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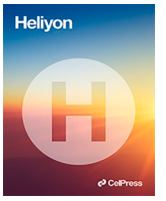

Research article

IL-34 and its receptors as predictors of brain metastasis and prognosis in lung adenocarcinoma: Unveiling insights through bioinformatic and immunohistochemical investigations

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ABSTRACT

Background: Brain metastasis (BM) is a prevalent form of metastasis in lung adenocarcinoma (LUAD), necessitating investigations into the underlying mechanisms. Interleukin 34 (IL-34) and its receptors, macrophage colony-stimulating factor-1 receptor (CSF-IR), Syndecan-1 (SDC-1), and protein-tyrosine phosphatase zeta receptor (PTPRZ1), are known to play pivotal roles in the metastasis of malignant tumors, thereby holding promise as potential biomarkers for studying BM in LUAD.

Methods: We performed immunohistochemistry to analyze the expression of IL-34, CSF-1R, SDC-1, and PTPRZ1 in 10 pairs of LUAD primary tissues and BMs, along with 96 unpaired primary tissues and 68 unpaired BMs. Subsequently, we evaluated the association between protein expression and the occurrence of BM. Furthermore, Kaplan-Meier survival curve analysis was conducted on both network and clinical data to explore the association between protein expression and patient prognosis and survival.

Results: At the protein level, the expression of IL-34 and its receptors showed significant variation between paired primary tumors and BMs in 10 LUAD patients. The levels of IL-34, CSF-1R, and SDC-1 expression are typically elevated in brain metastatic lesions of LUAD compared to primary LUAD tumors. Furthermore, patients with high CSF-1R expression in primary LUAD are at a greater risk of developing brain metastases. High expression of IL-34 and CSF-1R in primary LUAD lesions indicated poor disease-free survival (DFS) and overall survival (OS), while high expression of SDC-1 indicated poor OS. Cox multivariate analysis further revealed that CSF-1R and IL-34+CSF-1R positivity independently affected LUAD OS. These findings were further substantiated in unpaired samples.

Conclusions: Our results indicate significant alterations in the expression of IL-34 and its receptors, CSF-1R and SDC-1, between LUAD primary lesions and BMs, with increased expression observed in BMs. LUAD patients with positive CSF-1R expression in primary lesions exhibited a higher likelihood of developing BM, and high expression of IL-34, CSF-1R, and SDC-1 correlated with poor prognosis. These findings contribute novel insights towards identifying potential treatment or diagnostic targets for metastatic LUAD.

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1. Introduction

Brain metastasis (BM) is a leading cause of mortality among patients with malignant tumors. The incidence of BM from lung cancer is significantly greater than that from other cancers, such as melanoma, breast cancer, kidney cancer, and colorectal cancer. BM from lung cancer is typically associated with poor prognosis, high incidence, and short survival time, often lasting less than two months when left untreated. Approximately 20–40 % of non-small cell lung cancer (NSCLC) patients develop BMs at some point in the course of their disease [1]. The current treatment options for lung cancer BMs include surgery, radiotherapy, chemotherapy, and targeted immunotherapy, yet their efficacy remains limited. As a result, the search for novel therapeutic targets has emerged as an urgent priority in the treatment of lung cancer BMs.

IL-34 is a recently discovered cytokine whose dysregulation is implicated in a range of diseases, including infections, metabolic and neurological disorders, and tumors [2]. IL-34 exhibits predominant expression in the brain and skin where neurons and keratinocytes have been identified as the primary cell types expressing this cytokine, suggesting a critical role of IL-34 in stabilizing the microenvironment of the brain [3]. Moreover, it has been reported that IL-34 expression in lung cancer cells induces tumorigenesis and chemo-resistance by promoting immunosuppression within tumor-associated macrophages via CSF-1R [4]. However, it remains to be determined whether the expression of IL-34 in lung cancer cells is associated with the development of BM in patients with lung adenocarcinoma (LUAD).

IL-34 has been reported to bind to three receptors: CSF-1R, SDC-1, and PTPRZ1. Among these, CSF-1R is the main receptor for IL-34, and is crucial for the development of microglial macrophages in the central nervous system. Previous studies have also demonstrated that IL-34 binds to CSF-1R and enhances the invasion of cancer cells by activating multiple signaling pathways [5]. As CSF-1R kinase is a major target for cancer therapy, the development of small molecule CSF-1R inhibitors is under intensive focus, and several such inhibitors are currently under clinical trials [6]. On the other hand, SDC-1 and PTPRZ1 are expressed in the epithelium, cells of the central nervous system, and several other tissues. IL-34 combined with SDC-1 can regulate the IL-34-induced CSF-1R signaling pathway in macrophages cultured in vitro. Additionally, several studies have reported that PTPRZ1 can regulate the growth and migration of cancer cells [7,8].

Previous literature suggests that IL-34 and its receptors may play a collective role in cancer metastasis. Thus, this study aims to investigate the specific roles of IL-34 in BM of LUAD and the correlation and variations between IL-34 and its receptors in LUAD patients, with and without BM. By exploring the functions of IL-34 in this process, we hope to gain insights into the high prevalence of BM in LUAD. Furthermore, we believe that the innovative bioinformatics and immunohistochemical pipeline developed in this study could be applied to metastasis research of other cancer types.

2. Materials and methods

2.1. Clinical samples

Paraffin-embedded tissue samples were collected from patients diagnosed with lung adenocarcinoma (LUAD) who underwent surgical resection at Harbin Medical University Cancer Hospital between May 2007 and November 2016. A total of 96 primary lung lesion samples and 30 adjacent non-cancerous tissues were obtained from LUAD patients. Brain metastatic tissue samples were collected from 68 LUAD patients with brain metastases. Additionally, paired samples from 10 LUAD patients with brain metastases were collected, consisting of both primary tumor tissue and corresponding brain metastatic tissue. Brain metastasis diagnosis was confirmed through histopathological examination, aligning with the characteristics of the underlying primary tumor. This study received approval from the Institutional Research Ethics Committee at Harbin Medical University Cancer Hospital (Ethics 82373041), and written informed consent was obtained from all participating patients. None of the patients received any preoperative anti-tumor therapy. Clinical and pathological data, including age, sex, smoking history, tumor size, lymph node metastasis, clinical stage, histological differentiation, time of recurrence and metastasis, metastasis location, and overall survival (OS), were extracted from patients' medical records.

