Application of liquid biopsy in differentiating lung cancer from benign pulmonary nodules (Review)

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Received December 4, 2024; Accepted April 8, 2025

DOI: 10.3892/ijmm.2025.5547

Abstract. The diagnosis of malignant and benign pulmonary nodules has always been a prominent research topic. Accurately distinguishing between these types of lesions, particularly small or ground glass nodules, is crucial for the early detection and proactive treatment of lung cancer, ultimately leading to improved patient survival. Although various imaging methods and tissue biopsies have advanced the diagnostic efficacy of pulmonary nodules, each approach possesses inherent limitations. In recent years, there has been a growing interest in liquid biopsy as a non-invasive and easily obtainable alternative. Furthermore, in-depth investigations into the mechanisms underlying tumor initiation and progression have contributed to the development of circulating biomarkers for monitoring treatment response and efficacy in lung cancer. This review provides a comprehensive overview of the current landscape of pulmonary nodule diagnosis while highlighting the latest advancements in liquid biopsy techniques, such as extracellular vesicles, tumor-educated platelets, non-coding RNA, circulating tumor DNA and circulating antibodies.

Contents

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Abbreviations: GGNs, ground glass nodules; BPN, benign pulmonary nodules; CT, computerized tomography; PET, positron emission tomography; MRI, magnetic resonance imaging; AI, artificial intelligence; GGO, ground glass opacity; LDCT, low-dose computed tomography; SPN, solitary pulmonary nodules; LUAD, lung adenocarcinoma; CNNs, convolutional neural networks; LB, liquid biopsy; TB, tissue biopsy; EVs, extracellular vesicles; NSCLC, non-small cell lung cancer; AUC, area under the curve; TLDA, TaqMan low density array; TNM, tumor node metastasis; exLRs, EV-associated long RNAs; FGB, fibrinogen beta chain; FGG, fibrinogen gamma chain; EMT, epithelial-mesenchymal transition; NSE, neuron-specific enolase; CYFRA21-1, cytokeratin 19 fragment; SCC, squamous cell carcinoma antigen; IGHV4-4, immunoglobulin heavy variable 4-4; IGLV1-40, immunoglobulin lambda variable 1-40; TEPs, tumor-educated blood platelets; CEA, carcino-embryonic antigen; TEP ITGA2B, tumor-educated blood platelets integrin alpha 2b; PLR, platelet-to-lymphocyte ratio; SCHC, Sichuan Hospital of Cancer; ncRNA, non-coding RNA; pfeRNAs, protein functional effector RNAs; ctDNA, circulating tumor DNA; TAC1, tachykinin precursor 1; CDO1, cysteine dioxygenase type 1; HOXA7, homeobox A7; SOX17, SRY-box transcription factor 17; PTGER4, prostaglandin E receptor 4; RASSF1A, ras association domain family 1A; SHOX2, short stature homeobox gene 2; FUT7, fucosyltransferase 7; TAAb, autoantibodies targeting tumor-associated antigens; FCGR2A, Fc gamma receptor IIa; EPB41L3, erythrocyte B membrane protein band 4.1 like 3; LINGO1, nogo receptor-interacting protein-1; S100A7L2, S100 calcium binding protein A7 like 2; P53, tumor protein 53; PGP9.5, protein gene product 9.5; GAGE7, G antigen 7; GBU4-5, ATP-dependent RNA helicase 4-5; MAGEA1, melanoma-associated antigen A1; CAGE, cancer-associated gene; DCD, dermcidin; MID1IP1, MID1 interacting protein 1; PNMA1, paraneoplastic antigen MA1; TAF10, TATA-box binding protein associated factor 10; ZNF696, zinc finger protein 696

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Key words: pulmonary nodules, lung cancer, liquid biopsy, circulating biomarker, extracellular vesicles, non-coding RNA, tumor-educated platelets, circulating tumor DNA, circulating antibodies

1. Introduction

According to the Global Cancer Statistics 2020, lung cancer is the second most common cancer and the leading cause of cancer-related death (1). The distinction of pulmonary nodules and early diagnosis of lung cancer are crucial for improving prognosis and enabling surgical intervention. However, current screening methods, such as imaging and tissue biopsy, have limitations, such as false positives, radiation exposure and potential complications like hemorrhage, infection, pneumothorax and implantation metastasis. Therefore, there is an urgent need for more reliable, testable and safer biomarkers. Liquid biopsy has emerged as a non-invasive examination that can reveal important tumor features, including gene mutations and metabolic changes. It is increasingly recognized as a valuable alternative for diagnosing and differentiating pulmonary nodules. The present review aimed to discuss the current status of pulmonary nodule diagnosis and summarize the latest applications of liquid biopsy in identifying malignant and benign pulmonary nodules (BPN) (Table I; Fig. 1).

2. Current status in the diagnosis of pulmonary nodules

Pulmonary nodules, defined as rounded or abnormally cloudy lesions smaller than 30 mm in diameter, can be well or poorly demarcated and surrounded by an inflated lung on radiological imaging (2). They are classified as benign or malignant, with significant prognostic differences (3). Thus, improving existing methods or exploring new approaches for early diagnosis of pulmonary nodules is crucial. Tissue biopsy and imaging techniques, such as computerized tomography (CT), positron emission tomography (PET)/CT and magnetic resonance imaging (MRI), are currently the main diagnostic methods. The application of artificial intelligence (AI) tools on radiology images and digital pathology (4) can effectively enhance diagnostic efficiency, optimize treatment, evaluate prognosis and ultimately reduce mortality (5).

CT assessment of pulmonary nodules. Various CT characteristics (nodule size, growth rate, location, morphology, enhancement) are associated with the nature of pulmonary nodules (6). In cases where the probability of malignancy is low, such as with constant, perifissural, well-circumscribed nodules, satellite nodules with benign imaging features (stippled, laminated, dense central or popcorn patterns of calcification), or individual nodules without any risk factors (a mixed ground glass opacity <6 mm in diameter), further imaging follow-up may not be necessary if these features remain unchanged for two years or more. However, with nodules larger than 10-20 mm, the risk of malignancy increases significantly and medical follow-up is important (7). It should be noted that CT imaging for screening pulmonary nodules has limitations, as it can detect both invasive and inert tumors (8). Due to the high sensitivity of low-dose computed tomography (LDCT), numerous non-neoplastic solitary pulmonary nodules (SPN) are also detected, resulting in an increase in false positives and leading to follow-up repeat CT scans and potential issues with radiation exposure (9).

PET/CT assessment of pulmonary nodules. According to the research conducted by Dalli *et al* (10), PET/CT is a highly precise method for distinguishing between benign and malignant SPN. Similarly, ^{18}F-fluorodeoxyglucose PET/CT is more accurate than helical dynamic CT for diagnosing SPN. However, PET/CT may not be reliable for qualitative assessment and staging of pure ground-glass nodular lung adenocarcinoma (LUAD) (6).

MRI assessment of pulmonary nodules. MRI holds promise for longitudinally assessing lung disease and function, offering an alternative to LDCT in patients with lung cancer. With excellent soft tissue contrast and high spatial resolution, MRI provides both morphologic and functional information through diffusion-weighted and perfusion sequences (11). However, a critical challenge in lung MRI is its susceptibility to motion artifacts caused by cardiac and respiratory movements. To mitigate these effects, implementing breath-gated or breath-holding techniques during image acquisition is recommended (12).

AI diagnosis systems for pulmonary nodules. New techniques such as computational radiomics and deep learning-based AI show promise in differentiating between malignant and benign nodules (6). Computer-aided diagnosis systems traditionally rely on imaging characteristics, including image segmentation, feature value extraction and classification, and are now being enhanced with convolutional neural networks (CNNs)-based models. These models use a multi-view strategy to improve sensitivity for pulmonary nodules. CNN models are widely employed not only in CT images but also in cytopathological (13) and histological imaging (14). Certain studies utilize CNN models to identify key gene mutations or investigate the development mechanisms of cancer (15,16). However, nodule features may influence the predictive performance of CNN models. In addition, training complex CNN models with limited training sets can lead to overfitting (14).

3. Liquid biopsies for pulmonary nodules

Liquid biopsy is a non-invasive and reproducible method for real-time monitoring of tumors, allowing for the rapid retrieval of pathological information from patient body fluids. It offers valuable insights into the molecular mechanisms involved in tumorigenesis and progression, making it a promising alternative to tumor tissue samples in clinical settings (17). Various biomarkers, including extracellular vesicles (EVs), circulating tumor DNA (ctDNA), platelets, plasma RNA and circulating antibodies, have been identified as non-invasive markers for diagnosing pulmonary nodules (18). The integration of advanced technologies has further enhanced the efficient capture of biomarkers for liquid biopsy, revolutionizing clinical decision-making at various stages of lung cancer management (18-21).

EVs

EVs in lung cancer. EVs, which are endosome-derived nano vesicles (30-1,000 nm) involved in intercellular communication, play a role in the immune escape, metastasis, metabolic reprogramming and drug resistance of lung cancer (22). For

Table I. Application of liquid biopsy in the diagnosis of pulmonary nodules.

Class	Author/s, year	Biomarkers	Method	Deregulation	Diagnosis and differential diagnosis	Targets or biological processes	Sensitivity, %	Specificity, %	AUC	Other applications and advantages	(Refs.)
EV-associated miRNAs	Gao *et al* 2022	miR-106b-3p+ miR-125a-5p+ miR-3615+ miR-450b-5p	Sequencing RT-qPCR	NA	LUAD vs. HC and BPNs	ZNRF3+ KRAS and NF-kB pathways+ NA+YAP1	84.9	80.9	0.902	Recognizing patients with AIS and MIA	(31)
	Zhong *et al* 2021	miR-520c-3p+ miR-1274b	TLDA+ RT-qPCR	Up	NSCLC vs. BPNs	AKT1, AKT2+ CNN1	NA	NA	0.823	The highest efficiency of EV extraction and low albumin impurities	(37)
	Zhang *et al* 2023	miR-1290/ miR-29c-3p	Sequencing RT-qPCR	Up: miR-1290 Down: miR-29c-3p	LC vs. BPNs	IRF2/ PDGF-BB/ wnt pathway	97.37/ 89.47	89.47/ 84.21	0.934/ 0.868	Distinguishing between NSCLC and SCLC, and diagnosing early-stage lung cancer	(30)
	Chen *et al* 2022	miR-4732-5p+ miR-451a+ miR486-5p+ miR139-3p	Fucose-capture sequencing, RT-qPCR	Up: miR-4732-5p/ miR-451a/ miR486-5p Down: miR139-3p	LUAD vs. BPNs	XPR1+ATF2+ ribosomal proteins+ CHEK1	91.07	66.36	0.855	Developing a new method for extracting EV	(43)
	Zheng *et al* 2023	miR-4497	Sequencing RT-qPCR	Down	NSCLC vs. BPNs	GBX2	73.30	72.60	0.748	Diagnosing early-stage NSCLC and monitoring the degree of tumor malignancy	(48)
	Yang *et al* 2020	miR-21/ Let-7a ratio	RT-qPCR	Up	NSCLC vs. BPNs	PTEN	56.00	82.61	0.754	NA	(36)

Table I. Continued.

