the lung parenchyma beyond the edge of the main tumor [1]. STAS has received widespread attention since it was defined by Kadota et al. in 2015 [2]. Although a number of independent studies have validated STAS as a predictor of recurrence and survival in lung adenocarcinoma [2–6], the mechanisms underlying the presence of STAS have not been adequately studied.

The significance of STAS is predominantly due to its value in predicting the clinical outcomes in patients. However, some researchers have proposed that STAS may be a result of mechanical artifacts, including the spread through a knife during specimen section preparation [7]. Kadota et al. distinguished STAS from artifacts in the following way: Tumor floaters were favored by the presence of clusters of cells often randomly scattered over the tissue and at the edges of the tissue section. The presence of jagged edges of tumor cell clusters suggested tumor fragmentation or knife cuts during specimen processing rather than STAS. In addition, linear strips of cells that were lifted off of alveolar walls favored the presence of artifact. Identification of tumor cells distant from the main tumor was regarded as an artifact unless intraalveolar tumor cells could be demonstrated in a continuum of airspaces containing intraalveolar tumor cells back to the tumor edge [2].

Abbreviations: OS, overall survival; RFP, recurrence-free probability; STAS, spread through air spaces; TAMs, tumor-associated macrophages; TILs, tumor-infiltrating lymphocytes.

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Metovic demonstrated that STAS was not a pathologist-related artifactual event because of knife transportation of tumor cells during gross specimen handling and supported the notion that it was a phenomenon existing prior to surgical tissue processing [8]. To avoid confusion with the artificially detached cells during tumor dissection, it is important to collect as much biological evidence as possible with regard to STAS.

The molecular significance of STAS remains unclear, although some investigators have reported that STAS is associated with wild-type epidermal growth factor receptor *(EGFR)* [3,4,9], *ROS1* rearrangement [10], anti-anaplastic lymphoma kinase *(ALK)* arrangements [9], and immunohistochemically identified ALK expression [11]. The influence of tumor immunity on the progression of STAS has not yet been investigated. The presence of tumor-infiltrating lymphocytes (TILs) correlates with patient prognosis in solid malignancies, with outcomes dependent on the type and density of TILs [12–14]. Tumor-associated macrophages (TAMs) are a major component of the tumor immune microenvironment. TAMs, especially those with the M2 phenotype, release various growth factors, cytokines, and proteinases that create a favorable microenvironment for tumor progression, resulting in tumor cell dissemination and metastasis [15,16]. These associations have led to the hypothesis that the presence of TILs or TAMs may be correlated with the development of tumor STAS.

In this study, we analyzed a uniform cohort of Japanese patients with therapy-naïve, surgically resected lung adenocarcinoma, and investigated whether the type and density of TILs or TAMs were associated with the occurrence of STAS and with their clinical outcomes.

2. Materials and methods

2.1. Patients

This retrospective study was approved by the Institutional Review Board of Kagawa University. We reviewed data from patients ($n = 709$) with therapy-naïve lung adenocarcinoma who underwent surgical resection with systematic lymph node dissection at Kagawa University between 1999 and 2016. Cases with multifocal invasive carcinomas and stage IIIb–IV disease were excluded from the study.

Clinical data were collected from a prospectively maintained lung carcinoma database. Disease recurrence was confirmed by clinical, radiological, or pathological assessment. The disease TNM stage was assigned on the basis of the 8th edition of the *American Joint Committee on Cancer TNM Staging Manual* [17].

2.2. Histologic evaluation

Hematoxylin and eosin (H&E)-stained slides were reviewed by two pathologists who had no knowledge of the clinical outcome of the patients using an Olympus BX53 upright microscope (Olympus Corporation, Japan) with a standard 22-mm diameter eyepiece. Tumors were classified according to the 2015 WHO classification of lung carcinomas [1]. Presence of lymphatic and vascular invasion was noted if at least one tumor cell cluster was visible.

STAS is defined as small clusters of tumor cell nests within air spaces in the lung parenchyma beyond the edge of the main tumor [1–3]. They involve micropapillary patterns, solid nests, or single cells. The edge of the main tumor is defined as the outer border of the tumor, which is typically identified using low-power histologic examination. STAS was considered to be present when tumor cells were identified beyond the edge of the main tumor, even if they existed only in the first alveolar layer from the tumor edge.

