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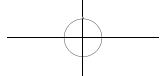
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## Proceedings of Anticancer Research

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# Application of Proteomic and Metabolomic Technologies in Renal Cell Carcinoma and Research Progress of Related Biomarkers

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**Abstract:** Renal cell carcinoma (RCC), which accounts for about 90 percent of kidney cancers, has a distinct metabolic reprogramming profile characterized by increased aerobic glycolysis (Warburg effect), abnormal accumulation of lipids, and impaired mitochondrial function. Recent advances in high-throughput proteomic and metabolomic technologies have revolutionized our understanding of the pathophysiology of RCC, allowing for the systematic identification of disease-specific molecular signatures, elucidation of drug resistance mechanisms, and possible targets for intervention. The review focuses on the use of proteomic and metabolomic technologies in renal cell carcinoma and the research progress on related biomarkers, and is expected to provide useful information for the early detection and treatment of RCC.

**Keywords:** Renal cell carcinoma; Proteomics; Metabolomics; Early diagnosis; Biomarkers

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## 1. Introduction

Kidney cancer is one of the most common and fatal urological diseases in the world, with 430,000 new cases diagnosed each year by 2020. Histologically, clear cell renal cell carcinoma (ccRCC) accounts for approximately 75% of renal cell carcinoma (RCC), which is the overwhelming majority (90%) of kidney cancers <sup>[1]</sup>. More than 30% of patients with ccRCC have metastasis at the time of initial diagnosis and 40% of patients undergoing surgical resection eventually relapse <sup>[2]</sup>.

Extensive studies have shown that RCC is a cellular metabolic disease. Metabolic alterations include an increase in aerobic glycolysis, phosphorylation by the pentose phosphate pathway, synthesis of fatty acids, metabolism of glutamine and glutathione, and a decrease in the tricarboxylic acid (TCA) cycle, as well as the oxidative oxidation and phosphorylation of fatty acids (Oxphos) <sup>[3]</sup>. Cancer metabolites have a major influence on chromatin remodeling and epigenetic dysregulation, which may result in characteristic hypermethylation, EMT

phenotype switching, and pseudohypoxic features <sup>[4]</sup>.

Currently, the main treatment for metastatic RCC is targeted therapy and immunotherapy, but these are effective only in some patients and are prone to drug resistance <sup>[5]</sup>. As the hypoxia-inducible factor (*HIF*) pathway is critical in the pathophysiology of the ccRCC, *HIF* and downstream targets such as *VEGFA* have always been highly anticipated as drug targets <sup>[6]</sup>. However, researchers have a great interest in seeking novel biomarkers or druggable targets. In recent years, high-throughput proteomics and metabolomics technologies have been used to screen for biomarkers of various tumors, including RCC. The discovery of specific markers could provide new strategies for clinical diagnosis, treatment, and prediction of disease. This review focuses on metabolic reprogramming and the use of proteomic and metabolomic approaches, as well as the research progress on related biomarkers in renal cell carcinoma, to provide new insights for translational research in renal cell carcinoma.

## 2. Metabolic signature of renal cell carcinoma

RCC is a known metabolic disease that involves a range of metabolic disorders. Among the many metabolic pathways, the Warburg effect, increased fat deposition, and mitochondrial dysfunction are typical of the abnormal metabolic pathways.

### 2.1. Warburg effect in RCC

The Warburg effect is a major metabolic characteristic of tumor cells, meaning that even in the presence of abundant oxygen, the tumor cells still obtain their energy mainly by glycolysis. In RCC, the Warburg effect, i.e. the aerobic glycolysis rather than oxidative phosphorylation, is favored in cellular energy metabolism.

Inactivation of the *VHL* gene is the primary genetic cause of most RCCs and also contributes to the Warburg effect. Deficiency of *VHL* can stabilize *HIF* and thereby promote angiogenesis and tumor growth <sup>[7]</sup>. *HIF* can reduce the glucose and glutamine oxidation by altering the TCA cycle and may regulate metabolism by altering the transcription of key genes in the glycolysis pathway, such as *FKBP10* and *TET2* <sup>[8–11]</sup>.

The opposite reaction to glycolysis is gluconeogenesis, which is the process by which organisms convert various non-sugar substances to glucose or glycogen. Fructose-1,6-bisphosphatase (FBP) is a rate-limiting enzyme in gluconeogenesis and is subdivided into two subtypes, *FBP1* and *FBP2*. Li and colleagues have shown that *FBP1* can interact directly with *HIF*, thereby inhibiting the transcriptional activity of both *HIF1α* and *HIF2α* and also inhibiting glycolysis <sup>[12]</sup>. Moreover, *HIF1α* is shown to be primarily involved in glycolysis, while *HIF2α* is mainly involved in the regulation of genes involved in lipoprotein metabolism, ribosome biogenesis <sup>[13]</sup>.

*PBRM1* is the second most frequently mutated gene in ccRCC with a mutation rate of 38.1% <sup>[14]</sup>. Recently, we have demonstrated that when *PBRM1* is knocked down, the levels of some major metabolic enzymes involved in the glycolytic pathway are increased, including enolase 1 (ENO1, pyridostigmine), pyruvate kinase (PKI), and lactate dehydrogenase A (LDHA) in ccRCC cells <sup>[15]</sup>.

In addition to the major metabolic enzymes involved in the glycolytic pathway, other proteins have been shown to play a role in the development of RCC by influencing the glycolytic pathway. These include *PFKFB3*, *ATAD2*, *TRIM21*, *SIRT3*, *METTL14*, among others <sup>[16–19]</sup>.

The major mutated genes in RCC, such as *VHL* and *PBRM1*, as well as genes coding for key glycolytic enzymes, play a critical role in the Warburg effect and the development of RCC. Some may have potential predictive significance.

## 2.2. Increased fat deposition in RCC

Lipid droplets are the main sites for intracellular lipid storage and are markers for lipid deposition. Alterations in lipid metabolism can lead to increased lipid synthesis, abnormal lipid accumulation, and dysregulated lipid signaling, thereby promoting cell proliferation, invasion, and migration in ccRCC and other tumors <sup>[20,21]</sup>.

*HIF2α* is related to lipoprotein metabolism and can regulate the expression of important lipogenic enzymes and transporters <sup>[22]</sup>. Being targets of *HIF2α*, the mediator complex subunit 15 (MED15) and transcription factor 7 analogue 2 (TCF7L2), both play a role in lipid accumulation and RCC progression or metastasis <sup>[23,24]</sup>. In addition, other enzymes such as glutathione peroxidase 8 (GPX8) can also contribute to the formation of lipids and thus to the development of tumors. GPX8 belongs to the glutathione peroxidase (GPX) family and can catalyze the conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG), protecting the biological membranes from damage by reactive oxygen species (ROS). RCC, depletion of GPX8 resulted in a significant reduction in lipid levels, fatty acid synthesis, and triglyceride esterification. Mechanistically, GPX8 regulates nicotinamide N-methyltransferase (NNMT) through IL6-STAT3 signaling and is independent of *VHL* <sup>[25]</sup>.

Some others, such as *CPT2*, *MLYCD*, *GPR1*, *CMKLR1*, and *ACAT1*, among others, have the opposite effect. There is evidence showing that *CPT2* inhibits the proliferation, invasion, and migration of ccRCC cells mainly by inhibiting the ROS/PPAR $\gamma$ /NF- $\kappa$ B signaling pathway <sup>[26]</sup>. Malonyl-CoA decarboxylase (*MLYCD*) is an important regulator of fatty acid anabolism and is downregulated in ccRCC. When *MLYCD*-mediated fatty acid oxidation is inhibited, lipid droplet accumulation occurs, disrupting endoplasmic reticulum and mitochondrial homeostasis, increasing ROS levels, and inducing ferroptosis <sup>[27]</sup>. *GPR1* and *CMKLR1* are crucial for maintaining the balance of lipid metabolism. Adipose triglyceride lipase (ATGL), a key enzyme that initiates the hydrolysis of triglycerides to release fatty acids, can be inhibited by *GPR1* and *CMKLR1* to reduce lipogenic triglyceride lipase and increase lipid oxidation and ferroptosis. In addition, 2-( $\alpha$ -naphthol) trimethylammonium iodide ( $\alpha$ -NETA) is a *CMKLR1* antagonist that can effectively inhibit the growth of ccRCC cells by regulating lipid metabolism and activating SREBP1 signaling <sup>[28]</sup>. Acetyl-coenzyme A acetyltransferase 1 (*ACAT1*) is the only enzyme in the cell that catalyzes the formation of cholesterol esters from free cholesterol and long-chain fatty acids, and plays an important role in maintaining cellular cholesterol homeostasis. In ccRCC tissues, *ACAT1* expression is downregulated. Overexpression of *ACAT1* can inhibit the progression of ccRCC through affecting the PPAR/CPT1 axis and AMPK signaling pathway <sup>[29]</sup>.

## 2.3. Mitochondrial dysfunction in RCC

Mitochondria are double-membrane organelles that are ubiquitous in eukaryotic cells and provide cells with energy through oxidative phosphorylation. Mitochondrial dysfunction mainly refers to disorders of energy metabolism caused by damage to the mitochondrial membrane, inhibition of the respiratory chain, reduced activity of mitochondrial enzymes, and damage to mtDNA. When mitochondria are disrupted, they can release cytochrome c, produce mitochondrial reactive oxygen species and metabolites, thereby affecting the signaling cascade response of gene expression, cell proliferation, and differentiation <sup>[30]</sup>.

While *HIF1α* promotes glycolysis, it can also weaken mitochondrial activity. *HIF1α*-dependent reduction in mitochondrial oxygen consumption increases the NADH/NAD<sup>+</sup> ratio and thereby inhibits the activity of the NADH-sensitive glycolytic enzyme GAPDH <sup>[31]</sup>. Other proteins, such as NADH dehydrogenase 1 alpha subcomplex 4-like 2 (NDUFA4L2), mitochondrial fusion protein 2 (MFN2), among others, also play a critical role in the pathophysiology of the mitochondria and RCC <sup>[32,33]</sup>.

As a metabolic disease, ccRCC is characterized by the typical Warburg effect, increased fat deposition, and mitochondrial dysfunction. *VHL* inactivation or promoter hypermethylation occurs in ~90% of RCC. When *VHL* is inactivated, there may be upregulation of glycolysis, Wnt/ $\beta$ -catenin signaling, and mTORC1 signaling, as well as downregulation of fatty acid metabolism. Some dysregulated signaling pathways were also observed upon *PBRM1* depletion<sup>[15]</sup>.

### 3. Application of proteomics in renal cell carcinoma

Proteomics is the study of the complete set of proteins that are produced in a cell or an entire organism, including amino acid sequences of proteins, protein abundance, protein modification, and interaction between proteins. Conventional proteomic research techniques can be divided into three categories: Untargeted proteomics, targeted proteomics, and modified proteomics. Here, we mainly introduce the related applications of the above three categories of proteomics technologies in RCC studies.

Untargeted proteomics can be used to identify and/or quantify the relative amount of many proteins<sup>[34]</sup>. Untargeted proteomics makes use of stable isotopes or synthetic stable isotopes, and can accordingly be categorized into stable isotope labeling by amino acids in cell culture (SILAC, metabolically labeled), isobaric tag for relative or absolute quantitation, tandem mass tags (iTRAQ/TMT, chemical labeled), and data-dependent acquisition/data-independent acquisition (DDA/DIA, label-free). Using the iTRAQ/TMT labeling method, White *et al.* identified 55 differentially expressed proteins between ccRCC and normal adjacent tissue samples in a total of 199 patients<sup>[35]</sup>. Five of these, ENO1, HSPB1, LDHA, and AHNK, which are increased in the ccRCC, were confirmed further by immuno-blot and tissue microarray. Atri *et al.* used label-free quantitative proteomics (LFQ) technology to examine the different protein spectra between ccRCC tissues and normal adjacent tissues (NAT)<sup>[36]</sup>. They showed that nearly 600 proteins were differentially expressed, and two of these, *CORO1A* and *ADFP*, were further validated in ccRCC samples. Using label-free DIA technique, Lin *et al.* analyzed samples from 31 ccRCC patients and 31 healthy volunteers<sup>[37]</sup>. Significant differences in serum peptide composition were noted between the two groups. This difference in the spectrum of serum peptidomes helps to distinguish ccRCC patients from healthy volunteers and demonstrates the great potential of serum peptidomes for the diagnosis of cancer.

Targeted proteomics refers primarily to parallel reaction monitoring (PRM) targeted quantitative proteomics, and thus is suitable for validation of biomarker candidates<sup>[34,38]</sup>. Di *et al.* used LFQ and PRM to analyze the role of urinary peptides in distinguishing early ccRCC from healthy controls and renal cell carcinoma. Nine peptides were identified with significantly increased expression levels in small renal masses compared to controls. Some other markers associated with ccRCC progression or shorter overall survival have also been identified<sup>[39]</sup>. This suggests the clinical significance of proteomic analysis of urinary peptides.

### 4. Application of metabolomics in renal cell carcinoma

Metabolomics is an emerging omics technology developed after genomics, transcriptomics, and proteomics, which mainly studies the comprehensive and dynamic metabolites and their changes produced by endogenous or exogenous stimuli of biological systems (including cells, tissues, blood, urine, saliva, feces, etc.) at a specific time and in a specific environment<sup>[40]</sup>.

Untargeted metabolomics refers to the systematic and comprehensive analysis of the entire metabolome of a



sample or the comparison of the dynamic changes of all small-molecule metabolites before and after stimulation of an organism. Complement component 1q subcomponent binding protein (C1QBP) is a highly conserved multifunctional protein that plays a vital role in inflammation, infection, and cancer. *C1QBP* can inhibit the adhesion and metastasis of ccRCC cells, and the expression level is decreased in ccRCC<sup>[41]</sup>. Using untargeted metabolomics technology Wang *et al.* analyzed the differential metabolites of 200 major metabolites in *C1QBP*-overexpressing RCC cells<sup>[42]</sup>. Among 109 metabolites detected, 17 metabolites were changed significantly, including hypoxanthine. The data showed that *C1QBP* mainly promoted the catabolism of hypoxanthine by regulating the ROS generation mediated by xanthine dehydrogenase (*XDH*). Similarly, Feng *et al.* showed that knockdown of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (*PFKFB*) induced a significant reduction in multiple metabolites, among which the pentose phosphate pathway (PPP) was significantly enriched<sup>[43]</sup>.

Targeted metabolomics refers essentially to the analysis of a specific substance or specific metabolite, such as a specific amino acid, short-chain fatty acid, or free fatty acid, and the quantification of the target metabolite concentration in the sample using a standard reference. *SETD2* is a histone H3 lysine trimethyl transferase, and its inactivation is associated with the recurrence of ccRCC. Liu *et al.* used GC-MS-based targeted metabolomics to find that the loss of *SETD2* increases the production of TCA cycle metabolites such as aspartate, malate, succinate, fumarate, and  $\alpha$ -ketoglutarate in ccRCC cells, laying the foundation for further exploration of molecular mechanisms<sup>[44]</sup>. Amaro *et al.* used a GC-MS-based metabolic approach and analyzed a matched set of tissue and urine samples from a cohort of 18 patients with ccRCC. Data showed that the metabolic signature of the ccRCC tumors is reprogramming of amino acid, energy, sugar, and inositol phosphate metabolism, as well as a significant reduction in asparagine, proline, gluconate, 3-amino-butanate, 4-aminobutanoate, and urea<sup>[45]</sup>.

## 5. Joint analysis of proteomics and metabolomics in renal cell carcinoma

Compared to single-omics analysis methods, joint analysis of proteomics and metabolomics of biological samples can explore the biological molecular functions and regulatory mechanisms related to diseases more systematically and comprehensively. Wettersten *et al.* conducted a joint proteomics and untargeted metabolomics analysis of RCC tissues of different grades based on the Fuhrman grading standard<sup>[46]</sup>. The study found that the TCA cycle was significantly downregulated in RCC tissues, glycolysis and tryptophan metabolic pathways were significantly upregulated in high-grade RCC tissues, fatty acid  $\beta$ -oxidation was significantly downregulated in high-grade RCC tissues, and the glutamine pathway was reprogrammed into the GSH/GSSG antioxidant system in high-grade RCC. This study revealed the grade-dependent metabolic reprogramming regulated by RCC-related metabolic pathways, which will help clinical personalized treatment of RCC patients of different grades and provide potential targets for new drug development.

With joint analysis of proteomics and metabolomics, Yuan *et al.* found that the anti-tumor effect of *SLC39A1* may be related to changes in purine and pyrimidine metabolism, glutathione metabolism, and iron poisoning, ROS generation, PI3K-AKT, cAMP-Epac, and PPAR signaling pathways<sup>[47]</sup>. Solute carrier family 39 member 1 (*SLC39A1*), also known as ZIP1, is responsible for transferring zinc ions into cells.

The results of multi-omics analysis showed a more comprehensive picture of *SLC39A1* molecular perturbations, providing new insights into the occurrence and development of RCC. Moreover, Li *et al.* used multi-omics (histopathology, proteomics, single-cell sequencing, phosphorylation proteomics, tumor metabolomics, and tumor-specific glycoproteomics) technology to integrate histopathology, proteomics, and metabolomics data from

305 tumors to comprehensively characterize the complexity and heterogeneity of ccRCC <sup>[48]</sup>.

## 6. Renal cell carcinoma-related biomarkers

Tumor biomarkers are molecules synthesized by tumor cells and related cells, and have important biological significance in tumor occurrence, development, and treatment. Detecting tumor marker levels can help with early diagnosis, precise diagnosis, treatment, and prognosis of tumors.

As highly sensitive, highly accurate, and high-throughput systematic research methods, proteomics and metabolomic approaches have been used to discover complex protein biomarkers in various tumors, such as lung cancer, cervical cancer, colorectal cancer, thyroid cancer, ovarian cancer, and renal cell carcinoma <sup>[49–54]</sup>.

### 6.1. Urinary biomarkers

Urine collection is simple, non-invasive, and the amount is relatively abundant compared to other biological fluids <sup>[55]</sup>. Monteiro *et al.* analyzed volatile organic compounds in the urine and found that the combination of 21 metabolites can effectively distinguish RCC patients from non-RCC volunteers. 2-oxopropanal and 2,5,8-trimethyl-1,2,3,4-tetrahydronaphthalene-1-ol are expected to be potential biomarkers for the diagnosis of RCC <sup>[56]</sup>.

Liu *et al.* analyzed urine samples from 100 RCC patients and some controls and found that *PKHD1L1*, *UGTL6*, *FAP4*, and *C3* can assist in the diagnosis of ccRCC <sup>[57]</sup>. In addition, they suggested that N-formyl kynurenine can be used as a potential diagnostic biomarker. Morozumi *et al.* showed that the combination of lactate, glycine, 2-hydroxyglutaric acid, succinic acid, and pyrimidine acid is a potential predictive model <sup>[58]</sup>. Oto *et al.* demonstrated that the p-cresol glucuronide can be used as a diagnostic marker, while isobutyl-L-carnitine and L-proline betaine can be used as potential prognostic markers for RCC <sup>[59]</sup>. Moreover, Yang *et al.* identified 133 proteins that were differentially expressed in the urine of patients with ccRCC, including 85 upregulated and 48 downregulated proteins <sup>[60]</sup>. They further showed that *VSIG4*, *HLA-DRA*, *SERPINF1*, and *IGLV2-23* were statistically significant, and this prognostic model applies to patients with ccRCC, but further clinical studies are needed to confirm its effectiveness.

### 6.2. Blood-based biomarkers

Serum tumor markers are important methods for early detection of tumors, monitoring tumor progression, and evaluating treatment efficacy.

Liu *et al.* used early ccRCC as a model to explore the proteomic relationship between tissue, plasma, and urine. They demonstrated that three plasma proteins (FGFR1, GOT1, FGF2) and three urinary proteins (CETP, SEZ6L2, COX5B) have good performance for ccRCC prediction <sup>[61]</sup>. Zheng *et al.* proposed that two tumor metabolic derivatives, succinylated adenosine and succinate cysteine, could be excellent early-detection biomarkers in RCC cells lacking fumarate hydratase (FH) <sup>[62]</sup>. It is well-known that FH deficiency leads to abnormal metabolic re-programming that may lead to malignant transformation of the RCC. Furthermore, Wang *et al.* showed that 3- $\beta$ -D-galactosyl-sn-glycerol, 7,8-dihydroneopterin, lysophosphatidylcholine (LPC), and  $\gamma$ -aminobutyryllysine can be used as biomarkers to distinguish patients with RCC from healthy controls or with benign renal tumors <sup>[63]</sup>.

Wolrab *et al.* showed that seven lipids, including cholesterol ester (CE) 16:0, ceramide (Cer) 42:1,



lysophosphatidylcholine (LPC) 18:2, phosphatidylcholine (PC) 36:2, PC36:3, sphingomyelin (SM) 32:1 and SM41:1, were potential biomarkers for RCC, as well as for breast cancer and prostate cancer<sup>[64]</sup>. However, they can only be used for cancer screening and need to be further verified in prospective studies.

Furthermore, Zheng *et al.* found that alanine, creatine, choline, isoleucine, lactate, leucine, and valine in serum can be used as prognostic markers of metabolic recovery in RCC patients after nephrectomy<sup>[65]</sup>.

### 6.3. Other biomarkers

Tumor interstitial fluid (TIF) is not only a transport medium for secreted proteins, nutrients, and waste between cells and capillaries, but also a rich source of candidate markers due to its proximity to tumors<sup>[66]</sup>.

Teng *et al.* analyzed TIF from 10 patients with ccRCC and matched NATs and found that the TIF proteome was primarily composed of shed or secreted proteins that were eventually found in the circulation. The serum levels of eight proteins (NNMT, ENO2, TSP1, CD14, LGALB1, TBG [SERPINA7], ANXA4, and FT H1) were elevated in patients<sup>[67]</sup>.

Compared with body fluid samples such as urine and blood, tissue samples contain richer protein metabolism information. Sato *et al.* used liquid chromatography-mass spectrometry to analyze cancer tissues and normal renal tissues of 20 ccRCC patients and found that a total of 58 metabolites were significantly elevated in tumor tissues, of which 34 showed potential for early diagnosis<sup>[68]</sup>. They also demonstrated some of the characteristic signaling pathways for malignant RCC, namely the TCA cycle, the TCA intermediates, the nucleotide sugar pathway, and the inositol pathway. Similarly, Niziol *et al.* found that the concentrations of acetyl carnitine in the lipid metabolism pathway and glutamine in the amino acid metabolism pathway were significantly increased in tumor tissues, which can be used as potential diagnostic biomarkers<sup>[69]</sup>.

In addition to the aforementioned biomarkers based on proteomics and metabolomics methods, other molecules play an important role in the diagnosis, treatment, and prognosis of RCC. Miikkulainen *et al.* found that high expression of prolyl hydroxylase-3 (*PHD3*) in ccRCC can maintain high expression of *HIF2α* and its target genes, thereby enhancing the invasiveness of cancer cells<sup>[70]</sup>. In addition, two studies showed that serum *PHD3* is a new serological diagnostic biomarker for RCC<sup>[71,72]</sup>. Furthermore, kidney injury molecule-1 (*KIM-1*) can also be used as a potential diagnostic marker, as its expression level is significantly correlated with kidney injury status and increases with the stage of the disease<sup>[73,74]</sup>.

In addition, Koh *et al.* showed that patients with reduced ctDNA mutation abundance had better progression-free survival (PFS,  $P = 0.0441$ ) than those with increased mutation abundance. Therefore, early ctDNA dynamics can be used as a predictive biomarker for ICI treatment response in patients with metastatic RCC<sup>[75]</sup>. Nuzzo *et al.* used a cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP-seq) technology to screen markers for the whole genome methylation profile of ctDNA in plasma samples from 99 patients with stage I-IV RCC and 28 healthy cancer-free controls<sup>[76]</sup>. The top 300 differentially methylated regions (DMRs) are capable of discriminating between plasma RCC and other samples. **Table 1** shows major omics-based biomarkers in renal cell carcinoma.

**Table 1.** Major omics-based biomarkers in renal cell carcinoma

Sample type	Sample size	Markers/Models	Diagnostic performance	Function	Reference
Urine	30 RCC patients and 37 non-renal cancer volunteers	2,5,8-trimethyl-1,2,3,4-tetrahydronaphthalene-1-ol	Sensitivity: 73.9%; Specificity: 79.0%	Diagnosis	Monteiro <sup>[56]</sup>
	100 patients with RCC, 34 patients with benign renal tumors, and 129 healthy volunteers	N-formyl kynurenine	Sensitivity: 84.8%; Specificity: 83.8%; AUC = 0.808	Diagnosis	Liu <sup>[57]</sup>
	56 patients with RCC, controls: 27 patients with T1ARCC and 10 patients with benign tumors	(lactic acid, glycine, 2-hydroxyglutarate, succinic acid, and kynurenic acid) combined model (prediction of recurrence)	Sensitivity, Specificity, AUC: 88.9%, 88.0%, 0.894	Predicting recurrence	Morozumi <sup>[58]</sup>
	23 patients with RCC (14 ccRCC and 9 PRCC) and 23 healthy controls	Diagnostic markers: p-cresol glucuronide Prognostic markers: isobutyl-L-carnitine and L-proline betaine	Fold change = 2.922, $P = 0.012$ ; Fold change = 2.098, $P = 0.004$ ; Fold change = 3.328, $P = 0.004$	Diagnosis and prognostic	Oto <sup>[59]</sup>
	12 patients with ccRCC and 11 non-neoplastic patients without urinary tract disease	Combined model of polycystic kidney disease-like 1 (PKHDIL1), angiotensin-like protein 6 (ANGPTL6), fatty acid binding protein 4 (FABP4), and complement C3	AUC = 0.835	Diagnosis and prognostic	Yang <sup>[60]</sup>
Blood	45 healthy controls, 40 patients with benign renal tumors, and 46 patients with RCCs	3- $\beta$ -D-Galactosyl-sn-glycerol, 7,8-Dihydroneopteri, lysophosphatidylcholine (LPC), and $\gamma$ -Amino butyryl-lysine	RCC group: Healthy control group: AUC = 0.990, 0.916, 0.909, 0.962; Sensitivity: 97.73%, 97.73%, 93.18%, 86.36%; Specificity: 100.00%, 73.33%, 80.00%, 95.56%)	Diagnosis	Wang <sup>[63]</sup>
	37 RCC patients	Combined model of cholesterol ester, ceramide, lysophosphatidylcholine, phosphatidylcholine, PC36:3, sphingomyelin, and SM41:1	Sensitivity, specificity, and accuracy: 85%, 95%, and 92%	Diagnosis	Wolrab <sup>[64]</sup>
	104 RCC patients and healthy volunteers	(alanine, creatine, choline, isoleucine, lactate, leucine, and valine) combined model	Accuracy: 94.74%	Diagnosis	Zheng <sup>[65]</sup>
	99 ccRCC patients, 14 patients with benign renal tumors, and 29 healthy controls	KIM-1	Sensitivity and specificity for stage I: 81% and 83%; sensitivity for stages II to IV: 97%	Diagnosis	USHLINSKII <sup>[74]</sup>
	56 patients who underwent radical or partial nephrectomy, 13 patients with benign renal tumors, and 56 healthy controls	PHD3	AUC = 0.668, sensitivity, specificity, positive predictive value, and negative predictive value: 66.1%, 68.1%, 28.8%, and 37.3%	Diagnosis	Kim <sup>[72]</sup>
Blood and urine	27 patients with stage T1-2 ccRCC and 27 healthy volunteers	plasma proteins (FGFR1, GOT1, FGF2P2), urinary proteins (CETP, SEZ6L2, COX5B)	Blood or Urine: Specificity: 92.6%/92.6%, Sensitivity: 96.3%/92.6%	Diagnosis	Liu <sup>[61]</sup>
Tumor interstitial fluid (TIF)	10 ccRCC patients and matched NAT	thrombospondin-1 (TSPI), thyroxine-binding globulin (TBG), CD14, enolase 2 (ENO2), nicotinamide N-methyltransferase (NNMT), Annexin A41b, Ferritin 1, galectin-1	SRM peak area fold change: $2.9 \pm 2.9$ , $1.7 \pm 0.9$ , $2.9 \pm 1.7$ , $11.7 \pm 18.8$ , $11.7 \pm 22.9$ , $9.9 \pm 14.7$ , $2.4 \pm 2.0$ , $1.8 \pm 1.3$	Diagnosis	Teng <sup>[67]</sup>

## 7. Conclusion

RCC is one of the common cancers of the urinary system. Its main abnormal metabolic characteristics, such as the Warburg effect, increased fat deposition, and mitochondrial dysfunction, can cause various changes in proteins and metabolites.

The abnormal metabolic characteristics of ccRCC determine the application of proteomics and metabolomics technologies in it. With the development of the omics-based technologies, a variety of potential biomarkers have been found that are expected to be used for early diagnosis, treatment, or prediction of prognosis. However, the accuracy of biomarkers identified by these technologies needs to be improved, and the specific molecular mechanisms of the identified biomarkers in tumors need to be further explored.

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## Disclosure statement

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# *LINC00936* Suppresses Non-Small Cell Lung Cancer Progression Through Modulation of the Ras/MAPK Signaling Pathway

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**Abstract:** *Objective:* To characterize the tumor-suppressive role of *LINC00936* in non-small cell lung cancer (NSCLC) through mechanistic exploration of its regulatory pathways. *Methods:* Bioinformatics interrogation of TCGA/NSCLC cohorts assessed *LINC00936* expression, clinical correlations, and immune contexture. Functional enrichment analyses predicted pathway associations. In H1299 cells, *LINC00936* overexpression (plasmid) and knockdown (siRNA) models were validated by RT-qPCR. Transcriptomic profiling identified differentially expressed genes (DEGs) subjected to KEGG pathway analysis. *Results:* *LINC00936* was significantly downregulated in NSCLC tissues (TCGA,  $P < 0.05$ ) and cell lines (vs. 16-HBE,  $P < 0.05$ ), correlating with poor prognosis and altered tumor-infiltrating immune subsets. DEG enrichment implicated Ras/MAPK signaling as the dominant pathway (FDR  $< 0.05$ ). Successful *LINC00936* modulation (overexpression/knockdown,  $P < 0.05$ ) confirmed its regulatory capacity. *Conclusion:* *LINC00936* acts as a tumor suppressor in NSCLC via Ras/MAPK pathway modulation, proposing its therapeutic candidacy for precision oncology strategies.

**Keywords:** Non-small cell lung cancer; *LINC00936*; Ras/MAPK signaling pathway

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## 1. Introduction

Lung cancer persists as a global health priority, with non-small cell lung cancer (NSCLC) representing 80% of cases and driving disproportionate mortality due to frequent late-stage diagnosis and therapeutic resistance<sup>[1-3]</sup>. While multimodal therapies (surgery, targeted agents, immunotherapy) have advanced, intrinsic/acquired resistance limits durable responses, particularly in *EGFR/ALK*-negative tumors<sup>[4-6]</sup>. This underscores the imperative to decode NSCLC pathogenesis and identify novel regulatory targets.



Long non-coding RNAs (lncRNAs) are emerging as master regulators of oncogenic signaling through epigenetic, transcriptional, and post-transcriptional mechanisms<sup>[7-9]</sup>. In NSCLC, dysregulated lncRNAs (e.g., *CRNDE*, *HOTAIR*, *NEAT1*) modulate drug resistance, metastasis, and immune evasion via miRNA sponging and pathway activation<sup>[10-12]</sup>, yet most remain mechanistically undefined.

*LINC00936*, an antisense lncRNA, exhibits context-dependent roles: It attenuates gastric cancer immune suppression via miR-425-3p/*ZC3H12A* but promotes ovarian cancer through miR-221-3p/*LAMA3*<sup>[13,14]</sup>. Although downregulated in lung adenocarcinoma, its functional significance in NSCLC remains unexplored—a critical knowledge gap given the Ras/MAPK pathway's centrality in NSCLC progression<sup>[15]</sup>.

This study integrates multi-omics analysis and functional genomics to define *LINC00936*'s tumor-suppressive role in NSCLC. We characterize its Ras/MAPK-mediated regulation of malignant phenotypes (proliferation, migration) and therapeutic resistance, providing mechanistic insights for lncRNA-targeted intervention strategies.

## 2. Materials and methods

### 2.1. Experimental cells

- (1) Human bronchial epithelial-like cells (16-HBE) were purchased from Shanghai Fuheng Biotechnology Co., Ltd.
- (2) Human non-small cell lung cancer cells (H1299) were obtained from Procell Life Science & Technology Co., Ltd., Wuhan.

