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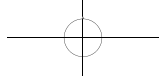
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Table of Contents

- 1 Zorifertinib for Non-small Cell Lung Cancer with EGFR-mutant after Leptomeningeal Metastasis on Double Dose Third-generation EGFR-TKI: A Case Report and Literature Review**
Ye Wang, Deliang Huang

- 9 Key Driving Pathways and Regulatory Mechanisms of Malignant Transformation of Mammary Gland Epithelial Cells under Long-Term Psychological Stress**
Rong Song, Na Huang, Ping Zhang, Yu Zhang, Yuan Sheng, Jiale Li, Rongtian Zhang

- 16 Thyroid Hormones in Prostate Cancer: A Systematic Review and Bibliometric Study**
Jinhai Wu, Xuejin Zhu, Yanfei Chen, Jing Li, Bin Wang

- 28 Exploring the Molecular and Immune Mechanisms Linking Hypothyroidism to Hepatocellular Carcinoma**
Jiahao Chen, Zhe Wang, Aoxiong Zhou, Xu Xie

- 42 Exploring Gastric Cancer-Related Genes and Clinical Significance Analysis Based on Bioinformatics**
Liansi Ye, Chuanxin Zou

- 52 A Narrative Study on the Reconstruction of Life Meaning in Breast Cancer Patients**
Yu Liang, Linqi Hu, Yanshan Zhou

- 65 Integrating Iron Overload Diagnosis with Electrocardiographic Abnormalities: Bridging Laboratory Findings to Primary Care Practice**
Xinqi Liu, Xinhan Liu, Roohollah Changizi, Fei Sun, Xinlian Jin

- 74 Comparative Study on the Diagnosis of Thoracic Wall and Rib Involvement in Lung Adenocarcinoma Using ^{99m}Tc-MDP SPECT/CT and MSCT**
Wenjin Zha, Qiaoying Li

- 81 Data Mining-Driven: Identification of Potential Traditional Chinese Medicine Categories Targeting Vasculogenic Mimicry in Esophageal Cancer**
Yunqin Wang, Yu Wang, Qian Zhang, Ruoshui Xia, Yanqing Liu, Jue Chen

92 Global Research Trends and Hotspots of Contrast-Enhanced Ultrasound in Tumor Diagnosis: A Bibliometric Analysis (2000–2025)

Xiaodi Chen, Zhiyang Lv

104 Synergistic Potential of Traditional Chinese Medicine and CART Cell Therapy: Immunoenhancement and Persistence Regulation Strategies

Keyi Yuan

115 Current Status and Prospects of Diagnosis and Intervention for HR-HPV Persistent Infection

Ping Tan, Yanbing Xiao

124 Revisiting IL-1 Antagonism in Lung Cancer Therapeutics: Lessons from Failure and Pathways to Precision Therapy

Sitong Feng, Cong Xu, Chuang Qi , Yi Li, Bo Shen

Zorifertinib for EGFR-mutant Non-small Cell Lung Cancer after Leptomeningeal Metastasis on Double-Dose Third-generation EGFR-TKI: A Case Report and Literature Review

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Abstract: *Background:* Leptomeningeal metastasis (LM) after third-generation EGFR-TKIs resistance carries a dismal prognosis. Limited blood–brain-barrier penetration rather than secondary EGFR mutations is the dominant resistance mechanism. We report a case managed with CNS-penetrant EGFR inhibition of zorifertinib. *Method:* A 53-year-old, never-smoking woman with EGFR L858R-mutant stage IVb non-small-cell lung cancer (NSCLC) developed LM after progression on osimertinib 160 mg and firmonertinib 160 mg. Salvage therapy with zorifertinib (200 mg BID) plus firmonertinib (80 mg qd) was initiated. *Results:* Within 14 days, the coma resolved. Karnofsky Performance Status improved from 20 to 70. Serial imaging at 3 and 5 months revealed stable disease with shrinkage according to RECIST 1.1. Only grade 1–2 diarrhea, rash, and transaminitis occurred and resolved with symptomatic care. *Conclusion:* The combination of zorifertinib plus firmonertinib provides durable intracranial control and rapid neurological recovery after third-generation EGFR-TKI failure. Prospective validation is warranted.

Keywords: Zorifertinib; Third-generation EGFR-TKI resistance; Leptomeningeal metastasis

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1. Introduction

Lung cancer persists as the most lethal malignancy globally, with non-small-cell lung cancer (NSCLC) constituting 80–85% of cases ^[1]. Epidermal growth factor receptor (EGFR) mutations are predominantly found in East Asian, female, non-smoking individuals with lung adenocarcinoma. Among the various EGFR mutations, the exon 19 deletion (19 del) and the exon 21 Leu858Arg (L858R) mutation are the two most common subtypes, together representing 85–90% of all EGFR mutations ^[2,3]. EGFR-mutant NSCLC patients are more likely to develop central

nervous system (CNS) metastases, especially leptomeningeal metastasis (LM), compared to those with wild-type EGFR. The incidence rate of LM is as high as 9.4% with the median overall survival being less than 1 year^[4].

Despite the efficacy of osimertinib, a third-generation EGFR-tyrosine kinase inhibitor (TKI), CNS metastases, particularly LM, remain a major challenge^[5,6]. While dose escalation to 160 mg osimertinib has shown investigator-assessed LM objective response rates of 41 % and median LM-progression-free survival (PFS) of 8.6 months in the BLOOM study, durable control is still limited^[7], which is mainly due to the limited penetration of most TKIs across the blood–brain barrier (BBB)^[8], and EGFR sensitive mutations are still the main type (69.2%) in the CSF of patients who experience CNS progression after treatment with third-generation EGFR TKIs^[9]. Osimertinib is a substrate of ABCB1 (P-glycoprotein, P-gp) and ABCG2 (breast cancer resistance protein, BCRP), which can actively pump osimertinib out of the BBB^[10]. Osimertinib 160 mg daily just achieves a CSF penetration rate of 16%, and this is still insufficient compared to the *in vitro* EGFR L858R IC₉₀, resulting in limitation of its sustained inhibition for leptomeningeal lesions^[5]. Though firmonertinib may have greater penetration of BBB compared to osimertinib or aumolertinib^[11,12] the PFS and intracranial-PFS of firmonertinib 160mg in patients with LM after resistance to third-generation EGFR-TKIs were only 4.3–5.5months^[9,12]. Whether the CSF concentration of firmonertinib is sufficient to fully address the issue of inadequate drug concentration still requires more data to support^[13].

Zorifertinib was specifically engineered to overcome the BBB limitations of existing EGFR inhibitors^[14]. In contrast to osimertinib and other TKIs that are substrates for P-gp/ABCB1 and BCRP/ABCG2 efflux transporters, zorifertinib is not recognised by either pump, resulting in a CSF-to-unbound plasma concentration ratio (UPR) reaching 1.11 (around 100 % BBB penetration), significantly exceeding the 2.5–16% observed for osimertinib. While firmonertinib demonstrated high brain penetration in mice, with a CSF-to-UPR of 3.31, which is also higher than osimertinib^[14–17]. In phase III EVEREST trial, zorifertinib significantly extended intracranial-PFS (15.2 vs. 8.3 months, HR = 0.467, $p < 0.001$), demonstrating excellent intracranial disease control, and intracranial PFS also favored zorifertinib for patients with LM (HR, 0.395)^[18]. The intracranial and extracranial response duration in a patient even lasted over 7 years^[19].

In the treatment dilemma of LM after resistance to third-generation TKIs, the combination of firmonertinib and zorifertinib shows a synergistic mechanism. Firmonertinib mainly acts to maintain systemic EGFR inhibition. It can irreversibly bind to EGFR, effectively blocking the EGFR signaling pathway and inhibiting tumor cell growth in the body, especially in extracranial lesions^[20]. On the other hand, zorifertinib focuses on compensating for insufficient intracranial exposure. This results in a synergistic effect of the two drugs in treating LM after resistance to third-generation TKIs.

This case reported a patient with EGFR-mutant (L858R) NSCLC who developed LM after progressing on osimertinib 160 mg, and we aimed to explore the efficacy and safety of subsequent salvage therapy with zorifertinib in combination with firmonertinib. This study has obtained informed consent from the patient.

2. Case presentation

2.1. Patient and baseline characteristics

A 53-year-old Chinese female non-smoker with well-controlled hypertension was diagnosed with invasive adenocarcinoma (T2bN2M1c, stage IVb) in July 8, 2022. Contrast-enhanced chest Computed Tomography (CT) revealed a 43 × 41 × 35 mm mass in the apical-posterior segment of the left upper lobe, accompanied by multiple

bilateral pulmonary nodules, and enlarged mediastinal and left hilar lymph nodes. Brain Magnetic Resonance Imaging (MRI) confirmed brain metastases. Genetic testing identified an EGFR exon 21 L858R mutation.

2.2. Therapeutic course

2.2.1. First-line therapy (July 19, 2022 – April 12, 2023)

On July 19, 2022, the patient initiated oral osimertinib 80 mg once daily. From July 21 to October 2022, bevacizumab was added to the regimen. She completed radiotherapy for brain metastases at a dose of 50 Gy in 10 fractions from August 28 to September 2, 2022. Re-evaluation in August 2022 showed a partial response (PR) in both intracranial and pulmonary lesions, with further tumor shrinkage observed in October 2022.

2.2.2. Intracranial progression and dose adjustment (April 13, 2023 – December 2024)

A cranial enhanced MRI on April 12, 2023, showed enlarged and newly emerged brain metastases. The dose of osimertinib was increased to 160 mg once daily starting from April 13, 2023. Follow-up in October 2023 revealed stable disease (SD) in both extracranial and intracranial disease. In August 2024, she experienced epileptic seizures, which were controlled with regular anti-epileptic treatment.

2.2.3. Confirmation of LM (December 2024)

In December 2024, the patient developed severe headache, vomiting, blurred vision and delirium. Cranial MRI indicated new LM, with positive cerebrospinal fluid (CSF) cytology and significantly elevated intracranial pressure. CSF genetic testing on January 13, 2025, still detected the EGFR exon 21 L858R mutation.

2.2.4. Salvage therapy and regimen adjustment (December 2024 – January 2025)

On December 24, 2024, she started oral firmonertinib 160 mg once daily. However, her symptoms did not improve and she developed stupor on January 9, 2025, and received dehydration and intracranial pressure-lowering therapy. She underwent 3 sessions of intrathecal pemetrexed injection on January 10, January 17, and February 7, however, she was still in a coma.

2.2.5. Combination therapy of zorifertinib and firmonertinib (from January 22, 2025)

The treatment regimen was adjusted to zorifertinib 200 mg twice daily combined with firmonertinib 80 mg once daily on January 22, 2025.

2.3. Efficacy assessments

After 7 days of combination therapy of zorifertinib and firmonertinib, her coma started to improve, and she fully recovered from the coma on February 4, 2025. Her headache and vomiting significantly improved, and her vision recovered. The performance status improved significantly, with the KPS increasing from 20 to 70 at 8 weeks. Imaging assessments showed that there were two target lesions before combination therapy: one intracranial target lesion with of 60 mm and one extracranial target lesion in left pulmonary with a maximal diameter of 27 mm. Re-evaluations around May and July, 2025 showed SD with shrinkage according to RECIST 1.1 criteria. The intracranial and extracranial target lesions decreased to 53.72 mm and 23.69 mm, respectively, on 1 July 2025 (**Figure 1**). As of the cut-off date of July 31, 2025, the PFS and intracranial-PFS were both more than 6 months, and the patient continues the combination therapy with sustained disease control and manageable AEs.

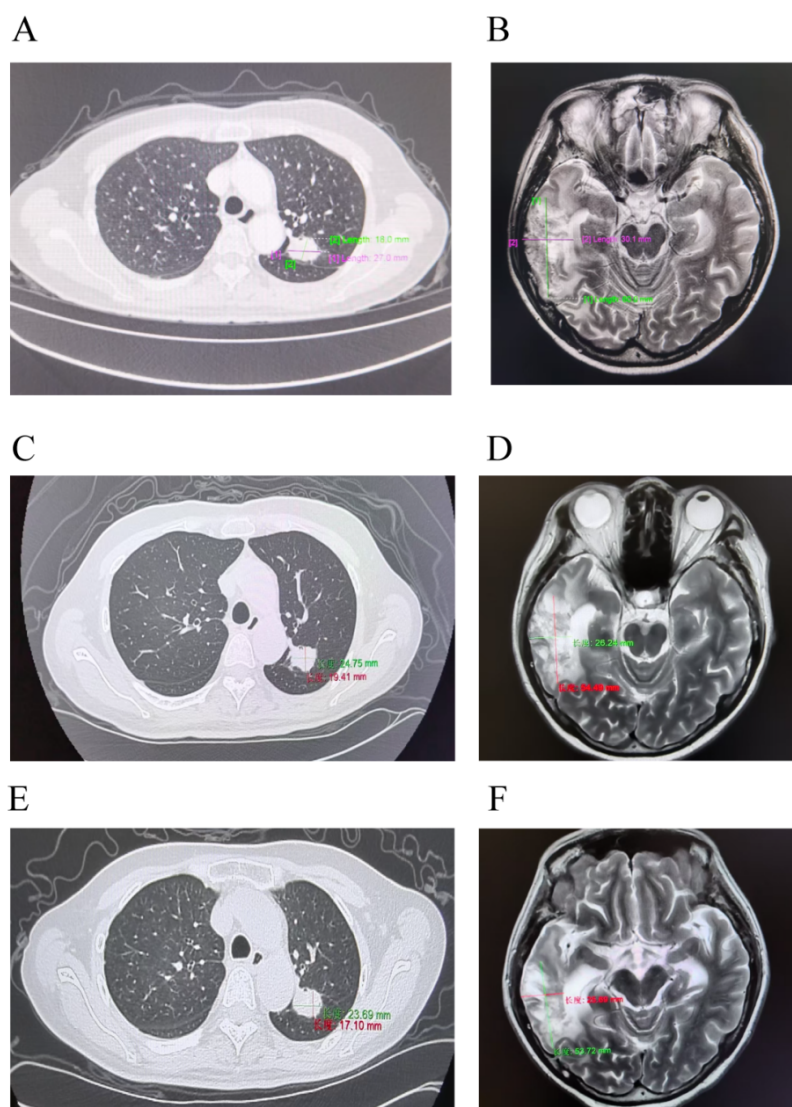


Figure 1. Follow-up imaging of lung and brain lesions in EGFR-mutant NSCLC treated with zorifertinib + firmonertinib combination therapy. (A) Chest CT before combination therapy. (B) Brain MRI before combination therapy. (C) Chest CT on 30 April 2025, stable pulmonary lesions at 3-month follow-up. (D) Brain MRI on 29 April 2025, residual leptomeningeal enhancement at 3-month follow-up. (E) Chest CT on 30 June 2025, continued stable disease at 5-month follow-up. (F) Brain MRI on 1 July 2025, further reduction of sulcal enhancement at 5-month follow-up. CT: Computed Tomography; LM: leptomeningeal metastasis; MRI: Magnetic Resonance Imaging.

2.4. Safety

During the combination therapy, grade 1 diarrhea, grade 2 Aspartate Aminotransferase (AST) elevation, and grade 2 scattered rash on the limbs and trunk occurred. All adverse events (AEs) improved with symptomatic treatment, and no grade ≥ 3 toxicities were observed.

3. Discussion

In this case, a female patient with EGFR L858R-mutant stage IVb NSCLC developed LM after disease progression on osimertinib (160 mg). Following the failure of salvage therapy with firmonertinib 160 mg monotherapy and intrathecal chemotherapy, she was switched to combination therapy with zorifertinib (200 mg BID) and firmonertinib (80 mg QD). Within 14 days, the patient's neurological symptoms improved, with the KPS increasing from 20 to 70 at 8 weeks. And per RECIST 1.1 criteria, the best overall response was SD with shrinkage; the therapeutic effect was sustained at 6 months, with no grade ≥ 3 AEs. The result indicates that the combination of zorifertinib and firmonertinib achieved durable control of LM after osimertinib resistance.

After progression on third-generation EGFR-TKIs, LM in EGFR-mutant NSCLC is driven by two converging factors. First, limited BBB penetration keeps intracranial drug concentrations below the therapeutic threshold. CSF-UPR reflects the distribution of the drug in the CSF, which is crucial for managing LM. The CSF-UPR of osimertinib is about 2.5%, 16%, 22% and 31.7%^[7,21,22]. Both the parent drug and its metabolites efficiently enter the CSF, which may be the basis for its CNS efficacy advantage. This higher brain penetration could contribute to its potential for treating CNS-related conditions, such as LM. Second, molecular profiling of cerebrospinal fluid reveals that the original EGFR-activating mutation (L858R or Ex19del) persists in 70%-80% cases, while classic resistance mutations such as C797S or MET amplification are less^[23,24].

The treatment of LM in NSCLC patients after third-generation EGFR-TKIs failure remains largely palliative. Intrathecal chemotherapy (ITC) is the historical standard for treating LM. According to the results of multiple studies, the objective response rates (ORR) of systemic therapy and intrathecal chemotherapy is 53%-76%, but the median overall survival (mOS) is 8-12 months^[25,26]. These data indicate that while ITC provides high CSF clearance and symptomatic benefit, its LM-specific ORR remains modest, underscoring a need for more effective CNS-penetrant strategies.

Dose-escalated third-generation EGFR-TKIs monotherapy or combination therapy is another choice. A prospective real-world study enrolled 48 EGFR-mutant NSCLC patients with LM, including 35 who had received prior third-generation EGFR-TKIs. All patients were treated with high-dose firmonertinib (240 mg QD) either as monotherapy or in combination regimens. The results demonstrated that the mOS in the overall cohort is 8.43 months, and it is 7.07 months in the prior third-generation EGFR-TKIs exposure subgroup^[27]. Another retrospective analysis of 105 Chinese EGFRm+ NSCLC patients with cytologically confirmed LM demonstrated that OS of high-dose third-generation EGFR-TKIs (osimertinib 160 mg, fumeitinib 160 mg, aumolertinib 165 mg) was 10.0 months in the third-generation EGFR-TKIs resistance group; combination with chemotherapy, anti-angiogenic therapy, or WBRT failed to prolong OS (12.3 vs 13.4 months)^[28].

Zorifertinib was specifically engineered to overcome the two major limitations of existing EGFR-TKIs in CNS disease: sub-therapeutic intracranial exposure and efflux-mediated clearance. Pre-clinical pharmacokinetic studies demonstrate that zorifertinib is not a substrate for P-gp or BCRP, thereby eliminating active efflux at the BBB^[18]. Consequently, its CSF-UPC ratio is 1.11, corresponding to 100 % BBB penetration, which is substantially higher than the 2.5–31.7% reported for osimertinib^[14]. This superior CNS exposure underpins the significantly prolonged intracranial antitumor activity of zorifertinib. In the treatment of EGFR mutation-positive (EGFRm+) NSCLC with CNS metastases. The Phase I BLOOM study demonstrated an overall ORR of 67% for zorifertinib, with an intracranial ORR as high as 87%^[16].

The Phase II CTONG1702 study further confirmed this advantage. In untreated EGFRm+ NSCLC patients with CNS metastases, zorifertinib showed an overall ORR of 80%, a mPFS of 15.8 months, and a median

intracranial PFS of 18.5 months^[29]. The EVEREST study is the first large-scale, global, registrational, prospective clinical trial focused on the treatment of brain metastases in lung cancer, achieving significant clinical findings. The EVEREST trial compared first-line zorifertinib with first-generation EGFR-TKIs in patients with advanced EGFRm⁺ NSCLC with CNS metastases. Patients enrolled were more clinically representative, being eligible regardless of the presence of CNS symptoms, and had a higher intracranial tumor burden and a higher proportion of patients with poor prognostic EGFR L858R in this study. The results showed that the zorifertinib group had particularly notable advantages in controlling intracranial lesions: investigator-assessed intracranial PFS (17.9 months vs 11.1 months), and intracranial ORR were all significantly better in the zorifertinib group. These benefits were consistent across subgroups, including those with the L858R mutation, LM and high intracranial tumor burden. The mOS was 37.3 months in patients who received subsequent third-generation EGFR-TKI treatments. This study confirms that zorifertinib is a superior first-line treatment option for patients with CNS metastases, significantly improving intracranial lesion control^[18].

The mechanism of combination treatment with firmonertinib and zorifertinib for EGFR-mutant non-small cell lung cancer (NSCLC) with leptomeningeal metastasis (LM) can be summarized as “synergistic extracranial-intracranial dual-pathway”: Firmonertinib (80 mg) maintains extracranial EGFR inhibition, providing sustained control of extracranial lesions. Zorifertinib (200 mg BID) directly compensates for insufficient intracranial drug exposure, continuously blocking EGFR signaling and overcoming resistance caused by the BBB.

In this case report, a 53-year-old female patient with EGFR L858R-mutant NSCLC progressed to LM after developing resistance to osimertinib. CSF testing still detected the L858R mutation, consistent with the characteristic feature of EGFR mutation-driven LM, also suggesting that the inadequate drug concentration, rather than the occurrence of a secondary mutation. After switching to the combination of firmonertinib 80 mg and zorifertinib 200 mg BID, the patient’s condition showed notable improvement: the coma resolved rapidly, and the KPS improved from 20 to 70. Although a partial response (PR) was not achieved, tumor regression within SD criteria suggests biological activity of the combination treatment, the CNS symptoms significantly improved, and the patient’s quality of life (QoL) showed significant improvement. In summary, the combination regimen, through the synergistic approach of “low systemic toxicity + high intracranial exposure,” continuously suppresses EGFR-driven progression, providing a new therapeutic paradigm for LM after resistance to third-generation TKIs. In this case, the AEs were mild to moderate (grade 1–2), including diarrhea, rash, and elevated AST. These AEs were alleviated with symptomatic treatment, and no grade ≥ 3 toxicities were observed. Overall, the treatment demonstrated good tolerability, making it suitable for long-term maintenance therapy.

This report is confined to a single patient, and the observed efficacy and safety profile require confirmation in larger, prospective cohorts. Furthermore, serial cerebrospinal fluid (CSF) cytological analysis was not conducted to monitor malignant cell clearance, and RANO-LM-based response assessment for LM was unavailable, potentially underestimating CNS-specific treatment effects. Future work should prospectively validate the zorifertinib–firmonertinib combination in larger LM cohorts while simultaneously integrating serial CSF pharmacokinetics and genomic profiling to establish a precision-based, penetration-driven treatment paradigm.

4. Conclusion

The regimen of zorifertinib plus firmonertinib theoretically sustains systemic EGFR inhibition and overcomes the BBB. This case provides proof-of-concept that such a penetration-driven strategy can achieve durable CNS

control and rapid symptom relief after third-generation EGFR-TKIs resistance. Multicenter, prospective trials are warranted to confirm efficacy, safety, and optimal dosing for EGFR-mutant NSCLC patients with LM.

Disclosure statement

The authors declare no conflict of interest.

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Key Driving Pathways and Regulatory Mechanisms of Malignant Transformation of Mammary Gland Epithelial Cells under Long-Term Psychological Stress

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Abstract: *Objective:* This study primarily focuses on analyzing the inductive effects of emotional disturbances on the malignant transformation process of mammary gland epithelial cells. *Methods:* A total of 42 patients with malignant transformation of mammary gland epithelial cells (breast cancer, observation group) and 42 patients without malignant transformation of mammary gland epithelial cells (non-breast tumors, control group) were selected as research subjects. The earliest consultation time was January 2022, and the latest was January 2024. The extent of psychological stress impact on these patients was compared. *Results:* Compared with the control group, the observation group experienced a higher frequency and intensity (LEU value) of adverse life events, with $P < 0.05$. The intensity of adverse life events in the observation group, except for mild events, was significantly higher than that in the control group ($P < 0.05$). In terms of the content distribution of adverse life events, the proportion of marital and family problems in the observation group was significantly higher than that in the control group ($P < 0.05$). The negative coping score and positive coping score in the observation group were significantly different from those in the control group ($P < 0.05$). Regarding social support, the objective support score in the observation group was higher than that in the control group ($P < 0.05$). *Conclusion:* During the malignant transformation process of mammary gland epithelial cells, long-term emotional disturbances have a significant impact, indicating a close relationship between psychological stress and the occurrence of breast cancer.

Keywords: Psychological stress; Malignant transformation of mammary gland epithelial cells; Emotional disturbances; Inductive mechanisms

Online publication: October 13, 2025

1. Introduction

Clinically, malignant transformation of mammary gland epithelial cells is classified as a malignant tumor and is

a disease with a relatively high prevalence among women, namely breast cancer. The masses in such patients are predominantly located in the upper outer quadrant, and as the mass volume increases, the affected area will also locally protrude ^[1]. In recent years, the number of breast cancer patients has been continuously increasing, posing a severe threat to the physical and mental health of women. During the disease's occurrence and development, traditional Chinese medicine theory posits a close association with psychological factors, particularly the stimulation of the mind and body by emotional trauma, that is, prolonged emotional disturbances ^[2]. Among them, the concept of emotional stress as a pathogenic factor is a unique etiological idea in traditional Chinese medicine. Therefore, this concept will be applied in the following study on the mechanism promoting malignant transformation of breast glandular epithelium, aiming to provide assistance for in-depth research on the disease.

2. Materials and methods

2.1. Clinical data

The research subjects primarily consisted of patients with malignant transformation of breast epithelium (breast cancer) and patients without malignant transformation of breast epithelium (non-breast tumors), with 42 cases in each group, named as the observation group and the control group, respectively. All patients were female. The age range in the control group was from 22 to 68 years old, with a median age of (47.25 ± 3.33) years old. In the observation group, the oldest patient was 67 years old and the youngest was 25 years old, with an average age of (47.21 ± 3.26) years old. The basic conditions of the two groups were similar, with no statistical significance ($P > 0.05$), making them comparable. The study was approved by the hospital's ethics committee.

2.2. Methods

2.2.1. Instruments

The main focus was on investigating and analyzing the emotional stress factors of the participants, including negative life events, coping styles, and social support.

2.2.2. Survey methods

Before patients filled out the relevant questionnaires, relevant workers were arranged to provide detailed information about the research purpose and clinical value. On the basis of obtaining understanding and trust, patients were required to sign an informed consent form. Patients were asked to answer the questions based on their personal actual situation. Workers should explain to patients the objectives of each scale survey and the precautions for filling them out to ensure that patients can independently and truthfully fill them out based on their personal actual situation.

2.3. Evaluation indicators

- (1) Conduct a systematic assessment of the number of adverse life events encountered between groups, the intensity of LEU values, scores for negative coping, scores for positive coping, and scores for social support.
- (2) Compare the intensity of adverse life events and the distribution of content of adverse life events between the two groups.

2.4. Statistical analysis

The data obtained were analyzed using SPSS version 23.0 statistical software, with a P -value of < 0.05 indicating statistical significance.

3. Results

3.1. Study on the number and intensity (LEU value) of adverse life events encountered

A comparison of relevant indicators between the groups showed statistical significance, with $P < 0.05$ (Table 1).

Table 1. Comparison of the number and intensity (LEU value) of adverse life events encountered by the two groups of patients ($n/\%$)

Group	n	Number of adverse life events (times)	Intensity (LEU Score)
Observation Group	42	2.84 ± 1.66	85.11 ± 47.85
Control Group	42	1.14 ± 0.13	44.58 ± 30.96
t -value		6.6166	4.6088
p -value		0.0000	0.0003

3.2. Analysis of the intensity of adverse life events in the two groups

Upon comparison, the observation group showed statistical significance in the absence, mild, and severe degrees of adverse life events compared to the control group, with $P < 0.05$ (Table 2).

Table 2. Comparison of the intensity of adverse life events in the observation group and the control group ($n/\%$)

Group	n	None	Mild	Moderate	Severe
Observation Group	42	6 (14.29)	11 (26.19)	16 (38.10)	9 (21.43)
Control Group	42	23 (54.76)	10 (23.81)	7 (16.67)	2 (4.76)
χ^2 -value		15.2201	0.0635	4.8496	5.1258
p -value		< 0.0001	0.8010	0.0276	0.0235

3.3. Comparison of the distribution of content of adverse life events between the observation group and the control group

The observation group had a higher proportion of marital and family issues compared to the control group, with $P < 0.05$ (Table 3).

Table 3. Distribution of content of adverse life events in the two groups of patients ($n/\%$)

Group	n	Marital/Family issues	Social/Environmental issues	Work/Academic issues	Interpersonal / Health issues
Observation Group	42	22 (52.38)	8 (19.05)	9 (21.43)	3 (7.14)
Control Group	42	12 (28.57)	10 (23.81)	11 (26.19)	9 (21.43)
χ^2 -value		4.9412	0.2828	0.2625	3.5000
p -value		0.0262	0.5948	0.6084	0.0613

3.4. Comparison of negative coping scores and positive coping scores between the two groups

All indicators in the observation group showed statistical significance compared to the control group, with $P < 0.05$ (Table 4).

Table 4. Analysis of negative coping scores and positive coping scores in the observation group and the control group ($n/\%$)

Group	n	Negative Coping Score (points)	Positive Coping Score (points)
Observation Group	42	39.22 ± 3.09	28.79 ± 4.21
Control Group	42	30.88 ± 3.74	37.56 ± 5.22
<i>t</i> -value		11.1411	8.4752
<i>p</i> -value		0.0000	0.0000

3.5. Study on social support scores in the observation group and the control group

A comparison of relevant indicators between the groups showed a significant difference in objective support scores, with $P < 0.05$ (Table 5).

Table 5. Comparison of social support scores between the two groups of patients ($n/\%$)

Group	n	Utilization Score (points)	Subjective Support Score (points)	Objective Support Score (points)	Total Score (points)
Observation Group	42	33.35 ± 4.42	18.88 ± 4.13	9.75 ± 3.29	35.74 ± 5.63
Control Group	42	33.33 ± 4.47	18.84 ± 4.15	7.74 ± 2.78	35.89 ± 5.68
<i>t</i> -value		0.0206	0.0443	3.0243	0.1216
<i>p</i> -value		0.9836	0.9648	0.0033	0.9036

4. Discussion

Currently, malignant transformation of breast epithelium, commonly known as breast cancer, poses a severe threat to women's physical and mental health after onset. It is also a malignant tumor with a relatively high clinical incidence rate, and the age of patients is tending to be younger. The vast majority of patients diagnosed with breast cancer experience significant psychological issues, particularly anxiety, inferiority, fear, and pessimism. Some patients even exhibit suicidal thoughts and behaviors, which severely impact the physical health and disease prognosis of the female population ^[3].

From the perspective of traditional Chinese medicine, breast cancer can be classified into various categories such as "Huahuashi" (flourishing stones), "Duru" (envious milk), and "Ruyan" (breast rock). However, in terms of predisposing factors, long-term emotional disturbances have always played a crucial role. In recent years, with the rapid advancement of technology, the pace of modern life and work has continuously accelerated, making individuals more prone to psychological and mental issues. Among these, social-psychological factors such as stressors and personality traits play a significant role in the occurrence and development of tumor diseases ^[4].

4.1. Negative life events

Life events are problems that modern individuals must confront and resolve in their social lives. These events are extensive and complex, encompassing aspects such as life, marriage, family, children, interpersonal relationships, daily work, learning, and unexpected incidents. Based on emotional experiences, life events can be further categorized into positive and negative events. Events that evoke pleasant emotions and contribute to positive emotional transformations are considered positive life events. Conversely, events that elicit negative emotional experiences are classified as negative life events. Additionally, negative life events can be further divided into major and minor events based on their intensity. Minor events are characterized by their cumulative and persistent nature. Based on long-term clinical research, it is understood that diseases triggered by negative life events are generally influenced by numerous factors, such as the intensity, quantity, timing, and nature of these events ^[5].

Combining the above research data, it was found that during the questionnaire survey conducted on 84 research participants, there were 42 cases in the observation group (malignant transformation of breast epithelium) and 42 cases in the control group (patients without malignant transformation of breast epithelium). No significant difference in age was observed between the two groups, with $P > 0.05$. However, in the survey of negative life events, it was found that the frequency and intensity of adverse life events experienced by patients in the observation group were significantly different from those in the control group, with $P < 0.05$. In particular, the number of negative events related to marriage and family was higher than that of other types of events. The reason for this is that all the research participants were women, who tend to be more emotionally expressive and sensitive. Compared to men, they pay more attention to marriage and family, making these the types of events that have the greatest impact on them ^[6].

4.2. Negative coping strategies

Coping strategies are generally considered to be the attitudes and behaviors adopted to address life events, consisting of both negative and positive components. Positive coping behaviors are more conducive to problem-solving and can significantly alleviate tension, thereby protecting one's physical and mental health to a certain extent. Negative coping behaviors, on the other hand, can exacerbate negative mental states, adversely affecting mental health and psychological well-being, and increasing the likelihood of illness. Based on the comparison of the aforementioned data indicators, it can be seen that the scores for positive coping in the observation group were significantly lower than those in the control group, while the scores for negative coping were higher. There were significant differences in scores between the two groups, with $P < 0.05$. This suggests that clinical breast cancer patients tend to adopt negative coping strategies when dealing with problems. In other words, when solving problems, if one chooses inactive means, they may fail to vent their inner emotions, thereby significantly increasing psychological pressure and adversely affecting the maintenance of internal balance in the body, thus making them more susceptible to illness. The analysis of the above indicators reveals that psychological stress, or emotional disturbance, significantly impacts the body's immune function. When subjected to intense mental stimulation, it inevitably leads to a continuous increase in the excitation level of the sympathetic nervous system, accelerating the formation of glucocorticoids and adrenal cortisol. Under the influence of stress hormones, various organs in the human body can be damaged, resulting in a higher risk of illness ^[7]. Therefore, even though negative life events may be unavoidable, the occurrence of diseases is still

somewhat related to the coping strategies employed. Actively coping can help reduce stress intensity, preventing adverse effects on the nervous system, immune system, and endocrine system, and facilitating quicker repair of damage.

4.3. Social support

Social support refers to the interaction between individuals, representing a behavioral process that promotes, assists, and provides support for various matters. Good social support encourages individuals to adopt a positive attitude when dealing with adverse events, thereby reducing psychological stress and ensuring the mental health of patients. However, being in unfavorable social relationships can increase psychological burdens and exacerbate the impact of adverse events. According to the research findings mentioned above, the observation group scored higher than the control group in terms of objective support, with $P < 0.05$. However, there were no significant differences between the groups in terms of subjective support, support utilization, and total scores, with $P > 0.05$.

In the aforementioned studies, although there is limited evidence linking social support to the occurrence and development of malignant transformation of breast epithelium, some reports still suggest a relationship between the two. In the study, all indicators (except for objective support) showed no significant differences between the groups compared to the control group, with $P > 0.05$. The reason is that currently, people have a strong sense of social belonging and mutual assistance, enabling patients to receive care from various sources. Meanwhile, the improvement in people's cultural literacy levels also allows them to make full use of support from multiple parties, enhancing the utilization of social support. Additionally, with the rapid development of modern healthcare, social and psychological factors have garnered attention. Medical professionals possess the ability to comfort and guide patients, and through emotional nursing, they can encourage patients to make rational use of assistance from various sources.

Generally speaking, long-term emotional stress plays a certain promoting role in the onset process of patients with malignant transformation of mammary epithelium, and the two are closely related. In the context of fierce social competition and the transformation of modern medical models, the importance of emotional factors has gradually become prominent. Therefore, it is essential to correctly understand the varying degrees of impact that emotions can have on diseases, and then actively take necessary preventive measures in clinical practice to provide valuable reference for disease prevention, diagnosis, and treatment.

5. Conclusion

In conclusion, this review underscores that long-term emotional disturbance acts as a significant contributing factor in the malignant transformation of mammary epithelial cells. The evidence strongly supports a close and potentially causal relationship between chronic psychological stress and the initiation of breast cancer. This highlights the critical importance of integrating psychological well-being and stress management into a holistic approach for breast cancer prevention and care.

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Disclosure statement

The authors declare no conflict of interest.

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Thyroid Hormones in Prostate Cancer: A Systematic Review and Bibliometric Study

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Abstract: Prostate cancer (PCa) is a prevalent malignancy in men, traditionally linked to androgen receptor signaling. Emerging evidence suggests thyroid hormones (THs, particularly T3/T4) play a complex role in PCa biology. THs regulate gene transcription via nuclear receptors TR α / β , modulating proliferation, apoptosis, and AR signaling, while non-genomic pathways through integrin α v β 3 activate MAPK/PI3K–Akt signaling, driving metabolic reprogramming, migration, and angiogenesis. Local DIO enzymes fine-tune T3/T4 levels, with DIO2 enhancing proliferation and DIO3 creating a low-TH microenvironment to facilitate immune evasion. Epidemiological studies associate hyperthyroidism or low TSH with elevated PCa risk, whereas experimental models show inconsistent effects, reflecting regulation by hormone levels, receptor distribution, and tumor molecular features. Bibliometric analyses reveal a shift from epidemiological studies to molecular, immune, and metabolic mechanistic research, though clinical translation remains limited. This review synthesizes current knowledge on THs in PCa, highlighting mechanistic insights, evidence gaps, and future directions, aiming to inform early detection, stratification, and therapeutic strategies.

Keywords: Prostate cancer; Thyroid hormones; Bibliometrics; CiteSpace; VOSviewer

Online publication: October 14, 2025

1. Introduction

Prostate cancer (PCa) is one of the most common malignant tumors in men, with increasing incidence and mortality rates ^[1]. Current research on PCa mainly focuses on the androgen signaling pathway, in which the interaction between androgens and the androgen receptor (AR) is pivotal to tumor initiation and progression ^[2]. However, recent studies have increasingly suggested that thyroid hormones (THs), particularly triiodothyronine (T3) and thyroxine (T4), may exert complex and incompletely understood effects in the initiation and progression of PCa as well as other malignancies ^[3].

THs are primarily synthesized and secreted by the thyroid gland. T4, as a prohormone, is converted in peripheral tissues into the biologically more active T3 by type 1 and type 2 deiodinases (DIO1, DIO2). T3 then

binds to thyroid hormone receptors (TR α/β), regulating downstream gene transcription and thereby influencing cell proliferation, differentiation, and metabolic processes ^[4].

In oncology, studies have revealed that alterations in physiological TH levels are closely associated with the development of multiple cancers. Evidence indicates that their effects in solid tumors, including breast, liver, and gastric cancers, are heterogeneous and depend on tumor type, hormonal milieu, and microenvironmental interactions ^[3]. For instance, in breast cancer, T3 induces the mRNA expression of growth factors TGF α and TGF β in ER-positive cells ^[5], while T4 activates the ERK1/2 signaling pathway to upregulate PD-L1 expression, enabling immune evasion ^[6]. In gastric and lung cancers, reduced TH levels are often linked to tumor progression, metastasis, and poor prognosis ^[7,8]. These findings suggest that the role of THs in cancer may be bidirectional: in some cases, T3/T4 promote tumor progression, whereas in others they exert inhibitory effects, depending on molecular characteristics and hormone levels.

In PCa, however, the role of THs remains controversial. Epidemiological studies have reported that hyperthyroidism or reduced thyroid-stimulating hormone (TSH) levels may be associated with an increased risk of PCa ^[9], whereas hypothyroidism may be associated with a reduced risk ^[10]. In animal models, an elevated T3/T4 ratio or administration of high-dose T3 was shown to suppress prostate tumor growth ^[11,12]. These observations indicate that the relationship between THs and PCa is not a straightforward causal relationship but is likely influenced by hormonal background, cancer molecular features, and the tumor microenvironment. Current evidence remains inconclusive. Therefore, this review aims to provide a systematic overview, integrating bibliometric analyses, of the research progress on THs, particularly T3 and T4, in PCa, exploring their mechanisms of action and potential clinical implications, with the goal of offering a theoretical basis for future research and therapeutic strategies.

2. Relationship between thyroid hormones and the prostate

In recent years, studies have increasingly recognized the prostate as an important target tissue of THs ^[13]. THs are involved in prostate growth, development, metabolic regulation, and potential processes of tumorigenesis through multiple mechanisms, including hormone transport, local activation, receptor-mediated signaling, and interactions with other endocrine factors.

2.1. Uptake and transport of thyroid hormones in the prostate

Studies have found that the entry of T3 and T4 into prostate cells mainly depends on specific transport proteins, including monocarboxylate transporters MCT8 (SLC16A2), MCT10 (SLC16A10), organic anion transporting polypeptides (OATPs), and members of the L-type amino acid transporter family. Among these, MCT8 is considered the most efficient and specific transporter of THs ^[14]. Expression of MCT8 has been detected in PCa cell lines such as LNCaP, DU145, and PC-3, suggesting that prostate cells possess the capacity for active THs uptake ^[14]. In addition, the high-affinity T3-binding protein μ -crystallin (CRYM) has been identified in both normal and cancerous prostate tissues. Within cells, CRYM binds T3, regulates its cytoplasmic accumulation and nuclear translocation, and simultaneously reduces T3 binding to TRs, thereby exhibiting potential antitumor activity ^[15].

2.2. Local activation and inactivation of thyroid hormones in the prostate

The activity of THs in the prostate is tightly regulated by the deiodinase system. The prostate expresses DIO1, which converts T4 into the more bioactive T3. However, its activity gradually declines with aging or androgen

deprivation, while sexual activity can enhance DIO1 activity through sympathetic nervous mechanisms, thereby maintaining local T3 levels in the prostate ^[16]. Conversely, type 3 deiodinase (DIO3) is upregulated under malignant or stress conditions, mediating the inactivation of T3 to produce reverse triiodothyronine (rT3). This creates a state of local hypothyroidism, which promotes tumor cell proliferation and immune evasion ^[17]. In animal models supplemented with T3, increased DIO3 expression accompanied by decreased DIO1 and DIO2 expression has also been observed, suggesting that THs in the prostate are subject to dynamic feedback regulation ^[12].