Class	Author/s, year	Biomarkers	Method	Deregulation	Diagnosis and differential diagnosis	Targets or biological processes	Sensitivity, %	Specificity, %	AUC	Other applications and advantages	(Refs.)
EV-associated long RNAs	Zhang *et al* 2023	SEC62+ ANXA4+ KIAA1217+ TMTC1+ KAZN+ AC009303.2+ HLA-E+ ARHGAP30+ CICP3+ CXCL8+ IVNS1ABP+ MTCYBP18+ CSRNP1+ GFI1B+ SNX29+ GOLGA3+ USP49+ RNU6-959P + SPIRE1 + LRRC49 + TMEM231 + PLTP+NPC2	Sequencing high-throughput screening and support vector machine analyses	Up: SEC62/ ANXA4/ KIAA1217/ TMTC1/ KAZN/ AC009303.2 Down: Others	LUAD vs. BPNs	Integrin α/ CAV1 pathway etc.	93.75	85.71	0.918	High diagnostic accuracy and classification of AIS vs. MIA/IAD	(51)
	Min *et al* 2022	RP5-977B1	Deep sequencing RT-qPCR	Up	NSCLC vs. HC	NA	82.86	84.93	0.890	Diagnosing early-stage NSCLC and predicting the prognosis of NSCLC	(53)
EV-associated protein	Kuang *et al* 2019	FGG+FGB	WB	Up	LC vs. BPNs	Adherence of tumor cells	81.40	70.00	0.794	NA	(56)
	Chang *et al* 2023	Versican	WB and ELISA	Up	NSCLC vs. HC and BPNs	Formation of an inflammatory	85.45	61.82	0.790	Positive correlation with TNM stage,	(57)

Table I. Continued.

Class	Author/s, year	Biomarkers	Method	Deregulation	Diagnosis and differential diagnosis	Targets or biological processes	Sensitivity, %	Specificity, %	AUC	Other applications and advantages	(Refs.)
						tumor microenvironment				lymph node metastasis, distant metastasis and mutation	
	Yang *et al* 2023	IGHV4-4+ IGLV1-40	WB and ELISA	Up	NSCLC vs. HC	NA	88.73	85.00	0.930	Diagnosing metastatic NSCLC and combining traditional biomarkers to improve diagnostic efficiency	(58)
	An *et al* 2019	Fibronectin	LC-ESI MS/MS	Up	NSCLC vs. HC	mTOR pathway	NA	NA	0.844	Increasing the tissue specificity	(61)
TEPs	Li et *al* 2021	Linc-GTF2H2-1+ RP3-466P17.2+ lnc-ST8SIA4-12	qPCR	Up: Lnc-ST8SIA4-12 Down: Linc-GTF2H2-1/ RP3-466P17.2	LC vs. HC	NA	82.60	87.10	0.921	Diagnosing early-stage LC and combining traditional biomarkers to improve diagnostic efficiency	(64)
	Xing *et al* 2019	ITGA2B	RNA-seq and PCR	Up	NSCLC vs. BPNs	Hematogenous metastasis of cancer	96.40	81.70	0.940	Predicting overall survival	(65)
miRNAs	Xi *et al* 2018	miR-17+ miR-146a+ miR-200b etc.	RT-qPCR	Up	NSCLC vs. BPNs	STAT3+ IRS2+ RhoE etc.	54.8-83.3	60.00-86.70	0.675-0.740	NA	(71)
	He *et al* 2018	miR-199a-3p+ miR-148a-3p+ miR-210-3p+	RT-qPCR	NA	LC vs. BPNs	Rheb etc.	34.00	90.20	NA	NA	(72)

Table I. Continued.

Class	Author/s, year	Biomarkers	Method	Deregulation	Diagnosis and differential diagnosis	Targets or biological processes	Sensitivity, %	Specificity, %	AUC	Other applications and advantages	(Refs.)
	Shen *et al* 2011	miR-378d+ miR-138-5p miR-21+ miR-210+ miR-486-5p	RT-qPCR	Up: miR-21/ miR-210 Down: miR-486-5p	LC vs. BPNs	PTEN+ JAK2/ STAT3 pathway+ TGF-β/ SMAD2 signaling pathway	75.00	84.95	0.860	NA	(73)
	Fan *et al* 2018	Five paired miRNA ratios (miR-15b-5p/ miR-146b-3p, miR-20a-5p/ miR-146b-3p, miR-19a-3p/ miR-146b-3p, miR-92a-3p/ miR-146b-3p, and miR-16-5p/ miR-146b-3p)	RT-qPCR	Up	NSCLC vs. BPNs	NA	70.00	90.00	0.870	NA	(74)
lncRNAs	Chen *et al* 2023	lncRNA THRIL	RT-qPCR	Up	LC vs. BPNs	miR-99a	87.34	83.78	0.912	Exploring the role of THRIL in the development of LC	(77)
	Jiang *et al* 2018	lncRNA XLOC_ 009167	RT-qPCR	Up	LC vs. HC	NA	78.70	61.80	0.740	Remains stable in whole blood under different conditions	(78)
pfeRNAs	Liu *et al* 2021	pfeRNAa to pfeRNAh	RT-qPCR	NA	NSCLC vs. BPNs	NA	77.10	74.25	0.840	Classification with vs. without pulmonary nodules	(81)

Table I. Continued.

Class	Author/s, year	Biomarkers	Method	Deregulation	Diagnosis and differential diagnosis	Targets or biological processes	Sensitivity, %	Specificity, %	AUC	Other applications and advantages	(Refs.)
ctDNA mutation	Jiang *et al* 2021	RNF213	NGS and IHC	Up	LC vs. BPNs	α-KGDD	NA	100.00	NA	High specificity	(84)
ctDNA methylation	Liang *et al* 2019	9 hypermethylated markers	High throughput DNA bisulfite sequencing	Up	LC vs. BPNs	NA	79.50	85.20	NA	Diagnosing early-stage LC	(87)
	Chen *et al* 2020	CDO1+ SOX17+ HOXA7	MOB and qMSP	Up	NSCLC vs. BPNs	Metabolism of cysteine+ Wnt/β-catenin signaling pathway+ NA	90.00	71.00	0.880	Using a new approach to extract DNA	(90)
	Fang *et al* 2022	Methylation level in FUT7 CpG_1-7	Mass spectrometry	Down	LC vs. BPNs	EMT and immune infiltration	71.43	56.65	0.658	Large number of clinical samples and classification LC vs. HC	(88)
Antibodies	Lastwika *et al* 2019	FCGR2A+ EPB41L3+ LINGO1+ S100A7L2	High-density protein arrays and ELISA	Up	NSCLC vs. BPNs (with the largest diameter 8-20 mm)	NA	91.70	57.10	0.780	Potentially useful for immunotherapeutic target selection	(97)
	Auger *et al* 2023	Annexin2+ DCD+ MID1IP1+ PNMA1+ TAF10+ ZNF696	High-throughput protein microarrays and custom Luminex immunobead assays	NA	'Actionable' vs. 'non-actionable' nodules	Tumor metastasis etc.	97.20	50.00	NA	Using a new approach to assay the candidate antibodies and high sensitivity	(99)
	Shome *et al* 2023	20 anti-microbial antibodies	Protein microarrays and ELISA	NA	LUAD vs. BPNs	NA	NA	NA	0.800	Large number of amples profiled sagainst hundreds of bacterial and viral antigens	(100)

Table I. Continued.

Class	Author/s, year	Biomarkers	Method	Deregulation	Diagnosis and differential diagnosis	Targets or biological processes	Sensitivity, %	Specificity, %	AUC	Other applications and advantages	(Refs.)
Combined indicators	Wang *et al* 2023	Plasma PGM5-AS1, SFTA1P, CTA-384D8.35, Log10CEA, Log10CA125, SCC and NSE	RT-qPCR	NA	NSCLC vs. HC	miRNA-423-5p, Hippo-YAP/TAZ signaling	NA	NA	0.970	Large number of clinical samples, outstanding diagnostic performance and clinical applicability	(52)
	Li *et al* 2019	lncRNA GAS5+CEA	RT-qPCR	NA	NSCLC vs. HC	IGF-1R	89.06	90.00	0.929	Diagnosing early-stage NSCLC and high specificity	(54)
	Peng *et al* 2019	Age of the patients, ctDNA mutations and serum biomarkers	Ultra-deep sequencing	NA	LC vs. BPNs	NA	80.00	99.00	NA	Excluding the noisy background of the cfDNA and high specificity	(85)
	Xing *et al* 2021	PTGER4, RASSF1A, SHOX2 and the diameter of pulmonary nodules	qMSP	NA	LC vs. BPNs	Cell cycle and apoptosis etc.	89.50	95.40	0.948	High diagnostic accuracy	(93)
	He *et al* 2023	100-feature cfDNA methylation, clinical features and CT imaging features	Targeted DNA methylation sequencing	NA	LUAD vs. BPNs	NA	98.00	0.500	0.900	Large number of clinical samples, high sensitivity, diagnosing early-stage LC and decision-making regarding surgery	(94)
	Xu *et a*l 2022	7AABs (P53, PGP9.5, SOX2, GAGE7,	ELISA	Up	LC vs. BPNs	TIF1γ etc.	96.40	79.10	0.960	Good performance and repeatability	(98)

Table I. Continued.

Class	Author/s, year	Biomarkers	Method	Deregulation	Diagnosis and differential diagnosis	Targets or biological processes	Sensitivity, %	Specificity, %	AUC	Other applications and advantages	(Refs.)
		GBU4-5, MAGEA1 and CAGE), clinical information and imaging data									

SEC62, SEC62 homolog, preprotein translocation factor; ANXA4, annexin A4; TMTC1, transmembrane and tetratricopeptide repeat containing 1; KAZN, Kazrin; HLA-E, human leukocyte antigen E; ARHGAP30, Rho GTPase-activating protein 30; CXCL8, C-X-C motif chemokine ligand 8; IVNS1ABP, influenza virus NS1A binding protein; MTCYBP18, mitochondrial cytochrome B pseudogene 18; CSRNP1, cysteine-serine-rich nuclear protein 1; GFI1B, growth factor independent 1B; SNX29, sorting nexin 29; GOLGA3, golgin A3; USP49, ubiquitin specific peptidase 49; RNU6-959P, RNA U6 small nuclear 959 pseudogene; SPIRE1, spire type actin nucleation factor 1; LRRC49, leucine-rich repeat-containing protein 49; TMEM231, transmembrane protein 231; PLTP, phospholipid transfer protein; NPC2, Niemann-Pick disease type C2; FGB, fibrinogen beta chain; FGG, fibrinogen gamma chain; IGHV4-4, immunoglobulin heavy variable 4-4; IGLV1-40, immunoglobulin lambda variable 1-40; ITGA2B, tumor-educated blood platelets integrin alpha 2b; RNF213, ring finger protein 213; CDO1, cysteine dioxygenase type 1; HOXA7, homeobox A7; SOX17, SRY-box transcription factor 17; FUT7, fucosyltransferase 7; FCGR2A, Fc gamma receptor IIa; EPB41L3, erythrocyte B membrane protein band 4.1 like 3; LINGO1, nogo receptor-interacting protein-1; S100A7L2, S100 calcium binding protein A7 like 2; DCD, dermcidin; MID1IP1, MID1 interacting protein 1; PNMA1, paraneoplastic antigen MA1; TAF10, TATA-box binding protein associated factor 10; ZNF696, zinc finger protein 696; GAS5, growth arrest-specific 5; PTGER4, prostaglandin E receptor 4; RASSF1A, ras association domain family 1A; SHOX2, short stature homeobox gene two; P53, tumor protein 53; PGP9.5, protein gene product 9.5; GAGE7, G antigen 7; GBU4-5, ATP-dependent RNA helicase 4-5; MAGEA1, melanoma-associated antigen A1; CAGE, cancer-associated gene; AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma; IAD, invasive adenocarcinoma; ZNRF3, zinc and ring finger 3; YAP1, Yes1-associated transcriptional regulator; CNN1, calponin h1; IRF2, interferon regulatory factor 2; PDGF-BB, platelet-derived growth factor-BB; XPR1, xenotropic and polytropic retrovirus receptor 1; ATF2, activating transcription factor-2; CHEK1, checkpoint kinase 1; GBX2, gastrulation brain homeobox 2; PTEN, phosphatase and tensin homolog; STAT3, signal transducers and activators of transcription; IRS2, insulin receptor substrate 2; RhoE, Rho family GTPase 3; Rheb, ras homolog enriched in brain; α-KGDD, α-KG-dependent dioxygenases; IGF-1R, insulin-like growth factor 1 receptor; TIF1γ, transcriptional intermediary factor 1γ; TLDA, TaqMan low density array; NGS, next-generation sequencing; IHC, immunohistochemical; AUC, area under the curve; MOB, nanoparticle-based DNA extraction; qMSP, quantitative methylation-specific PCR; EMT, epithelial-mesenchymal transition; NA, not applicable.