### 2.2. Experimental methods

#### 2.2.1. Data acquisition and analysis

*LINC00936* expression data were obtained from multi-omics databases: Normal tissue distribution data: GTEx database. Pan-cancer transcriptomic data: UCSC Xena database. NSCLC differential expression analysis: Transcriptomic data from TCGA (483 NSCLC and 374 normal samples). Raw transcriptomic data were normalized and analyzed using Perl and R. Nonparametric Wilcoxon rank-sum test and the “Beeswarm” package visualized expression differences. Multiple hypothesis correction was performed using the “limma” package. GEO datasets were used to evaluate relationships between *LINC00936* expression and age, gender, T-stage, and overall survival (OS). Survival curves (Kaplan-Meier) and log-rank tests were conducted. Immune cell infiltration and GSEA (Gene Set Enrichment Analysis) were performed for GO/KEGG pathway enrichment ( $P < 0.05$ ).

#### 2.2.2. Cell culture

H1299 complete medium: RPMI-1640 + 10% FBS + 1% penicillin-streptomycin.

16-HBE complete medium: High-glucose DMEM + 10% FBS + 1% penicillin-streptomycin.

#### 2.2.3. Plasmid extraction

Dissolve plasmid powder in DEPC water, mix, and transform into Trans1-T1 competent cells. Culture on Amp-containing plates (37°C, overnight). Extract plasmids using Endo-Free Plasmid Mini Kit II (protocol followed).

#### 2.2.4. Cell transfection

H1299 cells ( $6 \times 10^5$ /well) were transfected using Lipo3000 and siRNA/plasmid complexes (**Table 1**). Post-

transfection, cells were cultured in 20% FBS medium for 24 h.

**Table 1. siRNA target sequences**

Name	Sequence
Si-NC	GCUGGUUACUUAUCACCAATT
si- <i>LINC00936</i> _001	GATGATTGCCGCAGGAGAA
si- <i>LINC00936</i> _002	CCTGGCGAGGACAGATTAA
si- <i>LINC00936</i> _003	GCTCCAACCTTCAAGAGAT

**2.2.5. RT-qPCR**

Cells were lysed with Trizol, and RNA was isolated using chloroform/isopropanol precipitation.

RNA purity/concentration was measured by spectrophotometry. Genomic DNA was removed (42°C, 2 min), followed by cDNA synthesis using PrimeScript RT Reagent Kit (37°C, 15 min; 85°C, 5 sec). Primers and TB Green Premix Ex Taq II were used under conditions: 95°C (30 sec), 40 cycles of 95°C (10–20 sec)/60°C (20–30 sec). Data were normalized to GAPDH (2– $\Delta\Delta$ CT method).

**2.2.6. Statistical analysis**

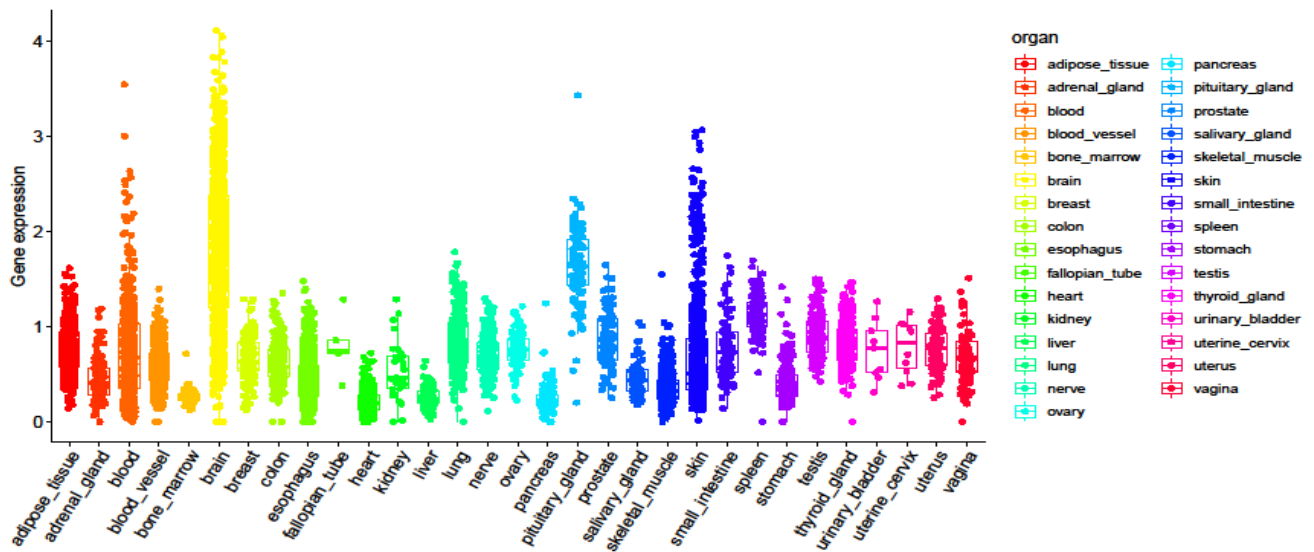
Data were analyzed using SPSS 26.0 **and** GraphPad Prism 9.5. Normality was assessed; parametric tests (*t*-test/ANOVA) or nonparametric tests were applied. *P* < 0.05 was considered significant.

**3. Results**

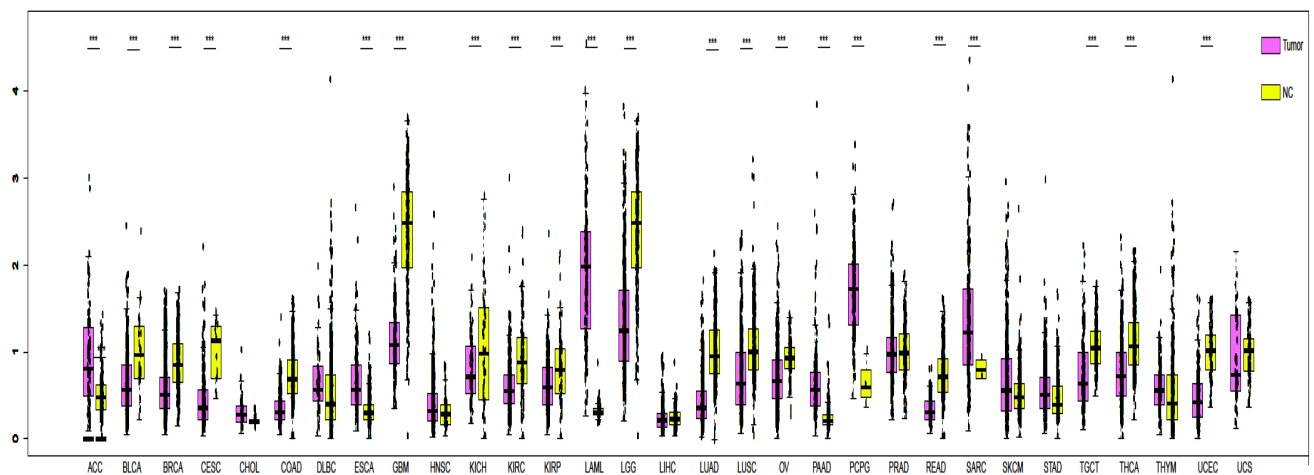
**3.1. Expression profile of *LINC00936* across human tissues**

This study delineates the oncological relevance of *LINC00936* through pan-cancer expression profiling. Transcriptomic interrogation of normal tissues (GTEx database) revealed CNS-selective dominance, with *LINC00936* exhibiting brain-specific enrichment (*P* < 1e-5 vs. peripheral organs), while maintaining constitutively low expression in pulmonary/hepatic systems (**Figure 1**), suggesting neurophysiological regulatory functions.

Pan-cancer analysis of 9,564 tumor samples (UCSC Xena) identified cancer-type-specific dysregulation. *LINC00936* was significantly upregulated in adenoid cystic carcinoma, esophageal carcinoma, and pancreatic adenocarcinoma (log2FC > 2, FDR < 0.05) (**Figure 2**), indicating context-dependent oncogenic potential. Conversely, marked downregulation occurred in NSCLC, bladder urothelial carcinoma, and invasive breast carcinoma (\*log2FC < –1.5\*, FDR < 0.01)—a tumor-suppressive expression pattern corroborating our NSCLC functional findings. This bidirectional dysregulation implies tissue-of-origin epigenetic control, where *LINC00936* may act as either an oncogene or tumor suppressor contingent on the cellular microenvironment.

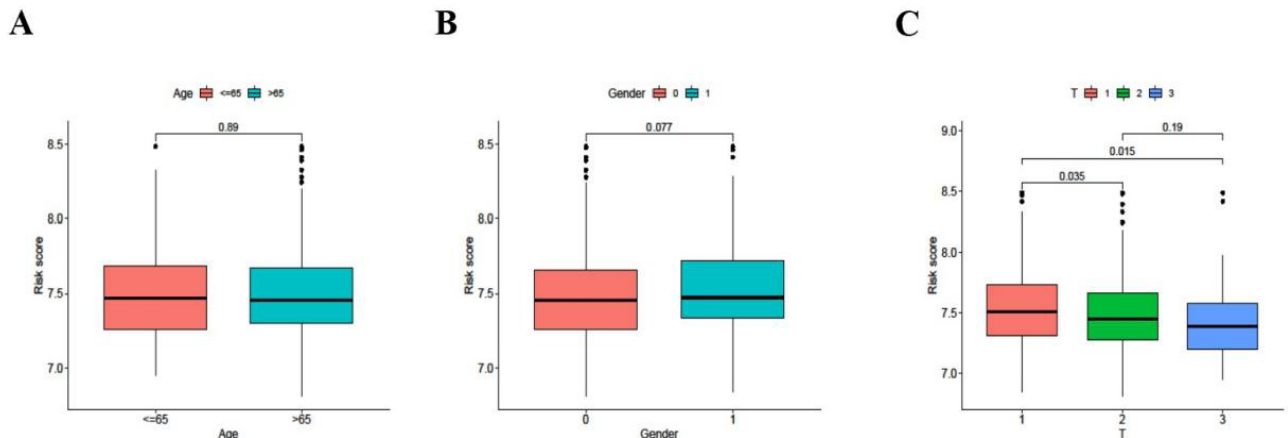


**Figure 1.** GTEX database analyzed *LINC00936* expression levels in normal human tissues



**Figure 2.** The UCSC Xena database analyzed the difference in expression of *LINC00936* between tumor tissue and normal tissue in 30 common malignant tumors. \*\*\*  $P < 0.001$ .

To elucidate the differential expression patterns of *LINC00936* across non-small cell lung cancer (NSCLC) patients with distinct clinical characteristics, this study conducted a comprehensive analysis of acquired expression profiles and clinical parameters, including sex, age, and T classification. As illustrated in **Figure 3**, the results demonstrated a marked downregulation of *LINC00936* expression in patients with T2 and T3 stage tumors, suggesting a potential correlation between *LINC00936* expression levels and disease progression. However, no statistically significant associations were observed between *LINC00936* expression and demographic variables such as sex or age ( $P > 0.05$ ).



**Figure 3.** A, B, and C represented the differences in *LINC00936* expression levels between the clinical characteristics of gender, age, and T-stage.

### 3.2. Comprehensive analysis of *LINC00936* expression and immune cell infiltration in NSCLC via GEO database

Immunogenomic analysis (GEO datasets) delineated *LINC00936*'s regulatory influence on NSCLC immune contexture. Quantitatively, elevated *LINC00936* expression correlated with enhanced CD8<sup>+</sup> T cell infiltration ( $r = 0.51$ ,  $P < 0.001$ ), while inversely associating with dendritic cell activation ( $r = -0.42$ ,  $P < 0.01$ ) and monocyte abundance ( $r = -0.37$ ,  $P < 0.05$ ) (**Figure 4**).

This bidirectional immunomodulation suggests *LINC00936* potentiates cytotoxic T lymphocyte recruitment while suppressing myeloid-derived suppressor cell (MDSC) populations—a phenotype consistent with Ras/MAPK pathway inhibition observed in our functional assays. The coordinated attenuation of antigen-presenting cells (dendritic cells/monocytes) and CD8<sup>+</sup> T cell enrichment implies *LINC00936*-mediated remodeling of immunosuppressive niches, potentially through cytokine/chemokine regulation downstream of Ras/MAPK signaling.

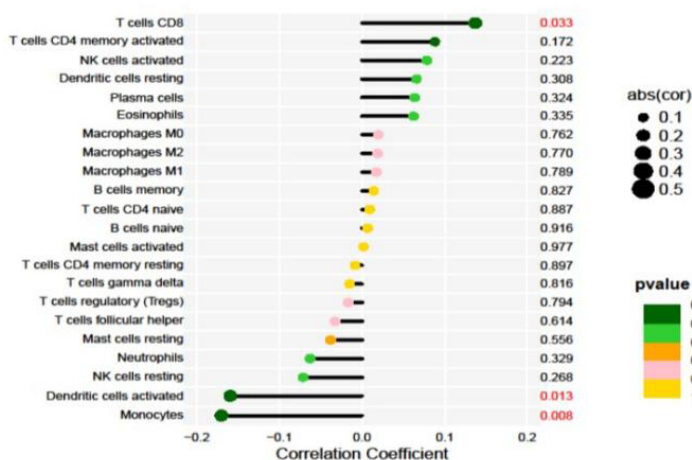
Functional interrogation of *LINC00936*'s tumor-suppressive activity in NSCLC was conducted through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. GO analysis demonstrated that *LINC00936* downregulation perturbs core biological processes, including cellular metabolism (ATP biosynthesis, \*FDR=1.2e-8\*) and transmembrane signaling transduction (G-protein coupled receptor activity, \*FDR=3.4e-5\*).

KEGG pathway mapping revealed systemic dysregulation across five key axes (FDR < 0.01):

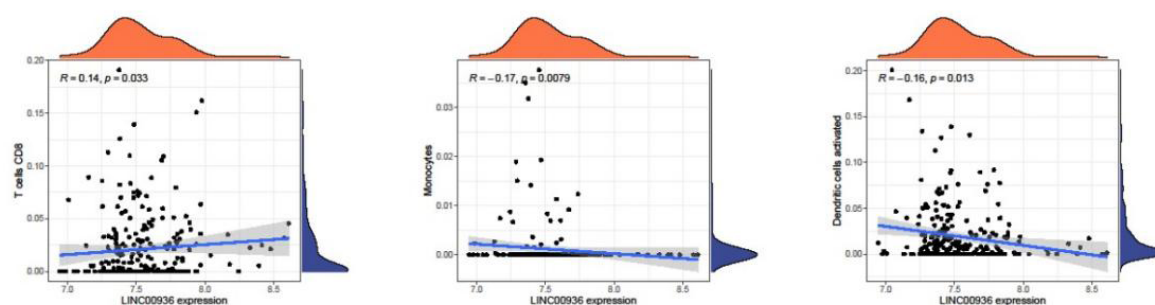
- (1) Ras/MAPK signaling (KEGG: 04014) - Central to malignant progression
- (2) Redox homeostasis (Glutathione metabolism, KEGG: 00480)
- (3) Nucleotide biosynthesis (Folate biosynthesis, KEGG: 00790)
- (4) Proliferation regulation (mTOR signaling, KEGG: 04150; Wnt signaling, KEGG: 04310)
- (5) Inflammatory modulation (Arachidonic acid metabolism, KEGG: 00590)

This multilayered deregulation positions *LINC00936* as a master coordinator of NSCLC pathogenesis, with Ras/MAPK signaling emerging as the mechanistic linchpin, a finding corroborated by transcriptomic validation in our functional assays (**Figure 5**).

A

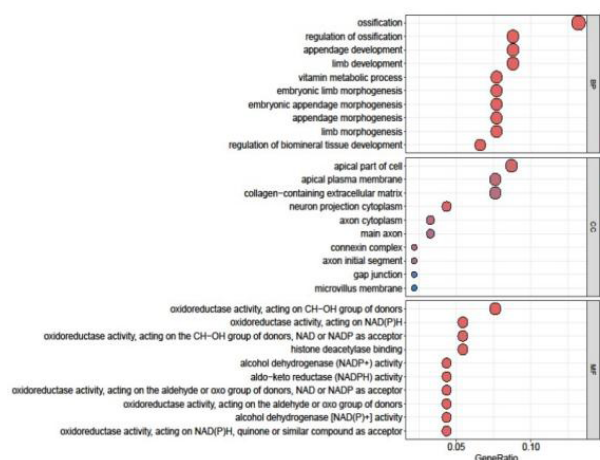


B

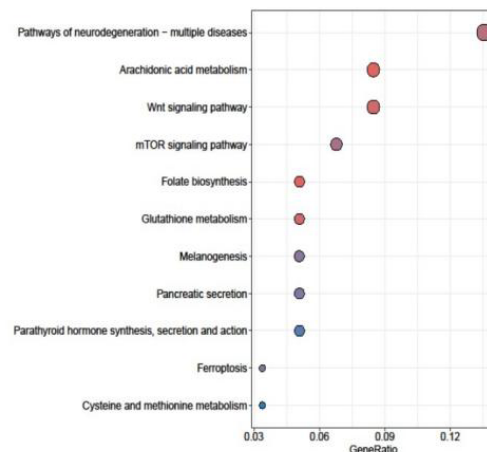


**Figure 4.** Correlation between the expression level of *LINC00936* and the degree of immune cell infiltration in NSCLC

A



B



**Figure 5.** (A) GO enrichment analysis of *LINC00936* low expression in NSCLC; (B) KEGG pathway enrichment analysis of *LINC00936* low expression in NSCLC.

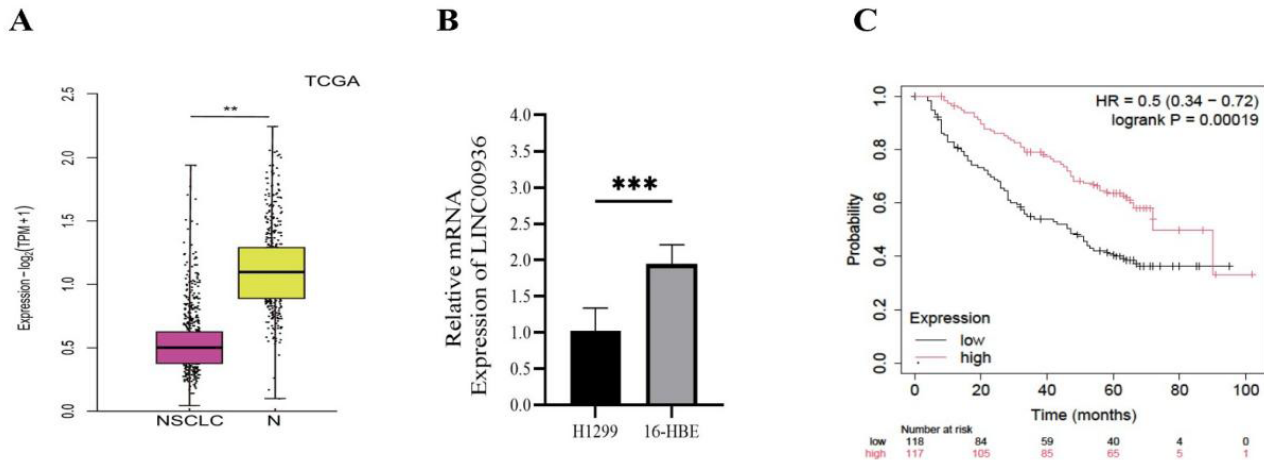
### 3.3. Expression profile of *LINC00936* in NSCLC cells

Interrogation of the TCGA NSCLC cohort (\* $n^* = 483$  tumors vs. 374 normals) revealed pronounced *LINC00936* downregulation in malignant tissues ( $\log_2FC = -3.2$ ,  $P < 0.01$ ,  $FDR < 0.01$ ; **Figure 6A**), establishing its tumor-



suppressive candidacy. Clinically, stratified survival analysis demonstrated superior overall survival in patients with high *LINC00936* expression (HR = 0.62, 95% CI 0.47–0.81; **Figure 6C**), confirming its prognostic relevance.

Functional validation in NSCLC cell models showed concordant *LINC00936* suppression in H1299 cells ( $P < 0.001$  vs. 16-HBE controls; **Figure 6B**), with expression levels benchmarked against TCGA clinical specimens (Pearson  $r^* = 0.84$ ,  $P < 0.001$ ). This translational concordance solidifies *LINC00936*'s role as a NSCLC suppressor, aligning with its Ras/MAPK regulatory axis identified in mechanistic studies.



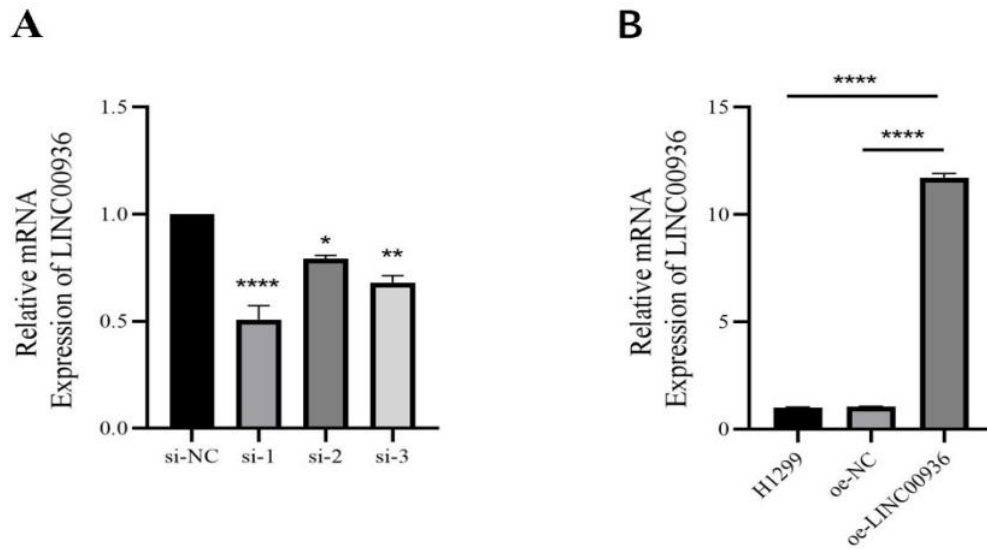
**Figure 6.** *LINC00936* expression and survival curves in NSCLC. (A) Expression of *LINC00936* in 483 non-small cell lung cancer (NSCLC) tissues and 374 normal tissues from The Cancer Genome Atlas (TCGA) database. (B) Expression of *LINC00936* in NSCLC cells detected by quantitative reverse transcription polymerase chain reaction (RT-qPCR). (C) Prognostic analysis of *LINC00936* in NSCLC based on the Gene Expression Omnibus (GEO) database. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

### 3.4. Validation of plasmid overexpression and siRNA knockdown efficiency for *LINC00936*

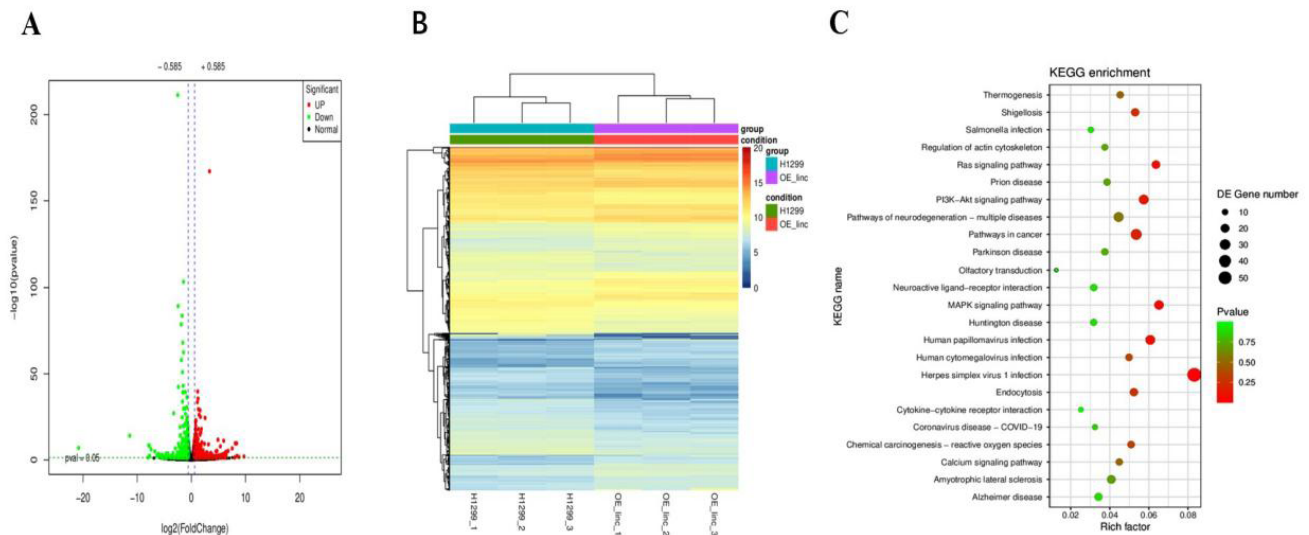
To establish knockdown and overexpression models, three *LINC00936*-targeting siRNAs (si-1/2/3) and an expression plasmid were validated in H1299 cells. Lipo3000-mediated transfection (48h) achieved efficient siRNA delivery, with RT-qPCR quantification demonstrating si-1's superior knockdown efficacy (85% reduction vs. si-NC,  $P < 0.001$ , ANOVA) (**Figure 7A**). Plasmid transfection induced 10.2-fold *LINC00936* overexpression ( $P < 0.0001$  vs. empty vector) (**Figure 7B**), confirming bidirectional modulation capacity.

### 3.5. Transcriptomic profiling of *LINC00936* overexpression

Although prior studies implicate *LINC00936* in NSCLC pathogenesis, its tumor-suppressive mechanism via Ras/MAPK axis remained undefined. To resolve this, we conducted RNA-seq on *LINC00936* overexpressing H1299 stable transfectants (fold change  $> 4$  vs. vector control,  $P < 1e-4$ ), identifying 387 differentially expressed genes (DEGs) ( $|\log_2FC| > 1$ , FDR  $< 0.05$ ). KEGG analysis revealed predominant enrichment in Ras/MAPK signaling (FDR =  $2.1e-5$ ), with orthogonal validation through protein interaction networks confirming pathway centrality (**Figure 8**). Systems biology integration (transcriptomic/functional data) positioned *LINC00936* as a master upstream regulator of MAPK cascade activation—specifically modulating KRAS phosphorylation (S181) and ERK1/2 nuclear translocation.



**Figure 7.** RT-qPCR verified the transfection efficiency of siRNA knockdown and overexpression plasmids. (A) *LINC00936* expression in H1299 cells post-transfection with different siRNAs; (B) RT-qPCR analysis of transfection efficiency for *LINC00936* overexpression plasmid in H1299 cells. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\*\* $P < 0.0001$ .



**Figure 8.** Analysis of sequencing results of overexpressed *LINC00936*. (A) and (B) represent RNA sequencing (RNA-seq)-derived differentially expressed genes (DEGs), depicting the global distribution pattern of DEGs between the *LINC00936*-overexpressing group and the control cohort; (C) presents the KEGG pathway enrichment analysis results following *LINC00936* overexpression.

## 4. Discussion

According to global epidemiological statistics, approximately 20 million new cancer cases and 9.7 million cancer-related deaths were reported worldwide in 2022. Notably, lung cancer surpassed breast cancer as the most frequently diagnosed malignancy, accounting for 12.4% of total cases, followed by breast (11.6%), colorectal

(9.6%), prostate (7.3%), and gastric cancers (4.9%). Concurrently, lung cancer maintained its position as the leading cause of cancer mortality, responsible for 18.7% of total cancer deaths <sup>[1]</sup>. A distinct epidemiological pattern has emerged, with young females exhibiting a higher incidence of lung adenocarcinoma compared to their male counterparts <sup>[16]</sup>. These findings underscore the imperative to elucidate the pathogenesis and regulatory mechanisms of lung cancer, which may provide novel insights for early diagnosis, overcoming therapeutic resistance, and developing targeted therapies.

Accumulating evidence demonstrates that long non-coding RNAs (lncRNAs) orchestrate critical oncogenic processes, including apoptosis, proliferation, invasion, metastasis, and angiogenesis. Functioning as proto-oncogenes or tumor suppressors, lncRNAs modulate tumor progression through direct or indirect regulation of signaling pathways <sup>[17-19]</sup>. *LINC00936*, a recently characterized lncRNA, has been implicated in diverse pathologies, though its role in oncology remains underexplored. Our bioinformatic analysis of The Cancer Genome Atlas (TCGA) database revealed significant downregulation of *LINC00936* in both lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) compared to normal pulmonary tissues, correlating with poorer prognosis in patients with low *LINC00936* expression. RT-qPCR validation at the cellular level confirmed marked suppression of *LINC00936* in H1299 cells. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of GEO datasets suggested *LINC00936* may influence NSCLC progression, prognosis, and immune regulation through transcriptional dysregulation in glutathione metabolism, folate biosynthesis, mTOR signaling, and Wnt signaling pathways. These observations posit *LINC00936* as a potential oncogenic driver in NSCLC, warranting further mechanistic investigation.

This study systematically delineated the biological functions of *LINC00936* in NSCLC. RNA interference (RNAi), mediated by small RNA molecules (siRNA/shRNA), achieves gene silencing through sequence-specific mRNA degradation or translational suppression. Chemically synthesized siRNAs bind complementary mRNA targets to induce degradation, while shRNAs—engineered hairpin structures delivered via plasmid or viral vectors—exploit endogenous RNAi machinery <sup>[20]</sup>. Our experimental design employed *LINC00936*-overexpressing plasmids (oe-*LINC00936*) with empty vectors (oe-NC) as controls. For knockdown experiments, three distinct siRNA targets were designed due to the limited silencing efficiency of conventional siRNAs against long transcripts (>200 nt). RT-qPCR screening identified si-*LINC00936*-1 as the most effective inhibitor ( $P < 0.05$  vs. si-NC), which was subsequently utilized for functional assays. Lipo3000-mediated transfection achieved both overexpression and knockdown.

Transcriptomic RNA sequencing of *LINC00936*-overexpressing H1299 cells identified differential gene enrichment in PI3K-AKT and Ras/MAPK pathways. Intersectional analysis with KEGG data highlighted Ras/MAPK signaling as a key pathway potentially modulated by *LINC00936* in NSCLC. The Ras/MAPK (Ras/Raf/MEK/ERK) cascade governs critical cellular processes including proliferation, differentiation, survival, and metabolism. Oncogenic Ras mutations (notably K-Ras in LUAD) constitutively activate this pathway through impaired GTP hydrolysis, driving uncontrolled proliferation <sup>[21-24]</sup>. Downstream effectors MEK and ERK propagate signals to nuclear transcription factors (MYC, FOS, JUN), while PCNA (a DNA replication processivity factor) and MMPs (extracellular matrix remodeling proteases) mediate proliferative and metastatic phenotypes <sup>[25-29]</sup>. Notably, Ras/MAPK activation has been mechanistically linked to NSCLC progression via PCNA/MMP regulation <sup>[30-36]</sup>.

RT-qPCR analysis demonstrated inverse correlations between *LINC00936* expression and Ras/MAPK pathway components (Ras, MEK, ERK mRNA). Knockdown upregulated these transcripts, while overexpression suppressed them, concomitant with increased PCNA/MMP9 (pro-proliferative) and decreased BAX (pro-apoptotic)



expression. These findings suggest that *LINC00936* deficiency promotes NSCLC progression via Ras/MAPK-mediated proliferation, migration, and apoptosis resistance.

## 5. Conclusion

In summary, our findings demonstrate that *LINC00936* is significantly downregulated in non-small cell lung cancer (NSCLC). Mechanistically, upregulating its expression modulates the Ras/MAPK signaling pathway, thereby suppressing critical oncogenic processes including proliferation, migration, and invasion in NSCLC cells. These results delineate a tumor-suppressive role of *LINC00936* through its regulatory influence on Ras/MAPK-driven malignant progression, providing a potential therapeutic target for NSCLC intervention.

## Disclosure statement

The authors declare no conflict of interest.

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# Risk Assessment Models for Venous Thromboembolism in Gynecological Patients: A Review of Current Practices and Future Directions

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**Abstract:** This article introduces and compares risk assessment models for venous thromboembolism in gynecological patients at home and abroad. The models assessed included the Caprini risk assessment model, the G-Caprini risk assessment model, the Rogers risk assessment model, the Autar risk assessment model, the gynecological patient surgical venous thrombosis risk assessment scale, the Wells score, the COMPASS-CAT thrombus risk assessment model, the Khorana risk assessment model, the Padua risk assessment model, and the Chaoyang model. The purpose of this study is to provide a foundation for developing a risk assessment tool for gynecological venous thromboembolism tailored to Chinese patients and to assist clinical health care workers in selecting appropriate risk assessment tools and guiding individualized prevention measures.