2.3. Expression of thyroid hormone receptors and signaling mechanisms in the prostate

Existing studies have shown that prostate epithelial cells express thyroid hormone nuclear receptors TR α 1, TR α 2, and TR β ^[18]. Using immunoblotting and mRNA analysis, TR β protein expression has been detected in multiple human PCa cell lines, including LNCaP, PC-3, and DU145 ^[19]. In addition to nuclear receptor-mediated mechanisms, THs can also activate non-genomic signaling pathways, such as integrin α v β 3-mediated signaling, which rapidly activates MAPK and PI3K/Akt pathways. These pathways contribute to metabolic reprogramming, proliferation, migration, and angiogenesis in prostate cells ^[20]. This non-classical mechanism appears to be enhanced in PCa, indicating its potential tumor-promoting role.

3. Association between thyroid hormones and prostate cancer

3.1. Epidemiological associations between thyroid function and PCa

Epidemiological studies provide important evidence for the potential association between thyroid hormones (THs) and prostate cancer (PCa) risk. Some epidemiological surveys have suggested that subclinical hypothyroidism is not significantly associated with PCa risk ^[21], although other researchers have proposed that untreated hypothyroidism may exert a protective effect against PCa ^[10]. For instance, in a prospective cohort study involving 3,649 patients with 20 years of follow-up, 7.8% of men were diagnosed with PCa. Serum TSH and free T4 were measured, and analysis revealed that higher TSH levels were associated with a reduced PCa risk (adjusted HR: 0.7 per 1 mIU/L increase), whereas higher free T4 was associated with an increased PCa risk (adjusted HR: 1.11 per 1 pmol/L) ^[22]. In addition, higher free T3 levels have also been closely associated with an increased risk of PCa ^[23]. Therefore, current findings remain somewhat controversial.

3.2. Potential mechanisms

3.2.1. Receptor-mediated gene transcription

THs regulate the expression of specific genes in PCa cells by binding to TR α and TR β . The binding of T3 to TR activates target gene transcription, thereby regulating cell proliferation, differentiation, and apoptosis ^[21]. In LNCaP cells, T3 markedly upregulates genes such as Cyclin D1 and MMP-2, which are associated with tumor proliferation and invasion ^[11]. Moreover, T3 has been shown to enhance AR expression by modulating NCOA4, an AR-associated protein, thereby increasing the androgen responsiveness of PCa cells ^[24]. These findings suggest that THs may influence tumor growth by upregulating AR and its co-activators, thereby modulating androgen responsiveness in PCa cells.

3.2.2. Non-genomic signaling pathways

In addition to the classical nuclear receptor pathway, THs have been found to activate intracellular signaling

cascades through membrane receptors such as integrin $\alpha\beta 3$, including the MAPK/ERK and PI3K/Akt pathways, thereby regulating cell proliferation, migration, and invasion^[25]. Specifically, the binding of T3 to integrin $\alpha\beta 3$ promotes MAPK pathway activation, which further enhances the invasive potential of PCa cells.

3.2.3. Role of the DIO enzyme family in PCa

The DIO enzyme family plays a crucial role in TH metabolism within PCa. DIO1 and DIO2 catalyze the conversion of T4 to T3, whereas DIO3 facilitates T3 inactivation. Studies have shown that DIO2 expression is upregulated in PCa cells, which may enhance tumor cell proliferation and invasion by increasing local T3 concentrations^[26]. Therefore, DIO2 activity may represent a key regulatory factor mediating the effects of THs in PCa.

4. Bibliometric analysis of studies on thyroid hormones and prostate cancer

4.1. Data sources and search strategy

We selected Web of Science Core Collection (WoSCC) as the data source^[27]. The literature was restricted to the period from January 1, 2015, to December 31, 2024. The search strategy was: TS = (“thyroid function” OR “thyroid hormone*” OR “Triiodothyronine” OR “Free T3” OR “Thyroxine” OR “Free T4” OR “Thyroid Stimulating Hormone” OR “TSH” OR “Thyrotropin” OR “Thyrotropin-Releasing Hormone” OR “TRH” OR “Thyroid Antibodies” OR “TPOAb” OR “TgAb” OR “TRAb” OR “TSHR-Ab”) AND (“prostate cancer” OR “prostate carcinoma” OR “prostate neoplasm*” OR “prostatic malignancy” OR “prostate tumor*” OR “prostate adenocarcinoma”). The document types were limited to original articles and reviews. A total of 141 publications were retrieved, including 95 original articles and 46 reviews. All publications were in English and focused on studies related to THs and PCa.

4.2. Analysis methods

The study extracted raw data from WoSCC, including publication count, citation frequency, h-index, publication year, country/region, institutions, authors, journals, references, and keywords. VOSviewer (version 1.6.19) was employed to visualize keywords and institutional collaborations, and to construct co-citation, co-occurrence, and collaboration networks in this research field^[28]. CiteSpace 6.2.R6 (64-bit) Basic was used to map the intellectual evolution and temporal distribution of document clusters, thereby revealing research progress and trends in the field of THs and PCa^[29]. To analyze annual research trends, R Studio 4.3.1 and the ggplot2 package^[30] were applied to perform statistical analysis and visualization of publication volume, further demonstrating the academic attention and developmental trends in this area.

4.3. Trends and changes in research output

Based on data from WoSCC, the number of publications on THs and PCa has exhibited fluctuations (**Figure 1**). From 2015 to 2017, the number of relevant publications remained relatively stable, at 15, 18, and 12 articles, respectively, indicating initial attention to this field. Between 2018 and 2020, the number of studies increased markedly, reaching 19 in 2020, reflecting strong academic interest in the relationship between THs and PCa. However, from 2021 onwards, the number of publications declined annually, with 11 articles in both 2021 and 2022, and an estimated 10 in 2024, suggesting a waning research momentum, possibly due to a plateau in the

field or a shift in focus to other areas. Overall, this field remains promising and valuable, and future progress may emerge through novel research approaches or interdisciplinary collaborations.

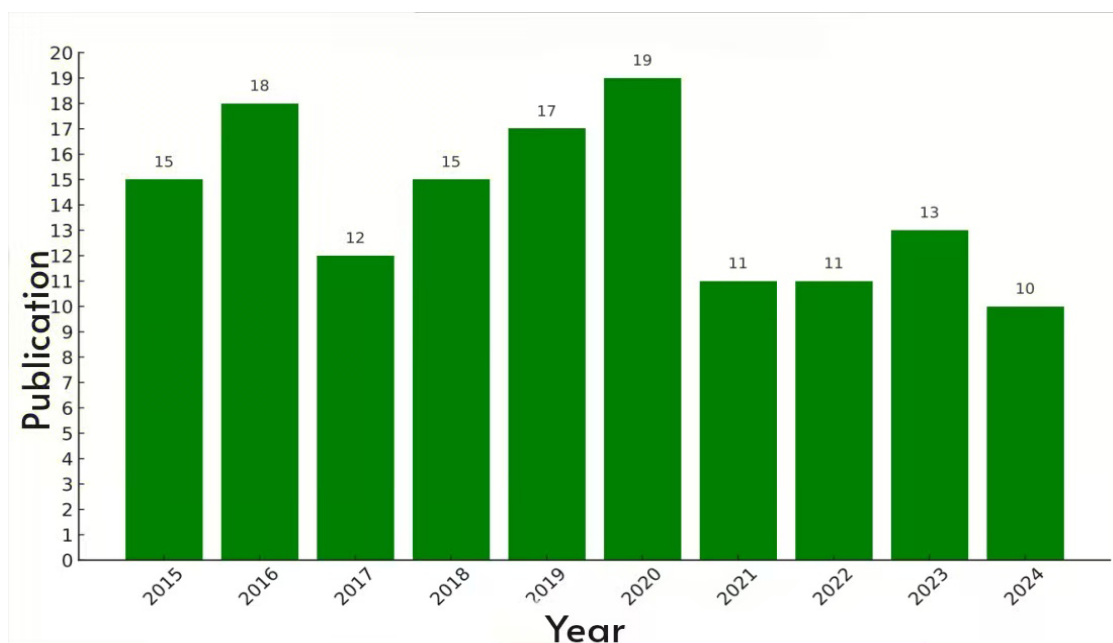


Figure 1. Trends in publications on THs and PCa research.

4.4. Dual-map overlay analysis of journals

The dual-map overlay generated by CiteSpace reveals the interdisciplinary knowledge flow characteristics in the field of THs and PCa research. The core paths are represented by two prominent knowledge transfer directions: the first is Molecular/Biology/Immunology → Molecular/Biology/Genetics (yellow path, $z = 5.367$, $f = 9810$), indicating that findings in immunology exert a profound influence on the field of genetics. For example, the interaction between thyroid antibodies (e.g., TPOAb/TgAb) and the PCa immune microenvironment (e.g., PD-1/PD-L1 signaling)^[31] has promoted research on epigenetic regulatory mechanisms in genetics, such as methylation of the THRB gene promoter^[32]. The second path is Medical/Clinical/Healthcare → Molecular/Biology/Genetics (green path, $z = 2.151$, $f = 4300$), indicating a relatively weak feedback from clinical medicine to basic research. This may result from barriers in translating clinical observational data (e.g., the correlation between TSH levels and PCa staging) into molecular mechanisms. On one hand, heterogeneity in thyroid function assessment standards (e.g., TSH cut-off values) complicates mechanistic studies; on the other hand, multi-omics integration techniques for clinical samples (e.g., single-cell sequencing) are not yet widespread, limiting in-depth analysis of molecular pathways. For instance, clinical observations of thyroid dysfunction in patients with castration-resistant prostate cancer (CRPC)^[33] have stimulated functional studies on DIO2/DIO3 metabolic enzymes, yet relevant translational outcomes remain limited^[34]. The dual-map overlay analysis (**Figure 2**) suggests that research on THs and PCa follows a “basic-to-clinical” model, with deep integration of immunology and genetics emerging as the dominant trend, while clinical translation remains constrained by data heterogeneity and technical barriers.

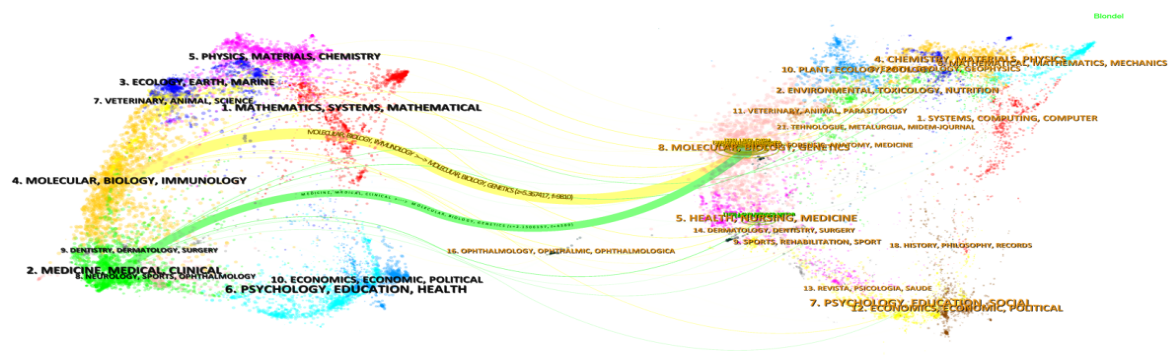


Figure 2. Dual-map overlay showing interdisciplinary knowledge flows in THs and PCa research.

4.5. Analysis of co-cited publications

Based on a co-citation network analysis of 141 publications (**Figure 3**), the field of THs and PCa research has formed three core knowledge clusters, exhibiting notable temporal evolution and translational potential. These clusters correspond to three major research themes:

- (1) THs Signaling Pathways and Tumor Proliferation: This cluster focuses on how THs promote tumor proliferation via classical nuclear receptors (e.g., THRA, THRB) and non-classical signaling pathways (e.g., PI3K/AKT, integrin $\alpha v \beta 3$).
- (2) Thyroid Dysfunction and Clinical Prognosis: Research in this cluster reveals a close relationship between thyroid dysfunction and clinical outcomes in PCa.
- (3) THs and Metabolic Reprogramming: Studies indicate that THs drive glycolysis via the AMPK/mTOR pathway^[35], while thyroid hormone disruptors (e.g., bisphenol A) can induce lipid metabolism abnormalities and promote dedifferentiated phenotypes^[36]. This suggests that THs not only contribute to tumor growth and metastasis but also play a critical role in the metabolic reprogramming of tumor cells.

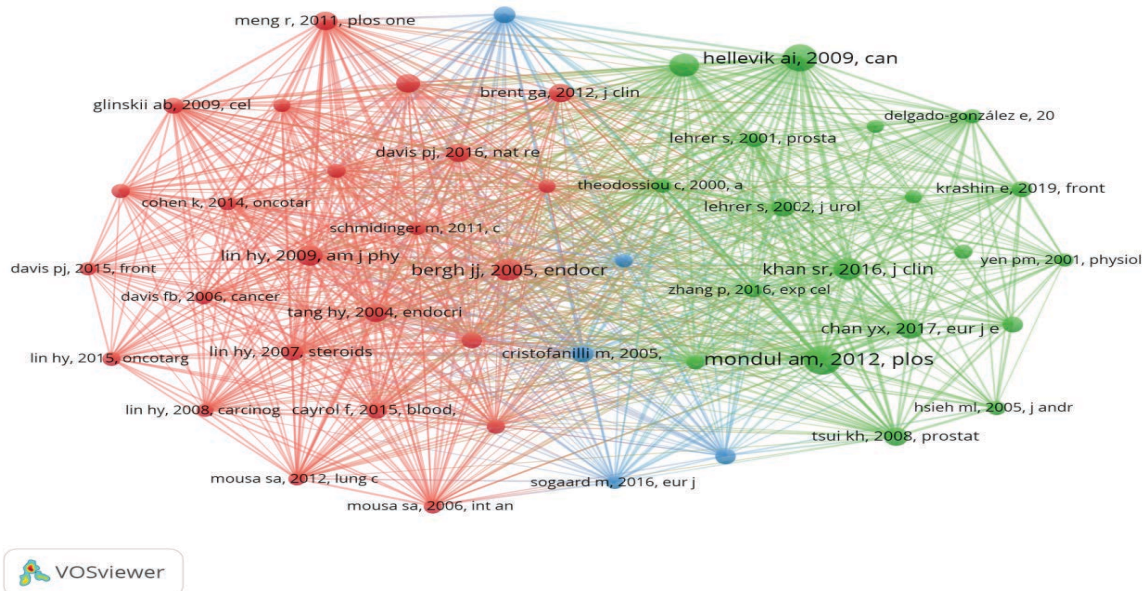


Figure 3. Visualization of the co-citation network of references.

4.6. Analysis of research hotspots and keywords

4.6.1. Co-occurrence analysis of keywords

Based on co-occurrence analysis of keywords from the selected publications using VOSviewer (**Figure 4**), the study identified five major research clusters, each representing distinct research directions and themes within the field. Each cluster reflects the specific focus of different disciplines on the relationship between THs and PCa.

- (1) Red cluster: Focuses on the molecular mechanisms of PCa development, with core keywords including “prostate cancer,” “thyroid hormone,” “expression,” “cell proliferation,” and “biomarker,” highlighting researchers’ strong interest in the role of THs in regulating gene expression and cell proliferation during PCa pathogenesis.
- (2) Yellow cluster: Primarily pertains to studies on thyroid hormone receptors, encompassing keywords such as “androgen receptor,” “nuclear receptor,” “estrogen receptor,” and “gene expression.” This cluster indicates that researchers are particularly interested in the potential regulatory roles of THs through interactions between nuclear receptors and sex hormone receptors within PCa cells, representing a significant current research direction.
- (3) Green cluster: Emphasizes the effects of THs on tumor cells, with core keywords including “in vitro,” “gene,” “proliferation,” “hypothyroidism,” and “angiogenesis.” This cluster reflects laboratory-based investigations into the effects of THs on PCa cell growth, differentiation, and tumor angiogenesis.
- (4) Blue cluster: Relates to epidemiological studies, with keywords such as “prostate cancer,” “cancer,” “breast cancer,” “mortality,” and “meta-analysis,” highlighting the role of THs across different cancer types, particularly in male populations and postmenopausal women.
- (5) Purple cluster: Focuses on the association between THs levels and cancer risk, with keywords including “triiodothyronine,” “thyroxine,” “serum triiodothyronine,” and “carcinoma.” This cluster reflects the ongoing academic interest in the relationship between THs levels and PCa risk.

Through co-occurrence analysis, the diversity and complexity of research in the field of THs and PCa are clearly observed, with clusters interweaving, indicating a shift toward multidisciplinary and integrative research directions.

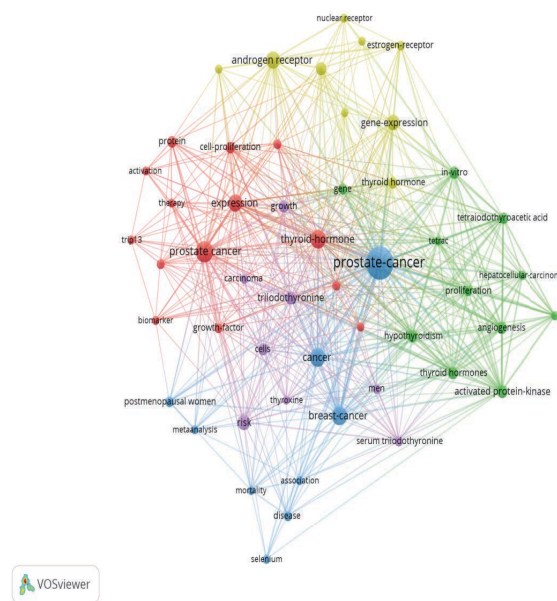


Figure 4. Co-occurrence network of keywords in THs and PCa research.

4.6.2. Burst analysis of keywords

Using CiteSpace, we conducted a burst analysis of keywords from the collected publications (Figure 5) and identified the 15 keywords with the most rapidly increasing citation frequencies between 2015 and 2024, revealing the research evolution and emerging hotspots in the field.

Between 2015 and 2017, research hotspots focused on the biological functions and mechanisms of THs, with keywords such as “postmenopausal women,” “thyroid hormone receptor,” and “cancer prevention” exhibiting strong bursts, reflecting substantial attention to the role of THs in different populations, particularly postmenopausal women, and their potential in cancer prevention [37,38,39].

During 2016–2017, the keywords “prostate cancer cells” and “carcinoma cells” emerged as bursts, indicating that research at the cellular level of PCa gradually became a focus, with attention shifting from clinical populations to cellular model studies.

After 2018, the period from 2019 to 2020 saw the most concentrated keyword bursts, particularly for “gene expression” and “thyroid hormones,” with burst strengths of 1.88 and 1.83, respectively. This indicates that considerable attention was given to the mechanisms by which THs regulate gene expression, cell growth, and their roles in tumorigenesis.

From 2020 onward, the keywords “beta” and “carcinoma” exhibited significant bursts, reflecting researchers’ efforts to explore the effects of THs on tumor progression, metastasis, and apoptosis. Meanwhile, the bursts of the keywords “breast” and “thyroid hormone” also signify the expansion of research in this field, with increasing focus on the roles of THs in other cancer types, such as breast cancer.

Through burst analysis, it can be observed that the research focus in this field has gradually expanded from fundamental molecular mechanisms to the clinical applications across different cancer types, particularly regarding the association between THs and cancer risk.

Top 15 Keywords with the Strongest Citation Bursts

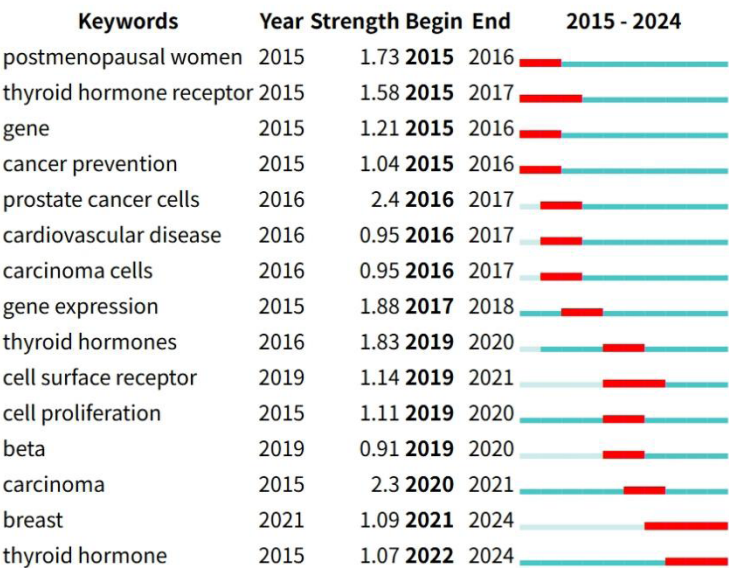


Figure 5. Burst analysis of keywords in research on THs and PCa.

5. Discussion

Existing studies consistently suggest that THs play a significant yet complex role in the initiation and progression of PCa. However, these effects are not unidirectional but rather dualistic. On one hand, epidemiological findings indicate that hyperthyroid states or elevated T3/T4 levels may increase PCa risk, whereas hypothyroidism may confer protective effects. On the other hand, certain cellular and animal studies have shown that T3 can inhibit tumor growth. This paradox suggests that multiple factors, including hormone concentration, receptor subtype distribution, local metabolic status, and tumor molecular subtype heterogeneity influence the role of THs in PCa.

Mechanistically, THs regulate the biological behaviors of PCa through multiple signaling pathways. The classical nuclear receptor pathway, mediated by TR α/β , modulates gene transcription, influencing cell cycle proteins, invasion-related factors, and AR expression, thereby indirectly promoting PCa cell proliferation and invasion. Concurrently, non-genomic mechanisms, particularly the MAPK/PI3K–Akt signaling pathway mediated by integrin $\alpha\beta 3$, exert rapid regulatory effects on metabolic reprogramming, cell migration, and angiogenesis in PCa. Moreover, the DIO enzyme family, key regulators of local TH metabolism, exhibits pathologically significant expression in prostate tissue: DIO2 upregulation increases local T3 levels, promoting proliferation, whereas DIO3 degrades T3 to establish a “locally hypothyroid” microenvironment, facilitating immune evasion by tumors. Collectively, these mechanisms indicate that THs may maintain a “dynamic balance” in PCa, rather than solely exerting pro- or anti-tumor effects.

Clinically, the relationship between THs and PCa holds potential translational value. Serum levels of TSH, FT3, and FT4 may serve as auxiliary biomarkers for prediction and prognosis; when combined with PSA and molecular subtyping, they could enhance the accuracy of individualized risk assessment. The influence of THs on the AR pathway and metabolic regulation suggests that thyroid functional status should be integrated into PCa stratification frameworks to optimize therapeutic decision-making. Furthermore, therapeutic strategies targeting DIO enzyme regulation or integrin $\alpha\beta 3$ blockade may represent novel treatment approaches for CRPC. Notably, thyroid function may also affect the efficacy and adverse effect management of androgen deprivation therapy (ADT) and novel AR pathway inhibitors, highlighting.

6. Conclusion

This study systematically reviews the research progress on the role of THs in the initiation and progression of PCa. Current evidence indicates that the effects of THs are bidirectional, potentially promoting tumor progression via nuclear receptors and non-genomic pathways, while also exerting anti-tumor effects under specific conditions. These outcomes depend on multiple factors, including hormone levels, receptor subtypes, local metabolic status, and the tumor microenvironment. Bibliometric analyses reveal that research hotspots in this field are gradually shifting from epidemiological associations toward molecular mechanisms and clinical translation. However, current studies are still limited by small sample sizes, heterogeneous stratification criteria, and lack of clinical validation. Future studies should focus on large-scale, multicenter prospective cohorts and multi-omics integrative analyses to elucidate the precise roles of THs in PCa and explore their potential as predictive biomarkers and therapeutic targets.

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Disclosure statement

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Exploring the Molecular and Immune Mechanisms Linking Hypothyroidism to Hepatocellular Carcinoma

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Abstract: *Background:* Hepatocellular carcinoma (HCC) is one of the most prevalent malignant tumors worldwide, and endocrine, metabolic, and immune factors influence its occurrence and progression. Hypothyroidism (HT) is a common endocrine disorder that may affect cancer risk; however, its relationship with HCC remains unclear. *Objective:* This study aimed to investigate the potential molecular and immune mechanisms underlying the association between HT and HCC, with a focus on the regulatory effects of HT-related genetic variants on the hepatic tumor immune microenvironment. *Methods:* Single-nucleotide polymorphisms (SNPs) associated with HT and HCC identified through Mendelian randomization were functionally annotated using the Ensembl Genome Browser and mapped to candidate genes. Functional enrichment and pathway analyses were performed with Metascape. Differentially expressed target genes between HCC and normal liver tissues were screened using GEPIA2, and their protein expression levels were validated in the Human Protein Atlas (HPA) database. The association between target gene expression and immune cell infiltration was further evaluated using TIMER2.0. *Results:* A total of 68 candidate genes were analyzed. Enrichment analysis revealed that these genes are involved in IFN- γ -mediated immune responses, PI3K/AKT and RAC1 signaling pathways, and other immune regulatory processes. Among them, HLA-DQA1, HLA-DPB1, HLA-DQA2, and PVT1 showed significant differential expression in HCC. HLA-DQA1, HLA-DPB1, and HLA-DQA2 were positively correlated with CD8⁺ T cells, regulatory T cells (Tregs), and M2 macrophages, suggesting that these genes may exert bidirectional effects on antitumor immunity and immunosuppression. PVT1 may influence the immune microenvironment by regulating myeloid cell recruitment and extracellular matrix remodeling. *Conclusion:* HLA-DQA1, HLA-DPB1, HLA-DQA2, and PVT1 may reduce the risk of HCC by enhancing IFN- γ -mediated antitumor immunity and modulating key signaling pathways, while also contributing to immune microenvironment remodeling. These findings provide mechanistic insights into the protective effects of HT on HCC and suggest potential targets for immunotherapeutic strategies.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent malignant tumors worldwide, with its incidence and mortality rates consistently ranking among the highest for malignant neoplasms globally ^[1]. Recent studies have revealed that the occurrence and progression of HCC are closely associated with multiple factors, including endocrine dysregulation, metabolic abnormalities, and immune imbalance ^[2]. Hypothyroidism (HT) is a common endocrine disorder in clinical practice, typically characterized by elevated serum thyroid-stimulating hormone (TSH) levels accompanied by reduced synthesis of triiodothyronine (T3) and/or thyroxine (T4). In iodine-sufficient regions, autoimmune diseases such as Hashimoto's thyroiditis are the leading causes of HT ^[3].

Previous studies have shown that thyroid hormones may regulate tumor initiation and progression through multiple mechanisms. For instance, thyroid hormones and TSH can directly participate in tumorigenesis by acting through cell surface receptor-mediated signaling, modulating estrogen signaling pathways, promoting angiogenesis, and regulating gene expression ^[4]. In addition, HT frequently coexists with chronic conditions such as diabetes and cardiovascular disease, which are closely associated with increased cancer risk ^[5]. Epidemiological studies have demonstrated substantial heterogeneity in the association between HT and various types of cancer. Some studies have suggested that HT is associated with increased risks of thyroid and breast cancers ^[6], whereas other large-scale cohort studies have not observed significant associations between HT and overall or site-specific cancer risks ^[7].

However, current observational studies investigating the association between HT and HCC remain controversial and are prone to confounding by factors such as age, underlying liver disease (e.g., hepatitis B or C infection), and metabolic status, making it difficult to accurately infer a causal relationship. Mendelian randomization is an epidemiological approach that uses genetic instrumental variables (IVs) to infer causal relationships between exposures and outcomes ^[8], thereby reducing confounding inherent in traditional observational studies. Recent studies employing Mendelian randomization have suggested an inverse causal association between HT and HCC ^[9]. However, thyroid hormone indices such as TSH and free thyroxine (FT4) have not shown significant causal relationships with HCC ^[9], indicating that the effects of HT on HCC may involve complex underlying biological mechanisms.

Based on these findings, the present study aims to further investigate the potential biological mechanisms of HT in HCC. We systematically analyze the differential expression patterns and immune infiltration characteristics of genes mapped by HT-related single-nucleotide polymorphisms (SNPs) in HCC tissues, thereby exploring the potential mechanisms through which these genes may influence HCC development by modulating the tumor immune microenvironment. Through this study, we aim to provide molecular and immunological evidence supporting the association between HT and HCC and to offer potential directions for the development of early intervention strategies and immunotherapeutic targets for HCC.

2. Materials and methods

2.1. Overall study design

This study aims to investigate the potential causal relationship between HT and HCC and to elucidate the underlying mechanisms (**Figure 1**). SNPs associated with HT and HCC were functionally annotated using the Ensembl database to identify their corresponding genes. Subsequently, the identified genes were subjected to pathway and functional enrichment analyses using the Metascape platform to uncover the potential biological processes and signaling pathways underlying the association between HT and HCC. Differential expression and prognostic significance of these genes in HCC and normal liver tissues were then analyzed using the GEPIA2 database, and protein-level validation was performed using immunohistochemistry data from The Human Protein Atlas (HPA) database. To further elucidate the immunological context, the TIMER2.0 platform was employed to systematically assess the infiltration levels of various immune cell populations, including CD8⁺ T cells, regulatory T cells (Tregs), macrophage subtypes (M0, M1, and M2), cancer-associated fibroblasts (CAFs), neutrophils, natural killer (NK) cells, and myeloid-derived suppressor cells (MDSCs).

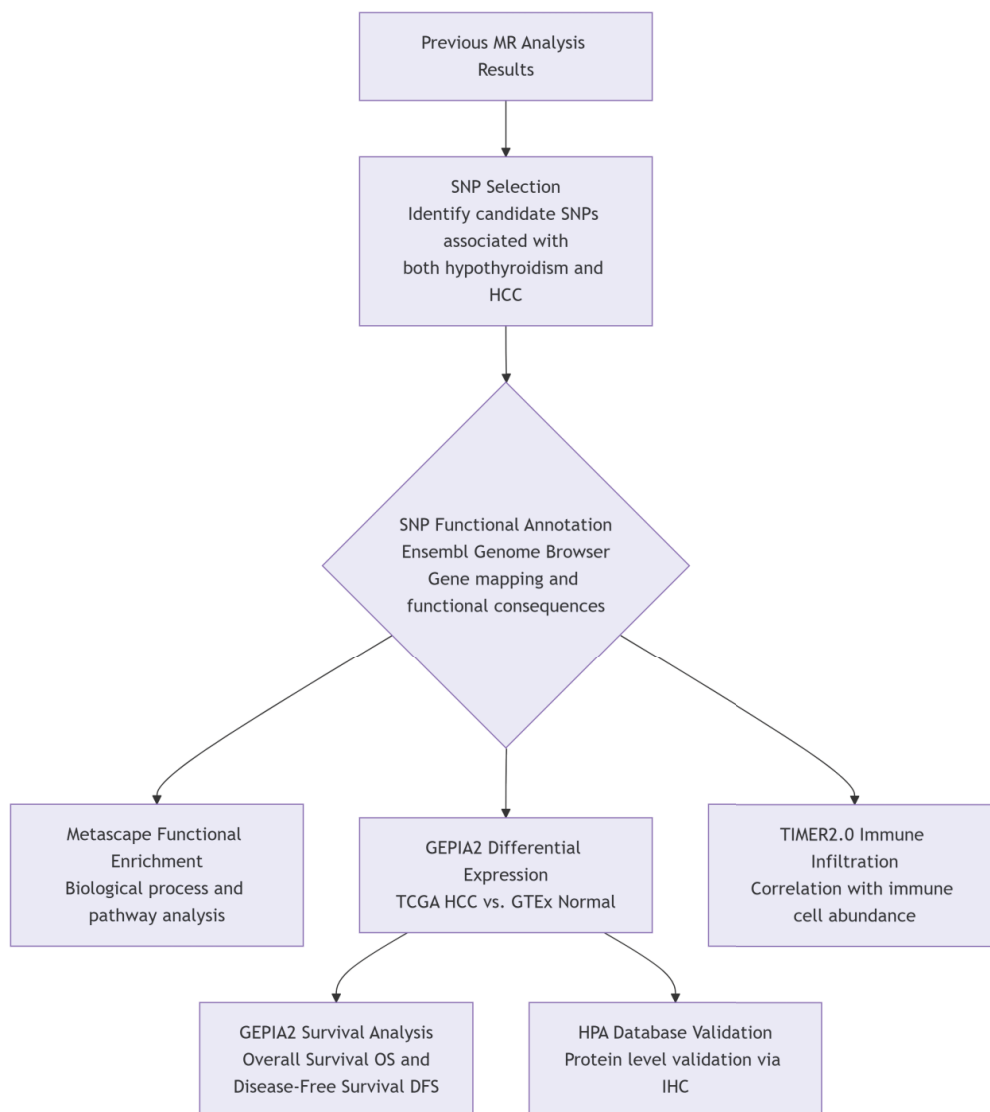


Figure 1. Experimental design flow chart.

2.2. Data sources

This study builds upon a previously published Mendelian randomization (MR) analysis ^[9], which indicated a significant inverse causal relationship between HT and HCC. Based on this, we further extracted the SNPs identified in the MR analysis that were associated with HT and significantly related to HCC, using these as candidate genetic variants for downstream analyses.

2.3. SNP functional annotation analysis

Functional annotation and gene mapping of the candidate SNPs were performed using the Ensembl Genome Browser (<https://www.ensembl.org/>) ^[10]. For each locus, we retrieved the chromosomal location, functional category (e.g., missense mutation, synonymous mutation, promoter region, intronic region, or regulatory region), and the corresponding target gene information.

2.4. Metascape functional enrichment analysis

Metascape (<http://metascape.org/gp/index.html#/main/step1>) is a comprehensive bioinformatics platform that integrates more than 40 distinct biological databases and provides various functions, including interactive analysis and gene annotation ^[11]. The study used Metascape to perform rapid gene expression profiling and functional enrichment analyses of the identified differentially expressed genes.

2.5. Differential expression analysis

Differential expression of candidate genes between HCC tissues (from The Cancer Genome Atlas, TCGA) and normal liver tissues (from the Genotype-Tissue Expression, GTEx, database) was analyzed using the GEPIA2 platform (<http://gepia2.cancer-pku.cn/>) ^[12]. The analysis was based on $\log_2(\text{TPM}+1)$ -normalized expression values, and a univariate differential expression test was performed. Subsequently, survival analyses were conducted using the GEPIA2 platform, including overall survival (OS) and disease-free survival (DFS). Kaplan–Meier curves were generated to visualize survival outcomes, and the significance of differences was assessed using the log-rank test, with p -values < 0.05 considered statistically significant.

2.6. Protein level validation

To validate the protein-level expression of the differentially expressed genes, immunohistochemistry (IHC) results were retrieved from The Human Protein Atlas (HPA) database (<https://www.proteinatlas.org/>) ^[13] to compare protein expression between HCC and normal liver tissues. The available HPA data were used to support the validation of transcriptional results obtained from GEPIA2 analyses.

2.7. Immune infiltration analysis

To investigate the potential roles of candidate genes in the tumor immune microenvironment, correlations between candidate gene expression and the infiltration levels of various immune cells were analyzed using the TIMER2.0 database (<http://timer.cistrome.org/>) ^[14]. The immune cell types analyzed included CD8⁺ T cells, regulatory T cells (Tregs), macrophage subtypes (M0, M1, and M2), cancer-associated fibroblasts (CAFs), neutrophils, natural killer (NK) cells, and myeloid-derived suppressor cells (MDSCs). Spearman correlation analysis was used to assess the relationships between gene expression and immune cell infiltration levels, with a significance threshold set at $p < 0.05$.

3. Results

3.1. SNP functional annotation analysis

To further investigate the potential functions and biological significance of the SNPs identified in the Mendelian randomization analysis of HT and HCC, we performed a systematic functional annotation of these SNPs. The 71 highly associated SNPs selected from the results were first deduplicated and then functionally annotated using the Variant Effect Predictor (VEP) tool provided by the Ensembl database, identifying the genomic location, potential functional effects, and associations with diseases or phenotypes for each SNP. After deduplication, 68 target genes were ultimately selected for subsequent analyses.

3.2. Functional enrichment analysis

3.2.1. Pathway and process enrichment analysis

Enrichment analysis of genes corresponding to the SNPs identified in the MR analysis revealed that these genes were primarily involved in immune regulation and cell signaling pathways (**Figure 2**). The most significant enrichment was observed in the autoimmune thyroid disease pathway (hsa05320, $\text{Log}_{10}P = -7.16$), suggesting a close association between HT-related genetic variants and immune function. Further analysis showed that the gene set was significantly enriched in the positive regulation of immune response (GO:0050778) and positive regulation of type II interferon production (GO:0032729), indicating that these genes may participate in antitumor processes by enhancing interferon signaling pathways. In addition, the RAC1 regulatory pathway (PID RAC1 REG PATHWAY) and PI3K/AKT signaling pathway (GO:0051896) were also significantly enriched, both of which are key drivers of cell proliferation and survival. Moreover, the gene set showed enrichment trends in the negative regulation of myeloid cell differentiation (GO:0045638), inflammatory response (GO:0006954), and macrophage efferocytosis (hsa04148), suggesting that these genes may play roles in modulating the inflammatory environment and immune cell functions.

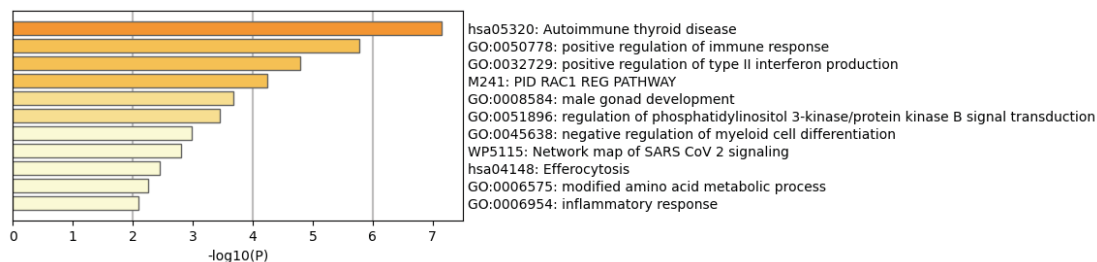


Figure 2. Pathway and process enrichment analysis.

3.2.2. DisGeNET11 disease association analysis

DisGeNET¹¹ analysis revealed that HT-related genes were significantly enriched in multiple autoimmune diseases (**Figure. 3**), including hypothyroidism (35 genes, 59%, $\text{Log}_{10}P = -44.00$, $\text{Log}_{10}q = -40.00$), Hashimoto's disease (13 genes, 22%, $\text{Log}_{10}P = -13.00$, $\text{Log}_{10}q = -9.50$), Graves' disease (18 genes, 31%, $\text{Log}_{10}P = -16.00$, $\text{Log}_{10}q = -13.00$), celiac disease (18 genes, 31%), and autoimmune chronic hepatitis (10 genes, 17%). Additionally, enrichment was observed for blood thyroid-stimulating hormone analysis (4 genes, 6.8%) and autoimmune hepatitis with central lobular necrosis (4 genes, 6.8%). These findings indicate that HT-related genes are highly associated with various autoimmune diseases and may participate in the negative regulation of HCC development by modulating the hepatic microenvironment through systemic immune regulation.

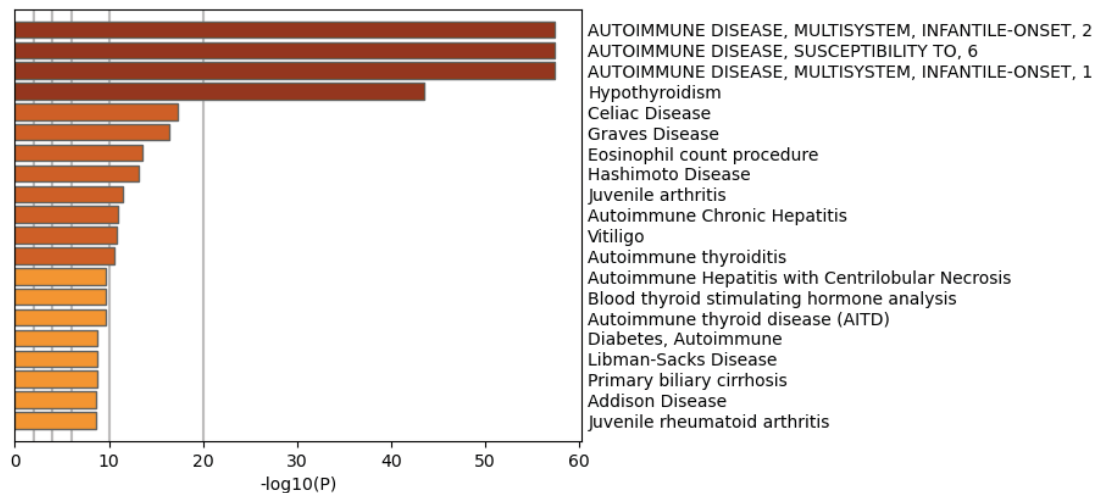


Figure 3. DisGeNET11 disease association analysis.

3.2.3. PaGenBase tissue/cell specificity analysis

PaGenBase analysis revealed that HT-related genes exhibited tissue- or cell-specific expression, including thyroid tissue (5 genes, 8.5%, $\text{Log}_{10}P = -4$, $\text{Log}_{10}q = -1.50$) and HL60 cells (3 genes, 5.1%, $\text{Log}_{10}P = -3$, $\text{Log}_{10}q = -0.68$). This suggests that some of the identified genes may participate in systemic or local immune and metabolic regulation through thyroid- or blood cell-specific functions.

3.2.4. TRRUST Transcription factor regulation analysis

TRRUST analysis indicated that RFX family transcription factors (RFXANK, RFXAP, RFX5) each regulated three genes (5.1%, $\text{Log}_{10}P = -5.40$ to -5.20 , $\text{Log}_{10}q = -2.60$ to -2.40). This suggests that the RFX family may play a critical role in regulating the expression of HT-related genes and modulating immune functions.