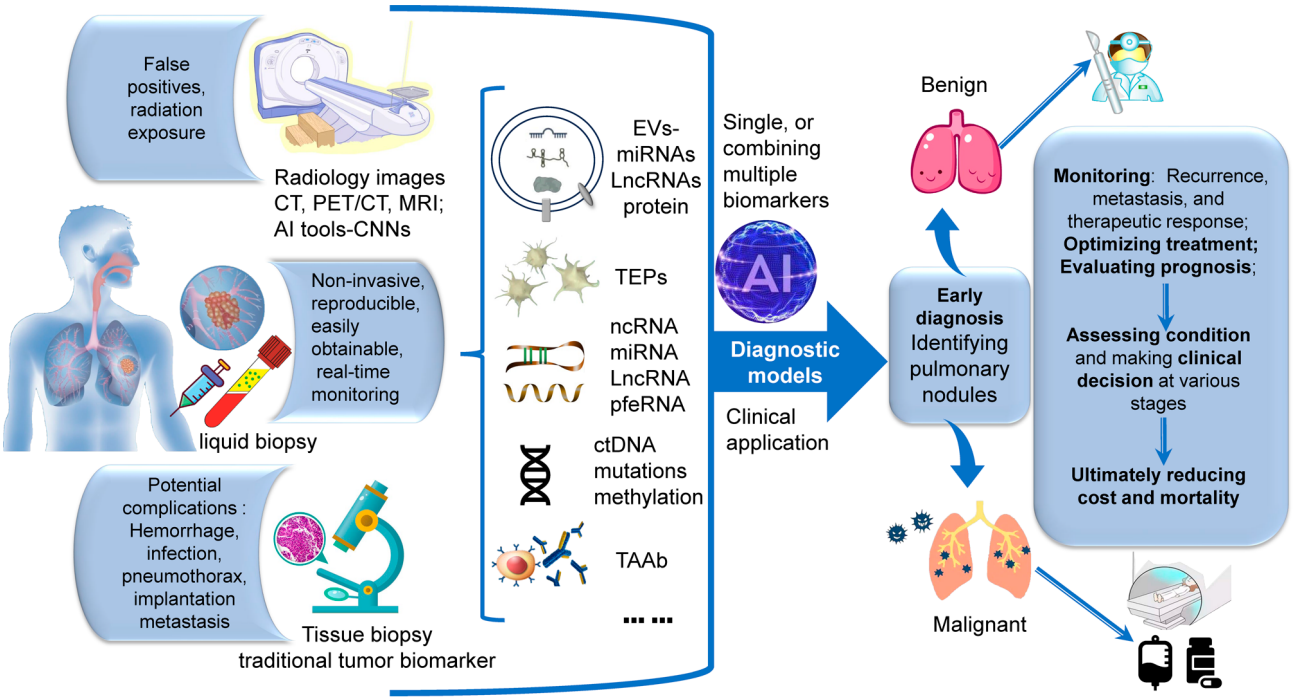

Figure 1. Combined use of multi-biomarkers in pulmonary nodules and lung cancer. Liquid biopsy serves as a crucial complement to traditional diagnostic methods in the identification and treatment of malignant and benign pulmonary nodules. CT, computerized tomography; PET, positron emission tomography; MRI, magnetic resonance imaging; AI, artificial intelligence; CNNs, convolutional neural networks; EVs, extracellular vesicles; TEPs, tumor-educated platelets; ncRNA, non-coding RNA; ctDNA, circulating tumor DNA; TAAb, autoantibodies targeting tumor-associated antigens.

instance, a study found that cancer cell-derived EVs containing circUSP7 inhibited the function of CD8+ T cells, promoting the progression of non-small cell lung cancer (NSCLC) and resistance to anti-programmed death 1 therapy (23). EVs carrying snail-1 released by cancer-associated fibroblasts induce epithelial-mesenchymal transition (EMT) (24). Cancer cells can utilize glycolysis and glutaminolysis to meet their metabolic needs (25). EVs carrying circSHKBP1 enhance glycolysis by sponging microRNA (miR)-1294, leading to increased expression of the glycolytic enzyme pyruvate kinase isozyme type M2 (PKM2) and ultimately affecting the function of NSCLC cells and macrophages (26). EVs derived from cancer-associated fibroblasts contained long intergenic non-coding RNA (LINC)01614, which enhanced glutamine uptake in lung cells by upregulating the expression of glutamine transporters (27). In the study of therapeutic resistance, hypoxia-induced EVs were found to transmit cisplatin resistance to sensitive NSCLC cells by delivering PKM2 (28). In addition, EV transfer of wild-type EGFR was also shown to promote resistance to the drug Osimertinib (29). Therefore, targeting the secretion and transfer of specific cargo in EVs, such as PKM2 and EGFR, may be a promising approach to overcome treatment resistance.

Application of EVs in the diagnosis of pulmonary nodules

i) EV-associated miRNAs: Numerous studies on EV miRNA have primarily focused on the early diagnosis of lung cancer. EV miR-29c-3p and miR-1290 have been identified as superior diagnostic biomarkers for distinguishing between lung cancer and benign BPN. Their expression levels show significant differences in lung cancer, with miR-29c-3p decreased and miR-1290 elevated. These miRNAs exhibit high sensitivity (89.47 and 97.37%), specificity (84.21 and 89.47%) and area under the curve (AUC) values (0.868 and 0.934) in discriminating lung cancer from BPNs. Furthermore, they demonstrate a strong discriminative ability between NSCLC and SCLC with AUC values of 0.842 and 0.810 (30). Utilizing multiple EV miRNAs enhances diagnostic accuracy. A diagnostic signature consisting of four EV-derived miRNAs (miR-106b-3p, miR-125a-5p, miR-3615 and miR-450b-5p) has been developed for the early detection of LUAD. In training cohorts, the signature exhibited an AUC value of 0.917, a sensitivity of 83.8% and a specificity of 87.1%. These diagnostic capabilities were further validated in test cohorts (31). Mechanistic studies have elucidated the roles of these miRNAs. EV miR-106b targets phosphatase and tensin homolog, promoting migration and invasion of lung cancer cells (32). In NSCLC, miR-125a-5p inhibits the expression of histone methyltransferase Suv39H1, leading to cancer suppression both *in vitro* and *in vivo* (33). Furthermore, miR-125a-5p exerts a tumor-inhibiting effect by targeting STAT3 (34). As the target RNA of two competing endogenous long non-coding (lnc) RNAs, miR-450b-5p demonstrates a tumor-suppressive function in NSCLC (35). Not only the quantity, but also the ratio of EV miRNAs, plays a significant role in distinguishing between benign and malignant pulmonary nodules. For instance, the miR-21/Let-7a ratio is markedly elevated in NSCLC compared to BPNs, with an AUC of 0.754 and a specificity of 82.61% (36).

Zhong *et al* (37) utilized TaqMan Low Density Array and reverse transcription-quantitative qPCR to identify and validate a distinct pattern of circulating EV miRNAs at multiple medical centers. They found that miR-520c-3p and miR-1274b were significantly elevated in patients with NSCLC compared to healthy controls and those with benign nodules. The developed panel effectively differentiated NSCLC from benign

nodules, with an AUC of 0.823, and was identified as an independent risk factor for NSCLC (37). Various intriguing findings warrant further exploration. miR-520c-3p is a tumor suppressor miRNA in NSCLC, regulating biological processes such as IL-8 (38) and PI3K/AKT signaling (39). It is higher in non-tumor tissues than in LUAD tissues. On the other hand, miR-1274b, associated with tumor growth and development (40), is upregulated in LUAD side population cells. However, as an apparent contradiction, both miR-520c-3p and miR-1274b were found to be upregulated in circulating EVs of patients with NSCLC compared to healthy controls and those with benign nodules. The upregulation of tumor-promoting miRNAs in both tissues and blood EVs is well understood, while it remains elusive how tumor-suppressive miRNAs are decreased in tumor cells but elevated in EVs. Endogenous RNAs, including miRNA targets, play a role in sorting miRNAs to EVs. The complex interactions between miRNAs and their targets can lead to the upregulation of both tumor-suppressive and tumor-promoting miRNAs in EVs (41). In addition, tumor-suppressive miRNAs in tumor cells could be eliminated through EVs and target tumor-associated immune cells in the microenvironment to promote immunosuppressive effects and induce tumor-promoting phenotypes. This dual mechanism also explains the elevation of tumor-suppressive miRNAs in EVs (42).

The main challenge in using EV-associated miRNAs for diagnosing lung nodules is the difficulty in effectively capturing and enriching tumor-derived EVs from complex blood systems. A recent study introduced a Glyexo-capture strategy using lentil lectin-magnetic beads to target exosome membranes. This dual-target method enhances the detection of valuable exosomal biomarkers with increased sensitivity and specificity. For instance, a miRNA panel consisting of miR-4732-5p, miR-451a, miR-486-5p and miR-139-3p showed promise in screening for early LUAD from BPNs, achieving an AUC of 0.855 with 91.07% sensitivity and 66.36% specificity (43). These miRNAs play crucial roles in inhibiting lung cancer through various pathways: miR-4732-5p targets xenotropic and polytropic retrovirus receptor 1 to suppress LUAD migration and metastasis (44), miR-451a inhibits invasion by targeting activating transcription factor-2 (45), miR-486-5p hinders the mTOR pathway by targeting ribosomal proteins (46) and miR-139-3p reduces lung squamous carcinoma viability by targeting checkpoint kinase 1 (47). In addition, exosomal miR-4497 is also a tumor suppressor marker, showing diagnostic efficacy in distinguishing NSCLC from BPNs with 73.3% sensitivity, 72.6% specificity and an AUC of 0.748. Importantly, miR-4497 demonstrates potential for monitoring tumor malignancy [size, tumor node metastasis (TNM) stage and metastasis] and overall survival (48).