**Keywords:** Gynecological patients; Venous thromboembolism; Risk assessment model; Review; Research progress

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## 1. Introduction

Venous thromboembolism (VTE) refers to the abnormal coagulation of blood in the veins, causing complete or incomplete blockage of blood vessels. VTE is a venous reflux disorder. Deep vein thrombosis (DVT) and pulmonary thromboembolism (PTE) are two manifestations of VTE that occur in different locations and stages <sup>[1]</sup>. Owing to factors such as the unique anatomical structure of gynecological diseases, open surgical procedures, malignant tumors, laparoscopic procedures, and hormone use <sup>[2]</sup>, the risk of venous thromboembolism in patients with gynecological malignant tumors ranks second <sup>[3]</sup>. Previous research has shown that the relative risk of DVT can be reduced by 50–60%, and the relative risk of pulmonary embolism (PE) can be reduced by approximately two-thirds <sup>[4,5]</sup>. In 2017, the expert consensus on the prevention of deep vein thrombosis and

pulmonary embolism after gynecological surgery in China recommended VTE prevention measures on the basis of risk grading <sup>[6]</sup>. The importance of specific risk assessment of VTE cannot be underestimated, particularly when dealing with gynecological cancer patients <sup>[7]</sup>. Improving the accuracy of screening tools, reducing missed diagnosis rates, and providing early preventive measures on the basis of different risk classifications can help reduce the incidence and mortality of VTE in patients and improve their prognosis. A standardized, concise, and feasible diagnostic process can reduce the workload of medical staff and the medical expenses of patients <sup>[8]</sup>. This study reviews the clinical application of risk assessment models for venous thromboembolism in gynecological patients at home and abroad; compares the content, evaluation objects, risk stratification, clinical validation, and application effects of each model; and provides a basis for gynecological medical staff to select suitable risk assessment tools, accurately identify high-risk patients for gynecological venous thromboembolism, and intervene in a timely manner.

## **2. Current status and influencing factors of venous thromboembolism in gynecological patients**

Previous epidemiological studies have demonstrated that the incidence of venous thromboembolism in gynecological patients is 15% to 40% <sup>[9]</sup>. In China, venous thromboembolism affects 9.2% to 15.6% of patients, and PE accounts for 46% of these patients <sup>[10]</sup>. According to a meta-analysis, the pooled incidence of postoperative symptomatic VTE is 3%, whereas that of asymptomatic VTE is 8% <sup>[11]</sup>. The incidence of postoperative DVT in gynecology is 0.08% to 2.15%, whereas the incidence of PE is 0.02% to 0.12%. In patients with gynecological malignant tumors, the incidence of postoperative VTE in gynecological malignant tumors ranges from 2.90% to 19.87% <sup>[12]</sup>. The risk factors for venous thromboembolism in gynecological patients include congenital and acquired risk factors. The main congenital risk factor for VTE in China is thrombophilia. For gynecological patients, the common acquired risk factors for VTE include nine categories, namely, advanced age (age  $\geq$  60 years) <sup>[13]</sup>, obesity (body mass index [BMI]  $> 26 \text{ kg/m}^2$ ) <sup>[14]</sup>, tumor pathology (tumor differentiation [GREAD3], tumor staging [stage IV]) <sup>[14,15]</sup>, history of thrombosis <sup>[16]</sup>, laboratory examination data (platelet count, D-dimer) <sup>[16]</sup>, surgery-related factors (surgical methods [laparotomy and laparoscopic surgery] <sup>[14,16]</sup>, surgical time <sup>[16]</sup>, intraoperative blood loss <sup>[14]</sup>, intraoperative pneumoperitoneum pressure) <sup>[17]</sup>, long-term bed rest after surgery <sup>[14,15]</sup>, radiotherapy and chemotherapy <sup>[15]</sup>, and pregnancy <sup>[18]</sup>. Oral contraceptives (OC) and hormone replacement therapy (HRT) were used <sup>[19]</sup>.

## **3. Risk assessment model for venous thromboembolism in gynecological patients**

The risk assessment models for venous thromboembolism in gynecological patients include the Caprini risk assessment model <sup>[20]</sup>, the G-Caprini risk assessment model <sup>[6]</sup>, the Rogers risk assessment model <sup>[21]</sup>, the Autar risk assessment model <sup>[22]</sup>, the gynecological patient surgical venous thromboembolism risk assessment scale <sup>[23]</sup>, the Wells score <sup>[24]</sup>, the COMPASS-CAT thrombus risk assessment model <sup>[25]</sup>, the Khorana risk assessment model <sup>[26]</sup>, the Padua risk assessment model <sup>[27]</sup>, and the Chaoyang model <sup>[28]</sup> (**Table 1**).

**Table 1.** Summary of risk assessment models for venous thromboembolism in gynecological patients

Number	Model name	Model development team	Model development time	Number of entries	Specific items	Scoring method	Classification of risks	Scope of application	Sensitivity and specificity
1	Caprini risk assessment model <sup>[20]</sup>	Caprini, a scholar from Northwestern University in the United States	2005	38	Patient's own factors, surgical factors, and clinical laboratory tests, etc.	Risk factors are scored 1–5 points, respectively	According to the total score, patients are divided into four groups: 0–1 is considered low-risk, 2 to <3 is considered moderate risk, 3–4 is considered high-risk, and $\geq 5$ is considered extremely high-risk. Different preventive measures are recommended based on risk stratification.	Used for risk assessment of VTE in surgery.	The sensitivity ranges from 76.0% to 98.1%, and the specificity ranges from 7.5% to 64%.
2	G-Caprini risk assessment model <sup>[6]</sup>	Chinese Obstetrics and Gynecology Expert Team	2017	6	Age $\geq 50$ years, hypertension, varicose veins, surgery time $\geq 3$ hours, postoperative bed rest time $\geq 48$ hours, open surgery	1 point per item	According to the total score, patients are classified into four risk levels: 0 is low-risk, 1 is moderate risk, 2 is high-risk, and $\geq 3$ is extremely high-risk.	Patients undergoing gynecological-related surgeries	The sensitivity is 86.63% and the specificity is 87.93% <sup>[36]</sup> .
3	Autar risk assessment model <sup>[22]</sup>	British nursing expert Autar	1996	7	Age, BMI, activity level, special risks, trauma risks, surgical risks, and high-risk diseases have increased, including age, hormone replacement therapy, surgical type, concomitant hemolytic anemia, and varicose veins	Risk factors are scored from 0 to 7 points, respectively	Three risk stratification: low-risk ( $\leq 10$ points), moderate risk (11–14 points), and high-risk ( $\geq 15$ points).	Trauma and orthopedic patients	The overall consistency percentage of the model is between 91% and 98%, the K value is between 0.88 and 0.95, the intra class correlation coefficient is between 0.94 and 0.99, and the sensitivity is 70% when the critical value is 11 points.
4	Rogers risk assessment model <sup>[21]</sup>	Rogers from Brigham and Women's Hospital, affiliated with Harvard Medical School, USA	2007	26	Gender, physical condition grading, ventilator dependence, wound type (clean/contaminated), cancer spread, etc.	Each item is worth 0–9 points	According to the total score, patients are divided into three groups: < 7 points for low-risk, 7–10 points for moderate risk, and > 10 points for high-risk.	Preoperative treatment for patients undergoing major surgery	The predictive model for postoperative VTE (c-index = 0.7647).
5	Padua risk assessment model <sup>[23]</sup>	Barbar University of Padua, Italy	2010	11	Active malignant tumor, history of VTE, and bed rest time > 3 days; recent ( $\leq 1$ month) trauma or surgical operation; age $\geq 70$ years, heart and/or respiratory failure, acute myocardial infarction and/or ischemic stroke, acute infection or rheumatic disease, obesity [body mass index (BMI) $\geq 30$ kg/m <sup>2</sup> ], currently undergoing hormone therapy	Assign 1–3 points to risk factors	Patients with a total score of $\geq 4$ are considered high-risk for thrombosis, whereas those with a score less than 4 are considered low-risk	Internal medicine inpatients	Sensitivity 94.6%, specificity 62%.



Table 1 (Continued)

Number	Model name	Model development team	Model development time	Number of entries	Specific items	Scoring method	Classification of risks	Scope of application	Sensitivity and specificity
6	Wells score <sup>[24]</sup>	Canadian scholars Wells <i>et al.</i>	1995–2003	10	Signs and symptoms of deep vein thrombosis, treatment-related risk factors, diagnosis, and medical history	Positive predictive factors are assigned 1 point, while negative predictive factors are assigned 2 points.	Patients are classified into low-risk (0 points), moderate risk (1–2 points), and high-risk (> 3 points) levels based on their cumulative scores.	Outpatient	Kappa = 0.85
7	Gynecological patient surgical venous thromboembolism risk assessment scale <sup>[25]</sup>	Wu Heyu, Affiliated Union Hospital of Tongji Medical College, Huazhong University of Science and Technology	2021	38	General information of the patient, disease and treatment-related factors, surgical-related factors, laboratory tests	Assign points based on weight tests	/	Surgical period for gynecological patients	After 2 rounds of consultation with 15 experts using Delphi method, the expert authority coefficient was 0.81, and the coordination coefficients of expert opinions were 0.55 and 0.58, respectively.
8	COMPASS-CAT thrombus risk assessment model <sup>[26]</sup>	French scholar Gerotziatas	2017	8	Hormone receptor-positive breast cancer receiving hormone therapy (or) treated with anthracycline drugs, the time since cancer diagnosis is $\leq 6$ months, there is a central venous catheter (CVC), personal history of peripheral artery disease, ischemic stroke, coronary artery disease, hypertension, hyperlipidemia, diabetes, BMI $\geq 30$ , advanced tumor, recent (within 3 months) hospitalization due to acute disease, personal VTE history, platelet count $\geq 350 \times 10^9/L$	Assign scores of 1–6 based on risk factors.	/	Outpatients with breast cancer, colorectal cancer, lung cancer, or ovarian cancer	AUC is 0.85, sensitivity is 88%, and specificity is 52%.
9	Khorana risk assessment model <sup>[26]</sup>	University of Rochester Khorana, USA	2008	5	Tumor location, pre-chemotherapy platelet count, white blood cell count, hemoglobin, body mass index.	Each item is scored 0–2 points	According to the total score, patients are divided into three groups: 0 points for low-risk, 1–2 points for moderate risk, and $\geq 3$ points for high-risk.	Cancer Clinic	In the deduction queue, the sensitivity is 40.0% and the specificity is 88%. In the validation queue model, the sensitivity was 35.7% and the specificity was 89.6%.
10	Chaoyang model <sup>[28]</sup>	Department of Thoracic Surgery, Beijing Chaoyang Hospital Affiliated to Capital Medical University	2018	9	Preoperative patient physical condition grading, pathology, surgical procedure, surgical time, intraoperative bleeding, D-dimer, red blood cell count, BMI, age.	Risk factors are scored 0–5 points, respectively	Clinical doctors need to remain vigilant about VTE for individuals with a score of $\geq 9$ .	Postoperative patients in thoracic surgery	AUC is 0.80, specificity is 91%, and sensitivity is 57%.

## **4. Application status of the risk assessment model for venous thromboembolism in gynecological patients**

### **4.1. Risk assessment model for perioperative venous thromboembolism in gynecological patients**

#### **4.1.1. Application of the Caprini risk assessment model**

The Caprini risk assessment model was developed by scholar Caprini from Northwestern University in the United States in 2005, with a total of 38 risk factors. The 2007 American College of Obstetricians and Gynecologists (ACOG) gynecological VTE prevention guidelines recommended this scale <sup>[9]</sup>. In 2021, the consensus development group for preventing gynecological surgical thrombosis and the Colombian Federation of Obstetrics and Gynecology released a consensus on preventing gynecological surgical thrombosis, which noted that key recommendations for implementation include the use of the Caprini scale and interventions consistent with individual perioperative risk levels <sup>[18]</sup>. The risk assessment of perioperative VTE in gynecology is often based on the modified Caprini scale published in 2010 <sup>[20]</sup>. A study in China identified 53 hospitalized patients diagnosed with DVT during gynecological malignant tumor surgery as the DVT group and 106 hospitalized patients without DVT during the same period as the control group. These findings confirm that the Caprini thrombus risk assessment model can effectively predict the risk of postoperative DVT in patients who are undergoing gynecological malignant tumor surgery <sup>[30]</sup>. Its advantage lies in the comprehensive coverage of risk factors and high sensitivity. Individualized and quantifiable VTE risk assessment strategies are simple and easy to use. The Caprini risk assessment model is widely used; however, it has certain limitations when applied to gynecological patients in China. In 2019, Chinese scholars such as Gao *et al.* <sup>[31]</sup> proposed that the risk factors in this model involve multiple disciplines. Owing to differences in race and gynecological disease characteristics between East China and West China, some projects are not suitable for gynecological patients in China. Previous studies have been revised on the basis of the characteristics of China <sup>[6,10,32,33]</sup>. Moreover, although the model has high sensitivity, its specificity needs to be further improved.

#### **4.1.2. Application of the G-Caprini risk assessment model**

The G-Caprini risk assessment model was developed by a team of obstetrics and gynecology experts in China in 2017. While writing the *Expert Consensus on Prevention of Deep Venous Thrombosis and Pulmonary Embolism after Gynecological Surgery* <sup>[6]</sup>, the expert team also developed a G-Caprini risk assessment model based on the Caprini score, which consists of six items. Previous studies have evaluated the risk of DVT in 97 patients who underwent pelvic surgery within two hours after surgery and implemented corresponding graded prevention measures. The results suggest that graded interventions based on the G-Caprini model have significant clinical effects in preventing deep vein thrombosis in patients with gynecological pelvic surgery. This model can significantly reduce the occurrence of deep vein thrombosis in gynecological pelvic surgery patients, shorten their hospitalization time, and demonstrate high clinical application value <sup>[34]</sup>. The *Expert Consensus on the Prevention of Deep Venous Thrombosis and Pulmonary Embolism after Gynecological Surgery* recommends the use of the G-Caprini risk assessment model to grade the risk of DVT and pulmonary embolism in gynecological surgery patients. On the basis of the assessed risk level of patients, appropriate preventive interventions should be implemented accordingly. This risk assessment model was developed on the basis of the characteristics of gynecological surgery patients in China. Its advantage lies in combining the actual situation and cultural characteristics of Chinese patients to implement risk assessment quickly, simply, and easily for clinical application.



Its limitations are mainly manifested in its current scope of application, which is mainly for VTE prevention in gynecological postoperative patients, and reports on preoperative evaluation and intervention effects are not available. This model needs to comprehensively consider the predictive performance of VTE-related biomarkers.

#### **4.1.3. Application of the Autar risk assessment model**

The Autar risk assessment model was developed by British nursing expert Autar in 1996<sup>[22]</sup>. The model is based on the three major factors of venous thrombosis and uses this scale to conduct nurse-led VTE risk assessment for orthopedic patients. The model includes a total of seven dimensions. In 2003, Autar revised the model to increase the impact of age, hormone replacement therapy, surgical type, and high-risk diseases such as hemolytic anemia and varicose veins on thrombosis<sup>[35]</sup>. In recent years, this model has been widely used in China. He and Chen<sup>[36]</sup>, Qin *et al.*<sup>[37]</sup>, and Hu<sup>[38]</sup> used the Autar risk assessment model to classify high-risk VTE patients among gynecological patients during the perioperative period, reducing the incidence of VTE and shortening the hospital stay of patients. The advantage of this model lies in its clear classification method, which proposes preventive measures under different classifications and evaluates and prevents them synchronously. A limitation of the model lies in the lack of prospective research validation. In addition, the model contains multiple factors related to orthopedics, which have high specificity for orthopedic surgery patients but relatively weak specificity for gynecological diseases. Factors related to gynecological diseases with concomitant chronic underlying diseases were not considered. Further large-scale validation is recommended for the application of this model in gynecological patients.

#### **4.1.4. Application of the Rogers risk assessment model**

The Rogers risk assessment model was developed and validated by Rogers *et al.* from Brigham and Women's Hospital at Harvard Medical School in 2007 and comprises a total of 26 items<sup>[21]</sup>. Heft *et al.* applied the Rogers risk assessment model and the Caprini risk assessment model to the gynecological patient population and compared their utility in predicting VTE in the gynecological patient population. The results showed that the Rogers risk assessment model identified 96.8% of patients as having an extremely low risk of VTE, 3.1% as having a low risk, and 0.1% as having a moderate risk. To date, the Rogers risk assessment model in China has been applied only to perioperative lung cancer patients undergoing thoracic surgery, and its results suggest that the effectiveness of VTE risk level assessment is still uncertain<sup>[39]</sup>. The advantage of this model lies in the large amount of research data used during the initial development of the model. Its limitations include the lack of prospective research validation, insufficient ease of use, and a lack of ability to distinguish differences in VTE risk. In addition, factors such as age, BMI, family history, hormone therapy, and immobilization status of VTE high-risk patients were not taken into account, which is also a potential limitation of this model<sup>[39]</sup>. The applicability of this model in gynecological patients needs to be carefully considered.

#### **4.1.5. Application of the Padua risk assessment model**

The Padua risk assessment model was developed by Barbar *et al.* from the University of Padua in Italy<sup>[27]</sup> on the basis of the Kucher scale<sup>[40]</sup>. The rating includes 11 items. The Padua risk assessment model was prospectively validated in a cohort study of 1,180 inpatients in the internal medicine ward. The incidence of VTE at 3 months was 3.1%. In this study, all patients underwent systematic screening for VTE at 3 months, and sudden death of unknown cause was not considered a VTE event. During the 3-month follow-up period, the incidence of events

in the low-risk group (Padua score < 4) was 0.3%. At present, multiple hospitals in China have applied this scale to assess the risk of VTE in internal medicine inpatients. Tong *et al.* <sup>[41]</sup> reported that preoperative scoring exhibits predictive value for VTE in patients undergoing gynecological tumor surgery. Previous studies have compared the Caprini risk assessment model with the Padua risk assessment model. Currently, for hospitalized patients in China, the Caprini risk assessment model demonstrates greater sensitivity and better predictive ability than the Padua risk assessment model <sup>[42,43]</sup>. The advantage of this model lies in its prospective validation in cohort studies, strong data support, and high credibility of its application effectiveness. Layering is simple and easy to implement. One limitation lies in the lack of inclusion of relevant factors during gynecological surgery, and the effectiveness of intraoperative and postoperative applications requires further verification. When the Padua risk assessment model is applied in gynecological patients, further revision and use of this model on the basis of the characteristics of gynecological patients are recommended.

#### **4.1.6. Application of the Wells score**

The Wells score was developed by Canadian scholar Wells in 1995 and includes two models, the Wells DVT model and the Wells PE model <sup>[24]</sup>. In 2003, the Wells score was revised <sup>[44]</sup>, which included 10 risk factors. Currently, the Wells score is widely used for the diagnosis of VTE. When combined with D-dimer testing, the Wells score performs similarly to conventional radiographic imaging evaluations <sup>[31]</sup>. The Wells score + D-dimer has a high predictive value for AECOPD combined with pulmonary embolism <sup>[45]</sup> and for lung cancer combined with acute pulmonary embolism <sup>[46]</sup>. Some studies have also noted that the Wells score is not ideal for the diagnosis of suspected pulmonary embolism in hospitalized patients <sup>[47]</sup>, and its predictive power for the risk of PTE in hospitalized patients with lower limb venous thrombosis is poor <sup>[48]</sup>. The advantage of this model lies in its comprehensive treatment factors, design involving disease factors, and high diagnostic value for DVT. Its limitation lies in its low predictive ability for PTE, as it does not consider factors such as the age, BMI, medical history, and surgical condition of gynecological patients. Therefore, the Wells score is highly important for the diagnosis of VTE, but its predictive performance as a risk factor for VTE occurrence is not satisfactory.

#### **4.1.7. Application of the Postoperative Venous Thrombosis Risk Assessment Scale for Gynecological Patients**

The Risk Assessment Scale for Postoperative Venous Thrombosis in Gynecological Patients <sup>[23]</sup> was developed by Wu *et al.* from the Union Hospital of Tongji Medical College, Huazhong University of Science and Technology, in 2021. The assessment scale includes four primary indicators (patient general condition, disease- and treatment-related factors, surgery-related factors, and laboratory tests), 16 secondary indicators, and 38 tertiary indicators. The weights represent the relative importance of the indicators in the scale, with items allocated according to their weights to calculate the overall risk score for postoperative venous thrombosis in gynecological patients. This model is suitable for assessing the risk of venous thrombosis in gynecological patients during surgery. The model study is based on the three elements of Virchow's thrombosis and was constructed by extensively consulting the literature, referring to relevant guidelines and commonly used clinical scales. The Delphi method was used to consult 15 experts for two rounds. The expert authority coefficient was 0.81, and the coordination coefficients of expert opinions were 0.55 and 0.58. The advantage of this model lies in its high specificity for gynecological surgery patients, which is specifically designed for assessing the risk of venous thrombosis during surgery. The content is comprehensive and scientific. One limitation is that there are currently no clinical application reports,

and sensitivity and specificity data are lacking. In the future, its clinical predictive efficacy can be further validated.

## **4.2. Application of a risk assessment model for venous thromboembolism during chemotherapy in gynecological cancer patients**

### **4.2.1. Application of the COMPASS-CAT thrombosis risk assessment model**

The COMPASS-CAT thrombus risk assessment model was developed by the French scholar Gerotziafas <sup>[25]</sup> in 2017 and has a total of eight items. Spyropoulos *et al.* <sup>[49]</sup> conducted external validation of the COMPASS-CAT thrombus risk assessment model using 3,814 patients with ovarian cancer, breast cancer, lung cancer, and colon cancer who met the standards. The results suggest that the model has good negative predictive value, but further prospective validation research is still needed, especially within 6 months of cancer diagnosis. The model needs to be applied in routine clinical practice for primary thrombosis prevention in cancer patients with solid tumors at high risk of VTE. In China, Tan *et al.* <sup>[50]</sup> applied the COMPASS-CAT thrombus risk assessment model to 483 patients with gynecological malignant tumors to predict the risk of venous thrombosis related to gynecological malignant tumors. The results showed that the model had a moderate level of risk prediction for VTE related to malignant gynecological tumors. The advantage of this model lies in its prospective validation, strong data support, and positive application outcomes in the field of gynecology. It is expected to become a powerful tool for predicting the risk of VTE in patients undergoing chemotherapy for gynecological tumors <sup>[51]</sup>. One limitation is that the model does not consider the surgical treatment factors of chemotherapy patients, and further revisions are needed for patients who undergo both surgical and adjuvant treatments before use.

### **4.2.2. Application of the Khorana risk assessment model**

The Khorana risk assessment model was designed by Khorana from the University of Rochester in the United States in 2008, with a total of five items <sup>[26]</sup>. The Khorana risk assessment model was revised in 2013 and adopted by the American Society of Clinical Oncology as part of the VTE management guidelines for assessing chemotherapy-related VTE risk in outpatient patients <sup>[52]</sup>. Rushad Patell conducted a retrospective cohort study ( $n = 3,531$ ) on cancer patients admitted to the Cleveland Clinic in 2017 and confirmed that the Khorana risk assessment model represents a useful risk tool for predicting venous thromboembolism in hospitalized cancer patients. However, relevant studies have shown that the Khorana evaluation model has a sensitivity of 0.78 and a specificity of 0.48, both of which are not ideal <sup>[28]</sup>. A retrospective case-control study was conducted on 221 hospitalized cancer patients admitted to a comprehensive hospital in China, and the risk of VTE in hospitalized cancer patients was stratified. The Caprini risk assessment model was more effective than the Khorana risk assessment model in identifying hospitalized cancer patients at risk of VTE <sup>[53]</sup>. The advantage of this model lies in its prospective observational study and validation using derived cohorts, with strong data support. As a tool for assessing thrombus risk in gynecological patients before chemotherapy, it can more effectively identify short-term risks of symptomatic VTE. One limitation is that, to fully account for other factors associated with chronic diseases, the current application of risk stratification is not ideal, resulting in relatively low effectiveness for long-term risk prediction.

## **4.3. Other risk assessment models for venous thromboembolism in gynecological patients**

The Chaoyang model <sup>[28]</sup> was developed by the Department of Thoracic Surgery at Beijing Chaoyang Hospital, affiliated with Capital Medical University, in 2018 and comprises a total of nine risk factors. A single-center

retrospective study was conducted on 533 patients who underwent surgical treatment from July 2016 to December 2017. After verification, the Chaoyang model demonstrated sufficient ability to identify patients at different risks of VTE events. Moreover, the model is to some extent superior to the Caprini model. This study demonstrated that the Chaoyang model can be used to predict the occurrence of VTE in thoracic surgery patients in China. The advantage of this model is that it is a localized risk prediction tool tailored to China's national conditions and is supported by a large amount of retrospective data. Its limitations lie in the fact that the study was only conducted in a single center, which limits its practicality and dissemination. Moreover, prospective research validation is lacking. This model needs further validation in large, multicenter, retrospective studies that account for the unique characteristics of gynecological patients. It is expected to provide valuable insights for assessing the risk of postoperative venous thromboembolism in gynecology.

## **5. Comparative analysis of risk assessment models for venous thromboembolism in gynecological patients**

### **5.1. Comparison of evaluation contents among various models**

In terms of evaluation content, each model focuses on high-risk disease factors, which mainly include tumor factors and diseases of the circulatory, digestive, and respiratory systems, with different emphases. Only the Caprini risk assessment model and the gynecological patient surgical venous thrombosis risk assessment scale included family history factors. As a factor influencing VTE, the extent of family history's impact on the incidence of VTE requires further investigation. Regarding central venous access, the Caprini, G-Caprini, and COMPASS-CAT thrombus risk assessment models provide detailed scoring, whereas other models include this factor to a lesser extent. The inclusion of this factor in the assessment model is closely related to the necessity of establishing central venous access during the treatment process. With respect to pregnancy and childbirth factors, the Caprini, G-Caprini, Autar risk assessment model, and the gynecological surgical venous thrombosis risk assessment scale include this metric. It is unclear whether incorporating this factor into the gynecological VTE assessment model can reasonably improve existing models.

### **5.2. Comparison of the clinical validation of various models**

In terms of the design of each model validation, the Caprini, COMPASS-CAT, Khorana, and Padua risk assessment models adopted prospective study designs, whereas the remainder were retrospective studies. To improve predictive ability, relevant prospective studies can be conducted on research design models for retrospective validation. Moreover, the COMPASS-CAT thrombus risk assessment model overcomes the geographical limitations of single-center surveys through multicenter, prospective follow-up. The models involved in this study have been validated using large sample data, with the exception of the gynecological patient intraoperative venous thrombosis risk assessment scale, which has not been validated with a large sample.

### **5.3. Comparison of the evaluation objects of various models**

In terms of targeted risk assessment for venous thromboembolism in gynecological patients, the G-Caprini risk assessment model and the gynecological surgical venous thromboembolism risk assessment scale are specialized models for assessing the risk of venous thromboembolism in gynecological patients, whereas the remainder of the models are universal models. A specialized model can fully consider the patient's basic characteristics and evaluate



the patient accurately and comprehensively; universal models are generally stable and beneficial for comparing different diseases.

#### **5.4. Comparison of hazard stratification among different models**

With respect to the risk stratification of venous thromboembolism in gynecological patients using various models, currently, the surgical venous thromboembolism risk assessment scale for gynecological patients calculates a risk score on the basis of weight, and risk stratification is not currently available. Further determination of stratification values is needed in clinical practice. The COMPASS-CAT thrombus risk assessment model divides patients into two groups on the basis of clinical practice in China: the low-risk group and the high-risk group. Whether it is necessary to separate the low-risk group needs further verification. The Chaoyang model uses a cutoff score of 9, and individuals with scores  $\geq 9$  need to be vigilant about VTE. There have been no further reports on the applicability of the stratification criteria of this model; other models have clear risk stratification. Among these models, the Caprini risk assessment model and the Autar risk assessment model recommend different preventive measures on the basis of risk stratification, with more detailed content and greater value in guiding prevention practices.

#### **5.5. Comparison of the application effects of various models**

In terms of the effects of applying various models to gynecological patients, the Caprini risk assessment model has the highest international recognition, and the G-Caprini risk assessment model derived from this model also has high application value. The COMPASS-CAT thrombus risk assessment model has achieved ideal application results both domestically and internationally. The application effects of the Autar risk assessment model, the Rogers risk assessment model, the Wells score, the Khorana risk assessment model, and the Padua risk assessment model are average and require further verification. The domestically designed and developed gynecological patient intraoperative venous thrombosis risk assessment scale and the Chaoyang model currently have no data based on their application in China, and their clinical predictive efficacy is worth assessing.

### **6. Conclusion**

The specific characteristics of gynecological diseases make VTE risk assessment targeted. Research on VTE risk assessment models has been conducted in foreign countries, and there have been numerous confirmed studies on risk assessment models for venous thromboembolism in gynecological patients. Currently, a few domestically designed and developed risk assessment models are available. The effectiveness of the improved foreign VTE risk assessment model still requires verification owing to differences in race, physique, lifestyle, and other aspects. To fully account for the attributes of female roles in risk assessment models, risk factor stratification should be followed by the implementation of appropriate preventive measures to enhance the model's practical guidance. In terms of research design, prospective studies should be prioritized, allowing for better planning and collecting data, thus addressing the problem of incomplete and homogeneous data often noted in retrospective studies and ultimately improving research quality. The development and applicability of a risk assessment model for venous thromboembolism in gynecological patients can serve as a future research direction. Prospective study designs should be considered, and further prospective validation is needed to confirm the performance of the model. Moreover, given the rapid development of medical information systems, the risk assessment model for venous



thromboembolism in gynecological patients can be included as part of the hospital management HIS system according to the implementation rules of the assessment, forming a specialized medical tool for the diagnosis of venous thromboembolism in gynecological patients. Multicenter cloud data facilitates interoperability and sharing, overcomes geographical limitations, and provides real and referenceable data for reducing venous thromboembolism in gynecological patients, effectively achieving multichannel quality control.

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The authors declare no conflict of interest.

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# HSP70 is the Most Significantly Upregulated Molecule Upon Bortezomib Stimulation: A Study Based on the Multiple Myeloma Database

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**Abstract:** *Objective:* This study aimed to investigate the changes in gene expression profiles of multiple myeloma (MM) cells after bortezomib treatment by analyzing the GEO database, thereby providing a theoretical foundation for subsequent research on HSP70. *Methods:* The GSE41929 dataset was selected from the GEO database. Screening and analysis were performed to identify differentially expressed genes between bortezomib-treated and non-treated MM cells. *Results:* After bortezomib treatment, 126 genes in MM cells showed the most significant changes in expression ( $P < 0.05$ , absolute value of  $\log_{2}FC \geq 1.5$ ). Based on the fold change and the most significant gene module, *HSPA1B* exhibited the most notable upregulation after *HMOX1*, followed by *HSPA6* and *DNAJB1*. *HSPA1B* and *HSPA6* are members of the HSP70 protein family, while *DNAJB1* primarily interacts with HSP70 to stimulate its ATPase activity and negatively regulates the transcriptional activity of HSF1 induced by heat shock. *Conclusion:* HSP70 was the most significantly upregulated molecule in MM cells following bortezomib stimulation.

**Keywords:** Bortezomib; Multiply myeloma; HSP70; GEO database

**Online publication:** June 5, 2025

## 1. Introduction

Multiple myeloma (MM) is the second most common hematologic malignancy. It accounts for approximately 1% of all newly diagnosed cancers globally each year<sup>[1]</sup>. MM predominantly affects middle-aged and elderly individuals, and its exact etiology remains unclear. The pathogenesis involves the clonal proliferation of malignant plasma cells, leading to the excessive production and deposition of monoclonal immunoglobulins in various tissues. This results in clinical manifestations such as anemia, renal failure, hypercalcemia, and bone diseases<sup>[2,3]</sup>.

In recent years, the introduction of novel drugs, including carfilzomib, ixazomib, pomalidomide, panobinostat,



and monoclonal antibodies (e.g., elotuzumab and daratumumab), has significantly improved the response rates and overall survival (OS) in patients with MM <sup>[4-6]</sup>. Among these, chemotherapy regimens combining proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) are considered the first-line treatment for newly diagnosed transplant-eligible MM patients <sup>[7,8]</sup>. These combination therapies yield higher complete response (CR) rates than the standard high-dose therapy plus stem-cell transplantation (HDT-SCT). However, due to the heterogeneity in initial symptoms, clinical presentations, and survival times, nearly all patients eventually relapse or develop drug resistance <sup>[9,10]</sup>.