2.2. Bioinformatics analysis

The Kaplan-Meier Plotter (https://kmplot.com/) online database was utilized to assess the association between genes and survival in breast, ovarian, lung, and gastric cancer samples. The clinical data encompassed gender, age, histology, stage, grade, TP53 mutation status, and chemotherapy information for all patients. Survival analysis was conducted to examine the relationship between IL-34, CSF-1R, SDC-1, and PTPRZ1 mRNA expression levels and survival outcomes in a cohort of 865 LUAD patients. The patient specimens were categorized into high- and low-expression groups based on the optimal cutoff. Hazard ratio (HR), 95 % confidence intervals (CIs), and log-rank p-values were employed to interpret the data.

2.3. Immunohistochemistry

The tissue slices were subjected to a dewaxing process using xylene and ethanol, followed by rinsing with distilled water. Prior to antigen retrieval in a citrate antigen repair buffer (pH 6.0), the slides were treated with 3 % hydrogen peroxide at room temperature for 10 min to block endogenous peroxidase activity. Subsequently, high-pressure heat-induced antigen retrieval was performed for 4 min, followed by natural cooling for 30 min. The slides were then incubated overnight at 4 °C with primary antibodies, and subsequently

incubated with secondary antibodies for 20 min at 37 °C. To visualize the staining, the sections were rinsed with Phosphate-buffered saline (PBS), stained with Diaminobenzidine (DAB), and counterstained with hematoxylin. Positivity was indicated by brown or yellow staining. The primary antibodies used were IL-34 (ab101443, Abcam, Cambridge, UK, dilution 1:250), CSF-1R (25949-1-AP, Proteintech, Wuhan, China, dilution 1:300), SDC-1 (10593-1-AP, Proteintech, dilution 1:1500), and PTPRZ1 (55125-1-AP, Proteintech, dilution 1:100).

Immunohistochemistry interpretation criteria: Two experienced pathologists, who were blinded to the clinicopathological data, independently analyzed all tissue sections. The cytoplasmic staining results were scored based on the following criteria: (a) percentage of immunoreactive cells: 0 (0 %), 1 (1–10 %), 2 (11–50 %), 3 (51–70 %), and 4 (≥71 %); (b) staining intensity: 0 (negative staining), 1 (weak staining), 2 (moderate staining), and 3 (intense staining). The final score for IL-34, CSF-1R, SDC-1, and PTPRZ1 expression was determined by summing the scores for the percentage of positive cells and the intensity score, resulting in a range from 0 to 7. This scoring method, which is relatively simple and reproducible, has been previously employed and shown to yield highly concordant results between independent evaluators [9]. For statistical analysis, a final staining score of 0 was categorized as negative staining, 1–4 as weakly positive expression, 5 as positive expression, and 6–7 as strongly positive expression.

2.4. Data processing and statistical analysis

All analyses were conducted using SPSS 25.0 for Windows (SPSS, Chicago, IL, USA). We assessed the correlation between the expression of IL34, CSF-1R, SDC-1, and PTPRZ1 and the clinicopathological characteristics of the patients using the chi-square test. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier (KM) method and compared using nonparametric log-rank test. Factors with a significance level (P-value) below 0.05 in the univariate analysis were included in the Cox model for multivariate analysis. A P-value below 0.05 was considered statistically significant.

3. Results

3.1. Differential expression of IL-34 and its receptors in paired samples of primary lung cancer lesions and brain metastases of 10 patients with LUAD

We collected a sample of 10 patients with LUAD who underwent surgical resection of both primary lung lesions and brain metastases (Table 1). Immunohistochemical staining was conducted on tissue sections to assess the expression of IL-34, SDC-1, CSF-1R, and PTPRZ1. Our findings demonstrated altered expression patterns of IL-34 and its receptors from the primary lung cancer lesions to the brain metastases (Fig. 1A). The immunohistochemistry score (IRS) was utilized to quantify the expression of these indicators and generate a heatmap (Fig. 1B). The statistical analysis revealed the following results: In comparison to the primary lung lesions, the expression of IL-34 was upregulated in 5 cases, downregulated in 3 cases, and unchanged in 2 cases. SDC-1 expression was upregulated in 7 cases and downregulated in 3 cases. CSF-1R expression was upregulated in 5 cases, downregulated in 2 cases, and unchanged in 3 cases. PTPRZ1 expression showed upregulation in 3 cases, downregulation in 2 cases, and remained unchanged in 5 cases (Table 2). Due to the small sample size, no statistically significant differences were observed. However, the expression of IL-34, SDC-1 and CSF-1R showed increase in brain metastases. Additionally, the expression of CSF-1R in the brain metastases of 3 patients remained unchanged, possibly due to the strong positive expression of CSF-1R in the primary lung cancer lesions. PTPRZ1 expression exhibited low levels in both primary lung cancer and brain metastases in the unchanged patients.

3.2. Higher expression of IL-34 and its receptor in LUAD patients with brain metastases compared to primary lung lesions

To further investigate differential expression of IL-34 and its receptor in primary lung lesions and bone metastases (BMs) of lung adenocarcinoma (LUAD), the sample size was expanded and a total of 96 unpaired lung cancer tissues and 68 BMs were collected. These samples were also subjected to immunohistochemistry (IHC) analysis. Furthermore, the expression levels of IL-34 and its

Table 1
Characteristics of 10 LUAD patients with brain metastases (BMs), including paired primary tumor origins and brain metastasis samples.

Characters		Number	%
Gender	male	6	60.0 %
	female	4	40.0 %
Age	<65y	8	80.0 %
	≥65y	2	20.0 %
Smoking history	positive	4	40.0 %
	negative	6	60.0 %
Lymph node metastasis	positive	4	40.0 %
	negative	6	60.0 %
Brain metastatic sites	cerebrum	7	70.0 %
	cerebellum	3	30.0 %
Chemotherapy	yes	5	50.0 %
	no	5	50.0 %