3.2.5. Transcription factor target analysis

Transcription factor target analysis revealed significant enrichment of PRDM5 target genes (3 genes, 5.1%, $\text{Log}_{10}P = -2.80$, $\text{Log}_{10}q = -0.46$), STAT4 01 (4 genes, 6.8%, $\text{Log}_{10}P = -2.70$), STAT5A 03 (4 genes, 6.8%, $\text{Log}_{10}P = -2.70$), and STAT6 01 (4 genes, 6.8%, $\text{Log}_{10}P = -2.70$); TERF2 and ZNF507 target genes included 3 and 5 genes, accounting for 5.1% and 8.5%, respectively. These results suggest that transcription factors such as STATs and PRDM5 may participate in cell signaling, immune regulation, and maintenance of cellular homeostasis by regulating HT-related genes.

3.3. Differentially expressed genes and protein level verification

Differential expression analysis of 68 selected genes between liver hepatocellular carcinoma (LIHC) and normal tissues was performed using GEPIA2 (**Figures 4–6**), which identified four significantly differentially expressed genes: HLA-DQA1, HLA-DPB1, PVT1, and HLA-DQA2. Subsequent analysis of OS and DFS revealed no significant association between these four genes and patient survival. Protein-level validation using The Human Protein Atlas indicated that HLA-DQA1 and HLA-DPB1 were markedly overexpressed in HCC tissues compared with normal liver tissues, consistent with the differential expression results obtained from GEPIA2. IHC images for PVT1 and HLA-DQA2 were unavailable.

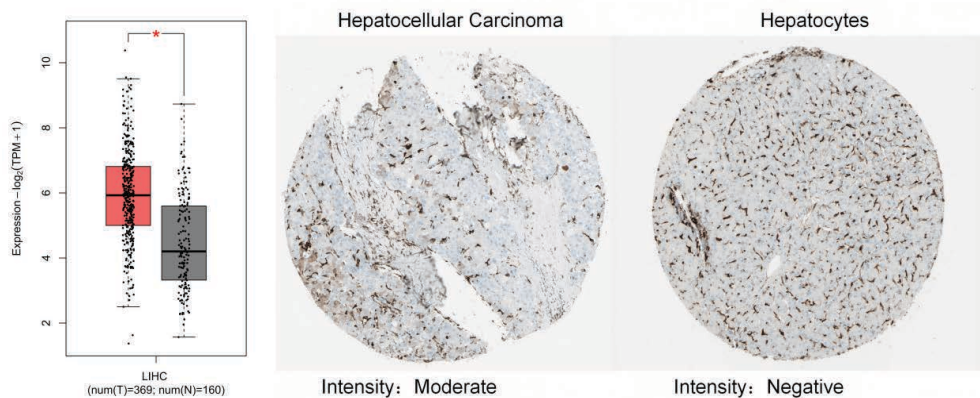


Figure 4. HLA-DPB1 differential expression analysis.

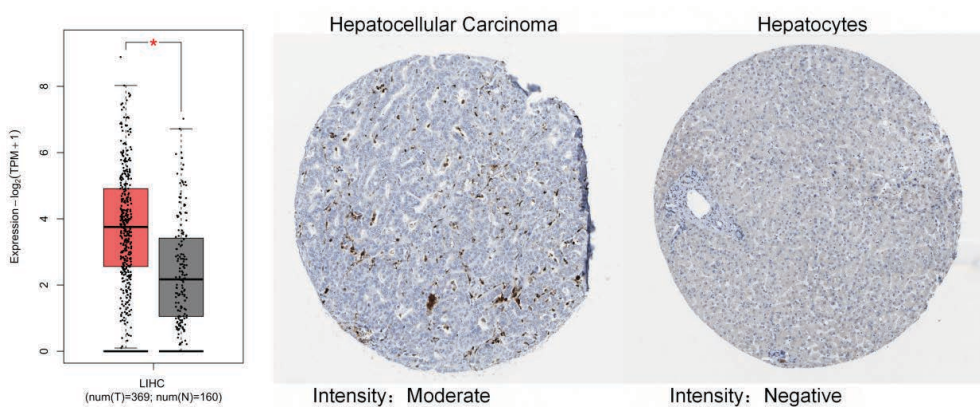


Figure 5. HLA-DQA1 differential expression analysis.

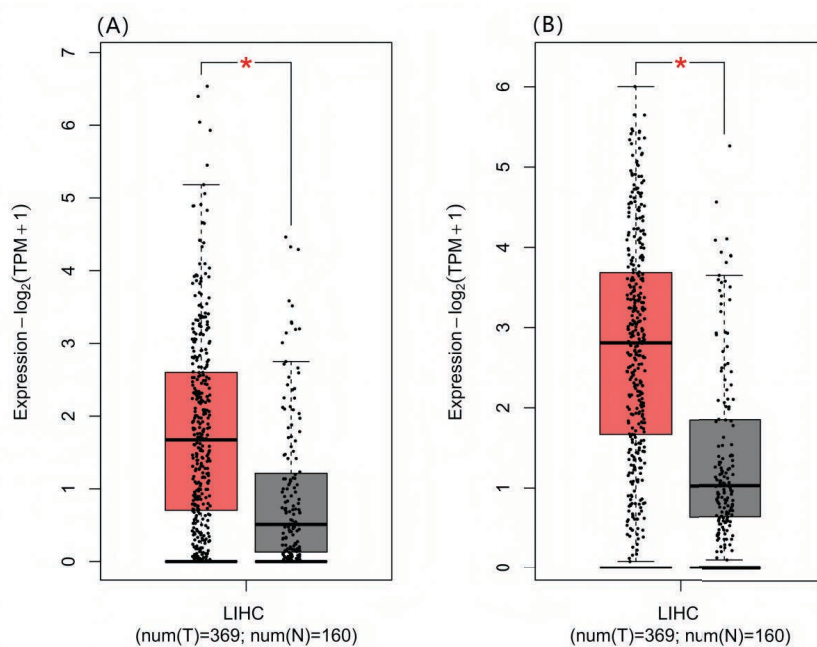


Figure 6. Differential expression of (A) HLA-DQA2 and (B) PVT1 in LHCC.

3.4. Immune infiltration analysis

Using the TIMER2.0 platform, correlations between the differentially expressed genes HLA-DQA1, HLA-DPB1, PVT1, and HLA-DQA2 and the infiltration levels of various immune cell types were analyzed (**Figures 7–10**). In HCC tissues, HLA-DQA1 expression was significantly positively correlated with Tregs (Rho = 0.674, $P = 5.64 \times 10^{-47}$), CD8⁺ T cells (Rho = 0.637, $P = 1.28 \times 10^{-40}$), M2 macrophages (Rho = 0.612, $P = 6.90 \times 10^{-37}$), and activated NK cells (Rho = 0.510, $P = 2.93 \times 10^{-24}$), while showing a significant negative correlation with tumor purity (Rho = -0.476, $P = 5.91 \times 10^{-21}$), suggesting higher expression in immune-infiltrated tumor microenvironments. HLA-DPB1 was significantly positively correlated with Tregs (Rho = 0.708, $P = 9.39 \times 10^{-54}$), M2 macrophages (Rho = 0.674, $P = 5.85 \times 10^{-47}$), and CD8⁺ T cells (Rho = 0.463, $P = 9.26 \times 10^{-20}$), and negatively correlated with tumor purity (Rho = -0.478, $P = 3.59 \times 10^{-21}$), indicating potential involvement in both immunosuppressive and immunoactive cellular processes. HLA-DQA2 was significantly positively correlated with Tregs (Rho = 0.462, $P = 1.27 \times 10^{-19}$), M1 macrophages (Rho = 0.331, $P = 2.97 \times 10^{-10}$), M2 macrophages (Rho = 0.479, $P = 3.35 \times 10^{-21}$), CD8⁺ T cells (Rho = 0.386, $P = 1.02 \times 10^{-13}$), and MDSCs (Rho = 0.237, $P = 8.28 \times 10^{-6}$), while negatively correlating with tumor purity (Rho = -0.243, $P = 4.90 \times 10^{-6}$). Overall, PVT1 exhibited weaker correlations with immune cell infiltration compared to HLA genes, showing positive associations with M0 macrophages (Rho = 0.261, $P = 8.8 \times 10^{-7}$), cancer-associated fibroblasts (Rho = 0.187, $P = 4.77 \times 10^{-4}$), and neutrophils (Rho = 0.143, $P = 7.62 \times 10^{-3}$), but no significant correlation with CD8⁺ T cells (Rho = -0.033, $P > 0.05$).

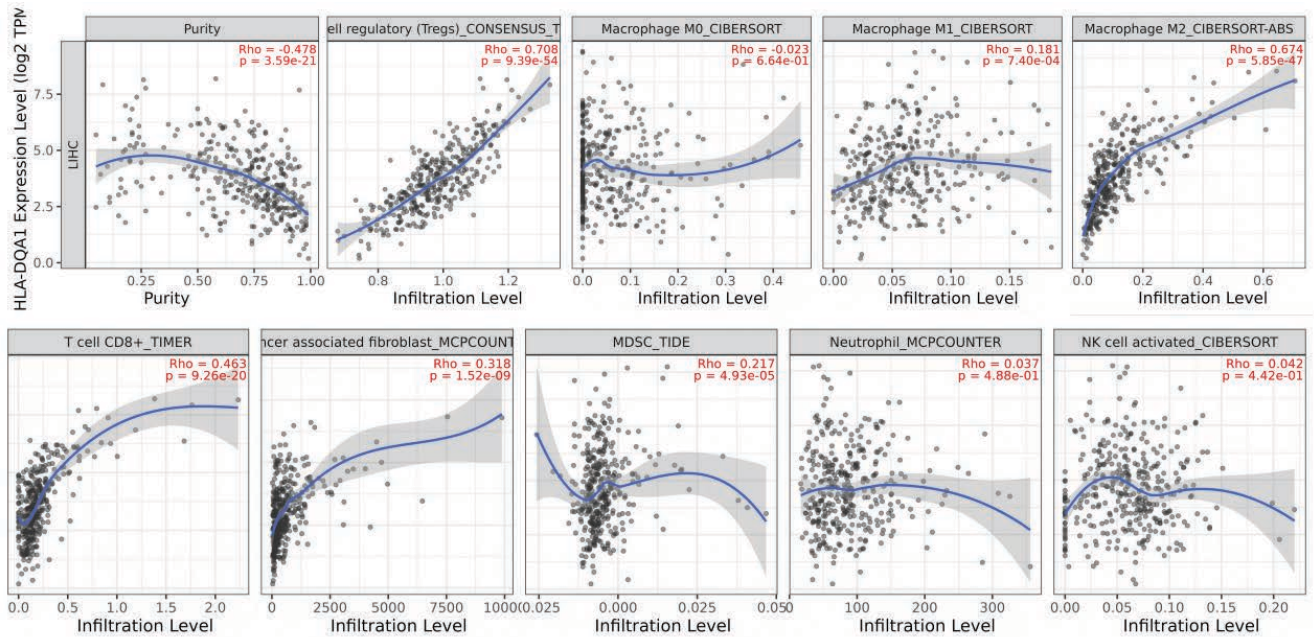


Figure 7. HLA-DQA1 Immune infiltration analysis.

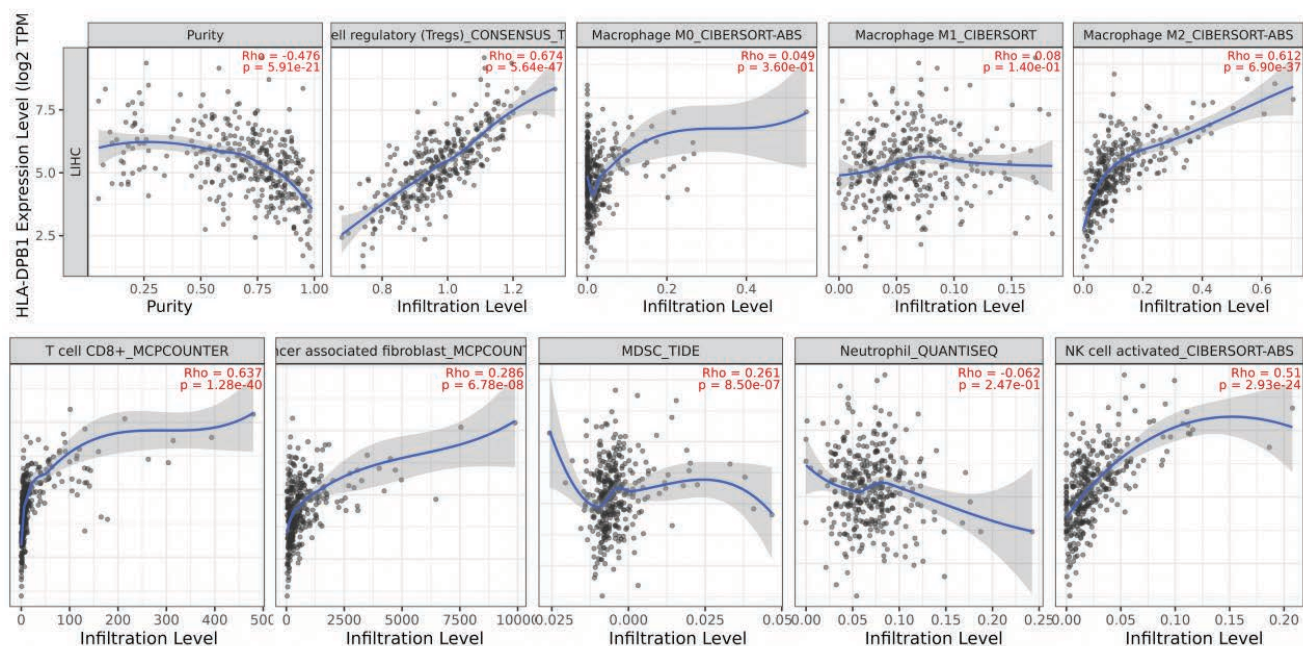


Figure 8. HLA-DPB1 Immune infiltration analysis.

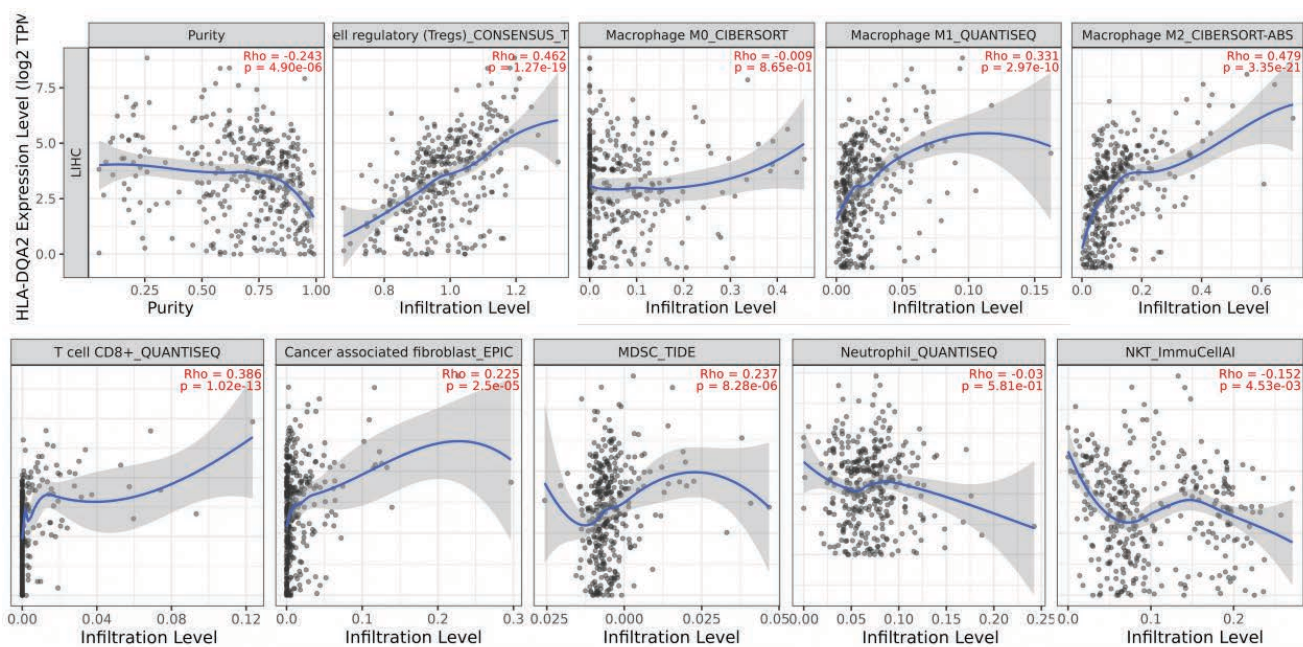


Figure 9. HLA-DQA2 Immune infiltration analysis.

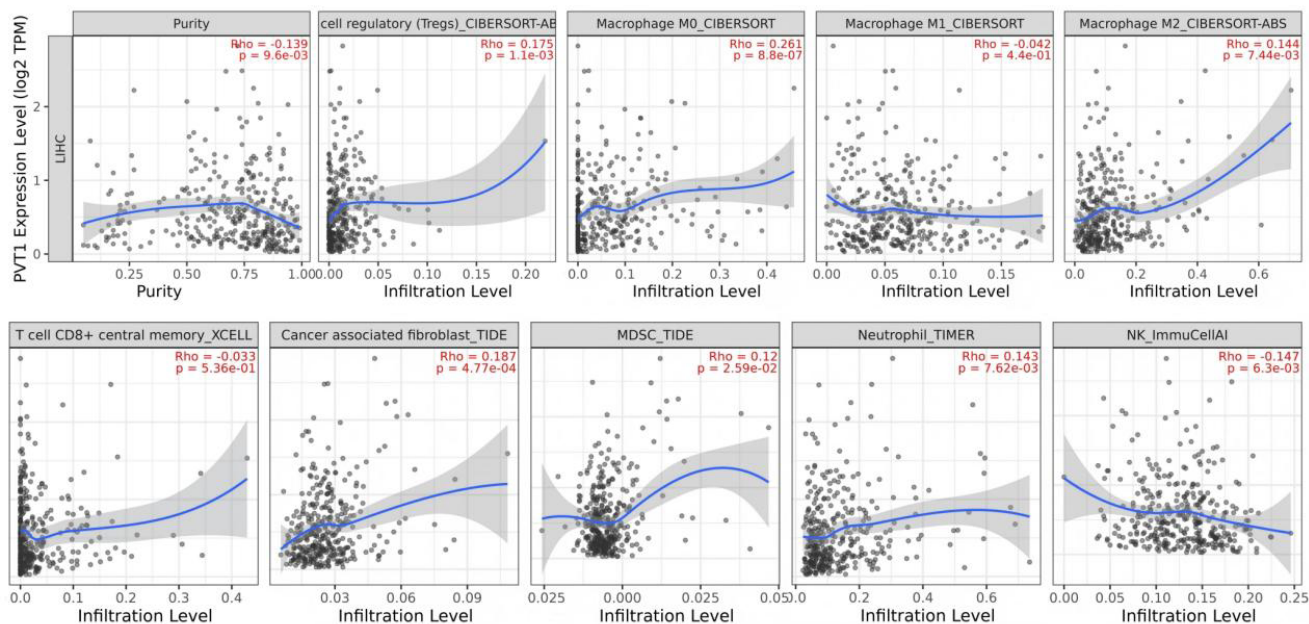


Figure 10. PVT1 Immune infiltration analysis.

4. Discussion

This study systematically explored the potential molecular mechanisms linking hypothyroidism (HT) and hepatocellular carcinoma (HCC) by integrating Mendelian randomization results with multi-level bioinformatic analyses. Initially, pathway enrichment analysis was performed to characterize the signaling features of HT-associated genes, followed by differential expression and immune infiltration analyses, which further identified HLA-DQA1, HLA-DPB1, HLA-DQA2, and PVT1 as key genes potentially involved in modulating the immune microenvironment of HCC.

4.1. The potential molecular mechanism of HT and HCC negative correlation

Pathway enrichment analysis indicated that HT-associated genes were primarily enriched in type II interferon (IFN- γ) signaling and positive regulation of immune response pathways. IFN- γ , a key cytokine in antitumor immunity, enhances antigen presentation, promotes cytotoxic T lymphocyte (CTL) activation, and induces tumor cell apoptosis^[15]. Thus, the genetic background associated with HT may enhance IFN- γ -mediated immune surveillance, improving the clearance of abnormal hepatocytes and thereby exerting a protective effect.

Additionally, enrichment of PI3K/AKT and RAC1 signaling pathways suggests that HT may suppress key axes promoting cell proliferation and migration, thereby attenuating HCC initiation and progression. This is consistent with previous studies indicating the role of thyroid hormones in PI3K/AKT regulation^[16], further supporting a molecular explanation for the inverse association between HT and HCC.

Disease association analysis using DisGeNET revealed that HT-associated genes are closely linked to multiple autoimmune diseases, particularly thyroid autoimmune disorders and autoimmune hepatitis. This suggests that HT may indirectly improve the hepatic microenvironment through heightened immune responses, thereby inhibiting HCC development. Tissue- and cell-specific analyses demonstrated that HT-associated genes exhibit specific expression in thyroid tissue and blood cells (HL60), further suggesting that they may mediate hepatoprotective

effects via systemic immune and metabolic regulation.

Transcription factor analysis indicated that RFX, STAT, and PRDM5 families play crucial roles in regulating HT-associated genes. The STAT family is involved in cell proliferation, apoptosis, and inflammation regulation, whereas the RFX family serves as a key regulator of MHC gene expression. These findings suggest that the genetic background associated with HT may modulate immune homeostasis and inflammatory responses through transcriptional regulatory networks, thereby exerting systemic inhibitory effects on HCC.

HT-associated genes were significantly enriched in inflammatory responses, negative regulation of myeloid cell differentiation, and macrophage efferocytosis. These processes are crucial for alleviating chronic inflammation, facilitating clearance of necrotic cells, and mitigating liver fibrosis, suggesting that HT may indirectly reduce HCC risk by improving the hepatic immune microenvironment.

4.2. The significance of differentially expressed genes and immune infiltration

4.2.1. The roles of the HLA-DQA1, HLA-DPB1, and HLA-DQA2 genes

HLA-DQA1, HLA-DPB1, and HLA-DQA2 are MHC class II molecules primarily responsible for presenting exogenous antigens and regulating CD4⁺ T cell activation ^[17]. In this study, these genes were found to be differentially overexpressed in HCC. Although no significant associations were observed in OS or DFS analyses, immune infiltration analysis revealed strong correlations with CD8⁺ T cells, Tregs, and M2 macrophages, suggesting the presence of both immune-activating and immunosuppressive signals within the tumor microenvironment. On one hand, high expression of MHC II molecules may enhance antigen presentation efficiency, thereby activating CD4⁺ T cells and indirectly stimulating CD8⁺ T cell-mediated antitumor responses, potentially inhibiting tumor growth in early stages or under specific conditions. On the other hand, aberrant or sustained high expression of HLA molecules may promote Treg recruitment or activate myeloid-derived suppressor cells, indirectly facilitating tumor immune tolerance and immune evasion. Previous studies have also indicated that abnormal expression of HLA II genes in cancers can impact antigen presentation pathways and T cell function ^[18]. Collectively, these findings suggest that HLA-DQA1, HLA-DPB1, and HLA-DQA2 may exert bidirectional regulation within the HCC immune microenvironment, simultaneously participating in antitumor immune activation and promoting immunosuppression and tumor escape. This dual role provides a potential mechanistic explanation for the inverse association between hypothyroidism and HCC and indicates that these genes may contribute to hypothyroidism-associated susceptibility to HCC via modulation of the tumor immune microenvironment.

4.2.2. The roles of PVT1 of the gene

PVT1, located on human chromosome 8q24.21, is a prototypical oncogenic long non-coding RNA (lncRNA) that promotes tumor progression through multiple mechanisms in various cancers ^[19], including acting as a miRNA sponge, stabilizing oncogenic proteins such as MYC, modulating epigenetic regulatory complexes, and mediating intercellular communication via exosomes to influence immune cell behavior. In this study, PVT1 was found to be significantly overexpressed in HCC, suggesting its potential involvement in hepatocarcinogenesis. Although no significant associations were observed in overall survival (OS) or disease-free survival (DFS) analyses, immune infiltration analysis using TIMER2.0 suggests that PVT1 may modulate the tumor immune microenvironment. Potential mechanisms include PVT1 promoting the recruitment of myeloid cells and their polarization toward immunosuppressive M2 macrophages via upregulation of chemokines or TGF- β signaling, or indirectly

modulating immune cell infiltration and function by activating cancer-associated fibroblasts (CAFs) to remodel the extracellular matrix and cytokine network. This effect likely occurs primarily at the stage of macrophage precursor recruitment, followed by polarization toward immunosuppressive phenotypes under tumor microenvironmental signals, suggesting that PVT1 may facilitate immune evasion in HCC through microenvironmental remodeling. Previous studies have demonstrated that PVT1 can competitively bind miRNAs, such as miR-143-3p and miR-214, to regulate downstream target gene expression ^[20], thereby promoting HCC cell proliferation, migration, and invasion, and stabilizing NOP2 to enhance tumor stem-like properties. Functional studies further indicate that PVT1 knockdown suppresses tumor cell proliferation and induces apoptosis ^[21], highlighting its potential as a therapeutic target for HCC.

4.3. Limitations

This study primarily relied on publicly available databases and bioinformatic analyses to systematically investigate the expression patterns and potential immunoregulatory roles of HT-related SNP-targeted genes in HCC. However, several limitations should be noted. First, the analyses were based solely on *in silico* approaches, lacking experimental validation; therefore, the observed gene expression differences and correlations with immune infiltration require confirmation through *in vitro* or *in vivo* experiments. Second, the molecular mechanisms underlying the causal relationship between HT and HCC remain incompletely elucidated. Although Mendelian randomization analyses suggest a negative association, prior literature indicates that HT-related hormonal indices (e.g., TSH, FT4) have not been shown to exert direct causal effects on HCC. Thus, further studies combining clinical samples and functional experiments are needed to clarify how HT may influence HCC development. Third, the immune microenvironment analysis was limited to gene expression correlations and cannot fully reflect the functional state or spatial distribution of immune cells within tumors.

Future research could be expanded in several directions. On one hand, functional experiments using clinical cohorts or *in vitro/in vivo* models should validate the roles of PVT1, HLA-DQA1, HLA-DPB1, and HLA-DQA2 in the HCC immune microenvironment, including their effects on macrophage polarization, Treg modulation, and antigen-presenting capacity. On the other hand, investigating the dynamic relationship between HT-related hormone levels and immune regulatory pathways may reveal the molecular mechanisms by which HT reshapes the HCC immune landscape. Multi-omics integrative analyses, such as transcriptomics, single-cell sequencing, and spatial omics, could further elucidate the complex interactions among tumor, immunity, and metabolism, providing theoretical foundations for early risk assessment and precision interventions in HT-associated HCC.

5. Conclusion

This study, based on Mendelian randomization results and bioinformatic analyses, systematically explored the potential mechanisms linking HT and HCC. HT-related genes may reduce HCC risk by enhancing IFN- γ -mediated antitumor immunity and suppressing the PI3K/AKT and RAC1 signaling axes. Key genes, including HLA-DQA1, HLA-DPB1, HLA-DQA2, and PVT1, potentially modulate the tumor immune microenvironment, influence myeloid cell polarization, and remodel the extracellular matrix, thereby exerting bidirectional regulation of both immune activation and immunosuppression. This study provides mechanistic insights into the protective effects of HT on HCC and identifies potential targets for immune-based interventions.

Disclosure statement

The authors declare no conflict of interest.

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Exploring Gastric Cancer-Related Genes and Clinical Significance Analysis Based on Bioinformatics

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Abstract: *Objective:* Employing bioinformatics methodologies to identify core genes intricately associated with the pathogenesis and progression of gastric cancer, and to evaluate their clinical significance. *Method:* Gene expression datasets GSE19826 and GSE13911 were acquired from the Gene Expression Omnibus (GEO). Differential gene expression analysis was conducted using GEO2R. Common differentially expressed genes (DEGs) were discerned via Venn diagram analysis on a bioinformatics platform. Functional enrichment analyses, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), were performed on these overlapping DEGs. A protein-protein interaction (PPI) network was constructed with the STRING database, and central hub genes were identified using Cytoscape software. The expression profiles, prognostic value, and immune infiltration correlations of these key genes were further examined utilizing the GEPIA, Kaplan-Meier plotter, Human Protein Atlas (HPA), and TIMER databases. *Results:* Analysis revealed 120 commonly differentially expressed genes. These genes were significantly enriched in biological pathways concerning muscle cell cytoskeleton regulation, nutrient absorption, and extracellular matrix receptor interactions. PPI network analysis highlighted 10 core genes, including COL1A1, COL1A2, BGN, THBS2, COL5A2, and TIMP1. These genes exhibited marked upregulation in GC tissues. Statistical evaluation confirmed a significant link between their elevated expression and unfavorable patient outcomes ($P < 0.01$). Furthermore, immune infiltration assessment indicated a positive correlation between the expression of these genes and macrophage infiltration within the tumor microenvironment, implying their involvement in modulating the immune response in GC, which could affect tumor behavior and clinical progression. *Conclusion:* The six genes identified may function as diagnostic biomarkers and represent promising therapeutic targets for gastric cancer.

Keywords: Gastric cancer; Biomarker; Bioinformatics; Differentially expressed genes; Immune microenvironment

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1. Introduction

Gastric cancer (GC) is a prevalent malignancy within the digestive tract, ranking among the most frequent cancers in China and representing the third primary cause of cancer-associated mortality globally ^[1]. Thanks to better understanding of the causes of GC and advancements in early screening and clinical diagnosis and treatment techniques, the overall incidence and mortality of GC have decreased significantly. However, in 2020, there were more than one million new cases and over 768,000 deaths worldwide. These figures are expected to increase by 2040, with 1.77 million new cases and 1.27 million deaths projected worldwide ^[2]. Probably because of the occult nature of early-stage gastric cancer, most patients have metastases at the time of initial diagnosis, reaching an advanced stage of diagnosis. This has a significant impact on treatment and prognosis. Therefore, adopting effective detection methods to improve early diagnosis, optimize treatment, reduce recurrence, and improve prognosis is the main challenge and prospect of GC patient management. With the rapid development of research on GC metabolic biomarkers, new GC biomarkers have been discovered, such as expression levels of various proteins and genes in GC samples, creating new opportunities for the diagnosis and monitoring of GC patients ^[3]. These findings may provide valuable targets for the early diagnosis and individualized treatment of GC. Therefore, the discovery of efficient tumor markers is of great significance for the diagnosis and treatment of GC and for improving patient survival rates. In this study, we screened and downloaded GC-related datasets from the GEO database, used bioinformatics methods to mine and analyze DEGs in GC tissues and adjacent normal tissues, selected the relevant key genes, analyzed their expression and survival curves, and the key was to identify possible GC diagnostic markers and potential therapeutic targets.

1.1 Data acquisition

The NCBI GEO database (<https://www.ncbi.nlm.nih.gov/geo>) was accessed to obtain the gene expression datasets GSE19826, comprising 12 samples (tumor/adjacent normal), and GSE13911, which includes 38 tumor and 31 normal samples.

1.2. Differential expression analysis

DEGs between GC tissues and matched normal tissues were identified using GEO2R. Thresholds were set at $|\log FC| > 1$ and an adjusted P -value < 0.05 . Common DEGs across datasets were visualized and selected through Venn diagram analysis on a bioinformatics platform.

1.3. Functional enrichment analysis

GO/KEGG analysis of overlapping DEGs was performed using the microbioinformatics platform ($P < 0.05$), with GO focusing on biological functions and KEGG focusing on signaling pathways.

1.4. PPI network construction

Construct the PPI network in the STRING database (with a confidence level of 0.4) and screen the Top 10 hub genes using the degree plugin of Cytoscape 3.9.0.

1.5. Validation of key genes

mRNA expression differences were analyzed by GEPIA, survival associations were evaluated by Kaplan-Meier plotter, protein expression was verified by HPA, and immune infiltration was analyzed by TIMER.

2. Results

2.1. Screening of differentially expressed genes

A total of 120 overlapping DEGs were consistently identified across the two analyzed datasets (**Figure 1** and **Figure 2**).

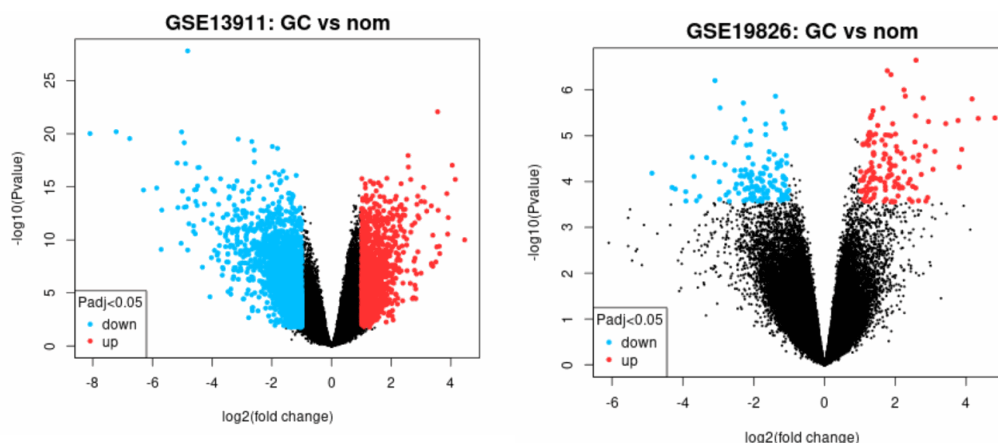


Figure 1. Volcano maps of the GSE19826 and GSE13911 datasets.

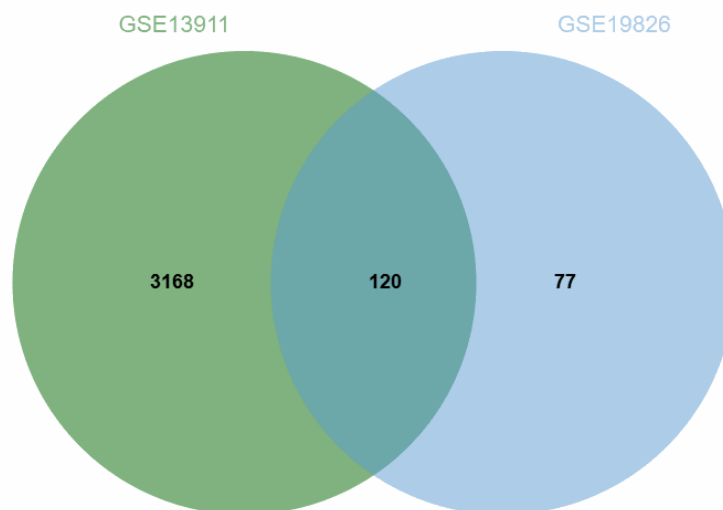


Figure 2. Venn diagrams of the GSE19826 and GSE13911 datasets DEGs.

2.2. Functional enrichment analysis of DEGs

DEGs are primarily involved in extracellular matrix formation (BP), collagen trimer formation (CC), and glycosaminoglycan binding (MF). The KEGG pathway is enriched in myocytoskeletal regulation and extracellular matrix receptor interaction (**Figure 3**).

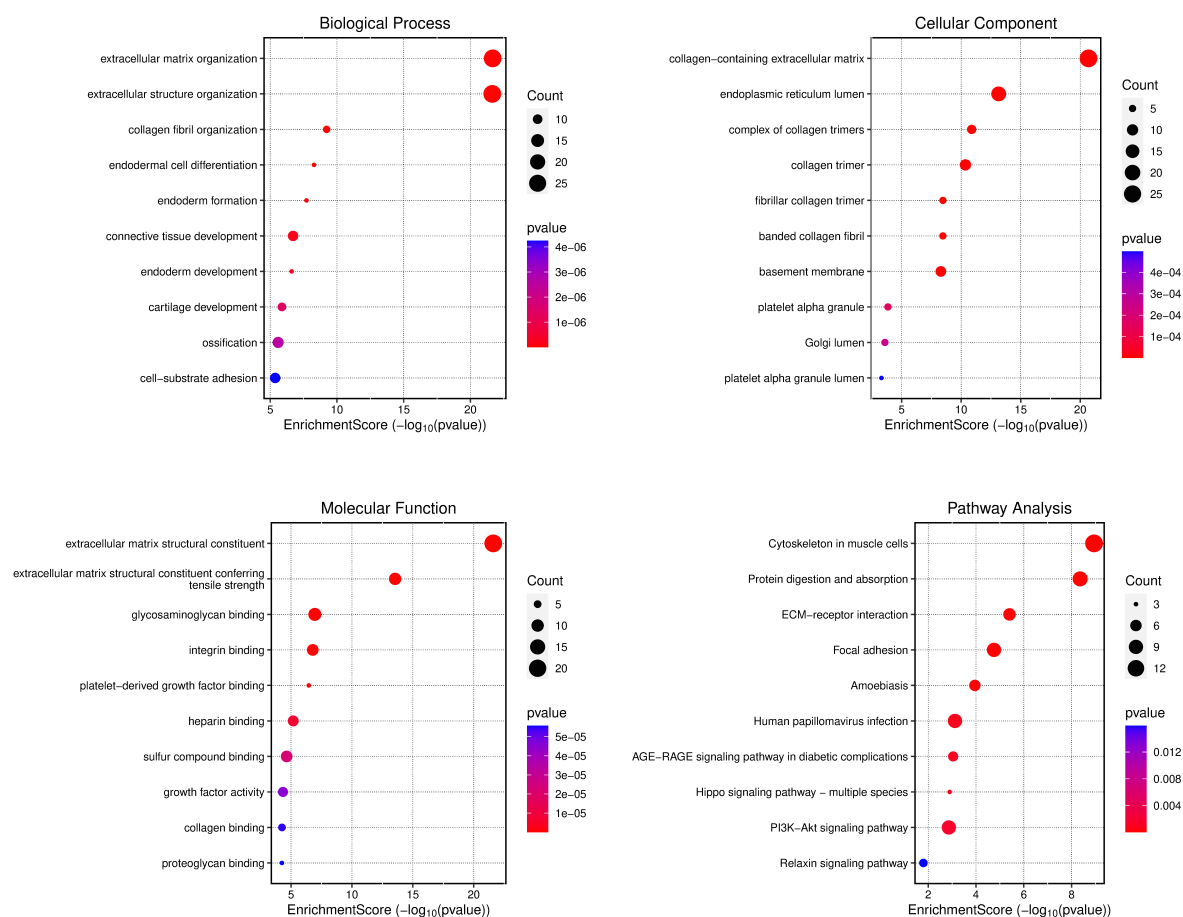


Figure 3. Enrichment analysis of overlapping differentially expressed genes based on GO and KEGG.

2.3. PPI network analysis

10 hub genes, such as COL3A1 and FN1 were screened out (Figure 4).

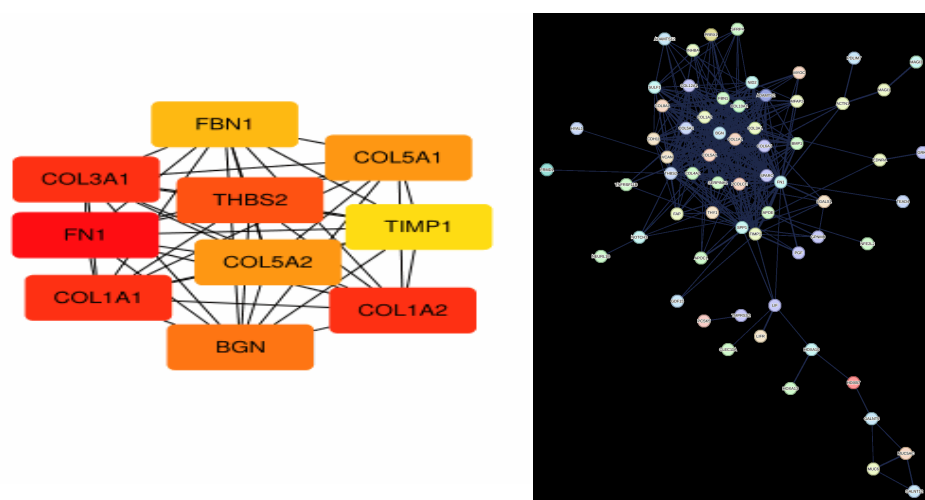


Figure 4. PPI network map of overlapping DEGs and key genes (A.PPI network B. Key genes - The redder the color, the more generation connection points).