2.3. Immunohistochemistry using tissue microarrays

Formalin-fixed, paraffin-embedded tumor specimens from patients who met the inclusion criteria of this study were used for tissue microarray construction. We marked one representative tumor area on H&E-stained slides and, using a tissue arrayer (Tissue Microprocessor KIN-2, Azumaya, Japan), we arrayed a cylindrical 3-mm tissue core from the corresponding paraffin block into a recipient block. In total, there were 709 available cases with adequate cores for immunohistochemical analysis.

We then took 4 μm sections from the tissue microarray blocks and stained them with anti-CD3 antibody (clone 2GV6, Ventana Medical Systems, Inc.; prediluted), anti-CD4 antibody (clone SP35, Ventana Medical Systems, Inc.; prediluted), anti-CD8 antibody (clone SP57, Ventana Medical Systems, Inc.; prediluted), anti-CD45 antibody (clone UCHL1, Leica; 1:200), anti-CD25 antibody (clone 4C9, Nichirei; prediluted), anti-CD20 antibody (clone L26, Dako; 1:400), and anti-CD68 antibody (clone KP1, Dako; 1:50), using a BenchMark ULTRA automated immunohistochemical slide staining system (Ventana Medical Systems, Inc.). Diaminobenzidine was used as the chromogen, and hematoxylin was used as the nuclear counterstain. Positive control tissues were stained in parallel with the study cases.

2.4. Immunohistochemical analysis and scoring of immune markers

For slides immunohistochemically stained with each immune marker, the three tumor areas with the highest density of immune cell infiltration, designated as hot spots, were photographed using an Olympus BX53 microscope equipped with a DP22 digital camera (Olympus Corporation, Japan), with a 20× objective. For each marker, immune cells were counted on each of the three photographs using the Patholoscope image analysis software (MITANI Corporation, Japan) or by manual counting method upon eyeball estimation.

The average count of the three areas was considered as the number of immune cells counted for each patient. Tumors were classified into the following four groups by immune cell count using quartiles: score 1, ≤25th percentile; score 2, >25th percentile and ≤50th percentile; score 3, >50th percentile and ≤75 percentile; and score 4, >75th percentile. As a reference, immune cell count scores of 1, 2, 3, and 4 of CD68 + TAMs are shown in Fig. 1A.

2.5. Statistical analysis

Associations between variables were analyzed using chi-square tests for categorical variables and Mann-Whitney *U* test for continuous variables. Multivariable analysis was conducted using linear regression analysis to evaluate the independent associations of STAS and clinicopathological factors with immune cell infiltration. Recurrence-free probability (RFP) was defined as the time from surgical resection to the date of disease recurrence. Overall survival (OS) was defined as the time from surgical resection to the date of death or last follow-up. RFP and OS were estimated using the Kaplan-Meier method, and nonparametric group comparisons were performed using log-rank tests. Multivariate analyses were performed using the Cox proportional hazards regression model. Multivariate models were built to include factors that were significant in the univariate analysis. Any associations between pathological factors were checked, and when strong associations were discovered, only one factor was included in the model. All statistical tests were two-sided, and used a 5% significance level. Statistical analyses were conducted using IBM SPSS Statistics for Windows (version 23.0; IBM Corporation, Armonk, NY).

3. Results

3.1. Patient clinicopathologic characteristics and their associations with STAS

The median age of the 709 patients was 70 years (range, 26–92 years), and more than half of the patients were men (n = 377; 53 %). Most patients (n = 536; 76 %) had pathological stage I disease. With regard to the surgical procedures, 560 (79 %) patients underwent