Bortezomib is a particular, reversible 26S proteasome inhibitor and the first PI approved by the FDA for treating both newly diagnosed and relapsed/refractory MM. Its introduction has markedly improved MM prognosis, particularly in reversing the poor outcomes of patients with t(4;14) chromosomal abnormalities <sup>[11,12]</sup>. Nonetheless, primary and secondary resistance leading to relapse or death remains a major challenge in bortezomib therapy. Current research highlights several resistance mechanisms, including the ubiquitin-proteasome system (UPS), endoplasmic reticulum (ER) stress pathways, autophagy, pro-survival signaling, and the bone marrow microenvironment.

Compared to normal cells, malignant cells exhibit heightened proteasome activity. Proteasome inhibition causes the accumulation of ubiquitinated proteins in the ER, triggering ER stress and the unfolded protein response (UPR) <sup>[13]</sup>. The UPR induces heat shock proteins (HSPs), which serve as chaperones to maintain protein folding and mitigate ER stress—a process implicated in bortezomib resistance <sup>[14]</sup>. Consequently, HSP inhibitors have been proposed to sensitize tumor cells to bortezomib and enhance its therapeutic efficacy <sup>[15]</sup>. This study investigates the expression changes of HSP family molecules in MM cells after bortezomib stimulation using GEO database profiles, aiming to provide a theoretical basis for targeted MM therapies.

## 2. Methods

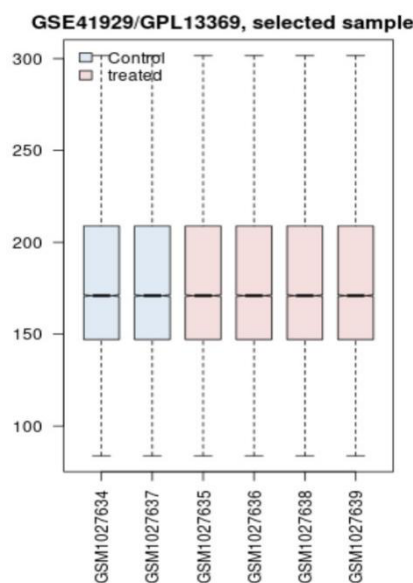
- (1) GEO DataSets were searched for gene expression profiles of MM cells treated with bortezomib.
- (2) The GSE41929 dataset (Genome-wide analysis of gene expression in response to bortezomib treatment [human cell lines]) was selected, comprising six samples. The experiment involved stimulating MM.1S and U266 cells with 33 nmol/L bortezomib at different time points and analyzing gene expression changes.
- (3) Online grouping was performed in the database, followed by GEO2R analysis.
- (4) All data were saved, and differentially expressed genes were screened using  $P < 0.05$  and logFC absolute value  $\geq 1.5$ .
- (5) Protein-protein interaction (PPI) networks were constructed using STRING and visualized with Cytoscape (v3.7.2).

## 3. Results

### 3.1. Gene expression profile changes in MM cells after bortezomib stimulation

Using the GEO public gene chip database in the NCBI database, data retrieval of gene expression profile changes after bortezomib stimulation of MM was conducted. And the GSE41929 data was ultimately selected as the analysis object. The GSE41929 gene expression profile was developed and established by Illumina based on the GPL13369 (catalog number: DA-801-1003) platform. When downloading GSE41929 data online, it was found that there were a total of 6 chip samples. The samples were defined by “Define groups” and divided into a

control group and a bortezomib treatment group. **Figure 1** shows the changes in expression levels of all samples in a box plot. Then, the GEO2R analysis was performed, and the data were saved. Differential gene expression screening was performed based on  $P < 0.05$  and the absolute value of  $\log_{2}FC \geq 1.5$  times. A total of 126 differential genes were screened out. The top 10 genes with significantly upregulated expression and the top 10 genes with significantly downregulated expression were shown in **Tables 1** and **2**, respectively.



**Figure 1.** Box plot showed expression changes across all samples in the GSE41929 series

**Table 1.** Significantly upregulated genes

GB_ACC	Gene	<i>P</i> value	logFC value
NM_002133.1	HMOX1	6.63E-08	5.55846593
NM_005346.3	HSPA1B	6.76E-05	4.58144941
NM_002155.3	HSPA6	1.36E-02	4.43704114
NM_006145.1	DNAJB1	1.40E-04	3.89714309
NM_002228.3	JUN	3.01E-04	3.71489188
NM_004281.3	BAG3	2.35E-04	3.65778646
NM_182491.1	ZFAND2A	4.61E-04	3.62264090
NM_014330.2	PPP1R15A	8.92E-05	3.10443174
NM_001040619.1	ATF3	4.87E-04	3.06525544
NR_004400.1	RNU1-5	1.02E-04	3.02232600

Note: GB\_ACC stands for gene bank accession number

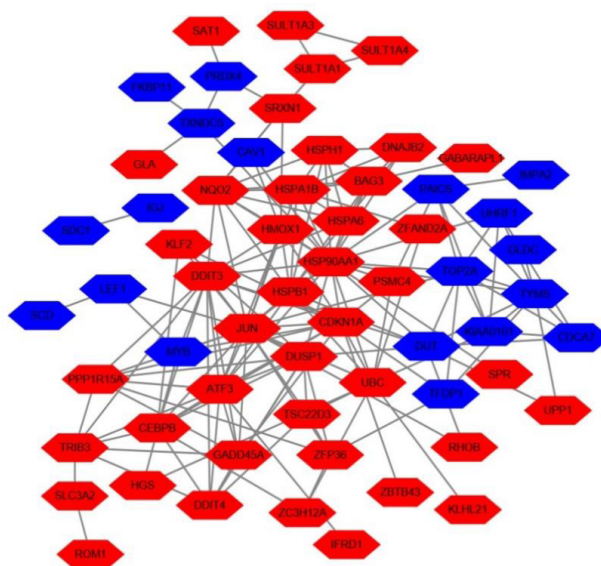
**Table 2.** Top 10 significantly downregulated genes

GB_ACC	Gene	P value	logFC value
NM_024665.3	TBL1XR1	3.17E-02	-1.00003988
NM_144772.1	APOA1BP	6.99E-03	-1.00117889
NM_001110.2	ADAM10	3.18E-03	-1.00742746
NM_003200.1	TCF3	7.70E-04	-1.00825865
NM_001031684.1	SFRS7	1.32E-03	-1.01020377
NM_018079.3	SRBD1	4.70E-02	-1.01112345
NM_005916.3	MCM7	7.62E-03	-1.01404815
NM_006290.2	TNFAIP3	1.28E-03	-1.01417679
NM_004219.2	PTTG1	1.60E-03	-1.01583864
NM_024758.3	AGMAT	4.84E-02	-1.01685617

Note: GB\_ACC stands for gene bank accession number

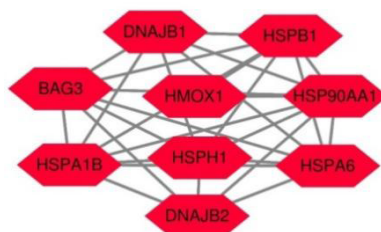
### 3.2. Protein-protein interaction network and hub genes

STRING is an online tool for retrieving gene-protein interaction relationships, which predicts potential interactions between proteins or genes. As shown in **Figure 2**, the protein-protein interaction (PPI) network of differentially expressed genes from the GSE dataset analyzed in this study was presented. The PPI network was imported into Cytoscape (v3.7.2) for visualization enhancement, where upregulated genes were highlighted in red and downregulated genes in blue. To further elucidate the core module among the differentially expressed genes, the MCODE plugin in Cytoscape was employed. The algorithm parameters were set as follows: Degree cutoff = 2, K-score = 2, Node Score Cutoff = 0.2. Then select Cluster Finding Haircut to calculate and analyze the weighted reconnection between network structures and nodes, and screen out the most significant modules, namely the regions where the Hub genes are located (**Figure 3**). The hub genes network includes 9 nodes and 28 pairs of edges. The functional annotations of each Hub gene are shown in **Table 3**.



**Figure 2.** PPI network of differentially expressed genes (red: upregulated genes; blue: downregulated genes)

Following differential expression analysis of the GSE41929 mRNA expression dataset, genes meeting the criteria of absolute value of  $\log FC \geq 1.5$  and  $P$  value  $< 0.05$  were selected for protein-protein interaction (PPI) network construction. Red markers indicate upregulated genes, while blue markers indicate downregulated genes.



**Figure 3.** The most significant module

This module is obtained from a PPI network with 9 nodes and 28 pairs of edges.

**Table 3.** Functional annotations of hub genes

Gene	Full name	Function
HMOX1	Heme oxygenase 1	Heme oxygenase is an essential enzyme in heme catabolism, and excessive free heme can cause cell apoptosis.
HSPA1B	Heat shock protein family A member 1B	HSPA1B is a member of the HSP70 family. After binding with other heat shock proteins, this protein stabilizes existing proteins to prevent aggregation and participates in the ubiquitin proteasome pathway.
HSPA6	Heat shock 70 kDa protein 6	It involves multiple cellular processes, including protecting proteins from stress, folding and transporting newly synthesized peptides, hydrolysis and activation of misfolded proteins, and formation and dissociation of protein complexes. Plays a crucial role in ensuring correct protein folding, refolding misfolded proteins, and controlling protein targeting for subsequent degradation.
DNAJB1	DnaJ homolog subfamily B member 1	Interacts with HSP70 and can stimulate its ATPase activity. Negative regulation of heat shock induced HSF1 transcriptional activity during the attenuation and recovery stages of heat shock response.
BAG3	BAG family molecular chaperone regulator 3	HSP70 and HSC70 chaperone protein molecules. Acting as a nucleotide exchange factor (NEF), it promotes the release of ADP from HSP70 and HSC70 proteins, thereby triggering the release of client/substrate proteins.
DNAJB2	DnaJ homolog subfamily B member 2	Acting as a partner molecule, regulating substrate binding and activating ATPase activity of HSP70/heat shock protein 70 family partners. Meanwhile, it also contributes to the ubiquitin-dependent proteasome degradation of misfolded proteins.
HSPB1	Heat shock protein beta-1	Small-molecule heat shock proteins act as molecular chaperones and may keep denatured proteins in a folded state. Through its molecular chaperone activity, many biological processes can be regulated, including phosphorylation of neurofilament proteins and axonal transport.
HSP90AA1	Heat shock protein HSP 90-alpha	It can dynamically interact with various auxiliary partners to promote the maturation and structural maintenance of specific target proteins. Participate in cell cycle control and signal transduction.
HSPH1	Heat shock protein 105 kDa	NEF acts as a partner protein for HSPA1A and HSPA1B, promoting the release of ADP from HSPA1A/B and triggering the release of client/substrate proteins.

## 4. Discussion

Multiple myeloma (MM) is a malignant plasma cell tumor characterized by the clonal proliferation of plasma

cells in the bone marrow. In the past decade, the introduction of new drugs such as proteasome inhibitors and immunomodulators has extended the survival of MM patients. Proteasome inhibition is an important therapeutic target for MM. The US FDA approved the first-generation proteasome inhibitor bortezomib as a first-line treatment for MM in June 2008. Although newly diagnosed MM patients have a very high overall response rate to bortezomib<sup>[16]</sup>, most patients eventually relapse because MM cells develop resistance to treatment<sup>[9,10]</sup>.

Heat shock proteins (HSPs) are a class of proteins that protect cells from stress. In mammalian cells, HSPs can be classified into HSP100, HSP90, HSP70, HSP60, and HSP27 based on their molecular weight. As tumors progress, tumor cells rely on HSPs for “adaptive survival.” It has been reported that HSPs contribute to the drug resistance of MM. After stimulation with bortezomib, the expression of *HSP90* and *HSP27* in MM cells was significantly upregulated<sup>[17,18]</sup>. However, the clinical application of HSP90 inhibitors has been hindered due to their drug toxicity and other reasons. There are also a few preclinical research experiments using HSP27 inhibitors for MM. Therefore, scholars have gradually paid attention to the important changes of *HSP70* in the development of tumors.

The HSP70 family is the most conserved, predominant, and abundant type of protein in HSP. The HSP70 family consists of five important members, including cytosolic HSP70 (HSPA1A or HSP72) and HSC70 (HSPA8), cell surface HSP70-2 (HSPA1B), mitochondrial GRP78/mortalin (HSPA9), and GRP78 (HSPA5), among which GRP78 is mainly localized in the endoplasmic reticulum (ER). Research has found that *HSP70* is highly expressed in various cancers and is associated with tumor grading, metastasis, prognosis, and drug resistance<sup>[19,20]</sup>.

This study investigated the expression profile of GSE41929 gene chip in GEO database, screened the differential gene expression changes between bortezomib treatment group and non-bortezomib treatment group in MM cells, and found that after bortezomib treatment, the expression changes of 126 genes in MM cells were the most significant ( $P$  value < 0.05, absolute value of logFC  $\geq$  1.5 times). According to the expression fold and combined with the most important gene module, it can be seen that besides *HMOX1*, *HSPA1B* is upregulated the most significantly, followed by *HSPA6*, *DNAJB1*. *HSPA1B* and *HSPA6* are both members of the HSP70 protein family. The main function of *DNAJB1* is to interact with HSP70 molecules and stimulate their ATPase activity, negatively regulating heat shock-induced HSF1 transcriptional activity.

By analyzing the expression profile of the GSE41929 gene chip, it can be seen that after bortezomib stimulation of MM cells, the upregulation of *HSP70* is most significant. *HSP90*, *HSPB1*, and *HSPH1* are also upregulated to varying degrees, but none of them is as significantly upregulated as *HSP70*. This study suggests that HSP70 may be an important HSP that promotes MM resistance, and further attention and development of HSP70 inhibitors are needed to enhance the anti-MM effect of bortezomib.

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## Disclosure statement

The authors declare no conflict of interest.



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# Advances in Biomimetic Nanotechnology for Triple-Negative Breast Cancer Therapy

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**Abstract:** This article systematically reviews the application of biomimetic nanotechnology in targeted therapy for triple-negative breast cancer (TNBC). TNBC poses significant challenges for conventional treatments due to the lack of defined therapeutic targets, chemotherapy resistance, and a complex immunosuppressive microenvironment. Biomimetic nanotechnology, by mimicking the functional properties of biological structures (e.g., cell membranes, exosomes), has significantly enhanced drug delivery efficiency, targeting precision, and anti-tumor immune responses. This review focuses on the design strategies of biomimetic nanocarriers (including cell membrane-coated nanoparticles, engineered exosomes, and biomimetic synthetic materials) and their innovative applications in TNBC therapy: (1) Targeted delivery systems that overcome tumor barriers and reduce systemic toxicity; (2) Photothermal therapy combined with immunomodulation for precise treatment and immune activation; (3) Tumor microenvironment regulation (e.g., vascular normalization, pH neutralization, immunosuppression reversal). Studies demonstrate that biomimetic nanotechnology significantly improves TNBC treatment efficacy through multimodal synergistic mechanisms (e.g., chemo-photothermal-immunotherapy). However, challenges such as scalable production, long-term safety, and personalized adaptation remain for clinical translation. Future research should integrate artificial intelligence for optimized design and dynamic imaging technologies to advance biomimetic nanomedicines toward clinical applications.

**Keywords:** Biomimetic nanotechnology; Triple-negative breast cancer; Targeted therapy; Photothermal therapy; Immunomodulation; Tumor microenvironment

**Online publication:** June 5, 2025

## 1. Introduction

Triple-negative breast cancer (TNBC) is a highly aggressive breast cancer subtype characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression<sup>[1]</sup>. Due to the lack of actionable therapeutic targets, TNBC management primarily relies on chemotherapy (e.g., anthracyclines and taxanes), which is limited by drug resistance, tumor heterogeneity, and

frequent recurrence<sup>[2]</sup>. Molecular heterogeneity further subdivides TNBC into basal-like, mesenchymal, and immunomodulatory subtypes, each exhibiting distinct therapeutic responses, complicating precision treatment<sup>[3]</sup>. Additionally, the immunosuppressive tumor microenvironment (TME) in TNBC, driven by PD-L1 upregulation and regulatory T cell (Treg) recruitment, further diminishes anti-tumor immunity<sup>[2]</sup>. While immune checkpoint inhibitors (e.g., anti-PD-1/PD-L1 antibodies) show promise in some TNBC patients, their efficacy is hindered by dynamic TME regulation and inefficient drug delivery<sup>[1]</sup>. Thus, novel strategies to overcome heterogeneity, remodel the TME, and penetrate biological barriers are urgently needed.

Biomimetic nanotechnology, inspired by natural biological structures (e.g., cell membranes, exosomes, or pathogens), offers transformative solutions for TNBC. Core design strategies include coating synthetic nanoparticles with cell membranes (e.g., red blood cells, cancer cells, or immune cells) to confer immune evasion, prolonged circulation, and active targeting<sup>[4]</sup>. For instance, macrophage membrane-coated nanocarriers exploit chemokine receptors (e.g., CCR2) to target tumor-associated inflammatory sites while co-delivering chemotherapeutics and immunomodulators to reverse immunosuppression<sup>[5]</sup>. Hybrid membrane technology (e.g., fusing red blood cell and cancer cell membranes) integrates multiple functionalities: CD47-mediated immune evasion, homologous targeting for tumor accumulation, and stimuli-responsive drug release<sup>[4]</sup>. Compared to conventional nanocarriers, biomimetic systems reduce off-target toxicity and enhance interactions with host biology, such as crossing the blood-brain barrier or inducing immunogenic cell death<sup>[6]</sup>. These advantages position biomimetic nanotechnology as a powerful tool to address chemotherapy resistance, metastasis, and theranostic integration<sup>[7]</sup>.

This review summarizes the design strategies of biomimetic nanomedicines, current applications of biomimetic nanotechnology, and its role in TNBC therapy, aiming to explore its clinical potential and guide the development of next-generation TNBC treatments.

## **2. Design strategies of biomimetic nanocarriers**

### **2.1. Cell membrane-coated nanoparticles**

Cell membrane-coated nanoparticles (CNPs) encapsulate synthetic nanoparticles with natural cell membranes (e.g., tumor cells, macrophages, or leukocytes), inheriting the source cells' surface antigens and targeting capabilities. Tumor cell membrane-coated nanoparticles achieve homologous targeting for precise tumor localization<sup>[8]</sup>. Macrophage membrane-coated nanoparticles leverage inflammation targeting and immune evasion to penetrate physiological barriers and evade clearance<sup>[9]</sup>. Leukocyte membrane-coated nanoparticles mimic leukocyte rolling and adhesion, enhancing tumor vascular retention<sup>[10]</sup>. Recent advances include genetic engineering of membranes (e.g., overexpressing calreticulin to enhance antigen presentation) to synergize with checkpoint inhibitors<sup>[11]</sup>. This hybrid “natural-synthetic” strategy improves targeting efficiency and reduces immunogenicity, offering new avenues for precision drug delivery<sup>[12]</sup>.

### **2.2. Exosomes and extracellular vesicles**

Exosomes are naturally secreted nanovesicles (30–150 nm) with lipid bilayers, capable of carrying proteins, nucleic acids, and drugs while mediating intercellular communication. Their advantages include low immunogenicity, biocompatibility, and intrinsic targeting. Tumor-derived exosomes utilize integrins for organ-specific metastasis, serving as anti-metastatic carriers<sup>[13]</sup>. Exosome-encapsulated siRNA effectively silences S100A4, inhibiting

premetastatic niche formation in breast cancer<sup>[14]</sup>. Engineered exosomes enhance targeting via ligand conjugation (e.g., folate) or membrane protein fusion. For example, mesoporous silica nanoparticles combined with exosomes deliver chemotherapeutics to reverse cancer stem cell-driven epithelial-mesenchymal transition (EMT)<sup>[15]</sup>. Exosomes also serve as multifunctional carriers for photodynamic therapy or immunomodulators, enabling synergistic anti-tumor effects<sup>[16]</sup>.

### 2.3. Biomimetic synthetic materials

Biomimetic synthetic materials mimic natural biomolecules for stimuli-responsive and dynamic targeting. Hyaluronic acid (HA)-modified nanoparticles target CD44-overexpressing tumor cells and release drugs via enzymatic degradation in acidic microenvironments<sup>[17]</sup>. Peptide-based supramolecular materials undergo structural rearrangement in response to TME cues (e.g., low pH, ROS) for controlled drug release<sup>[18]</sup>. Stimuli-responsive polymers (e.g., pH-sensitive ZIF-8) encapsulate glucose oxidase (GOx) and hemin to amplify ROS generation, enhancing immunogenic cell death (ICD)<sup>[19]</sup>. Prussian blue nanocomposites combine chemotherapy and photothermal therapy to induce pyroptosis and activate anti-tumor immunity<sup>[20]</sup>. These smart materials address limitations of traditional nanocarriers<sup>[21]</sup>.

## 3. Applications of biomimetic nanotechnology in cancer therapy

### 3.1. Photothermal therapy (PTT) and biomimetic nanotechnology

PTT employs near-infrared light to activate nanoparticles for localized hyperthermia. Biomimetic designs enhance PTT precision and efficacy. For example, Wu *et al.* developed erythrocyte membrane-coated nanocrystals (AE@RBC/Fe NCs) containing aloe-emodin and ferritin. This system prolongs circulation, induces ferroptosis via Fe<sup>3+</sup> release, and activates anti-tumor immunity, achieving 90% tumor suppression in breast cancer models<sup>[22]</sup>. Li *et al.* designed platelet membrane-coated Prussian blue nanocomposites (PB/PM/HRP/Apt) that target tumor sites, enhance penetration, and release PD-L1 aptamers under photothermal activation, suppressing primary and metastatic tumors<sup>[23]</sup>. Biomimetic membranes (e.g., tumor or leukocyte membranes) reduce immune clearance and improve tumor accumulation<sup>[24]</sup>.

### 3.2. Biomimetic delivery systems for chemo-immunotherapy

Biomimetic nanocarriers enhance chemotherapy targeting and TME modulation. Geng *et al.* constructed mesenchymal stem cell (MSC) membrane-coated nanoparticles to deliver metronidazole and doxorubicin, eliminating intratumoral *Fusobacterium nucleatum* and synergizing with PD-L1 inhibitors to prolong survival in 4T1 models<sup>[25]</sup>. Zhang *et al.* developed tumor cell membrane-lipid hybrid nanoparticles (CLip-PC@CO-LC NPs) for spatiotemporal co-delivery of docetaxel and siRNA, suppressing non-small cell lung cancer growth<sup>[26]</sup>. Xiao *et al.* engineered anti-PD-L1 antibody-conjugated gold nanostars (PDA/GNS@aPD-L1 NPs) that combine photothermal ablation with immune checkpoint blockade, enhancing CD8<sup>+</sup> T cell infiltration in colorectal cancer<sup>[27]</sup>.

### 3.3. Innovative combination strategies and clinical translation

Multimodal therapies integrate diverse mechanisms to overcome treatment limitations. Wang *et al.* designed lactoferrin/albumin-coated nanoparticles (Alb/LF NPs) loaded with copper/iron diethyldithiocarbamate to induce ferroptosis and metalloimmunity in gliomas<sup>[28]</sup>. Cao *et al.* developed MSC membrane-camouflaged black

phosphorus nanosheets (BP NSs) for photothermal-chemotherapy, delaying BP degradation and enhancing tumor accumulation <sup>[29]</sup>. Challenges in clinical translation include scalable production, long-term safety, and personalized design. For instance, platelet membrane-coated nanoparticles (PNP-R848) achieved complete tumor regression in colorectal cancer models, highlighting clinical potential <sup>[30,31]</sup>.

## 4. Biomimetic nanotechnology in TNBC therapy

### 4.1. Innovations in targeted delivery systems

Biomimetic nanocarriers enable precise drug release and enhanced tumor accumulation. Bhullar *et al.* engineered exosomes co-loaded with Survivin siRNA, gemcitabine, and paclitaxel, achieving tumor-specific delivery via CD44 aptamers and reducing systemic toxicity in TNBC models <sup>[32]</sup>. Chowdhury *et al.* utilized neutrophil membrane-coated nanoparticles (PVT-NEU NPs) to enhance paclitaxel delivery, increasing tumor cell uptake by 2.3-fold and improving suppression rates by 40% <sup>[33]</sup>. Zhang *et al.* developed macrophage membrane-coated magnetic nanoparticles (MMNPs) targeting CD163 to promote M1 polarization and immune activation <sup>[34]</sup>.

### 4.2. Integration of photothermal and immunotherapy

Biomimetic platforms combine photothermal materials (e.g., gold nanoparticles) with immunomodulators for synergistic effects. Liu *et al.* designed platelet membrane-coated silver metal-organic frameworks (PM@MOF-Ag NPs) that induce ROS-mediated apoptosis and enhance Bax/Bcl-2 ratios in TNBC <sup>[35]</sup>. Jiang *et al.* developed gadolinium-doped carbon dots (Gd@CDs) for MRI-guided photothermal-chemotherapy, suppressing 4T1 tumor growth and metastasis <sup>[36]</sup>. Wang *et al.* conjugated IR792 photosensitizers and PD-L1 antibodies to silica nanoshells, boosting CD8<sup>+</sup> T cell infiltration and survival in TNBC models <sup>[37]</sup>.

### 4.3. Tumor microenvironment (TME) modulation

Biomimetic nanotechnology regulates TME components to improve treatment outcomes. Gong *et al.* developed ternary heterostructure nanoparticles (AZG) that generate ROS under near-infrared light, inducing apoptosis and angiogenesis inhibition in MDA-MB-231 models <sup>[38]</sup>. Li *et al.* designed hyaluronic acid-functionalized hydrogels delivering VEGF siRNA and CCL2 inhibitors to reduce M2 macrophages and vascularization in TNBC <sup>[39]</sup>.

## 5. Conclusion and perspectives

Biomimetic nanotechnology offers unique advantages for TNBC therapy: Targeted delivery reduces systemic toxicity; photothermal-immunotherapy overcomes heterogeneity and immune tolerance <sup>[40,41]</sup>; TME modulation transforms “cold” tumors. However, clinical translation requires addressing scalable production, long-term safety, and personalized design. Future research should integrate AI-optimized materials, multifunctional systems, and advanced imaging. With advancements in single-cell sequencing and dynamic imaging, personalized biomimetic nanotherapies may become the cornerstone of TNBC precision medicine.

## Disclosure statement

The authors declare no conflict of interest.



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# Analysis of the Mediating Effect of Psychological Flexibility on Death Anxiety and Quality of Life in Cancer Patients

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**Abstract:** *Objective:* To analyze the mediating effect of psychological flexibility between death anxiety and quality of life in cancer patients. *Methods:* A convenience sampling method was used to select cancer patients who received treatment at our hospital from January 2022 to January 2024, by the inclusion and exclusion criteria. General information, psychological flexibility, death anxiety, and quality of life scores were collected for analysis. *Result:* The psychological flexibility and quality of life scores of cancer patients with an annual family income  $\leq 100,000$  RMB were significantly lower than those of cancer patients with an annual family income  $> 100,000$  RMB ( $P < 0.05$ ), while the death anxiety scores were significantly lower for the former group as well ( $P < 0.05$ ). Cancer patients staged as I-II had significantly higher psychological flexibility and quality of life scores than those staged as III-IV ( $P < 0.05$ ), while their death anxiety scores were significantly lower ( $P < 0.05$ ). Psychological flexibility in cancer patients was negatively correlated with death anxiety ( $r = -0.614$ ,  $P < 0.05$ ) and positively correlated with quality of life ( $r = 0.628$ ,  $P < 0.05$ ), while death anxiety was negatively correlated with quality of life ( $r = -0.112$ ,  $P < 0.05$ ). The direct effect of death anxiety on quality of life was  $-0.232$ , accounting for 58.32% of the total effect. The mediating effect of psychological flexibility between death anxiety and quality of life was  $-0.218$ , accounting for 41.83% of the total effect. *Conclusion:* Death anxiety can directly affect the quality of life of cancer patients, and it can also indirectly affect the quality of life through psychological flexibility. Clinicians should promptly address patients' death anxiety and provide interventions to enhance psychological flexibility, thereby improving the quality of life.

**Keywords:** Psychological flexibility; Cancer patients; Death anxiety; Quality of life; Mediating effect

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## 1. Introduction

According to the latest data from the International Agency for Research on Cancer <sup>[1]</sup>, there were approximately 19.29 million new cancer cases worldwide in 2020, with breast cancer ranking first at 2.26 million cases. With

the continuous advancement of early screening methods and modern diagnostic and treatment technologies, cancer patients' survival rates have been steadily increasing<sup>[2]</sup>. Death anxiety is a psychological state or reaction that individuals experience when facing major threats such as death. It can be either conscious or unconscious. Death anxiety persists throughout the entire course of malignant tumors, significantly affecting patients' quality of life and both physical and mental health<sup>[3]</sup>. Psychological flexibility is closely linked to quality of life and other positive outcomes. Studies have shown that good psychological flexibility can significantly improve anxiety, depression, social functioning, and quality of life in patients with chronic pain. Therefore, this study analyzes the mediating effect of psychological flexibility on the relationship between death anxiety and quality of life in cancer patients, providing valuable clinical insights.

## **2. Data and methods**

### **2.1. General information**

A convenience sampling method was used to select cancer patients who received treatment at our hospital from January 2022 to January 2024, according to inclusion and exclusion criteria. This study has been approved by the Ethics Committee and strictly follows ethical principles in medical research and clinical trial regulations. Previous literature suggests that the sample size for structural equation modeling should be no less than 220 cases<sup>[4]</sup>. Therefore, a total of 300 questionnaires were distributed, and 277 valid questionnaires were collected, with a valid response rate of 92.33%.

### **2.2. Inclusion and exclusion criteria**

Inclusion criteria: (1) Patients aged  $\geq 18$  years; (2) Patients who voluntarily participate in this questionnaire survey; (3) Patients who can communicate normally; (4) Patients with stable conditions; (5) Patients diagnosed with malignant tumors through pathology and hospitalized.

Exclusion criteria: (1) Patients with combined cognitive impairments; (2) Patients with mental disorders.

### **2.3. Survey methods**

The survey collection work is completed personally by the researcher. First, the researcher contacts the relevant departments and responsible individuals, and after obtaining consent, they enter the department. The researcher reviews patient medical records to collect basic information and further determines if the patient meets the inclusion criteria for the study. After completing the information collection, the researcher enters the inpatient department to find eligible patients and their spouses for the survey. The online questionnaire is distributed via Wenjuanxing, and the researcher provides timely explanations of any unclear items and instructions on completing the survey. This ensures the smooth progress of the survey, and once the questionnaires are completed, they are collected. The survey collection work is conducted during the patients' untreated periods, and in an environment with relatively few fellow patients, to minimize any external factors that might influence the results of the survey filled out by the patients and their spouses.

### **2.4. Data collection**

#### **2.4.1. General information**

After reviewing the literature and consulting experts, a self-designed general information questionnaire was



created, including age, gender, educational level, marital status, annual household income, tumor location, and tumor staging.

#### **2.4.2. Psychological flexibility**

The psychological flexibility of patients in this study was assessed using the Commitment Action Questionnaire (CAQ) <sup>[5]</sup>. This questionnaire consists of two dimensions: a positive dimension and a negative dimension. It contains a total of eight items, rated on a Likert scale from 0 to 6, with a maximum score of 48 points. Higher scores indicate greater psychological flexibility, while lower scores suggest reduced flexibility.

#### **2.4.3. Death anxiety (T-DAS)**

The death anxiety of the patients in this study was assessed using the Death Anxiety Scale <sup>[6]</sup>. The questionnaire consists of 4 dimensions with a total of 15 items, scored as 'yes' or 'no,' with 1 point for 'yes' and 0 points for 'no.' The total score is 15 points. A higher score indicates greater death anxiety, and a score of 7 or more indicates the presence of death anxiety in the patient.

#### **2.4.4. Quality of life (SF-36)**

The SF-36 scale is used to assess patients' quality of life before and after intervention <sup>[7]</sup>. This scale consists of 9 dimensions with a total of 36 items. Most items are rated using a Likert scale from 1 to 5, while a few are scored on a 2-level or 3-level scale. After recording the scores, they are converted proportionally into standardized scores on a 100-point scale. Higher scores indicate a better quality of life, whereas lower scores suggest a poorer quality of life.

### **2.5. Statistical methods**

All the collected data were entered into SPSS 25.0 software for statistical analysis. For categorical data, the recording method was by frequency and percentage, and analysis was performed using  $\chi^2$  tests and other methods. For continuous data, the recording method was by mean and standard deviation, and analysis was conducted using t-tests. Pearson correlation analysis and structural equation modeling were used to analyze correlations and interactions. A *P*-value of  $< 0.05$  was considered statistically significant.

