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ORIGINAL ARTICLE

Expression and Clinical Significance of Pim-1 and RON in Lung Cancer Patients with Benign and Malignant Pleural Effusion

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SUMMARY

Background: **This study aimed to investigate the expression differences and diagnostic value of Pim-1 and RON in lung cancer patients with benign and malignant pleural effusion.**
Methods: **Forty lung cancer patients with benign pleural effusion (BPE) and forty with malignant pleural effusion (MPE) were enrolled. The relative expression levels of Pim-1 and RON mRNA in pleural effusion were measured using real-time quantitative PCR (RT-qPCR). Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic performance of Pim-1 and RON.**
Results: **Compared to the BPE group, Pim-1 expression levels were significantly increased in the MPE group ($p < 0.05$). RON expression levels were also significantly elevated in the MPE group ($p < 0.01$). ROC curve analysis revealed that the areas under the curve (AUC) for diagnosing MPE using pleural effusion Pim-1 and RON were 0.646 (95% CI 0.526 - 0.766) and 0.809 (95% CI 0.715 - 0.904), respectively. The AUC for the combined detection of Pim-1 and RON was 0.853 (95% CI 0.773 - 0.934).**
Conclusions: **Pim-1 and RON are highly expressed in malignant pleural effusion, suggesting a role in lung cancer metastasis to the pleural cavity. Detection of Pim-1 and RON in pleural effusion demonstrates potential diagnostic value for MPE.**
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KEYWORDS

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INTRODUCTION

Lung cancer is one of the most common malignancies worldwide with high incidence and mortality rates. Global statistics from 185 countries reported approximately 1.8 million lung cancer deaths in 2022, accounting for 18.7% of all cancer deaths [1]. Its high invasiveness and early occult symptoms often lead to diagnosis at advanced stages, resulting in a poor prognosis. Pleural effusion is a common complication in lung cancer patients. Malignant pleural effusion (MPE) is present at initial diagnosis in 10 - 15% of non-small cell lung cancer (NSCLC) patients and ultimately develops in 50% of NSCLC cases due to pleural metastasis [2]. Differentiating between benign pleural effusion (BPE) and MPE is crucial for clinical management. MPE indicates tumor progression or metastasis and is associated with significantly shortened survival, whereas BPE requires different therapeutic strategies. Conventional cytological examination has limited sensitivity (50 - 60%) [3], attributed to overlapping cytological features between tumor cells, reactive mesothelial cells, and scarcity of malignant cells in early metastasis or post-treatment effusions. Therefore, identifying highly specific molecular biomarkers is urgently needed to improve diagnostic accuracy.

Pim-1, a serine/threonine kinase and proto-oncogene, promotes tumor cell proliferation, survival, and inhibits apoptosis [4]. RON (Recepteur d'Origine Nantais), a member of the MET receptor tyrosine kinase family, binds exclusively to macrophage-stimulating protein (MSP) and regulates cell motility, migration, invasion, and tubule formation [5]. Studies indicate that Pim-1 and RON are highly expressed in a variety of tumors and are closely related to tumor metastasis and poor prognosis [6-9]. This study aimed to analyze the expression differences of Pim-1 and RON in pleural effusion from lung cancer patients with BPE or MPE and evaluate their diagnostic value.

MATERIALS AND METHODS

Subjects

Eighty lung cancer patients with pleural effusion admitted to our hospital between January 2024 and March 2025 were enrolled. Based on cytological examination results, they were divided into: 1) malignant group (MPE, n = 40): cytopathology confirmed malignant cells in pleural effusion; and 2) benign group (BPE, n = 40): cytopathology showed no malignant cells in pleural effusion. All patients had radiologically or ultrasonographically confirmed pleural effusion.

The exclusion criteria were: 1) Patients receiving corticosteroids or immunosuppressants; 2) patients who had undergone radiotherapy or chemotherapy; 3) patients with major organ dysfunction, hematological, or immune diseases. There were no significant differences in age or gender between the two groups ($p > 0.05$). This study was approved by the hospital´s Ethics Committee, and informed consent was obtained from all participants.

Instruments and reagents

Instrument: ABI 7500 Real-Time PCR System (Applied Biosystems, USA).

Reagents: ReverTra Ace qPCR RT Master Mix with gDNA Remover (cDNA synthesis kit, FSQ-101, Toyobo Life Science, Japan).