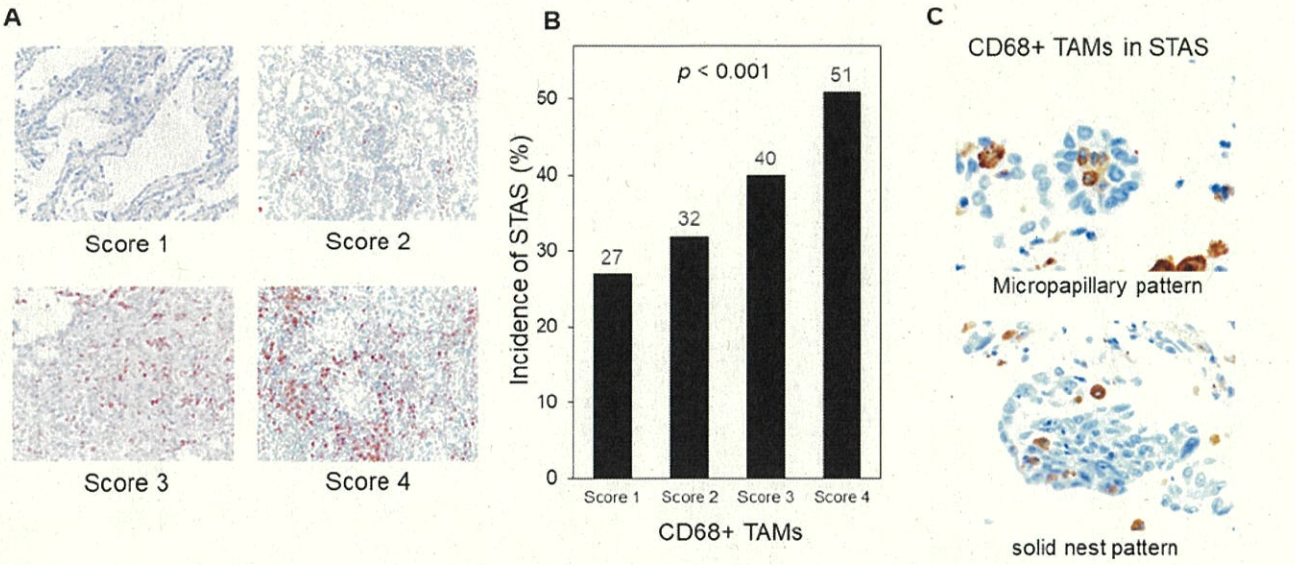

Fig. 1. Scoring of CD68+ tumor-associated macrophages (TAMs) and their association with the incidence of spread through air spaces (STAS).
(A) Tumors were classified into four groups by quantification of CD68 + TAMs, using quartiles: score 1, ≤25th percentile; score 2, >25th percentile and ≤50th percentile; score 3, >50th percentile and ≤75 percentile; and score 4, >75th percentile. The figure for score 1 shows CD68 + TAMs in a lepidic tumor, figure for score 2 shows CD68 + TAMs in an acinar tumor, and figures for scores 3 and 4 show CD68 + TAMs in solid tumors.
(B) The incidence of STAS linearly increased with the score of CD68 + TAMs (incidence of patients with STAS, 27 % for score 1, 32 % for score 2, 40 % for score 3, and 51 % for score 4; $p < 0.001$).
(C) Localization of CD68+ tumor-associated macrophages (TAMs) in spread through air spaces (STAS). CD68 + TAMs were found in micropapillary pattern and solid nest pattern of STAS.

lobectomy or more and 149 (21 %) underwent limited resection (segmentectomy or wedge resection). Eighty-nine patients received adjuvant therapy. During the study period, 147 patients experienced recurrence and 129 died. The median follow-up period for the patients who were alive at the time of the last follow-up was 60 months (mean ± SD, 48 ± 17).

STAS was observed in 262 patients (37 %) and occurred more frequently in male patients ($p < 0.001$). Frequency of STAS was significantly associated with a higher T status ($p < 0.001$), lymph node metastasis ($p < 0.001$), pathological stage ($p < 0.001$), lymphovascular invasion ($p < 0.001$), and histologic subtypes (micropapillary, solid or acinar patterns) ($p < 0.001$) (Supplementary Table 1).