ii) EV-associated long RNAs (exLRs): While previous studies have primarily focused on miRNAs, their limited presence in EVs hinders their effectiveness as biomarkers for lung cancer diagnosis (49). By contrast, exLRs, such as mRNAs, circRNAs and lncRNAs, are abundant in peripheral blood EVs and have shown promise as diagnostic biomarkers of lung cancer (50). Specifically, a panel of 23 exLRs, including 6 upregulated and 17 downregulated genes, identified in EVs can differentiate LUAD from BPNs with high sensitivity (93.75%), specificity (85.71%), and accuracy (88.24%). Furthermore, a signature of 17 exLRs, comprising 2 upregulated and 15 downregulated genes, can effectively distinguish between adenocarcinoma *in situ* and minimally invasive or invasive adenocarcinoma with exceptional sensitivity (93.33%), specificity (98.00%) and accuracy (96.25%) (51). In a recent study, a diagnostic model incorporating three exLRs (PGM5-AS1, SFTA1P and CTA-384D8.35) showed a strong predictive ability for NSCLC, with an AUC of 0.97 (52). Additionally, researchers found that EV lncRNA RP5-977B1 expression was elevated in NSCLC compared to healthy controls and patients with pulmonary tuberculosis, showing superior discriminatory power (AUC 0.890) over traditional markers carcino-embryonic antigen (CEA) (0.761) and cytokeratin 19 fragment (CYFRA21-1) (0.670). This comparative advantage was also observed in distinguishing early-stage NSCLC from controls (53). To improve diagnostic accuracy, a novel index combining gasdermin 5 and CEA was developed, achieving an AUC of 0.929, sensitivity of 89.06% and specificity of 90.00% for NSCLC diagnosis (54).

iii) EV-associated protein: EV-associated proteins, such as fibrinogen beta chain (FGB) and fibrinogen gamma chain (FGG), have been implicated in EMT progression of lung cancer (55). Researchers have demonstrated the utility of FGB and FGG levels in plasma EVs as diagnostic biomarkers for distinguishing benign and malignant pulmonary nodules. Compared to benign nodules, patients with lung cancer exhibited elevated FGB and FGG expression levels. When used individually, FGB showed a sensitivity of 0.628, specificity of 0.800 and AUC of 0.741, while FGG had a sensitivity of 0.535, specificity of 0.850 and AUC of 0.659. Combining FGB and FGG detection improved the diagnostic accuracy, with a sensitivity of 0.700 and AUC of 0.794, suggesting that these proteins could serve as sensitive biomarkers for distinguishing benign from malignant pulmonary nodules (56).

Plasma EV versican, a chondroitin sulfate glycoprotein was found to be significantly elevated in patients with NSCLC, with expression levels correlating with TNM stages and clinical parameters. Combining plasma versican and plasma EV versican showed superior diagnostic performance in identifying patients with NSCLC and those with metastasis compared to traditional biomarkers [neuron-specific enolase (NSE), CYFRA21-1 and squamous cell carcinoma antigen] (57). Yang *et al* (58) identified differential expression of immunoglobulin heavy variable 4-4 and immunoglobulin lambda variable 1-40 in serum EVs of patients with NSCLC, with the combination showing a high diagnostic capacity with a sensitivity of 88.73%, a specificity of 85.00% and AUC of 0.93. Recent research identified 150 altered EV proteins in patients with NSCLC, primarily involved in cell adhesion, differentiation, motility and osmoregulation, suggesting their potential as biomarkers for early NSCLC diagnosis [panel of FGB, FGG and von Willebrand factor] and metastasis prediction (panel of complement factor H related protein 5, complement component 9 and mannose-binding lectin 2) (59).

While EV biomarkers offer numerous advantages, challenges persist in their clinical application. EVs can be secreted by various cells and those from cells with pathological changes may be overshadowed by those from normal cells. Multi-level screening is an important strategy for enhancing tumor relevance and tissue specificity of EV markers. In a

study analyzing EV proteomes from paired tumor and adjacent tissues, exclusive proteins HIV-1 Tat interactive protein 2 and methyltransferase like 1 were identified as specific markers for LUAD. Furthermore, comparing plasma and tissue-derived EV proteins revealed plasma EV signatures that could distinguish between lung cancers even at early stages (60). In another study using lung cancer serum and cell culture supernatant, EV fibronectin emerged as a promising biomarker. It was able to effectively differentiate patients with NSCLC from healthy controls (AUC=0.844, P<0.001) and showed a significant increasing trend correlating with cancer progression (advanced NSCLC > early NSCLC > healthy controls, P<0.001) (61).

Tumor-educated blood platelets (TEPs). Platelets are rich in RNA species and functional spliceosomes, undergoing specific splicing in response to the tumor microenvironment, leading to changes in RNA content. There has been an increasing focus on the role of platelets in tumorigenesis, metastasis, immune evasion and chemotherapy resistance. As tumors progress, cancer cells can educate platelets, altering their transcriptome and molecular makeup (62,63). For instance, in patients with lung cancer, TEPs exhibit significant alterations in specific RNA species, including downregulation of linc-GTF2H2-1 and RP3-466P17.2, and upregulation of lnc-ST8SIA4-12, even at early stages of the disease. Integrating TEP lincRNA, CEA, NSE and CYFRA21-1 can effectively distinguish patients with advanced-stage lung cancer from early-stage ones with an AUC of 0.899 (64).

TEPs content can be transferred via MVs. Platelets can take up tumor-derived MVs and release their own protumoral MVs, thereby establishing a blood-based network for distributing tumor-derived RNA or protein. By analyzing changes in the platelet transcriptome through sequencing and proteomics, diagnostic models based on platelet activity can be developed and used in differentiating pulmonary nodules. TEP integrin alpha 2b (TEP ITGA2B) is a promising marker for improving the identification accuracy for patients with stage I NSCLC, distinguishing malignant tumours from BPNs. TEP ITGA2B levels were significantly elevated in patients with NSCLC, with an AUC of 0.940, sensitivity of 96.4% and specificity of 81.7% for identifying stage I NSCLC from BPNs. Compared to serum CEA, TEP ITGA2B demonstrated superior performance in distinguishing benign from malignant lung nodules, particularly in the case of SPN. Further research indicated that a nomogram incorporating ITGA2B and CEA may enhance diagnostic accuracy and predict overall survival (65).

Several studies have explored the association between pulmonary nodule with platelet characteristics, such as platelet-to-lymphocyte ratio (PLR). A higher PLR has been associated with an increased risk of positive nodules and lung cancer [odds ratio=1.29 (95% CI, 0.99-1.68)] (66). To enhance diagnostic accuracy and reduce bias, a multi-index diagnostic model called Sichuan Hospital of Cancer (SCHC), incorporating platelet features (platelet counts in platelet-rich plasma samples, plateletcrit in platelet-rich plasma samples and plateletcrit in whole-blood samples), age and nodule size, has shown promising results in distinguishing benign from malignant nodules. The SCHC model outperformed other clinical models (Veterans Affairs, Mayo Clinic, Brock University) by minimizing misclassification of malignant tumors and significantly improving reclassification metrics such as net reclassification improvement and integrated discrimination improvement. This platelet-based model could aid in accurately diagnosing early-stage malignancies and guiding optimal patient management in clinical settings (67).

ncRNAs. ncRNA refers to a group of RNA molecules that do not encode proteins but play important regulatory roles in cellular processes. Epigenetic-related ncRNAs include miRNAs, small interfering (si)RNAs, PiWi-interacting RNAs and lncRNAs.

miRNAs. miRNAs are small endogenous RNAs that target mRNAs, leading to post-transcriptional silencing and potentially influencing tumor development and metastasis (68). Dysregulation of specific miRNAs or miRNA groups is closely associated with cancer progression (69). Research has explored the use of circulating miRNAs for diagnosing pulmonary nodules. miR-499a (70) and a group of other miRNAs, including miRNA-17, -146a, -200b, -182, -221, -205, -7, -21, -145 and -210 (71), were significantly elevated in NSCLC compared to BPN, and they both have potential in differentiating between benign and malignant pulmonary nodules. In addition to single miRNAs, combining multiple miRNAs can enhance the accuracy of diagnosis. A panel of miRNAs (miR-199a-3p, -148a-3p, -210-3p, -378d and -138-5p) in blood has been validated for early diagnosis of LUAD from pulmonary nodules. The use of this miRNA panel alongside CT scans significantly reduces false positives. For instance, the false-positive rate of CT imaging for nodules and ground glass nodules was reduced from 33.1 to 3.2% when positive miRNA panel results were combined with nodule size (72). In addition, miR-21 and miR-210 levels were higher, while miR-486-5p levels were lower in patients with malignant lung nodules compared to benign ones. A combination of three miRNAs achieved an AUC of 0.86 in distinguishing lung cancer from BPN with 75.00% sensitivity and 84.95% specificity (73). Furthermore, paired miRNA ratios were used in the differential diagnosis of NSCLC and BPN. Five miRNA ratios (miR-92a-3p/miR-146b-3p, miR-20a-5p/miR-146b-3p, miR-19a-3p/miR-146b-3p, miR-15b-5p/miR-146b-3p and miR-16-5p/miR-146b-3p) showed higher expression levels in NSCLC compared to BPN, with a sensitivity of 0.70 and specificity of 0.90 (74).

LncRNA. LncRNAs, which are >200 nucleotides long and lack a protein-coding function (75), play a role in malignant behavior by affecting gene transcription (76). Specifically, the concentration of lncRNA THRIL is elevated in patients with lung cancer compared to those with BPN, showing promise in distinguishing between benign and malignant nodules (77). In patients with NSCLC, lncRNA XLOC_009167 levels were significantly higher than in healthy controls or individuals with pneumonia, with an AUC of 0.740, 78.7% sensitivity and 61.8% specificity in differentiating lung cancer from healthy controls. In addition, lncRNA XLOC_009167 was able to differentiate between lung cancer and pneumonia with 90.1% sensitivity, 50.0% specificity and an AUC of 0.701 (78).

Protein functional effector RNAs (pfeRNAs). PfeRNAs are a unique type of small ncRNA that directly binds and

regulates phosphorylated proteins involved in lung cancer tumorigenesis (79). Unlike miRNAs and siRNAs, pfeRNAs enhance the function of their target proteins instead of degrading them (80). Recent research has demonstrated that pfeRNAs can serve as diagnostic markers for pulmonary nodules. An 8-pfeRNA classifier (pfeRNAa to pfeRNAh) identified through deep sequencing can effectively differentiate between malignant and BPNs, with a sensitivity of 77.1% and specificity of 74.25% (81).

ctDNA. ctDNA is fragmented DNA from tumors found in the bloodstream, typically released by necrotic or apoptotic tumor cells in the tumor microenvironment (82). Mutated or methylated ctDNA can be identified using PCR or DNA sequencing to track lung cancer development. ctDNA exhibits strong tissue specificity and correlates well with tumor tissue DNA, making it a valuable biomarker for monitoring lung cancer progression.