Pleural effusion collection and processing

Pleural effusion was collected by thoracentesis performed by clinicians and was promptly delivered to the laboratory. Processing: 50 µL magnetic bead enzyme was added to the extraction kit. Then, 300 mL of fresh pleural effusion was aspirated. The kit was placed in an RNA extraction instrument. Total RNA was extracted and stored at -80°C.

Detection methods

Total RNA concentration and purity were measured. RNA samples were stored at -70°C until analysis. RT-qPCR was performed to measure Pim-1 and RON mRNA expression levels.

Reverse transcription (RT): RT was conducted using 20 µL reaction volume. Step 1: 65°C for 5 minutes, ice bath for 2 minutes. Step 2: 37°C for 50 minutes, 98°C for 5 minutes. cDNA was stored at -30°C. For Pim-1 qPCR analysis, the initial denaturation step was performed at 95°C for 2 minutes, followed by 45 cycles of 95°C for 10 seconds and 60°C for 32 seconds. For RON, the initial denaturation was carried out at 95°C for 1 minute, followed by 40 cycles of 95°C for 15 seconds and 60°C for 40 seconds. Melting curve analysis followed. Each sample was tested in duplicate. GAPDH was used as the endogenous control. Relative expression levels were calculated using the $2^{(-\Delta\Delta Ct)}$ method. The target gene primers are shown in Table 1.

Statistical analysis

Data analysis was performed using SPSS 26.0. Continuous data with non-normal distribution are presented as median (interquartile range, IQR) and were compared using the Mann-Whitney U test. ROC curve analysis assessed the diagnostic performance of Pim-1, RON, and their combination for MPE. A p-value < 0.05 was considered statistically significant.

RESULTS

Baseline clinical characteristics

There were no significant differences in age or gender between the MPE and BPE groups ($p > 0.05$). Among tumor markers, squamous cell carcinoma antigen (SCC) showed no significant difference ($p > 0.05$). All other markers (CEA, CA125, CA199, CA153, CYFRA 21-1) were significantly elevated in the MPE group ($p < 0.01$) (Table 2).

Expression levels of Pim-1 and RON

Pim-1 and RON expression levels were significantly higher in the MPE group compared to the BPE group (Pim-1: $p < 0.05$; RON: $p < 0.01$) (Table 3).

Diagnostic performance of Pim-1 and RON for MPE

ROC curve analysis showed that Pim-1 alone had an AUC of 0.646 (95% CI: 0.526 - 0.766, $p = 0.024$) for diagnosing MPE. RON alone had an AUC of 0.809 (95% CI: 0.715 - 0.904, $p < 0.001$). The combination of Pim-1 and RON achieved a higher AUC of 0.853 (95% CI: 0.773 - 0.934, $p < 0.001$) (Table 4, Figure 1).

Table 1. The sequence of target gene primers.

Primer name	Primer sequence (5' to 3')
PIM1-F	GGGGATCCTGCTGTATGATATGG
PIM1-R	TGCTGACATTCTGAAGAGACCCT
RON-F	GCCTAAGGGCATGGCATTTC
RON-R	TGTGGGGGTGGAACTCAGTA
β-actin-F	TGAGCGAGGCTACAGCTT
β-actin-R	TCCTTGATGTCGCGCACGATTT

Table 2. Clinical characteristics and tumor markers of the malignant and benign groups.

Parameter	Benign group (n = 40)	Malignant group (n = 40)	Z/χ^2 value	p-value
Age [years, M (IQR)]	73.50 (53.00, 65.00)	78.50 (49.00, 69.50)	-1.584	0.114
Male [n (%)]	23 (57.5%)	17 (42.5%)	1.8	0.18
CEA [µg/L, M (IQR)]	1.84 (1.13, 5.11)	146.41 (29.08, 1,500.00)	-5.865	< 0.001
CA125 [U/mL, M (IQR)]	553.3 (295.7, 1,664.9)	1,506.1 (601.4, 3,857.4)	-2.891	0.004
CA199 [U/mL, M (IQR)]	2.31 (2.00, 6.39)	278.31 (17.39, 1,200.00)	-5.274	< 0.001
CA153 [U/mL, M (IQR)]	6.0 (3.1, 16.7)	85.7 (10.0, 210.8)	-5.002	< 0.001
CYFRA 21-1 [ng/mL, M (IQR)]	2.29 (1.33, 4.16)	9.36 (4.74, 26.08)	-5.802	< 0.001
SCC [ng/mL, M (IQR)]	2.29 (1.33, 4.16)	1.0 (0.55, 4.16)	-0.716	0.474

CEA carcinoembryonic antigen, CA125 carbohydrate antigen 125, CA199 carbohydrate antigen 19-9, CA153 carbohydrate antigen 15-3, CYFRA 21-1 cytokeratin 19 fragment, SCC squamous cell carcinoma antigen. * Values below the detection limit were assigned the lower limit value; values above the detection limit were assigned the upper limit value.