3.2. Associations between the presence of STAS and immune cell infiltration

The associations between the presence of STAS and each type of immune cell infiltration are summarized in Table 1. A higher number of CD25+ TILs and CD68 + TAMs was identified in patients with STAS than in those without STAS ($p = 0.008$ and $p < 0.001$, respectively). The incidence of STAS increased linearly with the score of CD68+ TAMs (incidence of patients with STAS: 27 % for score 1; 32 % for score 2; 40 % for score 3; and 51 % for score 4; $p < 0.001$) (Fig. 1B). However, this trend was not observed for CD25+ TILs (Supplementary Fig. 1). Based on the association between STAS and CD68 + TAMs, all subsequent statistical analyses focused on CD68 + TAMs.

Table 1
Associations Between the Presence of STAS and Immune Cell Infiltration.

Marker	Immune cell count, median (25, 75 percentiles)				p
	STAS, absent		STAS, present		
CD3	456	(226, 770)	513	(241, 840)	0.13
CD4	123	(85, 200)	133	(73, 212)	0.99
CD8	171	(84, 328)	172	(62, 361)	0.79
CD45RO	268	(148, 442)	260	(138, 519)	0.77
CD25	32	(16, 54)	36	(20, 61)	**0.008**
CD20	157	(42, 437)	164	(35, 417)	0.59
CD68	11	(5, 24)	18	(8, 37)	**<0.001**

Significant *p*-values are shown in bold.
STAS, spread through air spaces.

To investigate the localization of CD68 + TAMs in the main tumor and in the tumor cells of STAS, we randomly selected 18 cases with prominent STAS and stained their samples with anti-CD68 antibody using whole blocks. Among them, eight patients (44 %) had CD68 + TAMs within tumor cells of STAS (Fig. 1C).

3.3. Associations of STAS and clinicopathologic features with CD68 + TAMs

The highest density of CD68 + TAMs was identified in male patients ($p < 0.001$), with a higher T status ($p < 0.001$), lymph node metastasis ($p = 0.004$), pathological stage ($p < 0.001$), lymphovascular invasion ($p < 0.001$), and histologic subtypes (micropapillary, solid, or acinar patterns) ($p < 0.001$) (Table 2). Multivariate linear regression analysis showed that STAS was an independent predictive factor for a higher number of CD68 + TAMs ($p = 0.014$) (Table 3).

3.4. Association between patient outcome and STAS or CD68 + TAMs

The univariate associations of patient outcomes (RFP and OS) with clinicopathologic factors and CD68 + TAMs are presented in Supplementary Table 2. Higher T status ($p < 0.001$), lymph node metastasis ($p < 0.001$), pathological stage ($p < 0.001$), lymphovascular invasion ($p < 0.001$), histologic subtypes (micropapillary, solid, or acinar patterns) ($p < 0.001$), and STAS ($p < 0.001$) were found to be significantly associated with a lower RFP. Older age ($p = 0.005$), male sex ($p = 0.001$), higher T status ($p < 0.001$), lymph node metastasis ($p < 0.001$), pathological stage ($p < 0.001$), lymphovascular invasion ($p < 0.001$), histologic subtypes (micropapillary, solid, or acinar patterns) ($p < 0.001$), and STAS ($p < 0.001$) were found to be significantly associated

Table 2
Associations of Clinicopathologic Features with CD68 + TAMs.

Variables	N	CD68 + TAMs		p
		Median	(25, 75 percentiles)	
Age, years				0.078
≤65	239	17	(6, 32)	
>65	470	13	(5, 28)	
Sex				<0.001
Female	332	11	(5, 23)	
Male	377	17	(7, 35)	
T status				<0.001
Tis	38	8	(5, 14)	
T1	478	13	(5, 27)	
T2	154	19	(7, 40)	
T3	24	18	(7, 49)	
T4	15	16	(10, 19)	
N status				0.004
N0	611	13	(5, 27)	
N1	43	18	(8, 38)	
N2	55	23	(9, 41)	
Pathological stage				<0.001
Stage 0	38	8	(5, 14)	
Stage I	536	13	(5, 28)	
Stage II	59	18	(7, 39)	
Stage III	76	19	(9, 40)	
Lymphovascular invasion				<0.001
Absent	442	11	(5, 22)	
Present	267	20	(8, 43)	
Histologic subtype				<0.001
AIS + MIA	136	9	(5, 18)	
Lepidic	90	10	(4, 18)	
Acinar	74	23	(16, 40)	
Papillary	270	14	(6, 33)	
Micropapillary	23	20	(6, 30)	
Solid	63	28	(13, 50)	
Others	53	11	(6, 30)	

Significant *p*-values are shown in bold.
TAMs, tumor-associated macrophages; AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma.