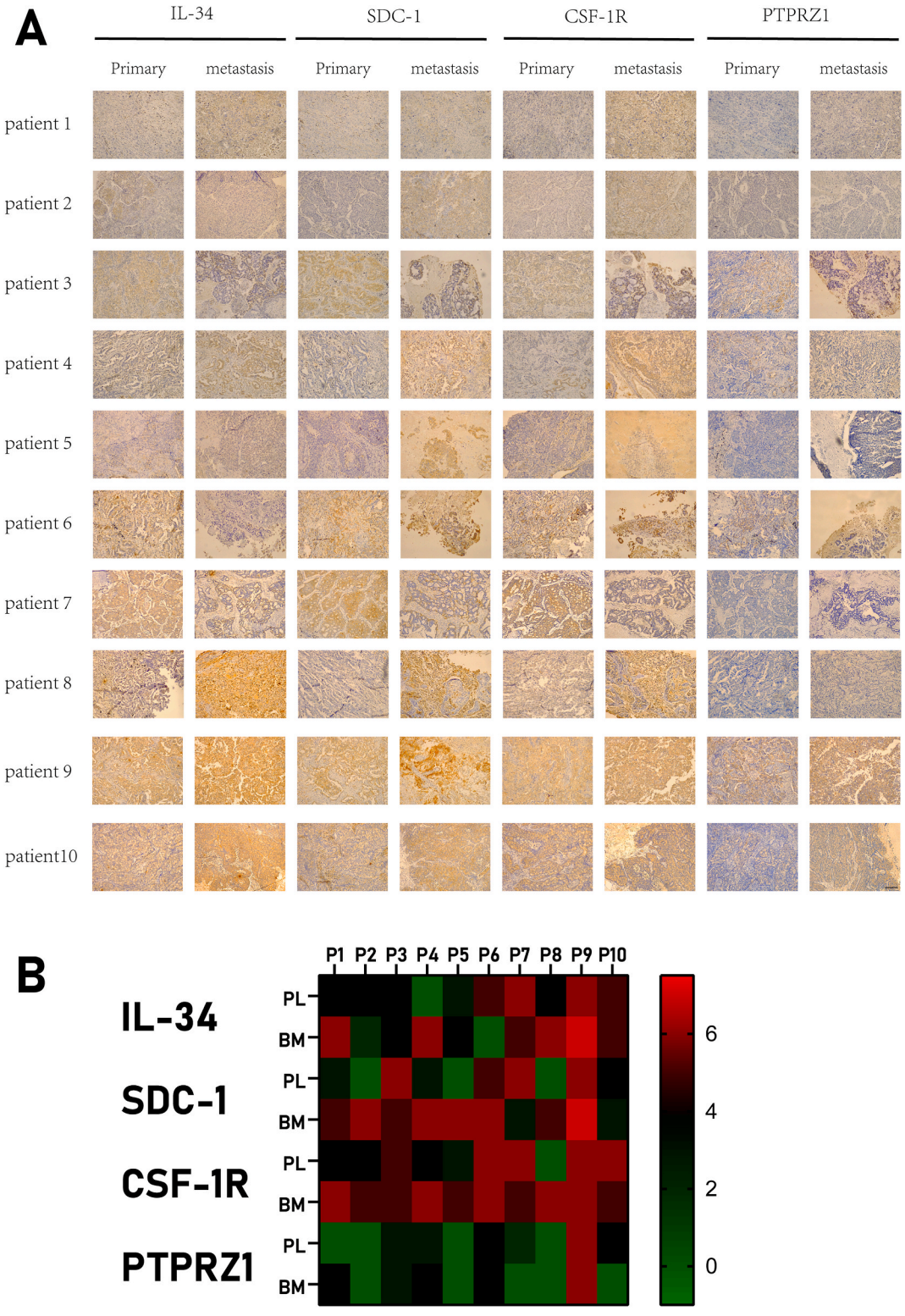

Fig. 1. Changes in the expressions of IL-34, CSF-1R, SDC1, and PTPRZ1 from primary lung lesions to brain metastases in 10 patients. (A) Immunohistochemical staining of IL-34, CSF-1R, SDC1, and PTPRZ1 in primary lung lesions and brain metastases of 10 patients. (B) Quantification of IL-34, SDC-1, CSF-1R, and PTPRZ1 expression in primary lung lesions and brain metastases using the immunohistochemistry score (IRS), presented as heat maps. PL: Primary lung lesions. BM: Brain metastatic lesions.

Table 2
The expression levels of IL-34, SDC1, CSF-1R, and PTPRZ1 in brain metastatic tissue compared to the original lung tissue for the 10 patients.

ID	IL-34			SDC-1			CSF-1R			PTPRZ1		
	P	M	Variation	P	M	Variation	P	M	Variation	P	M	Variation
P1	4	6	upregulated	3	5	upregulated	4	6	upregulated	0	4	upregulated
P2	4	2	downregulated	0	6	upregulated	4	5	upregulated	0	0	unchanged
P3	4	4	unchanged	6	5	downregulated	5	5	unchanged	3	3	unchanged
P4	0	6	upregulated	3	6	upregulated	4	6	upregulated	3	4	upregulated
P5	3	4	upregulated	0	6	upregulated	3	5	upregulated	0	0	unchanged
P6	5	0	downregulated	5	6	upregulated	6	6	unchanged	4	4	upregulated
P7	6	5	downregulated	6	3	downregulated	6	5	downregulated	2	0	downregulated
P8	4	6	upregulated	0	5	upregulated	0	6	upregulated	0	0	unchanged
P9	6	7	upregulated	6	7	upregulated	6	6	unchanged	6	6	unchanged
P10	5	5	unchanged	4	3	downregulated	6	5	downregulated	4	0	downregulated

P denotes expression level in original lung tissue and M denotes expression level in brain metastasis tissue.

receptor were examined in primary lung cancer tissues (Supplementary Fig. 1), BMs (Supplementary Fig. 2), and adjacent normal tissues. The IHC results indicated that IL-34 primarily localized in the cytoplasm and extracellular matrix of LUAD cells, while CSF-1R/SDC-1 and PTPRZ1 were mainly expressed on the cell membrane. Utilizing the immunohistochemistry scores detailed in the methods section, we defined negative and weak positivity as low expression, while positive and strong positive results as high expression. Interestingly, the expression of IL-34 and its receptors was found to be low in paracancerous lung tissues (Supplementary Fig. 1).

In contrast, IL-34 and CSF-1R were positively expressed in normal brain tissues, with a higher expression of CSF-1R in BMs (Supplementary Fig. 2). The correlation analysis between the expression level of IL-34 and its receptor and clinical/pathological features of LUAD revealed no significant association with age, sex, smoking, tumor size, lymph node metastasis, clinical stage, distant metastasis, or differentiation. Only SDC-1 expression demonstrated an association with smoking (Table 3), while the expression of IL-34 and its receptor in BMs showed no significant correlation with patients' gender, age, smoking status, or tissue differentiation (Table 4). Moreover, we performed statistical analysis on the positive expression ratio of IL-34 and its receptors in the aforementioned 96 lung cancer tissues and 68 BM tissues (Fig. 2), and observed significantly enhanced expression of IL-34/CSF-1R/SDC-1 in BMs compared to primary lung lesions (Fig. 2A–C). Notably, CSF-1R exhibited positive expression in all BMs, with only local metastatic lesions showing negative expression. However, no significant difference was detected in the expression of PTPRZ1 (Fig. 2D).