2.4. Expression and prognostic validation

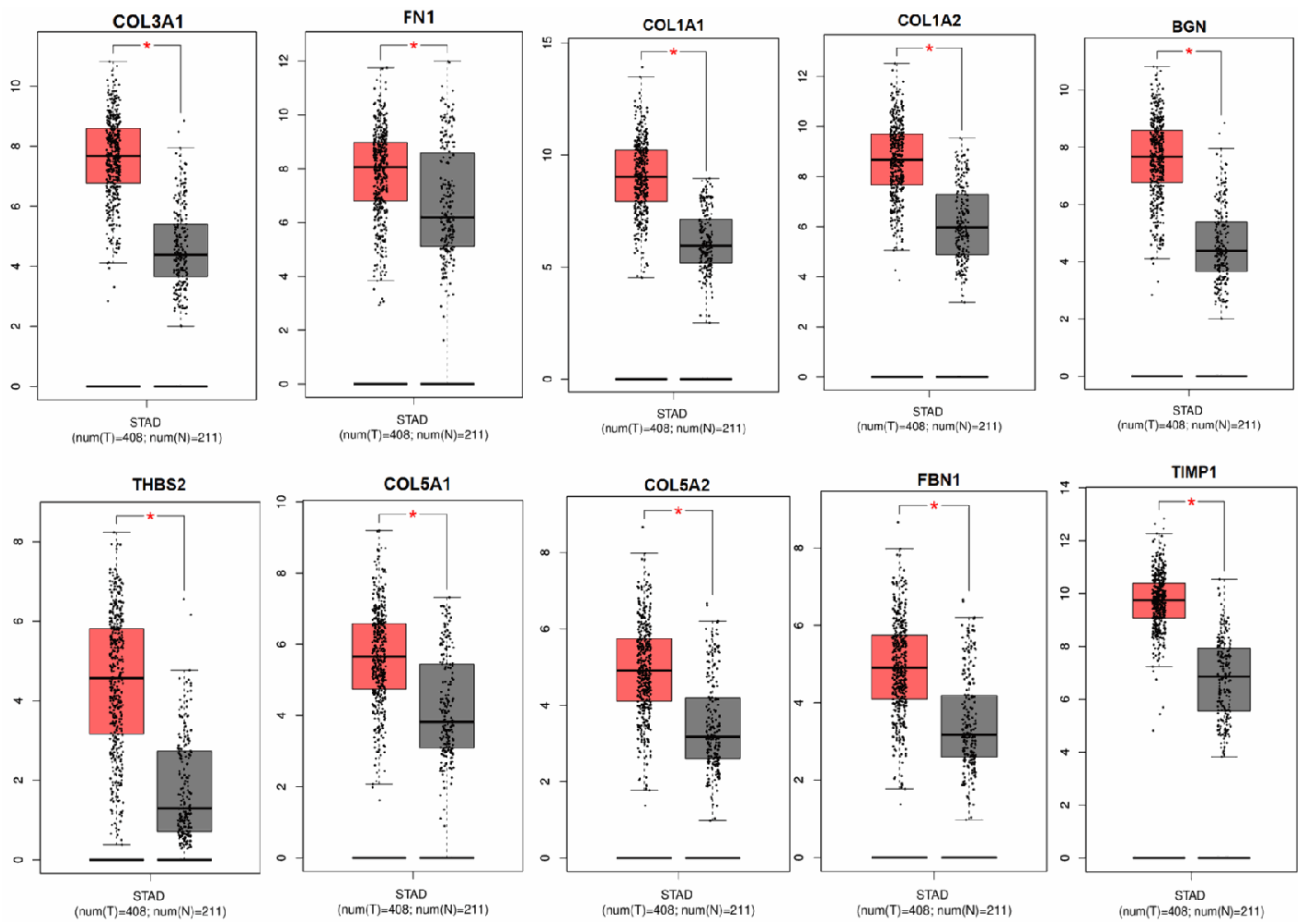


Figure 5. High expression of key genes in gastric cancer tissues (red - gastric cancer tissues, gray - normal gastric tissues).

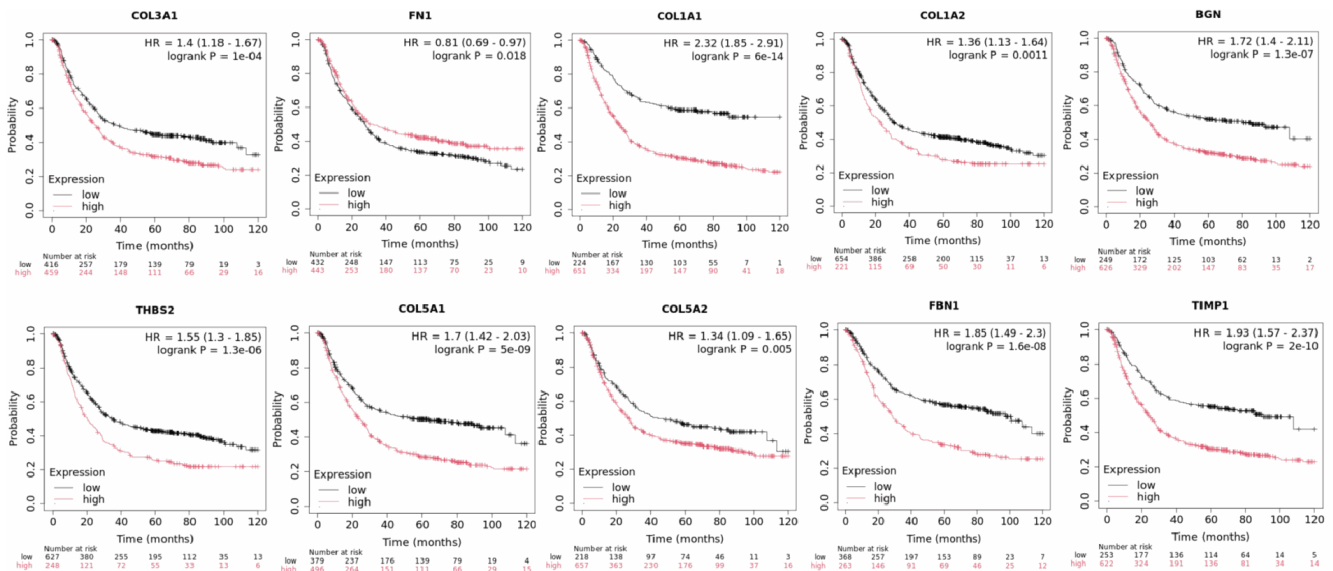


Figure 6. Kaplan-Meier survival curves of key genes (red - high expression, black - low expression).

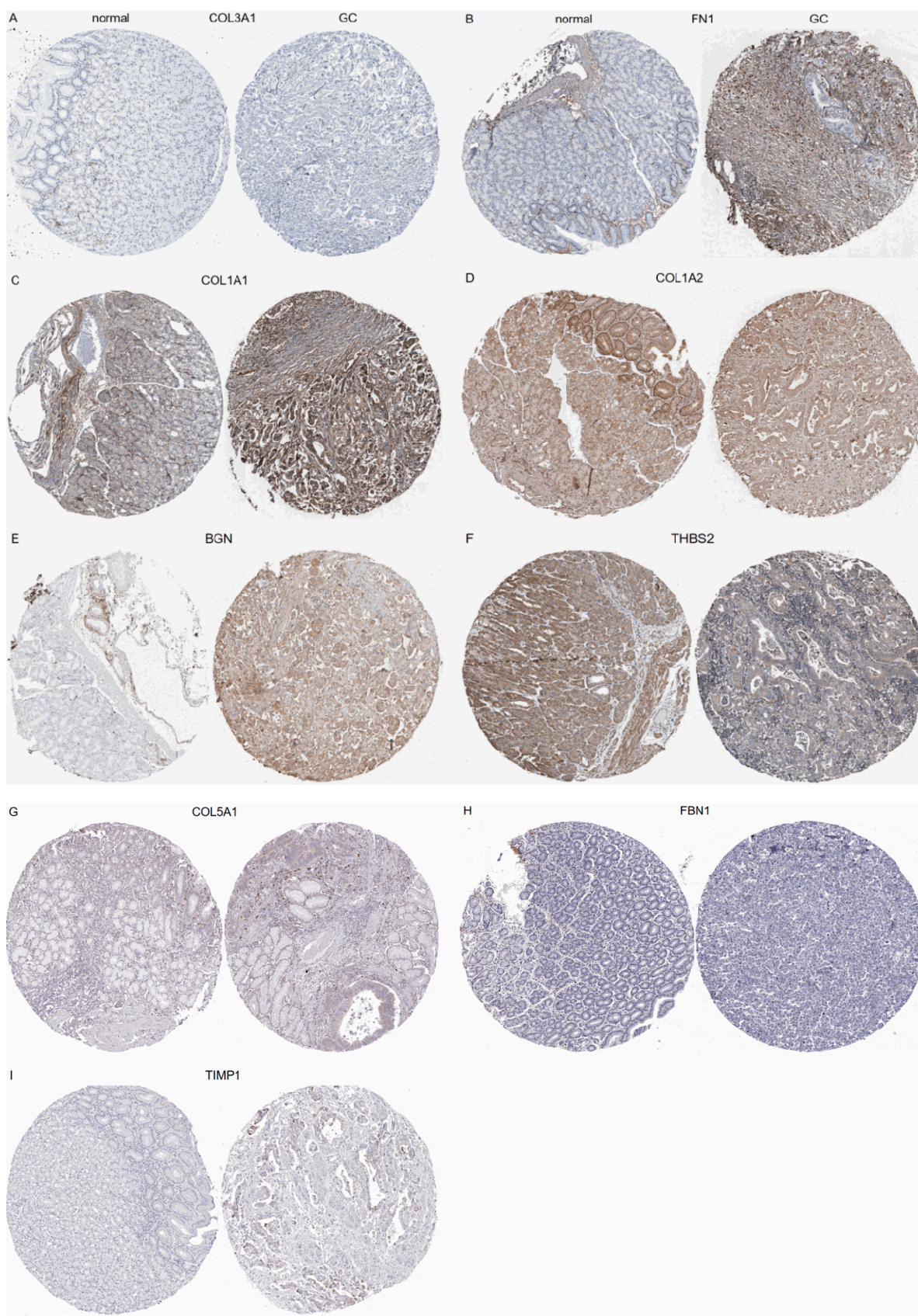


Figure 7. Immunohistochemical images of key genes in gastric cancer tissues and normal gastric tissues in the HPA database; (A-I) represent COL3A1, FN1, COL1A1, COL1A2, BGN, THBS2, COL5A1, FBN1, TIMP1, respectively.

GEPIA showed significantly high expression of 10 genes in gastric cancer tissues ($P < 0.01$, **Figure 5**). Survival analysis indicated that high expression of nine genes, including COL1A1, was associated with poor prognosis (**Figure 6**). HPA confirmed that six genes, including COL1A1, were specifically highly expressed at the protein level (**Figure 7**).

2.5. Immune infiltration analysis

TIMER showed that the key genes were significantly positively correlated with macrophage infiltration ($r > 0.3$, $P < 0.05$) and with infiltration of CD4+T cells, etc. (**Figure 8**)

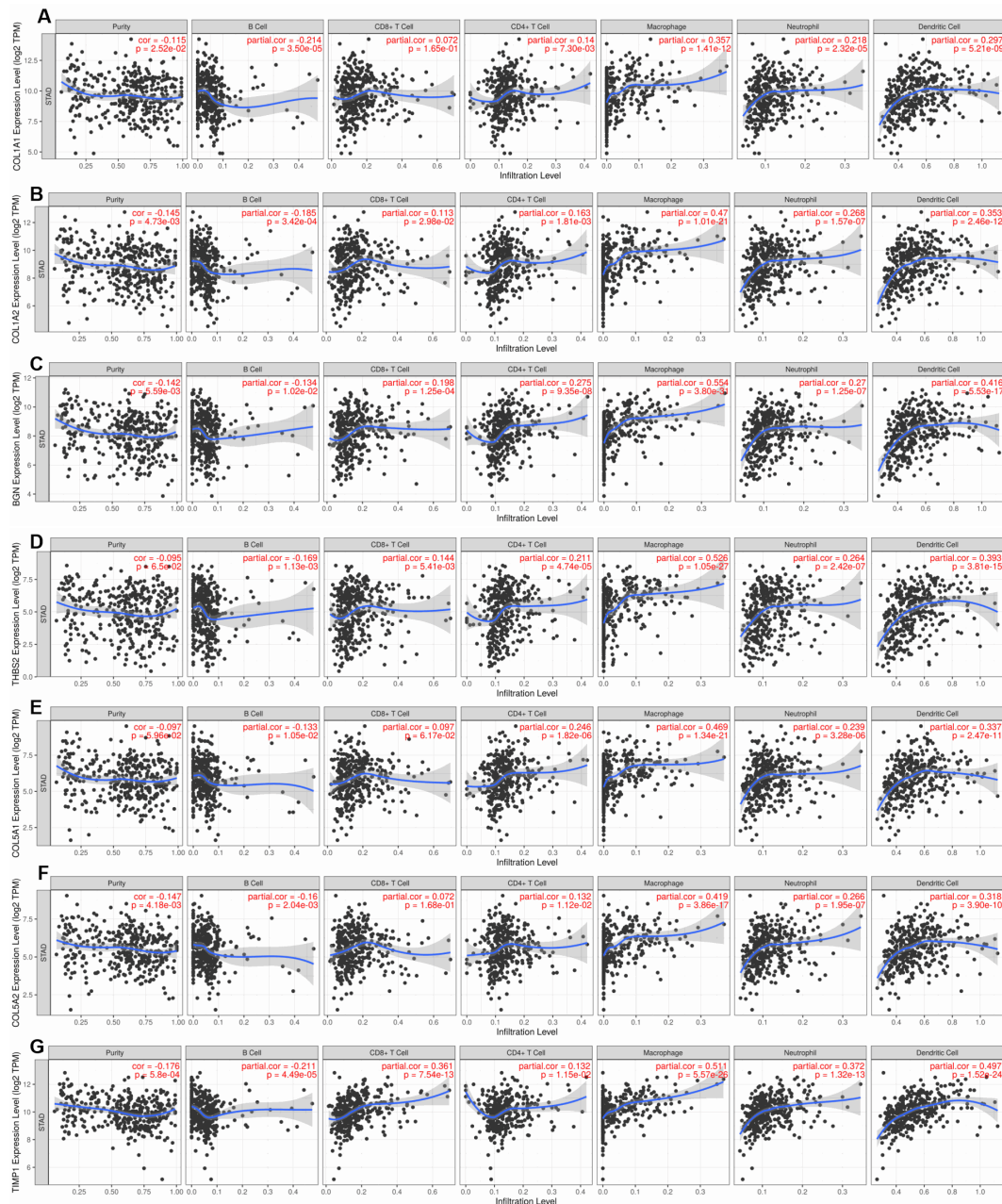


Figure 8. Correlation between the expressions of (A-G) COL1A1, COL1A2, BGN, THBS2, COL5A1, COL5A2, and TIMP1 in gastric cancer tissues and immune infiltrating cell populations (B cells, CD8+T cells, CD4+T cells, macrophages, neutrophils, dendritic cells). $P < 0.05$ was statistically significant.

3. Discussion

GC remains a leading cause of cancer mortality worldwide, posing a substantial public health challenge. The discovery of reliable biomarkers is thus critical for enhancing early detection rates and improving survival outcomes ^[3]. In this study, two microarray datasets (GSE19826 and GSE13911) from GEO were analyzed bioinformatically, yielding 120 common DEGs. Functional enrichment analysis indicated these genes were predominantly associated with extracellular matrix (ECM) organization and glycosaminoglycan binding. In healthy tissue, the ECM provides structural support and facilitates cellular communication, function, and morphology. Within tumors, the ECM additionally contributes to the tumor microenvironment (TME), fostering tumor progression, chemoresistance, and metastasis ^[4]. Glycosaminoglycans are common structural and functional components in the extracellular matrix, which can alter the physical properties and are actively involved in TME dynamics, regulating cancer cell proliferation, angiogenesis, invasion, and metastasis through interactions with growth factors and signaling pathways ^[5,6]. Pathway analysis further indicated enrichment in pathways such as regulation of the actin cytoskeleton, protein digestion and absorption, and ECM-receptor interaction. SERPINE1, a known robust prognostic marker in several cancers, has been closely linked to ECM-receptor interaction pathways in GC. Its high expression correlates with enrichment in these pathways, and in vitro studies show that SERPINE1 knockdown attenuates malignant behaviors in GC cells, potentially predicting prognosis and immunotherapy response ^[7,8]. From the common DEGs, 10 hub genes (COL3A1, FN1, COL1A1, COL1A2, BGN, THBS2, COL5A1, COL5A2, FBN1, TIMP1) were identified using STRING and Cytoscape. GEPIA database queries confirmed significant upregulation of their mRNA expression in GC tissues compared to normal stomach tissues from 408 patients. Survival analysis indicated that high expression of all except FN1 predicted poorer prognosis, underscoring their prognostic value. Protein-level validation via HPA confirmed high expression in GC for all genes except COL3A1, FBN1, and COL5A1, leading to the selection of COL1A1, COL1A2, BGN, THBS2, COL5A2, and TIMP1 as final candidate genes. Investigation into immune infiltration using TIMER revealed that these six genes correlated with infiltration levels of at least four types of immune cells, suggesting significant roles in GC pathogenesis.

COL1A1, COL1A2, and COL5A2 belong to the collagen family, crucial ECM structural components. In cancer, collagens contribute to tumorigenesis, progression, metastasis, and tissue fibrosis ^[9]. Upregulated COL1A1 and COL1A2 expression in colon cancer epithelium implicates them in angiogenesis and stromal formation during cancer progression ^[10]. Li *et al.* ^[11] reported elevated COL1A1 mRNA in premalignant and malignant tissues versus normal, and higher COL1A2 in malignant tissues, identifying them as prognostic factors in GC, consistent with our findings. COL1A1 can modulate proliferation and migration in GC cell lines, suggesting a role in early pathogenesis and potential as an early detection marker ^[12]. COL5A2 is implicated in focal adhesions and the PI3K-Akt pathway, essential for cell migration and angiogenesis in GC ^[13,14]. Reported dysregulation in various cancers and bioinformatics analyses suggest COL5A2 is a significant hub gene influencing GC prognosis ^[15]. While COL5A1 showed weak expression and was linked to poorer survival in our analysis, its role in GC requires further investigation due to the limited existing reports.

BGN, an extracellular matrix proteoglycan, is overexpressed in multiple cancers and predicts adverse outcomes. It associates with epithelial-mesenchymal transition (EMT) via integrating TGF β /Snail and TNF α /NF- κ B pathways within the TME ^[16]. EMT promotes the migration and invasion of tumor cells, thereby supporting the process of metastasis ^[17]. Bioinformatics techniques were utilized to detect and evaluate genes linked to gastric cancer, along with assessing their clinical relevance ^[18].

THBS2, which belongs to the family of matricellular proteins, plays a regulatory role in angiogenesis ^[19]. While some studies report lower THBS2 in GC correlating with better prognosis ^[20], others, including Zhang *et al.* ^[21], associate high THBS2 expression with poor prognosis and demonstrate that its knockdown inhibits GC cell proliferation and metastasis. It appears highly expressed during EMT, ECM remodeling, and invasion, indicating a complex role as a tumor regulator. TIMP1, an inhibitor of matrix metalloproteinases, can facilitate tumor invasion and metastasis when imbalanced ^[22]. In rectal cancer, TIMP1 levels distinguish patients from healthy controls and indicate disease progression ^[23]. In GC, elevated TIMP1 is associated with recurrence and poorer overall survival ^[24].

4. Conclusion

In summary, the six key genes COL1A1, COL1A2, BGN, THBS2, COL5A2, and TIMP1, which were screened and validated using bioinformatics methods in this study, may serve as biomarkers and potential therapeutic targets for GC diagnosis. However, the specific mechanisms remain unclear, and many studies have limitations, all of which require further validation of theoretical predictions in vivo and in vitro experiments.

Disclosure statement

The authors declare no conflict of interest.

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A Narrative Study on the Reconstruction of Life Meaning in Breast Cancer Patients

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Abstract: This study, conducted during an internship at the Tumor Hospital of S Province, employs a qualitative research paradigm. The primary research subjects were 11 hospitalized breast cancer patients, with data collected through semi-structured interviews and observation, followed by content analysis. The study aims to explore the disease experiences and life experiences of breast cancer patients, investigating what their illness means to them and whether it has led to a different understanding of the meaning of their lives. The findings reveal that the reconstruction of life meaning among breast cancer patients manifests as “new perceptions of existence, new attitudes toward life, and new life goals.”

Keywords: Breast cancer patients; Disease narrative; Life meaning

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1. Introduction

My internship at the Patient Service Center of S Province Cancer Hospital marked my first entry into the field of medical social work and the provision of medical social work services. Daily interactions with cancer patients instilled in me a deeper respect for life and a greater appreciation for health. During my internship, I came into contact with the largest group of patients, who were breast cancer patients. Through prolonged interactions and exchanges with them, I observed that the diagnosis and treatment of cancer constitute major crisis events, particularly for breast cancer patients, whose lives are profoundly affected. On the physiological level, breast cancer patients endure the pain of surgery and the side effects of adjuvant therapies such as chemotherapy and radiation therapy, including hair loss and nausea. Their personal appearance also changes due to mastectomy. On the psychological level, in addition to concerns about their health, they face significant psychological distress before and after diagnosis. In terms of social life, breast cancer patients also face challenges in reintegrating into society after surgery. The disease disrupts the existing connection between the patient and the world, leading to a sense of self-fragmentation and causing their lives to become chaotic and disordered.

The author of “Gentle at the Bedside,” Van den Berg, argues that healthy people have the deepest misunder-

standing of life and the shallowest understanding of its meaning^[1]. Healthy individuals are often preoccupied with important matters in their career plans, such as education, status, money, and career, but can these external factors truly signify the value of our lives? When lying in a hospital bed, the invading disease takes control of the body, disrupting the original life plans or routine, leaving one to wonder how to move forward. Therefore, breast cancer patients particularly need to integrate the fragments of themselves. This disease narrative, the recounting and re-enactment of the illness and treatment process, serves as a way for them to cope with the disease, integrate their selves, and reconnect the relationship and order between their bodies and the world. Sometimes, it is the only way. Their lives have been paused by the disease, granting them the time and opportunity to reexamine their past experiences, redefine their life direction, and reassess their life's value.

During adolescence, the author suffered from severe acne, medically known as “acne vulgaris,” which she believed to be a disease. From fifth grade to high school, the acne persisted for five years before finally disappearing. Although the acne has completely vanished, improper treatment left behind residual effects such as rough facial skin, thin epidermis, and enlarged pores. Before adolescence, I was particularly fond of snacks from the school canteen, unaware of their harmful effects. The acne episode during adolescence was a health crisis for me, leading to new insights about life and prompting positive changes in my behavior. Faced with the major life crisis of cancer, I became interested in how breast cancer patients perceive the meaning of life and what insights they gain from their experiences. This study aims to give breast cancer patients a voice, exploring and analyzing the insights gained from their disease experiences through their own narratives, and discussing and summarizing their understanding and reflections on the meaning of life after being diagnosed with breast cancer.

2. Research design

2.1. Research methodology

In social research, there are two fundamental yet mutually opposing methodological orientations: positivism and humanism. Positivism emphasizes the objective existence of social phenomena and the need to describe them as they are, while humanism emphasizes the subjective nature of human experience and the importance of understanding the subject's perspective to leverage the researcher's subjectivity in the research process. As Max Weber put it, this involves “committing to understanding,” or what Wright Mills referred to as “understanding people.” The interpretive approach seems most suitable for exploratory research on complex phenomena, especially when these phenomena are not well understood^[2]. This study adopts a humanistic methodological approach, listening to breast cancer patients' narratives about their disease experiences and life experiences to understand the meanings they assign to disease events and the life insights they gain. The research findings are the result of generative understanding achieved through dialogue between the researcher and breast cancer patients.

2.2. Research paradigm

In terms of research paradigms, qualitative research is a hallmark of the humanistic methodological approach. Qualitative research emphasizes gaining a natural and open understanding of the experiential world and contextual circumstances of research participants. Its key characteristics include providing vivid and comprehensive descriptions of phenomena within their natural contexts, avoiding constraints imposed by pre-determined constructs, and enabling a deep understanding of the meaning of experiences and phenomena. It explores individuals within their contextual frameworks to uncover the complexities of human life experiences^[3]. Each breast cancer patient's life experience is unique and individual, and their perceptions of their feelings after diagnosis and their past experiences vary greatly.

Quantitative research struggles to capture each unique individual's narrative of their own experiences and the construction of meaning, as well as describe the living environment and overall landscape influencing the meaning-making process of research participants. Therefore, to understand the subtle and nuanced shifts in the meaning of life experienced by breast cancer patients after diagnosis, qualitative research is the most appropriate research paradigm.

2.3. Research methods

2.3.1. Semi-structured interview method

This study employs semi-structured interviews to collect data. An interview outline is designed based on the research objectives and relevant literature to serve as a guide for the interview. During the interview process, questions are flexibly adjusted according to the actual situation. Given that interviewees may have different individual feelings, perspectives, or insights regarding the research questions, the relaxed conversational process allows them to express their subjective experiences with greater flexibility, thereby yielding their unique and rich life experiences and perspectives.

2.3.2. Observation method

Collect data by observing the facial expressions, body language, changes in daily life, and interactions with others of breast cancer patients during the interview process, serving as supplementary material for the interview data in this study.

2.3.3. Content analysis method

In order to present more comprehensive and diverse research results, the author attempts to interpret, judge, and explore the content related to the research questions by reading, feeling, analyzing, and understanding books related to breast cancer patients, and uses this as supplementary material for the research data. The theme of the author's research is "rebuilding the meaning of life," which requires a large amount of data to support the research. However, due to time and resource constraints, literary works are more intuitive and convenient. The literary works selected by the author describe real-life experiences that have occurred in reality, making them valuable as research materials for analysis.

2.4. Theoretical framework

Sachman analyzed different stages of disease experience, revealing how people in Western culture utilize their experiences of physical conditions to recognize disease symptoms and make positive changes. Sachman pointed out that when an individual believes they are ill, they undergo five distinct reaction stages: experiencing symptoms, accepting the role of being ill, seeking medical services, assuming the role of a dependent patient, and recovery and healing ^[4]. Lederer views the process of becoming ill as a complex psychological process. She proposed three mutually independent yet overlapping disease processes: the initial phase, the acceptance phase, and the recovery phase ^[5]. Sachman's theory of illness and medical care stages provides a comprehensive and detailed account of the disease process, vividly capturing the recurring emotions and inner struggles patients experience from the onset of illness through treatment and recovery. Lederer's three-stage theory of illness, however, is more concise, with its most distinctive feature being its precise explanation of the relationships between the three stages of illness. To uncover the meaning of life after illness from the disease narratives of breast cancer patients, this study integrates Sacks' illness and medical care stages theory and Lederer's three-stage theory of illness, combined with the author's experience interacting with breast cancer patients during their internship. The theoretical framework of this study is divided into three stages: the disease discovery stage, the disease treatment stage, and the disease adaptation stage (as shown in Figure 1). These three stages are both independent and overlapping. The interview

guidelines will be designed and refined around these three stages ^[6].



Figure 1: Theoretical framework of this study.

2.5. Research participants

The study participants are breast cancer patients. The selection criteria are determined based on the research objectives: women diagnosed with breast cancer through pathological examination and who are aware of their diagnosis; those who have undergone breast cancer resection surgery, are currently undergoing postoperative adjuvant therapy, and have a high expected survival rate; and those with no history of mental illness, and who possess adequate understanding and communication abilities ^[7].

3. Research process

3.1. Developing the interview guide

Based on the research objectives and questions, the researcher developed an interview outline through the analysis of relevant domestic and international literature. The outline covers three main areas: issues related to experiences prior to diagnosis, issues during treatment, and issues after treatment.

3.2. Selection of research participants

When selecting research subjects, the author referred to the selection criteria, comprehensively considered the homogeneity and heterogeneity of the sample, and ultimately determined 11 breast cancer patients as the research subjects for this study using purposive sampling. To protect the privacy of the interviewees, all research subjects are identified by numbers, and their basic information is shown in **Table 1**.

Table 1. Overview of basic information of respondents

Number	Age	Occupation	Educational attainment	Marital status	Status of children	Duration of illness
C1	32	Preschool teacher	Undergraduate	Married	1 woman	2 years 1 month
C2	45	Warehouse management	High school	Married	1 piece	3 months
C3	34	Sales	Specialist	Married	1 piece	2 years 2 months
C4	46	Workers	Technical school	Divorce	1 piece	5 months
C5	50	Chief Financial Officer	Undergraduate	Married	1 piece	1 year 1 month
C6	65	Accounting	Undergraduate	Married	1 woman	6 months
C7	51	Accounting	High school	Married	1 woman	1 year
C8	55	no	Elementary school	Married	1 girl 1 son	10 months
C9	57	No fixed job	High school	Married	2 girls 1 son	2 months
C10	43	No fixed job	Junior high school	Married	1 piece	Nine months
C11	56	Supermarket owner	High school	Married	2 sons	6 months

3.3. Collecting relevant information

Prior to the formal interviews, the researcher selected two participants for preliminary interviews to determine whether the interview outline needed revision and whether the interview results could address the research objectives. Each interview lasted approximately one hour, and participants were provided with answers to any additional questions based on their individual needs following the interview. Based on the insights gained from the pre-interviews and the preliminary analysis of the interview transcripts, the researcher discussed with two medical social workers from the internship hospital to refine the previously drafted interview questions, finalizing them as the formal interview outline. Additionally, drawing on the experience from the pre-interviews, the researcher organized key points regarding research ethics and data collection, which served as a reference for the formal interviews^[8].

Using the revised interview outline, the researcher conducted the formal interviews, interviewing a total of 11 breast cancer patients aged between 32 and 65 years old. Each interviewee was interviewed approximately one to two times, with varying interview durations ranging from 40 minutes to 159 minutes, totaling 876 minutes (**Table 2**). After each interview, the author reviewed the interview content, reflected on it in conjunction with observation notes, and identified key points for the next interview as well as issues requiring clarification. Additionally, since the researcher spent an extended period interning at the hospital, the participants regularly returned for treatment. The researcher continued to follow up with them after the interviews. This allowed the researcher to confirm the accuracy of the interview data and gain insights into the participants' reactions and circumstances post-interview, which was helpful for subsequent data analysis.

Table 2. Interview record form for respondents

Number	Interview location	Number of interviews	Duration of interviews (minutes)
C1	Breast ward	1	50
C2	Patient Service Center	1	58
C3	Patient Service Center	2	75
C4	Patient Service Center	2	105
C5	Breast ward	2	115
C6	Patient Service Center	2	123
C7	Patient Service Center	2	159
C8	Breast ward	1	50
C9	Breast ward	1	49
C10	Breast ward	1	40
C11	Breast ward	1	52

Drawing on some experience in selecting interviewees, the author began collecting literary works related to breast cancer patients using online platforms. After carefully reviewing the information provided in the works, the author once again employed the thematic sampling method to select seven literary works (**Table 3**) as the textual materials for the study, thereby supplementing the research data. To facilitate the use of the materials, the titles of the works are replaced with numbers. The author repeatedly read the selected literary works related to breast cancer patients, not only making reflective notes in the books but also marking content relevant to the research ques-

tions with a highlighter. These marked sentences will be used as supplementary materials for the interview data and analyzed together with the interview data in the later stages.

Table 3. Overview of literary works

Number	Title	Year of Publication
A1	“What Doesn’t Kill Me Makes Me Strong”	2016
A2	“Unfinished Life”	2011
A3	“Brilliant as Autumn Leaves”	2018
A4	“Mourning Breasts”	2010
A5	“All Good Times”	2018
A6	“Medical Journey Together”	2021
A7	“Life Like Summer Flowers”	2015

3.4. Data organization and analysis

The author first transcribed the interview recordings into text and saved them as electronic documents. Subsequently, each recording was re-listened to, and errors were corrected, redundant words were removed, and unclear or incomplete sentences were supplemented. All interview recordings were transcribed into verbatim transcripts totaling nearly 160,000 words. After saving the verbatim transcripts of the interviews, the author compiled the sentences marked in the text works into an electronic document to form verbatim transcripts. Based on the core concepts of grounded theory coding, the author used qualitative analysis software NVivo to further code and categorize the verbatim transcripts of the interviews and text works, extracting various themes reflected in the data ^[9].

3.4.1. Open coding

Initially, sentence-by-sentence coding was conducted, primarily based on the original language of the data and the researcher’s conceptualization of the content, aiming to accurately reveal the true meaning intended by the research subjects. After the first phase of coding, a total of 295 initial concepts were obtained. Due to the large number of initial concepts and some overlap, the researcher used NVivo’s node reorganization function to repeatedly summarize and refine these initial concepts, forming 35 categories frequently mentioned by the research subjects, such as valuing time, being tolerant of others, and following one’s interests. These categories were marked as free nodes, and representative original interview statements and initial concepts corresponding to each category were listed ^[10].

3.4.2. Main axis coding

This study conducted a detailed analysis of the connotations of the 35 basic categories and used them as a core to find the connections between the categories. After classification and integration, 17 main categories were finally formed, namely Perceiving Physical Abnormalities, Receiving a Diagnosis, Physical Suffering, Psychological Agony, Internal Acceptance, External Restructuring, Supportive Relationships, The Power of Love, The Importance of Life, health as the foundation, indifference to fame and fortune, understanding tolerance, understanding death, changing habits, focusing on the inner self, valuing family, and lowering expectations. Subsequently, the author marked these 17 main categories as sub-nodes, forming higher-level categories based on the free nodes.

3.4.3. Selective coding

In selective coding, 17 main categories were formed by integrating the main axis codes, from which six more systematic core categories were ultimately extracted, namely the sudden onset of disease, the bitterness of treatment, coexisting with disease, new understanding of existence, new attitudes towards life, and new life goals.

3.4.4. Establishing a relationship model

Coding and analyzing the disease narratives of breast cancer patients not only requires identifying the meaning of life after diagnosis but also summarizing the connections and mechanisms between the meaning of life and disease narratives. This study constructed a relational model of life meaning reconstruction for breast cancer patients based on typical core category relational structures (as shown in Figure 2). The process from the sudden onset of disease, through the bitterness of treatment, to living with the disease constitutes the entire narrative of breast cancer patients' disease. In this process, the author discovered the life meaning of breast cancer patients after diagnosis. The transition from their original life meaning to their current life meaning is a gradual and ascending process. Breast cancer patients not only experience post-traumatic growth and change during this process but also undergo transformation and elevation of their life meaning, primarily manifested in changes in existential cognition, attitudes toward life, and life goals. Breast cancer patients do not merely experience a change in their understanding of life's meaning after the conclusion of disease treatment. Throughout the entire disease journey, their understanding of life's meaning undergoes a gradual transformation. During the prolonged period of coexistence with the disease, breast cancer patients are better able to organize and integrate their understanding of life's meaning ^[11].

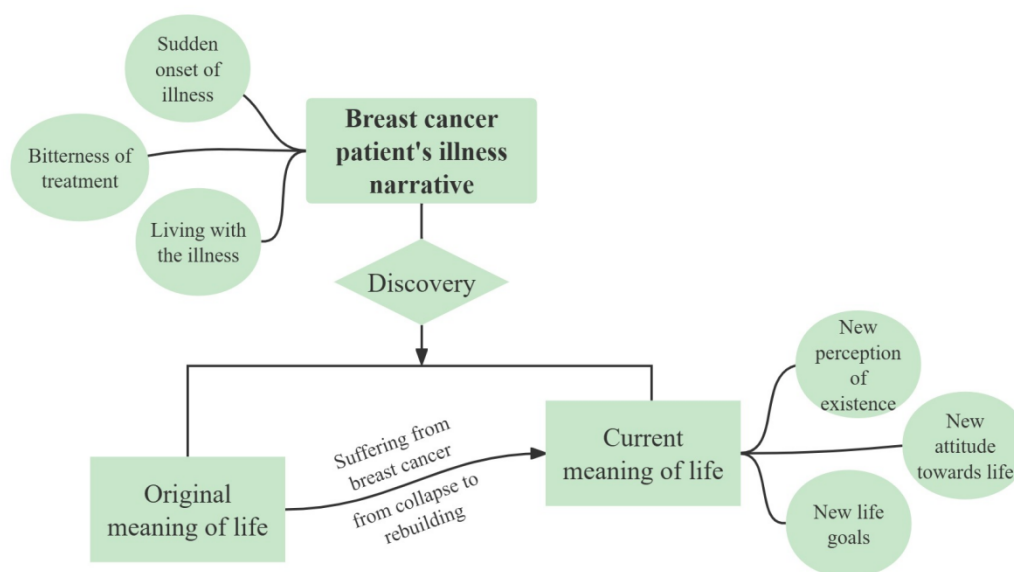


Figure 2. Model of the reconstruction of the meaning of life in breast cancer patients.

4. Research conclusions

After enduring the ordeal of life and death, breast cancer patients have gained new insights and interpretations of the meaning of their lives. They gradually break free from material constraints, place greater emphasis on family relationships while also prioritizing their own health and inner well-being, and actively live life for themselves.

4.1. Awareness: New existential recognition

When breast cancer patients learn of their diagnosis, they become aware of their existence. Family responsibilities and unfulfilled aspirations become their motivation to survive, and they quickly recognize the importance of individual life.

4.1.1. The power of love

Breast cancer patients express their love for their families primarily through fulfilling their family responsibilities, such as caring for their parents, accompanying their husbands, and spending time with their children. Family members are the source of confidence for breast cancer patients to continue treatment and provide them with the strength to keep living. Breast cancer patients also express a desire to enjoy life, with the desire to live fully becoming one of their motivations for survival. Some breast cancer patients express a desire to read and write, reflecting their love for themselves.

- C9: For the family, to fulfill one's duty to the elderly.
- C7: I feel like I haven't lived properly yet, I haven't truly enjoyed life, so how could this happen?

4.1.2. Life above all

Sometimes, simply being alive is the lowest standard, but it is also the highest standard. Only when faced with dire circumstances do people realize how fortunate it is to be alive. Breast cancer has robbed patients of their health, and the uncertainties of the future make life incredibly precious to them. In times of peace and tranquility, they took their lives for granted. The sudden onset of illness served as a wake-up call, prompting them to value and cherish their lives even more after narrowly escaping death.

- A2: As long as you're alive, you can talk about life.
- C11: We still haven't cherished life properly.

4.2. Insight: A new attitude toward life

Breast cancer patients cannot choose or change their disease, but they can choose how to approach it. After enduring the hardships of the disease, breast cancer patients are attempting to adopt a new attitude toward both their illness and life, thereby regaining the value and meaning of life.

4.2.1. Health is the foundation

From being healthy individuals forced into the role of patients, breast cancer patients lose their health and yearn to regain it, thereby realizing the importance of physical well-being. They once believed that illness was far removed from their lives, prioritizing higher life goals over their health. However, illness has made them aware that a healthy body is the foundation for achieving more in life.

- C3: Healthy people may never truly understand how much health is desired and how precious it is to a patient.
- C8: When you're not sick, you don't think much about it, but when you get sick, you realize how important health is. Only when your body is healthy can you think about other things.

4.2.2. Indifference to fame and wealth

While illness has made breast cancer patients more attentive to their health, it has also prompted them to reorder

the priorities in their lives. Money and fame are no longer their goals. They realize that fame and fortune are fleeting and that they should pursue long-lasting, sustainable life goals. Illness has also helped breast cancer patients see through some of their past relationships and distinguish their true friends.

- C7: When it comes to money, it's about working hard to earn it. But after getting sick, I realized it's not that important. After working hard for years, even though my position improved, the money I earned wasn't enough to cover the medical expenses.

- A2: Cancer has taught me that if there is someone in the afterlife whom I should dedicate myself to, you'll find that many of the world's social conventions are so trivial they're worth a casual smile.

4.2.3. Understanding tolerance

Disease brings crisis to the lives of breast cancer patients, but it is also an opportunity for transformation. After falling ill, they adjust their mindset, no longer judging others by their own unique standards, letting go of their own obsessions, and embracing others with a broad-minded attitude. They learn to let go of the trivial matters in life, experiencing the simplicity and beauty of tolerance.

- C2: Before, if my husband didn't put his shoes away properly, I would get angry. Now I don't care—it's just part of life, and I feel like I'm not so nitpicky anymore.

- C3: I don't feel that way anymore. For example, if someone lacks manners on the bus, I might think about it, but I won't scold them. I'll just think, "Why are they like that?" I feel my heart is bigger now, more open-minded, and more tolerant.

- C5: In both work and life, we should be tolerant of others and free our minds. When you tolerate others, you let go of your own burdens. Don't be too rigid.

4.2.4. Understanding death

Facing the threat of life and the approach of death is a shared experience for breast cancer patients. They are closer to death than before and have the opportunity to reflect on it, gaining a deeper understanding of the impermanence of life and its limitations. Breast cancer patients do not avoid discussing death. They understand that death is something everyone must experience, that it is inevitable and cannot be changed, but that one can choose how to face it. Before death arrives, they focus on what they can change, cherishing the time they have left and living each day to the fullest.

- A7: No one is afraid of death, and everyone will die; it is a certainty from the moment we are born.

- C5: I believe life is a process, and everyone will reach the end, some sooner than others. Therefore, living each day to the fullest makes life meaningful.

4.3. Action: New life goals

Life goals represent the objectives or aspirations that breast cancer patients currently or in the future wish to achieve or attain, as well as the direction they are committed to changing in their lives. Breast cancer has a profound impact on all aspects of a woman's physical and mental well-being, leading to varying degrees of change in their lifestyle habits, priorities, and relationships with family members.

4.3.1. Changing habits

In addition to actively changing their diets for health reasons, breast cancer patients also take proactive steps to

maintain their physical well-being by adjusting their schedules and exercising regularly. With a strong sense of responsibility for their health, they are willing to give up some of their previous preferences, paying attention to food combinations, taste, and whether foods are organic or eco-friendly, and no longer eat and drink indiscriminately. Some breast cancer patients had varying degrees of late-night work experience before falling ill. Staying up late can cause significant harm to the body. The disease has made them deeply aware of the negative impact of their previous late-night habits, prompting them to actively change their unhealthy sleep patterns. Before falling ill, they may have neglected exercise for various reasons. The disease has made them reevaluate the importance of exercise and rediscover its benefits.

- C6: I now force myself to eat eggs, even though I've never liked them my whole life. But since getting sick, I eat one every morning. This morning I really didn't want to eat an egg, so I fried one for myself.
- A6: I used to be someone who couldn't go a day without meat, but now I've become a vegetarian.
- A7: I made a promise with Vivi that we'll celebrate together every anniversary. After treatment, we'll be healthy again. I need to recover properly, get back to work step by step, and never stay up late again.
- C2: Health comes first; I must exercise regularly from now on.

4.3.2. Focus on the inner self

After going through an illness, breast cancer patients become more aware of what they truly want and need. They let go of many things they once carried, such as career prospects, making money, social status, and others' expectations. They follow their inner desires to live the life they want, pursue what truly interests them, develop new hobbies, and experience the beauty and comfort of travel and alone time. They truly live according to their own heart's wishes.