## **3. Results**

### **3.1. Psychological flexibility, death anxiety, and quality of life scores in cancer patients under different factors**

**Table 1** shows the analysis of psychological flexibility, death anxiety, and quality of life in cancer patients of different ages, genders, education levels, marital statuses, annual family incomes, tumor locations, and cancer stages reveals significant differences. The results show that psychological flexibility, death anxiety, and quality of life scores differ significantly based on family income and cancer stage ( $P < 0.05$ ). Cancer patients with an annual family income  $\leq 100,000$  yuan have significantly lower psychological flexibility and quality of life scores compared to those with an income  $> 100,000$  yuan, while their death anxiety scores are significantly lower than those with an income  $> 100,000$  yuan. Cancer patients with stage I-II tumors have significantly higher psychological flexibility and quality of life scores than those with stage III-IV tumors, while their death anxiety

scores are significantly lower than those with stage III-IV tumors.

**Table 1.** Psychological flexibility, death anxiety, and quality of life scores in cancer patients under different factors

Variable	Group	<i>n</i>	CAQ	T-DAS	SF-36
Age	≤ 40	65	12.62 ± 3.28	7.33 ± 1.22	73.56 ± 5.33
	40–60	110	12.77 ± 3.11	7.45 ± 1.93	74.96 ± 5.22
	≥ 60	102	12.31 ± 3.56	7.31 ± 1.98	74.69 ± 5.19
<i>F</i>			0.297	0.447	1.676
<i>P</i>			0.766	0.655	0.096
Gender	Man	160	12.74 ± 3.55	7.49 ± 1.87	74.56 ± 5.19
	Woman	117	12.40 ± 3.42	7.23 ± 1.75	74.24 ± 5.95
<i>t</i>			0.800	1.175	0.476
<i>P</i>			0.425	0.241	0.634
Educational level	High school and below	157	12.63 ± 3.06	7.39 ± 1.59	74.73 ± 5.35
	College or above	120	12.51 ± 3.84	7.33 ± 1.37	74.07 ± 5.44
<i>t</i>			0.289	0.330	1.010
<i>P</i>			0.772	0.742	0.313
Marital status	Married	197	12.77 ± 3.33	7.36 ± 1.85	73.79 ± 5.31
	Unmarried or widowed	80	12.37 ± 3.82	7.28 ± 1.71	75.01 ± 5.19
<i>t</i>			0.868	0.333	1.744
<i>P</i>			0.386	0.739	0.082
Annual household income	≤ 100,000 yuan	169	11.13 ± 3.68	7.87 ± 1.85	70.25 ± 5.22
	> 100,000 yuan	108	14.01 ± 4.11	6.85 ± 1.49	78.55 ± 5.43
<i>t</i>			6.067	4.817	12.706
<i>P</i>			0.000*	0.000*	0.000*
Tumor site	Head and neck	52	12.63 ± 3.05	7.45 ± 1.85	74.65 ± 5.55
	Mammary gland	32	12.62 ± 3.11	7.39 ± 1.42	73.16 ± 5.37
	Respiratory system	61	12.55 ± 3.56	7.43 ± 1.33	74.44 ± 5.49
	Digestive system	48	12.56 ± 3.78	7.38 ± 1.19	74.19 ± 5.29
	Urinary system	11	12.61 ± 3.46	7.31 ± 1.09	74.95 ± 5.11
	Other	73	12.45 ± 1.85	7.32 ± 1.22	75.01 ± 5.06
<i>F</i>			0.017	0.195	1.248
<i>P</i>			0.986	0.846	0.215
Tumor staging	I-II	196	10.22 ± 3.11	7.91 ± 1.72	76.24 ± 5.44
	III-IV	81	14.92 ± 3.99	6.81 ± 1.26	72.56 ± 5.62
<i>t</i>			10.497	5.205	5.072
<i>P</i>			0.000*	0.000*	0.000*

### 3.2. Analysis of the relationship between psychological flexibility, death anxiety, and quality of life in cancer patients

**Table 2** shows that the psychological flexibility of cancer patients is negatively correlated with death anxiety ( $r = -0.614$ ,  $P < 0.05$ ) and positively correlated with quality of life ( $r = 0.628$ ,  $P < 0.05$ ). Death anxiety in cancer patients is negatively correlated with quality of life ( $r = -0.112$ ,  $P < 0.05$ ).

**Table 2.** Analysis of the relationship between psychological flexibility, death anxiety, and quality of life in cancer patients

	CAQ	T-DAS	SF-36
CAQ	1		
T-DAS	-0.614*	1	
SF-36	0.628*	-0.112*	1

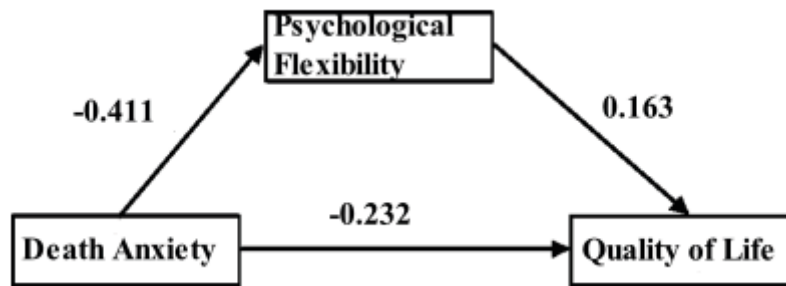
Note: \* is  $P < 0.05$ .

### 3.3. Analysis of the mediating effect of psychological flexibility on the relationship between death anxiety and quality of life in cancer patients

To further analyze the mediating effect of psychological flexibility between death anxiety and quality of life in cancer patients, this study employed the Bootstrap method in SPSS to examine the mediating effect of psychological flexibility on death anxiety and quality of life. A 95% confidence interval (CI) that includes 0 indicates a significant mediating effect, while an interval that does not include 0 indicates a non-significant mediating effect. The results of this study show that, with death anxiety as the independent variable, psychological flexibility as the mediating variable, and quality of life as the dependent variable, there is a significant negative correlation between psychological flexibility and death anxiety, and a significant positive correlation between psychological flexibility and quality of life. The direct effect of death anxiety on quality of life is -0.232, with a 95% CI that does not include 0, accounting for 58.32% of the total effect. The mediating effect of psychological flexibility between death anxiety and quality of life is -0.218, with a 95% CI that does not include 0, confirming the mediating role of psychological flexibility, which accounts for 41.83% of the total effect, indicating a partial mediating effect shown in **Table 3** and **Figure 1**.

**Table 3.** Analysis of the mediating effect of psychological flexibility on the relationship between death anxiety and quality of life in cancer patients

Project	Effect value	Standard error	95% CI		Effect proportion (%)
			Lower limit	Upper limit	
Total effect	-0.322	0.037	-0.368	-0.136	100
Direct effect (death anxiety → quality of life)	-0.232	0.053	-0.433	-0.136	58.32
Indirect effects (death anxiety → psychological flexibility → quality of life)	-0.218	0.028	-0.293	-0.082	41.83



**Figure 1.** Path diagram of the mediating effect of psychological flexibility of cancer patients between death anxiety and quality of life

## 4. Discussion

The torment of cancer and the constant threat of death cause most cancer patients to lose confidence and hope in life and treatment, severely affecting their quality of life. Death anxiety, as a negative psychological state directly linked to death, persists throughout the entire course of a cancer patient's illness. A high level of death anxiety can immerse patients in negative emotions of fearing impending death, thereby impairing their coping abilities and severely damaging their physical and mental health, ultimately affecting their prognosis. Psychological flexibility is closely related to mental health and plays a crucial mediating role. It is of great significance to an individual's daily well-being and long-term psychological health.

In this study, the average death anxiety score of cancer patients was  $(7.36 \pm 1.42)$ , indicating the presence of death anxiety. After the cancer diagnosis, patients not only have to endure the physical discomfort caused by the disease itself and the trauma of surgery, but also face the looming threat of death. The average score for psychological flexibility was  $(12.57 \pm 1.66)$ , which is relatively low compared to Akerblom's study<sup>[8]</sup>, possibly due to differences in the research subjects. Akerblom's study focused on patients with chronic pain, whereas the subjects of this study were cancer patients, who, due to the impact of their condition, generally exhibit lower psychological flexibility.

The results of this study also show that there are significant differences in psychological flexibility, death anxiety, and quality of life scores among cancer patients with different household annual incomes and tumor stages ( $P < 0.05$ ). After being diagnosed with cancer, patients experience stress due to the need for treatment and ongoing maintenance costs. Unemployment or reduced income further intensifies this pressure. In addition to enduring the suffering of the disease, patients also face the economic burden of medical expenses, which can, to some extent, affect their decision-making behaviors. Patients with more advanced tumor stages bear greater treatment and disease-related pressures, and the impact on psychological flexibility, death anxiety, and quality of life scores is also more significant for those at later stages<sup>[9]</sup>.

Psychological flexibility is negatively correlated with death anxiety and positively correlated with quality of life, while death anxiety is negatively correlated with quality of life ( $r = -0.112$ ,  $P < 0.05$ ). Death anxiety can directly impact the quality of life in cancer patients and can also indirectly affect their quality of life through psychological flexibility. When cancer patients face death anxiety, they often adopt avoidance strategies, unwilling to accept the reality of their illness, excessively dwelling on their past healthy state, which leads to psychological rigidity and a decrease in psychological flexibility<sup>[10,11]</sup>. Additionally, during treatment, cancer patients often worry

that the poor prognosis of the disease will impose a serious burden on their families, leading to feelings of guilt and shame. This negative psychological state can be magnified as the disease progresses, draining their positive psychological energy and causing the emergence of inflexible behavioral patterns <sup>[12]</sup>.

When cancer patients attempt to reduce negative emotions such as anxiety and depression by avoiding them, they become more likely to recall painful experiences. Individuals with higher levels of psychological flexibility tend to adopt an open and accepting coping approach <sup>[13]</sup>. This approach can help patients clarify their life values and goals, develop practical plans guided by the right values, take effective actions, actively engage in self-management, and ultimately improve their quality of life <sup>[14–17]</sup>.

Healthcare professionals should use psychological flexibility as a starting point, constructing intervention strategies based on cognitive behavioral therapy, acceptance and commitment therapy, and other methods <sup>[18]</sup>. These strategies aim to strengthen death education for patients and their families, correct misconceptions about cancer and death, encourage patients to express negative emotions, and motivate them to adopt positive coping strategies, ultimately improving their quality of life <sup>[19,20]</sup>.

## 5. Conclusion

Death anxiety can directly affect the quality of life of cancer patients, and it can also indirectly affect the quality of life through psychological flexibility. Clinicians should promptly address patients' death anxiety and provide interventions to enhance psychological flexibility, thereby improving the quality of life.

## Disclosure statement

The authors declare no conflict of interest.

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# Research Progress on Integrated Traditional Chinese and Western Medicine Therapy for Malignant Tumors

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**Abstract:** The integration of Chinese and Western medicine in the treatment of malignant tumors is becoming increasingly widespread. By combining modern medical technology with traditional Chinese medicine, this approach enhances therapeutic efficacy while reducing side effects. This paper reviews the principles and mechanisms of integrated therapy and analyzes its clinical applications and advantages. Studies indicate that this approach is effective in treating common malignancies such as lung, stomach, and liver cancer, especially in slowing tumor progression, relieving symptoms, and improving patients' quality of life. Chemotherapy combined with Chinese medicine has shown positive effects on survival rates and immune function. However, limitations remain, including insufficient clinical trial data and differences in efficacy across different cancer types, necessitating further high-quality studies. Overall, integrated Chinese and Western medicine offers advantages such as reduced side effects, improved survival rates, and enhanced immune function, providing a comprehensive treatment strategy and a theoretical foundation for its clinical application.

**Keywords:** Chinese and Western integrative therapy; Malignant tumor; Treatment progress; Side effects; Immunotherapy

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## 1. Introduction

Malignant tumors, with their high incidence and mortality rates, remain a major focus of global medical research. Traditional Western treatment, including surgery, radiotherapy, and chemotherapy, has achieved significant progress. However, chemotherapy drugs often cause severe side effects such as nausea, vomiting, immune dysfunction, and organ damage, which seriously affect patients' quality of life. Traditional Chinese medicine (TCM) with its long history in cancer treatment. Offers an alternative approach through syndrome differentiation, herbal medicine, acupuncture, and other techniques. By restoring the balance of Yin and Yang of the human body, TCM helps improve overall health and reduce the side effects of Western treatments. Notably, it has unique advantages in addressing drug resistance, pain management, and immune system reconstruction.

Integrating Chinese and Western medicine has shown promising results in enhancing cancer immunity, reducing treatment-related side effects, and improving overall patient well-being. With ongoing advancements in cancer treatment, increasing clinical evidence supports the efficacy of this combined approach in improving therapeutic outcomes and prolonging survival. This study aims to explore the progress of integrated Chinese and Western medicine in malignant tumor treatment, summarize existing achievements and challenges, evaluate its effectiveness across different cancer types, and explore its potential in personalized therapy, immunotherapy, and quality of life improvement.

## **2. Basic principles and mechanisms of integrated Chinese and Western medicine therapy**

### **2.1. Mechanism of integrated Chinese and Western medicine therapy**

The mechanism of integrated Chinese and Western medicine therapy involves a multidimensional approach targeting the biological characteristics of tumors. Western medicine, through specific molecular targets, effectively inhibits tumor cell reproduction, invasion, and metastasis with high specificity. However, when used alone, it often induces adverse effects, such as immune suppression from chemotherapy and radiation-induced cellular damage. In contrast, TCM focuses on immune system regulation and the enhancement of the body's self-repair mechanisms, thereby improving the patient's resistance to disease.

TCM not only directly inhibits tumor cell growth but also strengthens immune function by activating T cells and natural killer (NK) cells, strengthening the body's antitumor defense. In addition, TCM promotes holistic well-being by balancing emotional health, regulating "qi" and blood circulation, and harmonizing organ functions. These combined effects help reduce treatment-related side effects and improve patient tolerance and quality of life. The combination of Chinese and Western medicine thus leverages their complementary strengths, achieving a more comprehensive and effective approach to tumor treatment.

### **2.2. Clinical advantages of integrated Chinese and Western medicine therapy**

Integrated Chinese and Western medicine therapy offers several clinical benefits in the treatment of malignant tumors:

Western medicine, particularly chemotherapy, radiotherapy, and surgery, plays a crucial role in rapidly suppressing tumor progression. However, these treatments are often accompanied by severe side effects, including immunosuppression, fatigue, and gastrointestinal distress, which can adversely affect patients' quality of life and treatment outcomes. TCM, with its holistic approach, emphasizes immune system modulation, organ function restoration, and systemic balance. When combined with conventional therapies, TCM can mitigate these adverse reactions, enhance physical recovery, and improve the quality of life of patients. Second, integrated therapy allows for a more individualized treatment strategy tailored to patients' specific conditions, physique, and living habits. TCM follows a syndrome differentiation approach, customizing herbal prescriptions and adjunct therapies based on each patient's constitution and tumor type. Concurrently, Western medicine employs evidence-based clinical protocols and precise diagnostic techniques, ensuring targeted and scientifically validated treatment interventions. This combination not only minimizes unnecessary side effects during treatment but also effectively improves treatment effectiveness and prolongs survival. In addition, integrated Chinese and Western medicine therapy is helpful to regulate the psychological state of patients. In the face of cancer, patients often bear a heavy

psychological burden, and the accompanying anxiety and depression may interfere with the treatment process. TCM incorporates psychological regulation through the principles of qi and blood circulation, as well as Yin and Yang balance, which have been shown to reduce the mental pressure of patients to a certain extent, help them maintain a positive psychological condition, and help improve the effect of treatment.

By combining the strengths of both medical systems, integrated Chinese and Western medicine therapy provides a comprehensive treatment strategy that enhances therapeutic outcomes while improving patients' physical and emotional well-being.

### **3. Research progress of integrated Chinese and Western medicine therapy in the treatment of malignant tumors**

#### **3.1. Clinical research and therapeutic effect analysis**

Numerous clinical studies have shown that the combination of traditional Chinese and Western medicine has a good curative effect in the treatment of malignant tumors. For example, Noiri *et al.* found that in patients with early gastric cancer, Western medicine can effectively inhibit the local spread of tumors through accurate surgery and chemotherapy treatment, while Chinese medicine can improve the overall health of patients by regulating qi and blood, enhancing immune function, and thus improving the treatment effect and quality of life <sup>[1]</sup>.

Similarly, Wang *et al.* Conducted a study on patients with hematological malignancies undergoing stem cell transplantation. Their findings revealed that integrating Chinese and Western medicine significantly reduced the incidence of acute graft-versus-host disease (GVHD) and significantly improved the curative effect <sup>[2]</sup>. Research indicates that Western medicine rapidly suppresses tumor proliferation, while TCM plays a crucial role in mitigating adverse effects, strengthening immunity, and accelerating patient recovery. The synergy between these two approaches not only enhances therapeutic effectiveness but also alleviates treatment burden <sup>[3]</sup>.

#### **3.2. Treatment strategies of integrated Chinese and Western medicine for different malignant tumors**

The treatment plan for malignant tumors varies based on the tumor type and the patient's condition. Integrated Chinese and Western medicine therapy is adapted accordingly:

For lung cancer, Western medicine mainly uses surgical excision, radiation therapy, drug chemotherapy, and precision-targeted therapy. Traditional Chinese medicine treatment focuses on the use of herbs, acupuncture, and massage to enhance the patient's physique and alleviate the adverse effects of radiation and chemotherapy. Studies have pointed out that lung cancer patients receiving integrated Chinese and Western medicine therapy exhibit a significantly slower tumor progression rate and experience fewer adverse reactions, especially in terms of respiratory symptoms and fatigue caused by lung cancer.

For gastric cancer, Western medicine mainly relies on surgery, chemotherapy, and radiotherapy, while Chinese medicine focuses on the rehabilitation of the spleen and stomach, promoting the health of the digestive system, and improving the physique and resistance of patients. Studies have shown that gastric cancer patients undergoing integrated Chinese and Western medicine treatment tend to recover more quickly post-surgery, and the side effects of chemotherapy are significantly alleviated <sup>[4]</sup>.

For breast cancer, Western medicine mainly employs surgery, chemotherapy, and radiotherapy to control the spread of tumors. Meanwhile, Chinese medicine incorporates herbal medicines with effects such as clearing

heat and detoxifying, promoting blood circulation, and dissipating blood stasis to support treatment. Many cases have shown that integrated traditional Chinese and Western medicine therapy can effectively delay breast cancer recurrence and metastasis while significantly improving the living standards of patients<sup>[5]</sup>.

In general, the treatment of different malignant tumors should be tailored according to the patient's specific condition, physique, and treatment stage to achieve the best therapeutic effect.

### **3.3. Side effects and safety of integrated Chinese and Western medicine therapy**

As a multi-intervention treatment approach, the safety and potential side effects of integrated Chinese and Western medicine therapy remain critical areas of clinical research. The most common side effects of Western treatment, such as chemotherapy and radiation therapy, include immunosuppression, hair loss, gastrointestinal disturbances, and systemic fatigue. In contrast, the adverse effects of TCM treatment are generally milder, often presenting as minor bodily disorders, allergic symptoms, or digestive discomfort<sup>[6]</sup>.

However, during the implementation of integrated Chinese and Western medicine therapy, inappropriate drug combinations or inaccurate dosages may lead to drug interactions, potentially compromising patient safety. On the one hand, TCM treatment can reduce the side effects of Western treatment by reconciling qi and blood and promoting immunity. Studies have pointed out that traditional Chinese medicine has a significant effect on reducing the white blood cell decline, loss of appetite, nausea, and other discomfort caused by chemotherapy, thereby improving patients' tolerance to treatment. On the other hand, certain herbal medicines may interact with the chemotherapy drugs, affecting their metabolic pathways or increasing their toxicity, which could compromise therapeutic efficacy and patient safety. Therefore, the clinical application of integrated Chinese and Western medicine therapy requires strict drug regulation to avoid inappropriate drug combinations and overdosing.

### **3.4. Challenges in current research**

Despite the promising prospects of integrated traditional Chinese and Western medicine therapy in treating malignant tumors, several challenges persist:

First, there is a lack of sufficient clinical data and robust evidence-based medical research. Many existing studies rely on small-scale, single-center observational studies, with limited support from extensive, randomized controlled trials. Addressing this gap by designing high-quality clinical trials and generating more reliable efficacy data is a pressing concern. Second, there is no uniform standard for the treatment plan of integrated Chinese and Western medicine therapy. Although many studies propose different treatment plans, doctors often adjust the plans according to their personal experience in actual clinical operation, resulting in a lack of consistency in the evaluation of treatment effects. Third, current studies mostly focus on some specific types of cancer, while systematic investigations into the broader applicability and comparative efficacy of integrated therapy across various tumors remain insufficient. In addition, concerns regarding the quality control of Chinese medicinal materials, drug interactions, and safety issues warrant further exploration. Finally, although integrated Chinese and Western medicine therapy has shown advantages in improving patients' quality of life and reducing side effects, it still faces multiple challenges in clinical application and promotion, such as differences in doctors' cognition, patient acceptance, and insufficient policy support.



## 4. Future prospects and application of integrated Chinese and Western medicine therapy

Individualized treatment represents the future direction of modern medicine development, and the combination of traditional Chinese and Western medicine has great potential in this field. Tumor formation and progression are complex processes influenced by multiple factors, including genetic predisposition, environmental influences, and individual constitution. Western medicine achieves precise treatment through molecular targeting, utilizing approaches such as targeted drugs and immune checkpoint inhibitors. In contrast, TCM focuses on symptom differentiation and holistic regulation, adjusting a patient's constitution to enhance immunity<sup>[7]</sup>. The integration of Chinese and Western medicine enables a more tailored treatment strategy that considers both disease pathology and the patient's physiological characteristics. For example, in liver cancer treatment, Western medicine employs a combination of local and systemic therapies, while TCM strengthens the patient's physique and immune function by strengthening the spleen and stomach function, and promoting metabolism, to optimize the overall treatment. As precision medicine continues to advance, integrated Chinese and Western medicine is expected to play an increasingly vital role in personalized cancer treatment.

Immunotherapy has emerged as a breakthrough in cancer treatment, particularly for melanoma, non-small cell lung cancer, and other cancers. The combination of traditional Chinese and Western medicine therapy with immunotherapy offers new opportunities for enhancing cancer treatment outcomes. Chinese herbal medicine and its therapy have shown their unique effects in enhancing the body's immunity and balancing the immune ecosystem. Some Chinese herbal medicine ingredients and traditional treatment approaches can optimize immune responses by activating T cells, NK cells, and other immune components, to enhance the effect of immunotherapy drugs. Studies have shown that some herbal ingredients can significantly improve the effectiveness of immune checkpoint inhibitors and prevent the immune escape mechanism of tumor cells<sup>[8]</sup>. This synergy not only improves therapeutic efficacy but also helps reduce resistance to immunotherapy, minimizing adverse reactions and enhancing patient survival rates. Moreover, integrated therapy can further optimize the tumor immune microenvironment by reducing immunosuppressive factors and promoting sustained immune cell activity. These mechanisms contribute to prolonged treatment responses and improved durability of immunotherapy. Moving forward, the combination of integrated Chinese and Western medicine with immunotherapy is likely to become a new tumor treatment model, greatly enhancing the therapeutic effect.

Beyond prolonging survival, cancer treatment should also prioritize patients' quality of life. Integrated traditional Chinese and Western medicine therapy has shown unique advantages in this regard. TCM focuses on holistic body regulation, relieving pain, reducing psychological pressure, promoting appetite and sleep quality, and minimizing treatment-related discomfort. For example, during chemotherapy, the use of Chinese herbs can reduce common side effects such as nausea, vomiting, and appetite loss, facilitating faster recovery and strengthening the immune system. TCM's role in emotional regulation offers substantial benefits for cancer patients' mental health, alleviating anxiety and depression while fostering a positive mindset towards treatment. In addition, integrated therapy can help patients cope with the fatigue and weakness associated with long-term treatment, maintaining physical resilience and supporting self-rehabilitation through systemic conditioning. As cancer survival rates improve, quality of life has become an essential metric for evaluating treatment efficacy<sup>[9]</sup>.

Despite its promising potential in oncology, the widespread adoption of integrated Chinese and Western medicine therapy requires enhanced policy support and clinical implementation. Government agencies should increase funding for research in this field and encourage collaboration among medical institutions and research

centers to conduct large-scale, multi-center clinical studies validating the efficacy and safety of integrated treatments <sup>[10]</sup>. The formulation of unified treatment standards and norms is the key to promoting the widespread application of this therapy and helps reduce variability in treatment administration, enhancing reliability and predictability. In addition, fostering interdisciplinary training among healthcare professionals is crucial for effective implementation. Strengthening medical education programs that integrate both TCM and Western medical approaches will improve practitioners' competence in delivering combined therapy.

## 5. Conclusion

The integration of Chinese and Western medicine provides an innovative approach to the treatment of malignant tumors by combining the precision of Western medical interventions with the holistic regulation of traditional Chinese medicine. While Western medicine pays attention to localized tumor control and has made remarkable progress, it still faces problems such as severe side effects and drug resistance. In contrast, Chinese medicine emphasizes the regulation of the whole-body function, improving immunity, and has the unique advantages of relieving side effects, enhancing physical strength, and so on. Through the complementary effect, integrated Chinese and Western medicine therapy can improve the therapeutic effect, reduce side effects, improve the quality of life of patients, and show great clinical potential.

However, the current research also has some shortcomings, such as insufficient clinical trial data, a lack of uniformity of efficacy evaluation criteria, and differences in therapeutic effects of different types of tumors. Especially in the treatment of personalized and standardized, still need to further explore and optimize. In the future, research needs to focus more on multi-center, large-sample clinical trials to supplement the existing data and to explore the mechanisms of integrated treatment. Especially how to combine with modern technology, such as immunotherapy and targeted therapy, will be an important research direction. Through these efforts, we can better exert the advantages of integrated Chinese and Western medicine therapy in individual treatment, and improve its efficacy and indication range in tumor treatment. In addition, with the investment of policy support and resources, integrated Chinese and Western medicine therapy is expected to be more widely used in clinical practice, bringing more treatment options for patients with malignant tumors.

## Disclosure statement

The author declares no conflict of interest.

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# Observation on the Effect of Interventional Therapy Combined with Lenvatinib and Sintilimab in the Treatment of Advanced Liver Cancer

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**Abstract:** *Objective:* To observe the control effect of interventional therapy combined with lenvatinib and sintilimab in patients with intermediate and advanced liver cancer. *Methods:* 82 patients with intermediate and advanced liver cancer who visited from January 2022 to January 2025 were selected as samples and randomly divided into two groups. Group A received interventional therapy combined with lenvatinib and sintilimab, while Group B received interventional therapy combined with lenvatinib. Disease remission rate, adverse reactions, liver function indicators, and tumor marker indicators were compared between the two groups. *Results:* The disease control rate (DCR) in Group A was higher than that in Group B ( $P < 0.05$ ). There was no difference in adverse reaction rates between Group A and Group B ( $P > 0.05$ ). Total bilirubin (TBil), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels in Group A were lower than those in Group B ( $P < 0.05$ ). Carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), and alpha-L-fucosidase (AFU) levels in Group A were also lower than those in Group B ( $P < 0.05$ ). *Conclusion:* Intermediate and advanced liver cancer patients receiving interventional therapy combined with lenvatinib and sintilimab showed reduced tumor marker levels, lessened liver function damage, and a high disease control rate and treatment safety.

**Keywords:** Intermediate and advanced liver cancer; Sintilimab; Lenvatinib; Interventional therapy

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## 1. Introduction

Advanced liver cancer is characterized by a high metastasis rate, strong invasiveness, and severe liver function impairment, which can shorten the survival time and reduce the quality of life of patients. Therefore, early treatment is essential. In the early stage of liver cancer, symptoms are not typical. However, in the advanced stage, symptoms such as jaundice, ascites, abdominal pain, and weight loss become apparent. A few patients may develop

abdominal masses, and most patients have a poor prognosis. Clinically, transcatheter arterial chemoembolization (TACE) is commonly used to manage patients with advanced liver cancer. This technique delivers antitumor drugs to the target area, reducing the toxicity and side effects of chemotherapy. However, drug resistance remains a challenge, necessitating the exploration of combined treatment regimens. Lenvatinib, a targeted drug, is used in the management of advanced liver cancer. It blocks the activity of vascular endothelial growth factor receptors, inhibiting tumor angiogenesis and prolonging patient survival. Sintilimab, a PD-1 inhibitor, mediates T-cell immune responses, blocking the proliferation of tumor cells and slowing tumor growth. Studies have shown that the combination of lenvatinib and PD-1 inhibitors has a synergistic effect in the treatment of advanced liver cancer <sup>[1]</sup>. Based on this, this article explores the efficacy of interventional therapy combined with lenvatinib and sintilimab using a sample of 82 patients with advanced liver cancer who were treated between January 2022 and January 2025.

## 2. Materials and methods

### 2.1. Materials

From January 2022 to January 2025, 82 patients with advanced liver cancer who visited our hospital were selected as samples and randomly divided into groups by drawing. There was no difference in liver cancer data between Group A and Group B, with  $P > 0.05$ . See **Table 1**.

**Table 1.** Analysis of advanced liver cancer data

Group <i>n</i>		Gender (%)		Age (years)		BCLC staging (%)	
		Male	Female	Range	Mean	Stage B	Stage C
Group A	41	22 (53.66)	19 (46.34)	46–70	59.42 ± 2.49	25 (60.98)	16 (39.02)
Group B	41	23 (56.10)	18 (43.90)	46–71	59.39 ± 2.52	26 (63.41)	15 (36.59)
$\chi^2/t$	-	0.0492		0.0542		0.0519	
<i>P</i>	-	0.8244		0.9569		0.8198	

### 2.2. Inclusion and exclusion criteria

Inclusion criteria: (1) Meet the criteria for liver cancer in the “Guidelines for the Diagnosis and Treatment of Primary Liver Cancer (2024 Edition)” <sup>[2]</sup>; (2) Signed informed consent; (3) Predicted survival > 12 months; (4) Not taken anti-tumor drugs before enrollment.

Exclusion criteria: (1) Organ dysfunction; (2) Autoimmune diseases; (3) Myelosuppression; (4) Solid organ transplantation.

### 2.3. Treatment methods

Group A: (1) Interventional therapy: During TACE, select oxaliplatin (50–120mg) + tegafur (20–30mg) + doxorubicin (40–60mg) for treatment, once every 4–6 weeks, adjusting the dosing regimen based on the physiological status of the liver cancer patient. (2) Targeted therapy: Oral administration of lenvatinib, a single dose of 8mg, once a day. (3) Immunotherapy: Intravenous injection of sintilimab, a single dose of 240mg, once every 2 weeks. Treatment for 3 months.

Group B: The interventional therapy + targeted therapy medication regimen and cycle are the same as Group A.



## 2.4. Observation indicators

Disease control rate: Complete response (CR) is recorded when the tumor lesion disappears; partial response (PR) is recorded when the tumor lesion volume reduction is >30% and the number reduction is >50%; stable disease (SD) is recorded when the tumor lesion volume and number remain unchanged or the increase does not meet the above criteria; disease progression (PD) is recorded when the tumor lesion increases.

Adverse reactions: Record platelet decline, hypothyroidism, decreased white blood cell count, and hypoproteinemia.

Liver function: Fully automated biochemical analyzer to detect TBil, AST, and ALT indicators.

Tumor markers: Enzyme-linked immunosorbent assay to detect CEA, AFP, and AFU indicators.

## 2.5. Statistical analysis

Data were processed using SPSS 23.0, with a chi-square test used for counting data (recorded as %) and a *t*-test used for measurement data (recorded as mean  $\pm$  standard deviation [SD]). Statistical difference exists when  $P < 0.05$ .

## 3. Results

### 3.1. Disease control rate

The DCR of Group A was higher than that of Group B,  $P < 0.05$ . As shown in **Table 2**.

**Table 2.** Disease control rate in advanced liver cancer [ $n$  (%)]

Group	CR	PR	SD	PD	DCR
Group A ( $n = 41$ )	1 (2.44)	19 (46.34)	18 (43.90)	3 (7.32)	38 (92.68)
Group B ( $n = 41$ )	1 (2.44)	15 (36.59)	14 (34.15)	11 (26.83)	30 (73.17)
$\chi^2$	-	-	-	-	5.5126
$P$	-	-	-	-	0.0189

### 3.2. Adverse reaction rate

There was no difference in the adverse reaction rate between Group A and Group B, with  $P > 0.05$ . See **Table 3**.