3.3. Association of IL-34, CSF-1R, SDC-1, and PTPRZ1 expression with brain metastasis in primary LUAD tissues

Among the 96 LUAD patients, 21 developed postoperative brain metastasis (BM). To assess the relationship between IL-34, CSF-1R, SDC-1, and PTPRZ1 expression in primary lung tissues and postoperative BM, a correlation analysis was conducted (Fig. 3). Among the 21 patients with BM, 15 (71.43 %) exhibited IL-34 positivity in the primary lung lesions, while 6 (28.57 %) had IL-34-negative primary

Table 3
Correlation between IL-34, CSF-1R, SDC1, and PTPRZ1 expression levels and clinical/pathological features in patients with LUAD.

Characters		Total	$IL34^{high}$/ $IL34^{weak/absent}$	P-value	$CSF\text{-}1R^{high}$/ $CSF\text{-}1R^{weak/absent}$	P-value	$SDC1^{high}$/ $SDC1^{weak/absent}$	P-value	$PTPRZ1^{high}$/ $PTPRZ1^{weak/absent}$	P-value
		96	59/37		20/76		22/74		36/60	
Age	≥65	38	27/11	0.118	9/29	0.578	12/26	0.102	21/37	0.746
	<65	58	32/26		11/47		10/48		15/23	
Gender	male	48	27/21	0.294	9/39	0.615	15/33	0.052	22/26	0.092
	female	48	32/16		11/37		7/41		14/34	
Smoking history	Positive	39	22/17	0.401	9/30	0.654	14/25	0.012*	15/24	0.872
	Negative	57	37/20		11/46		8/49		21/36	
Primary Tumor size	T1	53	36/17	0.213	12/41	0.664	14/39	0.505	25/28	0.053
	T2	33	19/14		7/26		7/26		7/26	
	T3-4	10	4/6		1/9		1/9		4/6	
Lymph node metastasis	Positive	48	34/14	0.059	10/38	1.000	11/37	1.000	16/32	0.399
	Negative	48	25/23		10/38		11/37		20/28	
Tumor stage	I	38	23/15	0.609	9/29	0.828	9/29	0.989	15/23	0.227
	II	49	30/19		9/40		11/38		20/29	
	III	9	7/2		2/7		2/7		1/8	
Distant metastasis	Positive	70	47/23	0.123	18/52	0.053	17/53	0.601	26/44	0.906
	Negative	26	13/13		2/24		5/21		10/16	
Differentiation	Well-differentiated	61	37/24	0.831	12/49	0.711	16/45	0.308	23/38	0.956
	Poorly-differentiate	35	22/13		8/27		4/29		13/22	

Distant metastasis refers to the distant metastasis after TNM staging; *$P < 0.05$.

Table 4
Correlation between IL-34, CSF-1R, SDC1, and PTPRZ1 expression levels and clinical/pathological features in LUAD patients with brain metastases (BM).

Characters		Total	IL-34^{high}/ IL34$^{weak/absent}$	P	CSF-1R^{high}/CSF-1R$^{weak/absent}$	P	SDC-1^{high}/ SDC-1 $^{weak/absent}$	P	PTPRZ1^{high}/ PTPRZ1 $^{weak/absent}$	P
		68	57/11		20/76		22/74		36/60	
Age	≥65	26	22/4	0.889	22/4	0.046*	19/7	0.578	14/12	0.204
	<65	42	35/7		41/1		28/14		16/26	
Gender	male	38	33/5	0.447	35/3	0.847	26/12	0.889	15/33	0.385
	female	30	24/6		28/2		21/9		15/15	
Smoking history	negative	21	16/5	0.253	21/0	0.120	13/8	0.389	13/8	0.048*
	Positive	47	41/6		42/5		34/13		17/30	
Differentiation	Well	32	26/6	0.587	31/1	0.208	25/7	0.130	16/16	0.357
	Poorly	36	31/5		32/4		22/14		14/22	

*P < 0.05.

lung lesions. Among these patients, 7 (33.33 %) had primary lung lesions positive for CSF-1R, whereas 14 (66.67 %) had CSF-1R-negative primary lung lesions. Additionally, 3 (14.29 %) patients showed SDC-1-positive primary lung lesions, while 18 (85.71 %) had SDC-1-negative primary lung lesions. Furthermore, 7 (33.33 %) patients had PTPRZ1-positive primary lung lesions, while 14 (66.67 %) had PTPRZ1-negative primary lung lesions. The results indicated a higher percentage of positive IL-34 expression in the primary lung lesions of patients with postoperative BM. However, when considering the analysis results of 75 patients without BM, the expression proportion of IL-34 in patients without BM was similar, and no statistically significant difference was observed. On the other hand, the expression of CSF-1R displayed an association with brain metastasis ($P < 0.05$); the positive expression of CSF-1R in primary lung lesions was only 17.33 % in patients without BM, but increased to 33.33 % in patients with BM.

3.4. Association of high expression of IL-34, CSF-1R, and SDC-1 with poor prognosis in primary LUAD tissues

To investigate the expression of IL-34 in human LUAD tissues and its clinical significance in LUAD progression and survival, we performed an analysis using an online Web-open survival database. The analysis revealed that high expression of IL-34 was associated with shorter progression-free survival (PFS) (19.57 vs. 35.07 months) and overall survival (OS) (72 vs. 108.97 months) in LUAD patients (Fig. 4A). Similarly, high expression of CSF-1R was correlated with shorter PFS (15.43 vs. 38.13 months) and OS (54.17 vs. 117.33 months) in LUAD patients (Fig. 4C). The high expression of SDC-1 was also found to be correlated with shorter PFS (20.73 vs. 35 months) and OS (71.27 vs. 110.27 months) in LUAD patients (Fig. 4E). Conversely, low expression of PTPRZ1 was associated with shorter PFS (34.9 vs. 18.9 months) and OS (127 vs. 72 months) (Fig. 4G).

We further validated these findings in our cohort of 96 LUAD patients through immunohistochemistry. Consistent with the online data, the expression of IL-34 and CSF-1R showed a significant association with disease-free survival (DFS) and OS (Fig. 4B–D). Notably, high expression of SDC-1 was associated with poor OS but not DFS (Fig. 4F). In our cohort, the expression of PTPRZ1 was not found to be associated with DFS or OS (Fig. 4H).