- C3: What is my goal in job hunting now? Happiness must come first, and I must be willing to do this job. I must enjoy it and be able to bring some help to those around me, feeling a sense of fulfillment.
- A6: After returning to school, I decisively abandoned my original career plan—statistics and finance—because I didn't like it and wasn't good at it. Instead, I returned to the field where I felt most comfortable—the humanities—and became a Chinese language teacher for foreigners. It's not that I'm particularly talented in the humanities; I just wanted to use the time I had stolen from death twice to do something I was willing to do.
- A7: At that time, she always needed friends by her side. Over the years, her friends gradually noticed that she had changed. She no longer needed constant companionship; she would go shopping or dining alone and occasionally travel by herself.

4.3.3. Valuing family

Breast cancer patients reflect on the various relationships within their families before their illness and cherish and appreciate this familial bond even more. They actively strive to fill the gaps in their hearts. Faced with unforeseen circumstances and an uncertain future, breast cancer patients express their desire to spend more time with their parents and cherish every moment they have with them. The diagnosis of breast cancer serves as a turning point in the lives of patients, enabling them to discover previously unnoticed virtues in their husbands. They no longer look down on their husbands but gradually begin to value their presence. Breast cancer patients become more gentle and patient in their interactions with their children, paying attention to the ways they communicate with them. As a result, their children also grow and develop in the way their parents had hoped.

- C5: People say that companionship is the most sincere form of love. Spend more time with your parents.

When we were children, our parents were our world. Now, our children are our world. Go out for a walk, spend time with your parents, and make each day special.

- C2: After getting this illness, I realize that a husband and wife are the closest people. Look at our parents—they're old, and we can't rely on our children. We have siblings, but the one who's here with me is him. Now I speak to him more gently. Before, I looked down on him in every way, but now I think he's pretty good.

- C3: This includes how we treat our children. Before, if a child had an issue, I think all parents have scolded their children at some point. But now, we pay attention to how we do it.

4.3.4. Lowering expectations

Breast cancer patients, both in their personal lives and careers, have always set extremely high standards for themselves, striving for perfection in everything they do, often at the expense of their health. Now, they are beginning to adjust their expectations, lowering the bar for themselves and lightening their mental load. They are also letting go of the various demands and expectations they once placed on their husbands, relieving them of the invisible pressure they felt and allowing themselves to live a less strenuous life, free from the emotional toll these expectations once caused. Before their diagnosis, breast cancer patients placed great importance on their children's academic performance and rankings in class. However, the disease has led them to prioritize their children's physical and mental well-being, no longer forcing them to meet the goals set for them. They no longer impose excessive restrictions on their children through their words and actions. Additionally, breast cancer patients no longer impose strict demands on their colleagues and those around them, beginning to lower their internal standards and understanding the importance of maintaining a balance.

- C1: I don't care about many things anymore. I feel that my inner adjustment has been relatively good. It's because I wanted too much and felt burdened by too many things, wanting everything to be perfect.

- C3: In the past few years, my husband had some minor flaws, and I would point them out directly without holding back. But now I realize that true character is revealed in adversity. After over 30 years, he is just that kind of person—carefree and careless. I no longer mention it to him.

- C5: I don't judge others either. I used to be very strict, but now I'm not as strict anymore.

5. Discussion

As long as we are alive, people will always pursue the value and meaning of life. Narrative, as a method, provides a pathway for exploring the meaning of life. It enables individuals to confront their own experiences and the nature of their lives, address internal struggles, challenges, and complex emotions, and reinterpret the meaning of their lives from a new perspective. Medical social workers can use narrative methods to provide professional support to breast cancer patients in understanding the meaning of their lives and achieving positive growth and change. Combining the social work theories and methods learned, the author believes that three methods, film, theater, and painting, can be used to build a bridge for disease narratives, thereby making the narrative practices of medical social workers for breast cancer patients more scientific and practical.

5.1. Using visual narratives to guide breast cancer patients in reflecting on their lives

Visual media, such as photos and videos, can help breast cancer patients reflect on their lives. Medical social workers can organize important life events, such as birth, schooling, work, and marriage, based on a timeline, or group photos or videos around themes like family, friends, and travel to expand the breadth of patients' life stories from

different perspectives. By continuously extending the narrative through videos and photos and recreating existing memories, and through repeated recollection and reinforcement, past experiences can be integrated into current disease events, helping breast cancer patients deepen their understanding of the meaning of life.

5.2. Utilizing theater narrative to assist breast cancer patients in understanding the present

Theater believes that every person is unique, and every story is worth listening to. Medical social workers can use theater techniques such as “moving statues” and “three-part stories” to help breast cancer patients spontaneously use body movements and language to share stories and feelings about their current experiences with the disease. Through guiding both performers and audience members (all breast cancer patients) to reflect on the events, they can help them understand, recognize, and explore their current selves, facilitating the exchange and sharing of life experiences, thereby continuously elevating their understanding and appreciation of the meaning of life.

5.3. Applying a painting narrative to encourage breast cancer patients to face the future

Art is not only for appreciation but also a tool for communication and exchange. As an expressive art form, painting holds unique value and significance. Medical social workers can apply painting methods to encourage breast cancer patients to draw their future blueprints and plan their lives. Painting provides breast cancer patients with a channel to reflect on the meaning of life and explore their existence. Through the three stages of “painting,” “narrating the painting,” and “interpreting the painting,” breast cancer patients can unleash their creativity, reflect on their disease experiences and life experiences, and better understand the meaning of their lives and their future direction.

5.4. Utilizing narrative therapy to address individual differences among breast cancer patients

When individual breast cancer patients are unable to achieve change or growth through group narrative therapy and their quest for life meaning is hindered, medical social workers should employ narrative therapy to assist them. By encouraging breast cancer patients to share their experiences related to the disease, medical social workers can separate the issues that arise from the patients themselves, enabling them to gain the strength to solve problems and make changes, reflect on the meaning of their lives, and reinterpret their life stories, thereby fostering positive transformation.

6. Conclusion

When individuals treat a disease, the disease also acts like a doctor in treating the individual. The disease honestly tells them: Before falling ill, they were not living life to the fullest. Disease narratives can serve as a vehicle to combat disease and suffering, through which an individual’s life experiences are presented, interpreted, and reconstructed. Breast cancer patients, through disease narratives, reflect on their past life experiences and gain a new perspective on the meaning of life, thereby constructing new existential cognition, attitudes toward life, and life goals. This grants them a newfound strength and enables them to embody a new self.

This study only used a small number of breast cancer patients from hospitals and textual works as research subjects, which is insufficient to generalize to every breast cancer patient. The author was unable to further explore whether the age of breast cancer patients or the duration of their illness affects their understanding of the meaning of life. In this study, the participants’ sense of life meaning had already been largely reconstructed and they had

emerged from their low points. The author hopes to have the opportunity to conduct research with individuals who have experienced setbacks and undergone reconstruction, to present different inner meanings of life, and looks forward to more scholars joining this field of study and publishing related research in the future.

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Integrating Iron Overload Diagnosis with Electrocardiographic Abnormalities: Bridging Laboratory Findings to Primary Care Practice

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Abstract: *Objective:* To investigate the diagnostic status and electrocardiographic correlates in patients with biochemical evidence of iron overload. *Methods:* We conducted a retrospective cohort study of patients in our hospital with ferritin levels exceeding 500 ng/mL between January 1, 2011, and October 24, 2022 (corresponding to the pre-COVID-19 pandemic period in Beijing). Using ICD-10-CM coded medical records, we assessed the following: definitive diagnostic characterization (genetic or acquired), electrocardiographic (ECG) completion rates, and the prevalence of ECG abnormalities. Statistical analyses, encompassing chi-square tests and correlation studies, were performed using SPSS Statistics software (version 27.0). *Results:* Except for cases of malignancy, infectious diseases, hematological diseases, chronic diseases, for the unexplained diagnosis group found elevated ferritin during annual health checkup, there were 17 cases in the group with ferritin above 1,000 ng/ml and 36 cases in the group with ferritin ranging from 500 to 1,000 ng/ml, accounting for 23.2% and 25.8% of the entire ferritin analysis respectively, and the total proportion in the entire analysis was 24.0%. Among the cases indicating ferritin higher than 500ng/ml, 24.0% of the cases were of unknown diagnosis. ECG acquisition rate for was 55.7%, with 24% demonstrating abnormalities, including atrial fibrillation, sinus tachycardia arrhythmia, atrioventricular block, prolonged QT interval, T-wave inversion, and ST-segment depression. *Conclusion:* The study revealed that the proportion of unexplained diagnoses of ferritin overload remains relatively high, and the analysis of the ECG is also insufficient. There is a need to enhance clinicians' awareness and attention to iron overload in both diagnosis and ECG analysis.

Keywords: Ferritin; Electrocardiogram; ECG

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1. Introduction

Iron is an essential micronutrient for oxygen binding and transport in red blood cells, but it is also a stress-

responsive programmed cell death-inducing second messenger ^[1]. In the blood, iron mostly exists in the form of ferritin.

Iron overload, as elevated ferritin levels, is a condition involving excessive iron deposition in various organs such as the liver, heart, pancreas, joints, skin and reproductive system ^[2], causing liver damage ^[3], arrhythmia, abnormal blood sugar level, joint pain, skin pigmentation, and hypolibido. Studies have shown that excessive intracellular iron can interfere with the electrical function of the heart by generating a large number of free radicals or causing selective dysfunction of Na⁺ channels. Abnormal function of Na⁺ and K⁺ channels is associated with the etiology of prolonged QT syndrome, ventricular tachycardia, and atrial fibrillation ^[4].

Iron overload can be categorized into primary and secondary causes. Primary iron overload, so-called hereditary hemochromatosis, is due to genetic defects, like HFE mutations. It is a common disease in Caucasians but uncommon in Chinese. The genetic analysis of Chinese revealed the gene mutations in Chinese were different from Caucasians ^[5,6]. Secondary iron overload is a more diverse disease with many unclear areas to be explored. Secondary iron overload is more prevalent than primary iron overload and occurs as a consequence of various causes that differ significantly across geographic regions. The main causes of secondary iron overload are iron-loading anemia and chronic liver disease ^[7]. If iron overload has been excluded, evaluation for causes of hyperferritinemia should be pursued. Causes of hyperferritinemia include chronic liver disease, excessive infusion of red blood cells for treatment, ineffective hematopoiesis, malignancy, infections, kidney failure, and rheumatic conditions, such as adult-onset Still's disease or hemophagocytic lymphohistiocytosis ^[8,9].

As general practitioners, we encounter a wide range of diseases, including infections, tumors, chronic conditions, and health check-ups. Cases of unexplained elevated ferritin are frequently encountered. This study analyzed the diagnostic status and ECG findings in cases of elevated ferritin levels to raise awareness among clinicians.

2. Experimental design summary

Here, the study conducted an analysis on the diagnoses and ECG findings in outpatients and inpatients with elevated ferritin levels at our hospital over the past decade, from 2011 to 2022.

A ferritin level greater than 500 ng/mL is considered elevated ^[2]. We divided the cases into two groups for diagnostic analysis: the ferritin 500–1000 ng/mL group and the ferritin greater than 1000 ng/mL group.

A total of 12,238 ferritin tests were conducted in the laboratory. Among these, 11,920 tests (97.4%) showed ferritin levels below 500 ng/ml; 222 tests (1.81%) had ferritin levels between 500 and 1000 ng/mL, which corresponded to 155 patients (including repeated tests for the same individuals); and 96 tests (0.78%) had ferritin levels above 1000 ng/ml, corresponding to 66 patients.

The diagnostic categories were classified as follows: (1) Tumors; (2) Infections; (3) Hematologic diseases; (4) Other diseases, which include chronic conditions such as hyperlipidemia, diabetes mellitus, systemic lupus erythematosus, and other autoimmune diseases, cases not classified as tumors, infections, or hematologic diseases were grouped here; (5) Unknown causes, with many cases identified during health check-ups. Some cases underwent genetic testing for hemochromatosis (HH) and thalassemia, but no definitive diagnosis was ultimately confirmed.

ECG Categories: (1) Normal ECG, including sinus bradycardia; (2) Abnormal ECG, which includes atrial fibrillation, sinus tachycardia, arrhythmia, atrioventricular block, prolonged QT interval, T-wave inversion, ST-segment depression and other similar findings; (3) No ECG was done.

3. Statistical methods

The statistical software SPSS 27.0 was used for data processing. Measurement data were analyzed using Pearson's correlation and variance analysis.

4. Result

4.1. Age

There were a total of 221 cases, with an average age of approximately 50.79 years old and a standard deviation of 19.64 years old, which belonged to a non-normal distribution. Among them, the youngest age was 2 months old and the oldest age was 98 years old. Grouped by the degree of increase in ferritin, the average age of the ferritin 1000 ng/mL group (a total of 66 cases) was approximately 48.65 years old, and the average age of the ferritin 500–1000 ng/mL group (a total of 155 cases) was approximately 51.70 years old. The *P* value was 0.320, indicating that there was no significant statistical difference in the age data between the two groups of cases (**Figure 1, Table 1**).

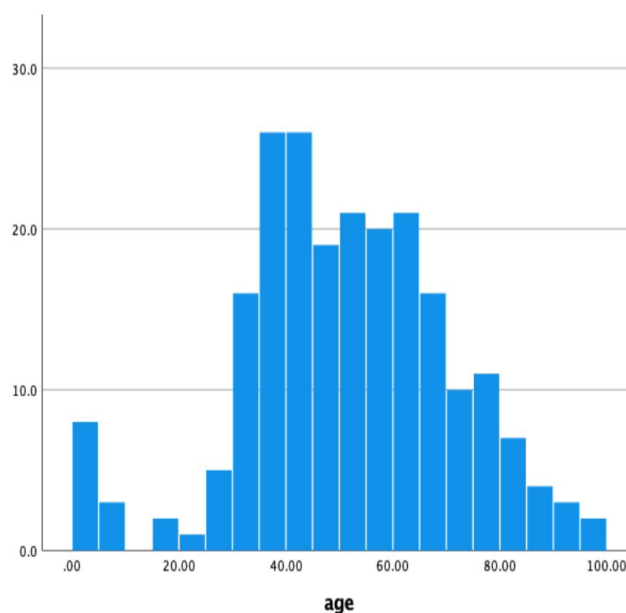


Figure 1. Variation of ferritin with age.

Table 1. Age distribution of patients by ferritin level group

Ferritin group	Count	Average value	Standard deviation	Standard error of the mean
> 1000 ng/mL	66	48.6553	21.56024	2.65388
500–1000 ng/mL	155	51.6984	18.76425	1.50718

4.2. Gender

Among them, there were 160 male cases and 61 female cases. Grouped by the degree of increase in ferritin, there were 51 males and 15 females in the ferritin > 1000 ng/mL group (a total of 66 cases), and 109 males and 46 females in the ferritin 500–1000 ng/mL group (a total of 155 cases). The *P* values were all < 0.001, showing significant statistical differences. Among the different degrees of elevated ferritin, the number of male cases was significantly higher than that of female cases (**Figure 2, Table 2**). The possible causes may be due to a female with

menses bleeding, which promotes iron metabolism and improves the condition of iron overload.

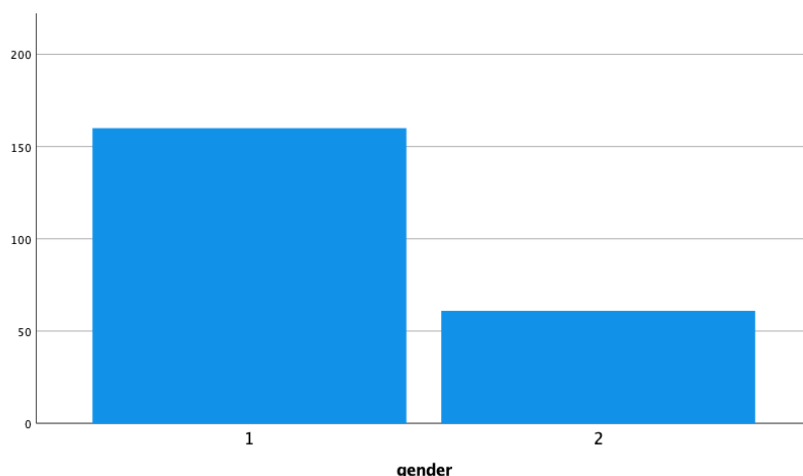


Figure 2. Variation of ferritin with gender.

Table 2. Distribution of gender by ferritin level group

Ferritin group		Gender		Total
		Male	Female	
> 1000 ng/mL	Count	51	15	66
	% within Group	77.3%	22.7%	100.0%
	% within Gender	31.9%	24.6%	29.9%
	% within Total	23.1%	6.8%	29.9%
500–1000 ng/mL	Count	109	46	155
	% within Group	70.3%	29.7%	100.0%
	% within Gender	68.1%	75.4%	70.1%
	% within Total	49.3%	20.8%	70.1%
Total	Count	160	61	221
	% within Group	72.4%	27.6%	100.0%
	% within Gender	100.0%	100.0%	100.0%
	% within Total	72.4%	27.6%	100.0%

4.3. Significant intergroup ferritin difference

The overall Pearson Chi-square value was 16.823, with a significance of 0.002, $P < 0.05$. There was a significant difference, indicating that there was a significant difference between groups. Among them, the Other diseases group (which includes chronic conditions such as hyperlipidemia, diabetes mellitus, systemic lupus erythematosus, and other autoimmune diseases, cases not classified as tumors, infections or hematologic diseases were grouped here), there was a significant statistical difference. In the Other diseases group, there were a total of 60 cases, only 6 cases (accounting for 10.0%) had a low proportion of ferritin greater than 1,000 ng/mL. The remaining 54 cases (accounting for 90.0%) had ferritin within the range of 500–1,000 ng/mL. It is indicated that the cases of ferritin

above 1,000 ng/mL in chronic diseases such as hyperlipidemia, diabetes, and systemic autoimmune diseases are significantly less than those in other diagnostic groups, such as tumors, infections, and the hematological diseases group. However, when comparing among the tumor group, the infection group, the blood group, and the healthy population group, there was no statistically significant difference (**Table 3**).

Table 3. Diagnosis & ferritin group

Diagnosis		Ferritin group		Total
		> 1000 ng/mL	500–1000 ng/mL	
Tumor group	Count	15 _a	24 _a	39
	Expected count	11.6	27.4	39.0
	% within Diagnosis Group	38.5%	61.5%	100.0%
	% within Ferritin Group	22.7%	15.5%	17.6%
Infectious diseases group	Count	22 _a	34 _a	56
	Expected count	16.7	39.3	56.0
	% within Diagnosis Group	39.3%	60.7%	100.0%
	% within Ferritin Group	33.3%	21.9%	25.3%
Hematological diseases group	Count	6 _a	7 _a	13
	Expected count	3.9	9.1	13.0
	% within Diagnosis Group	46.2%	53.8%	100.0%
	% within Ferritin Group	9.1%	4.5%	5.9%
Other diseases group	Count	6 _a	54 _b	60
	Expected count	17.9	42.1	60.0
	% within Diagnosis Group	10.0%	90.0%	100.0%
	% within Ferritin Group	9.1%	34.8%	27.1%
Unknown causes group	Count	17 _a	36 _a	53
	Expected count	15.8	37.2	53.0
	% within Diagnosis Group	32.1%	67.9%	100.0%
	% within Ferritin Group	25.8%	23.2%	24.0%
Total	Count	66	155	221
	Expected count	66.0	155.0	221.0
	% within Diagnosis Group	29.9%	70.1%	100.0%
	% within Ferritin Group	100.0%	100.0%	100.0%

Subscript letters denote homogeneous subsets of group categories where column proportions do not differ significantly at $\alpha = 0.05$ based on post-hoc pairwise comparisons.

Except for tumors, infectious diseases, hematological diseases, other diseases group, for the unknown causes group found during health checkup, there were 17 cases in the group with ferritin above 1,000 ng/mL and 36 cases in the group with ferritin ranging from 500 to 1,000 ng/mL, accounting for 23.2% and 25.8% of the entire ferritin analysis respectively, and the total proportion in the entire analysis was 24.0%. Among the cases indicating ferritin

higher than 500 ng/mL, 24.0% of the cases were of unknown diagnosis.

4.4. Electrocardiogram:

Among all the cases, 70 cases (31.7%) had normal electrocardiograms, 53 cases (24.0%) had abnormal electrocardiograms, and 98 cases (44.3%) had no electrocardiograms. In the group with ferritin above 1000, there were 17 cases (25.8%) with normal electrocardiograms, 16 cases (24.2%) with abnormal electrocardiograms, and 33 cases (50%) with no electrocardiograms. In the ferritin 500–1000 ng/mL group, 53 cases (34.2%) had normal electrocardiograms, 37 cases (23.9%) had abnormal electrocardiograms, and 65 cases (41.9%) had no electrocardiograms.

ECG acquisition rate was 55.7%, with 24% demonstrating abnormalities, including atrial fibrillation, sinus tachycardia, arrhythmia, atrioventricular block, prolonged QT interval, T-wave inversion, ST-segment depression and other similar findings. Current clinical practice demonstrates suboptimal recognition of cardiac sequelae in hyperferritinemia, with only half cases receiving electrocardiogram screening despite established associations with arrhythmogenic risks ^[10–15]. (Table 4 and Table 5)

Table 4. Electrocardiogram analysis of the ferritin group above 1000 ng/mL

Diagnosis		ECG			Total
		Normal ECG	Abnormal ECG	No ECG	
Tumor group	Count	3	7	5	15
	% within Diagnosis Group	20.0%	46.7%	33.3%	100.0%
	% within ECG Group	17.6%	43.8%	15.2%	22.7%
Infectious diseases group	Count	4	5	13	22
	% within Diagnosis Group	18.2%	22.7%	59.1%	100.0%
	% within ECG Group	23.5%	31.3%	39.4%	33.3%
Hematological diseases group	Count	1	0	5	6
	% within Diagnosis Group	16.7%	0.0%	83.3%	100.0%
	% within ECG Group	5.9%	0.0%	15.2%	9.1%
Other diseases group	Count	2	2	2	6
	% within Diagnosis Group	33.3%	33.3%	33.3%	100.0%
	% within ECG Group	11.8%	12.5%	6.1%	9.1%
Unknown causes group	Count	7	2	8	17
	% within Diagnosis Group	41.2%	11.8%	47.1%	100.0%
	% within ECG Group	41.2%	12.5%	24.2%	25.8%
Total	Count	17	16	33	66
	% within Diagnosis Group	25.8%	24.2%	50.0%	100.0%
	% within ECG Group	100.0%	100.0%	100.0%	100.0%

Table 5. Electrocardiogram analysis of the ferritin group 500–1000 ng/mL

Diagnosis		ECG			Total
		Normal ECG	Abnormal ECG	No ECG	
Tumor group	Count	5	7	12	24
	% within Diagnosis Group	20.8%	29.2%	50.0%	100.0%
	% within ECG Group	9.4%	18.9%	18.5%	15.5%
Infectious diseases group	Count	7	8	19	34
	% within Diagnosis Group	20.6%	23.5%	55.9%	100.0%
	% within ECG Group	13.2%	21.6%	29.2%	21.9%
Hematological diseases group	Count	2	3	2	7
	% within Diagnosis Group	28.6%	42.9%	28.6%	100.0%
	% within ECG Group	3.8%	8.1%	3.1%	4.5%
Other diseases group	Count	17	16	21	54
	% within Diagnosis Group	31.5%	29.6%	38.9%	100.0%
	% within ECG Group	32.1%	43.2%	32.3%	34.8%
Unknown causes group	Count	22	3	11	36
	% within Diagnosis Group	61.1%	8.3%	30.6%	100.0%
	% within ECG Group	41.5%	8.1%	16.9%	23.2%
Total	Count	53	37	65	155
	% within Diagnosis Group	34.2%	23.9%	41.9%	100.0%
	% within ECG Group	100.0%	100.0%	100.0%	100.0%

4.5. Special cases

A 3-year-old girl with transfusion-dependent β -thalassemia major exhibited severe growth retardation (< 3rd percentile for height, weight 3%, WHO growth charts) and iron overload (ferritin 2615.2 ng/mL), no electrocardiogram was done. Genetic testing was completed for some cases diagnosed as hereditary hemochromatosis. The genetic testing of two European Americans diagnosed with hemochromatosis showed the classic HFE gene mutation. But in one case, a Chinese patient with ferritin exceeding 500 ng/mL developed atrial fibrillation and underwent ablation treatment. Nuclear magnetic resonance indicates mild iron deposition. Typical hemochromatosis HFH examination was conducted and the result was negative. However, after genetic testing related to hemochromatosis in China, the result was a heterozygous mutation in exon 5 of SUGP2, which is different from the typical HFE gene mutation of hemochromatosis and is a special gene mutation of hemochromatosis in China. In a retrospective study, a case was found where atrial fibrillation occurred when the ferritin level was above 1000 ng/mL. After bloodletting therapy, the electrocardiogram returned to normal when the ferritin level recovered to around 300 ng/mL.

The application of genetic testing and other technologies to confirm the presence or absence of iron-related gene mutations, especially in the field of heterozygote gene testing, requires more research. For instance, a young Chinese male with a history of hyperlipidemia and fatty liver. Health checkup revealed elevated ferritin levels, fluctuating between 588 and 766 ng/mL. Transferrin saturation was normal. Abdominal MRI indicated mild iron

deposition in the liver. No classic hemochromatogenic gene variations were detected in the submitted samples (HFE, HJV, HAMP, TFR2, SLC40A1, DENND3, SUGP2 gene mutation detection and next-generation sequencing). Further next-generation sequencing detected a heterozygous variation of c.2724 + 3A > G in the intron 6 region of the ZYFVE16 gene. Literature-based research indicates that the ZYFVE16 genetic variant modulates systemic iron homeostasis and potentially promotes iron deposition. Subsequent functional studies will be undertaken to delineate its precise role in iron metabolism.

5. Discussion

There are many cases of unknown causes, which require more attention from us clinicians. For example, technologies such as genetic testing should be applied to confirm whether there are iron-related gene mutations^[16,17]. Especially in the aspect of heterozygous gene examination and gene mutations of hemochromatosis and iron metabolism in the Chinese population, more research is needed^[2,5].

The correlation analysis between ferritin values and abnormal electrocardiograms indicated that there was no linear correlation between the two. Studies have shown that the myocardium is more sensitive to iron than other muscle cells. So some patients may experience abnormal electrocardiograms when the ferritin level does not exceed 1000 ng/mL. In other diagnostic groups (chronic diseases, autoimmune diseases, etc.), although the proportion of ferritin above 1,000 ng/mL was relatively low, the proportion of abnormal electrocardiogram was the same as that in other groups. This suggests that whether the ferritin value is significantly elevated or slightly elevated, we need to conduct an electrocardiogram assessment and follow-up monitoring as early as possible^[14].

This study indicates that the etiology and diagnostic nature of ferritin overload have not received extensive attention in clinical practice. The status of electrocardiogram acquisition rate and follow-up is also very low. If the diagnosis is clear and the treatment is timely, ferritin can be controlled as early as possible, which can mitigate pathological iron accumulation and prevent damage to the myocardium, liver, and other irreversible end-organ damage^[18].

6. Conclusion

In summary, this study highlights two significant clinical challenges: a substantial proportion of hyperferritinemia cases remain undiagnosed, and concomitant electrocardiographic (ECG) analyses are frequently overlooked. These findings underscore the imperative to enhance clinical vigilance and improve comprehensive assessment for iron overload, encompassing both etiological investigation and routine ECG evaluation, to facilitate earlier diagnosis and intervention.

Disclosure statement

The authors declare no conflict of interest.

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Comparative Study on the Diagnosis of Thoracic Wall and Rib Involvement in Lung Adenocarcinoma Using ^{99m}Tc -MDP SPECT/CT and MSCT

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Abstract: *Objective:* To compare the diagnostic value of ^{99m}Tc -MDP SPECT/CT and MSCT in detecting thoracic wall and rib involvement in lung adenocarcinoma. *Methods:* A retrospective analysis was conducted on the imaging data of 78 thoracic wall and rib lesions from 66 patients, a total of 32 males and 34 females, aged (53.2 ± 5.6) years old with pathologically confirmed lung adenocarcinoma who underwent both ^{99m}Tc -MDP SPECT/CT and MSCT examinations from March 2017 to September 2023. The diagnostic efficacy of the two imaging modalities was compared using pathological confirmation or clinical follow-up as the gold standard. *Results:* Pathological confirmation or clinical follow-up revealed 74 lesions with thoracic wall bone involvement in lung adenocarcinoma (20 lesions confirmed by surgical pathology and 54 lesions confirmed by clinical follow-up) and 4 lesions without thoracic wall or rib involvement (2 lesions confirmed by surgical pathology and 2 lesions confirmed by clinical follow-up). The diagnostic sensitivity, specificity, and accuracy of ^{99m}Tc -MDP SPECT/CT were 97.3%, 50.0%, and 94.9%, respectively. Its diagnostic sensitivity and accuracy were higher than those of MSCT (72.3% and 74.4%, respectively), with statistically significant differences ($P < 0.05$). The specificity of ^{99m}Tc -MDP SPECT/CT was lower than that of MSCT (100.0%), but the difference was not statistically significant ($P < 0.05$). There were no statistically significant differences in the positive predictive value and negative predictive value between ^{99m}Tc -MDP SPECT/CT and MSCT ($P > 0.05$). ^{99m}Tc -MDP SPECT/CT examination revised the MSCT tumor staging in 14 patients [21.2% (14/66)] with lung adenocarcinoma. *Conclusion:* ^{99m}Tc -MDP SPECT/CT imaging demonstrates superior diagnostic efficacy compared to MSCT in detecting thoracic wall and rib involvement in lung adenocarcinoma. It offers more accurate tumor staging than MSCT, and an accurate diagnosis aids in clinical treatment decision-making.

Keywords: Lung adenocarcinoma; ^{99m}Tc -MDP SPECT/CT; Thoracic wall; Rib involvement

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1. Introduction

Lung cancer is one of the most common malignant tumors worldwide, with a high mortality rate. Non-small

cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancer cases, with lung adenocarcinoma being its primary histological subtype ^[1]. Patients with lung adenocarcinoma classified as T3 due to chest wall invasion have a poorer prognosis compared to those classified as T2 with visceral pleural invasion, with rib invasion of the chest wall being one of the factors contributing to a poor prognosis in lung adenocarcinoma ^[2,3]. The main prognostic factors affecting survival rates in lung adenocarcinoma patients with chest wall and rib invasion include the depth of chest wall infiltration, the extent of surgical resection, and the completeness of the resection ^[4]. Accurate diagnosis of chest wall and rib invasion in lung cancer patients is crucial for clinical staging of lung adenocarcinoma and for selecting appropriate surgical plans. Preoperative examination to establish a clear diagnosis is essential for determining the optimal treatment approach. MSCT examination is a conventional imaging method for diagnosing chest wall and rib invasion in lung adenocarcinoma, offering certain clinical value. However, its diagnostic value for early rib invasion is limited ^[5]. ^{99m}Tc-MDP SPECT/CT combines the advantages of functional imaging and anatomical imaging, effectively improving the accuracy of diagnosing bone lesions ^[6]. The use of ^{99m}Tc-MDP SPECT/CT imaging in diagnosing chest wall and rib invasion in lung adenocarcinoma has received relatively little attention, with limited evidence and reports on its diagnostic value. This study aims to compare the diagnostic value of ^{99m}Tc-MDP SPECT/CT and MSCT in detecting chest wall and rib invasion in lung adenocarcinoma, providing a theoretical basis for clinical treatment decisions.

2. Materials and methods

2.1. Clinical data

A retrospective analysis was conducted on 66 patients pathologically diagnosed with lung adenocarcinoma from March 2017 to September 2023, including 32 males and 34 females, with an average age of (53.2 ± 5.6) years old. All 66 patients with lung adenocarcinoma were highly suspected of having chest wall and rib invasion and underwent both ^{99m}Tc-MDP SPECT/CT and MSCT examinations. Inclusion criteria: (1) All cases were pathologically confirmed as lung adenocarcinoma; (2) CT images indicated that the lung mass was closely adjacent to the chest wall (with no gap between the lesion and the chest wall). Exclusion criteria: A history of thoracic trauma within the past 3 months.

2.2. Inspection methods

Whole-body and local tomographic fusion images were acquired using a Siemens Symbia T16 SPECT/CT machine. After intravenous injection of 740 MBq of ^{99m}Tc-MDP, the examinee was instructed to drink plenty of water and urinate frequently. Three hours after the injection of the imaging agent, the patient was asked to empty their bladder before the examination. The patient then lay supine on the examination table for a ^{99m}Tc-MDP SPECT/CT whole-body planar bone scan. The acquisition conditions for the whole-body bone scan were as follows: matrix size of 128x1024, energy peak at 140 keV, window width of 20%, and a scanning speed of 20 cm/min. The acquisition conditions for local bone tomographic fusion imaging were as follows: parallel acquisition with dual detectors, 20 seconds per frame, and a matrix size of 128x128. The CT scan conditions on the same machine were: voltage of 130 kV and automatic mA current. After post-processing, multi-axial SPECT/CT fusion images were obtained.

Lung CT images were acquired using a GE Lightspeed VCT machine. The MSCT acquisition conditions were as follows: the scanning range extended from the apex to the base of the lungs, with a voltage of 120 kV,

automatic mA current, slice thickness of 5 mm, and an interslice gap of 5 mm.

2.3. Image analysis and diagnostic criteria

The images were independently reviewed by one experienced nuclear medicine physician and one radiologist. A diagnosis of the presence or absence of chest wall bone invasion was made for each lesion. In cases where the two physicians had differing diagnoses for the same case, a final diagnosis was reached through joint discussion and consensus.

Diagnostic criteria for ^{99m}Tc -MDP SPECT/CT [7–10]: Osteolytic, osteoblastic, or mixed bone destruction in the ribs adjacent to the lung adenocarcinoma on the chest wall, with abnormal distribution of the imaging agent (increased or decreased uptake) in the corresponding area of destruction, and exclusion of lesions such as old bone callus and bone islands, is diagnosed as chest wall rib invasion. Abnormal distribution of the imaging agent (increased uptake) in the ribs adjacent to the lung adenocarcinoma on the chest wall on SPECT images, with no abnormal density in the corresponding area of increased uptake on the CT scan of the same machine, is diagnosed as chest wall rib invasion.

MSCT diagnostic criteria [7–10]: Osteolytic, osteogenic, or mixed bone destruction in the ribs adjacent to the chest wall in pulmonary adenocarcinoma, with the exclusion of conditions such as old rib callus and bone islands, is diagnosed as chest wall rib invasion.

Final diagnostic confirmation: Based on surgical or puncture pathology, or clinical follow-up with MSCT and ^{99m}Tc -MDP SPECT/CT imaging for over 6 months.

2.4 Statistical analysis

Statistical analysis was performed using IBM SPSS 24.0 software. Normally distributed measurement data were expressed as mean \pm standard deviation (SD), while categorical variables were expressed as frequencies (percentages). The χ^2 test was used to compare the diagnostic efficacy of the two imaging modalities for chest wall rib invasion in pulmonary adenocarcinoma. When over 20% of the cells had an expected frequency of less than 5, or at least one cell had an expected frequency of less than 1, Fisher's exact probability test was used. A P -value of less than 0.05 was considered statistically significant.

3. Results

3.1. Comparison of pathological or clinical follow-up results and diagnostic efficacy

A total of 78 lesions were identified in 66 patients. Pathological or clinical follow-up for over 6 months confirmed 74 lesions of chest wall rib invasion by pulmonary adenocarcinoma (20 lesions confirmed by surgical pathology and 54 lesions confirmed by clinical follow-up) and 4 lesions without chest wall rib invasion (1 rib bone island and 1 old callus confirmed by pathology, and 1 rib bone island and 1 old callus confirmed by clinical follow-up).

Among the 74 lesions confirmed as chest wall rib invasion by pulmonary adenocarcinoma through pathological or clinical follow-up, the miss rate of ^{99m}Tc -MDP SPECT/CT was lower than that of MSCT; the diagnostic sensitivity of ^{99m}Tc -MDP SPECT/CT was superior to that of MSCT, with a statistically significant difference ($P < 0.05$); the diagnostic accuracy of ^{99m}Tc -MDP SPECT/CT was higher than that of MSCT, with a statistically significant difference ($P < 0.05$); the specificity of MSCT was higher than that of ^{99m}Tc -MDP SPECT/CT, but the difference was not statistically significant ($P > 0.05$); there was no statistically significant difference in

the positive predictive value and negative predictive value between ^{99m}Tc -MDP SPECT/CT and MSCT ($P > 0.05$) (Table 1).

3.2. Comparison of tumor staging results between ^{99m}Tc -MDP SPECT/CT and MSCT

Compared to MSCT staging, ^{99m}Tc -MDP SPECT/CT revised the tumor staging of 14 patients, with an upward adjustment rate of 21.2% (14/66). Among these 14 patients, the T staging was altered, with 3 cases upgraded from T1c to T3, 5 cases upgraded from T2a to T3, and 6 cases upgraded from T2b to T3. The upward adjustment of staging in these 14 patients was diagnosed as positive by ^{99m}Tc -MDP SPECT/CT and negative by MSCT (10 patients were pathologically confirmed to have chest wall and rib involvement by lung adenocarcinoma, and 4 cases were clinically followed up and confirmed to have chest wall and rib involvement by lung adenocarcinoma).

Table 1. Comparison of ^{99m}Tc -MDP SPECT/CT and MSCT in diagnosing chest wall and rib involvement in lung adenocarcinoma

Imaging Modality	Diagnosis	Pathology/Follow-up Results		Sensitivity	Specificity	Accuracy	Positive Predictive Value	Negative Predictive Value
		+	-					
SPECT/CT	+	72	2	97.3%	50.0%	94.9%	97.3%	50.0%
	-	2	2	(72/74)	(2/4)	(74/78)	(72/74)	(2/4)
MSCT	+	54	0	72.3%	100.0%	74.4%	100.0%	16.7%
	-	20	4	(54/74)	(4/4)	(58/78)	(54/54)	(4/24)
χ^2 value				17.298	2.666	12.606	1.482	2.262
P value				< 0.001	0.102	< 0.001	0.223	0.132

4. Discussion

The 5-year survival rate for lung adenocarcinoma staged as T3 is lower than that for early-stage lung adenocarcinoma (T1-2). Patients with T3 tumor staging due to chest wall involvement in lung adenocarcinoma have a worse prognosis than those with T2 tumor staging due to visceral pleural involvement. Lung adenocarcinoma with involvement within the parietal pleura has a better prognosis than that with chest wall involvement, and involvement of the chest wall and ribs has a worse prognosis than involvement of superficial structures of the chest wall. Therefore, improving the accuracy of tumor staging in patients with lung adenocarcinoma and diagnosing chest wall and rib involvement is crucial for formulating clinical treatment plans and improving patients' quality of life and survival rates. MSCT is a conventional imaging method for diagnosing chest wall and rib involvement in lung adenocarcinoma, characterized by its ability to clearly display local rib contours and edge changes through spiral CT thin-slice scanning or reconstruction. However, it has the drawback of false positives^[11,12]. ^{99m}Tc -MDP SPECT/CT provides simultaneous information on bone phosphate metabolism, bone morphology, and anatomical structure. By combining the advantages of functional imaging and anatomical imaging, it effectively improves the diagnostic accuracy of bone lesions and has been widely recognized for its diagnostic value in skeletal lesions^[13].

Currently, few studies have evaluated the diagnostic value of ^{99m}Tc -MDP SPECT/CT in assessing chest wall and rib invasion by pulmonary adenocarcinoma. The results of this study indicate that the diagnostic sensitivity

of ^{99m}Tc -MDP SPECT/CT is 97.3%, which is higher than that of MSCT at 72.3%. The main reason for missed diagnoses by MSCT is that early-stage rib invasion may not yet exhibit bone morphology or density changes, rendering MSCT unable to detect abnormalities. Additionally, data show that the diagnostic specificity of MSCT is 100% (4/4), which is higher than that of ^{99m}Tc -MDP SPECT/CT at 50.0% (2/4). The reason for this is that MSCT, utilizing thin-slice scanning or reconstruction techniques, can more clearly display old bone calluses and bone island lesions compared to the integrated CT of SPECT/CT, resulting in higher specificity. The diagnostic accuracy of ^{99m}Tc -MDP SPECT/CT is 94.9%, which is higher than that of MSCT at 74.4%. This study preliminarily validates that the diagnostic efficacy of ^{99m}Tc -MDP SPECT/CT imaging in diagnosing chest wall and rib invasion by pulmonary adenocarcinoma is superior to that of MSCT. These findings are consistent with the results of a study by Dong et al. ^[14], which analyzed MSCT and SPECT/CT imaging data from 47 lung cancer patients and found that the diagnostic accuracy of SPECT/CT fusion imaging for chest wall bone invasion in lung cancer was 89.4%, significantly higher than that of MSCT at 72.3%. Based on the research, ^{99m}Tc -MDP SPECT/CT, compared to MSCT, can improve the diagnostic accuracy of chest wall and rib invasion by pulmonary adenocarcinoma and has good clinical application value.

