

Oncology Treatment Discovery

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Oncology Treatment Discovery

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From Mendelian Randomization to Bioinformatics Analysis: The Bridging Role of NOD2 in the Relationship Between Crohn's Disease and Pancreatic Cancer

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Abstract: *Background:* Pancreatic cancer (PC) is a leading cause of cancer death worldwide, with early diagnosis being difficult and prognosis poor. Crohn's disease (CD) is a chronic inflammatory bowel disease, and some studies suggest a potential link between CD and the development of pancreatic cancer. However, the exact biological mechanisms are unclear. This study investigates the causal relationship between CD and PC and focuses on the role of the NOD2 gene in pancreatic cancer. *Methods:* The study used Mendelian randomization (MR) to identify SNPs associated with both CD and PC, followed by functional annotation through the Ensembl database. Differential expression of these genes in pancreatic cancer was analyzed using the GEPIA2 platform. The study then used Metascape for gene enrichment and pathway analysis, and Kaplan-Meier Plotter to assess the relationship between gene expression and patient survival. Immunohistochemistry (IHC) was conducted to validate protein expression, and the TIMER 3.0 platform was used to examine immune cell infiltration related to NOD2. Finally, the study explored the relationship between NOD2 mRNA expression and clinical features using the cBioPortal platform. *Results:* Out of 91 candidate genes, 36 showed significant differential expression between pancreatic cancer and normal tissues. High expression of 9 genes was associated with poor prognosis. NOD2 was identified as a key gene with elevated expression in pancreatic cancer tissues, closely linked to immune cell infiltration. Further analysis showed that NOD2 expression correlated with tumor stage, lymph node metastasis, and distant metastasis, especially in advanced stages (T3, N1, Stage IIB). *Conclusion:* This study highlights the potential role of the NOD2 gene in linking Crohn's disease with pancreatic cancer, suggesting that NOD2 may contribute to pancreatic cancer development through immune and inflammatory processes. Elevated NOD2 expression is associated with clinical features of pancreatic cancer, making it a potential prognostic marker. Future research should focus on understanding NOD2's role in the immune microenvironment and its potential as a therapeutic target.

Keywords: Pancreatic cancer; Crohn's disease; Mendelian randomization; NOD2; Immune infiltration

Online publication: December 31, 2025

1. Introduction

Pancreatic Cancer (PC) is one of the leading causes of cancer-related mortality worldwide. It has a low rate of early diagnosis and a high recurrence rate, which leads to a very poor clinical prognosis. According to global cancer statistics, the five-year survival rate for pancreatic cancer is approximately 10%, with most patients diagnosed at advanced stages. Therefore, effective early screening and treatment methods have not yet made significant progress^[1]. Current research focuses on revealing the molecular characteristics of pancreatic cancer to identify new diagnostic biomarkers and therapeutic targets, with the aim of improving patient survival.

Crohn's Disease (CD) is a chronic inflammatory bowel disease characterized by persistent, non-specific inflammation in the intestines. The etiology is complex and likely closely related to genetic, immune, and environmental factors^[2]. Patients with CD are affected by abnormal immune system responses, which can lead to an imbalance between intestinal tissue damage and repair. Recent studies have suggested that CD may be linked to certain cancers, particularly those in the digestive system^[3]. While research has explored the relationship between CD and colorectal cancer^[4], the association between CD and pancreatic cancer has not been fully explained. Given the shared characteristics of chronic inflammation and immune system abnormalities between CD and pancreatic cancer, this provides a theoretical basis for further exploration of the potential biological relationship between the two.

A recent study using Mendelian randomization analysis explored the causal relationship between CD and pancreatic cancer^[5], revealing a possible causal connection. The study indicated that CD is a risk factor for pancreatic cancer. However, despite providing preliminary evidence for the relationship between CD and pancreatic cancer, the biological mechanisms involved remain unclear.

This study aims to further explore the causal relationship between CD and pancreatic cancer and uncover potential molecular mechanisms. By integrating existing genetic data, gene functional annotations, differential gene expression analysis, and enrichment analysis, the study hopes to identify candidate genes with potential prognostic value in pancreatic cancer, providing new ideas for future biological research and targeted therapies.

2. Materials and methods

2.1. Overall study design

This study's experimental design aims to further investigate the causal relationship between CD and PC using Mendelian randomization analysis, explore the biological links between these two diseases, and identify potential new therapeutic targets for PC. Initially, relevant SNPs associated with CD and PC are identified from the Mendelian randomization literature. These SNPs are then functionally annotated using the Ensembl database to pinpoint the related genes. The next step involves utilizing the GEPIA2 platform to analyze the significantly differentially expressed genes from the identified genes in PC as candidate genes. Subsequently, the differentially expressed genes undergo enrichment analysis on the Metascape platform to determine the biological processes and signaling pathways in which these genes participate. Following this, survival analysis is conducted using the Kaplan-Meier Plotter tool to identify genes that correlate with overall survival in PC patients. Genes with a P -value < 0.05 and a hazard ratio (HR) > 1 are selected as candidate prognostic markers for PC. A literature review is then conducted to identify potentially unexplored prognostic candidate genes. Immunohistochemical analysis is performed using the Human Protein Atlas database to validate the differential expression of these prognostic candidate genes in both normal pancreatic tissue and PC tissue. Finally, for the selected target genes, immune infiltration analysis is carried out using the TIMER 3.0 platform to evaluate their roles in the immune

microenvironment of PC. Furthermore, the relationship between the mRNA expression of these genes and the clinical pathological features of PC patients is analyzed through the cBioPortal platform (**Figure 1**).

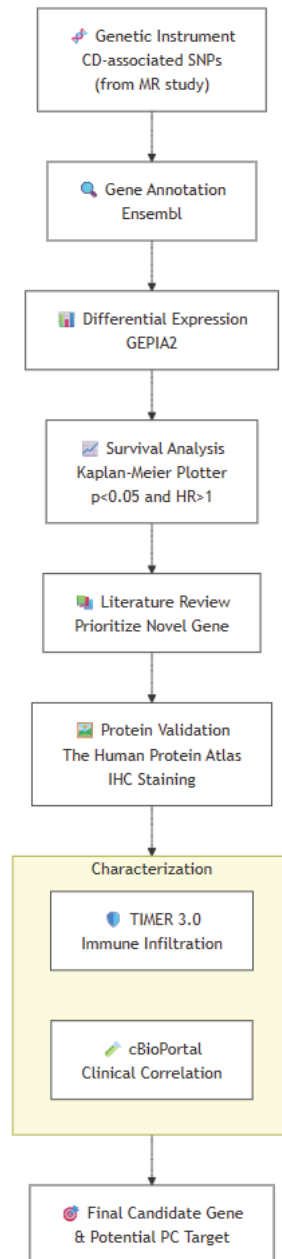


Figure 1. Experimental design flow chart.

2.2. Data sources

The causal relationship analysis between CD and PC discussed in this paper is based on the results of Mendelian randomization studies^[5].

2.3. SNP functional annotation analysis

The Ensembl Genome Browser (<https://www.ensembl.org/>) is a free and publicly accessible genome browsing tool, developed and maintained collaboratively by the European Bioinformatics Institute (EBI) and the Wellcome

Sanger Institute ^[6]. This platform provides a powerful resource for scientists and researchers worldwide to browse and analyze genomic data across multiple species, with particularly important applications in gene annotation, variant analysis, and transcriptomics. To further explore the relevant SNPs, the study used the Ensembl Genome Browser for functional annotation of the SNPs and to pinpoint related candidate genes.

2.4. Differential expression analysis

GEPIA2 (<http://gepia2.cancer-pku.cn/>) is an online platform for cancer gene expression analysis and biomarker research ^[7]. The study performed differential expression analysis on the transcription levels between pancreatic cancer tissue (from the TCGA database) and normal pancreatic tissue (from the GTEx database) using the GEPIA2 platform ^[10]. The analysis utilized $\log_2(\text{TPM}+1)$ normalized expression values and applied univariate differential testing for statistical analysis.

2.5. Gene enrichment analysis

Metascape is a free online bioinformatics platform that provides researchers with gene functional annotation, enrichment analysis, pathway analysis, and visualization tools, helping users understand the interactions between genes, genomes, and proteins ^[8]. For the selected differentially expressed genes, the study used the Metascape platform to perform gene enrichment analysis, aiming to identify the enrichment of these genes in biological processes, molecular functions, and cellular components, and to obtain relevant information about associated signaling pathways and biological functions.

2.6. Survival analysis

Kaplan-Meier Plotter is a widely used online tool for analyzing the relationship between gene expression and patient survival, particularly in cancer research ^[9]. This tool is based on the Kaplan-Meier survival analysis method, which assesses the association between gene expression and overall survival (OS), disease-free survival (DFS), and other clinical outcomes by plotting survival curves. The study used the Kaplan-Meier Plotter tool to conduct clinical prognosis analysis of these differentially expressed genes and evaluate their correlation with overall survival (OS) in pancreatic cancer patients.

2.7. Literature review

The study conducted a literature review on the candidate genes and, in conjunction with existing pancreatic cancer research, selected genes that have not been thoroughly studied as further candidates. These genes have not been fully explored in pancreatic cancer research and are thus considered potential avenues for future study.

2.8. Protein expression validation and immunohistochemical analysis

The Human Protein Atlas is an open, biomedical research platform aimed at providing expression data for all proteins in the human genome, as well as their distribution across different tissues, cells, and pathological states ^[10]. The platform, initiated and maintained by the Karolinska Institute in Sweden, consolidates extensive experimental data, playing a significant role, particularly in proteomics and clinical pathology research. To validate the expression of the candidate genes in pancreatic cancer, the study used the Human Protein Atlas database to search for and compare the immunohistochemical (IHC) staining results of these genes in normal pancreatic tissue and pancreatic cancer tissue, ensuring that their protein expression levels align with the differential expression results

obtained from the GEPIA2 platform.

2.9. Immune infiltration analysis

TIMER 3.0 (Tumor Immune Estimation Resource) is a powerful online platform for cancer immune microenvironment analysis ^[11]. It aims to assist researchers in assessing immune cell infiltration in tumors by integrating various cancer datasets and to explore the relationship between the immune microenvironment and cancer initiation and progression. The study performed immune infiltration analysis of the target genes on the TIMER 3.0 platform to evaluate their role in the immune microenvironment of pancreatic cancer.

2.10. Clinical pathological analysis

Finally, the study performed a correlation analysis between the mRNA expression of the target genes and the clinical pathological features (such as staging, prognosis, etc.) of pancreatic cancer patients using the cBioPortal platform, to evaluate the potential role of these genes in the initiation and progression of pancreatic cancer. cBioPortal is a widely used online platform for the visualization, analysis, and interpretation of cancer genomics data ^[12]. It was developed by the Memorial Sloan Kettering Cancer Center, a public genomic data platform in the United States, and is designed to provide an integrated, interactive tool to assist researchers and clinicians in analyzing genomic, transcriptomic, and clinical data across different cancer types, and their relationship with patient prognosis and treatment response.

3. Results

3.1. SNP functional annotation analysis

Based on the results of Mendelian randomization analysis in the existing literature, 82 SNPs associated with CD and PC were extracted. Functional annotation through the Ensembl database revealed that these SNPs are mapped to 91 genes. This analysis provides foundational data to support further investigation into the role of these genes in PC.

3.2. Differential gene expression

Differential expression analysis was performed on the 91 genes identified from the SNP functional annotation analysis using GEPIA2. The results showed that after filtering, 36 genes exhibited significant expression differences between pancreatic cancer and normal pancreatic tissue. These genes are: ACO2, ADCY3, APEH, ATG16L1, ATXN2, CARD9, CREM, ERAP2, FCGR2A, FOS, FUT2, HLA-B, HLA-DRA, IFNGR2, IKZF3, IL18R1, IRF1, JAK2, JAZF1, LSM14A, MTX1, NFATC1, NOD2, PHF5A, PHTF1, PLA2G4A, PLAU, PRDM1, RASGRP1, RMI2, RNF123, SBNO2, SH2B3, SKAP2, SLAIN2, TAGAP.

3.3. Functional enrichment analysis

3.3.1. Pathway and biological process enrichment analysis

In this enrichment analysis, the top 15 significantly enriched pathways and biological processes were identified. These pathways mainly reflected those related to immune response, inflammation, and viral response (**Figure 2**). The enriched terms were primarily concentrated in immune response, cell activation, and certain diseases such as inflammatory bowel disease, tuberculosis, and leishmaniasis, suggesting that CD may be potentially associated with the development of PC through these pathways. The specific analysis results are as follows:

- (1) T Cell Activation and SARS-CoV-2 (WP5098): This pathway included six differentially expressed genes (16.67%) and showed significant enrichment among all input genes. T cell activation is a central process of the adaptive immune response, and chronic immune activation in CD may increase the risk of PC through this mechanism.
- (2) Regulation of Adaptive Immune Response (GO:0002819): This process involved seven differentially expressed genes (19.44%) and exhibited a high degree of enrichment. Patients with CD often display abnormal immune activation, and dysregulation of adaptive immune response may represent an important mechanism underlying PC development.
- (3) Inflammatory Bowel Disease (hsa05321, KEGG Pathway): This pathway was enriched with five differentially expressed genes (13.89%). As CD itself is a typical inflammatory bowel disease, the enrichment suggests that intestinal inflammatory responses may indirectly influence PC development through systemic immune pathways.
- (4) Leishmaniasis (hsa05140, KEGG Pathway): This pathway involved five differentially expressed genes (13.89%). Although leishmaniasis is rarely directly related to PC, its immune evasion mechanisms may provide biological insight into immune escape in PC.
- (5) Regulation of Cell Activation (GO:0050865): This process was enriched with nine differentially expressed genes (25%), indicating that immune cell activation and its regulation may play critical roles in the pathogenesis of PC.
- (6) Tuberculosis (hsa05152, KEGG Pathway): This pathway was enriched with six differentially expressed genes (16.67%). As tuberculosis is a chronic immune-mediated disease, this suggests that immune system involvement in chronic inflammatory disorders may influence PC progression.
- (7) Response to Virus (GO:0009615): This process involved six differentially expressed genes (16.67%). The relationship between viral infection and cancer has been widely reported, and the persistent immune activation in CD may lead to abnormal antiviral responses, thereby promoting the development of PC.

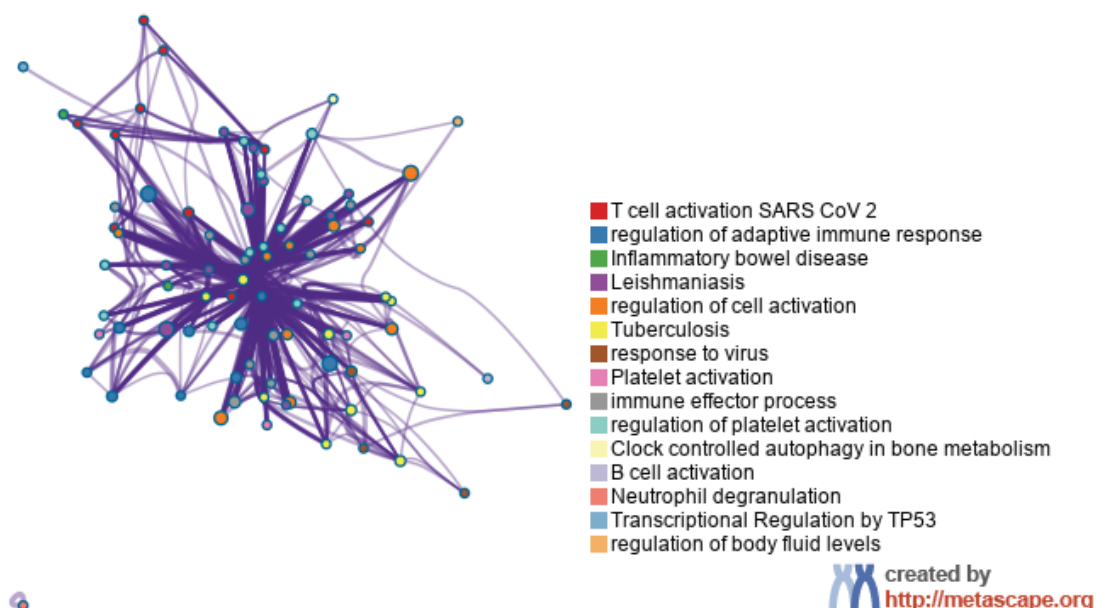


Figure 2. Top 15 enriched pathways and biological processes.

3.3.2. Protein-Protein Interaction (PPI) enrichment analysis

In the PPI enrichment analysis, the significant pathways identified were primarily related to T cell differentiation, regulation of cell activation, and the interferon- γ signaling pathway. The specific analysis results are as follows:

- (1) Th1 and Th2 Cell Differentiation (hsa04658): The Log₁₀(P) value for this pathway was -8.6, showing significant enrichment of the differentially expressed genes. Th1 and Th2 cells are two major T cell subpopulations involved in immune responses. CD may promote PC development by altering the balance of these T cell subpopulations.
- (2) Regulation of Cell Activation (GO:0050865): The Log₁₀(P) value for this pathway was -8.5, indicating significant enrichment of the differentially expressed genes in cell activation regulation. Activation of immune cells is fundamental to immune responses, and excessive or dysregulated activation of immune cells may influence the immune microenvironment of PC.
- (3) Interferon- γ Signaling Pathway (R-HSA-877300): The Log₁₀(P) value for this pathway was -8.5. Interferon- γ is an important immune cytokine, and aberrant activation of the interferon- γ signaling pathway may lead to immune tolerance or immune evasion, thus influencing PC development.

3.3.3. Quality control and association analysis

In the quality control and association analysis section, the results revealed multiple enriched pathways related to immune cell types and tissues, suggesting that these immune cells may play an important role in the relationship between CD and PC. The specific analysis results are as follows:

- (1) MANNO MIDBRAIN NEUROTYPES HMGL (M39051): Enrichment analysis showed 10 differentially expressed genes (28%) in this pathway, with a Log₁₀(P) value of -9.00, suggesting that midbrain immune system-associated cells may be involved in the immune microenvironment of PC.
- (2) HU FETAL RETINA MICROGLIA (M39266): This pathway was enriched with seven differentially expressed genes (19%), with a Log₁₀(P) value of -6.50, indicating that microglial cells may be involved in regulating the immune microenvironment of PC.
- (3) TRAVAGLINI LUNG EREG DENDRITIC CELL (M41697): This pathway was enriched with eight differentially expressed genes (22%), with a Log₁₀(P) value of -6.50. Dendritic cells play an important role in immune responses and may be involved in the immune dysregulation induced by CD, thereby increasing the risk of PC.