In the univariate analysis, the expression of IL-34 (HR for OS = 2.164, 95 % CI = 1.053–4.448, P = 0.036), CSF-1R (HR for OS = 2.541, 95 % CI = 1.316–4.907, P = 0.005), SDC-1 (HR for OS = 2.025, 95 % CI = 1.039–3.949, P = 0.038), co-expression of IL-34 and CSF-1R (HR for OS = 2.179, 95 % CI = 1.101–4.313, P = 0.025), distant metastasis (HR for OS = 3.946, 95 % CI = 1.518–10.25, P = 0.005), and lymph node metastasis (HR for OS = 2.393, 95 % CI = 1.238–4.628, P = 0.009) were identified as influencing factors for OS in LUAD patients (Table 5). In the multivariate analysis, high expression of CSF-1R (HR for OS = 8.548, 95 % CI = 1.553–47.04, P = 0.014) and co-expression of IL-34 and CSF-1R (HR for OS = 0.139, 95 % CI = 0.022–0.883, P = 0.037) were identified as independent prognostic factors influencing the survival of LUAD patients (Table 5).

3.5. Association of Co-expression of IL-34 and its receptor with poor prognosis in LUAD patients

We conducted an analysis of different phenotypic combinations of IL-34 and its receptor (Fig. 5). Our findings revealed that patients in the group with positive expression of both IL-34 and CSF-1R had poorer disease-free survival (DFS) (P = 0.010) and overall survival (OS) (P = 0.011) compared to those in the group with negative expression of both IL-34 and CSF-1R (Fig. 5A–B). Additionally, the IL-34- and SDC-1-positive group exhibited worse OS (P = 0.021) but comparable DFS (P = 0.124) when compared to the IL-34- and SDC-1-negative group and the IL-34- or SDC-1-positive group (Fig. 5C–D). Moreover, the IL-34-positive + PTPRZ1-negative group demonstrated inferior OS compared to the IL-34-negative + PTPRZ1-positive group and the IL-34-positive + PTPRZ1-positive/IL-34-negative + PTPRZ1-negative group (P = 0.035) (Fig. 5E); however, DFS was not significantly different (P = 0.185) (Fig. 5F).

4. Discussion

There is growing evidence suggesting heterogeneity in drug target expression among metastatic lesions, indicating that the molecular profiles obtained from primary tumors alone are insufficient for guiding metastasis treatment [10–12]. In our study, we

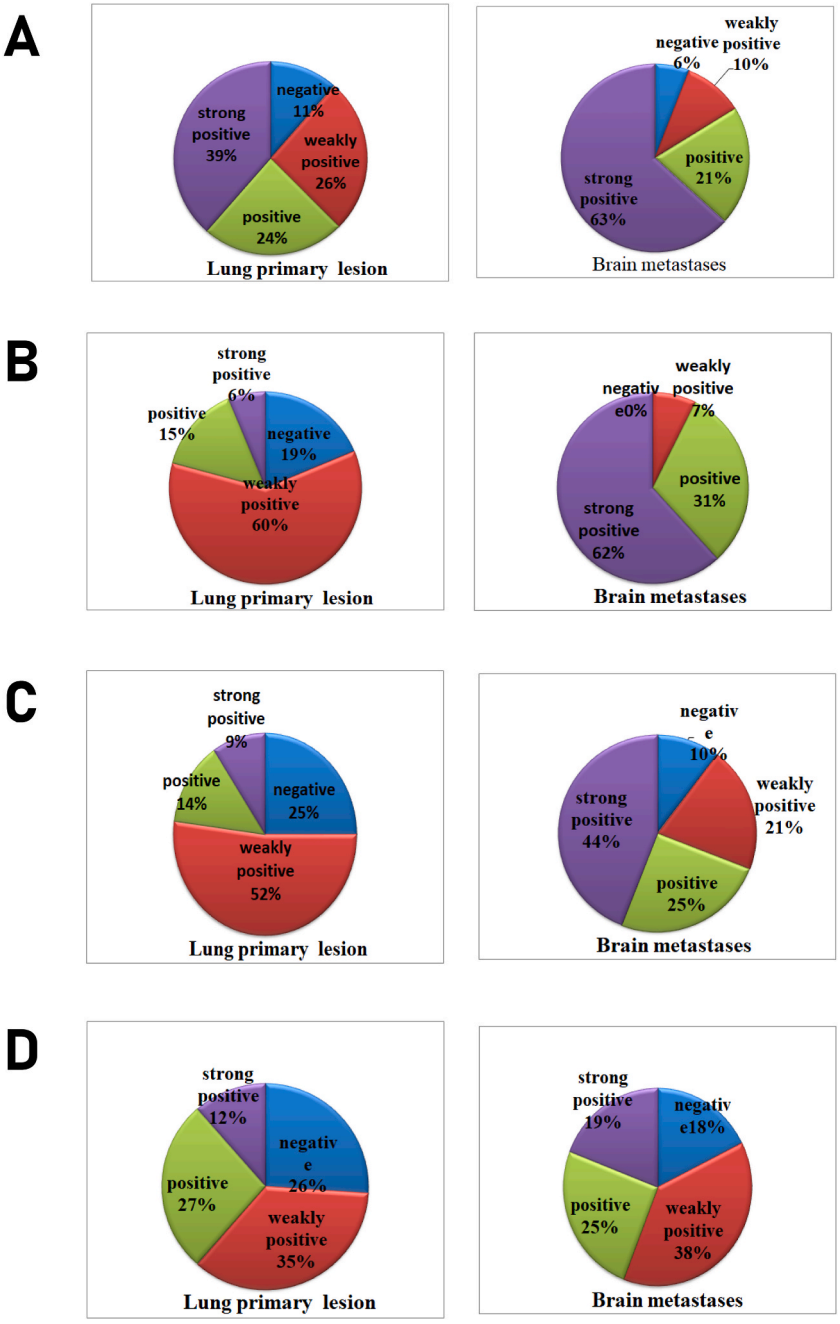

Fig. 2. Comparison of positive expression proportions of IL-34 and its receptors in primary lung cancer and brain metastases. The positive expression of IL-34 (A), CSF-1R (B), and SDC-1 (C) was significantly increased in brain metastases compared to primary lung adenocarcinoma, whereas the change in PTPRZ1 expression (D) was not statistically significant.

observed significant differences in the expression of IL-34 and its receptor between primary lesions and brain metastases (BM). Moreover, patients with positive IL-34 expression or negative PTPRZ1 expression in primary lesions exhibited a higher likelihood of developing BM. Additionally, high expression of IL-34, CSF-1R, and SDC-1 was associated with a poor prognosis. These findings contribute to the theoretical understanding of the mechanisms underlying BM and offer potential prognostic indicators for patients with LUAD.