Table 3. Comparison of Pim-1 and RON expression levels [median (IQR)].

Group	n	Pim-1 expression	RON expression
Malignant	40	1.003 (0.489, 1.943)	0.857 (0.215, 1.570)
Benign	40	0.558 (0.368, 1.249)	0.143 (0.061, 0.320)
Z-value		-2.252	-4.743
p-value		< 0.05	< 0.01

Table 4. Diagnostic performance of pleural effusion Pim-1 and RON alone and in combination for lung cancer with malignant pleural effusion.

Biomarker	AUC	Std. error	p-value	95% CI lower	95% CI upper
Pim-1	0.646	0.061	0.024	0.526	0.766
RON	0.809	0.048	< 0.001	0.715	0.904
Pim-1 + RON	0.853	0.041	< 0.001	0.773	0.934

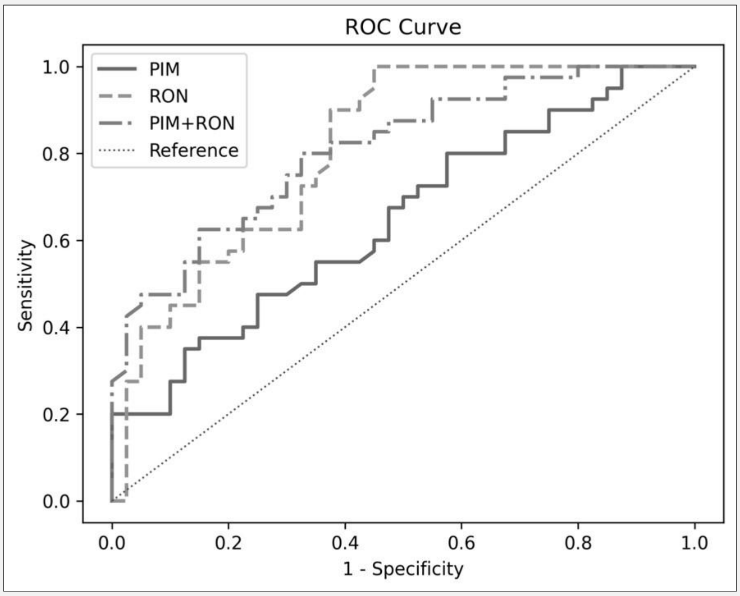

Figure 1. ROC curves for Pim-1, RON, and their combination in diagnosing lung cancer with malignant pleural effusion.

DISCUSSION

Pim-1 and RON, key molecules in tumor-related signaling pathways, play crucial roles in lung cancer proliferation, invasion, and drug resistance. Pim-1, a serine/threonine kinase, regulates cell cycle progression, inhibits apoptosis, and promotes metabolic reprogramming by phosphorylating downstream targets. Pim-1 is also implicated in immune suppression within the tumor microenvironment, potentially promoting immune escape by modulating T-cell function [10]. RON, belonging to the MET receptor tyrosine kinase family, binds MSP. Its aberrant activation induces epithelial-mesenchymal transition (EMT) via PI3K/AKT and MAPK/ERK pathways, enhancing lung cancer cell migration and invasion [11]. Knockdown of RON significantly reduces cancer cell viability and motility, highlighting its potential as a therapeutic target.

This study demonstrates significantly higher expression levels of both Pim-1 and RON in MPE compared to BPE. Pang et al. reported that Pim-1 was upregulated in 66.2% of NSCLC tissues and its overexpression correlated with advanced tumor stage ($p = 0.019$) and lymph node metastasis, indicating its critical role in promoting cancer progression and invasion [12]. Functionally, Pim-1 knockdown suppressed NSCLC cell proliferation, migration, and cell cycle progression by downregulating Cyclin D1 and CDK4, further supporting its oncogenic properties [13]. Similarly, RON is overexpressed in lung cancer tissues with further elevation in metastatic lesions. Its kinase-activated form correlates with total RON levels, and RON ablation impairs cell viability and motility, underscoring its role in lung cancer progression and metastasis [11]. Kim [14] linked high RON expression to solid growth patterns and pleural invasion in lung adenocarcinoma. Our ROC analysis confirms the diagnostic potential of both markers, with RON (AUC = 0.809) outperforming Pim-1 (AUC = 0.646). Importantly, their combination yielded a higher AUC (0.853), suggesting a multi-marker strategy enhances diagnostic accuracy for MPE.