3.3.4. Enrichment analysis of the DisGeNET database

In the enrichment analysis of the DisGeNET database, the significant disease pathways were primarily concentrated in the areas of immune system abnormalities and autoimmune diseases, revealing potential associations between CD and other immune-mediated diseases with PC (**Figure 3**). The analysis results suggest that the long-term activation of the immune system and chronic inflammatory responses may be key mechanisms by which CD increases the risk of PC. Specifically, the enrichment of immune-mediated chronic disease pathways, such as sclerosing cholangitis, ankylosing spondylitis, and celiac disease, indicates that immune dysregulation is closely related to the development of PC. In particular, the immune mechanisms of CD may promote immune evasion mechanisms in PC through chronic inflammation and immune responses. Furthermore, the enrichment of immune deficiency and immune cell-related pathways, such as common variant immune deficiencies and eosinophil counts, further supports the role of the immune system in immune evasion in PC.

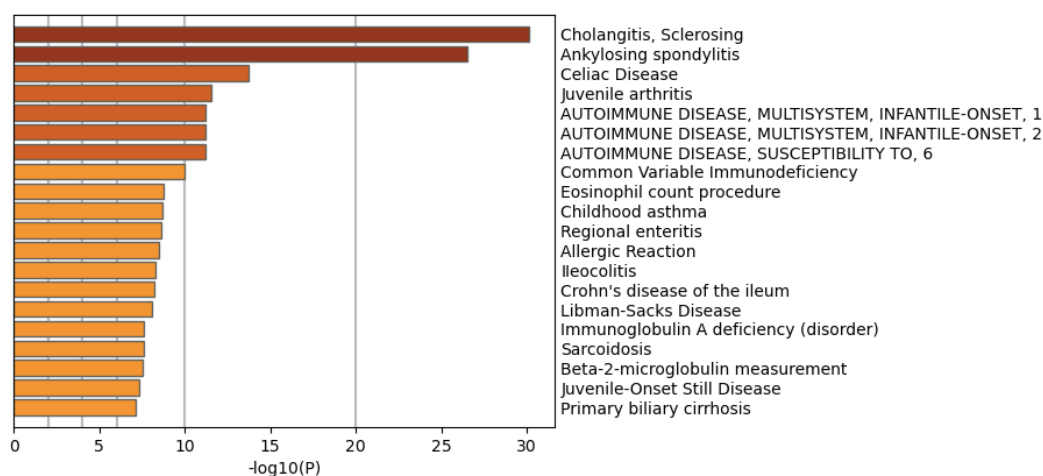


Figure 3. Disease pathways enriched in the DisGeNET database.

3.4. Survival analysis of candidate genes, literature review, and protein validation, screening of target genes

In the preliminary study, differential expression analysis of these genes in pancreatic cancer tissue was performed using the GEPIA2 platform, resulting in the identification of 36 differentially expressed genes. To further validate the prognostic value of these genes in PC, survival analysis was conducted.

3.4.1. Survival analysis

The study used the Kaplan-Meier Plotter platform to assess the overall survival (OS) of the 36 differentially expressed genes. The results showed that high expression of 9 genes was significantly associated with poor prognosis in pancreatic cancer patients ($p < 0.05$, $HR > 1$). These genes are: ACO2, ATG16L1, ERAP2, NOD2, PHF5A, PLAU, RMI2, SKAP2, and SLAIN2.

3.4.2. Literature review and protein expression validation

To further confirm the biological significance of these candidate genes in PC, the study conducted a literature review. ERAP2, PHF5A, PLAU, and SLAIN2 have already been extensively studied in PC, so they were excluded from further analysis. Subsequently, the study used The Human Protein Atlas database to validate the protein expression levels of the remaining candidate genes in normal pancreatic tissue and pancreatic cancer tissue. The results showed that the protein expression levels of ACO2, ATG16L1, RMI2, and SKAP2 did not show significant differences between normal pancreatic tissue and pancreatic cancer tissue, whereas NOD2 expression was significantly higher in pancreatic cancer tissue compared to normal pancreatic tissue. Based on the results from differential expression analysis, survival analysis, and protein expression validation (**Figures 4 and 5**), the study ultimately identified NOD2 as the target gene for further research.

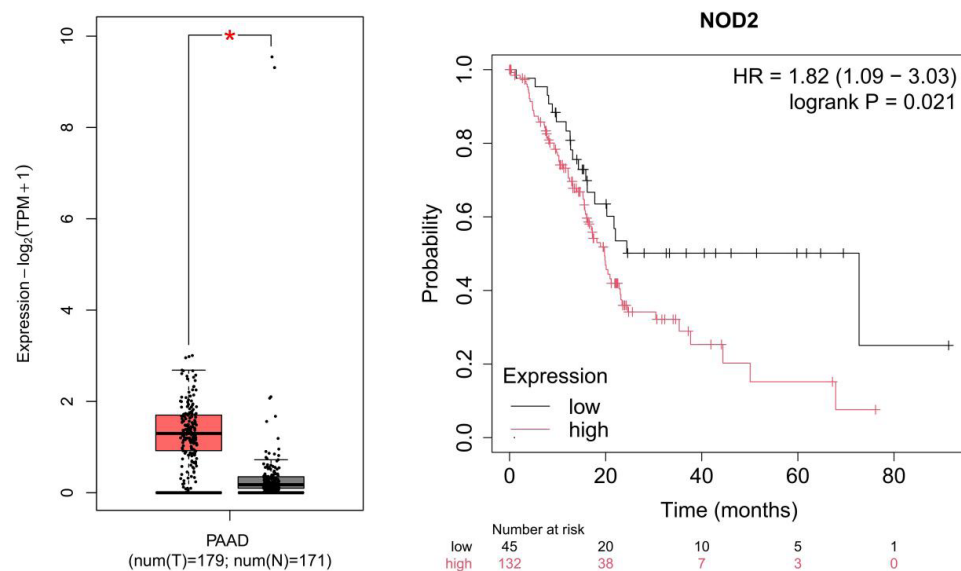


Figure 4. Differential expression and survival analysis results of NOD2.

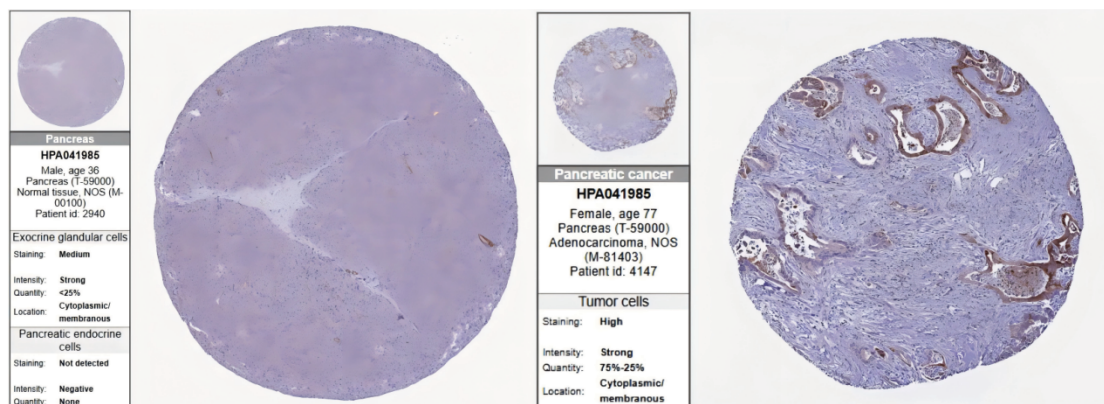


Figure 5. Protein expression level differences of NOD2 in normal pancreatic tissue and pancreatic cancer tissue.

3.5. SH2B3 immune infiltration analysis

The study analyzed the immune infiltration of the NOD2 gene in pancreatic cancer. The results showed that the expression of NOD2 in the pancreatic cancer immune microenvironment is closely related to the infiltration of various immune cell types (**Figure 6**). Specifically, the expression of NOD2 was significantly positively correlated with the infiltration of CD8⁺ T cells ($\text{Rho} = 0.377$, $P = 3.95\text{e-}07$), M2 macrophages ($\text{Rho} = 0.423$, $P = 8.91\text{e-}09$), dendritic cells ($\text{Rho} = 0.604$, $P = 2.67\text{e-}18$), cancer-associated fibroblasts ($\text{Rho} = 0.453$, $P = 5.32\text{e-}10$), and neutrophils ($\text{Rho} = 0.548$, $P = 1.00\text{e-}14$). These results suggest that the NOD2 gene may play a key role in immune surveillance and antitumor immune responses; it may regulate immune evasion mechanisms in the tumor microenvironment by promoting the infiltration of immunosuppressive immune cells (such as M2 macrophages and cancer-associated fibroblasts), thereby influencing the progression of PC.

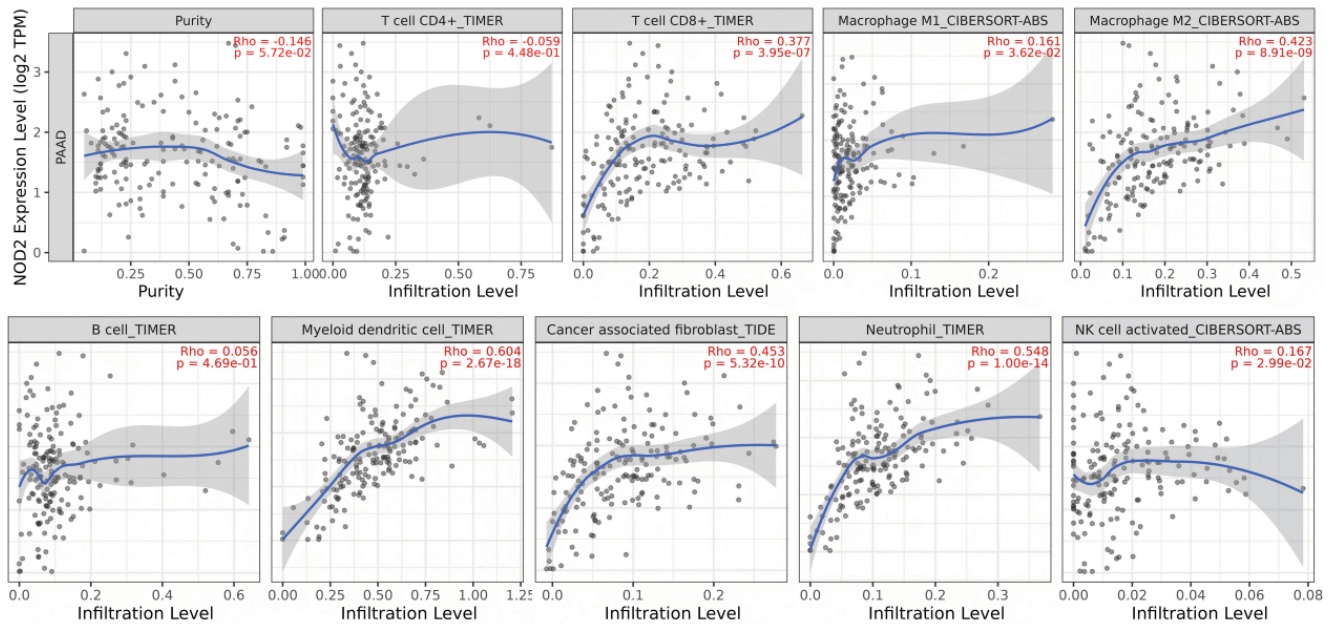


Figure 6. Immune infiltration analysis of NOD2 in pancreatic cancer.

3.6. NOD2 Clinical pathological correlation analysis

In this study, the expression pattern of the NOD2 gene in different clinical pathological stages of pancreatic cancer to determine its correlation with the clinical pathological features of PC (**Figure 7**).

3.6.1. AJCC pathological T stage (Tumor invasion depth)

According to the AJCC pathological T stage, pancreatic cancer is divided into four stages: T1, T2, T3, and T4. The mRNA expression of the NOD2 gene showed significant differences across the different T stages. Specifically, the mRNA expression level of NOD2 was highest in T3 stage pancreatic cancer samples, while the expression levels were relatively low in T1 and T2 stages, and significantly decreased in T4 stage. Regarding gene variation, amplifications (Amplification, red dots) and copy number gains (Gain, pink dots) of NOD2 were observed in all T stages. Particularly in T3, the distribution of NOD2 gene variations was denser, suggesting that the genetic changes in tumor cells at this stage are more complex.

3.6.2. AJCC pathological N stage (Lymph node metastasis)

In the AJCC pathological N stage, pancreatic cancer is divided into N0 (no metastasis), N1 (regional metastasis), N1b, and NX (stage unknown). The expression of NOD2 showed significant differences across the different N stages. Specifically, the expression of NOD2 was highest in N1 stage, followed by the N0 stage, while expression in the NX stage was significantly lower. Additionally, gene variations were more frequent in N0 and N1 stages, where gene amplifications and copy number gains were observed, while no gene variations were observed in the NX stage, suggesting fewer genetic changes in pancreatic cancer at this stage.

3.6.3. AJCC pathological M stage (Distant metastasis)

The M stage is used to assess whether pancreatic cancer has distant metastasis and is divided into M0 (no metastasis), M1 (metastasis present), and MX (stage unknown). The expression of NOD2 showed some variation

across the different M stages. In both M0 and M1 stages, the mRNA expression levels of NOD2 were higher, suggesting that the NOD2 gene may be closely related to the metastasis process in these stages. However, in the MX stage, NOD2 expression was significantly lower, indicating that there may be unknown biological mechanisms at play in pancreatic cancer at this stage. Gene variation analysis showed that in M0 and M1 stages, NOD2 amplifications, copy number gains, and some missense mutations (Missense, VUS, green dots) were more common, while gene variations were almost absent in the MX stage.

3.6.4. AJCC overall pathological stage

For the AJCC overall pathological stage (Stage I, Stage IA, Stage IB, Stage IIA, Stage IIB, Stage III, Stage IV), the study analyzed the expression of NOD2 across the different stages. The results showed that in Stage IIB, NOD2 had the highest mRNA expression level, showing strong gene activity. In Stage I, IA, IB, and IIA, the expression of NOD2 was slightly lower but still significantly higher than in Stage III and Stage IV. Further analysis of gene variation revealed that NOD2 gene variations (including amplifications, gains, and missense mutations) were most densely distributed in Stage IIB pancreatic cancer samples, suggesting that tumors at this stage may have a more complex genetic background.

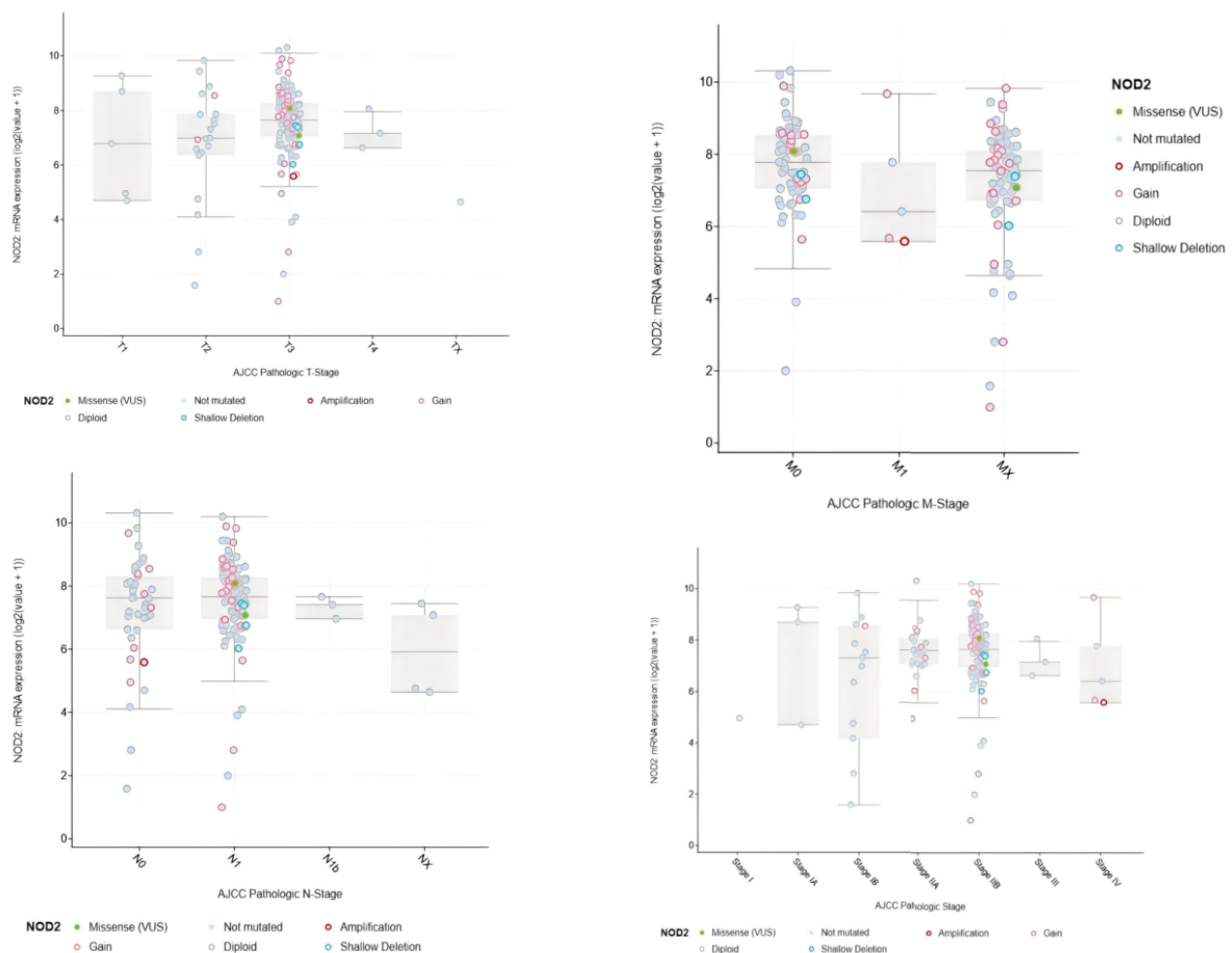


Figure 7. Correlation analysis of NOD2 mRNA expression in different pathological stages of pancreatic cancer.