Tumor-specific gene mutations. Gene mutations play a crucial role in tumor development by activating oncogenes and deactivating tumor suppressor genes. Mutations in ctDNA directly reflect tumor mutations, providing a valuable tool for distinguishing between malignant and BPNs. However, the sensitivity of ctDNA may be limited by interference from wild-type sequences (83). Recent studies utilizing targeted next-generation sequencing have identified specific gene mutations in ctDNA, such as RNF213, with high specificity of 100% in distinguishing between benign and malignant pulmonary nodules (84). Plasma ctDNA shows promise in early cancer detection, particularly when combined with clinical information and traditional biomarkers. Analysis of 65 lung cancer-related genes in plasma ctDNA revealed increasing mutation levels with tumor progression, particularly in driving genes. The ctDNA assay had a sensitivity of 69% and specificity of 96% for distinguishing lung nodule nature. Combining ctDNA, serum biomarkers and patient age boosted sensitivity to 80% and specificity to 99% (85).

ctDNA methylation. DNA methylation is a well-researched epigenetic modification, particularly in gene promoter regions, often leading to tumor suppressor gene inactivation and cancer development (86). Analyzing ctDNA methylation has emerged as a novel, sensitive and non-invasive method for early lung cancer detection and distinguishing lung cancers from BPN. DNA bisulfite sequencing was conducted on 309 pulmonary nodule tissue specimens to identify cancer-specific patterns. From 3,886 hypermethylated regions in the tissue, 71 regions found in plasma were refined to select 9 markers for a diagnostic model. In a validation set, the model had 79.5% sensitivity and 85.2% specificity in distinguishing lung cancer from BPNs (87). In addition to hypermethylation changes, ctDNA hypomethylation can also aid in diagnosing pulmonary nodules. Research indicates that methylation levels of seven sites of fucosyltransferase 7 in lung cancer were significantly lower than in normal controls. Furthermore, the levels of methylation at CpG-4 and CpG-7 were lower in lung cancer compared to BPN (88).

ctDNA is typically scarce and can be surrounded by a background of DNA from healthy tissues (89). To improve sensitivity and specificity, a combination of diagnostic markers and technological innovations was employed. A study involving 246 CT-detected patients with small pulmonary nodules (diameter, ≤3.0 cm) discovered elevated methylation levels of tachykinin precursor 1 (TAC1), cysteine dioxygenase type 1 (CDO1), homeobox A7 (HOXA7) and SRY-box transcription factor 17 (SOX17) in peripheral blood in cases of NSCLC compared to benign cases. The combination of SOX17, CDO1 and HOXA7 achieved a sensitivity of 90%, specificity of 71% and an AUC of 0.88 for diagnosis (90). To further promote the clinical application of these biomarkers, a new methylation analysis technique, multiplex digital methylation-specific PCR (MSP), was developed by Zhao *et al* (91), which increased the sensitivity from 88 to 90% and specificity from 60 to 82% for the combination of TAC1, CDO1, HOXA7 and SOX17. Another novel testing technology (92) utilized multi-locus qPCR to screen methylation markers, selecting the highest AUC marker as the anchor. This approach, combined with 10x4-fold cross-validations for each addition, resulted in the creation of two models: LunaCAM-D with 6 methylation markers to distinguish lung cancer from benign diseases and LunaCAM-S with 5 markers to classify lung cancer from healthy controls. In a recent study, methylated DNA (prostaglandin E receptor 4, ras association domain family 1A and short stature homeobox gene 2) was combined with a radiologic feature (pulmonary nodule diameter) to create prediction models. These models achieved an AUC value of 0.948 with sensitivity and specificity of 89.5 and 95.4%, respectively, for identifying malignant from BPN (93). Another study integrated ctDNA methylation, clinical features and CT imaging features to develop a composite model named PULMOSEEK Plus. This model demonstrated a sensitivity of ≥95% across all stages of lung cancer, an AUC value of 0.98 in early-stage lung cancer and an AUC value of 0.99 in indeterminate nodules (5-10 mm). By reclassifying pulmonary nodules with two cutoffs (0.65 and 0.89), unnecessary invasive procedures could have been reduced in 85% of BPN and delayed treatment avoided in 72% of malignant nodules (94).

Circulating antibodies. Autoantibodies targeting tumor-associated antigens (TAAb) are commonly found in the preclinical phase of lung cancer (95), indicating their potential as promising noninvasive biomarkers with satisfactory sensitivity and specificity (96). Studies have highlighted the effectiveness of multi-TAAb panels, such as the Fc gamma receptor IIa, erythrocyte B membrane protein band 4.1 like 3, Nogo receptor-interacting protein-1 and S100 calcium binding protein A7 like 2 combination, in accurately distinguishing indeterminate pulmonary nodules (8-20 mm) with an AUC value of 0.78, 91.7% sensitivity and 57.1% specificity (97). In a seven-TAAb panel (tumor protein 53, protein gene product 9.5, SOX2, G antigen 7, GBU4-5, melanoma-associated antigen A1 and cancer-associated gene), sensitivity and specificity were 59.7 and 81.1% for early lung nodule differentiation. In addition, integrating these seven autoantibodies and imaging features into a machine learning model markedly increased the AUC from 0.75 to 0.96 in distinguishing patients with pulmonary nodules (98). A recent study utilized high-throughput protein microarrays to identify TAAb and developed a random forest model with

Table II. Comparison of diagnostic performance and cost-effectiveness of various liquid biopsy biomarkers.

Biomarkers	Advantages	Limitations	Dynamic monitoring capabilities	Tumor localization accuracy	Diagnostic efficiency	Cost-effectiveness
EVs	High stability and information richness; overcoming the tumor of spatial heterogeneity	Lack of standardized processes; complex separation techniques	Moderate-high	Moderate-high	Moderate	Moderate
TEPs	Rapid turnaround times; easy to standardize	Lack of large and diverse studies; the mechanism of alternative splicing remains unclear; may be contaminated with other blood cells	High	Moderate	Moderate	Moderate
ncRNA	Highly tissue/cell specific; easy to detect	Low abundance; non-specific background signal interference	Low-moderate	Low-moderate	Low-moderate	Low-moderate
ctDNA	Real-time monitoring of tumor burden; allowing for early detection of recurrence	Highly degraded; low concentrations	Moderate	High	Moderate	Moderate-high
Circulating antibodies	Non-invasive; direct signaling of the immune response	Heterogeneity of immune responses; low specificity	Low	Low	Low	Low
Integrative approaches	High diagnostic efficiency	High diagnostic costs; lack of standardized processes	High	High	High	High

EVs, extracellular vesicles; TEPs, tumor-educated platelets; ncRNA, non-coding RNA; ctDNA, circulating tumor DNA.

Table III. Clinical application, combined application and cost-effectiveness of LB in diagnosis of pulmonary nodule and lung cancer.

Clinical application	Single diagnostic methods	Combined application
Early detection and screening	LDCT/PET-CT: Standard for lung cancer screening in high-risk individuals; Limitations: False positives and overdiagnosis	LDCT/PET-CT + LB (108,112): Detect genetic alterations and provide molecular evidence of malignancy even in early stages; improve the accuracy of early detection and reduces unnecessary invasive procedures
Overcoming tumor heterogeneity; comprehensive molecular profiling	TB: Gold standard for diagnosis; Limitations: Invasive, risky, provides a snapshot of the tumor's genetic profile at a specific site but may miss spatially heterogeneous mutations	TB + LB (113,114): Provide a comprehensive snapshot of the tumor's genetic landscape and improve the accuracy of molecular profiling; overcome the limitations of single-site TB, which may miss spatially heterogeneous mutations; useful when TB is contraindicated or fails to yield adequate material
Identification of targetable mutations; guiding targeted therapy and immunotherapy	TB: Identify actionable mutations for targeted therapy; assess PD-L1 expression and TMB, which are predictive of response to immune checkpoint inhibitors	TB + LB (115,116): Confirm or supplement tissue biopsy findings, enabling timely initiation of targeted therapies; provide dynamic assessment of TMB and PD-L1 status, complementing tissue-based analysis; bTMB could predict response to therapy and monitor the emergence of resistance mutations
Monitoring treatment response	CT/PET: Assess tumor size and metabolic activity during treatment; Limitations: May not detect early molecular changes	CT/PET + LB (117): Complementing imaging findings; real-time monitoring of tumor dynamics; indicate treatment efficacy or the emergence of resistance mechanisms
Post-treatment surveillance; detection of MRD	Imaging: Used for post-treatment surveillance; Limitations: May not detect microscopic residual disease	Imaging + LB (110): LB can detect MRD by identifying ctDNA in patients with no radiographic evidence; help to identify patients at risk of recurrence of disease, enabling early intervention

Cost-effectiveness of LB

- Population screening (108)
  The cost-effectiveness of LB in large-scale screening programs depends on the prevalence and the ability to detect early-stage disease accurately
- Reduced invasive and unnecessary procedures (118,119)
  LB is less invasive and costly, reducing repeated invasive procedures and complications, lowering associated healthcare costs and improving patient outcomes
- Faster turnaround time (120)
  Liquid biopsy results are often available more quickly than tissue biopsy, enabling faster treatment decisions and reducing costs associated with delayed care
- Personalized treatment (111)
  The combined approach enables more precise treatment selection, reducing the use of ineffective therapies and associated costs
- Challenges to cost-effectiveness (119)
  The cost of LB tests can be high, particularly for advanced genomic profiling. However, this cost is often offset by the benefits of personalized treatment and reduced need for repeated tissue biopsies

MRD, minimal residual disease; LDCT, low-dose computed tomography; PET, positron emission tomography; CT, computerized tomography; TB, tissue biopsy; TMB, tumor mutational burden; bTMB, blood-based tumor mutational burden; LB, liquid biopsy.

six autoantibodies (annexin 2, dermcidin, MID1 interacting protein 1, paraneoplastic antigen MA1, TATA-box binding protein associated factor 10, zinc finger protein 696) to detect high-risk pulmonary nodules suitable for LDCT scans (99). In addition, another study investigated antibody responses against bacterial and viral proteins in patients with lung cancer, finding more prevalent antibodies among BPNs than among LUAD. Then a panel of 20 antibodies was created to distinguish LUAD from BPNs with an AUC of 0.80 (100).

Challenges in clinical transformation of liquid biopsy. Each liquid biopsy method has distinct advantages, limitations, diagnostic performance and cost-effectiveness profiles, necessitating biomarker selection based on clinical context (Table II). However, all of these detection approaches face technical challenges in clinical translation. For instance, ultracentrifugation remains the gold standard for EVs isolation, its limitations-including being time-consuming, low-throughput and potentially damaging to vesicles-along with protein aggregate contamination, necessitate more efficient techniques such as nanosensors (101) and microfluidic chips (102). Similarly, TEPs face challenges in specificity due to blood cell contamination during isolation (103) and an incomplete understanding of spliceosome regulation (104), requiring further multicenter validation (105). ncRNAs, despite their detectability, have shortcomings of low abundance and interference in bodily fluids, demanding improved quantification methods (106). Although ctDNA enables real-time tumor monitoring, its low concentration necessitates costly high-sensitivity assays (e.g., nanoparticle-based DNA extraction/qMSP), complicating analysis (107). Circulating antibodies, while promising, risk false positives (cross-reactivity with infections/autoimmunity) and false negatives (immunosuppression), underscoring the need for multi-analyte diagnostic panels. Collectively, advancing isolation technologies, standardizing detection and integrating multi-omics approaches are critical for robust clinical translation of liquid biopsy.

4. Clinical applications, integrated diagnostic strategies and cost-effectiveness of liquid biopsy

Liquid biopsy is a transformative tool in lung cancer management, offering significant clinical benefits in several clinical applications, such as early detection and screening (108), providing comprehensive molecular profiling, guiding therapy (109), identifying minimal residual disease (110), and monitoring disease progression and treatment response (111). As an important complement or alternative to traditional diagnostic methods, liquid biopsy has significantly enhanced the precision and efficiency of lung cancer diagnosis and management. In particular, the integration of liquid biopsy with current diagnostic methods, such as imaging techniques, tissue biopsy and molecular profiling, can leverage the strengths of each method to provide a comprehensive understanding of the disease (108,112-117) (Table III). The cost-effectiveness of liquid biopsy is demonstrated through multiple key aspects: Population-based screening feasibility (108), reduction of unnecessary invasive procedures (118,119), accelerated diagnostic turnaround time (120) and facilitation of personalized treatment strategies (111). These advantages collectively contribute to significant cost reduction in the clinical management pathway for pulmonary nodules and lung cancer. Although the upfront costs of liquid biopsy can be high, its potential to reduce unnecessary treatments, complications and hospitalizations makes it a cost-effective option in numerous scenarios (119) (Table III). As technology advances and costs decrease, liquid biopsy is likely to become an integral part of lung cancer care, improving outcomes for patients and optimizing healthcare resource utilization.