Table 3
Multivariate Linear Regression Analysis for Predicting CD68 + TAMs.

Variables		Regression coefficient	95 % CI	*t*	*p*
Sex	Male vs. female	8.20	4.20 - 12.20	4.02	<0.001
Pathological stage	II-III vs. 0-I	3.28	−2.02 - 8.58	1.21	0.23
STAS	Present vs. absent	5.47	1.12 - 9.81	2.47	0.014

TAMs, tumor-associated macrophages; CI, confidence interval; STAS, spread through air spaces.

with a worse OS.

The RFP of patients with a CD68 + TAM score of 4 was significantly lower (5-year RFP, 67 %) than those with CD68 + TAM scores of 1, 2, or 3 (5-year RFP 76 %, 84 %, and 76 %, respectively; $p = 0.030$) (Supplementary Table 2). When CD68+ TAM scores 1, 2, and 3 were combined, the RFP for patients with a CD68+ TAM score of 4 was lower than that for patients with combined CD68+ TAM scores 1–3 (5-year RFP 67 % vs. 78 %; $p = 0.008$) (Fig. 2). However, there was no association between CD68 + TAM scores and OS ($p = 0.19$) (Supplementary Table 2). Among the patients with a CD68+ TAM score of 4, the RFP of patients with micropapillary, solid, and acinar patterns (5-year RFP 50 %, 63 %, and 42 %, respectively) was significantly lower than that in patients with other histologic subtypes (5-year RFP, 77 %; $p = 0.002$).

Multivariate analysis for RFP, after adjustment for pathological stage, lymphovascular invasion, and histologic subtypes, showed that STAS remained independently associated with a higher risk of recurrence (HR = 3.32, $p < 0.001$); however, CD68 + TAM scores were not independent predictors of recurrence (CD68 + TAM score 4 vs. scores 1–3; HR = 1.00, $p = 0.995$) (Table 4).

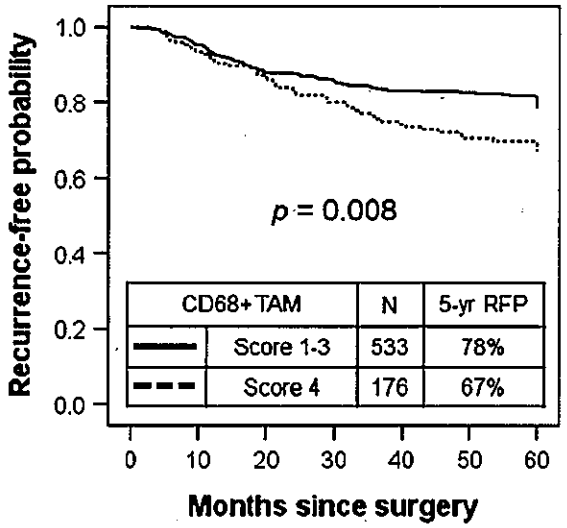

Fig. 2. Association between recurrence-free probability (RFP) and tumor-associated macrophages (TAMs). Five-year RFP for patients with CD68 + TAM score 4 was lower than that for patients with CD68 + TAM scores 1–3 (5-year RFP, 67 % vs. 78 %, respectively; $p = 0.008$).

4. Discussion

We performed comprehensive immunohistochemical analyses of tumor-infiltrating immune cells in an effort to identify significant predictors of tumor STAS using a uniform, large cohort of patients with surgically resected lung adenocarcinoma. We demonstrated that a higher than control density of CD68 + TAMs is an independent predictor of a higher rate of STAS, in addition to the correlations of STAS with the characteristics of aggressive tumor behavior such as larger tumor size, and higher lymph node metastasis, pathological stage, and lymphovascular invasion. Even though the presence of STAS was associated with CD68 + TAMs, STAS remained significantly associated with a higher risk of recurrence after adjustment for CD68 + TAMs, sex, pathological stage, and lymphovascular invasion.