4. Discussion

This study took the results of Mendelian randomization analysis between CD and PC as a starting point, combining multi-dimensional bioinformatics tools. By analyzing 82 SNPs associated with CD and PC, and performing functional annotation through the Ensembl database, the study identified 91 potential candidate genes. Subsequently, the differential expression of these genes in pancreatic cancer tissue was analyzed using the GEPIA2 platform, ultimately identifying 36 genes that showed significant expression differences between pancreatic cancer and normal pancreatic tissue.

Further enrichment analysis of the differentially expressed genes revealed several pathways significantly associated with immune response, cell activation, inflammation, and pathological responses, suggesting that CD may be closely related to PC through immune pathways. As CD is a chronic immune-mediated inflammatory disease, the sustained abnormal activation of its immune system may contribute to the imbalance of the immune environment, thereby providing favorable conditions for PC development. In particular, the abnormal activation of T cells and the loss of immune tolerance may promote tumorigenesis. Additionally, the enrichment of pathways related to inflammatory bowel disease further supports the potential role of chronic inflammation in CD patients, indicating that the systemic immune response associated with CD may influence the mechanisms underlying PC development. The enrichment of viral response pathways may suggest abnormalities in immune responses in CD patients, potentially leading to immune evasion of viral infections and thus creating an immune escape microenvironment conducive to the development of PC. These results offer new insights into the potential biological mechanisms linking CD and PC, particularly regarding the effects of immune responses and chronic inflammation on PC.

PPI Enrichment Analysis further revealed several important pathways closely related to immune response, providing further support for the hypothesis that CD affects PC development through immune pathways. Specifically, the enrichment results of Th1 and Th2 cell differentiation suggest that the immune system in CD may cause immune dysregulation by altering the balance of T cell subpopulations. This imbalance in immune cell function could provide a favorable immune environment for the development of PC, promoting tumor cell survival and expansion. Furthermore, the enrichment of the cell activation regulation process further revealed that excessive activation of immune cells could lead to changes in the immune microenvironment, thereby facilitating immune evasion in tumors. The enrichment of the interferon- γ signaling pathway suggests that aberrant activation of the interferon- γ signaling pathway may be closely related to immune tolerance and immune evasion mechanisms.

The results from quality control and association analysis also highlighted the crucial role of immune cells. The diversity of immune cells and immune regulation imbalance, particularly the enrichment of dendritic cells, NK cells, and macrophages, reflect the complex role of the immune system in the relationship between CD and PC. The functional imbalance of these immune cells may be an important mechanism by which CD leads to PC.

The enrichment analysis of the DisGeNET database suggests that the abnormal activation of the immune system, chronic inflammatory responses, and autoimmune diseases may be key mechanisms by which CD triggers PC. The long-term activation of the immune system and chronic inflammation may promote the development of PC. These findings provide strong support for further exploration of the role of the immune system in PC and offer insights for clinical immunotherapy research.

Among the numerous differentially expressed genes, NOD2 attracted particular attention. NOD2 is a key pattern recognition receptor predominantly expressed in intestinal epithelial cells and immune cells. It activates the downstream NF- κ B pathway through the recognition of muramyl dipeptide (MDP) in bacterial cell walls, thus

regulating immune and inflammatory responses ^[13]. The dysfunction of NOD2 is closely related to the onset of CD ^[14]. Common mutations in NOD2 in CD patients (such as R702W, G908R, and 3020insC) lead to functional deficiencies in immune responses, resulting in chronic intestinal inflammation, which is considered a potential trigger for colorectal cancer ^[15].

Although the role of NOD2 in CD has been extensively studied, there is insufficient evidence regarding its function in PC. Existing studies have shown that NOD2 plays an important role in other types of cancer (such as colorectal cancer, breast cancer, and lung cancer) ^[16, 17, 18], mainly by affecting immune system functions and changes in the microenvironment, thereby promoting cancer development and progression. In colorectal cancer, NOD2 affects the progression of intestinal carcinogenesis by regulating intestinal immune responses and microbial communities. This mechanism may also play a role in PC development, particularly through influencing the immune microenvironment of the pancreas.

Through the analysis of NOD2 gene immune infiltration in pancreatic cancer, the study found that the expression of NOD2 in the pancreatic cancer immune microenvironment was significantly associated with the infiltration of various immune cell types. Specifically, NOD2 expression showed a significant positive correlation with the infiltration of CD8+ T cells, M2 macrophages, dendritic cells, cancer-associated fibroblasts, and neutrophils. These results suggest that NOD2 may regulate immune evasion mechanisms in the tumor microenvironment by promoting the infiltration of immunosuppressive immune cells (such as M2 macrophages and cancer-associated fibroblasts), thus influencing the progression of PC. Particularly, M2 macrophages and cancer-associated fibroblasts, which commonly play immunosuppressive roles in the tumor immune microenvironment, promote tumor growth and metastasis. Therefore, NOD2 may enhance immune evasion and support tumor growth by regulating the function of these immune cells.

In the pathological correlation analysis, the study explored the expression pattern of NOD2 in different clinical pathological stages of pancreatic cancer and its relationship with clinical features of PC. The results showed significant differences in the expression of NOD2 across different pathological stages, particularly in the T stage, N stage, and M stage. In the T stage, NOD2 expression was highest in T3, while it was significantly reduced in T4, suggesting that NOD2 may play a key role in stages with greater tumor invasion depth. Additionally, gene variations were more densely distributed in T3, reflecting the more complex genetic changes in tumor cells at this stage. Regarding the N stage, NOD2 expression was highest in N1, and gene variations (such as amplifications and copy number gains) were more common in N0 and N1, indicating that NOD2 may be closely related to lymph node metastasis in PC. In the M stage, NOD2 expression was higher in both M0 and M1, suggesting a strong association with distant metastasis. In the AJCC overall pathological stage, NOD2 showed the highest expression in Stage IIB, with a denser distribution of gene variations, indicating a more complex genetic background in tumors at this stage.

Although this study reveals the potential role of NOD2 in the relationship between CD and PC, there are still some limitations. Firstly, these results need further validation through laboratory research and clinical samples. Secondly, this study primarily relies on publicly available databases and bioinformatics analysis, lacking direct clinical data support. Therefore, future research should focus on experimental validation of NOD2's role in PC through clinical samples and animal models, and further investigate its specific mechanisms in the immune microenvironment.

5. Conclusion

This study integrates Mendelian randomization analysis and bioinformatics methods to explore the potential causal relationship between CD and PC, revealing the key role of the NOD2 gene in this process. NOD2 may promote the occurrence and progression of PC by regulating immune and inflammatory responses. Particularly, NOD2's role in the immune microenvironment of pancreatic cancer may provide new insights into immune evasion mechanisms. The expression of NOD2 is closely associated with the prognosis of pancreatic cancer patients, and its potential as a prognostic biomarker and therapeutic target for PC warrants further investigation. These findings provide new biological evidence for the link between CD and PC, and open new directions for future research on early diagnosis and immunotherapy for pancreatic cancer.

Disclosure statement

The author declares no conflict of interest.

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Hyperthermia in Combination with Radiation versus Radiation Alone for Superficial Tumors: A Systematic Review and Meta-analysis

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Abstract: *Objective:* Aim to evaluate the efficacy of hyperthermoradiotherapy (HTRT) VS radiation therapy (RT) alone in patients with superficial tumors, mainly including breast cancer, head and neck cancer, and melanoma. The study undertook a systematic review and meta-analysis, and a preset subgroup analysis. *Methods:* A systematic literature search was conducted of the PubMed database and the bibliographies of related studies. *Results:* A total of 15 articles ($n = 1368$) met our eligibility criteria. The meta-analysis of all patients in 19 groups from 15 articles showed HTRT with significant improvement in complete response (CR) versus the RT group ($OR = 2.393$, 95% CI 1.749–3.274, $p = 0.000$) with high heterogeneity ($\chi^2 = 33.67$, $p = 0.014$, $I^2 = 46.5\%$). *Conclusion:* HTRT have significant improvement in CR versus RT alone in superficial tumors. A well-researched but maybe underutilized method, HT can have a major clinical impact by improving local tumor management.

Keywords: Hyperthermia; Radiotherapy; Superficial tumors; Complete response; Breast cancer; Local recurrence; Head and neck cancer

Online publication: December 31, 2025

1. Introduction

In the endeavor to augment tumoral temperature within the delineated interval of 40°C to 44°C, hyperthermia (HT) emerges as a potential efficacious therapeutic avenue. A high dosage of radiation therapy (RT), often requisite for maintaining local control, inherently carries an augmented likelihood of complications. HT enhanced the response to X-rays and photons in a murine tumor and normal skin with DNA damage and tumor hypoxia^[1]. Tumors' alpha/beta ratios witness reduction attributable to thermoradiobiological interactions impacting reparative

processes post RT-induced genotoxic insults, established as pivotal predictors discriminating risk variances between hyperthermoradiotherapy (HTRT) versus standalone RT ^[2]. In low-pH, hypoxic or nutrient-deprived conditions where radiation resistance could be observed in common, HT can bring direct cytotoxicity to gross tumors. HT causes protein denaturation as the predominant target, which could induce damage to all intracellular signaling pathways, including DNA repair and cell cycle sensitivity ^[3,4]. Combined radio(chemo)therapy and HT stabilized or improved the assessment of quality of life scale items 3 and 12 months after treatment in patients who were treated with palliative intent and curative intent ^[5]. Multimodal treatment has been suggested to improve outcomes in retroperitoneal soft tissue sarcoma, and multimodal neoadjuvant therapies, including regional HT are not related to postoperative morbidity ^[6,7]. Superficial water-filtered infrared-A radiation HT has high efficacy in clinical oncology when exposed to composite tissues with highly variable water and fat contents ^[8]. Abundant clinical investigations have documented that HTRT amplifies clinical outcomes—manifested through bolstered response metrics, localized dominance, and enhanced survival indices among patients with breast, cervix, head and neck cancers, melanoma, bone metastases, portraying negligible increments in normal tissue morbid sequelae ^[9–13]. HT offers a cost-effective approach to cancer treatment, making it particularly suitable for resource-limited settings. Its relatively low equipment and operational costs, compared with advanced radiotherapy or surgical interventions, enable wider accessibility in underdeveloped regions. Considering the basic research evidence, good clinical performance, and low economic burden of HT, we have decided to further study HT.

It is discernible that properly achieving thermal elevation in relatively surficial lesions appears plausible, accompanied by adequate acquisition of temperature distribution measurements, even amidst constraints posed by extant HT apparatus; thereby, superficial cancer typologies are accordingly prioritized for investigational pursuits. Aiming to evaluate the efficacy of HTRT VS RT alone in patients with superficial tumors, mainly including breast cancer, head and neck cancer, and melanoma, the study instituted comprehensive systematic review methodologies supplemented by meta-analytical assessment, complemented by prespecified subgroup dissections.

2. Methods

2.1. Search strategy

Compliant with the PRISMA standards, a comprehensive examination of literature within the confines of the PubMed database from the database's inception up to May 1, 2024, assessing the effects manifested by HTRT VS RT alone for superficial tumors. The keywords used were: HT, radiation, breast cancer, superficial tumor. The bibliographies of selected articles were also manually searched for any additional related reports.

2.2. Selection criteria

Studies had to meet the inclusion criteria, which were formulated as a priority. Firstly, 2-arm studies (randomized and non-randomized) treated superficial tumors with local HT and external radiation in the HTRT group versus RT alone as control. However, those using surgery and/or interstitial brachy therapy were excluded. Secondly, the present meta-analysis focused on studies that assessed the complete response (CR) rate. Thus, articles that did not provide data about CR were excluded. For articles with overlapping data from the same trial, we only included the most complete and recent article after analyzing all the data. The publication language was restricted to English.

2.3. Data extraction

The priority endpoint of concern was CR at the end of treatment, and all studies that reported CR after HTRT

or RT alone were considered. CR was defined as all target lesions disappeared and no new lesions appearing, extracted from every eligible article as the primary outcome in our research. Other extracted data included the first author, publication year, country, study design, accrual period, number of patients, age, follow-up, primary/recurrent disease, previous RT, RT dose, type of HT, HT-RT sequence and adverse events (AEs). The articles were extracted independently by two authors in case of discrepancy, and a consensus was reached between the authors.

2.4. Statistical analysis

The examination of the impact wielded by HTRT in contrast with RT upon CR necessitated computing the aggregated odds ratio (OR), alongside its associated 95% confidence interval (CI). An evaluation was conducted concerning a state of heterogeneity, which stood appraised through the implementation of both the χ^2 test and I^2 statistic. When heterogeneity was high ($I^2 > 75\%$), performing a random effects model was preferred. Subsequently, sources contributing to said heterogeneity underwent exploratory scrutiny via meta-regression analyses, in which potentially relevant factors examined individually were year of publication, country, RCT or not, number of patients, primary disease, previous RT, type of HT, interval between RT and HT, HT sessions per week, HT duration. Analyzed further were preplanned subgroup analyses, which encompassed three distinct subgroups: breast cancer, recurrent breast cancer, head and neck cancer. We performed the meta-analysis in Stata 14.0 software with the commands metan, metareg, and metabias.

3. Results

3.1. Included studies

A comprehensive examination yielded 1975 articles alongside a review of 26 bibliographical references relevant to the subject matter. Following the exclusion of studies not meeting eligibility parameters, an initial selection brought forth 29 articles. Observations revealed that four qualifying articles presented data derived from identical patient cohorts, necessitating reliance solely on findings supported by the most recent and updated datasets. Of these, two documents failed to provide pertinent data, while eight exhibited inappropriate experimental design. It is discernible from this sequence that ultimately, 15 articles (sample size: $n = 1368$), spanning publications from 1987 through to 2008, conformed to our stipulated criteria for inclusion in analysis. Details of the study screening are presented in **Figure 1**. Study characteristics extracted from each study are separately summarized in **Table 1**.

Table 1. Features of the 15 articles

Author, year	Country	RCT	Accrual period	Number of patients	Age (years)	Follow-up	Primary/recurrent disease status	Pathology	Previous RT	Previous RT dose
Scott et al. 1984 ^[14]	USA	No	NA	59	Average 59.4	Minimum 6 months	18/59 patients with squamous cell carcinoma of head and neck; 39/59 patients with adenocarcinoma of breast cancer; 2/59 patients with melanoma		0	NA
Dunlop et al. 1986 ^[15]	UK	No	NA	28	Female 33-82, male 29-70	NA	18/28 patients with adenocarcinoma of breast cancer		NA	NA
Perez et al. 1986 ^[16]	USA	No	1964–1984	164	NA	Minimum 6 months	Recurrent breast cancer	NA	75% patients > 50 Gy	
Howard et al. 1987 ^[17]	UK	No	NA	16	NA	4–31 weeks, mean 13 weeks.	6/16 patients with squamous cell carcinoma of the head and neck		NA	NA
Lindholm et al. 1987 ^[18]	Sweden	No	1980–1984	38	22-94, median 68	1–38 months	45/85 tumors: breast; 15/85 tumors: head and neck	66/85 tumors: adenocarcinoma; 12/85 tumors: squamous cell carcinomas	41/57 tumors (RT + HT); 16/28 tumors (RT)	24–70 Gy, median 49 Gy (RT + HT); 26–67 Gy, median 47 Gy (RT)
Arcangeli et al. 1987 ^[19]	Italy	No	1977–1984	55	NA	NA	81/119 tumours: squamous cell carcinoma of the head and neck; 39/119 tumours: melanoma		NA	NA
Egawa et al. 1989 ^[20]	Japan	Yes	1985–1987	92	20–82	1 month	33/92 patients with head and neck; 19/92 patients with breast; 24/92 patients with others	NA	18/44 (RT + HT); 15/48 (RT)	NA
Li et al. 1990 ^[21]	China	No	1980–1983	23	34–78, median 56	6–37 months	12 primary breast tumors and 52 recurrent tumors	NA	20/23 patients	40–65 Gy
Datta et al. 1990 ^[22]	India	Yes	NA	65	NA	18–28 months, median 21 months	Previously untreated squamous cell carcinoma of the head and neck		No previous RT	
Perez et al. 1991 ^[23]	USA	Yes	1981–1987	236	18–93	NA	97/236 patients with head and neck; 68/238 patients with breast or chest wall	84/236 adenocarcinoma; 75/236 squamous cell carcinomas	38% (RT + HT); 46% (RT)	50–60 Gy
Valdagni et al. 1994 ^[24]	Italy	Yes	1985–1986	41	37–84, median 61	4–80 months, median: 12 months (RT), 18 months (RT + HT)	Metastatic squamous cell lymph nodes of the head and neck		No previous RT	
Vernon et al. 1996 ^[25]	Europe and Canada	Yes	1988–1993	306	61 ± 13	Minimum 5 months	30/306 patients with primary breast cancer and 276/306 recurrent, MRC Bri: 30/30 primary disease.	NA	120/171 (RT + HT); 90/135 (RT). ESHO 56/56: recurrent disease with previous RT.	NA
Jones et al. 2005 ^[13]	USA	Yes	1994–2001	108	Median 52.4 (RT + HT); 59.3 (RT)	NA	37/56 patients with breast/breast wall (RT + HT); 33/52 patients with breast/breast wall (RT)	NA	22/56 (RT + HT); 17/52 (RT)	NA
Wahl et al. 2008 ^[26]	USA	No	1993–2005	81	26–70, Median 48	1–144 months, median 12 months	Locally recurrent breast cancer with previous RT	NA	100%	19.6–82G, median 60 Gy
Huigol et al. 2010 ^[27]	India	Yes	2005–2009	56	31–78, median 58	61.5% < 6 months (RT), 42.8% 6–12 months (RT + HT)	Head and neck cancer	NA	NA	NA

*Interval since the last treatment (RT, surgery, chemotherapy, immunotherapy, HT)

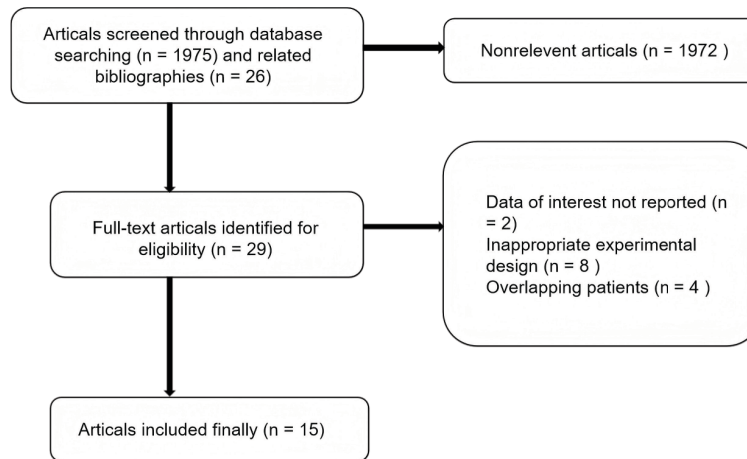


Figure 1. Flow diagram showing study eligibility and inclusion.