5. Conclusion and perspective

Liquid biopsy, a non-invasive and convenient diagnostic tool, is increasingly utilized in clinical practice. Circulating biomarkers provide insights into the mechanisms of lung cancer, aiding in early detection, screening, diagnosis, monitoring and treatment. However, the sensitivity and specificity of EVs, TEPs, ncRNA, ctDNA and TAAb as lung cancer biomarkers may be limited by low blood concentrations and potential interference from molecules secreted by normal cells. The heterogeneous and atypical nature of tumors necessitates a refined temporal and spatial variation map of these biomarkers, with research focusing on combining multiple biomarkers (the construction of clinical models, including imaging features and patient characteristics) or levels (the establishment of biomarkers from cells, tissue, peripheral circulation) for accurate differentiation of malignant tumour and BPN (Fig. 1). Furthermore, AI-driven analysis has the potential to efficiently analyze vast and complex datasets, thereby enabling the development of diverse and efficient diagnostic and predictive models (121). Standardization of liquid biopsy methods and critical level determination is essential for clinical application. Ultimately, liquid biopsy holds promise for enhancing early pulmonary nodule diagnosis.

Acknowledgements

Not applicable.

Funding

This study was funded by the Youth Interdisciplinary Special Fund of Zhongnan Hospital of Wuhan University (grant no. ZNQNJC2022008), the Natural Science Foundation of Hubei Province (grant no. 2021CFB415) and the National Natural Science Foundation of China (grant no. 81702273).

Availability of data and materials

Not applicable.

Authors' contributions

YT designed this study and revised the manuscript. MP, JG and YT wrote the manuscript. TA and HC generated the table and figure. LC, MC and JL participated in the literature search and collation. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
2. Larici AR, Farchione A, Franchi P, Ciliberto M, Cicchetti G, Calandriello L, Del Ciello A and Bonomo L: Lung nodules: Size still matters. Eur Respir Rev 26: 170025, 2017.
3. Groome PA, Bolejack V, Crowley JJ, Kennedy C, Krasnik M, Sobin LH and Goldstraw P; IASLC International Staging Committee; Cancer Research and Biostatistics; Observers to the Committee; Participating Institutions: The IASLC lung cancer staging project: Validation of the proposals for revision of the T, N, and M descriptors and consequent stage groupings in the forthcoming (seventh) edition of the TNM classification of malignant tumours. J Thorac Oncol 2: 694-705, 2007.
4. Viswanathan VS, Toro P, Corredor G, Mukhopadhyay S and Madabhushi A: The state of the art for artificial intelligence in lung digital pathology. J Pathol 257: 413-429, 2022.
5. Pei Q, Luo Y, Chen Y, Li J, Xie D and Ye T: Artificial intelligence in clinical applications for lung cancer: Diagnosis, treatment and prognosis. Clin Chem Lab Med 60: 1974-1983, 2022.
6. Kim TJ, Kim CH, Lee HY, Chung MJ, Shin SH, Lee KJ and Lee KS: Management of incidental pulmonary nodules: Current strategies and future perspectives. Expert Rev Respir Med 14: 173-194, 2020.
7. Ali K and Bal S: Management of Solitary Pulmonary Nodule. In: Recent concepts in minimal access surgery: Sharma D and Hazrah P (eds). Vol 1. Springer Singapore, Singapore, pp401-418, 2022.
8. Hasan N, Kumar R and Kavuru MS: Lung cancer screening beyond low-dose computed tomography: The role of novel biomarkers. Lung 192: 639-648, 2014.
9. Nooreldeen R and Bach H: Current and future development in lung cancer diagnosis. Int J Mol Sci 22: 8661, 2021.
10. Dalli A, Selimoglu Sen H, Coskunsel M, Komek H, Abakay O, Sergi C and Cetin Tanrikulu A: Diagnostic value of PET/CT in differentiating benign from malignant solitary pulmonary nodules. J BUON 18: 935-941, 2013.
11. Khalil A, Majlath M, Gounant V, Hess A, Laissy JP and Debray MP: Contribution of magnetic resonance imaging in lung cancer imaging. Diagn Interv Imaging 97: 991-1002, 2016.
12. Periaswamy G, Arunachalam VK, Varatharajaperumal R, Kalyan G, Selvaraj R, Mehta P and Cherian M: Comparison of ultrashort TE lung MRI and HRCT lungs for detection of pulmonary nodules in oncology patients. Indian J Radiol Imaging 32: 497-504, 2022.
13. Yang Y, Guan S, Ou Z, Li W, Yan L and Situ B: Advances in AI-based cancer cytopathology. Interdiscip Med 1: e20230013, 2023.
14. Li Y, Wu X, Yang P, Jiang G and Luo Y: Machine learning for lung cancer diagnosis, treatment, and prognosis. Genomics Proteomics Bioinformatics 20: 850-866, 2022.
15. AbdulJabbar K, Raza SEA, Rosenthal R, Jamal-Hanjani M, Veeriah S, Akarca A, Lund T, Moore DA, Salgado R, Al Bakir M, *et al*: Geospatial immune variability illuminates differential evolution of lung adenocarcinoma. Nat Med 26: 1054-1062, 2020.
16. Coudray N, Ocampo PS, Sakellaropoulos T, Narula N, Snuderl M, Fenyö D, Moreira AL, Razavian N and Tsirigos A: Classification and mutation prediction from non-small cell lung cancer histopathology images using deep learning. Nat Med 24: 1559-1567, 2018.
17. Li W, Liu JB, Hou LK, Yu F, Zhang J, Wu W, Tang XM, Sun F, Lu HM, Deng J, *et al*: Liquid biopsy in lung cancer: Significance in diagnostics, prediction, and treatment monitoring. Mol Cancer 21: 25, 2022.
18. Levy B, Hu ZI, Cordova KN, Close S, Lee K and Becker D: Clinical utility of liquid diagnostic platforms in non-small cell lung cancer. Oncologist 21: 1121-1130, 2016.
19. Zheng H, Wu X, Yin J, Wang S, Li Z and You C: Clinical applications of liquid biopsies for early lung cancer detection. Am J Cancer Res 9: 2567-2579, 2019.
20. Bao H, Min L, Bu F, Wang S and Meng J: Recent advances of liquid biopsy: Interdisciplinary strategies toward clinical decision-making. Interdiscip Med 1: e20230021, 2023.
21. Zhu Y, Li W, Lan F, Chen S, Chen X, Zhang X, Yan X and Zhang Y: DNA nanotechnology in tumor liquid biopsy: Enrichment and determination of circulating biomarkers. Interdiscip Med 2: e20230043, 2024.
22. El Andaloussi S, Mäger I, Breakefield XO and Wood MJA: Extracellular vesicles: Biology and emerging therapeutic opportunities. Nat Rev Drug Discov 12: 347-357, 2013.
23. Chen SW, Zhu SQ, Pei X, Qiu BQ, Xiong D, Long X, Lin K, Lu F, Xu JJ and Wu YB: Cancer cell-derived exosomal circUSP7 induces $CD8^+$ T cell dysfunction and anti-PD1 resistance by regulating the miR-934/SHP2 axis in NSCLC. Mol Cancer 20: 144, 2021.
24. You J, Li M, Cao LM, Gu QH, Deng PB, Tan Y and Hu CP: Snail1-dependent cancer-associated fibroblasts induce epithelial-mesenchymal transition in lung cancer cells via exosomes. QJM 112: 581-590, 2019.
25. Pavlova NN and Thompson CB: The emerging hallmarks of cancer metabolism. Cell Metab 23: 27-47, 2016.
26. Chen W, Tang D, Lin J, Huang X, Lin S, Shen G and Dai Y: Exosomal circSHKBP1 participates in non-small cell lung cancer progression through PKM2-mediated glycolysis. Mol Ther Oncolytics 24: 470-485, 2022.
27. Liu T, Han C, Fang P, Ma Z, Wang X, Chen H, Wang S, Meng F, Wang C, Zhang E, *et al*: Cancer-associated fibroblast-specific lncRNA LINC01614 enhances glutamine uptake in lung adenocarcinoma. J Hematol Oncol 15: 141, 2022.
28. Wang D, Zhao C, Xu F, Zhang A, Jin M, Zhang K, Liu L, Hua Q, Zhao J, Liu J, *et al*: Cisplatin-resistant NSCLC cells induced by hypoxia transmit resistance to sensitive cells through exosomal PKM2. Theranostics 11: 2860-2875, 2021.
29. Wu S, Luo M, To KKW, Zhang J, Su C, Zhang H, An S, Wang F, Chen D and Fu L: Intercellular transfer of exosomal wild type EGFR triggers osimertinib resistance in non-small cell lung cancer. Mol Cancer 20: 17, 2021.
30. Zhang Q, Zheng K, Gao Y, Zhao S, Zhao Y, Li W, Nan Y, Li Z, Liu W, Wang X, *et al*: Plasma exosomal miR-1290 and miR-29c-3p as diagnostic biomarkers for lung cancer. Heliyon 9: e21059, 2023.
31. Gao S, Guo W, Liu T, Liang N, Ma Q, Gao Y, Tan F, Xue Q and He J: Plasma extracellular vesicle microRNA profiling and the identification of a diagnostic signature for stage I lung adenocarcinoma. Cancer Sci 113: 648-659, 2022.
32. Sun S, Chen H, Xu C, Zhang Y, Zhang Q, Chen L, Ding Q and Deng Z: Exosomal miR-106b serves as a novel marker for lung cancer and promotes cancer metastasis via targeting PTEN. Life Sci 244: 117297, 2020.
33. Open Biology Editorial Team: Retraction 'Reduced miR-125a-5p level in non-small-cell lung cancer is associated with tumour progression'. Open Biol 10: 200205, 2020.
34. Zhong L, Sun S, Shi J, Cao F, Han X and Chen Z: MicroRNA-125a-5p plays a role as a tumor suppressor in lung carcinoma cells by directly targeting STAT3. Tumour Biol 39: 1010428317697579, 2017.
35. Ye P, Lv X, Aizemaiti R, Cheng J, Xia P and Di M: H3K27ac-activated LINC00519 promotes lung squamous cell carcinoma progression by targeting miR-450b-5p/miR-515-5p/YAP1 axis. Cell Prolif 53: e12797, 2020.
36. Yang G, Wang T, Qu X, Chen S, Han Z, Chen S, Chen M, Lin J, Yu S, Gao L, *et al*: Exosomal miR-21/Let-7a ratio distinguishes non-small cell lung cancer from benign pulmonary diseases. Asia Pac J Clin Oncol 16: 280-286, 2020.
37. Zhong Y, Ding X, Bian Y, Wang J, Zhou W, Wang X, Li P, Shen Y, Wang JJ, Li J, *et al*: Discovery and validation of extracellular vesicle-associated miRNAs as noninvasive detection biomarkers for early-stage non-small-cell lung cancer. Mol Oncol 15: 2439-2452, 2021.