The results of this study indicate that ^{99m}Tc -MDP SPECT/CT outperforms MSCT in tumor staging for pulmonary adenocarcinoma. Compared to MSCT tumor staging for pulmonary adenocarcinoma, ^{99m}Tc -MDP SPECT/CT revised the MSCT staging in 21.2% (14/66) of pulmonary adenocarcinoma cases. The T-staging changed in 14 patients, with all 14 patients experiencing an upward revision due to negative MSCT diagnoses (normal bone density in ribs adjacent to pulmonary adenocarcinoma lesions) but positive ^{99m}Tc -MDP SPECT/CT diagnoses (high MDP uptake in ribs adjacent to pulmonary adenocarcinoma lesions). The results of this study indicate that the bone morphology and density of the ribs adjacent to the chest wall in lung adenocarcinoma patients are normal. The ^{99m}Tc -MDP SPECT/CT demonstrated a high diagnostic accuracy of 100% (14/14) in identifying early-stage rib invasion. Compared to MSCT, the primary benefit of ^{99m}Tc -MDP SPECT/CT in tumor staging for rib invasion in lung adenocarcinoma involving the chest wall lies in its ability to assess early-stage chest wall and rib invasion. The reason for this is that during the early stages of rib invasion in lung adenocarcinoma, ^{99m}Tc -MDP SPECT/CT can detect abnormalities in bone phosphate metabolism before bone destruction becomes evident on the ribs and before MSCT can identify any abnormalities in bone density. Changes in bone phosphate metabolism occur earlier than abnormalities in bone morphology and anatomical structure, enabling the detection of early-stage chest wall and rib invasion in lung adenocarcinoma. The findings of this study are consistent with those reported by Zhang et al. ^[15] Their research showed that, after fully considering false-positive factors, SPECT/CT identified bone metastases in two cases where the vertebral morphology and density appeared normal but exhibited high ^{99m}Tc -MDP uptake, aligning with pathological confirmation. The results of this study suggest that ^{99m}Tc -MDP SPECT/CT outperforms MSCT in tumor staging for lung adenocarcinoma, providing crucial scientific evidence for clinical treatment decisions in lung adenocarcinoma.

5. Conclusion

In summary, this study preliminarily validated that ^{99m}Tc -MDP SPECT/CT imaging demonstrates superior diagnostic efficacy compared to MSCT in identifying rib invasion involving the chest wall in lung adenocarcinoma. The value of ^{99m}Tc -MDP SPECT/CT in tumor staging for rib invasion in lung adenocarcinoma involving the chest wall, as compared to MSCT, aids in formulating clinical treatment decisions. The added

value of ^{99m}Tc -MDP SPECT/CT imaging over MSCT in diagnosing rib invasion involving the chest wall in lung adenocarcinoma provides significant scientific evidence for precise staging and clinical treatment decisions in lung adenocarcinoma, thereby further improving patient prognosis and quality of life.

Disclosure statement

The authors declare no conflict of interest.

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Data Mining-Driven: Identification of Potential Traditional Chinese Medicine Categories Targeting Vasculogenic Mimicry in Esophageal Cancer

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Abstract: *Background:* Vasculogenic mimicry refers to a specialized tumor microvasculature independently formed by tumor cells, which facilitates the recurrence, metastasis, and therapeutic resistance in esophageal cancer. Within the framework of traditional Chinese medicine (TCM) theory, there is currently no clear conceptual classification or diagnostic-therapeutic principles for this phenomenon. *Objective:* To explore traditional Chinese medicine (TCM) herbs and syndrome factors related to the treatment of vasculogenic mimicry in esophageal cancer, and to provide a reference for clarifying the TCM clinical syndromes of vasculogenic mimicry in esophageal cancer. *Methods:* Based on public databases such as TCMSP, CNKI, and PubMed, TCM herbs related to esophageal cancer, clinical medications, and herbs inhibiting vasculogenic mimicry were retrieved. The herbs collected from multiple databases were standardized, collated, and cross-analyzed, and core herbs were screened for further analysis. *Results:* Among the public databases, herbs inhibiting vasculogenic mimicry and commonly used clinical herbs for esophageal cancer were mainly of the blood-activating and stasis-resolving type (Huoxue Huayu). In contrast, esophageal cancer-related herbs in the TCMSP database were mainly of the heat-clearing and toxin-resolving type (Qingre Jiedu). A total of 22 TCM herbs related to vasculogenic mimicry in esophageal cancer were identified, mainly blood-activating and stasis-resolving herbs, involving three syndrome factors: “blood stasis (Xueyu), Qi deficiency (Qixu), and Qi stagnation (Qizhi).” *Conclusion:* Vasculogenic mimicry can promote the progression of esophageal cancer, and blood-activating and stasis-resolving herbs may improve the prognosis of patients with esophageal cancer.

Keywords: Esophageal cancer; Vasculogenic mimicry; Recurrence and metastasis; Traditional Chinese medicine

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1. Introduction

Esophageal cancer is a common malignant tumor of the upper gastrointestinal tract in most economically underdeveloped countries and regions, including China, and surgical resection is the standard treatment except for cervical esophageal cancer ^[1]. Although postoperative adjuvant therapy as a supplement to surgical resection has improved the prognosis of some patients outside the T1N0 stage, a considerable proportion of patients still have regional lymph node recurrence and distant organ metastasis. This phenomenon suggests that current postoperative adjuvant treatment strategies do not cover some prognostic risk factors that have not yet been clearly defined. In previous studies, our research group found that the presence of vasculogenic mimicry (VM) had a certain adverse effect on the survival of esophageal cancer, and found that positive VM would affect the effect of postoperative adjuvant treatment. VM is a kind of highly aggressive tumor cells that remodel into vascular-like channels to provide nutrients to itself ^[2]. The formation of VM realizes the exchange of substances between the local microenvironment, including tumor cells, and the outside world, leading to tumor recurrence and distant metastasis. Therefore, intervening in VM is a potential strategy to reduce the risk of postoperative recurrence progression in patients with esophageal cancer with such pathological structures. However, Western medicine has limitations in inhibiting VM, and the intervention effect is not good, so it is necessary to combine traditional Chinese medicine (TCM) methods to form a systematic treatment strategy.

There is no clearly defined corresponding TCM pattern for vasculogenic mimicry in esophageal cancer. In view of the complex mechanism of VM and insufficient conventional treatment intervention, the use of drugs can be guided under the overall view of traditional Chinese medicine and the core idea of syndrome differentiation and treatment. Therefore, the potential TCM pattern can be inferred through the medication used for esophageal cancer related to VM. Previous studies have shown that extracts of Chinese herbs, such as those categorized as activating blood circulation and resolving stasis, can inhibit VM-related processes. Since there are few traditional Chinese medicine studies on esophageal cancer VM, this study plans to explore effective traditional Chinese herbs targeting VM and summarize the rules, and provide theoretical support for the comprehensive treatment of relevant patients through inferring patterns from medicines.

2. Methods

2.1. Literature search

PubMed, the China National Knowledge Infrastructure (hereinafter referred to as CNKI), the Wan fang Data Knowledge Platform (hereinafter referred to as Wan fang data), and VIP Chinese Science and Technology Journal Database (hereinafter referred to as VIP) were searched for Chinese herbal monomers or active ingredients that have been experimentally verified to inhibit vasculogenic mimicry. The keywords in PubMed were “Vasculogenic Mimicry” and “Herbs or Chinese Medicine or Chinese Traditional drug”. The search keywords of CNKI, Wanfang, and VIP were “Vasculogenic Mimicry” and “traditional Chinese medicine or formula, or prescription.”

CNKI, Wanfang data, and VIP searched for effective prescription studies for the clinical treatment of esophageal cancer, and the search keywords were “esophageal cancer” and “traditional Chinese medicine or formulas or prescriptions or empirical formulas “. The search time range was from 2014 to 2024, and the search fields included keywords, abstracts, subjects, titles, and titles.

2.2. Data analysis

Statistical analysis of the clinical data extracted from the literature was performed using Excel 2019 software.

2.3. Inclusion and exclusion criteria

2.3.1. Inclusion criteria

- (1) The disease described in the literature is esophageal cancer/esophageal squamous cell carcinoma;
- (2) The literature content confirms that the Chinese herb/formula related to vasculogenic mimicry is effective through experimental validation (in vivo or in vitro);
- (3) The literature format is research-based/clinical;
- (4) Studies on clinical formulas related to esophageal cancer must be published between 2014 and 2024.

2.3.2. Exclusion criteria

- (1) Non-esophageal cancer diseases documented in the literature;
- (2) The literature format is a review type;
- (3) Duplicate literature (different articles write the same professor's experience and integrate it into one article);
- (4) The full text of the literature is not available.

2.3.3. Flow chart

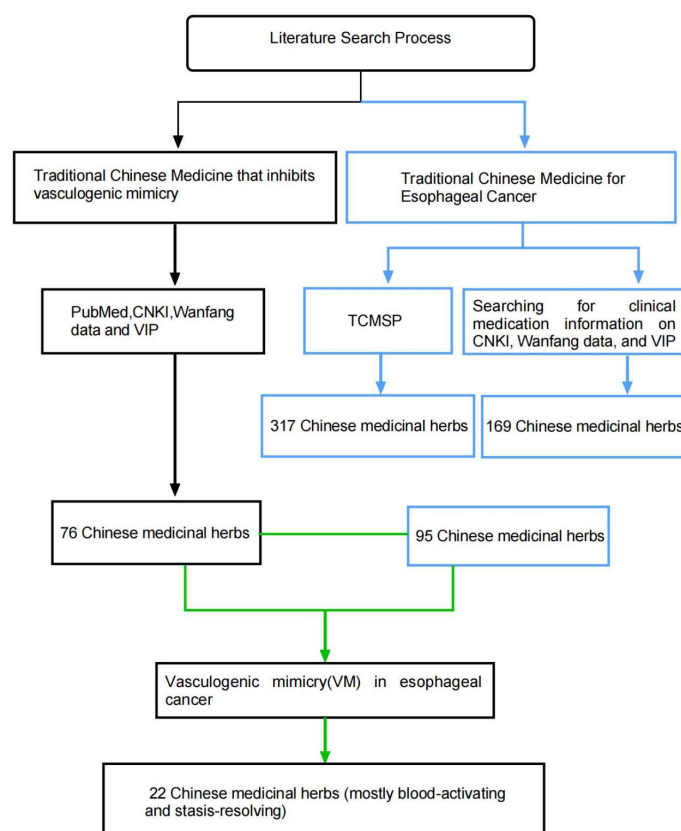


Figure 1. Flowchart of the literature selection process.

3. Results

3.1. The categories of traditional Chinese medicines related to inhibiting vascular mimicry are mainly blood-activating and stasis-resolving medicines

Traditional Chinese medicines that have been experimentally verified to effectively inhibit vascular mimicry were retrieved from PubMed, CNKI, and other databases. These included traditional Chinese medicine extracts curcumin, berberine, artemisinin, etc.; traditional Chinese herb pairs like *Astragalus membranaceus* (Huangqi) - *Atractylodes macrocephala* (Baizhu), *Panax ginseng* (Renshen), *Astragalus membranaceus* (Huangqi), and *Hedyotis diffusa* - *Scutellaria barbata* (Banzhilian) etc., as well as effective prescriptions such as anti-cancer prescriptions and spleen-strengthening and phlegm-reducing prescriptions. A total of 76 Chinese medicines were ultimately included. To explore whether these VM-inhibiting medicines share common patterns, the study analyzed the efficacy of the 76 Chinese medicines. The results revealed that the VM-inhibiting medicines primarily belong to the category of blood-activating and stasis-resolving medicines, with cold property and bitter taste (Figure 2).

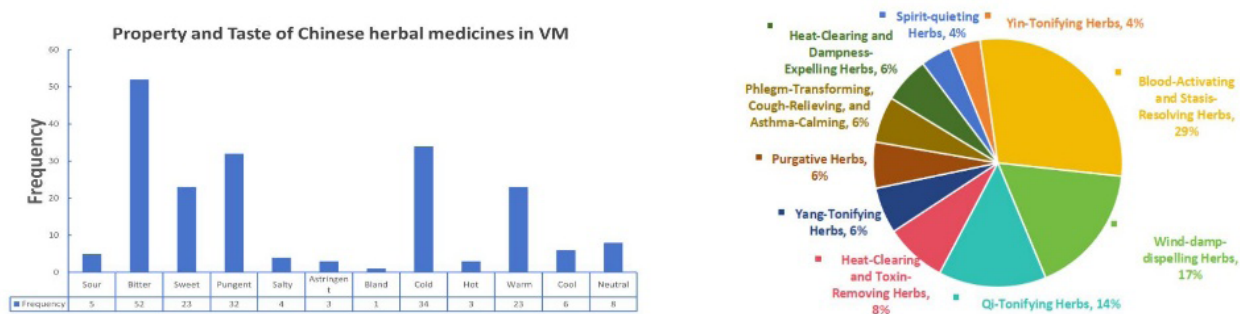


Figure 2. Literature search frequency map of property, taste and efficacy distribution of traditional Chinese medicine inhibiting VM.

3.2. Clinically effective Chinese medicines for esophageal cancer are primarily blood-activating and stasis-resolving medicines and Qi-regulating medicines

In order to understand the clinical use of traditional Chinese medicine in the clinical treatment of esophageal cancer, we collected and sorted out the effective prescriptions for the treatment of esophageal cancer in clinical practice, and searched the relevant prescriptions for the treatment of esophageal cancer from 2014 to 2024 in relevant databases. The prescriptions were primarily derived from the commonly used clinical prescriptions of renowned professors and scholars who have made significant contributions to anti-tumor therapy with Traditional Chinese Medicine, such as Huang Jinming, Pan Minqiu, Hua Baojin, Wang Xixing, Zheng Yuling, Xiong Jibo, Liu Yanqing, Li Zhigang, and Qi Lei. Ultimately, 51 clinically effective prescriptions were integrated, including a total of 752 flavors of traditional Chinese medicine. After cleaning the data, standardizing the names of the Chinese medicines, and removing duplicates, 187 flavors of traditional Chinese medicine were obtained after sorting. The analysis using Excel revealed that *Pinellia ternata* (Banxia), *Poria cocos* (Fuling), *Atractylodes macrocephala* (Baizhu), *Glycyrrhiza uralensis* (Gancao), *Citri Reticulatae Pericarpium* (Chenpi), *Astragalus membranaceus* (Huangqi), *Curcuma aromatica* (Yujin), *Angelica sinensis* (Danggui), *Curcuma zedoaria* (Ezhu), and *Amomum villosum* Lour (Sharen) were among the most frequently used medicines in the treatment of esophageal malignancies (**Figure 3**).

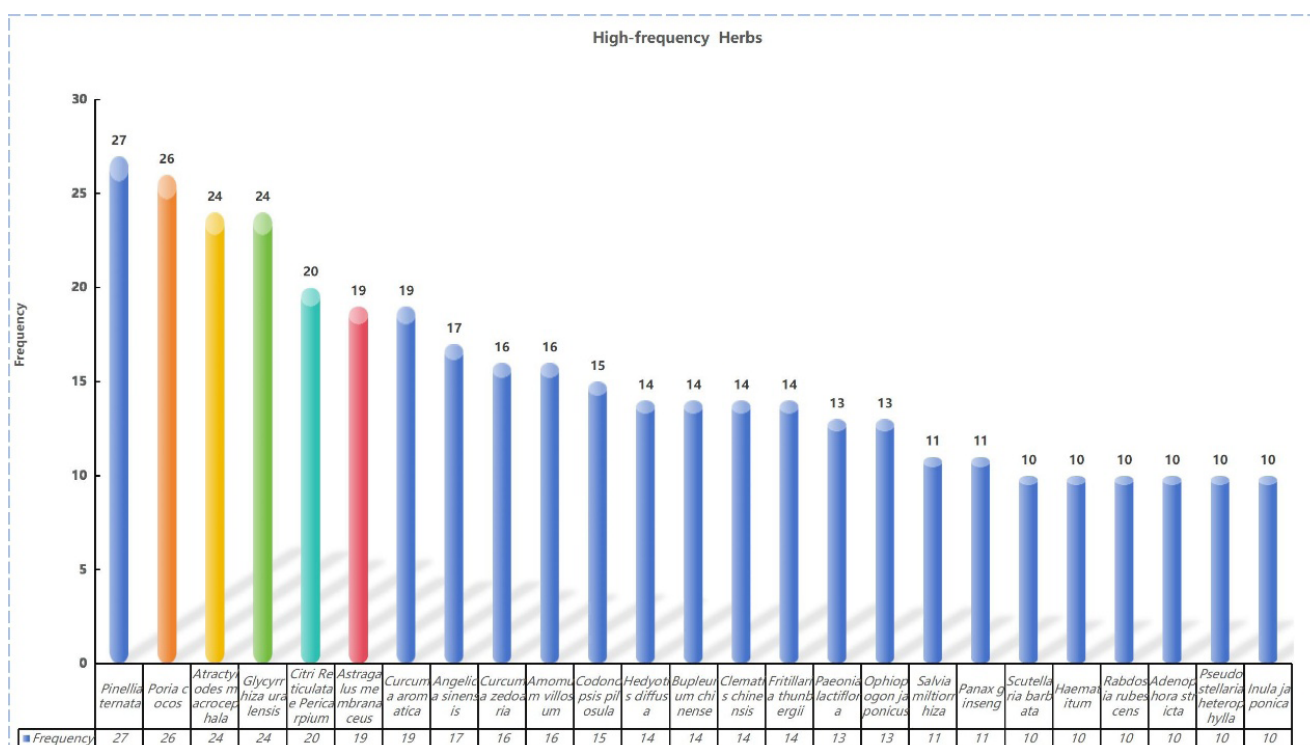


Figure 3. Frequency bar chart of commonly used Chinese herbal medicines in clinical practice (defined as commonly used Chinese herbal medicines with an application frequency of ≥ 10 times).

The above 187 Chinese medicines were standardized, and 18 were excluded, resulting in the final inclusion of 169 medicines. The analysis of 169 flavors of traditional Chinese medicine revealed that the primary efficacies of Chinese medicines used clinically to treat esophageal cancer are Blood-Activating and Stasis-Resolving medicines and Qi-Regulating medicines. The majority are bitter in taste, predominantly cold in property, and enter the Liver Meridian (**Figure 4**).

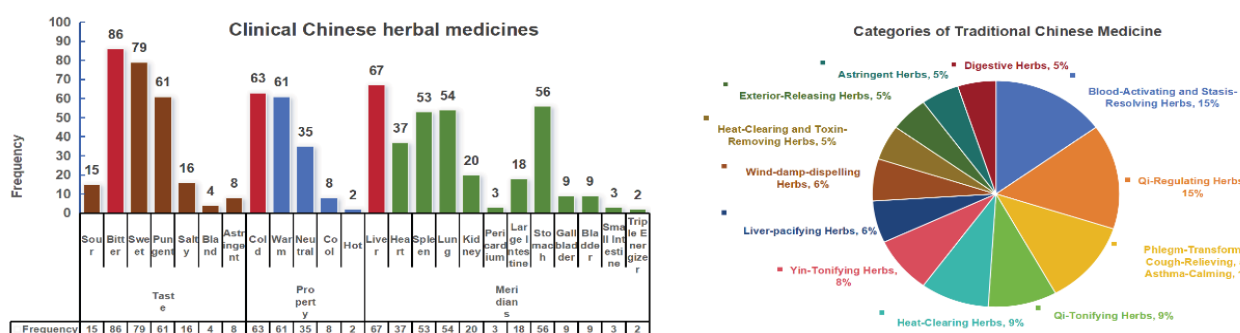


Figure 4. Literature search for the distribution frequency chart of traditional Chinese medicine in clinical use of esophageal cancer.

3.3. Chinese medicines inhibiting esophageal cancer vasculogenic mimicry are primarily blood-activating and stasis-resolving medicines

The study first intersected the Chinese medicines for esophageal cancer from two different sources—the TCMSP database and clinical data—ultimately obtaining 95 overlapping medicines, among which Blood-Activating and Stasis-Resolving medicines accounted for the highest proportion (Figure 5).



Figure 5. Efficacy of esophageal cancer-TCMSP and clinical TCM concentrated drugs.

In order to more accurately screen for Chinese medicines related to vasculogenic mimicry of esophageal cancer, the VM-related medicines were overlapped with the core medicines for esophageal cancer, resulting in an intersection of 22 medicines (Table 1). Statistical results revealed that among these medicines, 11 have the pattern element of blood stasis, accounting for 50% of this subset. The results showed that the corresponding effects of traditional Chinese medicine for esophageal cancer VM were mainly to promote blood circulation and reduce stasis, and the related TCM syndromes were mainly “blood stasis, Qi deficiency, and Qi stagnation”.

Table 1. 22 flavors of Chinese medicine properties, taste, return to meridians, efficacy, and TCM syndrome table

No.	Chinese Medicine	Taste	Propert	Meridians	Efficacy	TCM Pattern Elements
1	<i>Sophora flavescens</i>	Bitter	Cold	Heart, liver , Stomach, Large Intestine , Bladder Meridians	Heat-Clearing and Dampness-Expelling Herbs	Heat, Toxicity, Dampness
2	<i>Scleromitrium diffusum</i>	Bitter,Sweet	Cold	Stomach, Large Intestine, Small Intestine Meridians	Heat-Clearing and Toxin- Removing Herbs	Toxicity, Dampness, Blood stasis
3	<i>Polygonum cuspidatum</i>	Bitter	Cold	Liver, Gallbladder, Lung meridians	Dampness-Percolating and Diuresis-Promoting Herbs	Dampness, Phlegm, Blood stasis
4	<i>Coicis semen</i>	Sweet,bland	Cool	Spleen, Stomach, lung meridians	Dampness-Percolating and Diuresis-Promoting Herbs	Heat, Toxicity, Dampness
5	<i>Bupleurum chinense</i>	Bitter,Pungent	Cold	Liver and Gallbladder meridians	Exterior-Releasing Herbs	Qi stagnation, Qi deficiency
6	<i>Salvia miltiorrhiza</i>	Bitter	Cool	Heart, pericardium, liver meridian	Blood-Activating and Stasis- Resolving Herbs	Blood stasis, Heat
7	<i>Panax notoginseng</i>	Sweet,Bitter	Warm	Liver, Stomach Meridians	Blood-Activating and Stasis- Resolving Herbs	Blood stasis, Qi stagnation
8	<i>Curcuma aromatica</i>	Pungent,Bitter	Cold	Liver, Gallbladder, Heart meridians	Blood-Activating and Stasis- Resolving Herbs	Blood stasis, Dampness, Heat
9	<i>Sparganium stoloniferum</i>	Pungent,Bitter	Neutral	Liver and Spleen meridians	Blood-Activating and Stasis- Resolving Herbs	Blood stasis, Qi stagnation, Food accumulation
10	<i>Carthamus tinctorius</i>	Pungent	Warm	Heart, liver meridians	Blood-Activating and Stasis- Resolving Herbs	Blood stasis
11	<i>Ligusticum chuanxiong</i>	Pungent	Warm	Liver, Gallbladder, Pericardium Meridians	Blood-Activating and Stasis- Resolving Herbs	Blood stasis, Qi stagnation,Wind- Dampness
12	<i>Curcuma phaeocaulis</i>	Pungent,Bitter	Warm	Liver and Spleen meridians	Blood-Activating and Stasis- Resolving Herbs	Blood stasis, Qi stagnation, Food accumulation
13	<i>Commiphora molmo</i>	Pungent,Bitter	Neutral	Heart, Liver, Spleen meridians	Blood-Activating and Stasis- Resolving Herbs	Blood stasis
14	<i>Trichosanthes fructus</i>	Sweet,Bitter	Cold	Lung, Stomach, Large Intestine meridians	Phlegm-Transforming, Cough-Relieving, and Asthma-Calming Herbs	Phlegm, Heat
15	<i>Magnolia officinalis</i>	Bitter,Pungent	Warm	Spleen, Stomach, Lung, Large Intestine Meridians	Dampness-resolving Herbs	Phlegm, Dampness, Qi Stagnation, Food accumulation
16	<i>Rheum palmatum</i>	Bitter	Cold	Spleen, Stomach, Large Intestine, Liver, Pericardium meridians	Purgative Herbs	Heat, Toxicity, Blood stasis
17	<i>Paeonia lactiflora</i>	Bitter,sour	Cold	Liver and Spleen meridians	Blood-Enriching Herbs	Blood deficiency, Yin deficiency, Qi deficiency
18	<i>Atractylodes macrocephala</i>	Sweet,Bitter	Warm	Spleen, Stomach meridians	Qi-tonifying herbs	Qi deficiency, Phlegm, Dampness
19	<i>Panax quinquefolius</i>	Sweet,Bitter	Cool	Heart, lung, kidney meridians	Qi-tonifying herbs	Qi deficiency, Yin deficiency, Heat
20	<i>Astragalus membranaceus</i>	Sweet	Warm	Spleen, Lung meridians	Qi-tonifying herbs	Qi deficiency, Toxicity
21	<i>Panax ginseng</i>	Sweet,Bitter	Neutral	Lung, Spleen, Heart meridians	Qi-tonifying herbs	Qi deficiency
22	<i>Pseudostellaria heterophylla</i>	Sweet,Bitter	Neutral	Spleen, Lung meridians	Qi-tonifying herbs	Qi deficiency, Yin deficiency

4. Discussion

Through data mining, this study found that the proportion of traditional Chinese medicines for the treatment of esophageal cancer—whether from public databases or clinical experience—Blood-Activating and Stasis-Resolving medicines accounted for a higher proportion than other categories, involving the three pattern elements of “blood stasis, Qi deficiency, and Qi stagnation,” which is consistent with the core pathogenesis of esophageal cancer. Esophageal cancer belongs to the category of “dysphagia-occlusion syndrome” in traditional Chinese medicine, and the core pathogenesis is Qi stagnation, blood stasis, and phlegm coagulation intertwined in the esophagus, of which blood stasis runs through the disease, particularly in the advanced and late stages. Emotional disturbances and dietary irregularities can lead to Qi stagnation transforming into fire, which scorches fluids to form phlegm, phlegm and stasis, then bind together to form lumps^[3]. Blood stasis is not only a pathological product, but also hinders the flow of Qi and blood, leading to a deficiency of both Qi and blood and exacerbating stasis, thus forming a vicious cycle. The “Theory of the Origins of Diseases” also emphasizes the core role of Qi stagnation and blood stasis. Modern research has confirmed that patients with esophageal cancer often exhibit a hypercoagulable state and microcirculatory disorders. Blood-activating and Stasis-resolving Chinese medicines (e.g., *Pruni semen* (Taoren), *Carthamus tinctorius* (Honghua), *Radix paeoniae rubra* (Chishao), *Curcuma rhizoma* (Ezhu), etc.) can improve microcirculation, reduce blood viscosity, inhibit tumor proliferation, alleviate the toxic side effects of radiotherapy and chemotherapy, promote drug penetration, and enhance the sensitivity of radiotherapy and chemotherapy^[4–9]. Their combined use can significantly reduce tumor size and relieve obstruction^[10]. For esophageal cancer with the blood stasis pattern, Xuefu Zhuyu Decoction is often used as the basis, like *Semen impatientis* (Jixingzi) and *Herba artemisiae anomalae* (Liu Jinu), and other blood-breaking and stasis-removing drugs are used to relieve menstruation and pain^[11]. The combination of spleen-fortifying and stasis-dispelling Chinese medicines with concurrent radiotherapy and chemotherapy can reduce the incidence of radiation esophagitis and myelosuppression and improve the quality of life, and the mechanism may be related to the regulation of the immune microenvironment and the inhibition of inflammation^[12].

Radix notoginseng powder (San Qi Fen) combined with *Bletilla striata* powder (Baiji Fen) can control ulcerative bleeding and pain^[13]. Pharmacological studies have shown that the active ingredients of traditional Chinese medicine (such as tanshinone and curcumin) can induce apoptosis, inhibit proliferation, and angiogenesis by regulating signaling pathways such as PI3K/AKT and NF- κ B^[14,15]; *Cantharidin* exhibits significant toxicity to esophageal cancer cells^[16]; and *Spatholobi Caulis* (Jixueteng) can increase the CD4+/CD8+ ratio and enhance immunity^[17]. The treatment of blood circulation and blood stasis reduction embodies the idea of “tonifying by unblocking,” and the application requires treatment based on pattern differentiation, avoiding excessive use of stasis-breaking medicines that may consume qi, especially for those who are deficient in righteous Qi after surgery. It is appropriate to match qi-tonifying medicines to “remove stasis without harming righteousness.” Western medicine believes that VM is one of the culprits of its progression, and the effect of conventional postoperative adjuvant therapy is limited. Traditional Chinese medicine believes that the post-operative state is primarily a deficiency of both Qi and blood combined with residual static blood and the generation of phlegm-dampness, with “stasis, phlegm, and deficiency” running through the whole process^[18].

In previous studies, it was found that VM-positive patients have a higher likelihood of recurrence and metastasis than VM-negative patients, and stage II-III ESCC VM-positive patients derived minimal clinical benefit from postoperative adjuvant therapy. There was also no significant difference in efficacy between the three adjuvant treatment modalities. This may be related to the complex formation mechanism of VM; hypoxia

is a perfect inducer of VM formation, and tumor hypoxia activates HIF-1 α , upregulates the expression of genes such as VEGF and MMP-9, and promotes extracellular matrix (ECM) degradation and lumen formation^[19]. In addition to the activation of HIF-1 α promoting the formation of vascular-like structures in tumor cells, the plasticity of tumor cells themselves is enhanced under hypoxic conditions, which also endows tumor cells with endothelial cell-like characteristics^[20]. Changes in cellular properties make intercellular connections loose, and new connections between variant tumor cells and other tumor cells are required to form vascular-like channels composed of vascular endothelial cells to support tumor growth and metastasis^[21]. The hypoxic microenvironment promotes tumor cell metabolic reprogramming. Mitochondria are key sites of cellular metabolism. After the aerobic metabolic pathway is inhibited, it cannot meet the normal growth and reproduction of tumor cells, which will induce the Warburg effect, resulting in an increase in anaerobic glycolytic capacity and lactate accumulation in tumor cells^[22,23]. The above process is merely one part of the complex mechanism involved in the formation of VM, so studying drugs and related strategies to inhibit esophageal cancer VM from a modern medical perspective is challenging.

VM is a pathological phenomenon of highly aggressive tumors, and although there is no direct correspondence theory in traditional Chinese medicine, studies suggest that it is related to the concept of “diseased collaterals”^[24]. However, the author believes that vasculogenic mimicry is related to the diseased collaterals, but the concept of vascular mimicry cannot be simplistically equated with the disease network in general; rather, it should belong to a refined type of diseased collateral, namely, “toxic collaterals.” Toxic collaterals are a specific type of diseased collateral proposed based on the theory of diseased collaterals and the theory of “cancer toxin.” They refer to the pathological collaterals generated by cancer toxin, serving to extract essence and nutrients for the tumor and facilitate its metastasis and spread. Pathogenic collaterals are the product of pathological changes in the collaterals and are the basic pathogenesis of diseases^[25]. Toxic collaterals belong to the category of diseased collaterals, but they specifically refer to the hyperactive form created by the action of cancer toxin on the local collateral system. The formation of VM is thought to be the result of the accumulation of cancer toxin, which manifests as abnormal hyperactivity of the collateral pathways, accelerating tumor spread and nutrient supply. Therefore, VM can be regarded as a toxic collateral formed by cancer toxin acting on the collaterals, which is a specific manifestation of the diseased network in the tumor.

At present, cancer treatment has entered an era of multidisciplinary collaboration, and the combination of traditional Chinese and Western medicine forms a synergistic effect through complementary mechanisms. This integration is reflected not only at the technical level, but also in the innovation of treatment concepts - from allopathic therapy to homeostasis reconstruction. Academician Tong Xiaolin innovatively proposed the theory of “state-target syndrome differentiation”, which translates macroscopic theories into microscopic applications. This approach takes the disease as the reference, the state as the basis, and symptoms as targets, proposing specific disease target prescriptions/target drugs^[26]. However, due to the lack of research on VM in esophageal cancer and the corresponding symptoms and indicators have not been clearly reported. Therefore, we attempted to use data mining to identify traditional Chinese medicines targeting VM in esophageal cancer, reverse infer the symptoms/indicators corresponding to the disease, and then deduce the symptoms from the target medicines, and ultimately summarize the possible syndrome patterns corresponding to VM in esophageal cancer. This aims to maximize the potential and advantages of traditional Chinese medicine in the treatment of VM in esophageal cancer. However, this study still has certain limitations; the above research results have not been further experimentally and clinically verified. In the future, it will screen relevant genes and targeted drugs for in-depth research, effectively

transform the research results, and better serve clinical practice.

5. Conclusion

The corresponding traditional Chinese medicine syndrome pattern of vascular mimicry in esophageal cancer may be blood stasis and Qi deficiency, and blood-activating and stasis-reducing drugs are expected to improve the prognosis of esophageal cancer patients with VM-positive.

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Disclosure statement

The authors declare no conflict of interest.

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Global Research Trends and Hotspots of Contrast-Enhanced Ultrasound in Tumor Diagnosis: A Bibliometric Analysis (2000–2025)

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Abstract: *Objective:* To systematically evaluate global research trends on contrast-enhanced ultrasound (CEUS) in tumor diagnosis using bibliometric methods. *Methods:* Publications from January 2000 to **June 2025** were retrieved from the Web of Science Core Collection (SCI-EXPANDED). Only English-language articles and reviews were included. A total of 3,493 records were analyzed. VOSviewer 1.6.20 were used for bibliometric and visualization analyses, covering annual output, countries and institutions, authors, journals, keyword co-occurrence, collaboration networks, and co-citation patterns. *Results:* The number of publications demonstrated steady growth with acceleration after 2018, peaking in 2021 and 2023 (> 350 papers/year). Dietrich Christoph F. was the most productive and influential author, while Chinese scholars (e.g., Dong Yi, Wang Wen-Ping) and institutions such as Sun Yat-sen University and Fudan University emerged as leading contributors. European journals, particularly Ultrasound in Medicine and Biology and European Radiology, showed high academic influence. Keyword analysis revealed liver cancer, especially hepatocellular carcinoma, as the dominant research theme, with expanding applications in breast, renal, and prostate tumors. Collaboration networks highlighted strong partnerships between China and Europe, whereas North American participation remained limited. Co-citation analysis indicated that a small number of highly cited studies shaped the intellectual foundation of the field. *Conclusion:* CEUS research in tumor diagnosis has expanded rapidly, characterized by concentrated leadership, thematic diversification, and strengthening international collaboration. With advances in artificial intelligence, super-resolution imaging, and novel contrast agents, CEUS is expected to evolve from a diagnostic tool into an integrated platform for tumor detection, treatment monitoring, and personalized cancer care.

Keywords: Contrast-enhanced ultrasound; Tumor diagnosis; Bibliometrics; Research trends

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1. Introduction

Early diagnosis and accurate evaluation of tumors are crucial for improving patient survival and guiding individualized treatment strategies. Imaging techniques, as indispensable tools in oncology research and clinical practice, have significantly promoted the progress of precision oncology. Among them, ultrasound is widely applied due to its advantages of being noninvasive, safe, and capable of real-time imaging. However, conventional two-dimensional ultrasound is limited in spatial resolution and microvascular visualization, making it insufficient to fully capture tumor angiogenesis and tissue perfusion characteristics.

Contrast-enhanced ultrasound (CEUS), as a functional imaging technique, has developed rapidly in recent years. Through intravenous injection of microbubble contrast agents, CEUS enables real-time and dynamic monitoring of tumor blood perfusion, effectively compensating for the shortcomings of conventional ultrasound. Recent studies have demonstrated that CEUS plays an important role in the diagnosis, staging, and therapeutic assessment of various solid tumors, including those of the thyroid, breast, kidney, and liver.

For example, a recent meta-analysis by Gao et al. highlighted that CEUS exhibited significantly higher sensitivity, specificity, and area under the curve (AUC) compared with conventional ultrasound in detecting lymph node metastasis of thyroid cancer ^[1]. In the field of breast imaging, Zhu et al. reported that CEUS-based BI-RADS showed superior diagnostic efficacy over conventional BI-RADS, particularly for category 4 lesions, thereby reducing unnecessary biopsies ^[2]. Furthermore, Wu et al. (2024) demonstrated that high-frame-rate CEUS (H-CEUS) significantly improved the qualitative and quantitative characterization of solid renal tumors, providing better differentiation between benign and malignant masses ^[3].

Despite these promising advances, challenges remain in this field: research topics are fragmented, contributions vary across regions, and there is a lack of systematic global reviews to summarize research trends and frontiers. Bibliometric analysis offers a powerful approach to quantitatively map the knowledge structure of a discipline, identify influential scholars and institutions, and uncover emerging research hotspots. Such insights may facilitate a comprehensive understanding of the developmental trajectory of CEUS and provide valuable references for future innovations.

Based on this, the present study utilized the Web of Science Core Collection to retrieve relevant publications from 2000 to 2025, and employed VOSviewer for systematic bibliometric analysis. The aim was to delineate the global research landscape, identify core authors and institutions, and explore research hotspots and emerging trends, thereby providing constructive references for the academic development and clinical applications of CEUS.

2. Methods

2.1. Data source and search strategy

The data for this study were retrieved from the Web of Science Core Collection (WoSCC), with the Science Citation Index Expanded (SCI-EXPANDED, 1900–present) selected as the citation index. The search strategy was defined as follows: TS = (“contrast-enhanced ultrasound” OR “CEUS”) AND (“tumor” OR “tumour” OR “cancer” OR “carcinoma” OR “neoplasm” OR “oncology” OR “malignant”) AND (“diagnosis” OR “diagnostic” OR “detection” OR “screening”). The time span was set from January 2000 to June 30, 2025. Only articles and reviews published in English were included, while conference proceedings, early access publications, editorials, and letters were excluded. A total of 3,853 records were initially retrieved. After independent screening of titles and abstracts by two researchers, duplicates and irrelevant studies were removed, resulting in 3,493 publications (2,832 articles and 661 reviews) included for analysis.

2.2. Data analysis

Bibliometric analyses were conducted using VOSviewer 1.6.20. The analyses covered the following aspects: annual publication trends, geographical distribution of countries and regions, contributions of authors and institutions, high-frequency keywords and emerging research hotspots, collaboration networks, and co-citation patterns. This comprehensive bibliometric approach was designed to systematically map the global research landscape of CEUS in tumor diagnosis and to elucidate its developmental trajectories and emerging themes.

3. Results

3.1. Top 10 authors by publication output

Among the top 10 authors ranked by publication output, Dietrich Christoph F. ranked first with 77 articles, which have been cited 1,864 times (average 24.21 citations per article), indicating a strong academic influence. He was followed by Dong Yi (72 articles, 894 citations, average 12.42) and Wang Wen-Ping (58 articles, 1,197 citations, average 20.64). In terms of average citations, Piscaglia Fabio achieved the highest impact, with 40 articles cited 1,760 times (average 44.00 per article), followed by Lu Ming-De (31.00) and Xu Hui-Xiong (28.80). Overall, authors with higher publication outputs generally also demonstrated relatively high citation rates, suggesting their central contributions to this research field (**Table 1**).

Table 1. Top 10 authors by publication output

Author name	Total number of articles	Total citations	Average citations
Dietrich, Christoph F.	77	1864	24.2078
Dong, Yi	72	894	12.4167
Wang, Wen-Ping	58	1197	20.6379
Lu, Ming-De	55	1705	31
Xie, Xiao-Yan	55	1568	28.5091
Wang, Wei	52	1020	19.6154
Xu, Hui-Xiong	49	1411	28.7959
Luo, Yan	44	596	13.5455
Piscaglia, Fabio	40	1760	44
Goerg, Christian	37	234	6.3243

3.2. Top 10 institutions by publication output

At the institutional level, Sun Yat-sen University ranked first with 172 publications and 3,626 citations (average 21.08 citations per article), demonstrating its leading academic position in this field. Fudan University (166 publications, 2,935 citations, average 17.68) and Shanghai Jiao Tong University (139 publications, 2,410 citations, average 17.34) followed closely behind. Notably, although Peking University ranked eighth with only 61 publications, it achieved the highest average citation count of 23.15 among all institutions, highlighting the high quality and strong impact of its research output. Overall, multiple top-tier Chinese universities have formed a concentrated research force in this field (**Table 2**).

Table 2. Top 10 institutions by publication output

Institution name	Total number of articles	Total citations	Average citations
Sun Yat-sen University	172	3626	21.0814
Fudan Univ	166	2935	17.6807
Shanghai Jiao Tong Univ	139	2410	17.3381
Sichuan Univ	101	1310	12.9703
Zhejiang Univ	88	1225	13.9205
Huazhong Univ Sci & Technol	75	1157	15.4267
Chinese Peoples Liberat Army Gen Hosp	74	1059	14.3108
Peking Univ	61	1412	23.1475
Tongji Univ	60	893	14.8833
Iuliu Hatieganu Univ Med & Pharm	55	797	14.4909

3.3. Top 10 journals by publication output

Table 3 presents the top 10 journals ranked by publication output on contrast-enhanced ultrasound (CEUS) in tumor diagnosis. Among them, Ultrasound in Medicine and Biology published the highest number of articles ($n = 186$), followed by Clinical Hemorheology and Microcirculation ($n = 167$) and the Journal of Ultrasound in Medicine ($n = 127$). These three journals collectively accounted for nearly one-third of all publications in this field, indicating their central role in disseminating CEUS-related research.

Table 3. Top 10 journals by publication output

Journal Name	Total Number Of Articles	Total Citations	Average Citations
Ultrasound In Medicine And Biology	186	4301	23.1237
Clinical Hemorheology And Microcirculation	167	2821	16.8922
Journal Of Ultrasound In Medicine	127	2163	17.0315
Frontiers In Oncology	124	745	6.0081
Ultraschall In Der Medizin	96	5085	52.9688
European Radiology	92	3276	35.6087
Abdominal Radiology	89	1718	19.3034
European Journal Of Radiology	88	3035	34.4886
Medical Ultrasonography	74	854	11.5405
Diagnostics	70	606	8.6571

In terms of total citations, Ultraschall in der Medizin ranked first with 5,085 citations, despite contributing a smaller number of publications ($n = 96$). Its exceptionally high average citation rate (52.97 per article) highlights its strong academic influence and the high quality of its published works. Similarly, European Radiology (3,276 citations; average 35.61) and the European Journal of Radiology (3,035 citations; average 34.49) demonstrated significant impact, reflecting the leading position of European journals in radiological imaging research.