3.2. Metaanalysis

The meta-analysis of all patients in 19 groups from 15 articles showed that the addition of HT to RT resulted in significant improvement in CR versus the RT group ($OR = 2.393$, 95% CI 1.749–3.274, $p = 0.000$) with high heterogeneity ($\chi^2 = 33.67$, degrees of freedom (d.f.) = 18, $p = 0.014$, $I^2 = 46.5\%$; **Figure 2**). The application of the Egger test to individual trials did not reveal any publication bias (95% CI –0.810 to 3.604, $p = 0.199$; **Figure 3**). There was no factor that showed any significant relationship with heterogeneity in individual variable meta-regression analysis to explore the sources of heterogeneity (**Table 2**). We conducted three subgroup analyses in accordance with intended subsets and there was significant improvement in CR at each group ($OR = 2.170$, 95% CI 1.424–3.306, $p = 0.000$, and $\chi^2 = 17.10$, d.f. = 11, $p = 0.105$, $I^2 = 35.7\%$ for breast cancer; $OR = 4.980$, 95% CI 2.595–9.554, $p = 0.000$, and $\chi^2 = 1.92$, d.f. = 3, $p = 0.589$, $I^2 = 0.0\%$ for recurrent breast cancer; $OR = 2.994$, 95% CI 1.487–6.030, $p = 0.002$, and $\chi^2 = 14.56$, d.f. = 6, $p = 0.024$, $I^2 = 58.8\%$ for head and neck cancer; **Figure 4**).

Table 2. Results of meta-regression analysis

Covariate	Coefficient	95% CI		P
Year of publication	0.011	-0.038	0.060	0.640
Country (Asian or not)	0.314	-0.498	1.127	0.426
RCT	-0.286	-0.990	0.418	0.403
Number of patients	-0.003	-0.009	0.002	0.228
Primary disease (totally head and neck cancer)	0.680	-0.343	1.704	0.179
Previous RT	-0.516	-1.543	0.512	0.298
Type of HT (MV)	-0.239	-1.128	1.081	0.650
HT after RT	0.138	-1.672	1.948	0.872
Interval ≤ 30 min between RT and HT	0.172	-0.836	1.179	0.715
HT sessions (2/wk or not)	0.318	-0.564	1.199	0.454
HT duration ≥ 30 min	-0.124	-1.781	1.532	0.876

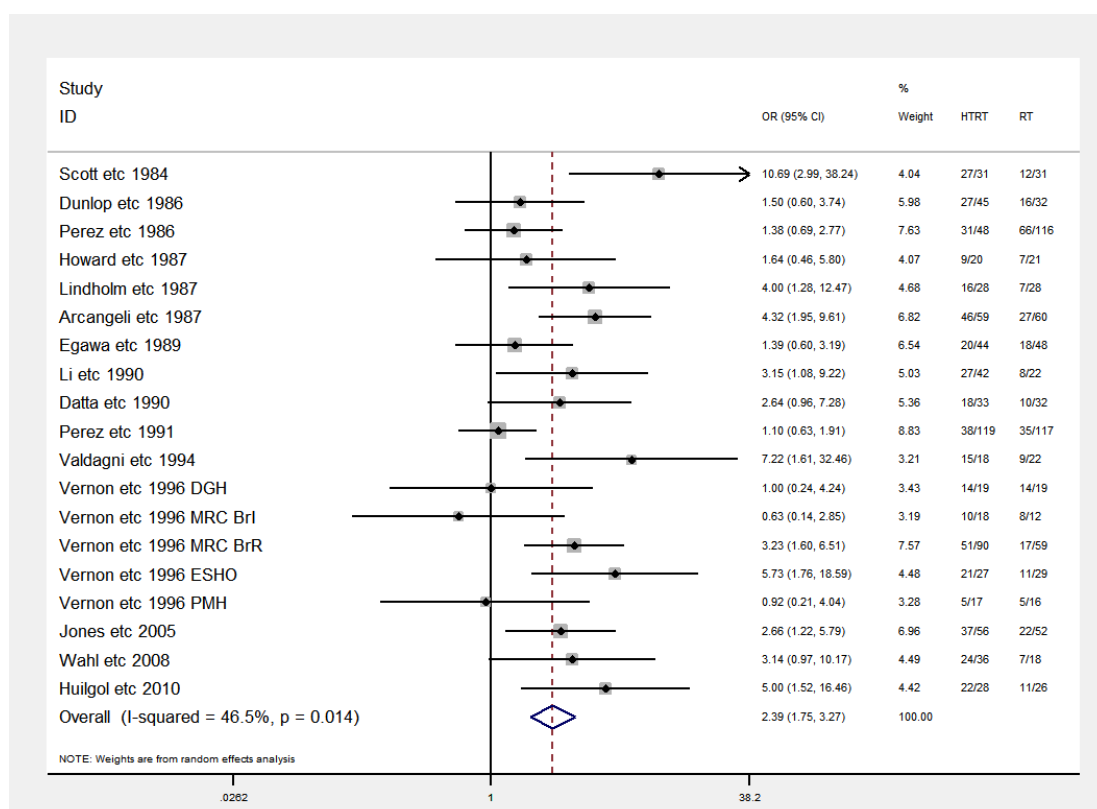


Figure 2. Forest plot of odds ratio (Hyperthermia + Radiation vs Radiation alone).Weights are from random effects analysis. Heterogeneity: $I^2 = 45.4\%$ (d.f. = 12), $p = 0.029$. The solid squares denote the mean difference, the horizontal lines represent the 95% confidence intervals (CIs), and the diamond denotes the weighted mean difference. Abbreviations: OR = odds ratio; HT = hyperthermia; RT = radiation therapy; d.f. = degrees of freedom.

* Complete response within 3 months of treatment.

** Complete response rates in the comparative study, including 56 tumors in 18 patients.

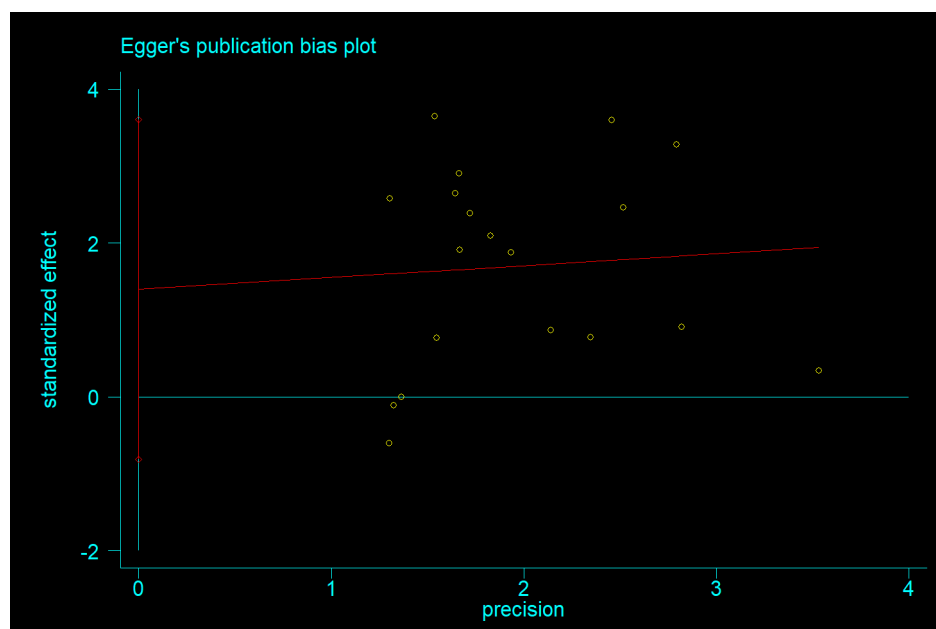


Figure 3. The Egger test for publication bias.95% confidence intervals (CIs): -1.445–3.550, $p = 0.379$.

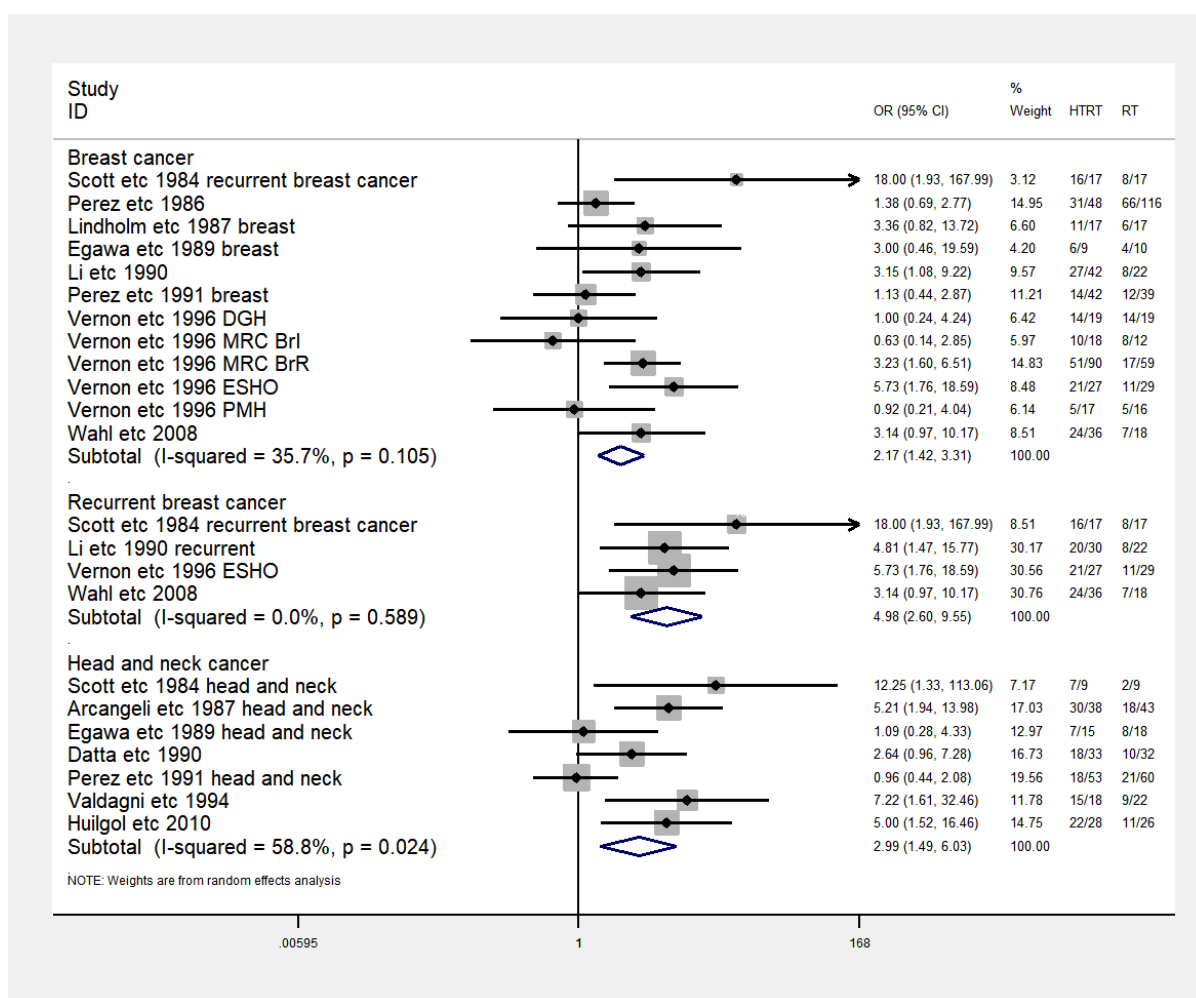


Figure 4. Forest plots of subgroup analyses based on different primary/recurrent disease status. Weights are from random effects analysis. Heterogeneity: breast cancer, $I^2 = 35.7\%$ (d.f. = 11), $p = 0.105$; recurrent breast cancer, $I^2 = 0.0\%$ (d.f. = 3), $p = 0.589$; head and neck cancer, $I^2 = 55.9\%$ (d.f. = 2), $p = 0.104$. The solid squares denote the mean difference, the horizontal lines represent the 95% confidence intervals (CIs), and the diamond denotes the weighted mean difference. Abbreviations: OR = odds ratio; HT = hyperthermia; RT = radiation therapy; d.f. = degrees of freedom.

3.3. Adverse events

By the overarching patient majority, both modalities of treatment were met with a level of tolerability not evoking substantial acute morbidity manifestations^[24]. The combined therapeutics manifested local erythema, thermal blistering, facial oedema, and pain occurrences alongside cessation instances, generally grade 1 to 2 in severity, resolved through conservative interventions^[13,15,22,23,25]. In patients treated by HTRT, instances of ulcerations appeared at a notable frequency (25% versus RT alone's 8%), and incidences of thermal burns were reported in 6% of thermally augmented recipients^[16]. Observedly, 15/33 tumors exhibited third or fourth-degree integumentary reactions subsequent to administration of hyperthermic treatment via 2450 MHz microwave sans coupling water bag system, juxtaposed against 3/24 under the regimen involving 915 MHz microwaves with a coupling deionized water bag system and refinement of microwave applicators, as well as the temperature control system^[18]. A discernible association emerges between the average maximum skin dose per treatment and the total skin reaction score^[17]. Notably, late arousal effects on skin manifest as skin atrophy with depigmentation or pigmentation,

telangiectasia, and fibrosis present minimal differential expression relative to varying treatment regimens ^[23,25,27]. Furthermore, HT integrated post-RT retains acceptable tolerance without significantly increasing either the clinically meaningful acute or long-term toxicity over HT alone.

4. Discussion

This study demonstrated the value of HT as a strategy for improving radiation efficacy with little additional harm. Improved local control in lesions less than 3 cm with HTRT has been elucidated as germane to precise thermal application. The difference between the number of HT treatments was examined with negative results. The group with recurrent breast cancer in this trial, for whom full-dose extra radiation could hardly be given ($OR = 4.980$, $p = 0.000$, and $\chi^2 = 1.92$, $I^2 = 0.0\%$), showed the most striking clinical benefit from adjuvant HT.

The phenomenon of hypoxic cellular environments engendering radiation resistance stands corroborated. Moderate HT alters tumorous microecology via augmentative shifts in vascular permeability combined with escalated oxygen tension levels. Despite enigmatic nuances veiling its mechanistic underpinnings, synergy in heightening radiative cytotoxicity remains incontrovertibly acknowledged. Combined HT and re-irradiation may be considered as a definitive treatment option for unresectable radiation-associated angiosarcoma of the breast, or as an adjuvant treatment, particularly in cases with positive resection margins or following surgery for local recurrence ^[28]. HTRT provides long-term, high local control rates with acceptable toxicity for patients with recurrent, newly diagnosed, unresectable, or resected breast cancer at high risk of relapse ^[29]. A systematic review supports the beneficial role of regional HT in the treatment of high-risk soft tissue sarcomas ^[30]. In some series, deep regional HT combined with initial transurethral resection and cisplatin-based chemoradiation has improved 5-year overall survival rates by up to 20% in bladder cancer ^[31]. HTRT with or without chemotherapy can improve local control and survival in various difficult-to-treat cancers and adequate reconstruction of HT applicators for treatment planning can further improve treatment quality.

When combined with standard therapies such as radiation or chemotherapy, HT can enhance tumor control rates and improve patient outcomes. This affordability and adaptability not only address economic constraints but also help reduce disparities in cancer care, ensuring that more patients, regardless of their location, can benefit from effective, comprehensive treatment strategies. There were several limitations in this study. First, it was difficult to establish a heat-response link because the recommended heat treatments in the different experiments varied greatly. Second, our study lacked recent studies about HT, maybe because of a lack of attention to HT in the medical field. A well-designed prospective multicentric trial is warranted to further improve HT performance and promote this technology, especially in underdeveloped areas.

5. Conclusion

A well-researched but maybe underutilized method, HT can have a major clinical impact by improving local tumor management. Additionally, the use of HT in conjunction with RT and chemotherapy may become more significant in the anti-cancer therapeutic arsenal.

Funding

Wenzhou Municipal Science and Technology Bureau (Project No.: Y2023913)

Disclosure statement

The authors declare no conflict of interest.

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Publisher's note

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Exploring the Treatment of Chemotherapy-Induced Myelosuppression with the Kidney-Tonifying, Essence-Replenishing, and Marrow-Fortifying Method Based on the Hematopoietic Stem Cell Homing Theory

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Abstract: Chemotherapy-induced myelosuppression is a common dose-limiting toxicity of chemotherapy for malignant tumors, and its core mechanism is closely related to hematopoietic stem cell homing disorder. Chinese medicine attributes chemotherapy-induced myelosuppression to the category of “medullary labor” and believes that “deficiency of kidney essence and drying up of the medulla oblongata” is the main pathogenesis of chemotherapy-induced myelosuppression, which is precisely the same as that of hematopoietic stem cell homing disorders revealed by modern medicine, which involves chemokines such as CXCL12/CXCR4 axis, adhesion molecules such as very late antigen-4 (VLA-4)/vascular cell adhesion molecule (VCAM), and other factors such as the CXCL12/CXCR4 axis, and the CXCL4/VCAM axis. This is highly compatible with modern medicine’s revelation of hematopoietic stem cell homing disorder. By activating signaling pathways such as *PI3K/Akt* and *Wnt/β-catenin*, up-regulating chemokine expression, enhancing cell adhesion, improving extracellular matrix remodeling, and alleviating oxidative stress, the method of tonifying the kidneys and filling in the marrow promotes the homing of hematopoietic stem cell through multiple pathways, which provides a new idea for the integration of Chinese and Western medicine in the treatment of chemotherapy-induced myelosuppression.

Keywords: Hematopoietic stem cell homing; Chemotherapy-induced myelosuppression; Kidney-tonifying; Essence-replenishing; Marrow-fortifying therapy

Online publication: December 31, 2025

1. Introduction

Malignant tumors constitute a major public health threat worldwide, with the evolving global disease burden attracting sustained attention from the international medical community ^[1]. According to GLOBOCAN data published by the WHO International Agency for Research on Cancer, China accounted for 25.5% of global new cancer cases and 26.5% of cancer-related deaths in 2022 ^[2].