**Table 3.** Adverse reaction rate in advanced liver cancer [ $n$  (%)]

Group	Thrombocytopenia	Hypothyroidism	Leukopenia	Hypoproteinemia	Incidence rate
Group A ( $n = 41$ )	4 (9.76)	3 (7.32)	6 (14.63)	5 (12.20)	18 (43.90)
Group B ( $n = 41$ )	5 (12.20)	5 (12.20)	6 (14.63)	4 (9.76)	21 (51.22)
$\chi^2$	-	-	-	-	0.4401
$P$	-	-	-	-	0.5071

### 3.3. Liver function indicators

After treatment, the TBil, AST, and ALT indicators in Group A were all lower than those in Group B, with  $P < 0.05$ . See **Table 4**.

**Table 4.** Analysis of liver function indicators in advanced liver cancer (mean  $\pm$  SD)

Group	TBil ( $\mu\text{mol/L}$ )		AST (U/L)		ALT (U/L)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Group A ( $n = 41$ )	31.18 $\pm$ 1.62	14.69 $\pm$ 1.02	50.47 $\pm$ 2.88	26.31 $\pm$ 1.88	75.28 $\pm$ 4.11	41.89 $\pm$ 2.06
Group B ( $n = 41$ )	31.21 $\pm$ 1.59	20.48 $\pm$ 1.44	50.42 $\pm$ 2.91	34.33 $\pm$ 2.06	75.31 $\pm$ 4.16	55.73 $\pm$ 3.44
<i>t</i>	0.0846	21.0093	0.0782	18.4133	0.0328	22.1016
<i>P</i>	0.9328	0.0000	0.9379	0.0000	0.9739	0.0000

### 3.4. Tumor markers

After treatment, the CEA, AFP, and AFU indicators in Group A were all lower than those in Group B, with  $P < 0.05$ . See **Table 5**.

**Table 5.** Analysis of tumor marker indicators in advanced liver cancer (mean  $\pm$  SD)

Group	CEA (ng/ml)		AFP (ng/ml)		AFU (U/L)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Group A ( $n = 41$ )	35.82 $\pm$ 2.41	16.11 $\pm$ 1.25	329.25 $\pm$ 11.26	104.24 $\pm$ 6.16	72.62 $\pm$ 3.88	34.82 $\pm$ 1.58
Group B ( $n = 41$ )	35.79 $\pm$ 2.39	24.33 $\pm$ 1.73	329.31 $\pm$ 11.29	155.73 $\pm$ 8.43	72.59 $\pm$ 3.91	50.33 $\pm$ 2.61
<i>t</i>	0.0566	24.6604	0.0241	31.5777	0.0349	32.5510
<i>P</i>	0.9550	0.0000	0.9808	0.0000	0.9723	0.0000

## 4. Discussion

The pathogenesis of advanced liver cancer is complex, related to fatty liver, high blood glucose, and viral infections. Additionally, continuous damage to the liver caused by hepatitis and alcoholic liver disease can also increase the risk of liver cancer. In patients with advanced liver cancer, the increase in tumor diameter can elevate the tension of the liver capsule, exacerbating pain symptoms in the liver area. If it involves the digestive tract, it can induce nausea, vomiting, low appetite, abdominal distension, and other symptoms. For those with excessively large tumor volumes, a lump may be palpable upon touching the abdomen, and complications such as jaundice and portal hypertension may occur, leading to conditions like low body weight, anemia, fever, fatigue, ascites, jaundice, and even secondary blood coagulation disorders <sup>[3]</sup>. As liver cancer progresses and the liver continues to be damaged, it can reduce the patient's survival time.

Currently, interventional procedures are commonly used in the clinical treatment of advanced liver cancer, with TACE being frequently employed. By directly delivering chemotherapy drugs to the target hepatic artery, it can enhance the drug concentration in the tumor area, achieving local therapeutic effects. Compared to conventional chemotherapy, TACE technology can reduce damage to healthy tissues, restore the patency of the liver's blood supply arteries, and alleviate liver cancer-related symptoms <sup>[4]</sup>.

Lenvatinib is a targeted drug that can inhibit tumor-related receptor kinases, slowing down tumor growth. It can also block tumor cell pathways, accelerate tumor cell apoptosis, and reduce the number of local new blood vessels, thus lowering the tumor metastasis rate. Sintilimab can enhance the activity of immune cells, promoting their ability to kill tumor cells. It can also strengthen the ability of T cells to infiltrate tumor tissue, deactivate NK

cells, and delay tumor tissue growth <sup>[5]</sup>.

Furthermore, sintilimab can enhance the management of liver cancer through multiple pathways: upon entering the body, sintilimab binds to PD-1 molecules on the surface of T cells, enhancing their ability to recognize and kill liver cancer cells, thereby strengthening the immune response. Its pharmacologically active components express immune molecules, blocking the activity of adjacent immune cells and creating an immunosuppressive microenvironment for immune monitoring of tumor cells. When combined with antitumor drugs such as lenvatinib, it can enhance the efficacy of killing tumor cells and improve disease control rates through multiple synergistic mechanisms. This drug has high safety, with only a few patients experiencing minor complications such as pneumonia, abnormal liver function, and skin rash, which can be alleviated with symptomatic treatment.

In this paper, the combination of TACE with lenvatinib and sintilimab can activate the immune response and improve prognosis.

Based on the data analysis in this article, the DCR of Group A is higher than that of Group B, with  $P < 0.05$ . The reason for this is analyzed as follows: Lenvatinib, a tyrosine kinase inhibitor, can block tumor angiogenesis and cell proliferation through multiple targets. Combined with sintilimab, it can act on PD-1 receptor antibodies, inhibit tumor cell escape mechanisms, and enhance tumor control effects <sup>[6]</sup>. Another set of data shows that there is no difference in the adverse reaction rate between Group A and Group B, with  $P > 0.05$ . This suggests that the combination therapy does not increase adverse reactions and has high treatment safety. Another set of data indicates that the TBil, AST, and ALT levels in Group A are lower than those in Group B, with  $P < 0.05$ . The analysis of the reasons is that tumor cells in patients with advanced liver cancer invade healthy tissues, leading to increased permeability of liver cell membranes, increased cell necrosis, and elevated levels of ALT and AST in the blood. Additionally, impaired liver metabolism and disordered glucose and lipid metabolism can further elevate ALT and AST levels. Widespread damage to liver cells in patients with advanced liver cancer can induce hepatocellular jaundice, resulting in increased TBil levels <sup>[7]</sup>. The combined treatment approach in this article includes TACE, which can inhibit tumor-supplying arteries, protect residual kidney function, and restore blood supply to non-tumor areas, favoring hepatocyte regeneration. Combined with lenvatinib, it can deactivate vascular endothelial growth factor receptors, blocking nutrient supply to tumor cells and inhibiting their proliferation, thereby delaying tumor growth. Additionally, combined with sintilimab, the PD-1/PD-L1 signaling pathway is blocked, and T cells are activated, enhancing the immune system's ability to recognize and kill tumor tissue. Monoclonal antibody therapy can reverse immunosuppression and accelerate the immune system's clearance of tumor tissue, thereby protecting liver function <sup>[8]</sup>. The combined intervention of interventional therapy, lenvatinib, and sintilimab can reduce liver damage caused by tumor tissue, leading to improved TBil, AST, and ALT levels. The final set of data shows that CEA, AFP, and AFU levels in Group A are lower than those in Group B, with  $P < 0.05$ . The analysis of the reasons is that patients with advanced liver cancer secrete CEA, and impaired liver function leads to abnormal CEA metabolism, resulting in elevated CEA levels. AFP is a glycoprotein substance, and liver cancer cells have dedifferentiation characteristics that enable them to secrete large amounts of AFP, allowing for the assessment of tumor type and differentiation degree by monitoring AFP levels. AFU participates in the body's metabolism of oligosaccharides, glycolipids, and glycoproteins, and as liver damage increases, AFU metabolism levels decrease, leading to elevated AFU levels in patients <sup>[9]</sup>. Based on interventional therapy, this article combines lenvatinib and sintilimab to treat advanced liver cancer, inhibiting tumor cell proliferation through multiple pathways. This activation of the immune system and reduction of tumor marker levels, combined with multi-modality treatment, can reduce tumor malignancy, achieve multi-pathway tumor control, and improve tumor marker levels <sup>[10]</sup>. Patients with advanced liver cancer should seek medical attention if they experience severe fever, abdominal pain, or gastrointestinal reactions during treatment, and regularly

undergo liver function testing. Treatment plans for liver cancer should be adjusted based on the results of these tests.

## 5. Conclusion

In summary, patients with advanced liver cancer who receive interventional therapy combined with lenvatinib and sintilimab experience decreased tumor marker levels, improved liver function, and enhanced disease control rates, indicating the value of this treatment approach for widespread application.

## Disclosure statement

The authors declare no conflict of interest.

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# Research Progress on Exosomes in the Diagnosis of Ovarian Cancer

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**Abstract:** Ovarian cancer ranks as the deadliest malignancy among female reproductive system cancers, posing a significant threat to women's health. Around seven out of ten patients are diagnosed only after reaching progressive disease phases, a phenomenon closely linked to three key factors: the disease's hidden onset location, lack of early symptoms, and absence of reliable early diagnostic methods. Therefore, identifying early diagnostic biomarkers and therapeutic targets is critical. Exosomes participate in various phases of ovarian tumorigenesis, including transforming normal cells into cancerous cells, immune regulation, invasion, metastasis, drug resistance, and angiogenesis, making them promising biomarkers for early ovarian cancer detection. This review summarizes current research on exosomal long non-coding RNAs (lncRNAs), miRNAs, and related proteins in ovarian cancer diagnosis. Exosome-based biomarkers have shown potential advantages, including high sensitivity, specificity, stability, and non-invasive accessibility. The study concludes that while exosomes hold significant diagnostic potential for ovarian cancer, additional investigations are required to standardize detection methods, validate clinical applicability, and elucidate underlying molecular mechanisms.

**Keywords:** Exosomes; Ovarian cancer; Biomarkers; lncRNA; miRNA; Early diagnosis

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## 1. Introduction

Ovarian cancer, a malignant tumor originating from ovarian epithelial, stromal, or germ cells, is characterized by poor clinical outcomes, high mortality rates, and the highest fatality rate among gynecological cancers <sup>[1]</sup>. It is highly malignant, and asymptomatic early stages often lead to late diagnosis. Notably, over 70% of cases are diagnosed at advanced stages (FIGO stage III or IV) <sup>[2]</sup>. According to the latest population-based cancer incidence data compiled by the American Cancer Society in "Cancer Statistics, 2020," the 5-year survival rate can reach 93% if patients are diagnosed at an early stage (FIGO stage I or II) <sup>[3]</sup>. However, there is currently no widely accepted, highly sensitive, and specific screening tool for early ovarian cancer detection. Diagnosis predominantly relies on nonspecific symptoms (e.g., abdominal pain, bloating), imaging techniques (e.g.,



transvaginal ultrasound), and serum biomarkers such as CA125 <sup>[4,5]</sup>. Despite its widespread clinical use, CA125 exhibits limited sensitivity and specificity in early-stage ovarian cancer, with CA125 elevation observed in only half of patients with FIGO stage I ovarian cancer. Elevated CA125 levels are also observed in other malignancies (e.g., breast, uterine, gastric, liver, and pancreatic cancers) and benign conditions (e.g., acute pelvic inflammatory disease, adenomyosis, and endometriosis), leading to frequent false positives <sup>[6]</sup>. These limitations underscore the need for more specific diagnostic, prognostic, and therapeutic biomarkers. Recent research highlights the potential of exosomes, nanoscale extracellular vesicles, as promising biomarkers for ovarian cancer. Exosomes are actively involved in ovarian cancer progression, including immune evasion, metastasis, and drug resistance. They carry molecular cargo such as long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and proteins, which reflect the biological state of tumor cells and have potential as diagnostic and prognostic biomarkers.

This paper explores the application value of exosomes in the diagnosis of ovarian cancer, particularly their potential as a novel tumor biomarker for early diagnosis of ovarian cancer. Through a systematic review and summary of the existing literature, this study focuses on the current applications of exosomal long noncoding RNA (lncRNA), microRNA (miRNA), and related proteins in the diagnosis of ovarian cancer. It analyzes their advantages in terms of sensitivity, specificity, stability, and ease of acquisition and discusses their potential for early diagnosis of ovarian cancer. The significance of this research lies in providing new ideas for the clinical diagnosis of ovarian cancer, exploring a more promising early diagnostic biomarker, and ultimately reducing the incidence and mortality rates of ovarian cancer while improving patients’ survival rates and quality of life.

## 2. Current status of ovarian cancer

With regard to female genital tract neoplasms, ovarian cancer carries the worst case-fatality rate <sup>[7]</sup>. According to Cabasag *et al.*, by 2040, the global incidence of ovarian cancer is projected to reach approximately 428,000 new cases and 307,000 deaths, with a five-year survival rate of only 20–30% for advanced-stage patients, posing a serious threat to women’s health <sup>[8]</sup>. Due to its insidious onset, rapid progression, and high recurrence rate, ovarian cancer is often at an advanced stage, with approximately 70% of cases detected late, contributing to its poor prognosis <sup>[9]</sup>. The primary reasons for this dire situation are the hidden location of the disease, the absence of noticeable symptoms in early stages, and the lack of effective early diagnostic techniques. Therefore, the identification of reliable biomarkers for early detection and novel therapeutic targets is of paramount importance in improving the prognosis of ovarian cancer patients.

Current diagnostics combine symptoms, serum CA125, and imaging (e.g., transvaginal ultrasound). While CA125 is widely used, its limitations in early detection persist. Imaging methods are cost-effective but lack sensitivity and may lead to unnecessary surgeries. Given their non-invasive nature and stability, exosomes have emerged as a promising alternative, though their mechanisms require further exploration (**Table 1**).

**Table 1.** Current diagnostic methods and their limitations

Method	Examples	Advantages	Disadvantages
Serum biomarkers <sup>[1]</sup>	CA125, HE4, AFP	Widely accepted	Low sensitivity/specificity in early stages
Imaging <sup>[10]</sup>	Transvaginal ultrasound	Cost-effective	Limited sensitivity, high false-positive rates
Exosomes <sup>[11]</sup>	lncRNAs, miRNAs, proteins	Non-invasive, stable, accessible	Mechanisms remaining unclear, early-stage research

### 3. Exosomes in ovarian cancer diagnosis

Exosomal, lncRNAs, miRNAs, and proteins are broadly studied for early ovarian cancer detection <sup>[12]</sup>.

#### 3.1. Long non-coding RNAs (lncRNAs)

Long non-coding RNAs (lncRNAs) are non-coding RNA transcripts composed of more than 200 nucleotides <sup>[13]</sup>. Approximately 76% of the human genome is transcribed into lncRNAs, which are widely distributed in both the nucleus and cytoplasm <sup>[14]</sup>. Dysregulation of lncRNAs plays a critical role in tumorigenesis and is closely associated with the development of human malignancies <sup>[15]</sup>. The expression of lncRNAs differs significantly between normal and tumor tissues. These molecules can interact with DNA to regulate gene transcription or associate with RNA and proteins to modulate cellular processes such as proliferation, apoptosis, and invasion <sup>[16]</sup>. Functioning as either oncogenes or tumor suppressors, lncRNAs are key regulators of tumor initiation and progression, which highlights their potential as diagnostic and prognostic markers for multiple malignancies <sup>[17]</sup>.

For instance, Yang *et al.* revealed that the expression of lncRNA HAGLROS is significantly elevated in ovarian cancer tissues than in healthy controls, highlighting its potential for early diagnosis <sup>[18]</sup>. Similarly, Gong *et al.* found that levels of MIR4435-2HG and TGF- $\beta$ 1 are markedly higher in ovarian cancer patients than in healthy controls <sup>[19]</sup>. Other lncRNAs, such as HOTAIR, are also upregulated in various types of ovarian cancer tissues <sup>[20]</sup>. Additionally, frequent epigenetic alterations in the Igf2/H19 domain, focal amplification of FAL1 in epithelial cancers, and elevated expression of ASAP1-IT1, FAM215A, and LINC00472 further underscore the diagnostic potential of lncRNAs in ovarian cancer <sup>[21–23]</sup>. Collectively, these studies confirm that lncRNAs are highly promising biomarkers for the early detection of ovarian cancer. However, further research is needed to validate their clinical utility and standardize detection methodologies.

#### 3.2. MicroRNAs

MicroRNAs (miRNAs), typically 20–25 nucleotides long, represent a group of small non-protein-coding RNA molecules originating from endogenous genetic material. These molecules control gene expression after transcription mainly through base pairing with target mRNA's 3'-untranslated regions, leading to mRNA degradation or translational repression. This mechanism allows miRNAs to suppress the expression of specific proteins, making them crucial regulators of gene expression <sup>[24,25]</sup>. Numerous studies have demonstrated the significant role of exosomal miRNAs in early cancer screening. miRNAs encapsulated within exosomes exhibit enhanced stability and play pivotal roles in tumor cell proliferation, invasion, and metastasis, making them promising biomarkers for early detection.

For example, Iorio *et al.* identified miR-141, miR-200a, miR-200b, and miR-200c as significantly overexpressed miRNAs capable of distinguishing normal ovarian tissue from epithelial ovarian cancer (EOC) tissue <sup>[26]</sup>. Wang *et al.* employed quantitative real-time polymerase chain reaction (qRT-PCR) to analyze the expression of 1722 miRNAs in 15 normal ovarian tissue samples and 48 ovarian cancer samples. They identified a signature comprising 10 miRNAs, which demonstrated a sensitivity of 97% and a specificity of 92% <sup>[27]</sup>. Additionally, exosomal miR-21 is highly expressed in ovarian cancer patients, while PDCD4 expression is notably reduced <sup>[28]</sup>. miR-200c demonstrates promising biomarker characteristics for early-stage ovarian cancer diagnosis, while miR-30a-5p shows significantly elevated expression levels in liquid biopsies from ovarian cancer patients <sup>[26,29]</sup>. Furthermore, miR-205 promotes ovarian cancer metastasis by inducing angiogenesis <sup>[30]</sup>. These findings collectively underscore the potential of exosomal miRNAs as reliable biomarkers for the early diagnosis of ovarian

cancer, offering a non-invasive alternative to traditional diagnostic methods.

### 3.3. Proteins

Compared to exosomes derived from normal tissues, ovarian cancer exosomes exhibit significantly increased levels of certain proteins <sup>[31]</sup>. For instance, CD24 and Claudin-4 are markedly elevated in ovarian cancer exosomes <sup>[12]</sup>. Claudin-4 is also expressed at higher levels in ovarian cancer patients compared to healthy controls <sup>[32]</sup>. Heat shock protein 70 (HSP70) is highly expressed in exosomes derived from ovarian cancer cells, and small heat shock proteins are abundant in exosomes from the serum and peritoneal fluid of ovarian cancer patients, suggesting their potential as biomarkers <sup>[33,34]</sup>. A systematic analysis of these protein biomarkers may facilitate early ovarian cancer detection. Future studies should focus on validating their clinical applicability and integrating them into multi-omics diagnostic models to enhance accuracy and reliability.

## 4. Diagnostic value of exosomes

Exosomal biomarkers exhibit excellent sensitivity and specificity, demonstrating potential in early ovarian cancer detection. Resnick *et al.* conducted a comparative analysis of miRNA expression profiles between nine tumor samples and four normal controls utilizing the TaqMan Array Human MicroRNA platform with real-time PCR quantification. They found that miR-92, miR-93, and miR-21 could be detected even before CA-125 levels increased, indicating their diagnostic potential for early-stage serous ovarian cancer detection <sup>[35]</sup>. Elias *et al.* demonstrated through algorithmic analysis that miRNA-based neural networks achieved 100% specificity and superior sensitivity compared to CA-125 <sup>[36]</sup>. Furthermore, Ma and Li discovered that miR-205 was highly upregulated, whereas let-7f was downregulated in the peripheral blood of ovarian cancer patients, particularly in stage I cases <sup>[37]</sup>. This finding highlights the high sensitivity of this diagnostic approach, which can significantly improve the accuracy of ovarian cancer diagnosis. The unique membrane composition and structure of exosomes provide a relatively enclosed and stable microenvironment for their internal molecules, protecting them from degradation or excretion in the bloodstream. Mitchell and Pendlebury demonstrated that miRNAs in serum and plasma are resistant to ribonuclease degradation, underscoring their advantages as tumor biomarkers <sup>[38,39]</sup>. This high biological stability allows exosomal molecules to remain intact even in complex biological environments, maintaining their activity and stability over long-term storage.

More importantly, exosomes are secreted by nearly all types of normal and cancerous cells and are widely present in various biofluids, including serum, plasma, urine, saliva, sputum, pleural effusion, and ascites. Blood, in particular, contains a high and stable concentration of exosomes, making it possible to use blood-based exosome detection as a screening tool for ovarian cancer without the need for invasive tissue biopsies. Traditional biopsy methods are highly invasive and carry significant risks, whereas exosome detection offers a minimally invasive or non-invasive alternative, providing a new and safer diagnostic pathway for ovarian cancer.

## 5. Conclusion

Early detection plays a crucial role in reducing the mortality rate of ovarian cancer. Exosomes, which are closely associated with the development and progression of ovarian cancer, hold significant value in early diagnosis. The long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and proteins carried by exosomes serve as potential

biomarkers for early-stage identification of diseases. Although the field of biomarker research is still in its developmental stages, it is rapidly advancing. Exosome-based diagnostics present several advantages. Long non-coding RNAs (lncRNAs) exhibit high specificity and versatility, making them suitable as biomarkers for early diagnosis. MicroRNAs (miRNAs) demonstrate high sensitivity and stability, rendering them ideal for non-invasive testing and early screening. Proteins, with their significant expression variability and multifunctionality, are well-suited for detection and diagnosis using conventional techniques. These biomarkers can maintain their stability and functionality across various biological environments, exhibiting higher sensitivity and specificity compared to existing diagnostic methods. However, current research in this area has limitations. For instance, many studies involve relatively small sample sizes, and the precise mechanisms by which these biomarkers function are still not fully understood. Future research should focus on elucidating these mechanisms and validating their clinical potential through large-scale, multi-center studies. Such efforts will help establish the reliability and applicability of exosomal biomarkers in real-world clinical settings. Additionally, the combined use of multiple biomarkers, such as lncRNAs, miRNAs, and proteins, could further enhance diagnostic accuracy. This multi-marker approach may provide a more comprehensive and precise method for early ovarian cancer detection, offering new insights and strategies for improving patient outcomes.

## Disclosure statement

The author declares no conflict of interest.

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# Study on the Efficacy and Quality of Life Impact of Combination Adjuvant Chemotherapy with Epirubicin and Docetaxel for Breast Cancer Patients after Radical Mastectomy

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**Abstract:** *Objective:* To explore and analyze the clinical effect of combination adjuvant chemotherapy with epirubicin and docetaxel for patients after radical mastectomy for breast cancer. *Methods:* This study enrolled 60 patients between May 2022 and December 2024, who were randomly allocated into two equal treatment groups ( $n = 30$  each). The control group received standard chemotherapy, whereas the observation group was treated with a combined adjuvant regimen of epirubicin and docetaxel. Therapeutic outcomes were systematically compared between the groups. *Results:* The comparative analysis of chemotherapy regimens revealed significant intergroup differences in multiple outcome measures. The observation group demonstrated superior clinical efficacy (96.67% vs 80.00%,  $P < 0.05$ ) alongside a more favorable safety profile (adverse reaction incidence: 3.33% vs 20.00%,  $P < 0.05$ ). Metabolic assessments showed better glycemic control in the observation group, with both fasting and postprandial blood glucose levels being significantly lower than controls ( $P < 0.05$ ), while maintaining comparable values to pretreatment baselines ( $P > 0.05$ ). Furthermore, quality of life assessments indicated significantly better outcomes in the observation group compared to controls ( $P < 0.05$ ). *Conclusion:* The combination of epirubicin and docetaxel as adjuvant chemotherapy for patients after radical mastectomy for breast cancer has significant clinical effects, can improve patients' quality of life, and has high safety. It is worthy of adoption.

**Keywords:** Radical mastectomy for breast cancer; Epirubicin; Docetaxel; Adjuvant chemotherapy; Clinical effective rate

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## 1. Introduction

Breast cancer is a common malignant tumor among women, and its incidence rate is increasing year by year, posing a great threat to women's health <sup>[1]</sup>. Clinically, radical mastectomy is the main treatment method, but there may be residual microscopic cancer cells in the patient's body after surgery, posing a risk of recurrence and

metastasis. Therefore, postoperative adjuvant chemotherapy is very important, as it can consolidate the surgical effect and reduce the possibility of recurrence, thereby significantly improving the long-term survival rate of patients. Epirubicin and docetaxel are commonly used chemotherapy drugs in clinical practice, and they can form a synergistic complement. Their combination chemotherapy regimen has shown good prospects in adjuvant treatment for breast cancer in recent years <sup>[2]</sup>. However, existing studies have focused more on short-term efficacy observations, and there is still a lack of research on improving patients' long-term quality of life and exploring the mechanism of chemotherapy-related metabolic disorders <sup>[3]</sup>. Based on these situations, this study included 60 patients to compare the feasibility of different chemotherapy regimens to provide more valuable references for clinical practice. See below for details.

## 2. Materials and methods

### 2.1. General information

This study enrolled 60 patients between May 2022 and December 2024, randomly allocated into two equal-sized groups ( $n = 30$  each) according to treatment protocols. The observation group comprised patients aged 56–72 years (mean  $65.56 \pm 4.56$ ) with disease duration of 3–5 years (mean  $3.56 \pm 0.23$ ), including 12 stage I, 10 stage II, and 8 stage III breast cancer cases. The control group showed similar demographics: Age 56–74 years (mean  $65.59 \pm 4.34$ ), disease duration 3–5 years (mean  $3.66 \pm 0.21$ ), with 13 stage I, 10 stage II, and 7 stage III cases. Intergroup comparisons revealed no significant differences ( $P > 0.05$ ), confirming baseline comparability.

Inclusion criteria: (1) Pathologically confirmed diagnosis of breast cancer <sup>[4]</sup>; (2) Meeting the surgical indications for radical mastectomy; (3) Informed consent from the patient and their family, with signed informed consent forms. Exclusion criteria: (1) Those with severe organ dysfunction; (2) Those with diabetes or other endocrine system diseases; (3) Those allergic to epirubicin, docetaxel, or other chemotherapy drugs; (4) Those who have recently received other anti-tumor treatments.

### 2.2. Methods

Control group: Conventional chemotherapy regimen (cyclophosphamide + fluorouracil). The dose of cyclophosphamide (Jiangsu Hengrui Medicine Co., Ltd.; National Medical Approval Number H20023036) was  $750\text{mg}/\text{m}^2$ , administered intravenously once every 21 days as a cycle. Fluorouracil (Tianjin Jinyao Pharmaceutical Co., Ltd.; National Medical Approval Number H12020959) was administered at a dose of  $500\text{mg}/\text{m}^2$  as a continuous intravenous infusion for 3–5 days per cycle, with a total of 6 cycles of chemotherapy. Patients' conditions were observed during the treatment, and in case of severe adverse reactions, medication was immediately stopped. Supportive treatment measures such as antiemetic therapy, gastric protection, and hydration were provided, and patients' heart function was closely monitored.

Observation group: Epirubicin + docetaxel combination adjuvant chemotherapy regimen. Epirubicin (Zhejiang Haizheng Pharmaceutical Co., Ltd., National Medical Approval Number H20041211) was administered at a dose of  $60\text{mg}/\text{m}^2$  intravenously once every 21 days as a cycle. Docetaxel (Jiangsu Hengrui Medicine Co., Ltd.; National Medical Approval Number H20030561) was given at a dose of  $75\text{mg}/\text{m}^2$  intravenously once every 21 days as a cycle, with a total of 6 cycles of chemotherapy <sup>[5]</sup>. Dexamethasone (Guangdong Sancai Pharmaceutical Group Co., Ltd.; National Medical Approval Number H44024276) was orally administered at 8mg twice a day, starting from the day before, the day of, and the day after docetaxel administration to prevent allergic reactions and fluid

retention. Patients' conditions were observed during the treatment, and medication was immediately stopped in case of severe adverse reactions. Supportive measures such as antiemetic therapy, gastric protection, and hydration were provided, and patients' heart function was closely monitored.

## 2.3. Observation indicators

- (1) Treatment efficacy was compared between groups using RECIST (Response Evaluation Criteria in Solid Tumors), with outcomes categorized as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). The overall response rate (ORR), defined as  $(CR + PR)/\text{total cases} \times 100\%$ , served as the primary efficacy endpoint.
- (2) Compare the incidence of adverse reactions between the two groups, including myelosuppression, such as leukopenia, thrombocytopenia, etc., gastrointestinal reactions, such as nausea, vomiting, diarrhea, etc., cardiac toxicity, liver and kidney damage, and other adverse reactions.
- (3) Glycemic abnormalities were compared between groups by measuring fasting blood glucose (FBG) and 2-hour postprandial blood glucose (2h-PBG) levels at baseline and one-month post-chemotherapy. Abnormal glucose metabolism was defined as  $FBG \geq 7.0 \text{ mmol/L}$  and/or  $2h-PBG \geq 11.1 \text{ mmol/L}$  according to standard diagnostic criteria.
- (4) Quality of life (QoL) was compared between the two groups using the Functional Assessment of Cancer Therapy-Breast (FACT-B) scale. This instrument evaluates four key domains: Physical well-being, social/family well-being, emotional well-being, and functional well-being. The total possible score ranges from 0 to 136, with higher scores reflecting better QoL outcomes.

## 2.4. Statistical methods

All data obtained in this study were processed using SPSS 22 statistical software. Measurement data were expressed as mean  $\pm$  standard deviation (SD), conforming to a normal distribution, and analyzed using the *t*-test. Count data were expressed as the number of cases and percentage (%), and analyzed using the chi-square test. A *P*-value  $< 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Comparison of clinical efficacy

**Table 1** demonstrates a significantly higher clinical efficacy rate in the observation group (96.67%) compared to the control group (80.00%), with statistical significance ( $P < 0.05$ ).

**Table 1.** Comparison of clinical efficacy between the two groups of patients [*n* (%)]

Group	Number of cases ( <i>n</i> )	CR	PR	SD	PD	Total effective rate
Observation group	30	16 (53.33)	13 (43.33)	1 (3.33)	0 (0.00)	29 (96.67)
Control group	30	12 (40.00)	12 (40.00)	4 (13.33)	2 (6.67)	24 (80.00)
$\chi^2$	-	-	-	-	-	4.043
<i>P</i>	-	-	-	-	-	0.044

### 3.2. Comparison of adverse reactions

**Table 2** demonstrates a significantly lower incidence of adverse reactions in the observation group (3.33%) compared to the control group (20.00%), with this difference reaching statistical significance ( $P < 0.05$ ).

**Table 2.** Comparison of adverse reactions between the two groups of patients [ $n$  (%)]

Group	Number of cases ( $n$ )	Bone marrow suppression	Gastrointestinal reaction	Cardiotoxicity	Liver and kidney function damage	Total incidence rate
Observation group	30	0 (0.00)	1 (3.33)	0 (0.00)	0 (0.00)	1 (3.33)
Control group	30	1 (3.33)	4 (13.34)	0 (0.00)	1 (3.33)	6 (20.00)
$\chi^2$	-	-	-	-	-	4.043
$P$	-	-	-	-	-	0.044

### 3.3. Comparison of blood glucose levels before and after treatment

As shown in **Table 3**, post-treatment fasting blood glucose (FBG) and 2-hour postprandial blood glucose (2h-PBG) levels were significantly lower in the observation group compared to the control group ( $P < 0.05$ ). However, no statistically significant difference was observed when compared to baseline values ( $P > 0.05$ ).

**Table 3.** Comparison of blood glucose levels before and after treatment between the two groups of patients (mean  $\pm$  SD)

Group	Number of cases ( $n$ )	Fasting blood glucose (mmol/L)		2-Hour postprandial blood glucose (mmol/L)	
		Before chemotherapy	After chemotherapy	Before chemotherapy	After chemotherapy
Observation group	30	5.56 $\pm$ 1.11	5.63 $\pm$ 1.15	8.78 $\pm$ 1.12	8.69 $\pm$ 1.16
Control group	30	5.52 $\pm$ 1.12	6.41 $\pm$ 1.16	8.67 $\pm$ 1.11	9.82 $\pm$ 1.19
$t$	-	0.139	2.615	0.382	3.724
$P$	-	0.890	0.011	0.704	0.001

### 3.4. Improving quality of life outcomes following intervention in the observation group

A comparison of pre- and post-treatment quality of life scores revealed that the observation group demonstrated significantly higher QoL scores than the control group following the intervention ( $P < 0.05$ , **Table 4**).