By contrast, some journals with relatively high output, such as Frontiers in Oncology ($n = 124$), showed

lower average citations (6.01), suggesting that while these journals contribute substantially to volume, their academic influence is relatively limited compared with specialized radiology or ultrasound journals.

Overall, the distribution pattern suggests that CEUS-related tumor diagnosis research is mainly disseminated in specialized ultrasound and radiology journals, with European journals demonstrating particularly strong academic impact. This reflects both the interdisciplinary nature of CEUS and the regional research advantages in Europe.

3.4. Co-occurrence analysis of keywords

Table 4 summarizes the top 10 keywords by co-occurrence frequency. The most frequent term was “contrast-enhanced ultrasound” ($n = 1024$), followed by its variant spelling “contrast-enhanced ultrasound” ($n = 847$). Other frequently co-occurring keywords included “hepatocellular carcinoma” ($n = 776$), “diagnosis” ($n = 722$), “ultrasonography” ($n = 593$), and “sonography” ($n = 452$). General oncological and imaging-related terms such as “cancer” ($n = 447$), “CT” ($n = 429$), “lesions” ($n = 425$), and the abbreviation “CEUS” ($n = 378$) also appeared among the top 10. These findings indicate that research on CEUS in tumor diagnosis is strongly linked to imaging modalities, clinical diagnosis, and liver cancer, especially hepatocellular carcinoma.

The co-occurrence network map (**Figure 1**) visualizes these relationships and highlights the clustering of keywords into distinct research themes. Four major clusters were identified:

The green cluster, centered on “contrast-enhanced ultrasound,” “diagnosis,” and “cancer,” emphasizes clinical diagnostic applications and differentiation between benign and malignant lesions. The blue cluster focuses on liver-related research, particularly hepatocellular carcinoma, intrahepatic cholangiocarcinoma, cirrhosis, and surveillance, reflecting the central role of CEUS in liver oncology. The yellow cluster is associated with comparative imaging modalities such as CT and MRI, highlighting CEUS as an alternative or complementary diagnostic tool.

The red cluster emphasizes technical aspects, including contrast agents, perfusion, microbubbles, angiogenesis, and breast cancer applications, representing the development of CEUS methodologies and therapeutic monitoring.

Together, the keyword analysis reveals that CEUS research in tumor diagnosis is dominated by studies on liver cancer and hepatocellular carcinoma, while also extending to breast, kidney, and prostate tumors, as well as broader applications in lesion characterization and differential diagnosis. Moreover, the interplay between technical development and clinical application underscores the interdisciplinary nature of CEUS research.

Table 4. Top 10 keywords by co-occurrence frequency

Rank	Frequency	Centrality	Time	Keyword
1	1024	0	2005	contrast-enhanced ultrasound
2	847	0.01	2006	contrast enhanced ultrasound
3	776	0	2005	hepatocellular carcinoma
4	722	0.01	2005	diagnosis
5	593	0.01	2007	ultrasonography
6	452	0.02	2005	sonography
7	447	0.01	2007	cancer
8	429	0.02	2005	ct
9	425	0.01	2005	lesions
10	378	0.01	2010	ceus

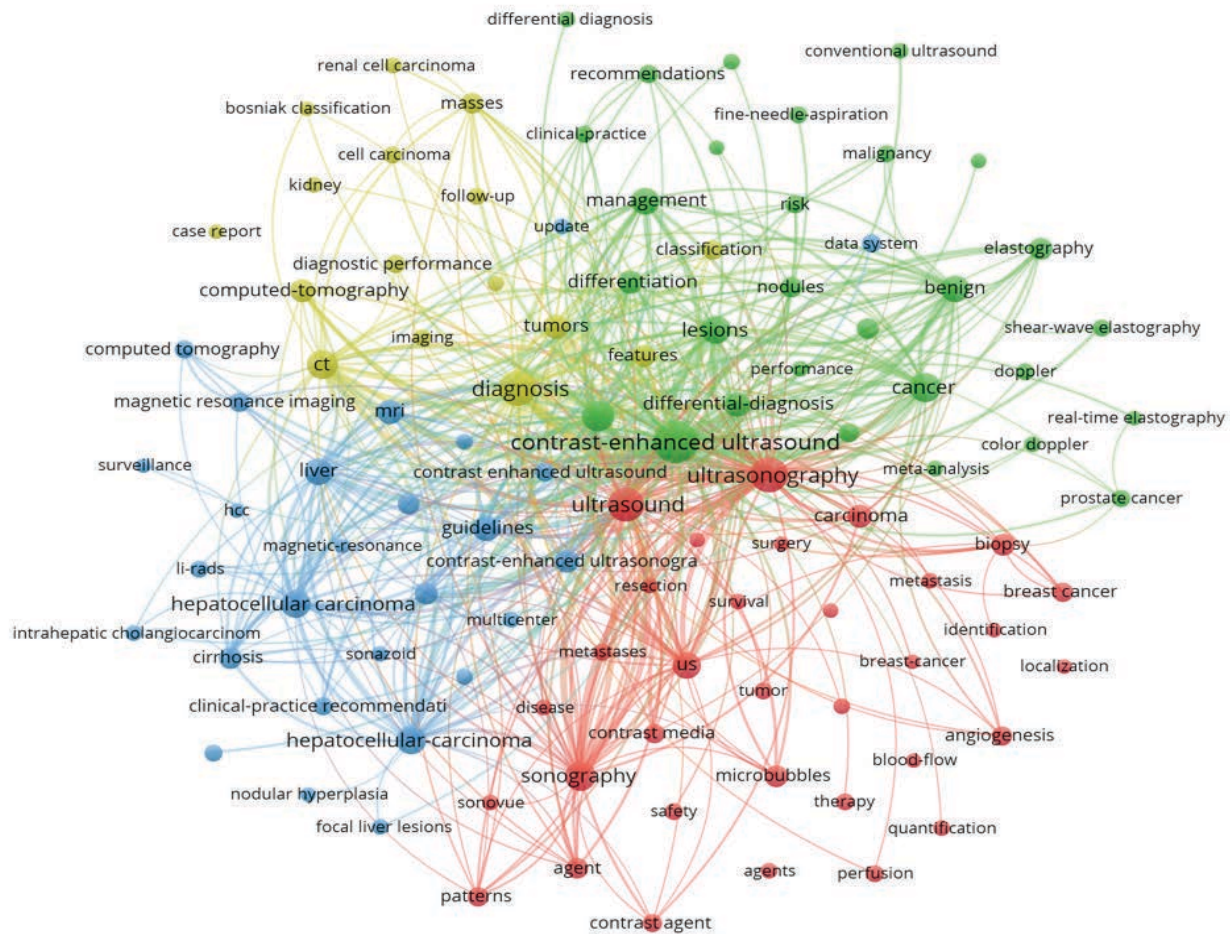


Figure 1. Co-occurrence network of keywords.

3.5. Author collaboration network

The author collaboration network illustrates the academic linkages among researchers and the formation of research communities. Node clusters of different colors represent tightly connected groups of scholars, with node size corresponding to publication output and edges indicating the strength of collaboration. From the overall structure, the network exhibits a multi-core distribution, with Chinese and European/American scholar groups being the most prominent. The group led by European scholars such as Dietrich, Piscaglia, and Jenssen primarily focuses on methodological innovation and international collaboration, whereas the Chinese clusters, represented by Wang Wei, Liu Guangjian, and Xu Xiaoyan, are more oriented toward applied research and clinical promotion. The network also highlights the presence of bridging scholars who connect different research groups and play key roles in fostering cross-national cooperation. These findings suggest that the field is gradually transitioning from isolated research efforts to cross-regional and cross-institutional collaboration, which facilitates the international dissemination of research outcomes (**Figure 2**).

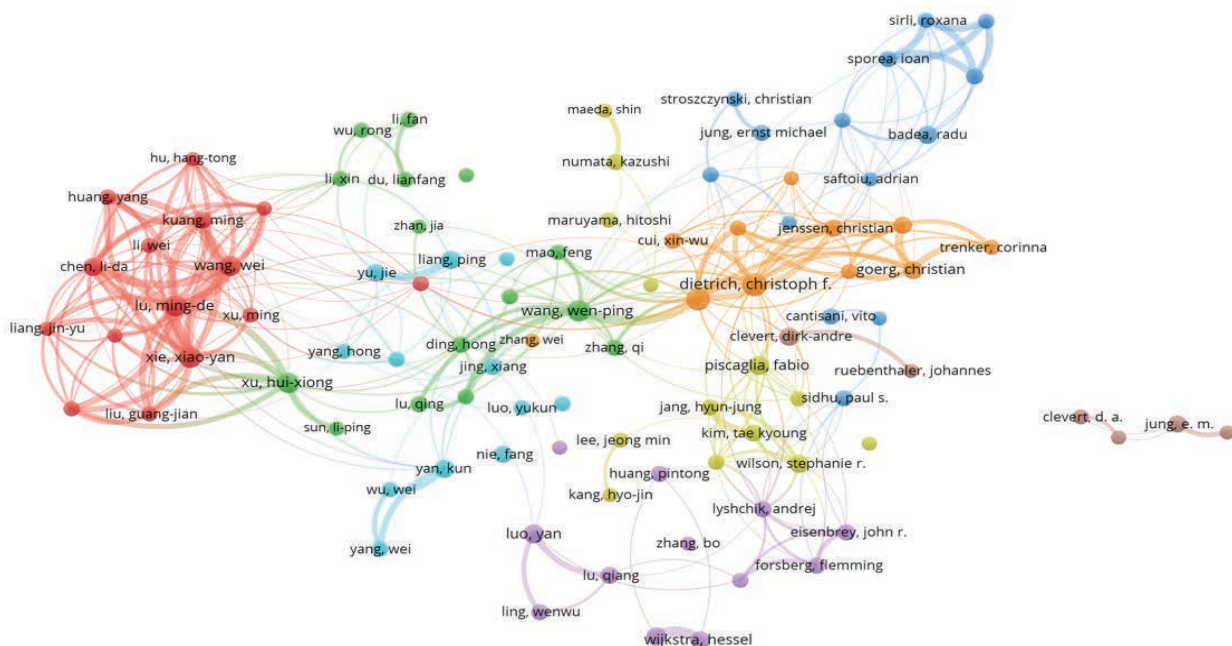


Figure 2. Author collaboration network.

3.6. Institutional collaboration network

The institutional collaboration network reflects patterns of academic cooperation at the organizational level. Chinese universities such as Fudan University, Shanghai Jiao Tong University, Sun Yat-sen University, and Zhejiang University occupy central positions in the network, underscoring their importance in both research output and collaborative capacity. Western institutions, including Stanford University, the University of California system, and several European medical centers, also demonstrate high node weights, indicating their substantial international influence. The clustering of different colors reveals the formation of several tightly connected groups, with collaborations among Chinese universities being the most frequent, highlighting the characteristics of regional academic alliances. In contrast, cross-national collaborations are more concentrated between international medical research institutions and leading Chinese universities. This collaborative model not only promotes the diversification and internationalization of research but also facilitates the bidirectional exchange of clinical experience and experimental techniques (**Figure 3**).

3.7. National collaboration network

The national collaboration network reveals the global distribution of research power and patterns of cooperation. In the network, countries such as China, the United States, and Germany exhibit larger nodes, reflecting their central roles in publication output and international influence. The collaboration between China and the United States is particularly strong, with both also maintaining close ties with Germany, Italy, and Japan, thereby forming a tightly connected international network. European countries constitute a regional collaboration circle characterized by frequent intra-regional partnerships. Notably, several emerging countries, including Romania, India, and South Korea, have become increasingly active in recent years. Although their overall publication volume remains limited, collaboration with core countries has enhanced their research visibility. Overall, the structure of international collaboration is characterized by a small number of core countries driving the field while

engaging broad participation from multiple nations. This model not only facilitates the sharing and dissemination of academic achievements but also provides a solid foundation for cross-national clinical applications and standardized research.

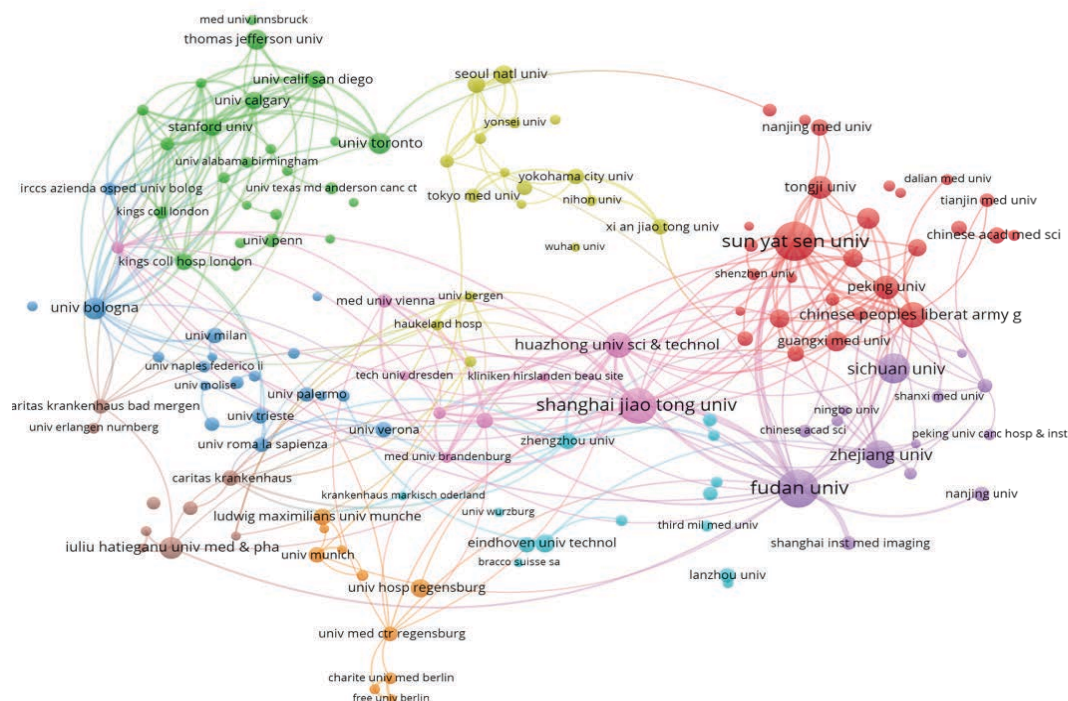


Figure 3. Institutional collaboration network.

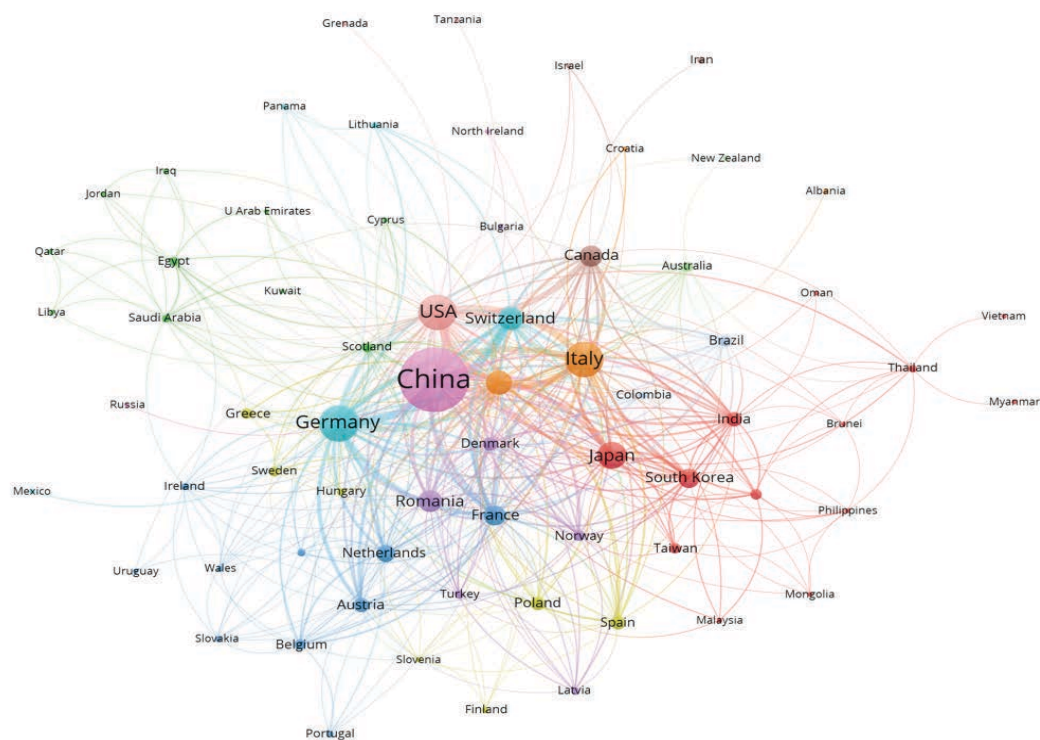


Figure 4. National collaboration network.

3.8. Annual publication trends

The annual publication trend reflects the overall developmental trajectory of this field. From 2005 to the present, the number of publications has shown a steady upward trend, with an accelerated increase observed after 2018, reaching stage-specific peaks in 2021 and 2023, with more than 350 articles published annually. The close fit between cumulative publications and the exponential growth model ($R^2 = 0.9488$) indicates that the development of this field follows an exponential growth pattern. This trend suggests that the field has not only maintained continuous academic attention but has also achieved new breakthroughs in methodological innovation, clinical application, and interdisciplinary integration. In light of the growing global demand for medical imaging and precision diagnostics in recent years, it can be anticipated that research activity in this area will remain at a high level, with continued growth in scientific output (**Figure 5**).

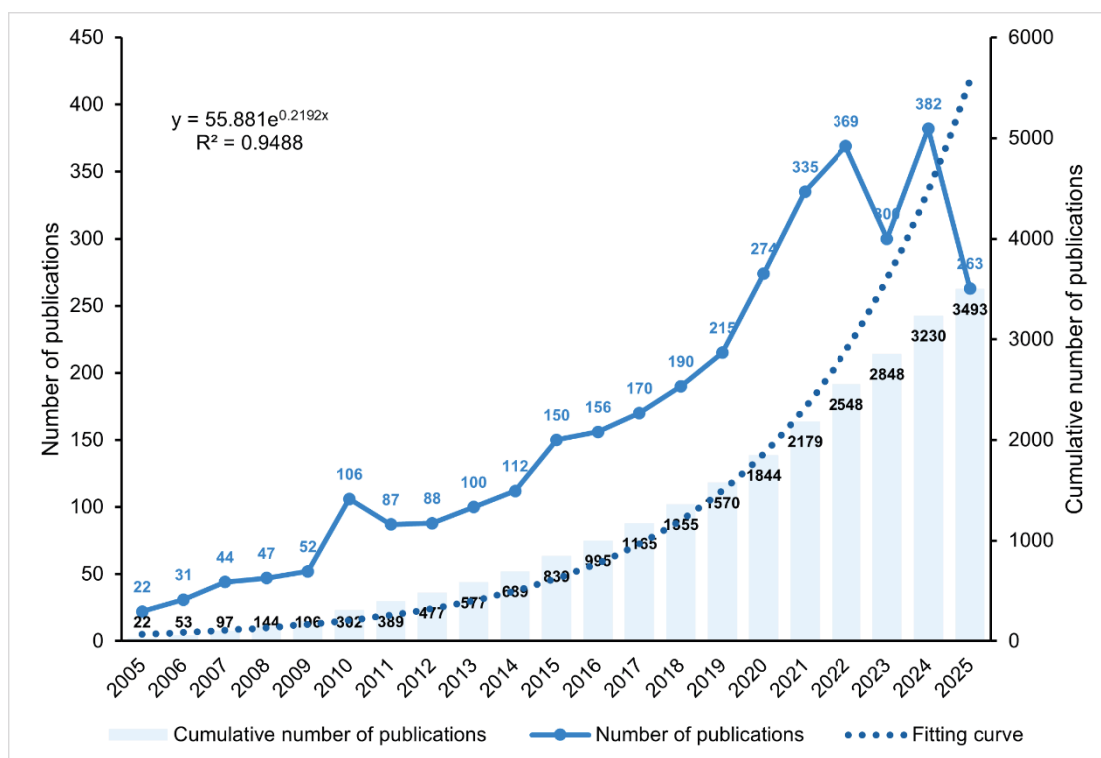


Figure 5. Annual publication trends.

3.9. Co-citation analysis

The co-citation analysis reveals the intellectual foundation of the field and the clustering of core references. In the network, nodes of different colors represent groups of publications with high co-citation frequencies, reflecting several relatively independent yet interconnected research themes. The green and blue clusters are mainly centered on imaging methodologies and clinical diagnostic studies, whereas the red cluster focuses on emerging applications and methodological refinements. Node size indicates citation frequency, while the thickness of the connecting lines reflects co-citation strength. For example, seminal works such as Claudon (2013) and Dietrich (2020) occupy central positions, underscoring their pivotal roles in advancing theoretical frameworks and methodological development in this field. The overall network demonstrates a core-periphery structure, suggesting that research hotspots are driven by a limited number of highly influential references, gradually diffusing and branching into diverse directions. This pattern implies that future investigations are likely to continue building upon these highly

Beyond oncology, new applications have been explored in the urinary system, from kidneys to bladder ^[8]. These developments reflect the cross-cancer and multi-organ potential of CEUS.

Technological innovation has further expanded CEUS capabilities. The 2023 update of DCE-US standards emphasized its use for treatment monitoring in oncology ^[4]. In parallel, super-resolution CEUS techniques are enabling microvascular analysis at unprecedented resolution, providing novel insights into liver tumor vascularity and therapeutic monitoring ^[9]. Collectively, these advances demonstrate that CEUS is evolving beyond a diagnostic modality toward an integrated platform for diagnosis, therapy monitoring, and potentially therapeutic delivery.

Collaboration networks illustrate the global and regional dynamics of CEUS research. Chinese and European scholars, often anchored by leaders such as Dietrich, form the backbone of international collaboration, while North American integration remains limited. National collaborations are driven by China and the United States, complemented by Germany, Italy, and Japan, whereas emerging countries such as India, South Korea, and Romania are improving visibility through collaborations with core nations. This model of a few core countries driving the field while engaging broader global participation fosters both knowledge dissemination and standardization.

The upward trend in publications, particularly post-2018, signals strong academic momentum and reflects growing clinical relevance. The exponential growth trajectory suggests CEUS research is in an accelerated development phase, with its findings increasingly influencing guidelines and clinical practice.

Several limitations must be acknowledged. This study relied on a single database, which may exclude regional or non-English publications. Moreover, bibliometric indicators such as publication and citation counts measure academic impact but do not necessarily capture clinical utility or translational outcomes. As artificial intelligence, deep learning, and molecular imaging converge with CEUS, future evaluations will require methods that better reflect interdisciplinary integration.

Looking forward, CEUS research offers broad opportunities. New contrast agents and molecular probes may extend their role into theranostics ^[4,9]. Artificial intelligence–driven image analysis will likely enhance reproducibility and diagnostic precision. Clinically, CEUS has strong potential for early tumor detection, therapy response monitoring, and long-term surveillance, particularly in resource-limited settings where it offers a cost-effective solution. High-quality multicenter prospective studies and global collaborative frameworks will be essential to strengthen the evidence base and promote guideline adoption.

In conclusion, CEUS research in tumor diagnosis is undergoing rapid expansion, characterized by concentrated leadership, thematic diversification, and strengthened collaboration. With ongoing technological innovation, clinical translation, and global cooperation, CEUS is expected to play an increasingly central role in oncologic imaging and personalized cancer care.

5. Conclusion

This study provides a comprehensive overview of the global research landscape of CEUS in tumor diagnosis. The field has grown rapidly over the past two decades, with China emerging as a major contributor. Research themes highlight both technological innovation and clinical application, particularly in hepatocellular carcinoma, while extending to other tumor types. Looking ahead, advances in artificial intelligence, novel contrast agents, and strengthened multicenter collaborations are expected to drive CEUS from a diagnostic modality toward a

comprehensive imaging tool, playing an increasingly important role in personalized cancer management.

Disclosure statement

The authors declare no conflict of interest.

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Synergistic Potential of Traditional Chinese Medicine and CART Cell Therapy: Immunoenhancement and Persistence Regulation Strategies

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Abstract: CAR-T cell therapy demonstrates tremendous potential for tumor treatment, yet faces challenges in solid tumor therapy due to immune suppression, T-cell exhaustion, and cytokine release syndrome (CRS) induced by the tumor microenvironment (TME). Traditional Chinese medicine (TCM) holds substantial potential to enhance CAR-T efficacy and mitigate adverse reactions due to its multi-targeted advantages. TCM active ingredients and formulations can synergistically amplify CAR-T anti-tumor effects while reducing adverse events through multiple mechanisms, including reversing T-cell exhaustion, prolonging CAR-T cell persistence, improving TME hypoxia and fibrosis, modulating gut microbiota, and suppressing CRS. This benefits patient treatment and recovery. Combining TCM with CAR-T therapy can increase objective response rates, prolong cell persistence, and reduce CRS incidence. Future efforts will focus on exploring the precise mechanisms and standardized protocols for TCM-enhanced CAR-T treatment through high-quality clinical trials and multi-omics technologies, driving its clinical translation and application.

Keywords: Traditional Chinese Medicine; CAR-T cell therapy; Tumor microenvironment; T cell exhaustion; Cytokine storm

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1. Introduction

CAR-T cell therapy is a cancer immunotherapy that genetically engineers T cells to express chimeric antigen receptors (CARs), enabling them to precisely recognize and eliminate tumor cells. This approach has significantly improved overall survival rates and quality of life for patients with malignant hematologic malignancies and is now widely adopted in clinical practice. However, its application in solid tumor treatment remains challenging, primarily due to the immunosuppressive properties of the tumor microenvironment (TME).

Within the TME, T cells highly express inhibitory receptors such as PD-1 and CTLA-4. These molecules suppress the PI3K/AKT signaling pathway, leading to T cell exhaustion and diminishing the therapeutic efficacy of CAR-T cells. Hypoxic conditions and tissue fibrosis within the TME further hinder effective CAR-T cell infiltration, limiting treatment outcomes. Conversely, CAR-T therapy may also trigger severe adverse reactions such as cytokine release syndrome (CRS), compromising patient safety and recovery outcomes.

Traditional Chinese medicine shows promising applications in synergizing CAR-T therapy, particularly in addressing some limitations of CAR-T in treating solid tumors. Leveraging their multi-component, multi-targeted properties, certain TCM formulas that fortify the body's foundation can enhance overall immune status through mechanisms like replenishing qi and nourishing blood, or tonifying yin and warming yang. These formulas suppress excessive inflammatory responses, regulate fibrosis and abnormal angiogenesis within the tumor microenvironment (TME), thereby promoting CAR-T cell infiltration and cytotoxicity. The World Health Organization (WHO) has incorporated traditional Chinese medicine into its cancer supportive care framework. Emphasizing holistic concepts and syndrome differentiation, TCM reduces healthcare costs while prioritizing mind-body integrated rehabilitation models, offering unique Eastern wisdom and solutions for global cancer prevention and treatment.

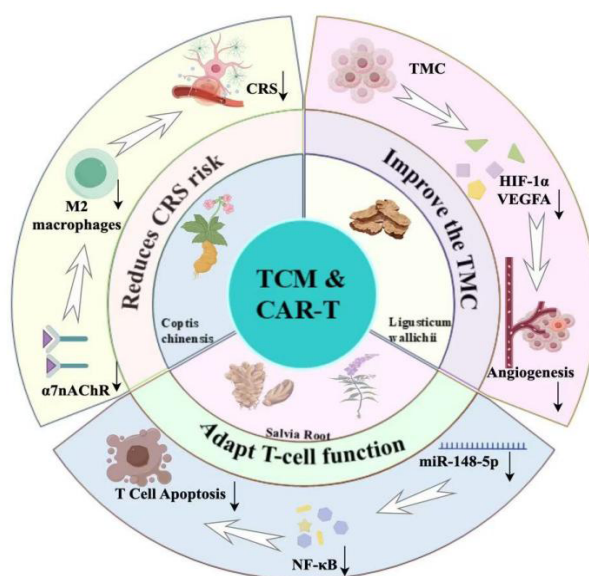


Figure 1. Traditional Chinese medicinal materials and CAR-T.

2. Traditional Chinese medicine modulates T cell function: Alleviating exhaustion and prolonging survival

2.1. Reversal of T-cell exhaustion by traditional Chinese medicine components

T cell exhaustion is one of the core mechanisms underlying immune dysfunction in chronic infections and the tumor microenvironment (TME). Its occurrence is closely associated with the presence of immunosuppressive cells in the TME, such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and M2 macrophages, as well as the cytokines they release, including transforming growth factor-beta (TGF- β) and interleukin-10 (IL-10). This state is primarily characterized by sustained high expression of inhibitory receptors

(such as PD-1 and CTLA-4), metabolic abnormalities including mitochondrial dysfunction, and epigenetic alterations. It represents an adaptive response of T cells to prolonged antigenic stimulation. While T cell exhaustion provides some protective effect in suppressing autoimmune reactions, it also significantly impairs T cell tumor clearance capacity, limiting the efficacy of immunotherapies such as CAR-T cell therapy.

Recent studies have demonstrated that multiple active components of traditional Chinese medicine exhibit potential to reverse T cell exhaustion in in vivo experiments and animal models. The underlying mechanisms involve multiple pathways, including regulating key inhibitory receptors, improving T cell metabolic dysfunction, modulating exhaustion-associated transcription factors, and reshaping the tumor microenvironment. Shi *et al.* ^[1] discovered in a systemic candidiasis infection model that paeoniflorin promotes memory T cell formation by upregulating SOCS1/SOCS3 expression, thereby inhibiting excessive activation of the cytokine/JAK/STAT pathway. Yu *et al.* ^[2] confirmed in a Hepa1-6 subcutaneous tumor-bearing mouse model that combined treatment with bufadienolide and either pyruvate (PA) or oxalic acid (OX) suppressed lactate dehydrogenase A (LDHA) expression, enhanced the activity and stability of NK1.1 and NKG2D receptors in NK cells, and elevated serum levels of perforin, TNF- α , and IFN- γ , ultimately significantly inhibiting tumor growth. Zhang *et al.* ^[3] discovered in a cardiomyocyte injury model that Tanshinone IIA (TIIA) inhibits NF- κ B pathway activation by regulating miR-148-5p, thereby reducing T cell apoptosis. These studies suggest that active components of traditional Chinese medicine can reverse T cell exhaustion through multiple pathways, providing theoretical support for combining traditional Chinese medicine with immune-targeted therapies to enhance tumor treatment efficacy.

2.2. Traditional Chinese Medicine Enhances CAR T-Cell Persistence

Insufficient CAR-T cell persistence is a key limiting factor affecting the long-term efficacy of this therapy, primarily associated with mechanisms such as T cell exhaustion, immune desertification, and metabolic dysregulation, which reduce tumor clearance capacity and limit treatment outcomes. Recent studies have revealed that multiple classical Chinese herbal formulas can optimize CAR-T cell function through multiple mechanisms, prolonging their survival in vivo and enhancing antitumor activity. Gao *et al.* ^[4] demonstrated in an IL-6-induced HCCLM3 hepatocellular carcinoma epithelial-mesenchymal transition (EMT) model and H22 tumor-bearing mouse model that Biejia Jianwan promotes CD8⁺ T cell infiltration into the tumor microenvironment by upregulating CCL5 chemokine expression ^[5], simultaneously modulating the CCL5–CCR5 axis to enhance T-cell chemotaxis, suppressing PD-L1 expression to alleviate immunosuppressive microenvironments, and downregulating the EMT-related transcription factor TWIST via the JAK/STAT3 signaling pathway to sustain T-cell activity. Zhang *et al.* ^[6,7] discovered in a total parenteral nutrition (TPN)-induced intestinal mucosal immune injury model that Yupingfeng Powder modulates gut microbiota in Peyer's patches (PPs), enhances antigen-presenting cell function, and subsequently activates naive T cells via MHC-antigen peptide complexes and co-stimulatory signals (CD28-B7). This promotes cytokine secretion (e.g., IL-2), induces T cell clonal expansion, and increases the proportion of central memory T cell precursors (CD62L⁺CD127⁺). This formula also significantly reduces apoptosis rates in activated T cells (from 13.1% in the model group to 6.2% in the Yupingfeng group), promoting cell survival by regulating Bcl-2/Bax expression, enhancing CCR9/ α 4 β 7-mediated migration and retention of memory T cells in the small intestinal lamina propria, and maintaining levels of key survival factors like IL-7 and IL-15. This multifaceted approach supports the long-term persistence of Tcm cells. The synergistic multi-component, multi-target regulatory mechanism of

traditional Chinese medicine offers a unique approach to enhancing CAR-T therapy persistence. Its integration with modern immunotherapy holds promise as a novel strategy to overcome tumor resistance and recurrence.

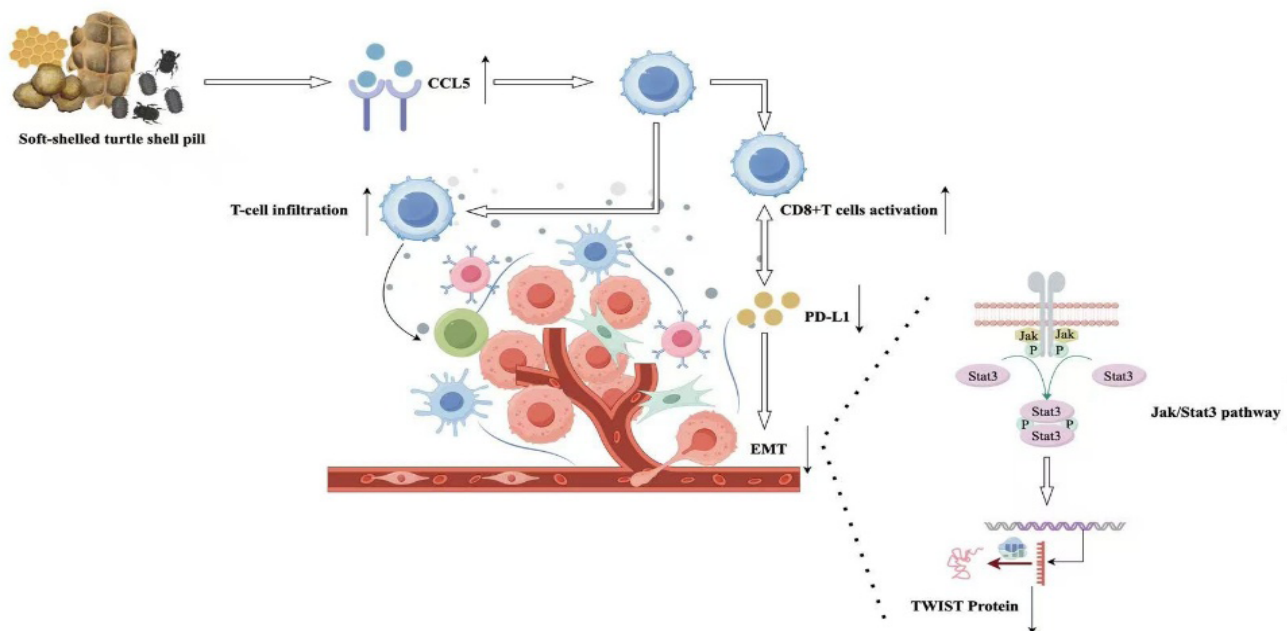


Figure 2. Biejia Jianwan suppresses TME to maintain T cell activity.

3. Traditional Chinese medicine improves tumor microenvironment: Targeted intervention in hypoxia and fibrosis

3.1. Blood-activating and stasis-resolving herbs alleviate hypoxia

Hypoxia within the tumor microenvironment (TME) is a key factor contributing to radiation resistance, chemotherapy resistance, and immunosuppression. Its mechanism is primarily associated with the sustained activation of hypoxia-inducible factor-1 α (HIF-1 α), which not only promotes tumor cell invasion and metastasis but also creates an immunosuppressive microenvironment by recruiting myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs), thereby diminishing the efficacy of immunotherapies such as CAR-T cells and NK cells. Traditional Chinese medicines that promote blood circulation and resolve stasis offer a potential strategy to alleviate tumor hypoxia through their multi-targeted mechanism of action,” improving microcirculation–modulating immunity–reprogramming metabolism” [8]. Among these, chuanxiongzone and tanshinone IIA provide novel insights for reversing TME immunosuppression by enhancing tumor blood flow perfusion and inhibiting hypoxia-related signaling pathways through distinct mechanisms.

Liu *et al.* [9] discovered in an in vitro model of A549 lung cancer stem-like cells (CSLCs) that chuanxiongzone significantly suppressed the expression of the vascular mimicry (VM)-associated protein EphA2 ($P < 0.05$) and downregulated the protein levels of hypoxia-inducible factor HIF-1 α and vascular endothelial growth factor VEGFA ($P < 0.01$). suggesting that chuanxiongzone may exert anti-angiogenic effects by modulating the hypoxia-angiogenesis signaling axis in the tumor microenvironment (TME). Ren *et al.* [10] similarly demonstrated in the A549 CSLCs model that Tanshinone IIA significantly suppressed VEGF expression under both normoxic and hypoxic conditions (48.6% reduction in normoxia, 62.3% reduction in hypoxia) by

modulating the VE-cadherin/MMP-9/Integrin β 1 signaling cascade to remodel the tumor microenvironment. The combination of blood-activating and stasis-resolving Chinese herbal medicines with anti-angiogenic drugs (e.g., bevacizumab) holds potential for synergistic inhibition of tumor neovascularization. However, caution is warranted to avoid the risk of “vascular pruning” caused by excessive vascular normalization. Currently, Salvia and Ligusticum Injection is widely used as an adjunctive therapy for cardiovascular and cerebrovascular diseases. Future research may explore its potential value as an adjunct to tumor chemotherapy and immunotherapy, a translational direction warranting further investigation.

3.2. Anti-fibrotic effects promote CAR T-cell infiltration

In solid tumor therapy, the physical and biochemical barriers formed by tumor tissue fibrosis severely impede CAR-T cell infiltration and function. Overactivated cancer-associated fibroblasts (CAFs) form physical barriers by secreting abundant extracellular matrix (ECM) proteins such as collagen I/III and fibronectin^[11], impeding CAR-T cell infiltration while inducing immunosuppression through factors like TGF- β and IL-6. Research indicates that targeting the fibrotic tumor microenvironment significantly enhances CAR-T cell penetration and activity. Traditional Chinese medicine components like crocin and notoginsenosides offer novel strategies for improving CAR-T cell infiltration through their anti-inflammatory, anti-fibrotic, and ECM remodeling effects. Crocin, the core active component of safflower, serves as the primary active ingredient in this blood-activating and stasis-resolving agent. Zheng *et al.*^[12] investigated the safflower pigment through a PI3K/AKT/mTOR signaling pathway model. They discovered that safflower pigment exerts anti-inflammatory effects by inhibiting the PI3K/AKT signaling pathway (downregulating p-PI3K, PI3K, AKT, and p-AKT protein expression), reducing the secretion of pro-inflammatory factors such as VEGF, and modulating the ratio of apoptosis-related proteins Bcl-2/Bax. Concurrently, Cheng *et al.*^[13] observed in tumor microenvironment studies that Notoginsenoside R1 reduces ECM stiffness by inhibiting the release of matrix metalloproteinases (MMPs) associated with NETs formation, thereby promoting CD8⁺ T cell infiltration. Furthermore, notoginsenosides may also mitigate ECM degradation via the TNF- α /MMP-2 axis with a certain probability^[14].

Traditional Chinese medicine exhibits a certain degree of alleviating effect on fibrosis within the TME. Future approaches could combine traditional Chinese medicine with CAR-T targeting the TME to dissolve barriers formed by tumor fibrosis, thereby promoting CAR-T infiltration.

4. Immunomodulatory strategies of traditional Chinese medicine to reduce CRS risk

4.1. Intervention in cytokine storms

Cytokine release syndrome (CRS) is the most common and potentially life-threatening complication in CAR-T cell therapy, severely limiting its clinical application and adoption. Its pathogenesis involves an overactivated immune system triggering explosive release of inflammatory factors such as IL-6, IFN- γ , and TNF- α , leading to systemic inflammatory responses and multi-organ dysfunction. Current clinical interventions primarily rely on monoclonal antibodies, which face limitations such as single-target specificity and difficulty in blocking upstream signaling pathways. Consequently, traditional Chinese medicine (TCM) formulas demonstrate unique advantages in CRS prevention and treatment through multi-targeted interventions. Their strategy primarily focuses on inhibiting key inflammatory cytokines, modulating core inflammatory signaling pathways,

stabilizing immune cell function to suppress excessive activation, protecting endothelial cells, and reducing vascular leakage, critical steps in CRS development, thereby inhibiting CRS progression at multiple levels.

Xin *et al.* ^[15] found that berberine inhibits the anti-inflammatory function of M2 macrophages and reduces IL-10 secretion by activating $\alpha 7$ nicotinic acetylcholine receptors ($\alpha 7$ nAChR). This mechanism may involve $\alpha 7$ nAChR-mediated inhibition of the JAK2-STAT3 signaling pathway or downregulation of NF- κ B activity through cholinergic anti-inflammatory pathways, thereby suppressing IL-10 transcription and release. This action helps reverse immunosuppressive microenvironments and restore pro-inflammatory/anti-inflammatory balance, demonstrating potential value in infection- or tumor-associated immune regulation. Hao *et al.* ^[16] demonstrated that Gansui Banxia Tang inhibits excessive neutrophil recruitment by targeting the CXCL2/CXCR2 pathway. During CRS, activated macrophages massively secrete CXCL2, which binds to CXCR2 on neutrophil surfaces to promote their infiltration into inflammatory sites. This activates NF- κ B and MAPK pathways, further amplifying the release of inflammatory mediators like IL-1 β and IL-8. The terpenoid esters in Gan Sui directly inhibit CXCL2 transcription, while Pinellia alkaloids downregulate CXCR2 expression, thereby blocking this positive feedback loop through dual-targeted inhibition.