As a primary treatment modality for malignancies, chemotherapy exerts its effects through broad-spectrum cytotoxic mechanisms. While effectively inhibiting the growth of malignant cells, it also causes non-selective damage to normal tissues with high metabolic activity. Among chemotherapy-related toxicities, myelosuppression stands out as a key dose-limiting factor due to its high incidence and complex clinical management. Typical manifestations include leukopenia, anemia, and thrombocytopenia ^[3]. Clinical studies report that the cumulative incidence of Grade ≥ 3 myelosuppression reaches 60.9% in small cell lung cancer patients treated with etoposide plus platinum-based regimens ^[4].

Current clinical management of Chemotherapy-induced myelosuppression (CIM) primarily follows NCCN guidelines, emphasizing growth factor support and blood component transfusion strategies. However, the time-sensitive limitations of pharmacological interventions and variations in patient tolerance remain significant therapeutic challenges. Consequently, exploring Traditional Chinese Medicine (TCM)-based prevention and treatment strategies for CIM holds considerable clinical importance.

Although bone marrow suppression following tumor chemotherapy is a concept derived from modern medicine, ancient TCM texts describe analogous conditions under the term “myelole” based on symptom location and clinical manifestations ^[5]. *Zhang’s Medical Compendium* states, “The source of blood lies in the kidneys,” elucidating the kidney’s role as the foundation of innate constitution—storing essence, generating marrow, and transforming essence and marrow into blood. Thus, for CIM patients, a common TCM pathogenesis chain is: “Chemotherapy drug toxicity invades internally, depleting kidney essence → marrow sea becomes depleted → blood transformation lacks its source.” Contemporary Western medical research on myelosuppression pathogenesis primarily focuses on impaired Hematopoietic Stem Cell (HSC) function and imbalances in the bone marrow microenvironment ^[6]. HSCs migrate directionally to injury sites via chemokine-mediated “homing” mechanisms, a process bearing substantial parallels with the TCM “kidney-essence-marrow-blood” pathogenesis chain. This paper explores the scientific rationale for treating CIM with kidney-tonifying, essence-replenishing, and marrow-nourishing therapy from this integrative perspective.

2. HSC homing impairment as a key mechanism in CIM

2.1. Definition of HSC homing

HSC homing refers to the biological process whereby endogenous human hematopoietic stem cells migrate from peripheral blood or the bone marrow microenvironment and colonize specific niches within the bone marrow during physiological homeostasis, tissue repair, or pathological injury. Its core mechanisms rely on chemokine signaling, adhesion molecule cascades, and precise regulation by the bone marrow microenvironment.

2.1.1. Chemokine guidance: the core role of the CXCL12-CXCR4 axis

The chemokine CXCL12, also known as stromal cell-derived factor-1 (SDF-1), secreted by bone marrow stromal cells (BMSCs), serves as the principal guiding signal for HSC homing. Binding of CXCL12 to the G protein-coupled receptor CXCR4 on HSC surfaces activates downstream signaling pathways, including *PI3K* and *Rho*

GTPases, driving cytoskeletal reorganization and polarization to facilitate directed migration^[7]. A decreasing concentration gradient of CXCL12 from the bone marrow toward blood vessels establishes a chemotactic gradient that guides HSCs from the circulation into the bone marrow. Furthermore, CXCL12 enhances HSC adhesion to endothelial cells by increasing the affinity of chemokine receptors such as very late antigen-4 (VLA-4) on CXCR4^[8].

2.1.2. Cascade of adhesion molecules

HSC homing depends on the sequential activation of multiple adhesion molecules. The process initiates with the rolling phase, where HSCs reversibly adhere through L-selectin on their surface, binding to P-selectin and E-selectin on vascular endothelial cells^[8]. This is followed by the stable adhesion phase, wherein integrins (e.g., VLA-4/ $\alpha 4\beta 1$, $\alpha \nu\beta 3$) bind to vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) on endothelial cell surfaces, enabling firm HSC adhesion to the vascular wall. The interaction between VLA-4 and VCAM-1 is particularly critical for HSC transendothelial migration^[9]. Finally, during the transendothelial migration phase, integrin $\alpha \nu\beta 3$ binds to fibronectin in the extracellular matrix, while matrix metalloproteinases degrade the basement membrane, collectively facilitating HSC penetration through the vascular wall into the bone marrow microenvironment^[10].

2.1.3. Biomechanical regulation: corticotropin-releasing hormone (CRH)-THBS2-Integrin $\alpha \nu\beta 3$ feedback loop

Emerging research highlights the importance of biomechanical properties such as HSC adhesion and deformability in homing. CRH binds to the CRHR1 receptor on HSC surfaces, activating a *RhoA*-dependent *YAP* nuclear translocation mechanism that upregulates the extracellular matrix protein THBS2. THBS2 binding to integrin $\alpha \nu\beta 3$ promotes F-actin polymerization, further enhancing *YAP* nuclear translocation. This establishes a mechanical feedback loop that significantly improves HSC adhesion, migration, and deformability^[11], offering a novel perspective on HSC mechanical remodeling within the bone marrow microenvironment.

2.1.4. Microenvironmental signal interactions

Various cells within the bone marrow microenvironment precisely regulate HSC homing through the secretion of specific factors. CXCL12-Abundant Reticular cells and arterial endothelial cells secrete CXCL12, multipotent growth factors, and insulin-like growth factor 1, providing crucial support for HSC migration and survival^[12]. Osteoblasts maintain HSC pluripotency by secreting CXCL12 and Jagged-1 (a *Notch* signaling pathway ligand), while interacting with integrin $\alpha \nu\beta 3$ on HSC surfaces to promote adhesion^[12]. Additionally, endothelial cells not only express adhesion molecules like VCAM-1 and ICAM-1 but also secrete SDF-1, collectively guiding HSCs through the vascular wall into the bone marrow microenvironment^[7].

2.1.5. Dynamic regulatory mechanisms

The HSC homing process is finely tuned by multiple dynamic mechanisms. Firstly, the local concentration of CXCL12 is tightly regulated: the G protein-coupled receptor GPR182 dynamically controls CXCL12 levels through endocytosis and degradation, thereby influencing HSC migration direction^[13]. Concurrently, dipeptidyl peptidase IV cleaves CXCL12, reducing its activity and modulating HSC homing efficiency^[14]. Secondly, adhesion molecule activity undergoes dynamic changes; for instance, proteases released by neutrophils (e.g., elastase) can cleave VCAM-1, disrupting HSC-endothelial adhesion and promoting HSC mobilization from bone

marrow into circulation ^[15]. Finally, synergistic interactions exist between mechanical and biochemical signals: the CRH-THBS2 pathway interfaces with the classical CXCL12-CXCR4 signaling axis, integrating mechanical stimuli with chemotactic signals to precisely coordinate HSC homing pathways ^[11].

2.2. HSC homing is closely linked to CIM

2.2.1. Chemotherapy-induced damage to the bone marrow microenvironment

Chemotherapeutic agents such as cytarabine and cyclophosphamide directly damage BMSCs and the extracellular matrix, disrupting vascular niche integrity and chemokine secretion, thereby impeding HSC homing. For example, cytarabine induces matrix cell apoptosis by inhibiting DNA synthesis, consequently reducing SDF-1 secretion ^[16]. The cyclophosphamide metabolite acrolein damages endothelial cells via oxidative stress, compromising niche integrity and impairing HSC migration and adhesion ^[17].

2.2.2. Abnormalities in chemokine signaling pathways

Chemotherapy significantly disrupts key signaling pathways essential for HSC homing. Firstly, the CXCL12/CXCR4 axis becomes dysregulated. CXCL12, secreted by BMSCs, binds to the CXCR4 receptor on HSCs, guiding their migration towards the bone marrow and maintaining their quiescent state. Chemotherapy disrupts this pathway through dual mechanisms: (1) Induction of stromal cell apoptosis, downregulating SDF-1 expression and weakening homing signals ^[18-19]; (2) Inhibition of CXCR4 function, potentially via direct suppression of its expression or interference with SDF-1 binding through phosphorylation ^[20]. For instance, cytarabine indirectly downregulates CXCR4 transcription by inducing DNA damage, thereby impairing HSC migration ^[16].

Secondly, adhesion molecule dysfunction occurs. HSC homing relies on the interaction between VLA-4 (integrin $\alpha 4\beta 1$) on HSCs' surface and VCAM-1 in the bone marrow microenvironment. Chemotherapy downregulates the expression of VLA-4 on HSCs' surface and/or microenvironmental VCAM-1, disrupting HSC-stromal adhesion and causing HSC retention in peripheral blood ^[18].

2.2.3. Disruption of inflammatory responses

Chemotherapy-induced inflammatory responses release abundant proinflammatory cytokines (e.g., TNF- α , IL-6), which significantly interfere with HSC homing through multiple pathways. Inflammatory factors suppress SDF-1 secretion: TNF- α and IL-6 inhibit SDF-1 transcription in BMSCs by activating the *NF- κ B* signaling pathway, thereby reducing its secretion and attenuating the key chemotactic homing signal ^[21]. Simultaneously, the inflammatory environment exerts complex effects on HSC survival and proliferation: acute inflammatory factors transiently activate HSC proliferation, whereas chronic inflammation persistently activates apoptotic pathways, ultimately reducing HSC numbers ^[22]. Furthermore, inflammatory mediators disrupt adhesion molecule function by downregulating VLA-4 expression on HSCs and VCAM-1 expression in the bone marrow microenvironment, further impairing HSC adhesion and migratory capacity ^[23].

2.2.4. Impairment of intrinsic HSC function

Chemotherapy drugs not only disrupt the bone marrow microenvironment but also directly impair intrinsic HSC functions. Firstly, chemotherapy induces HSC apoptosis and senescence. Chemotherapeutic agents activate the *p53* signaling pathway via DNA damage, triggering HSC apoptosis. For example, cytarabine inhibits DNA polymerase, leading to Caspase-3-dependent HSC apoptosis ^[24]. Additionally, chemotherapy induces oxidative stress, propelling

HSCs into a senescent state characterized by cell cycle arrest and functional decline. Concurrently, chemotherapy-induced autophagy dysfunction exacerbates HSC impairment: chemotherapeutic drugs inhibit Beclin1-dependent autophagy pathways, causing mitochondrial damage and increased HSC apoptosis. Moderate autophagy is crucial for clearing damaged organelles, thereby sustaining HSC survival and homing capacity^[22].

2.2.5. Pathways of HSC homing impairment leading to bone marrow suppression

HSC homing impairment ultimately leads to bone marrow hematopoietic failure through a triple pathological network of “cell behavior disorder-molecular signaling dysregulation-microenvironment vicious cycle,” specifically manifested as:

(1) Cascade of HSC Retention and Apoptosis

Homing impairment causes HSCs to persist in peripheral blood or the spleen, triggering a chain reaction. For instance, the cyclophosphamide metabolite acrolein downregulates VLA-4 expression via oxidative stress, disrupting HSCs’ adhesion to VCAM-1 and impairing rolling and anchoring capacity^[25]. Chemotherapy-induced iron overload and reactive oxygen species (ROS) accumulation inhibit the mitochondrial respiratory chain, reducing membrane potential, decreasing ATP production, and shifting metabolism toward inefficient glycolysis, thereby triggering caspase-3-dependent apoptosis^[26]. Furthermore, chemotherapy induces HSC senescence via the p38 MAPK-*p16INK4a* pathway, and secreted inflammatory cytokines like IL-6 further suppress stromal repair^[27].

(2) Molecular Networks Underlying Delayed Hematopoietic Reconstitution

Insufficient bone marrow HSCs lead to defective hematopoietic progenitor cells. Downregulation of the *Notch* ligand by stromal cells reduces Intracellular Notch nuclear translocation and *Hes1* transcription in HSCs, causing premature differentiation and depletion of the stem cell pool^[28]. Retained HSCs may undergo abnormal differentiation in ectopic microenvironments like the spleen, associated with dysregulated activation of the CXCL12/CXCR4 axis^[29]. Chemotherapy also disrupts megakaryocyte-endothelial cell contacts, reducing thrombopoietin secretion; clinical studies confirm that thrombopoietin receptor agonists (e.g., eltrombopag) shorten platelet recovery time by activating the JAK2/STAT5 pathway^[30].

(3) Vicious Cycle of Impaired Microenvironment Repair

Impaired homing suppresses microenvironment regeneration, creating a “damage-failed repair” positive feedback loop. Chemotherapy induces stromal cell senescence, increasing secretion of small extracellular vesicles carrying resistance proteins like ABCB4, which inhibit HSC homing and promote tumor resistance^[31]. Increased numbers of bone marrow adipocytes secrete leptin or adiponectin, inhibiting HSC migration and proliferation; PPAR- γ agonists promote adipogenesis, further delaying reconstruction^[32]. Chemotherapy disrupts sinusoidal endothelial integrity, reducing Ang-1/VEGF secretion; studies indicate that CRH activates the *RhoA/YAP* pathway to promote HSC secretion of THBS2, enhancing endothelial adhesion and improving vascular niche repair^[25].

3. “Deficiency of kidney essence and drying of the sea of marrow” is the primary pathomechanism of CIM

3.1. Physiological relationship between the kidney and the bone marrow

The kidney, considered the foundation of innate constitution, governs storage and conservation. Its essence serves as the source for life’s transformation and nurturing, embodying the capacity to refine essence into blood. As stated in *Suwen*: Great Treatise on Yin-Yang Correspondences and Manifestations, “The kidney generates bone marrow”^[33], clearly indicating that kidney essence is the fundamental source for the transformation and generation

of the marrow sea. Kidney essence is the shared origin of both marrow and blood. *Ling Shu*: Decision on Qi notes, “The middle burner receives qi and extracts its essence, transforming it into red substance; this is called blood”^[34]. However, this process fundamentally relies on the warming action of kidney yang and the nourishing power of kidney essence. The innate essence, inherited from parents, is stored within the kidneys; the acquired essence, derived from food and water, is transported by the spleen and also stored in the kidneys. These two essences intermingle and transform, descending into the bones to establish the mechanism of “essence transforming into marrow, marrow generating blood.” This aligns with the principle in *Essentials of Integrating Chinese and Western Medical Classics*: “When essence is abundant, marrow is plentiful; when essence is deficient, marrow is depleted”^[35], clearly demonstrating that the prosperity or decline of kidney essence is the pivotal mechanism governing bone marrow abundance or deficiency. The function of the kidney essence transforming into marrow and generating blood can also be understood through the theory of “essence and blood sharing the same origin.” Bone marrow resides within bone cavities and is nourished and enriched by kidney essence.

3.2. Pathogenesis of medicinal toxins depleting kidney essence

Chemotherapy drugs fall under the category of “medicinal toxins” in TCM theory, characterized by a violent nature, heavy taste, and turbid substance. While such toxic substances possess potent efficacy in overcoming pathological obstacles, their indiscriminate action readily damages vital Qi. *Treatise on the Origins and Manifestations of Various Diseases*: Symptoms of Improper Medication Use states: “Medicines have both synergistic and antagonistic effects; if used improperly, they inevitably lead to disaster”^[36]. This theory reveals that using toxic medicines to attack pathogens is a double-edged sword: excessive use may eliminate pathogens but will deplete vital qi. Chemotherapy toxicity first invades the Middle Jiao, impairing the pivotal functions of the spleen and stomach. It then descends along the Triple Burner pathways to the Shao Yin, ultimately depleting kidney essence. This progression aligns with the principle in *Jingyue Quanshu*: Deficiency and Debility that “injuries to the five Zang organs, when exhausted, inevitably reach the kidneys”^[37], revealing the fundamental truth that organ deficiency ultimately stems from kidney essence depletion.

The mechanism by which drug toxicity injures the kidneys centers on the depletion of kidney yin, which impairs the storage of kidney essence. Drug toxins possess a violent nature: they exhibit fiery properties that readily scorch true yin and possess a destructive force that easily depletes vital qi. *Suwen*: The Great Treatise on the Five Constant Policies advises: “When treating disease with potent toxins, remove six parts out of ten... Do not overdo it, lest you injure the healthy Qi”^[33]. Overuse of chemotherapy drugs is akin to removing the fuel from beneath the cauldron, damaging both yin and yang within the kidneys. The failure to contain the ministerial fire leads to depletion of kidney yin. Furthermore, as Ye Tianshi stated: “Initially, disease resides in the meridians; prolonged disease enters the collaterals”^[38]. The cumulative toxicity of chemotherapy over time damages the kidney collaterals and impairs their storage and concealment functions.

The proliferation of secondary syndromes stems from kidney essence deficiency, triggering a chain reaction among the Zang-fu organs. Kidney essence, as the root of life, houses true Yin and true Yang. Chemotherapy damages kidney essence, disrupting the mutual support between Yin and Yang. When yin damage affects yang, symptoms include aversion to cold, cold limbs, and frequent nocturnal urination. When yang damage affects yin, symptoms manifest as restless insomnia, tidal fevers, and night sweats. Furthermore, depletion of essence and marrow leads to soreness in the lower back and knees, loose teeth, and hair loss. Failure of essence to transform into Qi manifests as mental fatigue, weakness, shortness of breath, and reluctance to speak. These diverse

symptoms all originate from kidney essence damage, causing Yin-Yang imbalance.

3.3. Pathological process of kidney essence deficiency leading to bone marrow exhaustion

Ling Shu: Treatise on the Sea states: “When the marrow sea is abundant, one becomes light and vigorous, possessing great strength”^[34]. Although this refers specifically to cerebral marrow, bone marrow shares the same origin and is similarly derived from kidney essence. Chemotherapy drugs deplete the true yin within the kidneys, damaging both the innate essence and hindering the return of the acquired essence to its storehouse, thereby obstructing the transformation of essence into marrow. Clinically, patients often exhibit dry hair, loose teeth, and dull nails, indicating not merely blood deficiency but fundamentally reflecting essence depletion failing to generate marrow, resulting in insufficient marrow to nourish the body. Symptoms such as nighttime fever subsiding by morning and bone-steaming night sweats indicate essence depletion, marrow reduction, and the manifestation of floating deficient yang.

The toxic nature of these drugs, once they enter the collaterals, is violent and aggressive. Initially, they predominantly deplete qi and injure yin. Over time, they penetrate deeper into the Lower Jiao, plundering the kidney’s true yin and severely damaging the innate essence. Deficiency of essence leads to a depleted source for marrow transformation, an empty marrow sea, and a failure of the marrow collaterals to receive nourishment from the essence. Kidney essence deficiency manifests in several ways: Firstly, the marrow channels become depleted, akin to a dried-up riverbed where water cannot flow, preventing the transport of vital substances through the marrow pathways to nourish bone marrow and generate new blood. Secondly, the mechanisms of transformation and production are obstructed. Kidney essence is the foundation for marrow generation; its depletion weakens the capacity to produce marrow, slows new marrow generation, and impairs the source for hematopoietic reconstruction. Thirdly, internal disturbance by deficient fire consumes vital substances, leading to concurrent essence and marrow depletion. Fourthly, the essence gate becomes unstable, disrupting opening and closing mechanisms, causing leakage of vital substances and ultimately resulting in the insidious depletion of marrow essence.