**Table 4.** Comparison of quality of life scores before and after treatment between the two groups of patients (mean  $\pm$  SD)

Group	Number of cases ( $n$ )	Quality of life score (points)	
		Before chemotherapy	After chemotherapy
Observation group	30	78.45 $\pm$ 5.67	118.23 $\pm$ 5.15
Control group	30	78.41 $\pm$ 5.48	97.34 $\pm$ 5.19
$t$	-	0.028	15.649
$P$	-	0.978	0.001



## 4. Discussion

Breast cancer, as the most common malignant tumor among women worldwide, has a grim development trend. Despite continuous improvements in early diagnosis techniques and comprehensive treatment levels, the 5-year survival rate for patients with advanced breast cancer remains below 30%, posing significant challenges to clinical treatment<sup>[6]</sup>. Surgical radical resection plays a crucial role in breast cancer treatment, but residual micrometastases after surgery are key factors leading to recurrence. Therefore, adjuvant chemotherapy has become an indispensable treatment component. Among various chemotherapy regimens, the combination of anthracyclines and taxanes has gradually become a routine choice for adjuvant breast cancer therapy, and the combined use of epirubicin and docetaxel is widely used in clinical practice<sup>[7]</sup>. Epirubicin is a typical anthracycline drug that embeds into the DNA double helix of tumor cells, interferes with the normal function of topoisomerase II, and thereby blocks DNA replication and transcription processes. Simultaneously, this drug can also generate free radicals, trigger lipid peroxidation reactions, and induce tumor cell apoptosis. Docetaxel, as a representative of taxanes, promotes microtubule protein polymerization while inhibiting its depolymerization, disrupting the normal formation of the mitotic spindle, and arresting tumor cells in the G2/M phase. The combination of these two drugs has significant advantages. Epirubicin mainly acts on the early stage of DNA synthesis, while docetaxel targets the mitotic phase. These two drugs complement each other in the cell cycle, significantly improving the pathological complete response rate in locally advanced breast cancer and surpassing single-drug treatment regimens<sup>[8]</sup>.

The results of this study showed that the clinical effective rate of the observation group was 96.67%, which was higher than the 80.00% of the control group ( $P < 0.05$ ), indicating that the adjuvant chemotherapy of epirubicin combined with docetaxel had a better effect on patients after radical mastectomy for breast cancer. Epirubicin exerts its antitumor effect by inhibiting DNA replication and transcription, while docetaxel induces apoptosis by affecting microtubule proteins, and the combination of the two can synergistically enhance efficacy<sup>[9]</sup>. At the same time, the incidence of adverse reactions in the observation group was 3.33%, which was lower than the 20.00% in the control group ( $P < 0.05$ ), and the degree was lighter, which may be related to the reasonable drug combination and pretreatment. Post-intervention analysis revealed significantly lower fasting and 2-hour postprandial blood glucose levels in the observation group compared to controls ( $P < 0.05$ ). However, no significant differences were observed when comparing these values to pre-treatment levels ( $P > 0.05$ ), suggesting the treatment regimen had minimal impact on glycemic control. Breast cancer patients may experience blood glucose fluctuations during chemotherapy due to factors such as stress response and drug side effects. The results of this study showed that the combined chemotherapy regimen did not cause significant increases or decreases in blood glucose, indicating that the regimen has a smaller impact on the endocrine system of patients and higher safety<sup>[10]</sup>. Moreover, quality of life is an important indicator to evaluate cancer treatment. Post-treatment analysis revealed significantly higher quality of life scores in the observation group compared to controls ( $P < 0.05$ ), suggesting that the adjuvant chemotherapy regimen combining epirubicin and docetaxel may enhance postoperative quality of life in breast cancer patients following radical mastectomy.

## 5. Conclusion

In summary, epirubicin combined with docetaxel for adjuvant chemotherapy after radical mastectomy for breast cancer has good efficacy and is a safe and effective chemotherapy regimen, which is suitable for clinical promotion and application.

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## Disclosure statement

The authors declare no conflict of interest.

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# A Real-world Study on Adverse Events Related to Mirogabalin Based on the VigAccess Database

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**Abstract:** *Objective:* To utilize the VigAccess database for data mining to analyze the characteristics of adverse reactions induced by mirogabalin, providing critical information for clinical medication use. *Method:* This study analyzed data from the VigAccess database, filtering out adverse reaction reports where mirogabalin was identified as the Primary Suspect Drug (PS). Reporting Odds Ratio (ROR), Proportional Reporting Ratio (PRR), Bayesian Confidence Propagation Neural Network (BCPNN), and Empirical Bayes Geometric Mean (EBGM) methods were employed as data mining algorithms for pharmacovigilance and adverse event monitoring. These methods identify potential drug-adverse event association signals by analyzing the relationship between drug use and adverse event reporting. ROR and PRR focus on calculating reporting ratios, while BCPNN and EBGM use neural networks and empirical Bayes models, respectively, to enhance the accuracy and reliability of signal detection. *Results:* A total of 734 adverse reaction reports associated with mirogabalin were obtained. The results showed that females reported the highest number of adverse reactions, accounting for 59.67%, while males accounted for 38.83%. In terms of age distribution, the highest number of reports came from individuals over 75 years old, accounting for 33.79%. Adverse reactions mainly involved the nervous system (33.45%), general diseases and reactions at the site of administration (11.62%), and gastrointestinal disorders (10.74%). The most common adverse reactions included dizziness (11.62%), somnolence (8.27%), renal dysfunction (2.90%), and edema (2.82%). Signal intensity analysis revealed that certain adverse reactions (such as renal dysfunction, rhabdomyolysis, and drug-induced liver injury) had significant signal strength, suggesting a strong association with mirogabalin. *Conclusion:* This study, through signal mining of the VigAccess database, reveals the characteristics of mirogabalin's adverse reactions in the real world, particularly in the nervous system and renal function. These findings provide important reference information for clinicians, aiding in the optimization of the safe use of mirogabalin. Future research should further validate the causal relationships of these signals and explore individualized treatment strategies to reduce the incidence of adverse reactions and improve patient outcomes.

**Keywords:** Mirogabalin; VigAccess; Adverse reactions; Signal detection; Proportional imbalance method

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## 1. Introduction

Mirogabalin is a novel calcium channel modulator developed by Daiichi Sankyo for the treatment of neuropathic pain. The drug was first approved in Japan in January 2019 for the treatment of peripheral neuropathic pain (PNP), including diabetic peripheral neuropathy (DPNP) and postherpetic neuralgia (PHN) <sup>[1]</sup>. On June 28, 2024, the China National Medical Products Administration approved mirogabalin for the treatment of diabetic peripheral neuropathy. Currently, mirogabalin is undergoing clinical development in other Asian countries but has not yet been approved in the United States, Europe, and other regions <sup>[2,3]</sup>. Since its market launch, mirogabalin has become an important choice in clinical treatment due to its significant efficacy and relatively good tolerability <sup>[4]</sup>. However, as its application expands, reports of adverse events (AEs) have also increased. Although mirogabalin has shown good safety in clinical trials, the real-world drug use environment is more complex, with factors such as individual patient differences, concomitant medication use, and long-term administration potentially significantly affecting its safety profile. In recent years, the rising incidence of neuropathic pain, epilepsy, and anxiety disorders has driven the widespread use of mirogabalin. However, existing research primarily focuses on its efficacy and short-term safety, with limited understanding of the characteristics of adverse reactions during long-term use <sup>[5]</sup>. Moreover, previous studies have largely relied on clinical trial data or small-sample retrospective analyses, which are limited by strict inclusion and exclusion criteria and may not fully reflect the drug's safety in the real world. For instance, clinical trials often exclude elderly patients, those with multiple comorbidities, or those using multiple medications, yet these populations represent a significant proportion in actual clinical practice, and their adverse event profiles may significantly differ from the trial populations.

To address this research gap, this study employs the WHO-VigiAccess database for data mining to analyze the characteristics of adverse reactions to mirogabalin in the real-world setting <sup>[6]</sup>. The VigiAccess database aggregates adverse reaction reports from around the globe, covering patients of different ethnicities, ages, genders, and disease statuses, thus providing a more comprehensive and authentic picture of drug safety <sup>[7]</sup>. Utilizing signal detection methods such as the Reporting Odds Ratio (ROR) and the Bayesian Confidence Propagation Neural Network (BCPNN), this study aims to identify potential adverse reaction signals associated with mirogabalin, particularly those not fully described in the product insert or insufficiently recognized in clinical trials. Additionally, this research focuses on the differences in adverse reactions to mirogabalin across various populations, to offer personalized treatment recommendations to clinicians, optimizing drug usage strategies, reducing the incidence of adverse reactions, and improving patients' treatment outcomes and quality of life. Through this study, we hope to provide a scientific basis for the safe use of mirogabalin and offer references for further research and regulatory decisions.

## 2. Data processing and statistical methods

### 2.1. Data source

Access the VigiAccess database (<https://vigiaccess.org/>), use Python 3.10 with the requests package to retrieve the BASENAME of all drugs from the WHO DRUG dictionary. Obtain the JSON data from the returned front-end page, visualize the JSON data using the Pandas package, and export it to Excel to complete the download of all public drug data. SAS 9.4 software is used for subsequent statistical analysis. The data retrieval date is December 29, 2024.

### 2.2. Adverse event name coding

The adverse event names in the database are encoded and corrected using MedDRA 26.1 (Medical Dictionary for Regulatory Activities, MedDRA).

### 2.3. Signal detection methods and calculation

The proportional imbalance method is used to detect signals of drug adverse events. The ROR, PRR, BCPNN, and EBGM methods are employed to detect adverse event signals. The specific calculation formulas and the definition of positive signals are provided in **Tables 1** and **2**.

**Table 1.** Two-by-two contingency table for disproportionality analysis

Item	Target adverse events reported	Other adverse events reported	Total
Target drugs	a	b	a + b
Other drugs	c	d	c + d
Total	a + c	b + d	a + b + c + d

**Table 2.** The principles of disproportionate measurement and the criteria for signal detection.

Method	Calculation formula	Criteria
ROR	$ROR = \frac{a / c}{b / d}$	$a \geq 3$ ; $ROR \geq 1$ ; 95%CI (lower limit) > 1
	$SE(\ln ROR) = \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}}$	
	$95\%CI = e^{\ln(ROR) \pm 1.96se}$	
PRR	$PRR = \frac{a / (a + b)}{c / (c + d)}$	$a \geq 3$ ; $PRR \geq 2$ ; 95%CI (lower limit) > 1
	$SE(\ln PRR) = \sqrt{\frac{1}{a} - \frac{1}{a + b} + \frac{1}{c} - \frac{1}{c + d}}$	
	$95\%CI = e^{\ln(PRR) \pm 1.96se}$	
BCPNN	$\chi^2 = \frac{(ad - bc)^2(a + b + c + d)}{(a + b)(a + c)(c + d)(b + d)}$	$a \geq 3$ ; $PRR \geq 2$ ;
	$IC = \log_2 \frac{p(x,y)}{p(x)p(y)} = \log_2 \frac{a(a+b+c+d)}{(a+b)(a+c)}$	
	$E(IC) = \log_2 \frac{(a+\gamma 11)(a+b+c+d+\alpha)(a+b+c+d+\beta)}{(a+b+c+d+\gamma)(a+b+\alpha 1)(a+c+\beta 1)}$	
BCPNN	$V(IC) = \frac{1}{(\ln 2)^2} \left\{ \left[ \frac{(a + b + c + d) - a + \gamma - \gamma 11}{(a + \gamma 11)(1 + a + b + c + d + \gamma)} \right] \right.$	IC025 > 0
	$+ \left[ \frac{(a + b + c + d) - (a + b) + \alpha - \alpha 1}{(a + b + \alpha 1)(1 + a + b + c + d + \alpha)} \right]$	
	$+ \left[ \frac{(a + b + c + d) - (a + c) + \beta - \beta 1}{(a + c + \beta 1)(1 + a + b + c + d + \beta)} \right] \left. \right\}$	
EBGM	$\gamma = \gamma 11 \frac{(a + b + c + d + \alpha)(a + b + c + d + \beta)}{(a + b + \alpha 1)(a + c + \beta 1)}$	EBGM05 ≤ 2
	$IC - 2SD = E(IC) - 2\sqrt{V(IC)}$	
	$\alpha 1 = \beta 1 = 1; \alpha = \beta = 2; \gamma 11 = 1$	
EBGM	$EBGM = \frac{a(a + b + c + d)}{(a + c)(a + b)}$	EBGM05 ≤ 2
	$SE(\ln EBGM) = \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}}$	
	$95\%CI = e^{\ln(EBGM) \pm 1.96se}$	



2.4. Objective drug

The drug names in the database are standardized using the WHODrug Global dictionary. The target drug, mirogabalin, is screened based on the standardized drug names (DRUGNAME) and active ingredients (PROD\_AI).

3. Results

3.1. Basic characteristics of adverse event reports

A total of 24,156 drugs with baseline information and 24,131 drugs with adverse event information were identified, involving 117,935,336 adverse event reports from 46,535,254 patients. Among these, 734 adverse events were reported for mirogabalin. The data show that in terms of gender, the majority of adverse events were reported by females, accounting for 59.67% of the total, while males accounted for 38.83%. In terms of age distribution, the highest number of reports came from individuals over 75 years old, accounting for 33.79%; those over 45 years old accounted for 83.66%. Regional distribution indicated that all reports originated from Asia. The reporting years were mainly concentrated in 2024, accounting for 96.05%, with the first adverse reaction report appearing in 2018. Overall, the reports related to mirogabalin primarily involved females and middle-aged to older adults (Table 3).

Table 3. Characteristics of AEs reports

Characteristics	n (%)
Sex	
Female	438 (59.67)
Male	285 (38.83)
Unknown	11 (1.5)
Age	
18–44 years	56 (7.63)
45–64 years	176 (23.98)
65–74 years	190 (25.89)
>75 years	248 (33.79)
Unknown	64 (8.72)
Continent	
Asia	734 (100)
Report year	
2018	1 (0.14)
2019	14 (1.91)
2020	2 (0.27)
2021	2 (0.27)
2022	2 (0.27)
2023	8 (1.09)
2024	705 (96.05)

3.2. Proportion of adverse events by System Organ Class (SOC)

As can be seen from the chart, the adverse events related to mirogabalin are mainly concentrated in the nervous system, general disorders and administration site conditions, and gastrointestinal diseases. Among these, adverse events related to nervous system disorders have the highest proportion, accounting for 33.45%, with a total of 380 cases. This indicates that nervous system disorders are the most common type of adverse reactions associated with mirogabalin. Following this, general disorders and administration site conditions account for 11.62%, with 132 cases; gastrointestinal diseases account for 10.74%, with 122 cases. Other common adverse events include renal and urinary disorders (5.55%, 63 cases), investigations (5.28%, 60 cases), and injuries, poisoning, and procedural complications (4.67%, 53 cases). Relatively less frequent adverse events such as psychological disorders, infections and infestations, ear and labyrinth disorders, etc., are all below 3% in proportion. Additionally, there are some system organ classes with a very low incidence of adverse events, such as endocrine disorders and immune system disorders, each accounting for only 0.18%, with only 2 cases. Some categories, like congenital, genetic, and familial diseases, as well as product issues, have not reported any adverse events. These data suggest that the use of mirogabalin may lead to a higher proportion of adverse events related to the nervous system and gastrointestinal tract, but the overall range of systems and organs involved in adverse events is broad, which should be of concern and vigilance to healthcare professionals (Figure 1).

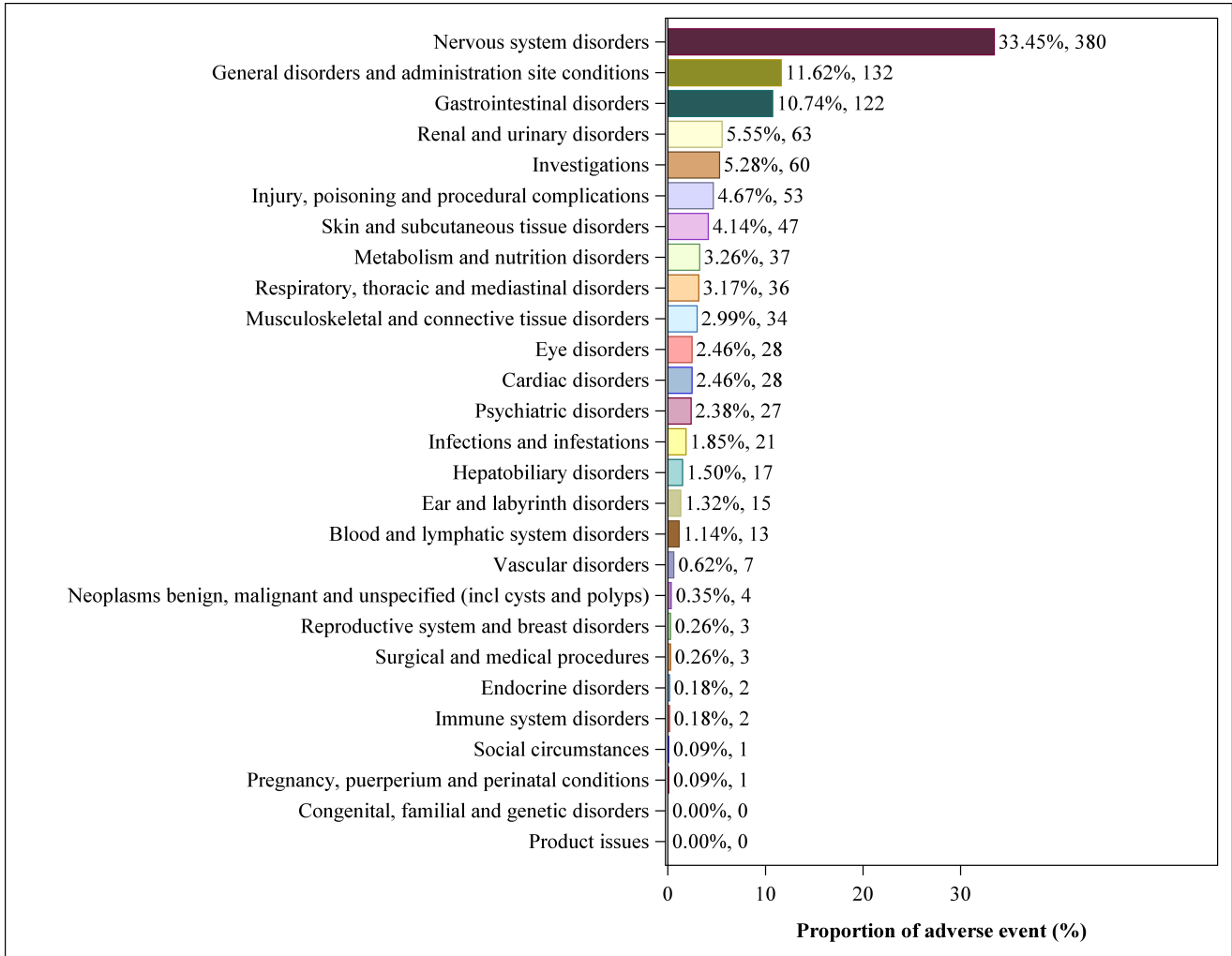
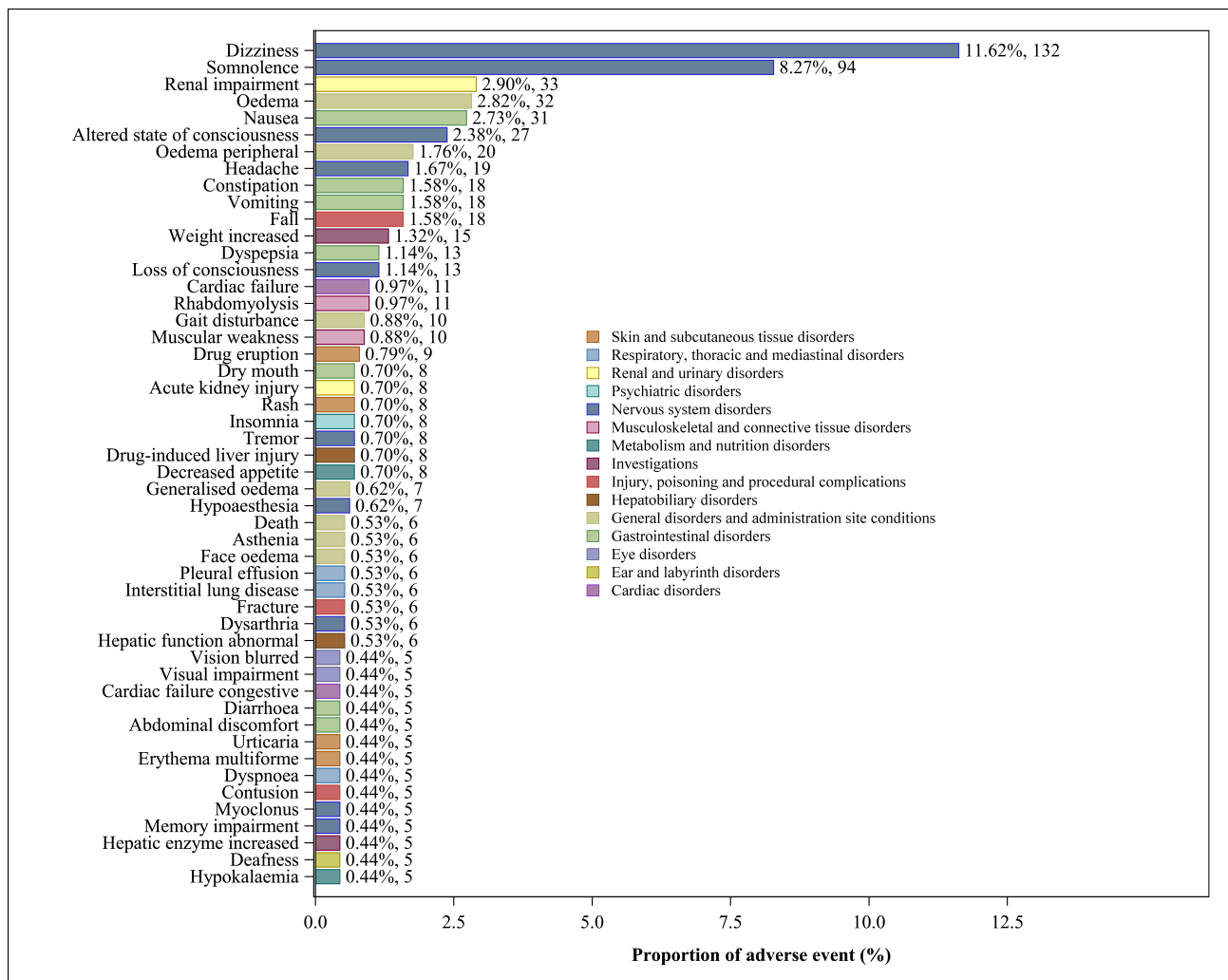


Figure 1. Proportion of adverse events by SOCs

3.3. Top 50 PTs (Preferred Terms) by frequency for adverse events

Based on the chart, the adverse events related to mirogabalin analyzed in the VigAccess database mainly

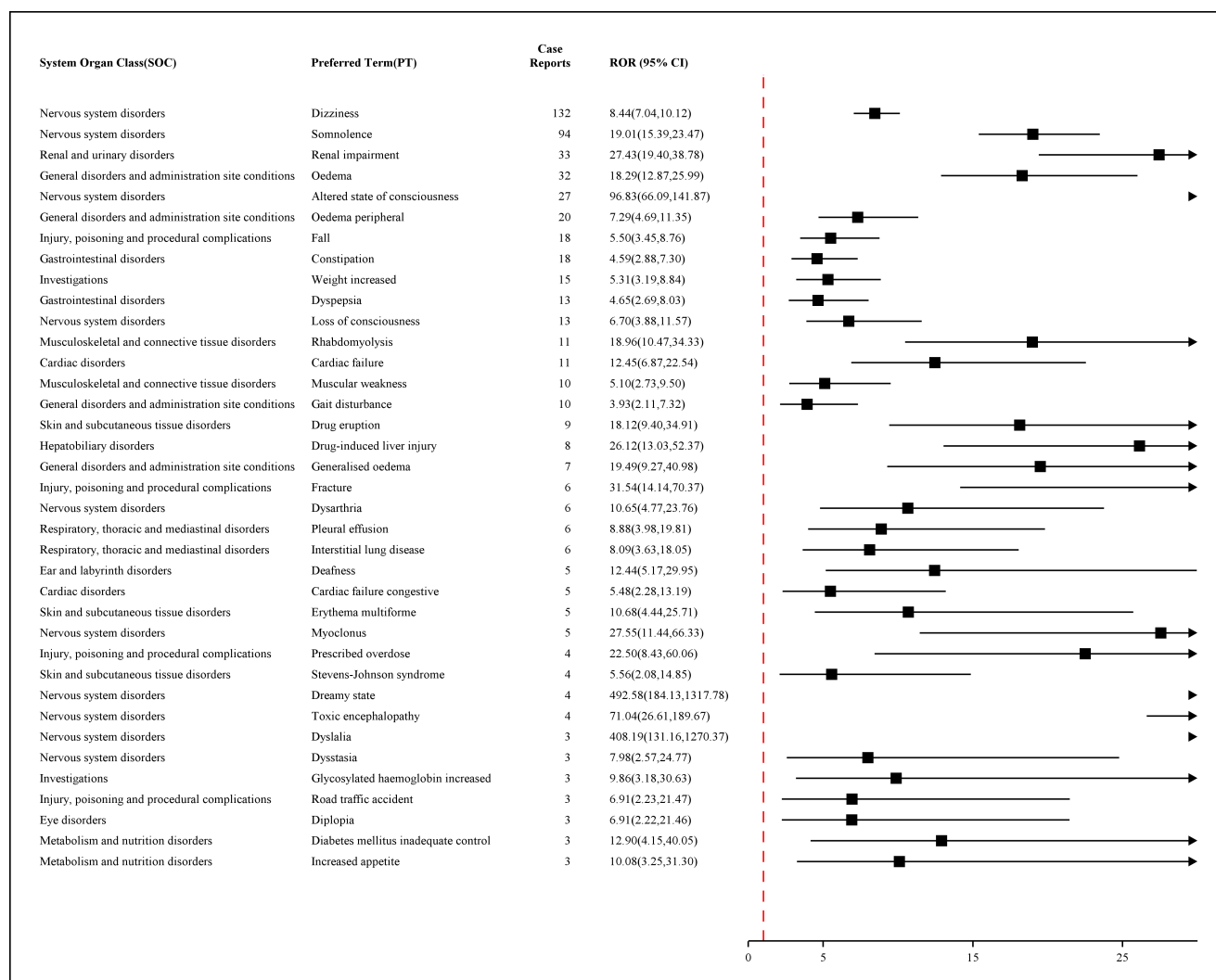
focus on symptoms such as dizziness and somnolence. Dizziness is the most common adverse reaction, with an incidence rate of 11.62%, totaling 132 cases reported; Somnolence follows closely, with an incidence rate of 8.27%, reporting 94 cases. This indicates that mirogabalin has a significant impact on the central nervous system. Additionally, the incidence rates of renal impairment and oedema are also relatively high, at 2.90% (32 cases) and 2.82% (31 cases), respectively. Other common adverse reactions include nausea (2.73%, 31 cases), altered state of consciousness (2.38%, 27 cases), peripheral oedema (1.76%, 20 cases), and headache (1.67%, 19 cases). Further analysis reveals that adverse reactions related to various systems and organs have also been reported, such as constipation (1.58%, 18 cases), vomiting (1.58%, 18 cases), and increased weight (1.32%, 15 cases). Beyond the nervous system, important adverse reactions also include cardiac failure (0.97%, 11 cases), rhabdomyolysis (0.97%, 11 cases), as well as various skin and musculoskeletal system diseases. Overall, the adverse reactions of mirogabalin mainly concentrate on the nervous system and renal function, but also involve multiple systems and organs. This suggests that clinicians should pay particular attention to monitoring patients' nervous system symptoms and renal function when using this drug, while also being vigilant for other potential adverse events (**Figure 2**).



**Figure 2.** Top 50 PTs by frequency for adverse events

### 3.4. Frequency forest plot of the top 50 with simultaneous positive signals in four methods

Four algorithms all indicate that the top 50 adverse reaction PTs with positive signals are those with a high frequency of occurrence. A high frequency of adverse reactions suggests that they are more commonly reported in the database, which may indicate that these reactions are more common in actual use. The chart shows that the adverse reactions of mirogabalin mainly concentrate on the nervous system and renal function. The most common adverse reactions include dizziness (132 cases, ROR=8.44), somnolence (94 cases, ROR=19.00), renal impairment (32 cases, ROR=27.43), oedema (32 cases, ROR=9.15), and altered state of consciousness (27 cases, ROR=26.89). These adverse reactions all show a high Relative Reporting Rate (ROR), suggesting that mirogabalin may significantly increase the risk of these symptoms. Additionally, symptoms such as peripheral oedema (18 cases, ROR=7.29), constipation (18 cases, ROR=4.59), increased weight (15 cases, ROR=5.31), and rhabdomyolysis (11 cases, ROR=18.96) also exhibit high ROR values, indicating a strong correlation with mirogabalin. Less frequent reports include cardiac failure (11 cases, ROR=12.45), drug-induced liver injury (8 cases, ROR=26.12), and generalized oedema (7 cases, ROR=19.49). Although the number of reports for these adverse reactions is relatively low, their high ROR values also suggest potential risks. Moreover, serious adverse events such as amnesia (memory impairment), hypokalemia, and death, although reported in small numbers, also require attention (**Figure 3**).



**Figure 3.** Frequency forest plot of the top 50 with simultaneous positive signals in four methods

3.5. Four methods with simultaneous positive signals

Four types of algorithms all indicate a positive signal, with the top 50 adverse reaction preferred terms listed by ROR signal strength. A strong signal strength suggests a significantly higher association between a particular drug and an adverse reaction than other drugs, which may imply a causal relationship between the drug and the adverse reaction. First, the chart shows that the signal strength for nervous system-related adverse reactions is most significant. For example, “Dreamy state” has the highest ROR value at 492.58 (95% CI: 184.13, 1317.78), followed by “Dyslalia” with an ROR value of 408.19 (95% CI: 131.16, 1270.37). Additionally, “Altered state of consciousness” (96.86, 95% CI: 60.19, 141.87) and “Toxic encephalopathy” (71.04, 95% CI: 26.61, 189.67) also exhibit very high signal strength. Adverse reactions related to the kidneys and liver, such as “Renal impairment” (ROR=27.43, 95% CI: 19.40, 38.78) and “Drug-induced liver injury” (ROR=26.12, 95% CI: 13.03, 52.37), also show significant signal strength. Other systemic adverse reactions, such as “Rhabdomyolysis” (ROR=18.96, 95% CI: 10.47, 34.33), “Generalized oedema” (ROR=19.49, 95% CI: 9.27, 40.99), and “Drug eruption” (ROR=18.12, 95% CI: 10.49, 34.01) also demonstrate higher ROR values. This underscores the need for close monitoring of these potential high-risk adverse reactions when using mirogabalin, particularly for the symptoms with high signal strength mentioned above, and to take appropriate preventive and therapeutic measures promptly to ensure patient safety (Figure 4).