In pathological practice, macrophages are frequently identified in virtually all types of solid malignancies, including lung carcinomas. The phenotypes of classically activated type I or M1 macrophages, derived from healthy or inflamed tissues, include the ability to kill microorganisms and tumor cells, present antigens, and produce high levels of T-cell stimulatory cytokines [18,19]. The phenotypes of alternatively

Table 4
Multivariate Analysis of Recurrence-Free Probability.

Variables		Hazard ratio	95 % CI	*p*
Recurrence-free probability				
Pathological stage	II-III vs. 0-I	2.44	1.71 – 3.48	<0.001
Lymphovascular invasion	Present vs. absent	2.40	1.52 – 3.79	<0.001
Histologic subtypes	SOL/MIP/ACI vs. others	1.47	1.03 – 2.09	0.033
STAS	Present vs. absent	3.32	2.18 – 5.07	<0.001
CD68 + TAMs	Score 4 vs. scores 1–3	1.00	0.71 – 1.42	0.995

CI, confidence interval; SOL, solid; MIP, micropapillary; ACI, acinar; STAS, spread through air spaces; TAMs, tumor-associated macrophages.

activated type II or M2 macrophages, which may be developed by exposure to IL-4 and IL-10 in tumors, however, have poor antigen-presenting capability, and produce factors that suppress T-cell proliferation and activity [18,19]. In specific microenvironments, M2 TAMs can release growth factors, cytokines, chemokines, or enzymes that regulate tumor growth, angiogenesis, invasion, or metastasis [20, 21]. In resected lung adenocarcinomas, we demonstrated that TAMs were associated with aggressive biological behavior, such as larger tumor size, lymphovascular invasion, and lymph node metastasis. We demonstrated that a higher density of CD68 + TAMs was an independent predictor of STAS. STAS was also correlated with larger tumor size, lymph node metastasis, higher pathological stage, and lymphovascular invasion. Assuming these findings reflect biologically significant correlations between STAS and TAMs, the development of tumor STAS may be promoted by immunological functions of TAMs.

Previous studies have found that TAMs are associated with unfavorable clinical outcomes in lung cancer [22–24]. As the most common immunohistochemical TAM marker, CD68 has been used as a pan-macrophage marker, whereas CD163 has been used as an M2 marker. Chen et al. found that the median survival for non-small cell lung carcinoma (NSCLC) patients with a high density of tumor-infiltrating macrophages, detected by anti-CD68 antibodies, was significantly shorter than for those with a low density of tumor-infiltrating macrophages [22]. Jackute et al. demonstrated that M2 macrophages, detected by immunohistochemical double staining of CD68 and CD163, predominated over M1 macrophages in tumor tissue. High levels of infiltration of M2 macrophages in tumor tissue were associated with reduced OS in patients with NSCLC [23]. Cao et al. reported that NSCLC patients with low infiltration of all macrophages and M2 macrophages had a better OS than those with high infiltration of all macrophages, as determined by labelling with CD68, and M2 macrophages identified by labelling with CD163 in tumor islets. M2 macrophages were more abundant than M1 macrophages in the tumor microenvironment [24]. In lung carcinomas, the majority of total TAMs, detected using CD68 as a pan-macrophage marker, were M2 macrophages detected by CD163; therefore, the prognostic value of CD68 may be similar to that of CD163. In lung adenocarcinomas, we found that a high density of CD68 + TAMs was associated with a higher risk of recurrence in univariate analysis; however, CD68 + TAMs were not independent predictors of recurrence in multivariate analysis after adjusting for STAS. These results suggest a strong association between a high density of CD68 + TAMs and the presence of STAS.