In summary, medicinal toxins deplete kidney essence, causing essence deficiency and marrow depletion alongside impaired storage functions. This core pathological outcome aligns closely with modern medical findings of bone marrow microenvironment disruption, signaling pathway dysfunction, and HSC functional failure—all manifestations of HSC homing disorders.

4. HSC homing impairment: the modern biological essence of “kidney essence deficiency and drying of the marrow sea”

4.1. Deep coupling between the kidney essence-to-marrow conversion mechanism and the HSC homing microenvironment

The classical assertion in *Suwen*: Yin-Yang Correspondence and Phenomenon Theory that “the kidney generates bone marrow” reveals scientific implications transcending its era in the field of hematopoietic regulation. When delving into the modern implications, we find that kidney essence, as the primordial source of life, nourishes bone marrow through a physiological process that fundamentally achieves precise regulation of HSCs by constructing the bone marrow microenvironment. Research by Tang Chao revealed that normal BMSCs secrete the chemokine SDF-1, forming molecular pathways that guide HSC homing. This aligns with the principle in *Essentials of Medical Classics Integrating Chinese and Western Medicine*: “When essence is abundant, marrow is plentiful.”

Robust kidney essence ensures the functional integrity of the bone marrow microenvironment, comprising osteoblasts, endothelial cells, and others. The SDF-1/CXCR4 signaling axis, as a key chemotactic guidance system, effectively mediates the directed migration of HSCs toward bone marrow vascular niches and maintains their quiescent state ^[16]. Chemotherapy drugs damage stromal cells, significantly reducing SDF-1 secretion and disrupting the chemotactic gradient essential for HSC homing. This molecular-level alteration concretely embodies the TCM theory that “deficiency of essence leads to depletion of marrow.” The TCM theory of “essence and blood sharing the same origin” also finds a new interpretation within the adhesion molecule system. The VLA-4/VCAM-1 interaction, essential for HSC homing, can be viewed as the biological vehicle through which kidney essence transforms into Qi and blood, sustaining normal intercellular communication and positioning. Chemotherapy downregulates VLA-4 and VCAM-1 expression, causing failure of HSC adhesion to stromal cells, resulting in HSC retention in peripheral blood and failure to return to their niche. This pathological process resonates with the theory in *Ling Shu*: Jue Qi that “the Middle Jiao requires kidney essence to nourish the transformation of fluids into blood” ^[34]: abundant kidney essence ensures the integrity of the adhesion molecule network, guaranteeing effective HSC homing; deficient kidney essence leads to inadequate marrow nourishment, causing disruption in adhesion molecule expression or function.

4.2. Cascading damage to the homing signal network via pathways of drug-induced kidney injury

The rampant toxicity of drugs, with its fierce nature akin to a blazing fire penetrating the Lower Jiao, not only scorches kidney yin but also directly depletes the essence within the kidneys. Pharmaceutical toxins directly damage HSCs’ genetic material, activating *p53*-dependent apoptosis and senescence cascades, causing stem cell pool depletion ^[24], corresponding to the depletion of true yin and deficiency of essence and marrow. Simultaneously, the inflammatory cytokine storm persistently suppresses the secretion of the key homing factor SDF-1 by BMSCs via the *NF-κB* signaling axis, while disrupting the adhesion molecule network that anchors HSCs within the bone marrow niche ^[21,23]. This manifests as deficient fire disturbing the essence of the bone marrow.

The profound depletion of kidney essence and the emptying of the marrow sea represent the inevitable culmination of pathological progression. At the modern biological level, this manifests as a vicious cycle of failed HSC homing. HSCs trapped in peripheral blood, deprived of the nourishment and protection of the bone marrow microenvironment, become exposed to metabolic toxins such as iron overload and ROS accumulation, accelerating their apoptotic process ^[26]. The dwindling reserve of residual HSCs within the bone marrow leads to downregulation of critical signaling pathways like *Notch*, forcing them into abnormal differentiation to meet urgent hematopoietic demands, further depleting the stem cell pool ^[28]. Compounding this, the damaged bone marrow microenvironment becomes infiltrated by adipose tissue, which abnormally secretes factors like leptin. Rather than supporting repair, these factors become pathological signals inhibiting hematopoietic reconstruction ^[32]. These pathological mechanisms result in the failure of kidney essence to store and protect, profoundly suppressing and extinguishing the fundamental function of bone marrow hematopoiesis—a state recognized in TCM as “essence gate not secure, biochemical mechanisms suppressed.”

4.3. Pathological resonance between essence deficiency and marrow exhaustion with HSC functional failure

Extreme deficiency of kidney essence ultimately diminishes HSC survival capacity. Cytarabine activates the

p53 pathway, triggering caspase-3-dependent apoptosis, corresponding to the depletion of true yin followed by false fire scorching the marrow, leading to a sharp reduction in the number of HSCs. Chemotherapy-induced iron overload and ROS accumulation cause collapse of the HSC mitochondrial membrane potential, shifting energy metabolism from efficient oxidative phosphorylation to inefficient glycolysis^[23,26]. This phenomenon aligns with the traditional understanding of “insufficient transformation of kidney essence into Qi,” diminished kidney qi fails to provide adequate warmth and propulsion, leading to functional decline of HSCs due to energy depletion. The activation of HSC senescence programs represents the ultimate manifestation of marrow depletion. The *p38* MAPK-*p16INK4a* pathway-driven cell cycle arrest resembles marrow duct obstruction, causing HSCs to lose their self-renewal capacity. Furthermore, factors secreted by senescent cells, such as IL-6, further inhibit matrix repair^[27]. This phenomenon resonates with Wang Qishi’s admonition in *Li Xu Yuan Jian*: “When essence and blood cannot be rapidly replenished, the primordial energy must be urgently preserved.” The abrupt depletion of kidney essence severs the root of marrow generation, leaving HSC homing and regenerative functions like water without a source. Ultimately, the marrow channels become depleted, leading to terminal decline.

5. Kidney-tonifying method to restore essence and enrich marrow for CIM treatment

The core pathological mechanism of CIM is closely linked to impaired HSC homing. TCM’s theory of kidney essence deficiency offers a unique perspective to elucidate this pathogenesis. The kidneys govern bones and produce marrow; kidney essence serves as the material foundation for marrow generation. Chemotherapy drugs, as agents that attack pathogens, readily damage kidney essence, leading to depletion of the marrow sea and a lack of source for Qi and blood production. Modern research indicates that HSC homing relies on precise regulation of cytokine networks (e.g., CXCL12/CXCR4, SCF/*c-Kit* pathways) and stromal cell function within the bone marrow microenvironment^[7]. The method of tonifying the kidneys, replenishing essence, and enriching marrow can promote HSC homing through multi-target regulation, thereby improving bone marrow suppression.

5.1. Pathological correlation between kidney essence deficiency and HSC homing impairment

In TCM theory, the physiological process of “the kidney stores essence, essence generates marrow, and marrow transforms into blood” exhibits functional homology with HSC proliferation, differentiation, and homing within the bone marrow microenvironment. As stated in *Ling Shu*: Decision on Qi, “Essence is the foundation of the body.” Sufficient kidney essence nourishes the bone marrow microenvironment, ensuring normal HSC homing. Kidney essence deficiency disrupts BMSC function, leading to insufficient chemokine secretion and abnormal adhesion molecule expression, thereby hindering HSC-directed migration to bone marrow niches. Modern research reveals that chemotherapeutic agents like cyclophosphamide significantly downregulate CXCL12 expression in BMSCs and reduce the activity of the HSC homing receptor CXCR4, causing abnormal circulation of HSCs in peripheral blood and preventing their homing^[17]. The method of “tonifying kidney essence and replenishing marrow” centers on “nourishing kidney essence and enriching the marrow sea.” By replenishing kidney essence, this approach improves the secretory function of stromal cells and restores normal regulation of signaling pathways such as CXCL12/CXCR4, thereby creating a suitable microenvironment for HSC homing.

5.2. Molecular mechanisms of kidney-essence-replenishing and marrow-enhancing methods in promoting HSC homing

5.2.1. Chemokine system regulation: Activation of the SDF-1/CXCR4 axis

Kidney-tonifying formulas (e.g., Jisheng Shenqi Tang, Shenfu Tang) significantly upregulate the expression of SDF-1 and its receptor CXCR4 in the bone marrow microenvironment, forming a chemotactic gradient that guides the directed migration of HSCs. Animal studies demonstrate that after Jisheng Shenqi Tang intervention, SDF-1 and CXCR4 protein expression significantly increase in the liver tissue of cirrhotic rats. Concurrently, levels of hematopoietic factors such as EPO and G-CSF in peripheral blood are upregulated, promoting BMSC migration and homing. This process relies on activation of the *PI3K/Akt* signaling pathway, which enhances HSC migratory capacity by regulating actin reorganization and cytoskeletal remodeling^[39]. Furthermore, SDF-1 binding to CXCR4 activates the *RhoA/YAP* pathway, promoting the expression of extracellular matrix proteins and further enhancing HSC adhesion and mechanical remodeling capabilities^[25].

5.2.2. Enhanced adhesion molecule expression: CD44/CD62L-mediated cell adhesion

The kidney-tonifying method enhances HSC-myeloid endothelial cell interactions by modulating surface adhesion molecule expression. For instance, Shenshutang significantly increased the surface expression of CD44 and CD62L on HSCs, molecules that mediate HSC adhesion to and transendothelial migration through BMSCs, respectively. Flow cytometry analysis revealed that after Shenfu Tang intervention, the proportion of the Sca-1+ cells in the bone marrow of transplanted mice significantly increased, with CD44 and CD62L expression rates elevated by approximately 20%–30% compared to the control group^[40].

5.2.3. Signal pathway activation: Synergistic effects of Wnt/ β -Catenin and PI3K/Akt

Traditional Chinese kidney-tonifying herbs regulate HSC proliferation, migration, and differentiation by activating signaling pathways such as *Wnt*/ β -catenin and *PI3K/Akt*. Zouguiwan-containing serum significantly upregulates *Wnt* and *Oct4* gene expression while suppressing tumor suppressor genes like *PI6INK4a*, thereby maintaining stem cell self-renewal capacity^[41]. Icaritin promotes osteoblast differentiation of BMSCs by activating the *BMP-2/Smad* pathway while enhancing their migratory capacity^[42]. Osteoblasts, as key components of the bone marrow microenvironment, when increased in number and function, help maintain and improve niche structures and signaling molecules that support HSC homing.

5.2.4. Epigenetic regulation: Interaction between miRNA and Notch pathway

Researchers studying the proprietary formula “Kidney-Tonifying, Blood-Activating, Nutrient-Regulating, Phlegm-Resolving Decoction” found it promotes BMSC homing and differentiation by downregulating *miRNA-139-5p* expression, thereby releasing its inhibition on the *Notch* pathway. Animal studies demonstrated that after intervention with this formula, *Notch* pathway activity in the bone marrow of asthma model mice significantly increased, with BMSC migration capacity improving by approximately 40%^[43]. Additionally, Zuoguiwan can delay HSC senescence by regulating DNA methylation and histone modifications to maintain the expression of pluripotency-related genes^[41].

6. Conclusion

CIM represents a common challenge in tumor therapy, with its core mechanism in modern medicine closely linked

to impaired HSC homing. Building upon the TCM pathogenesis theory of “kidney essence deficiency leading to depletion of the marrow sea,” this paper substantiates the scientific rationale for treating CIM with the method of tonifying the kidney, replenishing essence, and nourishing marrow. This therapeutic approach cultivates innate essence and nourishes the marrow as the source of transformation. At the modern biological level, it manifests as multi-target regulation of the HSC homing microenvironment: upregulating SDF-1/CXCR4 chemotactic axis expression, enhancing the function of adhesion molecules like VLA-4/VCAM-1, activating key signaling pathways including *PI3K/Akt* and *Wnt/β-catenin*, improving bone marrow stromal function, and mitigating oxidative stress and inflammatory damage. These actions synergistically promote directed migration, anchoring, and homing of HSCs, thereby accelerating hematopoietic reconstitution. This study integrates the TCM theoretical chain of “kidney-essence-marrow-blood” with the modern mechanisms of HSC homing. It not only deepens the understanding of the action mechanisms of the kidney-tonifying, essence-nourishing, and marrow-strengthening method but also provides new theoretical foundations and research approaches for the integrated Chinese and Western medicine prevention and treatment of CIM.

Currently, this method is widely applied in treating CIM induced by cancers such as lung cancer, breast cancer, and lymphoma, with commonly used formulas including Liuwei Dihuang Wan, Guilu Erxian Tang, Zuogui Wan, and Yougui Wan. However, existing research still faces several critical issues: Firstly, studies on HSC homing mechanisms largely remain confined to animal experiments, with limited clinical monitoring data on HSC dynamic changes. Secondly, the complex composition of TCM formulations makes the synergistic interactions between individual compounds and compound combinations unclear. Finally, efficacy evaluation standards remain inconsistent, particularly lacking reliable quantitative indicators for assessing HSC homing efficiency.

Looking ahead, future research should integrate advanced technologies like single-cell sequencing and in vivo imaging to explore how kidney-tonifying herbs precisely regulate the HSC homing microenvironment. Concurrently, multi-center, large-scale clinical studies should be promoted to provide more robust evidence for standardizing this therapeutic approach. From the traditional theory of “the kidneys govern bone and produce marrow” to the modern biological mechanism of HSC homing, the method of tonifying the kidneys, replenishing essence, and nourishing marrow not only validates the foresight of TCM theory but also pioneers a distinctive pathway for supportive treatment in malignant tumors.

Funding

This article was supported by the Scientific Research Program of Administration of Traditional Chinese Medicine of Hebei Province, entitled "Distribution Law of TCM Syndrome Elements in Tumor Therapy-Induced Thrombocytopenia and Observation on Clinical Efficacy of Shengban Fuyuan Decoction"(No.T2026091).

Disclosure statement

The authors declare no conflict of interest.

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Application and Nursing Care of Mid-Length Catheters in the Infusion of Oxaliplatin in Gastrointestinal Tumor Patients Refusing Central Venous Catheterization

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Abstract: *Objective:* To investigate the application effectiveness and nursing care of mid-length catheters (MCs) in the infusion of oxaliplatin in gastrointestinal tumor patients who refuse central venous catheterization. *Methods:* A total of 71 patients with gastrointestinal tumors who were treated in our hospital from August 2024 to June 2025 were selected. All of them refused central venous catheterization due to subjective willingness and voluntarily accepted MC insertion for oxaliplatin chemotherapy. The MC insertion status of the patients was recorded, and the incidence of catheter-related complications during chemotherapy and the quality of life before and after intervention were observed. *Results:* The catheterization success rate among the 71 patients was 97.18%; the average catheterization time was (18.25 ± 1.12) minutes, and the average catheter indwelling time was (12.64 ± 4.58) days; a total of 5 catheter-related complications occurred during chemotherapy, with an overall incidence rate of 7.04%, all of which were mild to moderate complications, and no severe complications occurred; the quality of life score after intervention was significantly higher than that before intervention ($P < 0.05$). *Conclusion:* The application of mid-length catheters in the infusion of oxaliplatin in gastrointestinal tumor patients who refuse central venous catheterization offers advantages such as a high catheterization success rate, long indwelling time, low complication rate, and improved quality of life for patients. Combined with targeted nursing measures, it can further ensure medication safety.

Keywords: Medium-length catheter; Gastrointestinal tumor; Nursing; Complications

Online publication: December 31, 2025

1. Introduction

Chemotherapy is an important approach in the comprehensive treatment of gastrointestinal tumors. Oxaliplatin, as a third-generation platinum-based chemotherapy drug, exhibits certain intravenous irritancy. Clinically, it is

often recommended to administer the drug through central venous catheterization or peripherally inserted central catheters (PICCs) ^[1]. However, some patients with gastrointestinal tumors subjectively refuse central venous catheterization due to concerns about catheter-related trauma, infection risks, limitations in daily activities, and financial burdens, resulting in clinical dilemmas in selecting venous access routes ^[2]. Although direct peripheral venous infusion of oxaliplatin is simple to perform, the incidence of venous irritation reactions can be as high as 30% to 50%, which not only increases patient discomfort but may also affect the chemotherapy process due to deteriorating vascular conditions ^[3]. The medium-length catheter (MC), as a novel venous access device, combines the ease of operation of peripheral venous catheters with the safety and reliability of central venous catheters. It features minimal catheter-related trauma, convenient nursing care, and lower costs, and has gradually been applied in patients requiring medium- to long-term intravenous therapy ^[4]. Currently, further validation is needed regarding the application effects, complication prevention and control, and targeted nursing measures of medium-length catheters in chemotherapy for patients with gastrointestinal tumors. This study selected 71 patients with gastrointestinal tumors who refused central venous catheterization, employed MC insertion for oxaliplatin infusion, and implemented targeted nursing interventions to systematically explore its clinical application value and provide a safer and more feasible venous access solution for such patients.

2. Materials and methods

2.1. General information

Seventy-one patients with gastrointestinal tumors who were treated in our hospital from August 2024 to June 2025 were selected, including 42 males and 29 females; aged 38 to 74 years old, with an average age of (56.32 ± 9.45) years old; disease types: 48 cases of colorectal cancer and 23 cases of gastric cancer; clinical stages: 18 cases in stage II, 35 cases in stage III, and 18 cases in stage IV; chemotherapy cycles: 6 to 12, with an average of (8.45 ± 1.03) cycles.

2.2. Inclusion and exclusion criteria

Inclusion criteria: (1) Diagnosed with gastrointestinal tumors via histopathological examination and requiring an oxaliplatin-based chemotherapy regimen; (2) Aged 18 to 75 years; (3) Explicitly refusing central venous catheterization due to subjective willingness and voluntarily accepting the insertion of a medium-length catheter; (4) Having clear consciousness and being able to cooperate with catheter insertion and nursing procedures; (5) Expected to undergo ≥ 2 chemotherapy cycles and requiring medium- to long-term intravenous access support.

Exclusion criteria: (1) Severe coagulation disorders; (2) Infection, rash, ulcer, or severe skin diseases at the puncture site; (3) Superior vena cava syndrome or a history of venous thrombosis in the limb where the catheter is to be inserted; (4) Mental illness or cognitive impairment that prevents cooperation; (5) Allergy to catheter materials or local anesthetics.