This study has limitations: the single-center design and relatively small sample size may limit generalizability. Future multi-center studies with larger cohorts are needed. Further stratification of the MPE group by histological subtype (e.g., adenocarcinoma vs. squamous cell carcinoma) would also be valuable.

In conclusion, this study reveals the overexpression of Pim-1 and RON in MPE associated with lung cancer and highlights their combined potential as auxiliary diagnostic biomarkers for distinguishing MPE from BPE, offering a non-invasive approach.

Ethical Approval Statement:

This study was approved by the Ethics Committee of Yangming Hospital Affiliated to Ningbo University (Yuyao People's Hospital) (2023-12-003).

Declaration of Interest:

All authors declare that they have no conflicts of interest.

References:

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024;74 (3):229-63. (PMID: 38572751)

2. Porcel JM, Light RW. Pleural effusions due to pulmonary embolism. Curr Opin Pulm Med 2008;14(4):337-42. (PMID: 18520269)

3. Hooper C, Lee YC, Maskell N; BTS Pleural Guideline Group. Investigation of a unilateral pleural effusion in adults: British Thoracic Society Pleural Disease Guideline 2010. Thorax 2010;65 (Suppl 2):ii4-17. (PMID: 20696692)

4. Brasó-Maristany F, Filosto S, Catchpole S, et al. PIM1 kinase regulates cell death, tumor growth and chemotherapy response in triple-negative breast cancer. Nat Med 2016;22(11):1303-13. (PMID: 27775704)

5. Chen YQ, Zhou YQ, Angeloni D, Kurtz AL, Qiang XZ, Wang MH. Overexpression and activation of the RON receptor tyrosine kinase in a panel of human colorectal carcinoma cell lines. Exp Cell Res 2000;261(1):229-38. (PMID: 11082293)

6. Jin Y, Tong DY, Chen JN, et al. Overexpression of osteopontin, αvβ3 and Pim-1 associated with prognostically important clinicopathologic variables in non-small cell lung cancer. PLoS One 2012;7(10):e48575. (PMID: 23119061)

7. Hu CY, Xu XM, Hong B, et al. Aberrant RON and MET Co-overexpression as Novel Prognostic Biomarkers of Shortened Patient Survival and Therapeutic Targets of Tyrosine Kinase Inhibitors in Pancreatic Cancer. Front Oncol 2019;9:1377. (PMID: 31867280)

8. Gurska LM, Okabe R, Schurer A, et al. Crizotinib Has Preclinical Efficacy in Philadelphia-Negative Myeloproliferative Neoplasms. Clin Cancer Res 2023;29(5):943-56. (PMID: 36537918)

9. Yang H, He K, Dong W, et al. PIM-1 may function as an oncogene in cervical cancer via activating the EGFR signaling. Int J Biol Markers 2020;35(3):67-73. (PMID: 32914663)

10. Cao L, Wang F, Li S, Wang X, Huang D, Jiang R. PIM1 kinase promotes cell proliferation, metastasis and tumor growth of lung adenocarcinoma by potentiating the c-MET signaling pathway. Cancer Lett 2019;444:116-26. (PMID: 30583073)

11. Kanteti R, Krishnaswamy S, Catenacci D, et al. Differential expression of RON in small and non-small cell lung cancers. Genes Chromosomes Cancer 2012;51(9):841-51. (PMID: 22585712)

12. Pang W, Tian X, Bai F, et al. Pim-1 kinase is a target of miR-486-5p and eukaryotic translation initiation factor 4E, and plays a critical role in lung cancer. Mol Cancer 2014;13:240. (PMID: 25342548)

13. Ntzifa A, Marras T, Kallergi G, Kotsakis A, Georgoulias V, Lianidou E. Comprehensive liquid biopsy analysis for monitoring NSCLC patients under second-line osimertinib treatment. Front Oncol 2024;14:1435537. (PMID: 39497713)

14. Kim YA, Sung WJ. The immunohistochemical expression of c-MET and RON in lung adenocarcinoma with clinicopathologic correlation. Indian J Pathol Microbiol 2025;68(1):23-9. (PMID: 38847203)