Our study had several limitations. First, TAMs were stained with CD68, which is known as a pan-macrophage marker, but were not stained using a specific CD163 antibody, which is a distinct M2 macrophage marker. However, as described in previous studies [23,24], TAMs are mainly M2 macrophages in the distinct tumor microenvironment. This finding supports the hypothesis that the prognostic and biological significance of CD68+ total macrophages is at least partly consistent with that of CD163 + M2 macrophages. Second, since this study was based on immunocytochemistry using tissue microarray, selection bias was inevitable in selecting tumor areas for tissue microarray construction. To minimize this selection bias, we adopted a relatively large tissue core, of 3 mm, and evaluated three tumor areas with the highest density of immune cell infiltration in each core. We stained 18 case samples with prominent STAS with anti-CD68 antibody using whole blocks, and demonstrated that CD68 + TAMs were found in the area of tumor STAS as well as in the area of the main tumor. Third, as immunohistochemical analysis cannot confirm the mechanism underlying STAS by immune cells, further experiments using cell lines and animal models are warranted.

5. Conclusions

We demonstrated that the incidence of STAS increased linearly with the number of CD68 + TAMs, and that a higher density of CD68 + TAMs was an independent predictor of a higher incidence of STAS. The presence of STAS was also correlated with aggressive tumor behavior. Even though the presence of STAS was associated with CD68 + TAMs, STAS remained significantly associated with a higher risk of recurrence after adjustment for other clinicopathologic prognosticators and levels of CD68 + TAMs. If these results reflect biological correlations between STAS and TAMs, the development of tumor STAS may be promoted by the immunological functions of TAMs.

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CRediT authorship contribution statement

Chihiro Yoshida: Formal analysis, Investigation, Visualization, Writing - original draft. **Kyuichi Kadota:** Conceptualization, Funding acquisition, Methodology, Writing - review & editing. **Toshihiro Ikeda:** Data curation. **Emi Ibuki:** Resources. **Tetsuhiko Go:** Validation. **Reiji Haba:** Project administration. **Hiroyasu Yokomise:** Supervision.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

None.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2021.06.009.

References

[1] W. Travis, E. Brambilla, A. Burke, et al., WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart, International Agency for Research on Cancer, Lyon, France, 2015, https://doi.org/10.1097/JTO.0000000000000663.
[2] K. Kadota, J. Nitadori, C.S. Sima, et al., Tumor spread through air spaces is an important pattern of invasion and impacts the frequency and location of recurrences after limited resection for small stage I lung adenocarcinomas, J. Thorac. Oncol. 10 (2015) 806–814, https://doi.org/10.1097/JTO.0000000000000486.
[3] A. Warth, T. Muley, C.A. Kossakowski, et al., Prognostic impact of intra-alveolar tumor spread in pulmonary adenocarcinoma, Am. J. Surg. Pathol. 39 (2015) 793–801, https://doi.org/10.1097/PAS.0000000000000409.
[4] S. Shiono, N. Yanagawa, Spread through air spaces is a predictive factor of recurrence and a prognostic factor in stage I lung adenocarcinoma, Interact. Cardiovasc. Thorac. Surg. 23 (2016) 567–572, https://doi.org/10.1093/icvts/ivw211.
[5] K. Masai, H. Sakurai, A. Sukeda, et al., Prognostic impact of margin distance and tumor spread through air spaces in limited resection for primary lung cancer, J. Thorac. Oncol. 12 (2017) 1788–1797, https://doi.org/10.1016/j.jtho.2017.08.015.
[6] H. Uruga, T. Fujii, S. Fujimori, et al., Semiquantitative assessment of tumor spread through air spaces (STAS) in early-stage lung adenocarcinomas, J. Thorac. Oncol. 12 (2017) 1046–1051, https://doi.org/10.1016/j.jtho.2017.03.019.
[7] E. Thunnissen, H.J. Blaauwgeers, E.M. de Cuba, et al., Ex vivo artifacts and histopathologic pitfalls in the lung, Arch. Pathol. Lab. Med. 140 (2016) 212–220, https://doi.org/10.5858/arpa.2015-0292-OA.
[8] J. Metovic, E.C. Falco, E. Vissio, et al., Gross specimen handling procedures do not impact the occurrence of spread through air spaces (STAS) in lung cancer, Am. J. Surg. Pathol. 45 (2021) 215–222, https://doi.org/10.1097/PAS.0000000000001642.
[9] J.S. Lee, E.K. Kim, M. Kim, et al., Genetic and clinicopathologic characteristics of lung adenocarcinoma with tumor spread through air spaces, Lung Cancer 123 (2018) 121–126, https://doi.org/10.1016/j.lungcan.2018.07.020.
[10] Y. Jin, P.L. Sun, S.Y. Park, et al., Frequent aerogenous spread with decreased E-cadherin expression of ROS1-rearranged lung cancer predicts poor disease-free survival, Lung Cancer 89 (2015) 343–349, https://doi.org/10.1016/j.lungcan.2015.06.012.
[11] K. Kadota, Y. Kushida, S. Kagawa, et al., Limited resection is associated with a higher risk of locoregional recurrence than lobectomy in stage I lung