In this study, we present novel findings regarding the expression of IL-34 and its receptor in both primary lung lesions and brain metastases (BM), based on a comprehensive analysis of immunohistochemical staining results from 10 matched pairs of primary lung cancer and brain metastatic tissues. Our results demonstrated a significant alteration in the expression of IL-34, CSF-1R, and SDC-1,

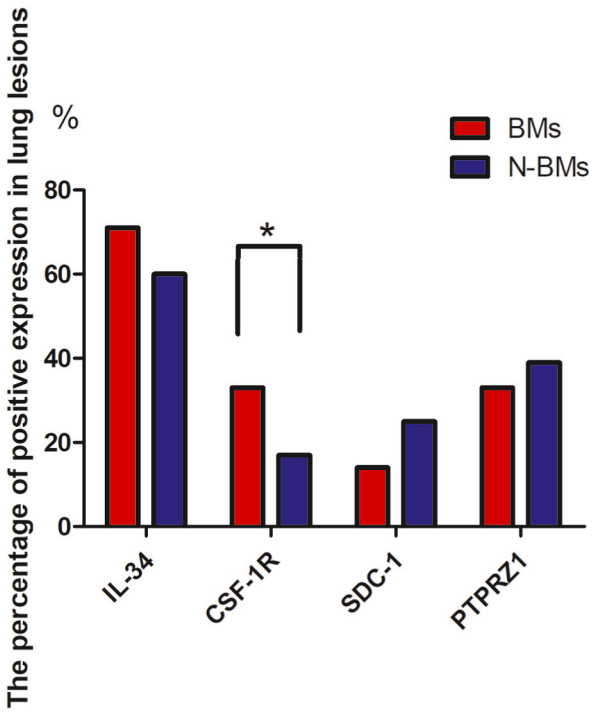

Fig. 3. Proportion of positive expression of IL34, CSF-1R, SDC-1, and PTPRZ1 in primary lung lesions of LUAD patients with and without brain metastasis. BMs: Brain metastasis. N-BMs: Non-brain metastasis. *P < 0.05.

with a marked increase observed in BM compared to primary lesions. However, the expression of PTPRZ1 remained unchanged in the majority of patients (5/10). Specifically, we observed consistently low PTPRZ1 expression in both primary lung lesions and BMs. These findings align with the characterization of PTPRZ1 as a tumor suppressor gene in cancer, despite its role in promoting cancer in certain tumor types. Our results suggest a correlation between the expression of IL-34 and its receptor and the development of BM. Moreover, the higher expression of IL-34, CSF-1R, and SDC-1 in brain metastatic lesions compared to primary lung cancer lesions, coupled with the low expression of PTPRZ1, may contribute to the propensity for lung cancer to metastasize to the brain.

Immunohistochemistry results revealed significant alterations in the expression of IL-34, CSF-1R, and SDC-1 in brain metastases (BM) compared to primary lung cancer lesions. The percentage of positive and strongly positive expression was markedly increased, while the percentage of weak positive and negative expression was significantly decreased. Specifically, CSF-1R expression was universally positive (100 %) in BMs. Evarietkotter et al. demonstrated similar findings in breast cancer BMs, with significantly higher expression of CSF-1R and IL-34 compared to normal brain tissue [13].

Notably, there was no significant difference in the expression ratio of PTPRZ1 between primary lung cancer and BMs. Thus, our study highlights, for the first time, the altered expression ratio of IL-34 and its receptor in both primary lung lesions and BMs. Similar expression differences have been observed in brain metastatic lesions of brain glioma [13]. In brain tissue, the survival of microglia is dependent on IL-34, and IL-34LacZ/LacZ mice exhibit reduced microglia numbers in specific brain regions, particularly the cortex and hippocampus [2]. These findings suggest that the increased expression of IL-34, CSF-1R, and SDC-1 in BM may promote the survival and formation of metastatic foci of LUAD cells by altering the brain tissue microenvironment. Notably, CSF-1R appears to play a critical role in the microenvironment of BM, particularly in the activation of microglia and the promotion of BM [14]. Blocking CSF-1R eliminates the effects of CSF-1 and IL-34 through this receptor, while not affecting IL-34 signaling through PTPRZ1 and SDC-1 [14,15]. Therefore, targeting IL-34 and its receptor, particularly CSF-1R, represents a significant breakthrough in the treatment and prevention of BM in LUAD.

In a cohort of 96 patients with LUAD, 21 patients developed BMs during follow-up. We subsequently investigated the association between the expression of IL-34, CSF-1R, SDC-1, and PTPRZ1 in primary lung lesions and the occurrence of postoperative BM. Our analysis revealed that 7 out of 21 patients exhibited positive CSF-1R expression in their primary lung lesions, suggesting an increased probability of developing BM. These findings highlight the potential of high CSF-1R expression as a predictor for BM in LUAD, further supporting the role of CSF-1R in the microenvironment of BM.

This study aimed to explore the relationship between the expression of IL-34, CSF-1R, SDC-1, and PTPRZ1 and the prognostic survival of LUAD patients using data from a bioinformatics technology database. Our analysis revealed that patients with high expression of IL-34, CSF-1R, and SDC-1, and low expression of PTPRZ1 had a poor prognosis. Previous research by Ken-Ichiro Seino et al. conducted immunohistochemistry staining on IL-34 in a heterogeneous group of 332 lung cancer patients and reported a correlation between high IL-34 expression and poor overall survival [4]. In our study, we focused specifically on clinical tissue sections