2.3. Methods

All patients received a chemotherapy regimen featuring oxaliplatin. Catheter placement method: The catheter placement procedure was performed by specialized intravenous therapy nurses who had undergone specific training, utilizing the ultrasound-guided modified Seldinger technique. The basilic vein was the first choice, followed by the median cubital vein and the cephalic vein. The patient was placed in a supine position with the upper limb abducted at 90 degrees, and routine disinfection and draping were performed. The target vessel was

located using an ultrasound probe. After the anesthesia took effect, the puncture needle was inserted into the target vessel along the long axis of the vessel under ultrasound guidance. After blood return was observed, the puncture needle was secured, and a guidewire was slowly inserted before the puncture needle was withdrawn. A dilator was inserted along the guidewire to dilate the subcutaneous tissue and then withdrawn. A medium-length catheter was slowly inserted to the predetermined length along the guidewire, after which the guidewire was withdrawn. After blood return was observed upon aspiration, the catheter was flushed with saline in a pulsatile manner to confirm its patency. Ultrasound examination confirmed that the catheter tip was located in the subclavian vein. The external portion of the catheter was secured in a “U” shape and covered with a sterile transparent dressing. Finally, the catheter was sealed with saline using pulsatile positive pressure.

Nursing measures:

- (1) Pre-catheterization nursing: Before catheter placement, the advantages of inserting a central venous catheter were re-emphasized to the patient. If the patient refused, they signed an informed consent form refusing central venous catheter placement. The advantages of the medium-length catheter (MC), the catheter placement procedure, possible discomfort, and coping methods were explained in detail to the patient and their family. The risks associated with peripheral intravenous infusion were compared to alleviate the patient's anxiety. The patient was informed of key points for cooperation during catheter placement and signed an informed consent form for medium-length catheter insertion.
- (2) Nursing care during catheter placement: Adjust the temperature of the ward to 22–24 °C. Provide soft cushions to support the patient's limbs and distract their attention. During catheter placement, closely observe the patient's complexion, facial expressions, and vital signs, communicate promptly, and address any discomfort. Ensure sterile operation to prevent contamination.
- (3) Nursing care after catheter placement: Prevention and management of complications: Phlebitis: Encourage appropriate arm movement after catheter placement; promptly provide symptomatic treatment if phlebitis occurs. Thrombosis formation: Instruct patients to perform functional exercises such as clenching and releasing their fists with the upper limb on the puncture side to avoid prolonged immobilization; closely observe for limb swelling, pain, and increased skin temperature, regularly monitor vascular ultrasound, and promptly administer anticoagulant therapy if thrombosis is detected. Catheter occlusion: Strictly adhere to flushing and sealing protocols to avoid drug incompatibilities; in case of occlusion, never forcefully inject; first, attempt to aspirate the catheter with a syringe to clear the blockage; if unsuccessful, use urokinase for thrombolysis. Infection: Strengthen care at the puncture site and strictly adhere to sterile procedures; observe for redness, swelling, heat, pain, and purulent discharge at the puncture site, regularly monitor blood tests, promptly remove the catheter and administer antibiotics if infection is detected. Blood or fluid leakage: After catheter placement, press the puncture site for more than 5 minutes and closely observe for any bleeding; if significant bleeding occurs, promptly change the dressing and apply pressure bandaging. Inform patients to avoid exposure to cold stimuli to prevent peripheral neurotoxicity; in case of drug extravasation, immediately stop the infusion, retain the catheter, aspirate residual drugs, apply a 50% magnesium sulfate cold compress, and, if necessary, administer a local block. Provide patients and their families with an MC maintenance manual and explain relevant precautions in detail. Establish a follow-up file and conduct regular follow-ups to understand catheter usage and the occurrence of complications.

2.4. Observation indicators

2.4.1. Catheterization-related indicators

Success rate of catheterization, catheterization time (from the start of disinfection to completion of fixation), duration of catheter indwelling (from successful catheterization to catheter removal), and selection of catheterization site.

2.4.2. Incidence of complications

Phlebitis, thrombosis, catheter occlusion, infection, bleeding and exudation, catheter displacement/dislodgement, etc.

2.4.3. Quality of life

Assessed using the Quality of Life Questionnaire-Core 30 (QLQ-C30) for cancer patients, which includes five dimensions such as physical functioning, role functioning, and emotional functioning. The total score is 100, with higher scores indicating better quality of life.

2.5. Statistical methods

Data are presented as mean \pm standard deviation (SD) for t-tests and continuous variables, all analyzed using statistical software (SPSS 24.0). A *P*-value less than 0.05 is considered statistically significant.

3. Results

3.1. Catheterization-related indicators

Out of the 71 patients, catheter placement was successful in 69 cases, resulting in a success rate of 97.18%. Catheter placement failed in 2 cases, with one failure attributed to vasospasm and the other to difficulty in guidewire insertion. After the failures, catheter placement was successfully performed on the contralateral upper limb. The average catheter placement time was (18.25 ± 1.12) minutes, and the average catheter indwelling time was (12.64 ± 4.58) days, with the longest indwelling time being 16 days and the shortest being 3 days. There were no interruptions in chemotherapy due to catheter-related issues.

3.2. Incidence of catheter-related complications

During chemotherapy, a total of 5 catheter-related complications occurred, with an overall incidence rate of 7.04%. All complications were mild to moderate in severity, and no severe complications were reported. See **Table 1**.

Table 1. Incidence of catheter-related complications

Complication Type	Number of Cases	Incidence Rate (%)
Thrombosis	1	1.41
Catheter Occlusion	2	2.82
Hemorrhage/Seepage	2	2.82
Total Incidence	5	7.04

2.3. Quality of life scores

The quality of life scores after intervention were significantly higher than those before intervention ($P < 0.05$). See **Table 2**.

Table 2. Quality of life scores (mean \pm SD, points)

Time	<i>n</i>	Physical Function	Role Function	Emotional Function	Cognitive Function	Social Function	Total Score
Before Intervention	71	54.23 \pm 7.33	55.34 \pm 6.32	54.42 \pm 7.41	57.53 \pm 6.43	56.25 \pm 6.34	55.54 \pm 6.45
After Intervention	71	82.32 \pm 7.34	84.43 \pm 7.84	85.34 \pm 6.33	84.23 \pm 6.42	82.35 \pm 6.45	83.64 \pm 7.55
t-value		22.817	24.341	26.734	24.760	24.316	23.844
<i>P</i> Value		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

4. Discussion

Patients with gastrointestinal tumors require long chemotherapy cycles, and the intravenous irritation caused by oxaliplatin necessitates that the intravenous access be safe and durable to a certain extent. Although central venous catheterization can meet the demands of chemotherapy, some patients refuse it due to psychological concerns, worries about their quality of life, and other reasons, resulting in a clinical dilemma of “no suitable intravenous access”^[5]. Studies have shown that more than 43% of patients are not clinically suitable for PICC^[6]. As a venous access tool that falls between peripheral intravenous catheters and central venous catheters, medium-length catheters have their tips located in the axillary vein or the lower segment of the subclavian vein. They not only avoid direct irritation of peripheral veins but also do not require entry into the central veins, offering unique advantages in terms of safety, operational convenience, and cost-effectiveness.

Research indicates that medium-length catheters have lower incidences of thrombosis and catheter-related bloodstream infections compared to PICC, and their indwelling time can meet the needs of short-term chemotherapy, making them suitable for patients who refuse central venous catheterization^[7]. Another study has confirmed that ultrasound-guided insertion of medium-length catheters can improve puncture success rates and reduce vascular injury^[8]. The results of this study indicate that among 71 patients, the success rate of MC catheter placement was 97.18%, with an average catheter placement time of (18.25 \pm 1.12) minutes and an average catheter indwelling time of (12.64 \pm 4.58) days. This confirms that MC catheter placement is a simple procedure with a high success rate, eliminating the need for frequent changes of intravenous access and reducing the pain caused by repeated punctures in patients. The incidence of complications is a core indicator for evaluating the safety of intravenous access. Sheng et al.^[9] pointed out that compared to short peripheral intravenous catheters, MCs have a longer indwelling time and lower incidences of phlebitis and exudation. Compared to central venous access devices, their insertion process is safer, with lower risks of catheter-related bloodstream infections and thrombosis. Researchers such as Li Xiuyun^[10] have also noted that, in meeting patients’ treatment needs, medium-length catheters can avoid unnecessary central venous catheter placement and reduce catheter-related bloodstream infections. In this study, the overall incidence of catheter-related complications was only 7.04%, with no serious complications such as severe infections, catheter rupture, or pulmonary embolism, indicating a low incidence of complications and good safety in the application of medium-length catheters. The study results also indicate that patients experienced a higher quality of life after the intervention. Prior to catheter placement,

diversified education was provided to alleviate patient anxiety. During catheter placement, specialized intravenous therapy nurses performed the procedure to ensure safety. After catheter placement, standardized maintenance and continuous management were implemented to reduce complications, while attention was also given to patients' psychological states and living needs, thereby improving their quality of life.

5. Conclusion

In summary, medium-length catheters (MCs) offer several advantages in the infusion of oxaliplatin in gastrointestinal tumor patients who refuse central venous catheterization, including a high catheter placement success rate, long indwelling time, low incidence of complications, and improved patient quality of life. When combined with targeted nursing measures, they can further ensure treatment safety and possess clinical value. However, this study has certain limitations. Firstly, it is a single-center study with a relatively small sample size, which may introduce selection bias. Secondly, the observation period was relatively short, and long-term follow-up of patients was not conducted. Therefore, the long-term complication rate of MCs still requires further investigation. In the future, large-sample, multicenter studies could be conducted to clarify the clinical value of MCs.

Disclosure statement

The authors declare no conflict of interest.

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Effect of Laparoscopic Radical Resection and Open Surgery for Liver Cancer and Their Impact on Inflammatory Factor Levels

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Abstract: *Objective:* To evaluate the therapeutic effects of laparoscopic radical resection and open surgery for liver cancer. *Methods:* A total of 80 liver cancer patients admitted to the hospital from January 2023 to January 2025 were selected and equally divided by a random number table. The observation group underwent laparoscopic radical resection, while the reference group underwent open surgery. Perioperative indicators and inflammatory factor levels were compared between the two groups. *Results:* The observation group exhibited superior perioperative indicators. One day after surgery, the observation group showed excellent inflammatory factor levels, lower fibrosis factor levels, excellent liver function indicators, and a reduced complication rate, with a statistically significant difference between the groups ($P < 0.05$). *Conclusion:* Laparoscopic radical resection for liver cancer is minimally invasive, promotes postoperative recovery, reduces postoperative inflammatory responses, inhibits the progression of liver fibrosis, protects liver function, and demonstrates high surgical safety.

Keywords: Laparoscopic radical resection for liver cancer; Open surgery; Liver cancer; Inflammatory factor levels

Online publication: December 31, 2025

1. Introduction

Liver cancer is a gastrointestinal tumor with a relatively high incidence rate. It lacks typical symptoms in the early stages of the disease, has strong concealment, and is mostly diagnosed in the middle to late stages, making surgical treatment challenging ^[1]. Open surgery is a commonly used curative treatment for this disease, allowing for the resection of liver cancer lesions under direct vision. The surgical procedure is relatively simple, but the precision of the operation is not optimal, which can easily lead to adverse events such as intraoperative bleeding, thereby affecting the quality of the surgery. In comparison, laparoscopic surgery offers significant minimally invasive advantages, reducing intraoperative blood loss, avoiding risk factors for postoperative complications, shortening the patient's rehabilitation period, and achieving better surgical prognosis ^[2]. Based on this, this study selected 80 patients with liver cancer to evaluate the therapeutic advantages of laparoscopic radical resection of liver cancer

for patients with this disease.

2. Materials and methods

2.1. General information

Eighty patients with liver cancer admitted to the hospital from January 2023 to January 2025 were included and equally divided using a random number table. The observation group consisted of 40 patients, including 22 males and 18 females; their ages ranged from 40 to 74 years, with a mean age of (55.16 ± 4.78) years old; the tumor diameters ranged from 1.5 to 7.1 cm, with a mean diameter of (4.12 ± 0.59) cm; in terms of TNM staging, 19 cases were stage I and 21 cases were stage II. The reference group consisted of 40 patients, including 24 males and 16 females; their ages ranged from 41 to 76 years, with a mean age of (55.27 ± 4.62) years old; the tumor diameters ranged from 1.6 to 7.3 cm, with a mean diameter of (4.19 ± 0.63) cm; in terms of TNM staging, 17 cases were stage I and 23 cases were stage II. When comparing the data between the two groups, $P > 0.05$.

Inclusion criteria: Diagnosed with liver cancer according to the “Diagnostic and Treatment Guidelines for Primary Liver Cancer (2017 Edition)”^[3]; pathological diagnosis of liver cancer; meeting surgical indications; having relatively complete basic information; highly informed about the study. Exclusion criteria: Presence of cardiopulmonary, hepatic, or renal dysfunction; intrahepatic or distant metastasis of tumor lesions; suffering from other malignant tumors; having surgical contraindications; significant fluctuations in vital signs; withdrawing from the study midway.

2.2. Methods

The reference group underwent laparotomy: The patient was assisted to assume a supine position with the body in a “humanoid” shape, and tracheal intubation and general anesthesia were performed. Based on the location of the tumor lesion, the incision was made at the right subcostal margin or the midline of the abdomen. The abdominal cavity was entered through the incision, and the ligaments around the liver parenchyma were dissected to expose the tumor lesion. The liver parenchyma was separated using an ultrasonic scalpel, and the tumor lesion was completely resected, with attention paid to controlling intraoperative bleeding. Evaluate for the presence of bile leakage. If no abnormalities were found, a drainage tube was placed, and the abdomen was closed routinely.

The observation group underwent laparoscopic radical resection of liver cancer: The position and anesthesia method were the same as above. The observation port was located 1 cm below the umbilicus, and a 10 mm trocar was inserted; the operating ports were located at the anterior axillary line below the xiphoid process, the subcostal region, and the midline of the left and right clavicles, with 5 mm trocars inserted to create a pneumoperitoneum with a pressure value of 8–12 mmHg. A comprehensive exploration of the patient’s abdominal cavity should be conducted, and the choice of radical resection method should be differentiated based on tumor staging. If the tumor is located in segments II to III, a left lateral lobectomy should be performed under laparoscopic guidance, and a linear stapler should be used during the process of transecting the liver parenchyma. If the tumor is located in segment IV or is a large-volume tumor, a left hemihepatectomy should be performed under laparoscopic guidance. The resection margin line should be determined using laparoscopic ultrasound, the liver parenchyma should be separated, hemostasis should be achieved by electrocoagulation, the left hepatic pedicle should be transected using a linear stapler, the left hepatic vein and residual liver tissue should be fully resected, and the left hemihepatectomy should be completed. If the tumor is located in segments V to VIII, a partial hepatectomy should be performed

based on the tumor diameter.

2.3. Observation indicators

- (1) Perioperative indicators: Evaluate indicators such as incision length, intraoperative blood loss, and postoperative time to initiate a liquid diet.
- (2) Level of inflammatory factors: Venous blood (5ml, fasting) should be collected before surgery and on postoperative day 1, centrifuged for 10 minutes at a speed of 3500 r/min, and the supernatant should be taken to evaluate interleukin-6 (IL-6), IL-2, and tumor necrosis factor- α (TNF- α) using enzyme-linked immunosorbent assay.
- (3) Fibrosis factors: At the same time intervals, blood should be collected, centrifuged, and the supernatant taken to evaluate hyaluronic acid (HA), procollagen type III peptide (PIIIP), and procollagen type I carboxy-terminal peptide (PICP) using an automated enzyme-linked immunosorbent assay reader with an immunoluminescence method.
- (4) Liver function indicators: At the same time period, after blood collection, a fully automated biochemical analyzer was used to evaluate aspartate aminotransferase (AST), total bilirubin (TBil), and alanine aminotransferase (ALT).
- (5) Complication rate: Observe the incidence probabilities of refractory ascites, abdominal cavity infection, encapsulated effusion in the liver section, and other conditions.

2.4. Statistical analysis

Data were processed using SPSS 28.0 software. Measurement values were compared/tested using t-values, while count values were compared/tested using chi-square (χ^2) values. Statistical significance was considered as $P < 0.05$.

3. Results

3.1. Comparison of perioperative indicators between groups

The perioperative indicators in the observation group were superior to those in the reference group ($P < 0.05$) (Table 1).

Table 1. Comparison of perioperative indicators between groups (mean \pm SD)

Group	Number of cases (<i>n</i>)	Incision length (cm)	Intraoperative blood loss (mL)	Time to liquid diet postop (days)	Time to ambulation postop (days)	Postoperative hospital stay (days)
Observation Group	40	6.35 \pm 1.48	198.75 \pm 16.45	4.27 \pm 1.51	1.44 \pm 0.52	12.45 \pm 2.66
Control Group	40	22.16 \pm 3.18	271.36 \pm 22.71	6.62 \pm 1.78	2.81 \pm 0.75	15.37 \pm 2.74
t-value	-	28.508	16.376	6.367	9.494	4.836
P-value	-	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

3.2. Comparison of inflammatory factor level between groups

One day after surgery, the inflammatory factor levels in the observation group were excellent, with a significant difference between groups ($P < 0.05$) (Table 2).

Table 2. Comparison of inflammatory factor level between groups (mean \pm SD)

Group	Number of cases (<i>n</i>)	IL-6 (pg/mL)		IL-2 (μ g/L)		TNF- α (ng/mL)	
		Preop	Postop 1d	Preop	Postop 1d	Preop	Postop 1d
Observation Group	40	9.45 \pm 1.78	11.12 \pm 2.19	5.30 \pm 1.06	4.47 \pm 1.05	3.29 \pm 0.57	4.22 \pm 0.78
Control Group	40	9.41 \pm 1.82	13.37 \pm 2.25	5.29 \pm 1.10	3.43 \pm 0.86	3.32 \pm 0.63	5.91 \pm 1.78
t-value	-	0.099	4.532	0.041	4.846	0.223	5.500
P-value	-	0.921	< 0.001	0.967	< 0.001	0.824	< 0.001

3.3. Comparison of fibrosis factors between groups

One day after surgery, the fibrosis factors in the observation group were lower, with a significant difference between groups ($P < 0.05$) (Table 3).