adenocarcinoma with tumor spread through air spaces, Am. J. Surg. Pathol. 43 (2019) 1033–1041, https://doi.org/10.1097/PAS.0000000000001285.
[12] J. Galon, A. Costes, F. Sanchez-Cabo, et al., Type, density, and location of immune cells within human colorectal tumors predict clinical outcome, Science 313 (2006) 1960–1964, https://doi.org/10.1126/science.1129139.
[13] F. Pagès, A. Berger, M. Camus, et al., Effector memory T cells, early metastasis, and survival in colorectal cancer, N. Engl. J. Med. 353 (2005) 2654–2666, https://doi.org/10.1056/NEJMoa051424.
[14] K. Kadota, J.I. Nitadori, P.S. Adusumilli, Prognostic value of the immune microenvironment in lung adenocarcinoma, Oncoimmunology 2 (2013) e24036, https://doi.org/10.4161/onci.24036.
[15] A. Sica, P. Larghi, A. Mancino, et al., Macrophage polarization in tumour progression, Semin. Cancer Biol. 18 (2008) 349–355, https://doi.org/10.1016/j.semcancer.2008.03.004.
[16] N. Linde, M. Casanova-Acebes, M.S. Sosa, et al., Macrophages orchestrate breast cancer early dissemination and metastasis, Nat. Commun. 9 (2018) 21, https://doi.org/10.1038/s41467-017-02481-02485.
[17] M.B. Amin, S. Edge, F. Greene, et al., AJCC Cancer Staging Manual, 8th ed., Springer, New York, 2017, pp. 431–456.
[18] C.E. Lewis, J.W. Pollard, Distinct role of macrophages in different tumor microenvironments, Cancer Res. 66 (2006) 605–612, https://doi.org/10.1158/0008-5472.CAN-05-4005.
[19] A. Mantovani, S. Sozzani, M. Locati, et al., Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes, Trends Immunol. 23 (2002) 549–555, https://doi.org/10.1016/S1471-4906(02)02302-5.
[20] B. Ruffell, N.I. Affara, L.M. Coussens, Differential macrophage programming in the tumor microenvironment, Trends Immunol. 33 (2012) 119–126, https://doi.org/10.1016/j.it.2011.12.001.
[21] J. Condeelis, J.W. Pollard, Macrophages: obligate partners for tumor cell migration, invasion, and metastasis, Cell. 124 (2006) 263–266, https://doi.org/10.1016/j.cell.2006.01.007.
[22] J.J. Chen, P.L. Yao, A. Yuan, et al., Up-regulation of tumor interleukin-8 expression by infiltrating macrophages: its correlation with tumor angiogenesis and patient survival in non-small cell lung cancer, Clin. Cancer Res. 9 (2003) 729–737.
[23] J. Jackute, M. Zemaitis, D. Pranys, et al., Distribution of M1 and M2 macrophages in tumor islets and stroma in relation to prognosis of non-small cell lung cancer, BMC Immunol. 19 (2018) 3, https://doi.org/10.1186/s12865-018-0241-0244.
[24] L. Cao, X. Che, X. Qiu, et al., M2 macrophage infiltration into tumor islets leads to poor prognosis in non-small-cell lung cancer, Cancer Manag. Res. 11 (2019) 6125–6138, https://doi.org/10.2147/CMAR.S199832.