38. Tang CP, Zhou HJ, Qin J, Luo Y and Zhang T: MicroRNA-520c-3p negatively regulates EMT by targeting IL-8 to suppress the invasion and migration of breast cancer. Oncol Rep 38: 3144-3152, 2017.
39. Gulhane P and Singh S: MicroRNA-520c-3p impacts sphingolipid metabolism mediating PI3K/AKT signaling in NSCLC: Systems perspective. J Cell Biochem 123: 1827-1840, 2022.
40. Xu X, Lu X, Sun J and Shu Y: microRNA expression profiling of side population cells in human lung cancer and preliminary analysis. Zhongguo Fei Ai Za Zhi 13: 665-669, 2010 (In Chinese).
41. Squadrito ML, Baer C, Burdet F, Maderna C, Gilfillan GD, Lyle R, Ibberson M and De Palma M: Endogenous RNAs modulate microRNA sorting to exosomes and transfer to acceptor cells. Cell Rep 8: 1432-1446, 2014.
42. Qi Y, Jin C, Qiu W, Zhao R, Wang S, Li B, Zhang Z, Guo Q, Zhang S, Gao Z, *et al*: The dual role of glioma exosomal microRNAs: Glioma eliminates tumor suppressor miR-1298-5p via exosomes to promote immunosuppressive effects of MDSCs. Cell Death Dis 13: 426, 2022.
43. Chen X, Yu L, Hao K, Yin X, Tu M, Cai L, Zhang L, Pan X, Gao Q and Huang Y: Fucosylated exosomal miRNAs as promising biomarkers for the diagnosis of early lung adenocarcinoma. Front Oncol 12: 935184, 2022.
44. Hu Y, Bai J, Zhou D, Zhang L, Chen X, Chen L, Liu Y, Zhang B, Li H and Yin C: The miR-4732-5p/XPR1 axis suppresses the invasion, metastasis, and epithelial-mesenchymal transition of lung adenocarcinoma via the PI3K/Akt/GSK3β/Snail pathway. Mol Omics 18: 417-429, 2022.
45. Shen YY, Cui JY, Yuan J and Wang X: MiR-451a suppressed cell migration and invasion in non-small cell lung cancer through targeting ATF2. Eur Rev Med Pharmacol Sci 22: 5554-5561, 2018.
46. Ding L, Tian W, Zhang H, Li W, Ji C, Wang Y and Li Y: MicroRNA-486-5p suppresses lung cancer via downregulating mTOR signaling in vitro and in vivo. Front Oncol 11: 655236, 2021.
47. Zheng X, Zhang Y, Wu S, Jiang B and Liu Y: MiR-139-3p targets CHEK1 modulating DNA repair and cell viability in lung squamous carcinoma cells. Mol Biotechnol 64: 832-840, 2022.
48. Zheng B, Peng M, Gong J, Li C, Cheng H, Li Y and Tang Y: Circulating exosomal microRNA-4497 as a potential biomarker for metastasis and prognosis in non-small-cell lung cancer. Exp Biol Med (Maywood) 248: 1403-1413, 2023.
49. Jin X, Chen Y, Chen H, Fei S, Chen D, Cai X, Liu L, Lin B, Su H, Zhao L, *et al*: Evaluation of tumor-derived exosomal miRNA as potential diagnostic biomarkers for early-stage non-small cell lung cancer using next-generation sequencing. Clin Cancer Res 23: 5311-5319, 2017.
50. Li Y, Zhao J, Yu S, Wang Z, He X, Su Y, Guo T, Sheng H, Chen J, Zheng Q, *et al*: Extracellular vesicles long RNA sequencing reveals abundant mRNA, circRNA, and lncRNA in human blood as potential biomarkers for cancer diagnosis. Clin Chem 65: 798-808, 2019.
51. Zhang Y, Liu W, Zhang H, Sun B, Chen T, Hu M, Zhou H, Cao Y, Han B and Wu L: Extracellular vesicle long RNA markers of early-stage lung adenocarcinoma. Int J Cancer 152: 1490-1500, 2023.
52. Wang N, Yao C, Luo C, Liu S, Wu L, Hu W, Zhang Q, Rong Y, Yuan C and Wang F: Integrated plasma and exosome long noncoding RNA profiling is promising for diagnosing non-small cell lung cancer. Clin Chem Lab Med 61: 2216-2228, 2023.
53. Min L, Zhu T, Lv B, An T, Zhang Q, Shang Y, Yu Z, Zheng L and Wang Q: Exosomal LncRNA RP5-977B1 as a novel minimally invasive biomarker for diagnosis and prognosis in non-small cell lung cancer. Int J Clin Oncol 27: 1013-1024, 2022.
54. Li C, Lv Y, Shao C, Chen C, Zhang T, Wei Y, Fan H, Lv T, Liu H and Song Y: Tumor-derived exosomal lncRNA GAS5 as a biomarker for early-stage non-small-cell lung cancer diagnosis. J Cell Physiol 234: 20721-20727, 2019.
55. Wang H, Meyer CA, Fei T, Wang G, Zhang F and Liu XS: A systematic approach identifies FOXA1 as a key factor in the loss of epithelial traits during the epithelial-to-mesenchymal transition in lung cancer. BMC Genomics 14: 680, 2013.
56. Kuang M, Peng Y, Tao X, Zhou Z, Mao H, Zhuge L, Sun Y and Zhang H: FGB and FGG derived from plasma exosomes as potential biomarkers to distinguish benign from malignant pulmonary nodules. Clin Exp Med 19: 557-564, 2019.
57. Chang W, Zhu J, Yang D, Shang A, Sun Z, Quan W and Li D: Plasma versican and plasma exosomal versican as potential diagnostic markers for non-small cell lung cancer. Respir Res 24: 140, 2023.
58. Yang P, Zhang Y, Zhang R, Wang Y, Zhu S, Peng X, Zeng Y, Yang B, Pan M, Gong J and Ba H: Plasma-derived exosomal immunoglobulins IGHV4-4 and IGLV1-40 as new non-small cell lung cancer biomarkers. Am J Cancer Res 13: 1923-1937, 2023.
59. Luo B, Que Z, Lu X, Qi D, Qiao Z, Yang Y, Qian F, Jiang Y, Li Y, Ke R, *et al*: Identification of exosome protein panels as predictive biomarkers for non-small cell lung cancer. Biol Proced Online 25: 29, 2023.
60. Hoshino A, Kim HS, Bojmar L, Gyan KE, Cioffi M, Hernandez J, Zambirinis CP, Rodrigues G, Molina H, Heissel S, *et al*. Extracellular vesicle and particle biomarkers define multiple human cancers. Cell 182: 1044-1061.e18, 2020.
61. An T, Qin S, Sun D, Huang Y, Hu Y, Li S, Zhang H, Li B, Situ B, Lie L, *et al*: Unique protein profiles of extracellular vesicles as diagnostic biomarkers for early and advanced non-small cell lung cancer. Proteomics 19: e1800160, 2019.
62. Schlesinger M: Role of platelets and platelet receptors in cancer metastasis. J Hematol Oncol 11: 125, 2018.
63. Denis MM, Tolley ND, Bunting M, Schwertz H, Jiang H, Lindemann S, Yost CC, Rubner FJ, Albertine KH, Swoboda KJ, *et al*: Escaping the nuclear confines: Signal-dependent pre-mRNA splicing in anucleate platelets. Cell 122: 379-391, 2005.
64. Li X, Liu L, Song X, Wang K, Niu L, Xie L and Song X: TEP linc-GTF2H2-1, RP3-466P17.2, and lnc-ST8SIA4-12 as novel biomarkers for lung cancer diagnosis and progression prediction. J Cancer Res Clin Oncol 147: 1609-1622, 2021.
65. Xing S, Zeng T, Xue N, He Y, Lai YZ, Li HL, Huang Q, Chen SL and Liu WL: Development and validation of tumor-educated blood platelets integrin alpha 2b (ITGA2B) RNA for diagnosis and prognosis of non-small-cell lung cancer through RNA-seq. Int J Biol Sci 15: 1977-1992, 2019.
66. Tian T, Lu J, Zhao W, Wang Z, Xu H, Ding Y, Guo W, Qin P, Zhu W, Song C, *et al*: Associations of systemic inflammation markers with identification of pulmonary nodule and incident lung cancer in Chinese population. Cancer Med 11: 2482-2491, 2022.
67. Zu R, Wu L, Zhou R, Wen X, Cao B, Liu S, Yang G, Leng P, Li Y, Zhang L, *et al*: A new classifier constructed with platelet features for malignant and benign pulmonary nodules based on prospective real-world data. J Cancer 13: 2515-2527, 2022.
68. Lu TX and Rothenberg ME: MicroRNA. J Allergy Clin Immunol 141: 1202-1207, 2018.
69. He B, Zhao Z, Cai Q, Zhang Y, Zhang P, Shi S, Xie H, Peng X, Yin W, Tao Y and Wang X: miRNA-based biomarkers, therapies, and resistance in Cancer. Int J Biol Sci 16: 2628-2647, 2020.
70. Ge N, Mao C, Yang Q, Han B, Wang Y, Xu L, Yang X, Jiao W and Li C: Single nucleotide polymorphism rs3746444 in miR-499a affects susceptibility to non-small cell lung carcinoma by regulating the expression of CD200. Int J Mol Med 43: 2221-2229, 2019.
71. Xi KX, Zhang XW, Yu XY, Wang WD, Xi KX, Chen YQ, Wen YS and Zhang LJ: The role of plasma miRNAs in the diagnosis of pulmonary nodules. J Thorac Dis 10: 4032-4041, 2018.
72. He Y, Ren S, Wang Y, Li X, Zhou C and Hirsch FR: Serum microRNAs improving the diagnostic accuracy in lung cancer presenting with pulmonary nodules. J Thorac Dis 10: 5080-5085, 2018.
73. Shen J, Liu Z, Todd NW, Zhang H, Liao J, Yu L, Guarnera MA, Li R, Cai L, Zhan M and Jiang F: Diagnosis of lung cancer in individuals with solitary pulmonary nodules by plasma microRNA biomarkers. BMC Cancer 11: 374, 2011.
74. Fan L, Sha J, Teng J, Li D, Wang C, Xia Q, Chen H, Su B and Qi H: Evaluation of serum paired MicroRNA ratios for differential diagnosis of non-small cell lung cancer and benign pulmonary diseases. Mol Diagn Ther 22: 493-502, 2018.
75. Huang Z, Wang Z, Xia H, Ge Z, Yu L, Li J, Bao H, Liang Z, Cui Y and Xu Y: Long noncoding RNA HAND2-AS1: A crucial regulator of malignancy. Clin Chim Acta 539: 162-169, 2023.
76. Karger A, Mansouri S, Leisegang MS, Weigert A, Günther S, Kuenne C, Wittig I, Zukunft S, Klatt S, Aliraj B, *et al*: ADPGK-AS1 long noncoding RNA switches macrophage metabolic and phenotypic state to promote lung cancer growth. EMBO J 42: e111620, 2023.