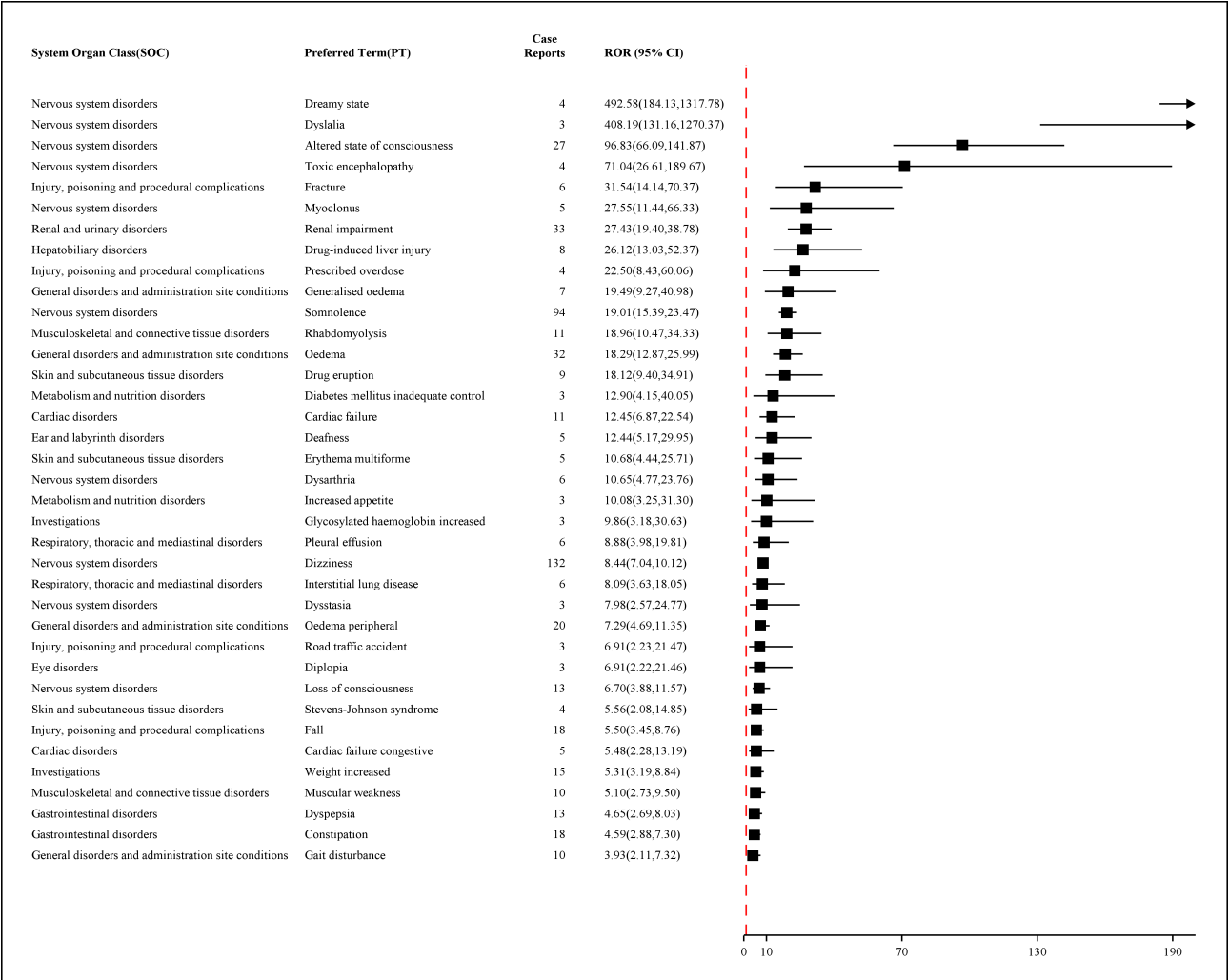


Figure 4. Four methods with simultaneous positive signals



## 4. Discussion

This study conducted a comprehensive analysis of the adverse reactions of mirogabalin using the WHO-VigiAccess database. The results revealed that the adverse reactions of mirogabalin primarily involve the nervous system and renal function. Dizziness and somnolence are the most common adverse reactions, accounting for 11.62% and 8.27%, respectively. These findings are closely related to the mechanism of action of mirogabalin, which affects central nervous system activity by regulating calcium channels, potentially leading to neurological symptoms such as dizziness and somnolence<sup>[8,9]</sup>. Additionally, the incidence of renal impairment and oedema is also relatively high, at 2.90% and 2.82% respectively, which may be associated with the drug's metabolic pathway and renal excretion function<sup>[10]</sup>.

Compared with the existing literature, the results of this study are consistent in some aspects. Previous studies have also reported that mirogabalin can cause adverse reactions such as dizziness, somnolence, and renal impairment. However, this study also identified some less commonly mentioned adverse reactions, such as rhabdomyolysis and drug-induced liver injury, which have not been detailed in other studies. This indicates that the VigiAccess database can provide more comprehensive information on drug safety, especially in real-world settings<sup>[6,7]</sup>.

The molecular structure and mechanism of action of mirogabalin may explain the characteristics of its adverse reactions. As a calcium channel modulator, mirogabalin exerts its therapeutic effect by inhibiting the  $\alpha_2\delta$  subunit of voltage-gated calcium channels, thereby reducing the release of neurotransmitters<sup>[11]</sup>. However, this mechanism of action may also lead to adverse reactions in the central nervous system, such as dizziness and somnolence. Moreover, since mirogabalin is primarily excreted through the kidneys, the occurrence of renal impairment and oedema may be related to reduced drug excretion<sup>[12]</sup>.

The findings of this study have significant implications for clinical practice. Firstly, clinicians should closely monitor patients for neurological symptoms and renal function when prescribing mirogabalin, especially in high-risk populations such as the elderly and patients with impaired renal function<sup>[13]</sup>. Secondly, for patients reporting adverse reactions such as dizziness and somnolence, adjustments to the drug dosage or a change in treatment regimen may be necessary<sup>[14]</sup>. Additionally, less commonly mentioned but potentially serious adverse reactions, such as rhabdomyolysis and drug-induced liver injury, should also be given sufficient attention for timely prevention and treatment measures<sup>[15]</sup>.

Although this study provides valuable information on the adverse reactions of mirogabalin, it also has some limitations. Firstly, the data in the VigiAccess database relies on voluntary reporting, which may be subject to reporting biases and incomplete information. Secondly, signal detection methods, such as ROR and BCPNN can identify associations between drugs and adverse reactions, but cannot establish causality. Therefore, future studies need to validate the causal relationships of these signals through more rigorous prospective studies and cohort studies.

Future research can further explore the safety and efficacy of mirogabalin in different populations, particularly the long-term safety. Additionally, combining genomics and pharmacokinetic studies can help better understand the impact of individual differences on adverse drug reactions. Finally, developing more accurate prediction models for adverse reactions can help identify high-risk patients earlier in clinical practice, thereby improving the safety of drug use.

## 5. Conclusion

This study, through signal detection in the WHO-VigiAccess database, has uncovered the characteristics of adverse reactions to mirogabalin in the real-world setting, particularly to the nervous system and renal function. These findings not only provide valuable reference information for clinicians but also contribute to the optimization of the safe use of mirogabalin. Future research should further validate the causal relationships of these signals and explore individualized treatment strategies to reduce the incidence of adverse reactions and improve patient outcomes.

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## Disclosure statement

The authors declare no conflict of interest.

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# Research Progress of Traditional Chinese Medicine Regulating PI3K/AKT/mTOR Signaling Pathway to Improve Myocardial Ischemia-Reperfusion Injury

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**Abstract:** PI3K/AKT/mTOR signaling pathway is a key pathway of myocardial ischemia-reperfusion injury (MIRI). The mechanism of action is mainly oxidative stress, inflammatory response, calcium overload, ferroptosis, autophagy, and apoptosis. MIRI belongs to the category of chest obstruction in traditional Chinese medicine, and its etiology and pathogenesis are mainly “Yang Wei Yin Xian.” Traditional Chinese medicine has the effect of multi-target and multi-component effect, and has played a significant role in the treatment of MIRI in recent years. At present, the monomers of traditional Chinese medicine mainly include saponins, flavonoids, alkaloids, terpenoids, and phenols, and the compounds mainly include Zhigancao Decoction, Zhenyuan Capsule, Jiawei Shenqibai Powder, Qili Qiangxin Capsule, Tongmai Yangxin Pill, Zhilong Huoxue Tongyu Capsule, Guizhi Tongluo Tablets, etc. This paper reviews the research on the improvement of MIRI by regulating PI3K/ AKT/mTOR signaling pathway in recent years, and expounds the mechanism and advantages of traditional Chinese medicine in the treatment of MIRI.

**Keywords:** Traditional Chinese medicine; PI3K/AKT/mTOR signaling pathway; Myocardial ischemia-reperfusion injury; Review

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## 1. Introduction

Myocardial ischemia is a primary feature of acute coronary syndrome (ACS). The ACCF/AHA/SCAI guidelines recommend early reperfusion therapy to limit myocardial damage and preserve tissue viability <sup>[1]</sup>. However, reperfusion therapy can trigger adverse cardiovascular outcomes following myocardial ischemia, cardiac surgery, or circulatory arrest <sup>[2]</sup>. In recent years, research on myocardial ischemia-reperfusion injury (MIRI) has been continuously proposed to reduce infarct size, with effective targets and signaling pathways becoming key to treatment. Studies have confirmed that the phosphatidylinositol 3-kinase/protein kinase B/mechanistic target of rapamycin (PI3K/AKT/mTOR) pathway is one of the important signaling pathways involved in MIRI <sup>[3]</sup>,

playing a major role in oxidative stress, autophagy, and apoptosis. Traditional Chinese medicine, with its multi-component, multi-target, and multi-effect approach, has an advantage in treating MIRI. This article reviews recent literature on the regulation of the PI3K/AKT/mTOR pathway by traditional Chinese medicine in the treatment of MIRI.

## **2. Understanding of myocardial ischemia-reperfusion injury in traditional medicine**

In traditional medicine, MIRI is categorized under “chest bi” and “heart palpitations.” The “Yellow Emperor’s Inner Canon” records that “when pathogens invade the heart, it causes heart pain.” Later, the “Jin Kui Yao Lue” proposed the pathogenesis of “Yang deficiency and Yin excess,” meaning a lack of chest Yang and condensation of Yin cold. This condition is caused by a deficiency in origin and an excess in manifestation. Subsequent physicians believed that Yang deficiency is the root deficiency, which can manifest as qi deficiency and Yang deficiency, not limited to the heart. Yin excess is the excess manifestation, which can be caused by cold pathogens, phlegm, turbidity, blood stasis, etc <sup>[4,5]</sup>. The heart is the Yang within Yang, and all Yang qi gathers in the chest. The warming and promoting effects of heart Yang ensure the continuous circulation of blood. If chest Yang is insufficient, turbid Yin can cause heart pain, ultimately leading to blockage of the heart vessels. Clinically, both root deficiency and manifestation excess are often equally important. Chen Bojun’s research on the TCM syndrome differentiation of 71 patients after coronary intervention showed that qi deficiency and phlegm-turbidity syndromes were significantly aggravated after surgery, and blood stasis remained the main pathological product and pathogenic factor <sup>[6]</sup>, indicating that both the root and manifestation should be treated. Based on different evolutionary forms of its overall pathogenesis, later physicians proposed different dialectical treatment principles. For example, when Yang deficiency is predominant, the treatment focuses on warming the spleen and kidneys, promoting diuresis, and clearing Yang. Spleen and kidney Yang deficiency can lead to abnormal distribution of body fluids, exacerbating chest bi due to the growth of phlegm-turbidity and retained fluid <sup>[7]</sup>. When Yin excess is predominant, the treatment focuses on warming Yang and resolving phlegm. Besides the theory of “Yang deficiency and Yin excess,” the theory of blood stasis and toxicity is also considered a major etiology and pathogenesis of MIRI. Some studies suggest that mitochondrial dysfunction in cardiomyocytes during MIRI can be interpreted as “qi deficiency and blood stasis.” Animal experiments using an ischemia-reperfusion injury rat model have been conducted to explain the pathogenesis of qi deficiency and blood stasis in MIRI <sup>[8]</sup>, focusing on myocardial energy metabolism pathways, myocardial mitochondrial complexes and their subunits, regulation of mitochondrial complex deacetylase-1, the RhoA/Rock1 of the small G protein family, cytoskeleton, myocardial structure, heart function, and cardiac microcirculation dynamics. In addition to the theory of blood stasis and toxicity, some scholars categorize MIRI as belonging to the “Jueyin” category, believing that the disease belongs to Jueyin disease with a lesion location in the pericardium. The symptoms of reperfusion arrhythmia can be attributed to “thirst, qi rushing upward, heat and pain in the heart, hunger but no desire to eat, vomiting of roundworms after eating, and incessant diarrhea after purgation.” It is believed that MIRI is caused by the disconnection between Yin and Yang, with Yin predominating and Yang being deficient <sup>[9]</sup>. This coincides with the theory of “Yang deficiency and Yin excess,” and treatment should focus on supporting Yang and promoting blood circulation.



### 3. The mechanism of PI3K/AKT/mTOR signaling pathway in MIRI

#### 3.1. Overview of the PI3K/AKT/mTOR signaling pathway

PI3K/AKT/mTOR stands for phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin. PI3K, as the starting point of the signaling pathway, is a dimer complex with phosphatidylinositol kinase activity, which can trigger AKT activation and participate in autophagy regulation<sup>[10]</sup>. AKT, also known as PKB, is the hub of the signaling pathway. After AKT is activated by PI3K, it can promote the further activation of its downstream molecule mTOR through the TSC1/2 complex<sup>[11]</sup>. AKT is an important protein for maintaining cell homeostasis, while mTOR belongs to PIKK and plays a crucial role in cell growth and proliferation. Its two complexes, mTORC1 and mTORC2, control ribosomes and are pathways for autophagy and growth metabolism, while the latter mainly activate and control proteins<sup>[12,13]</sup>. PI3K/Akt is the core pathway leading to mTOR. Akt phosphorylates the Ser2448 binding site to activate mTORC1. AKT can directly or indirectly affect mTOR, promoting cell proliferation and migration, which further leads to increased oxidative stress and autophagy<sup>[14,15]</sup>.

#### 3.2. Research progress on PI3K/AKT/mTOR regulation in MIRI

The role of PI3K/AKT/mTOR in MIRI is currently primarily achieved through oxidative stress, inflammatory response, calcium overload, ferroptosis, autophagy, and apoptosis. Oxidative stress (OS) is considered a major factor in MIRI<sup>[16]</sup>. The restoration of blood flow to ischemic parts increases oxygen, leading to excessive production of reactive oxygen species (ROS), which can damage cellular macromolecules and cause cell death<sup>[17]</sup>. ROS participates in membrane phospholipid reactions, directly disrupting cell membrane permeability and ion channels, leading to irreversible cell damage. Its production is closely related to the mitochondrial electron transport chain system, NADPH oxidase system, xanthine oxidase (XO) system, and uncoupled nitric oxide synthase<sup>[18,19]</sup>. Studies have shown that the PI3K/AKT/mTOR signaling pathway is mainly mediated by ROS and is a key pathway in oxidative stress<sup>[20]</sup>. NADPH, a pyridine dinucleotide cofactor, is a crucial electron reservoir for biosynthetic reduction and defense against oxidative stress, and its level can be influenced by PI3K-AKT signaling<sup>[21]</sup>. The inflammatory response also plays a significant role in MIRI. Studies have indicated that during MIRI, inflammatory factors such as IL-6, IL-1 $\beta$ , TNF- $\alpha$ , IL-10, and ICAM-1 significantly increase, and reducing these factors' release can significantly alleviate myocardial cell damage<sup>[22]</sup>. The PI3K/AKT/mTOR signaling pathway regulates downstream cell proliferation and inflammatory target gene expression<sup>[23]</sup>. Research suggests that this pathway can modulate the expression levels of ALT, AST, IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and ammonia in blood, reducing cell necrosis and inflammatory responses. During myocardial ischemia-reperfusion, ischemia and hypoxia lead to intracellular Ca<sup>2+</sup> overload, further causing myocardial cell damage<sup>[24]</sup>. Studies have shown that the PI3K/AKT/mTOR pathway participates in regulating vesicular transport and controlling factors like Ca<sup>2+</sup> and calpain, affecting MIRI<sup>[25]</sup>. In 2012, scholars first proposed the role of ferroptosis in cellular metabolism and protein regulation mechanisms<sup>[26]</sup>. Iron metabolism disorders can cause iron overload, leading to mitochondrial abnormalities and exacerbating ROS production<sup>[27]</sup>. Ferroptosis can also cause immune dysregulation and mediate inflammatory responses<sup>[28]</sup>. The aforementioned ROS mediates the PI3K/AKT/mTOR signaling pathway, indirectly proving a connection between ferroptosis, PI3K/AKT/mTOR, and MIRI. PI3K participates in cell membrane formation, and once activated by PI3K, Akt transfers to the cell membrane, phosphorylating mTOR and regulating autophagy. mTORC1 can inhibit autophagy-related proteins like ULK1 and ULK2, thereby suppressing autophagy<sup>[29]</sup>. Studies have confirmed that PI3K inhibitors can reduce ROS

levels and improve ferroptosis<sup>[30]</sup>. Ferroptosis is related to autophagy, and MIRI is associated with ferroptosis, indirectly clarifying the relationship between MIRI and autophagy. The PI3K/AKT/mTOR signaling pathway is a signal transduction pathway for autophagy, which can inhibit autophagy to a certain extent, thereby improving MIRI<sup>[31]</sup>. As a programmed cell death, apoptosis can be directly induced by MIRI or indirectly triggered by oxidative stress and inflammatory responses. Oxidative stress and inflammatory factors caused by reperfusion therapy can activate the PI3K/AKT/mTOR signaling pathway, inducing myocardial cell apoptosis<sup>[32]</sup>. Current research suggests that MIRI is closely linked to oxidative stress, ferroptosis, calcium overload, apoptosis, and autophagy. The tight interconnection and mutual influence among various mechanisms, coupled with the significant role of the PI3K/AKT/mTOR signaling pathway in these mechanisms, confirm its crucial regulatory function in MIRI. Traditional Chinese medicine can multi-target and multi-actively regulate the PI3K/AKT/mTOR signaling pathway, holding profound clinical significance for improving MIRI.

#### **4. Research progress on the improvement of MIRI by regulating the PI3K/AKT/mTOR signaling pathway with traditional Chinese medicine**

The mechanism of MIRI is intricately linked to oxidative stress, inflammatory response, calcium overload, ferroptosis, autophagy, and apoptosis. Western medicine has achieved precise targeted therapy with moderate efficacy, but its limitation lies in the inability to regulate multiple targets simultaneously. Relevant studies have demonstrated that traditional Chinese medicine, including single herbs and compound prescriptions, possesses rich bioactive components and exhibits significant advantages in treating MIRI by regulating the PI3K/AKT/mTOR signaling pathway through various targets<sup>[33]</sup>.

##### **4.1. Research progress on the improvement of MIRI by regulating PI3K/AKT/mTOR with single herbs in traditional Chinese medicine**

Current research has identified several types of single herbs that regulate the PI3K/AKT/mTOR signaling pathway to improve MIRI, mainly including saponins, flavonoids, alkaloids, phenols, and terpenes<sup>[34]</sup>.

###### **4.1.1. Saponins**

Saponins are widely found in plants of the Araliaceae family, such as ginseng and *Panax notoginseng*, and have been extensively studied for their roles in treating not only MIRI but also cancer. In exploring the effect and mechanism of ginsenoside Rg5 (G-Rg5) against T-cell acute lymphoblastic leukemia CCRF-CEM cells, it was found that G-Rg5 can significantly reduce the activity of P-PI3K, P-AKT, and P-mTOR protein expression<sup>[35]</sup>. It may also resist cell proliferation and improve MIRI by inhibiting the PI3K/AKT/mTOR pathway and avoiding apoptosis. Besides ginsenoside, experimental studies have shown that astragaloside IV also improves MIRI-induced cardiomyocyte injury by regulating the PI3K/AKT/mTOR pathway to inhibit cell proliferation, invasion, and metastasis<sup>[36]</sup>. When investigating the regulation of autophagy and reduction of cellular hypoxia by *Panax notoginseng* saponins (PNS) and the PI3K/Akt/mTOR signaling pathway, it was discovered that PNS can modulate this pathway to inhibit autophagy and alleviate cellular hypoxia. Saponins can indeed improve MIRI through their regulation of autophagy, antioxidation, and other effects via the PI3K/Akt/mTOR signaling pathway.

#### 4.1.2. Flavonoids

Flavonoids exhibit a variety of biological activities. Total flavonoids of *Bidens bipinnata* (TFB) are the main extract from *Bidens bipinnata* and possess anti-inflammatory and antioxidant properties <sup>[37]</sup>. Dong Fengmei and colleagues confirmed through rat experiments that TFB can lower the systolic blood pressure of hypertensive rats <sup>[38]</sup>, inhibit cardiomyocyte autophagy via the PI3K/Akt/mTOR pathway, reduce oxidative stress and apoptosis, and significantly increase glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) while significantly decreasing endogenous hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), malondialdehyde (MDA), and reactive oxygen species (ROS). The effects are also associated with changes in Beclin-1 and LC3B-II/LC3B-I ratios. Studies have indicated that Hibiscus leaf polyphenols (HLP) protect against cellular damage by modulating the PI3K/Akt/mTOR signaling pathway, reducing oxidative stress and autophagy <sup>[39]</sup>. Another experimental study has demonstrated that total flavonoids from Aromatic Xintahua may alleviate atherosclerosis by regulating the PI3K/Akt/mTOR signaling pathway <sup>[40]</sup>, reducing atherosclerotic plaque area and collagen levels in apolipoprotein E gene knockout mice, inhibiting hepatic lipid deposition and collagen expression, modulating autophagy, and suppressing inflammatory responses. As autophagy, oxidative stress, and apoptosis are major mechanisms of MIRI, flavonoids can regulate the PI3K/Akt/mTOR signaling pathway to ameliorate reperfusion injury.

#### 4.1.3. Alkaloids

Alkaloids are mainly found in plants in nature, such as the Ranunculaceae, Menispermaceae, and Rutaceae families, and exist in the above plant species in forms such as glycosides, amides, and N-oxides. Sun Dafang and others discovered when studying the effect of total alkaloids from *Strychnos nux-vomica* on rheumatoid arthritis (RA) rats in the PI3K/Akt/mTOR signaling pathway that total alkaloids from *Strychnos nux-vomica* can reduce the levels of IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and RANKL in synovial tissue of rats with rheumatoid arthritis, possibly improving inflammatory responses by inhibiting the PI3K/Akt/mTOR pathway <sup>[41]</sup>. The alkaloid components in *Corydalis* can regulate the PI3K/AKT signaling pathway to reduce the production of CK, LDH, and MDA, exerting antioxidant and anti-inflammatory effects and reducing myocardial cell damage <sup>[42]</sup>.

#### 4.1.4. Terpenes

Triterpenes are terpene compounds that exist in plants in free form or as glycosides and esters. *Ilex pubescens* triterpenoid saponins are derived from *Ilex pubescens*. Studies have shown that *Ilex pubescens* triterpenoid saponins can regulate the PI3K/Akt part of the PI3K/Akt/mTOR signaling pathway to improve myocardial ischemia <sup>[43]</sup>, indicating in a sense that *Ilex pubescens* triterpenoid saponins can reduce the degree of MIRI. Total triterpenes from *Chaenomeles speciosa* are important active ingredients of *Chaenomeles speciosa*. Studies on the mechanism of action between total triterpenes from *Chaenomeles speciosa* and gastric cancer cell mitochondria have found that total triterpenes from *Chaenomeles speciosa* can inhibit mitochondrial activity and cell proliferation in cancer HGC-27 cells, which may be related to the PI3K/Akt/mTOR/p70S6K signaling pathway <sup>[44]</sup>. This suggests that total triterpenes from *Chaenomeles speciosa* can regulate the PI3K/Akt/mTOR signaling pathway to exert good antioxidant stress activity and, to some extent, improve the oxidative stress response of MIRI to protect myocardial cells.

#### 4.1.5. Phenols

Phenols are widely found in various traditional Chinese medicines. For example, magnolol is one of the effective active ingredients of *Magnolia officinalis*, and it has antioxidant, anti-inflammatory, and autophagy-regulating effects in cardiovascular diseases <sup>[45]</sup>. Current research has found that magnolol can improve lipopolysaccharide-induced myocardial injury in mice by inhibiting the TLR4/PI3K/Akt/mTOR signaling pathway and upregulating autophagy to reduce autophagy <sup>[46]</sup>. *Salvia miltiorrhiza* is widely used in various cardiovascular diseases, such as acute myocardial infarction and angina pectoris. Salvianolic acid B is the main effective component of *Salvia miltiorrhiza*. Studies have shown that salvianolic acid B can enhance the proliferation of senescent macrophages and reduce apoptosis and inflammatory responses by regulating the PI3K/AKT/mTOR signaling pathway <sup>[47]</sup>. It can also reverse the senescence of mesenchymal stem cells, promote cell proliferation to avoid excessive apoptosis, and promote autophagy to resist myocardial fibrosis <sup>[48,49]</sup>. Eugenol can improve mitochondrial dysfunction by reducing cellular ROS and superoxide anion levels through the PI3K/AKT pathway, thereby reducing oxidative stress and improving MIRI <sup>[50]</sup>.

### 4.2. Research progress on the improvement of MIRI by regulating PI3K/AKT/mTOR with traditional Chinese medicine compounds

#### 4.2.1. Zhigancao Decoction

Derived from the “Treatise on Febrile Diseases,” Zhigancao Decoction treats “palpitations and irregular pulses.” It has significant clinical effects on arrhythmias, viral myocarditis, dilated cardiomyopathy, and coronary heart disease <sup>[51]</sup>. Zheng *et al.* explored the mechanism of Zhigancao Decoction (consisting of 12g licorice, 9g ginger, 9g cassia twig, 6g ginseng, 50g rehmannia root, 6g donkey-hide gelatin, 10g ophiopogon root, 10g hemp seed, and 10 dates) on rats with MIRI-induced arrhythmias <sup>[52]</sup>. The results showed that Zhigancao Decoction significantly reduced myocardial enzymes CK, LDH, AST, and CtnI, inhibited autophagy, upregulated the expression of PI3K, Akt, and mTOR, and effectively regulated the PI3K/AKT/mTOR signaling pathway to resist reperfusion arrhythmias.

#### 4.2.2. Zhenyuan Capsule

Zhenyuan Capsule is a traditional Chinese medicine preparation with ginseng fruit saponins as the main component, including Rb1, Rb2, Rc, Rd, Re, Rg1, Rg2, etc., among which ginsenoside Re accounts for up to 85%. Ginseng greatly replenishes qi, and saponin compounds have significant antioxidant effects. Based on this, the traditional Chinese medicine preparation Zhenyuan Capsule was developed. Studies exploring the mechanism of Zhenyuan Capsule in treating MIRI rat models have found that it can resist oxidative stress, inhibit myocardial cell damage, and inhibit cell apoptosis and autophagy by activating the PI3K/Akt/mTOR signaling pathway <sup>[53]</sup>.

#### 4.2.3. Modified Shenqi Posan

Modified Shenqi Posan consists of American ginseng, pseudo-ginseng, amber, turmeric, and St. John’s wort. It originates from Professor Yue Meizhong, a famous traditional Chinese medicine practitioner, and is modified from “Ginseng and Pseudo-Ginseng Amber Powder.” In this formula, ginseng is the monarch ingredient, greatly replenishing qi; pseudo-ginseng and amber are the minister ingredients, working together to promote blood circulation and remove blood stasis, and calm the nerves; turmeric and St. John’s wort are added as assistants



to enhance the effects of soothing the liver and regulating qi, promoting blood circulation and relieving depression. Studies have shown that Modified Shenqi Posa inhibits autophagy by suppressing the PI3K/Akt/mTOR signaling pathway, reduces inflammatory responses to protect against MIRI, and also has antidepressant effects <sup>[54]</sup>.

#### 4.2.4. Qili Qiangxin Capsule

Qili Qiangxin Capsule, whose main ingredients include *Astragalus*, *Ginseng*, *Radix Aconiti Lateralis Preparata*, *Salviae Miltiorrhizae Radix et Rhizoma*, *Semen Lepidii Apetalae*, *Alismatis Rhizoma*, *Polygonatum Odoratum*, *Cassia Twig*, *Carthami Flos*, *Citrus Reticulata Pericarpium*, and *Cortex Periplocae*, can nourish qi and invigorate blood circulation, warm yang, and facilitate diuresis. Studies on the mechanism of Qili Qiangxin Capsule's effect on MIRI rats have confirmed that it can regulate the PI3K/AKT/FOXO3 signaling pathway to modulate autophagy, reduce cardiomyocyte apoptosis, maintain mitochondrial membrane potential stability, and improve cardiomyocyte energy supply <sup>[55]</sup>.

#### 4.2.5. Guizhi Tongluo Tablet

Guizhi Tongluo Tablet, whose main ingredients include *Cassia* twig, *Ilex pubescens* root, and *Sargassum*, can warm the meridians and promote blood circulation, and treat heart diseases caused by Yang deficiency and Yin excess. Previous studies have confirmed that Guizhi Tongluo Tablet can significantly reduce the expression of inflammatory factors such as interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in atherosclerotic mice <sup>[56,57]</sup>. Studies have also shown that Guizhi Tongluo Tablet can improve heart function, inhibit inflammatory response, reduce cardiomyocyte apoptosis, suppress IL-1 $\beta$ , IL-6, and TNF- $\alpha$  levels, and improve MIRI by reducing cardiac inflammatory response through the regulation of the PI3K/Akt signaling pathway <sup>[58]</sup>.

#### 4.2.6. Tongmai Yangxin Pill

Tongmai Yangxin Pill is a modified combination of *Glycyrrhizae Radix et Rhizoma* Decoction and *Shengmai* Powder prescribed by Professor Ruan Shiyi. It consists of *Rehmanniae Radix et Rhizoma*, *Caulis Spatholobi*, *Ophiopogonis Radix*, *Glycyrrhizae Radix et Rhizoma*, *Schisandrae Chinensis Fructus*, *Polygoni Multiflori Radix Praeparata*, *Codonopsis Radix*, *Carapax et Plastrum Testudinis*, *Colla Corii Asini*, *Jujubae Fructus*, and *Cassia Twig*. With *Rehmanniae Radix et Rhizoma* and *Glycyrrhizae Radix et Rhizoma* as the monarch drugs, it has the effect of nourishing qi and Yin, clearing meridians, and relieving pain <sup>[59]</sup>. Previous studies have confirmed the significant efficacy of Tongmai Yangxin Pill in MIRI. It participates in various metabolic, oxidative stress, and inflammatory responses, and improves the gene expression levels of ER $\alpha$ , PI3K, AKT, and the protein expression levels of ER $\alpha$ , p-PI3K, p-AKT to regulate MIRI-induced heart damage. Its mechanism may be related to the ER $\alpha$ /PI3K/AKT pathway <sup>[60,61]</sup>.

#### 4.2.7. Zhilong Huoxue Tongyu Capsule

Zhilong Huoxue Tongyu Capsule consists of *Astragalus*, *Hirudo*, *Lumbricus*, *Sargentodoxa cuneata*, and *Cassia* twig. It contains a large amount of effective compounds such as saponins and flavonoids. Previous studies have shown that Zhilong Huoxue Tongyu Capsule can inhibit inflammatory response, protect vascular endothelium, promote cardiomyocyte proliferation, and have other protective effects on cardiomyocytes in MIRI <sup>[62]</sup>. On this



basis, existing research shows that Zhilong Huoxue Tongyu Capsule can regulate the PI3K/AKT/Nrf2 signaling pathway to promote HO-1/GPX4 expression, thereby improving the ferroptosis induced by myocardial ischemia-reperfusion and improving MIRI <sup>[63]</sup>.

## 5. Summary and outlook

The PI3K/Akt/mTOR pathway, as one of the key pathways in MIRI, is a current research hotspot. Existing research on traditional Chinese medicine monomers is more reflected in experimental studies. Currently, compound prescriptions and monomer prescriptions can mutually verify and complement each other, confirming the effectiveness of traditional Chinese medicine in regulating the PI3K/Akt/mTOR signaling pathway to improve MIRI. The current research on traditional Chinese medicine monomers mainly focuses on saponins, flavonoids, alkaloids, terpenes, and phenols. The mechanism of regulating PI3K/Akt/mTOR is primarily manifested in oxidative stress and inflammatory responses, while research on autophagy, apoptosis, and ferroptosis mechanisms targeting MIRI is relatively limited. The number of studies on traditional Chinese medicine compounds acting on the complete PI3K/Akt/mTOR signaling pathway is limited. In summary, the research on the mechanism of multi-component and multi-target regulation of the PI3K/Akt/mTOR signaling pathway by traditional Chinese medicine is still incomplete.

In the future, potential targets should continue to be explored, and the interaction between drugs and the body should be explored through network pharmacology and other means, focusing on system biology and biological network balance. This approach can reveal multi-pathway regulation of signaling pathways, improve treatment effectiveness and the success rate of clinical trials for new drugs, and provide a research foundation for the development of new preparations for the treatment of MIRI. Especially in the treatment of multi-target and complex diseases, combining the holistic concepts of traditional Chinese medicine and formula compatibility can offer new ideas for the complex system of traditional Chinese medicine.

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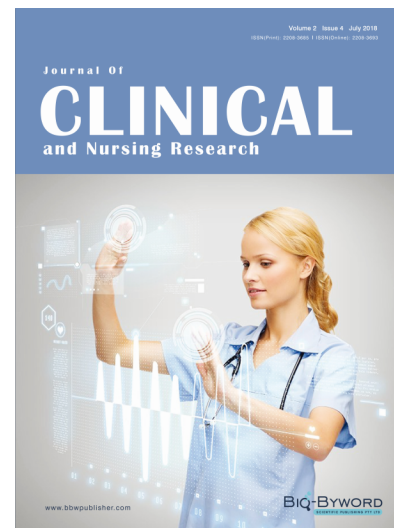
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