Traditional Chinese medicine (TCM) offers a holistic regulatory strategy for CRS prevention and treatment distinct from monoclonal antibody drugs, operating through a multidimensional synergistic mechanism: “antagonizing cytokines, inhibiting signaling pathways, stabilizing immune cells, protecting target organs.” Current evidence primarily stems from basic research and small-scale clinical observations. The precise targets, pharmacokinetic properties, and synergistic effects with conventional anti-CRS drugs require validation through high-quality randomized controlled trials (RCTs) and in-depth mechanistic studies. Integrating TCM into CRS prevention and control systems holds promise for effectively managing toxicity while maximizing the antitumor efficacy of CAR-T cells, thereby broadening the therapeutic window.

4.2. Gut microbiota modulation of CRS association

In recent years, the role of gut microbiota in immune regulation has garnered increasing attention, with its association with cytokine release syndrome (CRS) emerging as a research hotspot. Studies indicate that dysbiosis of the gut microbiota compromises intestinal barrier function, allowing harmful bacteria and their metabolites to enter the systemic circulation. This activates inflammatory signaling pathways, promotes the release of pro-inflammatory cytokines (such as IL-6 and TNF- α), and ultimately triggers a cytokine storm. During CAR-T therapy, pre-treatment chemotherapy-induced gut microbiota disruption exacerbates CRS severity, providing theoretical support for traditional Chinese medicine (TCM)-based microbiota modulation as a CRS intervention ^[17]. Intervention strategies, exemplified by TCM formulas improving microbiota composition and rhubarb restructuring the intestinal microenvironment, demonstrate significant CRS mitigation effects by multi-target regulation of the microbiota-immune interaction network.

TCM formulas regulating microbiota structure offer unique advantages in CRS prevention and treatment. Liu *et al.* ^[18] found that spleen-tonifying formulas like Sijunzi Tang increase the abundance of probiotics (e.g., bifidobacteria, lactobacilli), promote short-chain fatty acid (SCFA) production, thereby enhancing regulatory T cell (Treg) function and suppressing excessive inflammatory responses. Zhang *et al.* ^[19] discovered that rhubarb and its active components exert CRS intervention by restructuring the intestinal microenvironment. Anthraquinone components of rhubarb (e.g., emodin, aloe-emodin) downregulate the TLR4/NF- κ B pathway to reduce intestinal macrophage activation while promoting tight junction protein expression (occludin, ZO-1) to

repair chemotherapy-damaged intestinal barriers.

Current individual variations hinder the establishment of standardized treatment protocols, and the interaction mechanisms among herbal components, microbiota, and hosts require further elucidation. Future research integrating metagenomic sequencing and metabolomics could establish predictive models for “Chinese herbal medicine-microbiota-CRS” interactions. Exploring combined regimens of Chinese herbal medicine and probiotics may achieve synergistic effects in CRS prevention and treatment.

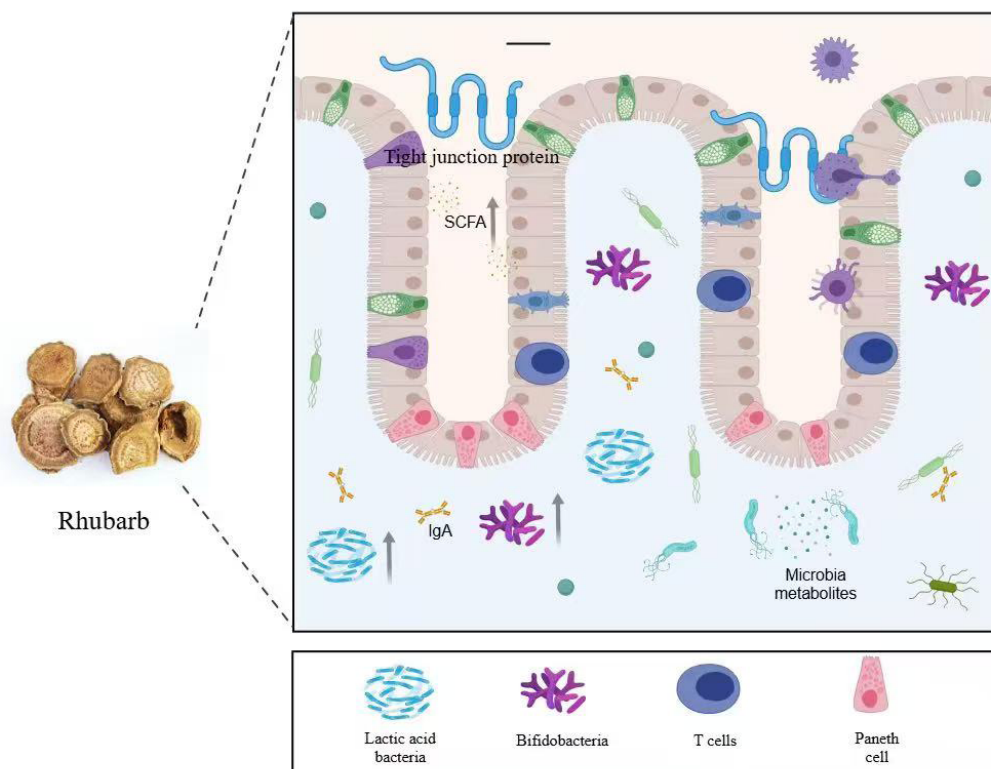


Figure 3. Rhubarb acts on the gut microenvironment to inhibit CRS occurrence.

5. Clinical Translation Pathways for Combination Therapy

5.1. Current Clinical Evidence

As applied research continues to advance, multiple clinical observations and prospective studies have preliminarily demonstrated the feasibility and clinical value of combining traditional Chinese medicine with CAR-T therapy in treating malignant tumors. Notably, studies on *Salvia miltiorrhiza* combined with CAR-T therapy for B-cell lymphoma and Yu Pingfeng San pre-treatment to reduce cytokine release syndrome (CRS) incidence provide crucial practical evidence for optimizing cell immunotherapy through integrated Chinese and Western medicine. These clinical data not only validate the potential of TCM in enhancing therapeutic efficacy and mitigating adverse effects but also guide the design of subsequent large-scale clinical trials.

A clinical study by Xiao *et al.* ^[20] demonstrated that co-administering Danshen significantly optimized CAR-T therapy outcomes in B-cell lymphoma patients. The objective response rate in the Danshen group increased to 85% (vs. 67% in the control group), with CAR-T cell expansion peaks averaging 1.5 times higher

and persistence extending beyond 28 days. Regarding safety, the incidence of grade 3 or higher CRS in the Danshen group decreased to 14%, significantly lower than the 31% in the control group. This effect may be related to Danshen's inhibition of the IL-6/JAK/STAT3 signaling pathway. The results indicate that Danshen can optimize CAR-T therapy through a dual mechanism of “enhancing efficacy and reducing toxicity.” Shuai *et al.* ^[21] found that pre-treatment with Yupingfeng Powder significantly reduced the CRS incidence to 25.6% compared to 46.5% in the control group ($p = 0.032$). Mechanistic studies revealed that serum IL-6 and IFN- γ peak levels decreased by 30%–40% in the pretreated group, while the proportion of CD4+CD25+Foxp3+ Treg cells increased twofold. This aligns with the role of astragaloside IV in Yupingfeng San, which modulates Th17/Treg balance. Long-term follow-up data revealed no significant difference in progression-free survival (PFS) between groups, indicating that Yupingfeng San reduced toxicity without compromising antitumor efficacy.

In summary, the combination of TCM and CAR-T therapy shows great promise in improving treatment outcomes and quality of life for patients with malignant tumors. However, current studies remain limited by small sample sizes and insufficient standardization. Future efforts should focus on conducting larger-scale phase III randomized controlled trials (RCTs) and deepening the exploration of its mechanisms of action. This will facilitate the transition of TCM from empirical use to evidence-based medical practice in the field of cellular immunotherapy, ultimately achieving precise synergy between TCM and CAR-T therapy.

5.2. Future research directions

Current preclinical and preliminary clinical studies combining traditional Chinese medicine with CAR-T cell therapy have demonstrated promising results, though their mechanisms of action and efficacy remain unclear, posing challenges for widespread adoption. Moving forward, traditional Chinese medicine will integrate more closely with other fields. For instance, a dynamic monitoring system based on immune cell subset analysis could establish a ternary model linking “TCM components-immune subsets-therapeutic efficacy prediction.” Single-cell sequencing technology could be employed to dynamically track changes in the immune microenvironment during treatment, enabling precise evaluation and personalized dosing for combined TCM-CAR-T therapy. For instance, astragaloside IV reduces the proportion of PD-1+ exhausted T cells, enhancing CAR-T persistence ^[22]. Concurrently, nanogel technology offers novel approaches for the targeted delivery of TCM bioactive components. This system protects small-molecule TCM components from rapid clearance and enables their enrichment at tumor sites via the EPR effect or active targeting. It may even enable controlled release of TCM immunomodulators for synergistic action with CAR-T cells. For instance, pH-responsive nanogels loaded with *Tripterygium wilfordii* homoside selectively suppress tumor-associated macrophages (TAMs) in the tumor microenvironment, enhancing CAR-T infiltration ^[23]. Future developments may include dual-drug delivery systems simultaneously transporting CAR-T cells and TCM immunomodulators.

This technological breakthrough transforms traditional empirical knowledge into digital standards, propelling TCM from “vague experience” toward “precision medicine.” Ultimately, it enables personalized prevention and treatment of chronic and complex diseases (such as fibrosis and tumors), positioning TCM as a vital complement to global healthcare systems.

6. Conclusion

Theoretically, the synergistic therapy combining traditional Chinese medicine (TCM) with CAR-T cells

demonstrates unique comprehensive advantages. Through its multi-component, multi-target holistic regulatory mechanism, it overcomes the limitations of existing monotherapy across multiple dimensions. On one hand, TCM effectively modulates the tumor immune microenvironment by suppressing cancer-associated fibroblast activation and extracellular matrix deposition, thereby enhancing CAR-T cell infiltration into tumor sites. On the other hand, TCM components regulate T cell differentiation states, inhibit the expression of T cell exhaustion-related molecules, and promote the formation of central memory T cell subsets, ultimately prolonging CAR-T cell persistence in vivo. Regarding toxicity management, TCM formulas with heat-clearing, blood-cooling, detoxifying, and stasis-resolving effects significantly inhibit the activation of inflammatory signaling pathways like NF- κ B and reduce levels of key inflammatory cytokines such as IL-6 and TNF- α . This mitigates the severity and duration of cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). This dual-regulatory effect of “reducing toxicity while enhancing efficacy” embodies a therapeutic philosophy integrating holistic perspectives with precision medicine, aligning with contemporary trends in personalized healthcare.

Practical application in this field still faces several challenges. The complexity and standardization of TCM components pose difficulties, as existing formulations lack unified quality control standards. Significant variations in active ingredient content make it challenging to ensure consistent and reproducible therapeutic outcomes. Second, although basic research suggests multiple potential mechanisms of action, a translation gap persists between laboratory evidence and clinical validation. Rigorous large-scale, multicenter randomized controlled trials (RCTs) are urgently needed to clarify the actual clinical benefits and safety of TCM synergistic therapy. Furthermore, the specific molecular targets of TCM-CAR-T cell interactions, pharmacokinetic characteristics, and optimal timing and protocols for combination therapy lack an established evidence-based medical framework. High-quality research is required to advance these areas, thereby promoting the standardization and internationalization of integrated Chinese-Western medicine approaches in tumor immunotherapy.

Disclosure statement

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Current Status and Prospects of Diagnosis and Intervention for HR-HPV Persistent Infection

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Abstract: Persistent infection with high-risk human papillomavirus (HR-HPV) is the core pathogenic factor of cervical cancer (CC). Although HPV vaccination is an effective primary prevention method for CC, the global vaccination rate is generally insufficient (target population vaccination rate in China < 5%), far from meeting the requirements for herd immunity (80%) and the WHO target (90%). However, only about 10% of HR-HPV infections progress to persistent infections. Therefore, identifying and intervening in the “HR-HPV persistent infection” population can systematically narrow the scope of prevention and control, reduce prevention and control costs, and provide a new path for low-income countries to explore suitable prevention and control models for CC. Based on this understanding, the team has pioneered a systematic method for identifying HR-HPV persistent infections and a tiered intervention system based on drug classification, which has achieved good results in both basic research and clinical observations. This article will summarize the current research status of “HR-HPV persistent infection” in relation to CIN and CC, as well as the team’s relevant concepts and research results, to provide a reference for the identification and intervention of “HR-HPV persistent infection.”

Keywords: HR-HPV; Persistent infection; Diagnosis and intervention; Cervical cancer prevention

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1. Introduction

Human papillomavirus (HPV) infection has become a consensus in the academic community that, especially persistent infection with high-risk HPV (HR-HPV), leads to cervical cancer (CC). CC severely affects patients’ quality of life and health, and is one of the major public health issues globally, as well as the primary burden of HPV-related diseases worldwide. The prevention and control model for CC is divided into a three-tier prevention system. Among them, the HPV vaccine, which has been available for many years, has not achieved an optimistic vaccination status. Taking China as an example, the vaccination rate among target populations is still less than 5%, far from meeting the requirements for achieving herd immunity (80%) and the WHO vaccination target (90%). This leaves the vast majority of unvaccinated individuals and some who have failed

to be protected at risk of HPV infection and developing CC, seriously affecting the actual effectiveness of the HPV vaccine in preventing CC.

The HPV infection rate among the general female population in China is approximately 17.70%, with an HR-HPV infection rate of about 13.12%^[1]. Only about 10% of HR-HPV infections will progress to persistent infections, and “persistent HR-HPV infection” is the primary factor leading to cervical cancer (CC). Therefore, by focusing solely on the diagnosis and intervention of “persistent HR-HPV infection,” it is possible to systematically narrow the intervention population, reduce prevention and control costs, and explore a prevention and control model for CC that is suitable for low-income countries.

In recent years, “persistent HR-HPV infection” has received increasing attention from the academic community. Relevant literature and academic conferences have demonstrated the correlation and research progress of persistent HR-HPV infection with cervical intraepithelial neoplasia (CIN) and cervical cancer (CC) at different levels and in different fields^[2]. Our team has also been deeply involved in this field for many years, conducting extensive research on the diagnosis and intervention of persistent HR-HPV infection, ranging from basic to clinical levels. We have proposed a systematic diagnostic method, a drug classification system, and a hierarchical intervention concept based on lactic acid bacteria. This article provides a review of the current research status of “persistent HR-HPV infection” in relation to CIN and CC, as well as our team’s relevant concepts and research results. The aim is to provide a reference for the diagnosis and intervention of “persistent HR-HPV infection,” reduce or avoid the widespread over-intervention of HPV in clinical practice, alleviate the economic burden and psychological anxiety of infected individuals, and offer insights for low-income countries in exploring appropriate prevention and control models for CC.

2. HPV infection and cervical cancer

HPV is a small, circular, double-stranded DNA virus that prefers complex squamous epithelium, with a size of approximately 8kb and no envelope. It is widely present in nature. Currently, over 200 different subtypes have been identified, of which more than 60 subtypes are associated with infections of the reproductive tract system. The International Agency for Research on Cancer (IARC) classifies HPV subtypes and the risk of inducing cervical cancer (CC) into low-risk HPV (LR-HPV) subtypes and high-risk HPV (HR-HPV) subtypes. Low-risk subtypes mainly cause various warts, while high-risk subtypes can lead to various cancers, such as CC, vulvar cancer, vaginal cancer, oropharyngeal cancer, etc. Among them, CC is directly related to persistent HR-HPV infection and is the primary burden of HPV-related tumors globally.

The infection rate of HR-HPV among women in China is 13.12%^[1]. Studies have shown that approximately 80% of women will be infected with at least one HPV subtype during their lifetime^[3], which is particularly prevalent among sexually active individuals. Most HPV infections are transient, with about 90% of infections being cleared by the body’s immune system within 1-2 years without any intervention^[4]. However, a portion (about 10%) of infections, especially HR-HPV infections such as HPV16 and HPV18, persist, and persistent infection is closely associated with the development of various malignancies^[5]. Persistent HR-HPV infection can lead to the development of normal cervical tissue into CIN and eventually progress to invasive cancer^[6]. Although persistent HR-HPV infection is a necessary condition for the development of CC, it is not the only condition, suggesting that other factors, such as vaginal microbiota and immune status may also play a role in cancer susceptibility and progression^[7].

3. Persistent HPV infection and cervical cancer

Persistent infection with HR-HPV is the primary pathogenic and initiating factor in the development of cervical cancer (CC). The main mechanism involves HPV interfering with host cell cycle regulation through oncogenic proteins E6 and E7. E6 protein induces the degradation of p53 tumor suppressor protein, while E7 protein leads to the inactivation of retinoblastoma (Rb) protein, collectively promoting cell immortalization^[8]. During persistent infection, HPV also evolves sophisticated mechanisms to evade immune surveillance and evoke host immunity, thereby maintaining the stability of the viral genome. Furthermore, HPV infection often occurs in the transformation zone of cervical squamous epithelium and columnar epithelium, where the specific microenvironment provides favorable conditions for persistent infection. The complex interplay between HPV and the host constitutes the molecular basis for its persistent infection and further progression to malignant tumors.

It has become a consensus in academia that persistent HR-HPV infection leads to cervical cancer (CC). Accurate diagnosis is the prerequisite for intervention and treatment. Therefore, accurately determining the status of persistent HPV infection is crucial for risk stratification and clinical management of CC. However, to date, there is no unified standard for defining persistent HPV infection. Currently, commonly used time thresholds in clinical studies include 6 months, 12 months, and 24 months^[9]. Studies have shown that the duration of HPV infection is positively correlated with the risk of CC transformation. When the infection persists for more than 12-24 months, the risk of carcinogenesis increases significantly. Additionally, HPV viral load is also considered an important indicator for assessing disease severity and clinical prognosis^[9]. However, current guidelines do not require routine HPV testing for newly diagnosed CC cases, which may lead to insufficiently precise risk stratification and management for some cases.

Due to the lack of concepts and methods for judging “persistent infection” of HPV, there is a phenomenon of including “transient infection” in both clinical research and clinical treatment, which inevitably leads to widespread deviations in research results and excessive clinical intervention, affecting the rigor of research and increasing the economic burden and anxiety of patients^[7]. Although current guidelines do not mandate HPV testing for newly diagnosed CC, a large body of evidence indicates that only a minority of HR-HPV infections (about 10%) will develop into persistent infections, which is the main cause of CC. Therefore, distinguishing between transient and persistent infections can significantly enhance the predictive value of cervical lesion progression. Especially in regions with limited medical resources, establishing a reliable basis for judging persistent infections is particularly important, as these areas often struggle to implement standardized CC screening. Secondly, from a public health perspective, intervening only on individuals identified as having persistent infections can avoid overtreatment of those with transient infections. Furthermore, new strategies for developing specific treatments targeting persistent infections, such as targeted therapies targeting E6/E7 oncoproteins, also require accurate judgments of persistent infections as a prerequisite. Therefore, establishing a unified and standardized diagnostic criterion for persistent infections is of great significance for optimizing CC prevention strategies and should become an important part of the CC prevention and control system.

4. Detection techniques and judgment methods for persistent HPV infection

Persistent HPV infection typically refers to the state where HR-HPV persists in the cervix (or other infected sites) for a duration exceeding a certain time threshold and is not naturally cleared by the human immune

system. Currently, it is mostly defined as the detection of the same type of HPV in cervical exfoliated cells from two consecutive tests, separated by 6-12 months, in the same individual^[10]. Currently, commonly used HR-HPV detection techniques in clinical practice mainly include DNA-level PCR typing detection, RNA-level mRNA detection, and epigenetic marker methylation detection. PCR typing technology can accurately identify 14 high-risk HPV types through real-time fluorescent quantitative PCR. However, DNA detection cannot distinguish between active and latent infections, while HR-HPV E6/E7 mRNA detection can better differentiate between persistent and transient infections. Methylation detection shows good specificity (79.77%) in non-type 16/18 HR-HPV infections and is of great value in guiding clinical intervention. In addition, novel detection technologies such as the Xpert® HPV system achieve a sensitivity of 86.4% in detecting HR-HPV in urine samples, providing possibilities for non-invasive detection.

There is still significant controversy regarding the criteria for determining the duration of HR-HPV infection. Existing studies have adopted various standards such as 6 months, 12 months, and 24 months. Among women aged 18-26, the incidence of persistent infection lasting for 6 months is 35.5 per 1000 person-years, which is significantly higher than that of the 27-45 age group (29.0 per 1000 person-years). Longitudinal studies have shown that 37 out of 72 individuals infected with HR-HPV developed into a persistent infection state. It is worth noting that persistent infection lasting for 6 months is significantly associated with the risk of cervical intraepithelial neoplasia grade 2 or higher (CIN2+) (HR=2.31), while follow-up data for 24 months indicates that bacterial vaginosis (BV) may affect the persistent infection state of HR-HPV. These differences in time standards reflect varying understandings among different studies regarding the threshold for cancer risk associated with persistent infection.

Currently, there is no unified standard for determining persistent HPV infection both domestically and internationally. In light of this, our team, combining relevant research findings and new technological advancements, has taken the lead in proposing seven methods for determining persistent HR-HPV infection, namely^[11]: 1. Patients with clear records of HR-HPV infection who remain non-negative for the same HPV subtype after a one-year follow-up; 2. Patients who were initially screened positive for HR-HPV and remain positive for the same HPV subtype after a one-year follow-up; 3. HPV typing and integration detection technology, with positive HPV integration; 4. Cervical tissue P16/Ki67 detection technology, with single or dual positive results; 5. Cell/tissue immunohistochemical broad-spectrum HPV-L1 staining detection technology, with negative detection results; 6. Gene methylation detection technology, with positive detection results; 7. HPV E6/E7 gene/protein detection technology, with positive detection results. These methods provide reference and guidance for the current determination of persistent HR-HPV infection.

5. Current status of intervention for HPV infection

Currently, the prevention of HPV infection is primarily achieved through vaccination, which is also one of the most powerful weapons in the WHO's strategy to eliminate CC. However, to date, the global coverage rate of HPV vaccines remains unsatisfactory, with significant vaccination gaps particularly in low- and middle-income countries. Furthermore, existing vaccines do not cover all high-risk types, and preventive vaccines are ineffective for those who are already infected.

For individuals with persistent HPV infection, active intervention is an important approach to prevent the infection from leading to CIN/CC. Currently, there are many methods used clinically to treat HPV infection, such

as pharmacotherapy, surgery, physical therapy, immunotherapy, etc. For those without concurrent cervical lesions (CIN, cervicitis, etc.), pharmacotherapy remains the primary clinical treatment method. Based on the mechanism of action of commonly used anti-HPV drugs in clinical practice, the team categorizes them into three types ^[12]:

- (1) The first type includes immunomodulators or enhancers, such as interferon, Paiteling, Baofukang Suppository, etc., which can enhance the anti-HPV ability of local tissues and cells through local immune regulation.
- (2) The second type consists of ligand-receptor drugs, such as Ruilintang, which achieve the goal of completely eliminating the HPV virus through specific antigen-antibody binding, forming a “cocktail therapy” of humoral immunity + cellular immunity + phagocytic immunity. Additionally, the positive-negative charge adsorption type belongs to another category of ligand-receptor drugs, including almost all anti-HPV biological protein gels, which are dressings containing β -lactalbumin and carbomer components. Their main mechanism of action is to form a protective gel film on the vaginal wall, isolating bacteria and viruses. The β -lactalbumin in the gel utilizes the effect of positive and negative charges to destroy the protein structure of HPV, promoting the inactivation of HPV. Carbomer encapsulates the inactivated HPV and facilitates its smooth excretion from the body, thereby eliminating the HPV infection in the body. Studies have shown that the long-term negative conversion rate of anti-HPV biological protein dressings in the treatment of HPV infection is superior to that of recombinant human interferon alpha-2b gel.
- (3) The third type includes gene therapy drugs, such as Ruibeisheng, which eliminate the invading HPV virus by removing adenine from the HPV virus DNA structure, hindering the binding of ribosomes and protein synthesis elongation factor 2 (EF-2). Clinical studies have reported that all types of drugs have certain anti-HPV effects, and some drugs also have the effect of treating CIN.

Clinically, almost all drugs for treating HPV infection are administered vaginally. Prolonged vaginal administration may alter the vaginal microenvironment, leading to “vaginal microecological imbalance” and resulting in various manifestations of vaginitis, thereby reducing patients’ treatment experience, even disrupting treatment and affecting the continuity and ultimate effectiveness of therapy. Therefore, in prolonged vaginal administration therapy, maintaining or correcting vaginal microecological imbalance is a crucial clinical consideration, which requires the intervention of exogenous lactic acid bacteria (bacilli/cocci). On the other hand, numerous studies have shown that a high proportion of women with HPV infection, especially those with persistent HR-HPV infection, exhibit vaginal microecological imbalance. This condition is observed in almost all types of vaginal microecological imbalance, except for vaginal candidiasis, indicating a clear correlation between vaginal microecological imbalance characterized by reduced lactic acid bacteria and persistent HPV infection. This suggests that the combined use of lactic acid bacteria preparations in the treatment of HPV infection through vaginal administration can not only exert its anti-HPV effect but also correct or maintain vaginal microecological imbalance, achieving a dual purpose. Based on this understanding, our team proposed the “Lactic Acid Bacteria Three-Pronged Approach” several years ago as a methodological system for graded intervention in persistent HPV infection.

6. Lactic acid bacteria and their functions

Lactic acid bacteria (LAB) are a general term for a group of bacteria capable of utilizing fermentable carbohydrates

to produce large amounts of lactic acid. They are widely distributed in nature and exhibit rich species diversity. LAB belongs to the Gram-positive bacteria category, typically does not form spores, and reproduces through binary fission. They are primarily divided into the genera *Lactobacillus* and *Coccus* based on morphology.

Except for a very small minority, the vast majority of LAB are essential and have important physiological functions in the human body. LAB are widely present in the human oral cavity, intestines, and vagina. Lactic acid bacteria in the vagina play a role in maintaining and repairing the vaginal microecology. *Lactobacillus* capsules for vaginal use made from live lactobacilli (brand name: Dingjunsheng) and *Lactobacillus* vaginal capsules made from live enterococcus (brand name: Yanhua) have been widely used clinically for the treatment and repair of vaginal microecological disorders.

In addition to its role in maintaining and improving vaginal microecology, LAB also exhibits a certain anti-HPV effect. In recent years, the role of LAB in anti-HPV infection has received increasing attention. Relevant studies have shown that LAB forms a multi-level anti-HPV defense network through mechanisms such as microecological remodeling, immune activation, and epigenetic regulation, playing a preventive and adjuvant therapeutic role in HPV infection. The main mechanisms are as follows ^[13,14]:

- (1) Acidic and antibacterial substances synergistic defense: LAB-metabolized lactic acid and acetic acid can stabilize the vaginal pH at 3.8-4.5, creating an acidic environment that is unfavorable for HPV survival. At the same time, the secreted hydrogen peroxide (H₂O₂) and bacteriocins (such as Lactocin 160, Reuterin) can directly destroy the HPV capsid protein structure and inhibit the fusion of the virus with host cells. Studies have shown that the bacteriocin produced by *Lactobacillus crispatus* can reduce the viral load of HPV16 by up to 67%.
- (2) Immune regulation: LAB stimulates vaginal epithelial cells to secrete β -defensins, LL-37, and other antimicrobial peptides through the TLR2/4 signaling pathway, directly neutralizing HPV particles.
- (3) Adaptive immune enhancement: LAB metabolites (such as lipoteichoic acid) can activate dendritic cells (DCs), promote the secretion of IL-12 and IFN- γ , and drive the differentiation of HPV-specific CD8⁺ T cells. LAB can enhance systemic mucosal immune responses by regulating the gut-vaginal axis.
- (4) Competitive occupancy: LAB preferentially binds to the heparan sulfate proteoglycan (HSPG) receptors on vaginal epithelial cells through surface adhesins (such as Mub protein, S-layer protein), occupying and blocking the interaction between HPV L1 protein and host cells. Animal experiments have shown that colonization with *L. crispatus* can reduce the HPV infection rate in mice by 52%.
- (5) Epigenetic regulation: LAB metabolites butyrate and propionate can upregulate the expression of host cell tumor suppressor genes (such as p53, p21) by inhibiting histone deacetylase (HDAC), while silencing HPV E6/E7 oncogenes, blocking the progression of cervical intraepithelial neoplasia.
- (6) Biofilm protection: LAB forms a biofilm on the vaginal wall to resist the invasion of external pathogens. Nanoscale imaging technology research has confirmed that LAB biofilms can physically block HPV viruses from contacting epithelial cells and continuously release antimicrobial substances.

The aforementioned characteristics of LAB provide an evidence-based basis for the treatment of HPV infection by combining LAB with other anti-HPV drugs.

7. Lactic acid bacteria three-pronged approach

Based on the fact that LAB has dual effects of maintaining/repairing vaginal microecology and anti-HPV, the

team, after long-term non-systematic clinical observation and exploration, has proposed the concept and method system of “Lactobacillus Three-Pronged Approach” for the graded treatment of HR-HPV persistent infection, which is based on lactic acid bacteria and combined with other anti-HPV drugs with different mechanisms of action. Using Hela cells as a carrier, experimental studies were conducted on HPV18 E6/E7 gene expression and cell viability, and the following main research results were obtained ^[15,16,17]:

- (1) LAB fermentation broth has a significant effect on down-regulating HPV18 E6/E7 gene expression in HeLa cells and inhibiting cell proliferation activity.
- (2) Meifukang, Ruilintaka, and Ruibeisheng all have effects on down-regulating HPV18 E6/E7 gene expression in HeLa cells and inhibiting cell proliferation activity.
- (3) The effect of LAB fermentation broth combined with three anti-HPV drugs is superior to that of single-drug treatment. This indicates that the idea of using LAB as a basis, combined with other anti-HPV drugs to treat persistent HPV infection, is feasible.

The “Lactobacillus Three-Pronged Approach” consists of three schemes:

- (1) First-line scheme: LAB combined with immunomodulatory drugs;
- (2) Second-line scheme: LAB combined with ligand-receptor drugs;
- (3) Third-line scheme: LAB combined with gene therapy drugs. Currently, the team is conducting a regional multicenter study, hoping to objectively evaluate the effect of the Lactobacillus Three-Pronged Approach in treating HR-HPV persistent infection in the real world through multicenter and large sample data.

8. Conclusion and outlook

Cervical cancer (CC) prevention and control is a major public health issue of global concern. Persistent human papillomavirus (HPV) infection is the primary factor leading to CC. Therefore, blocking HPV infection is the first step in preventing CC. According to the early prevention strategy of the World Health Organization (WHO), HPV vaccination is one of the simplest and most effective means to prevent HPV infection, and it should be vigorously promoted, advocated, and implemented. However, to date, no vaccine has achieved 100% protection efficacy, and existing HPV vaccines cannot prevent all HPV infections. Even with vaccination, regular cervical screening is still necessary. In addition, according to data from the National Immunization Program Information Management System, from 2018 to 2020, the cumulative full-course vaccination rate of HPV vaccine among women aged 9 to 45 in China was only 2.24% ^[18]. The coverage rate in rural areas may be even lower due to economic and educational resource constraints, and the first-dose vaccination rate among girls aged 9 to 14 is only 4%, far below the WHO target (90%), hindering the achievement of herd immunity against HPV. Therefore, in the process of implementing primary prevention, scientific concepts should be upheld, and the protective effect of HPV vaccines should not be exaggerated. Overemphasizing the protective effect and vaccination compliance rate of HPV vaccines may, on the one hand, deviate from the scientific spirit; on the other hand, it may lead to a huge financial burden and commitment trap. Most HPV infections are transient (90%), and only a small percentage (10%) of HR-HPV can develop into a persistent infection. Intervening only in a small number of HR-HPV persistent infections may systematically reduce the intervention population and lower intervention costs. Therefore, in the absence of vaccine protection (non-vaccination) or in cases of protection failure, paying attention to the diagnosis and intervention of HR-HPV persistent infection is undoubtedly one of the effective measures to prevent cervical cancer. This should become an important

component of CC prevention and a distinctive feature of China's prevention and control efforts.

The “Guidelines for Cervical Cancer Screening in China (Part I)” has recommended HR-HPV nucleic acid testing as the primary screening method for cervical cancer (CC)^[19], indicating that HPV is the preferred item for CC screening. Since persistent HR-HPV infection is the primary cause of CC, it is logically and from a preventive medicine perspective, completely correct and necessary to diagnose and intervene in persistent HR-HPV infection. Given that persistent HR-HPV infection accounts for only a small proportion, intervening solely on this may systematically reduce prevention and control costs, achieving optimal cost-effectiveness.

China is a populous country with relatively limited public health resources. With less than six years remaining before the WHO's global goal to eliminate cervical cancer (CC) is reached, there are obvious difficulties in achieving the “90-70-90” strategic targets, whether in terms of funding, awareness, or operational aspects. Therefore, if we can correctly examine the HPV vaccine from a scientific and practical perspective, and focus our limited funds, manpower, and financial resources on the diagnosis and intervention of HR-HPV persistent infection, three-tier screening for CC, and CIN management, it will be possible to systematically narrow the target population, reduce prevention and control costs, improve prevention and control effectiveness, and embark on a path of CC prevention and control that is suitable for China's national conditions.

CC prevention should have Chinese characteristics, wisdom, and solutions!

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Revisiting IL-1 Antagonism in Lung Cancer Therapeutics: Lessons from Failure and Pathways to Precision Therapy

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Abstract: Despite compelling preclinical and epidemiological evidence (e.g., reduced lung cancer incidence in the CANTOS trial), IL-1 β inhibition with canakinumab failed to achieve the expected therapeutic effect in the Phase III clinical trials (CANOPY series) of non-small cell lung cancer (NSCLC). This perspective analyzes the disconnect between mechanistic promise and clinical outcomes. IL-1 β drives NSCLC progression by promoting immunosuppression, angiogenesis, and metastasis. However, CANOPY-2 showed no overall survival (OS) benefit, though a trend emerged in patients with an elevated baseline of high-sensitivity C-reactive protein (hs-CRP). Similarly, CANOPY-1 and adjuvant CANOPY-A missed primary endpoints for progression-free survival (PFS) and disease-free survival (DFS), respectively. These failures highlight limitations of IL-1 monotherapy in advanced, immunosuppressive microenvironments and underscore inadequate patient selection. We propose that IL-1 antagonism retains therapeutic potential but requires refined strategies: biomarker-driven enrichment (e.g., inflammation signatures like hs-CRP), rational combinatorial regimens informed by successful multi-target agents (e.g., cadonilimab), and early-stage intervention. Repositioning IL-1 blockers through precision approaches could unlock their value in immuno-oncology.

Keywords: Canakinumab; Clinical trial failure; Biomarker-driven enrichment

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1. Introduction

The IL-1 β inhibitor canakinumab demonstrated significant anti-tumor potential in preclinical models and

epidemiological studies (e.g., reduced lung cancer incidence in the CANTOS trial)^[1]. Nevertheless, its failure in the Phase III clinical trials (CANOPY series) of non-small cell lung cancer (NSCLC) underscores critical gaps in patient selection and therapeutic context^[2]. This viewpoint contends that IL-1 blockade retains mechanistic promise but requires redefined clinical strategies, including biomarker-driven enrichment, optimized combinatorial regimens, and early-stage intervention. Integrating insights from successful multi-target agents (e.g., cadonilimab in gastric cancer), we propose a roadmap to reposition IL-1 antagonists within the evolving landscape of immuno-oncology.

2. IL-1 β in lung cancer: Mechanistic promise and clinical setbacks

IL-1 β , a pivotal mediator of inflammation, drives lung cancer progression by fostering immunosuppression, angiogenesis, and metastatic niche formation^[3]. Preclinical studies highlight its role in recruiting myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs), while dampening cytotoxic T-cell activity. Despite this rationale, Phase III trials of the IL-1 β inhibitor canakinumab (CANOPY series) in NSCLC yielded disappointing outcomes^[4]. In CANOPY-2 (NCT03631199), combining canakinumab with docetaxel failed to improve overall survival (OS; HR = 0.93, p = 0.29), though a trend emerged in patients with elevated baseline of high-sensitivity C-reactive protein (hs-CRP; ≥ 10 mg/L). Similarly, CANOPY-1 (NCT03626545), which tested canakinumab alongside pembrolizumab and chemotherapy in first-line NSCLC, showed no significant progression-free survival (PFS) or OS benefit, underscoring the limitations of IL-1 monotherapy in advanced, immunosuppressive microenvironments. CANOPY-A (NCT03447769), evaluating adjuvant canakinumab post-resection, also missed its disease-free survival (DFS) endpoint, likely due to residual tumor heterogeneity. These failures suggest that unselected patient populations and late-stage intervention may obscure IL-1 β 's therapeutic potential, rather than invalidating its mechanistic relevance.

3. Unmasking failure: The critical role of biomarkers and timing

The CANOPY trials' shortcomings stem from a "one-size-fits-all" approach, neglecting the heterogeneity of IL-1 β -driven tumorigenesis. For instance, KRAS-mutant NSCLCs exhibit inflammasome hyperactivity and inflammatory cytokine profiles (e.g., IL-6, CXCL1/2), rendering them plausible candidates for IL-1 blockade^[5]. Retrospective analyses of CANOPY-2 hinted at OS benefits in patients with elevated hs-CRP, mirroring the CANTOS trial's finding that CRP reduction correlated with reduced lung cancer incidence^[6]. However, the critical absence of prospective biomarker stratification (notably tumor IL-1 β expression, peripheral cytokine quantification, or inflammasome-related transcriptional signatures) precluded rigorous validation of these mechanistic hypotheses. Additionally, the trials focused on advanced NSCLC, where entrenched immunosuppressive networks (e.g., VEGF, TGF- β) may overwhelm single-pathway inhibition. Conversely, CANTOS revealed canakinumab's striking cancer-preventive efficacy through a 67% reduction in lung cancer incidence among high-risk cardiovascular cohorts, strongly implying that therapeutic interception during premalignant/early-stage disease may achieve superior clinical impact.

4. Rebuilding the strategy: biomarkers, combinations, and early intervention

To resurrect IL-1 antagonists, future trials must prioritize biomarker-driven patient enrichment and

combinatorial synergy. Prospective validation of IL-1 β pathway biomarkers, such as tumor NLRP3 expression, dynamic CRP monitoring, or inflammatory gene signatures, could identify responsive subsets, including KRAS/TP53-mutant tumors with cytokine-rich microenvironments^[7,8]. Mechanistically, IL-1 β blockade may synergize with PD-1 inhibitors by reversing T-cell exhaustion and MDSC-mediated suppression, as seen in preclinical models. Clinically, combining canakinumab with anti-angiogenics (e.g., bevacizumab) or KRAS-G12C inhibitors could address pathway redundancy, akin to cadonilimab's success in gastric cancer through dual PD-1/CTLA-4 targeting. Early-stage applications, such as neoadjuvant or adjuvant therapy for resectable NSCLC with elevated inflammatory markers, offer a strategic niche, leveraging IL-1 β 's role in micrometastatic progression. Furthermore, repurposing IL-1 inhibitors for cancer interception in high-risk populations (e.g., COPD patients with precancerous lesions) aligns with CANTOS' preventive insights, bridging cardiology and oncology paradigms.

5. Lessons from cadonilimab: Multi-target synergy and adaptive design

The breakthrough of cadonilimab, a PD-1/CTLA-4 bispecific antibody, in gastric cancer (COMPASSION-15 trial) underscores the power of multi-target strategies to broaden efficacy and mitigate resistance^[9]. By engaging two immune checkpoints, cadonilimab achieved responses across PD-L1 expression levels, including PD-L1-negative tumors—a lesson applicable to IL-1 antagonists. Combining IL-1 β inhibition with complementary targets (e.g., IL-6R, VEGF) could counteract compensatory cytokine activation, while dynamic biomarker monitoring (e.g., serial CRP/IL-6 measurements) might enable adaptive therapy adjustments. Additionally, cadonilimab's favorable safety profile compared to combination checkpoint inhibitors highlights the importance of balancing efficacy and tolerability in IL-1-based regimens. Translating these principles, future IL-1 trials should adopt modular designs, integrating biomarker stratification, combination partners, and stage-specific contexts to unlock its full potential.

6. Conclusion

The inability of IL-1 β blockade in NSCLC trials reflects not mechanistic futility but a disconnect between biological rationale and clinical execution. By embracing precision oncology, prospectively defining responsive subsets, optimizing combinatorial logic, and shifting to early-stage or preventive settings, canakinumab and next-generation IL-1 inhibitors may yet carve a niche in lung cancer therapy. As cadonilimab redefined dual checkpoint inhibition, a similarly innovative framework, rooted in biomarker-driven adaptation and multi-modal synergy, could resurrect IL-1 antagonism as a strategic component of the immuno-oncology arsenal.

Disclosure statement

The authors declare no conflict of interest.

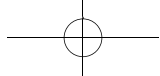
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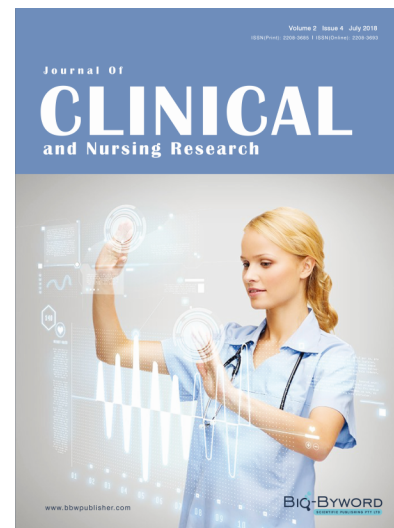
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