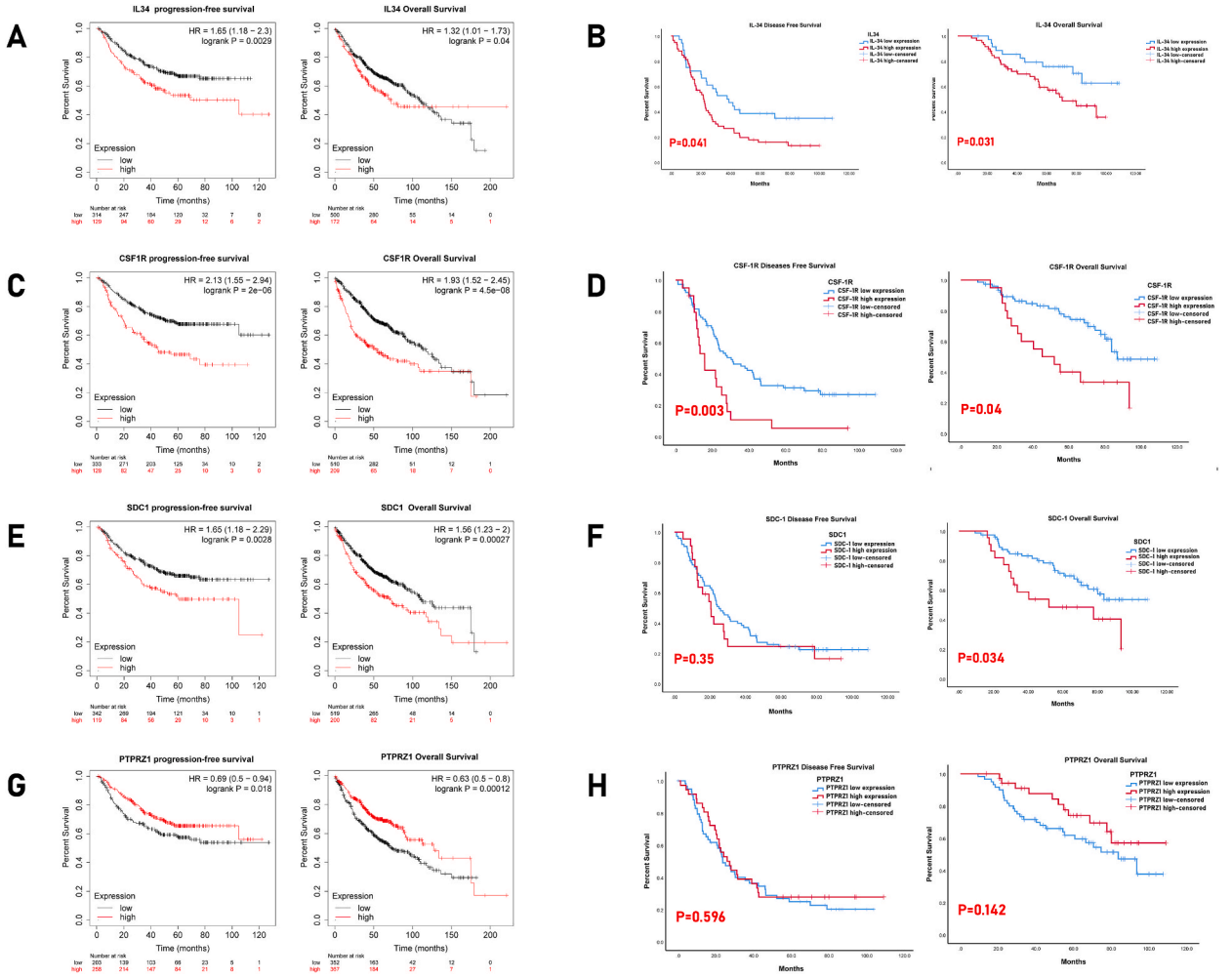

Fig. 4. Associations of IL-34, CSF-1R, SDC-1, and PTPRZ1 expressions in LUAD tissue with survival outcomes. (A, C, E, G) Kaplan-Meier survival curves illustrating progression-free survival (left) and overall survival (right) for LUAD patients with high and low mRNA expression levels of IL-34, CSF-1R, SDC-1, and PTPRZ1, analyzed using Online survival analysis software. (B, D, F, H) Kaplan-Meier survival curves depicting disease-free survival (left) and overall survival (right) in 96 LUAD patients based on high and low protein expression levels of IL-34, CSF-1R, SDC-1, and PTPRZ1.

Table 5
Univariate and multivariate analyses for overall survival (OS) in patients with LUAD.

Characteristics	Univariate			Multivariate		
	HR	95%CI	P value	HR	95%CI	P value
AGE(>65 vs. <65)	1.355	0.721–2.544	0.345	ND		
GENDER(male vs.female)	1.177	0.624–2.219	0.615	ND		
Smoking staus(nonsmoking vs. smoker)	0.845	0.433–1.649	0.621	ND		
Primary Tumor size	1.17	0.759–1.803	0.477	ND		
Lymph node metastasis	2.393	1.238–4.628	0.009**	1.783	0.802–3.965	0.156
Differentiation	0.789	0.419–1.488	0.464	ND		
Distant metastasis	3.946	1.518–10.256	**0.005****	2.246	0.740–6.815	0.153
IL34 expression (high vs. low)	2.164	1.053–4.448	**0.036***	1.833	0.781–4.299	0.164
Csf-1r expression (high vs. low)	2.541	1.316–4.907	**0.005****	8.548	1.553–47.042	**0.014***
Sdc-1 expression (high vs. low)	2.025	1.039–3.949	**0.038***	1.625	0.776–3.402	0.198
IL34 +CSF-1R + expression vs. others	2.179	1.101–4.313	**0.025***	0.139	0.022–0.883	**0.037***
IL34 +SDC-1+ expression vs. others	1.868	0.908–3.840	0.089	ND		
PTPRZ1 expression (high vs. low)	0.596	0.297–1.199	0.147	ND		

Distant metastasis refers to the distant metastasis after TNM staging. HR: hazard ratio. CI: confidence interval.*P < 0.05. **P < 0.01.