77. Chen X, Zhu X, Yan W, Wang L, Xue D, Zhu S, Pan J, Li Y, Zhao Q and Han D: Serum lncRNA THRIL predicts benign and malignant pulmonary nodules and promotes the progression of pulmonary malignancies. BMC Cancer 23: 755, 2023.
78. Jiang N, Meng X, Mi H, Chi Y, Li S, Jin Z, Tian H, He J, Shen W, Tian H, *et al*: Circulating lncRNA XLOC_009167 serves as a diagnostic biomarker to predict lung cancer. Clin Chim Acta 486: 26-33, 2018.
79. Fackche NT, Mei Y, Ito T, Garner M and Brock M: Abstract 1822: A mitochondrial pfeRNA associates with far upstream element binding protein 1 (FUBP1) to promote lung adenocarcinoma tumorigenesis. Cancer Res 79 (Suppl 13): S1822, 2019.
80. Brock M and Mei Y: Protein functional effector sncRNAs (pfeRNAs) in lung cancer. Cancer Lett 403: 138-143, 2017.
81. Liu W, Wang Y, Huang H, Fackche N, Rodgers K, Lee B, Nizam W, Khan H, Lu Z, Kong X, *et al*: A cost-effective and non-invasive pfeRNA-based test differentiates benign and suspicious pulmonary nodules from malignant ones. Noncoding RNA 7: 80, 2021.
82. Ponomaryova AA, Rykova EY, Solovyova AI, Tarasova AS, Kostromitsky DN, Dobrodeev AY, Afanasiev SA and Cherdyntseva NV: Genomic and transcriptomic research in the discovery and application of colorectal cancer circulating markers. Int J Mol Sci 24: 12407, 2023.
83. Schwarzenbach H, Hoon DSB and Pantel K: Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer 11: 426-437, 2011.
84. Jiang N, Zhou J, Zhang W, Li P, Liu Y, Shi H, Zhang C, Wang Y, Zhou C, Peng C, *et al*: RNF213 gene mutation in circulating tumor DNA detected by targeted next-generation sequencing in the assisted discrimination of early-stage lung cancer from pulmonary nodules. Thorac Cancer 12: 181-193, 2021.
85. Peng M, Xie Y, Li X, Qian Y, Tu X, Yao X, Cheng F, Xu F, Kong D, He B, *et al*: Resectable lung lesions malignancy assessment and cancer detection by ultra-deep sequencing of targeted gene mutations in plasma cell-free DNA. J Med Genet 56: 647-653, 2019.
86. Hung CS, Wang SC, Yen YT, Lee TH, Wen WC and Lin RK: Hypermethylation of CCND2 in lung and breast cancer is a potential biomarker and drug target. Int J Mol Sci 19: 3096, 2018.
87. Liang W, Zhao Y, Huang W, Gao Y, Xu W, Tao J, Yang M, Li L, Ping W, Shen H, *et al*: Non-invasive diagnosis of early-stage lung cancer using high-throughput targeted DNA methylation sequencing of circulating tumor DNA (ctDNA). Theranostics 9: 2056-2070, 2019.
88. Fang Y, Qu Y, Ji L, Sun H, Li J, Zhao Y, Liang F, Wang Z, Su J, Liu J, *et al*: Novel blood-based FUT7 DNA methylation is associated with lung cancer: Especially for lung squamous cell carcinoma. Clin Epigenetics 14: 167, 2022.
89. Crowley E, Di Nicolantonio F, Loupakis F and Bardelli A: Liquid biopsy: Monitoring cancer-genetics in the blood. Nat Rev Clin Oncol 10: 472-484, 2013.
90. Chen C, Huang X, Yin W, Peng M, Wu F, Wu X, Tang J, Chen M, Wang X, Hulbert A, *et al*: Ultrasensitive DNA hypermethylation detection using plasma for early detection of NSCLC: A study in Chinese patients with very small nodules. Clin Epigenetics 12: 39, 2020.
91. Zhao Y, O'Keefe CM, Hsieh K, Cope L, Joyce SC, Pisanic TR, Herman JG and Wang TH: Multiplex digital methylation-specific PCR for noninvasive screening of lung cancer. Adv Sci (Weinh) 10: e2206518, 2023.
92. Wang Z, Xie K, Zhu G, Ma C, Cheng C, Li Y, Xiao X, Li C, Tang J, Wang H, *et al*: Early detection and stratification of lung cancer aided by a cost-effective assay targeting circulating tumor DNA (ctDNA) methylation. Respir Res 24: 163, 2023.
93. Xing W, Sun H, Yan C, Zhao C, Wang D, Li M and Ma J: A prediction model based on DNA methylation biomarkers and radiological characteristics for identifying malignant from benign pulmonary nodules. BMC Cancer 21: 263, 2021.
94. He J, Wang B, Tao J, Liu Q, Peng M, Xiong S, Li J, Cheng B, Li C, Jiang S, *et al*: Accurate classification of pulmonary nodules by a combined model of clinical, imaging, and cell-free DNA methylation biomarkers: A model development and external validation study. Lancet Digit Health 5: e647-e656, 2023.
95. Seijo LM, Peled N, Ajona D, Boeri M, Field JK, Sozzi G, Pio R, Zulueta JJ, Spira A, Massion PP, *et al*: Biomarkers in lung cancer screening: achievements, promises, and challenges. J Thorac Oncol 14: 343-357, 2019.
96. Zhang X, Liu M, Zhang X, Wang Y and Dai L: Autoantibodies to tumor-associated antigens in lung cancer diagnosis. Adv Clin Chem 103: 1-45, 2021.
97. Lastwika KJ, Kargl J, Zhang Y, Zhu X, Lo E, Shelley D, Ladd JJ, Wu W, Kinahan P, Pipavath SNJ, *et al*: Tumor-derived autoantibodies identify malignant pulmonary nodules. Am J Respir Crit Care Med 199: 1257-1266, 2019.
98. Xu L, Chang N, Yang T, Lang Y, Zhang Y, Che Y, Xi H, Zhang W, Song Q, Zhou Y, *et al*: Development of diagnosis model for early lung nodules based on a seven autoantibodies panel and imaging features. Front Oncol 12: 883543, 2022.
99. Auger C, Moudgalya H, Neely MR, Stephan JT, Tarhoni I, Gerard D, Basu S, Fhied CL, Abdelkader A, Vargas M, *et al*: Development of a novel circulating autoantibody biomarker panel for the identification of patients with 'actionable' pulmonary nodules. Cancers (Basel) 15: 2259, 2023.
100. Shome M, Gao W, Engelbrektson A, Song L, Williams S, Murugan V, Park JG, Chung Y, LaBaer J and Qiu J: Comparative microbiomics analysis of antimicrobial antibody response between patients with lung cancer and control subjects with benign pulmonary nodules. Cancer Epidemiol Biomarkers Prev 32: 496-504, 2023.
101. Sina AAI, Vaidyanathan R, Dey S, Carrascosa LG, Shiddiky MJA and Trau M: Real time and label free profiling of clinically relevant exosomes. Sci Rep 6: 30460, 2016.
102. Fang S, Tian H, Li X, Jin D, Li X, Kong J, Yang C, Yang X, Lu Y, Luo Y, *et al*: Clinical application of a microfluidic chip for immunocapture and quantification of circulating exosomes to assist breast cancer diagnosis and molecular classification. PLoS One 12: e0175050, 2017.
103. Morales-Pacheco M, Valenzuela-Mayen M, Gonzalez-Alatriste AM, Mendoza-Almanza G, Cortés-Ramírez SA, Losada-García A, Rodríguez-Martínez G, González-Ramírez I, Maldonado-Lagunas V, Vazquez-Santillan K, *et al*: The role of platelets in cancer: From their influence on tumor progression to their potential use in liquid biopsy. Biomark Res 13: 27, 2025.
104. Didychuk AL, Butcher SE and Brow DA: The life of U6 small nuclear RNA, from cradle to grave. RNA 24: 437-460, 2018.
105. Najafi S, Asemani Y, Majidpoor J, Mahmoudi R, Aghaei-Zarch SM and Mortezaee K: Tumor-educated platelets. Clin Chim Acta 552: 117690, 2024.
106. Khan J, Lieberman JA and Lockwood CM: Variability in, variability out: best practice recommendations to standardize pre-analytical variables in the detection of circulating and tissue microRNAs. Clin Chem Lab Med 55: 608-621, 2017.
107. Sánchez-Herrero E, Provencio M and Romero A: Clinical utility of liquid biopsy for the diagnosis and monitoring of EML4-ALK NSCLC patients. Adv Lab Med 1: 20190019, 2020.
108. Lennon AM, Buchanan AH, Kinde I, Warren A, Honushefsky A, Cohain AT, Ledbetter DH, Sanfilippo F, Sheridan K, Rosica D, *et al*: Feasibility of blood testing combined with PET-CT to screen for cancer and guide intervention. Science 369: eabb9601, 2020.
109. Cescon DW, Bratman SV, Chan SM and Siu LL: Circulating tumor DNA and liquid biopsy in oncology. Nat Cancer 1: 276-290, 2020.
110. Chaudhuri AA, Chabon JJ, Lovejoy AF, Newman AM, Stehr H, Azad TD, Khodadoust MS, Esfahani MS, Liu CL, Zhou L, *et al*: Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling. Cancer Discov 7: 1394-1403, 2017.
111. Fagery M, Khorshidi HA, Wong SQ, Vu M and Ijzerman M: Health economic evidence and modeling challenges for liquid biopsy assays in cancer management: A systematic literature review. PharmacoEconomics 41: 1229-1248, 2023.
112. Kammer MN, Lakhani DA, Balar AB, Antic SL, Kussrow AK, Webster RL, Mahapatra S, Barad U, Shah C, Atwater T, *et al*: Integrated biomarkers for the management of indeterminate pulmonary nodules. Am J Respir Crit Care Med 204: 1306-1316, 2021.
113. Siravegna G, Marsoni S, Siena S and Bardelli A: Integrating liquid biopsies into the management of cancer. Nat Rev Clin Oncol 14: 531-548, 2017.
114. Jamal-Hanjani M, Wilson GA, McGranahan N, Birkbak NJ, Watkins TBK, Veeriah S, Shafi S, Johnson DH, Mitter R, Rosenthal R, *et al*: Tracking the evolution of non-small-cell lung cancer. N Engl J Med 376: 2109-2121, 2017.
115. Mok TS, Wu YL, Ahn MJ, Garassino MC, Kim HR, Ramalingam SS, Shepherd FA, He Y, Akamatsu H, Theelen WSME, *et al*: Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. N Engl J Med 376: 629-640, 2017.

116. Gandara DR, Paul SM, Kowanetz M, Schleifman E, Zou W, Li Y, Rittmeyer A, Fehrenbacher L, Otto G, Malboeuf C, *et al*: Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. Nat Med 24: 1441-1448, 2018.
117. Oxnard GR, Paweletz CP, Kuang Y, Mach SL, O'Connell A, Messineo MM, Luke JJ, Butaney M, Kirschmeier P, Jackman DM and Jänne PA: Noninvasive detection of response and resistance in EGFR-mutant lung cancer using quantitative next-generation genotyping of cell-free plasma DNA. Clin Cancer Res 20: 1698-1705, 2014.
118. Rolfo C, Mack P, Scagliotti GV, Aggarwal C, Arcila ME, Barlesi F, Bivona T, Diehn M, Dive C, Dziadziuszko R, *et al*: Liquid biopsy for advanced NSCLC: A consensus statement from the international association for the study of lung cancer. J Thorac Oncol 16: 1647-1662, 2021.
119. Pennell NA, Mutebi A, Zhou ZY, Ricculli ML, Tang W, Wang H, Guerin A, Arnhart T, Dalal A, Sasane M, *et al*: Economic impact of next-generation sequencing versus single-gene testing to detect genomic alterations in metastatic non-small-cell lung cancer using a decision analytic model. JCO Precis Oncol 3: 1-9, 2019.
120. Leighl NB, Page RD, Raymond VM, Daniel DB, Divers SG, Reckamp KL, Villalona-Calero MA, Dix D, Odegaard JI, Lanman RB and Papadimitrakopoulou VA: Clinical utility of comprehensive cell-free DNA analysis to identify genomic biomarkers in patients with newly diagnosed metastatic non-small cell lung cancer. Clin Cancer Res 25: 4691-4700, 2019.
121. Xie H, Jia Y and Liu S: Integration of artificial intelligence in clinical laboratory medicine: Advancements and challenges. Interdiscip Med 2: e20230056, 2024.