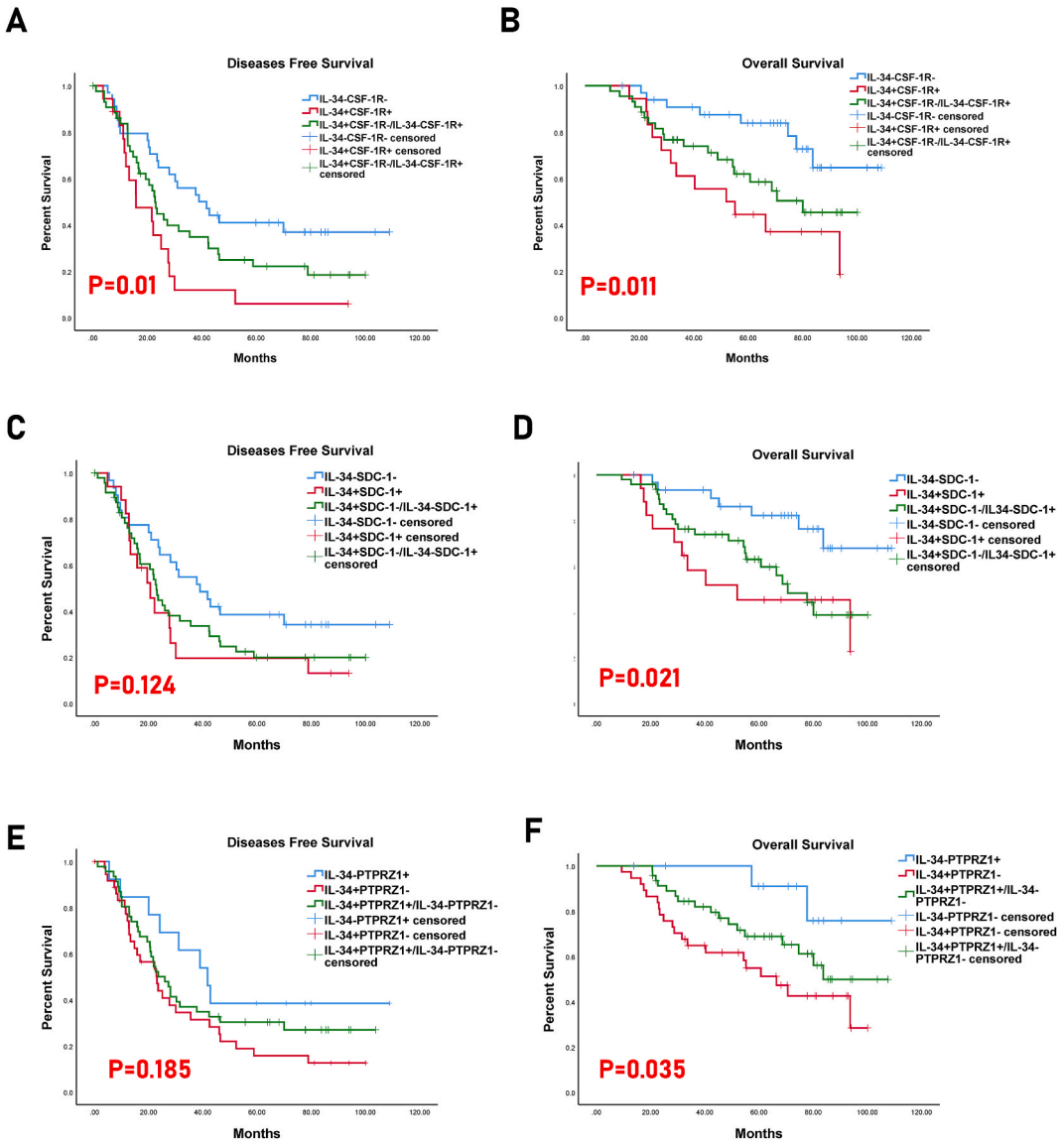

Fig. 5. Kaplan-Meier survival curves illustrating disease-free survival (left) and overall survival (right) in 96 LUAD patients based on different combinations of IL-34, CSF-1R, SDC-1, and PTPRZ1 expressions. (A–B) Disease-free survival (left) and overall survival (right) curves for LUAD patients with different combinations of IL-34 and CSF-1R. (C–D) Disease-free survival (left) and overall survival (right) curves for LUAD patients with different combinations of IL-34 and SDC-1. (E–F) Disease-free survival (left) and overall survival (right) curves for LUAD patients with different combinations of IL-34 and PTPRZ1.

from 96 LUAD patients and found that high expression of IL-34, CSF-1R, and SDC-1 was associated with shorter overall survival (OS). Additionally, high expression of IL-34 and CSF-1R correlated with shorter disease-free survival (DFS). However, no significant changes in DFS and OS were observed with alterations in PTPRZ1 expression. Furthermore, we explored different combinations of IL-34 and its receptor expressions and observed that co-expression of IL-34 and CSF-1R was associated with enhanced malignant potential in LUAD cells and poorer prognosis for patients [16]. These findings underscore the close association of IL-34 and its receptor expression with poor prognosis in LUAD patients and suggest their potential as prognostic predictors.

It is noteworthy that as research on IL-34 advances, additional receptors for IL-34 besides CSF-1R, SDC-1, and PTPRZ1 may be discovered. For instance, TREM2 has recently been identified as an additional receptor for IL-34 [17]. Exploring the relationship between these novel receptors of IL-34 and poor prognosis in patients with LUAD could enhance our understanding of the role of IL-34 in LUAD. However, this study does not encompass such an investigation as, at the time of data collection from patients, only CSF-1R, SDC-1, and PTPRZ1 were recognized as IL-34 receptors.

Finally, it is important to highlight that our assessment was limited to immunohistochemistry on tumor tissues, allowing us to discern variations between primary lung tumors and brain metastases solely at the tissue level. Further investigation into the

expression of IL-34 and its receptors in specific cell types within the tumor microenvironment, including tumoral cells and macrophages, presents an intriguing avenue for exploration. However, such analysis would necessitate single-cell sequencing or spatial sequencing, marking our future research directions.

5. Conclusion

In conclusion, our study reveals a significant association between the expression of IL-34 and its receptor in the primary lesions of LUAD patients and the occurrence of BM, as well as poor prognosis. The increased expression of IL-34 and its receptors, CSF-1R and SDC-1, in lung cancer BM tissues, coupled with the low expression of PTPRZ1 in brain metastatic tissues, may contribute to the initiation and progression of BM. These findings not only provide a theoretical foundation for prognostic assessments in LUAD patients and predicting the likelihood of BM, but also highlight the possibility of divergence in treatment strategies between BM and primary LUAD. As a result, further investigation into the underlying mechanisms of IL-34 in the development and progression of BM is warranted.

Data availability statement

The data utilized in this study is available athttps://data.mendeley.com/drafts/jwxsp9s7my.

CRediT authorship contribution statement

Jianxiong Geng: Writing – original draft, Validation, Investigation, Data curation, Conceptualization. **Shanqi Xu:** Investigation, Conceptualization. **Yingyue Cao:** Project administration, Methodology, Conceptualization. **Fang Liu:** Software, Resources. **Xingmei Ren:** Methodology, Investigation, Conceptualization. **Dehai Che:** Software, Methodology. **Bo Pan:** Visualization, Project administration, Data curation. **Yan Yu:** Writing – review & editing, Visualization, Conceptualization.

Declaration of competing interest

The authors declare no competing financial interests or personal relationships that could have influenced the work presented in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33791.

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