Table 3. Comparison of fibrosis factors between groups (mean \pm SD)

Group	Number of cases (<i>n</i>)	HA (μ g/L)		PHIP (ng/L)		PICP (μ g/L)	
		Preop	Postop 1d	Preop	Postop 1d	Preop	Postop 1d
Observation Group	40	73.21 \pm 7.15	87.65 \pm 8.19	8.46 \pm 1.95	10.21 \pm 1.53	110.75 \pm 18.63	128.51 \pm 10.97
Control Group	40	73.26 \pm 7.11	101.53 \pm 12.48	8.49 \pm 1.74	12.34 \pm 1.59	110.62 \pm 18.43	140.16 \pm 12.76
t-value	-	0.031	5.881	0.073	6.105	0.031	4.379
P-value	-	0.975	< 0.001	0.942	< 0.001	0.975	< 0.001

3.4. Comparison of liver function indicators between groups

One day after surgery, the liver function indicators of the observation group were superior, with a P -value < 0.05 in the inter-group comparison (Table 4).

Table 4. Comparison of liver function indicators between groups (mean \pm SD)

Group	Number of Cases (<i>n</i>)	AST (U/L)		TBil (μ mol/L)		ALT (U/L)	
		Preop	Postop 1d	Preop	Postop 1d	Preop	Postop 1d
Observation Group	40	58.16 \pm 4.81	40.32 \pm 3.98	88.42 \pm 9.15	39.05 \pm 4.16	62.45 \pm 9.11	41.25 \pm 3.78
Control Group	40	58.19 \pm 4.77	46.77 \pm 4.05	88.48 \pm 9.11	45.19 \pm 4.20	62.31 \pm 9.08	50.37 \pm 3.92
t-value	-	0.028	7.184	0.029	6.569	0.069	10.592
P-value	-	0.978	< 0.001	0.977	< 0.001	0.945	< 0.001

3.5. Comparison of complication rates between groups

The observation group had a lower complication rate, with a P -value < 0.05 in the inter-group comparison (Table 5).

Table 5. Comparison of complication rates between groups (n/%)

Group	Number of cases (n)	Refractory ascites	Intra-abdominal Infection	Wrapped pericaval effusion	Pulmonary infection	Reactive pleural effusion	Overall incidence rate
Observation group	40	0	0	1 (2.50)	1 (2.50)	0	5.00 (2/40)
Control group	40	1 (2.50)	1 (2.50)	3 (7.50)	2 (5.00)	1 (2.50)	20.00 (8/40)
χ^2 -value	-	-	-	-	-	-	4.114
P-value	-	-	-	-	-	-	0.043

4. Discussion

Liver cancer is a malignant tumor of the digestive tract with a high mortality rate, primarily caused by hepatitis B or C virus infection, leading to malignant transformations from hepatitis or cirrhosis^[3]. For patients with liver cancer without metastasis, radical surgery can be performed to completely remove the tumor, thereby improving the long-term quality of life of patients. Open surgery is a conventional treatment method that allows complete removal of the tumor under direct vision, with a high radical resection rate. However, it is highly invasive, involves significant intraoperative blood loss, and is prone to causing liver function damage, thereby prolonging postoperative recovery time and increasing the long-term recurrence rate^[4].

Laparoscopic radical resection of liver cancer is a novel surgical approach for this disease. It utilizes laparoscopy for surgical operations, allowing for an expanded surgical field while clearly displaying the tumor's location, size, and the distribution of surrounding blood vessels, thereby facilitating the rational formulation of a surgical plan. Compared to open surgery, laparoscopic radical resection of liver cancer offers a broader exploration range, enabling observation of areas such as the pelvic rectum at the junction of the stomach, esophagus, and liver, thus facilitating the detection of occult lesions^[5]. This surgical technique employs a four-port method for operation, allowing for simultaneous diagnosis and treatment, which can shorten the time for disease diagnosis and treatment and offers significant therapeutic advantages.

The results showed that the perioperative indicators of the observation group were significantly better than those of the reference group ($P < 0.05$). The reasons for this analysis are as follows: Laparoscopic surgery utilizes linear staplers during the operation, enabling proper handling of ductal stumps and reducing adverse events such as intraoperative bleeding^[6]. During the surgery, the liver parenchyma can be separated to the level of the hepatic segmental duct, which is then clamped, and the tumor lesion is excised to achieve therapeutic goals. This approach minimizes damage to the surrounding liver tissue, resulting in better surgical outcomes. The inflammatory factor levels of the observation group on the first day after surgery were better than those of the reference group, while the fibrosis factor levels were lower than those of the reference group ($P < 0.05$). Inflammatory factors can assess the level of postoperative inflammatory response in patients and predict the degree of their stress response, thus allowing for the evaluation of the patient's inflammatory mediator levels and the determination of the negative impact of surgery on the patient's body^[7].

Among inflammatory factors, IL-6 and TNF- α can be used to evaluate the severity of inflammation. Both are pro-inflammatory factors that are highly involved in the onset and development of inflammation, with their levels showing a positive correlation with the inflammatory response^[8]. IL-2 regulates the activity of leukocytes, participates in the body's tumor surveillance process and antibody response process, has a strong effect on B lymphocytes, accelerates their proliferation, activates T cells, and thereby enhances the immune anti-cancer

effect. After hepatectomy, liver fibrosis is a stress response with a relatively high incidence. Evaluating fibrosis factors can predict surgical hazards early and thus assess surgical efficacy ^[9]. After laparoscopic surgery, the aforementioned indicators are superior to those after open surgery, indicating that laparoscopic surgery induces a milder inflammatory response, can suppress stress responses, stabilize patients' postoperative vital signs, and facilitate patient recovery. The liver function indicators of the observation group one day after surgery were superior to those of the reference group ($P < 0.05$). The reason for this analysis is that laparoscopic surgery can preserve hepatic blood flow, reduce hepatic tissue hypoxia or ischemia, and thereby mitigate the extent of liver function damage ^[10]. Additionally, the precision and minimally invasive nature of laparoscopic surgery can reduce damage to liver tissue, resulting in excellent liver function indicators. The complication rate in the observation group was lower than that in the reference group ($P < 0.05$). The reason for this analysis is that laparoscopic surgery provides a clear field of view, allowing for continuous monitoring of surgical procedures and causing minimal damage to the tissues surrounding the liver, thus resulting in a lower risk of complications.

5. Conclusion

In conclusion, laparoscopic radical hepatectomy for liver cancer patients offers superior treatment outcomes compared to open surgery, achieving better perioperative indicators, reducing the degree of inflammatory and stress responses, protecting patients' liver function, and demonstrating high surgical safety benefits.

Disclosure statement

The author declares no conflict of interest.

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Bio-Byword Scientific Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Clinical Application Research of Gene Signature Based on the STAT3/P53 Pathway in Prognosis Prediction for Osteosarcoma Patients

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Abstract: *Objective:* To construct a gene signature centered on the STAT3/P53 signaling pathway and clarify its clinical value in prognosis assessment for osteosarcoma patients through direct comparison between the control group and the observation group, providing a reference for personalized treatment. *Methods:* Eighty osteosarcoma patients admitted to the orthopedics department of our hospital from May 2024 to October 2025 were selected as the study subjects. The expression levels of 12 core genes (CDKN1A, BCL2, MDM2, etc.) in the STAT3/P53 pathway in tumor tissues were detected using real-time fluorescent quantitative PCR (qRT-PCR). Using the median of the gene signature risk score as the cutoff, patients were divided into a high-risk group (observation group, $n = 40$) and a low-risk group (control group, $n = 40$). Clinical and pathological characteristics, core gene expression patterns, treatment responses, and short-term prognosis outcomes were compared between the two groups. *Results:* There were no statistically significant differences between the observation group and the control group in terms of age, gender, and tumor location (all $P > 0.05$). However, significant differences were observed in the maximum tumor diameter, Enneking stage, and LDH level (all $P < 0.001$). The expression levels of oncogenes in the observation group were significantly higher than those in the control group, while the expression levels of tumor suppressor genes were significantly lower. The chemotherapy response rate in the observation group was significantly lower than that in the control group ($\chi^2 = 12.170$, $P = 0.001$). After 3 months of follow-up, the recurrence and metastasis rate in the observation group was significantly higher than that in the control group, with a statistically significant difference ($\chi^2 = 8.658$, $P = 0.003$). *Conclusion:* The gene signature based on the STAT3/P53 pathway can effectively distinguish between high-risk and low-risk osteosarcoma patients, with significant differences observed between the two groups in terms of clinical characteristics, gene expression, and short-term prognosis. This gene signature provides a reliable basis for prognostic prediction and the formulation of treatment plans.

Keywords: Osteosarcoma; STAT3/P53 pathway; Gene signature; Control group; Observation group; Prognostic evaluation

Online publication: December 31, 2025

1. Introduction

Osteosarcoma is the most common primary malignant bone tumor in adolescents and young adults, with a slightly higher incidence in males than females. Its pathological feature is the excessive proliferation of abnormal osteogenic mesenchymal cells, predominantly occurring in the metaphysis of long bones ^[1] (with half of the cases involving the area around the knee joint). Ten percent of cases involve axial bones such as the pelvis, and patients with axial bone involvement are generally older. Despite the standard treatment regimen of neoadjuvant chemotherapy combined with surgery, some patients still experience recurrence or distant metastasis in clinical practice, leading to a poor prognosis. Moreover, traditional indicators such as the Enneking staging system and LDH levels struggle to accurately capture the molecular malignant characteristics of tumors, lacking a basis for individualized treatment ^[2]. The functional imbalance of the STAT3/P53 pathway is closely related to the malignant progression of osteosarcoma, and a gene signature combining multiple genes offers greater advantages in risk stratification compared to a single biomarker ^[3]. This study, involving 80 patients treated from 2024 to 2025, aimed to verify the clinical utility of the gene signature related to this pathway through a comparative study, providing new insights for precision medicine.

2. Materials and methods

2.1. General information

Eighty patients with osteosarcoma admitted to the orthopedics department of our hospital from May 2024 to October 2025 were selected as the study subjects. Firstly, the expression levels of core genes in the STAT3/P53 pathway in the tumor tissues of osteosarcoma patients were detected by qRT-PCR. Referring to the 12 core genes in the STAT3/P53 pathway (CDKN1A, BCL2, MDM2, BAX, CCNB1, CYCS, FAS, GADD45A, JUN, MYC, P21, TP53I3) screened in previous studies, the gene signature risk score for each patient was calculated as follows: RiskScore = (0.32 × CDKN1A expression level) + (0.28 × BCL2 expression level) + (0.41 × MDM2 expression level) - (0.35 × BAX expression level) + (0.29 × CCNB1 expression level) + (0.33 × CYCS expression level) - (0.27 × FAS expression level) + (0.30 × GADD45A expression level) + (0.26 × JUN expression level) + (0.38 × MYC expression level) - (0.31 × P21 expression level) + (0.25 × TP53I3 expression level). Using the median risk score (0.62) as the cutoff point, patients were divided into a high-risk group (observation group, score ≥ 0.62, *n* = 40) and a low-risk group (control group, score < 0.62, *n* = 40).

Inclusion criteria: (1) Patients diagnosed with primary osteosarcoma by pathological biopsy, with the pathological type being classic osteoblastic osteosarcoma; (2) Patients who were initially treated and had not received radiotherapy, chemotherapy, targeted therapy, or immunotherapy; (3) Patients with complete clinical data, including baseline data such as tumor location, size, Enneking stage, and LDH level; (4) Patients and their family members provided informed consent and signed the informed consent form. Exclusion criteria: (1) Patients with primary malignant tumors in other locations; (2) Patients with severe liver or kidney dysfunction or cardiopulmonary dysfunction; (3) Patients with unclear pathological diagnosis or missing clinical data; (4) Patients who withdraw from treatment midway or lose contact during follow-up.

2.2. Experimental materials

Reagents included TRIzol total RNA extraction reagent, Prime Script RT reverse transcription kit, TB Green Premix ExTaq qRT-PCR kit, and primers synthesized by Sangon Biotech (Shanghai) Co., Ltd., with sequences listed in Table 1. Instruments included a real-time fluorescent quantitative PCR instrument, high-speed

refrigerated centrifuge, nucleic acid protein detector, and super clean bench.

Table 1. qRT-PCR primer sequences for core genes

Gene Name	Forward Primer (5'-3')	Reverse Primer (5'-3')	Product Length (bp)
CDKN1A	GGGATGAGTTGGGAGGT	CAGGGTTTCTCTTCCTCT	186
BCL2	GGTGGGGTCATGTGTGT	GGTTCAGGTACTCAGTC	212
MDM2	CCGAAGTTTGTGAAGGAG	GGAGACAAGTTGTAGGG	198
BAX	GCTACAGGGTTTCATCC	CAGTTGAAGTTGCCGTC	205
MYC	CTCCTCGGACACGCTGCTG	CAGCAGCTCGAATTCTTCC	220
FAS	TGCCACCTCTCTTTCCT	GCTGTCCTGCTTGTCTGT	195
GAPDH	GAAGGTGAAGGTCGGAG	GAAGATGGTGATGGGATT	226

2.3. Gene detection methods

- (1) Sample Collection and Processing: After surgical resection of the tumor tissue, 0.5 cm³ of tumor parenchyma (avoiding necrotic areas) was taken under sterile conditions, rapidly frozen in liquid nitrogen for 10 minutes, and then stored in a -80°C ultra-low temperature freezer for later use.
- (2) RNA Extraction and Quality Detection: Total RNA was extracted using TRIzol reagent, and its purity (A260/A280 ratio of 1.8–2.0) and concentration were verified using a nucleic acid protein detector. The integrity was confirmed by agarose gel electrophoresis (clear bands of 28S and 18S with a ratio of approximately 2:1).
- (3) Reverse transcription reaction: Take 1 µg of qualified RNA and construct a 20 µL reaction system using the Prime Script RT kit (containing buffer, enzyme mixture, primers, etc.). Perform reverse transcription at 37°C for 15 minutes, inactivate the enzyme at 85°C for 5 seconds, and store at 4°C.
- (4) qRT-PCR detection: Using cDNA as the template, a 20 µL system containing TB Green premix, upstream and downstream primers, and the internal reference gene GAPDH was prepared. After initial denaturation at 95°C for 30 seconds, 40 cycles were performed (denaturation at 95°C for 5 seconds, annealing and extension at 60°C for 34 seconds). Three replicates were set up, and the relative gene expression levels were calculated using the 2^{-ΔΔCt} method.

2.4. Treatment plan and evaluation indicators

- (1) Treatment Plan: All patients received a standard neoadjuvant chemotherapy regimen (methotrexate 12 g/m² on day 1, cisplatin 100 mg/m² on day 2, and doxorubicin 75 mg/m² on day 3), with each cycle lasting 3 weeks. After two cycles, surgical treatment (limb salvage surgery or amputation) was performed, followed by four additional cycles of the original chemotherapy regimen.
- (2) Evaluation Indicators: (a) Clinical and pathological characteristics: average age, gender, tumor location, maximum tumor diameter, Enneking stage, and LDH level; (b) Core gene expression levels: comparison of the relative expression levels of oncogenes (BCL2, MDM2, MYC, CCNB1) and tumor suppressor genes (BAX, FAS, P21, CDKN1A) between the two groups; (c) Therapeutic response: Assessment was conducted after 2 cycles of chemotherapy according to the RECIST1.1 criteria ^[4], categorizing responses into complete remission (CR, complete disappearance of the tumor), partial

remission (PR, reduction in the maximum diameter of the tumor by $\geq 30\%$), stable disease (SD, tumor size reduction or increase), and progressive disease (PD, tumor enlargement by $\geq 20\%$ or the appearance of new lesions). The response rate = (CR + PR)/total number of cases $\times 100\%$. (d) Short-term prognosis: Patients were followed up for 3 months post-surgery, and recurrence and metastasis were recorded through imaging examinations and pathological biopsies.

2.5. Statistical methods

Data analysis was performed using SPSS26.0 software. Measurement data were expressed as mean \pm standard deviation (SD), and comparisons between groups were made using independent sample t-tests. Count data were expressed as the number of cases [$n(\%)$], and comparisons between groups were made using the χ^2 test. A P -value < 0.05 was considered statistically significant.

3. Results

3.1. Comparison of clinicopathological characteristics between the two groups

There were no statistically significant differences in age, gender, or tumor location between the observation group and the control group (all $P > 0.05$). However, significant differences were observed in the maximum tumor diameter, Enneking stage, and LDH levels between the two groups (all $P < 0.001$). See **Table 2**.

Table 2. Comparison of clinicopathological characteristics between the two groups

Clinical Characteristics		Observation Group ($n = 40$)	Control Group ($n = 40$)	χ^2	P
Age (years)		17.62 \pm 4.21	17.58 \pm 4.20	0.043	0.967
Gender	Male	24 (60.0)	25 (62.5)	0.053	0.818
	Female	16 (40.0)	15 (37.5)		
Tumor Location	Extremities	35 (87.5)	37 (92.5)	0.139	0.709
	Trunk	5 (12.5)	3 (7.5)		
Maximum Tumor Diameter	≥ 5 cm	27 (67.5)	9 (22.5)	16.364	< 0.001
Enneking Stage	Stage I–II	10 (25.0)	30 (75.0)	20	< 0.001
	Stage III–IV	30 (75.0)	10 (25.0)		
LDH Level	Normal	12 (30.0)	29 (72.5)	14.459	< 0.001
	Elevated	28 (70.0)	11 (27.5)		

3.2. Comparison of core gene expression levels between the two groups

The expression levels of oncogenes in the observation group were significantly higher than those in the control group, while the expression levels of tumor suppressor genes were significantly lower. See **Table 3**.

Table 3. Comparison of core gene expression levels between the two groups

Group	Pro-oncogene Expression Level				Tumor Suppressor Gene Expression Level			
	BCL2	MDM2	MYC	CCNB1	BAX	FAS	P21	CDKN1A
Observation (<i>n</i> = 40)	2.41 ± 0.48	2.63 ± 0.51	2.25 ± 0.42	2.18 ± 0.39	0.43 ± 0.12	0.39 ± 0.11	0.46 ± 0.13	0.51 ± 0.14
Control (<i>n</i> = 40)	1.01 ± 0.23	1.00 ± 0.21	1.02 ± 0.19	1.04 ± 0.12	1.03 ± 0.12	1.02 ± 0.11	1.03 ± 0.24	1.04 ± 0.23
<i>t</i> -value	16.636	18.691	16.876	17.670	22.361	25.613	14.830	12.450
<i>P</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

3.3. Comparison of treatment responses between the two groups

The chemotherapy response rate in the observation group was significantly lower than that in the control group ($\chi^2 = 12.170$, $P = 0.001$). Specific data can be found in **Table 4**.

Table 4. Comparison of treatment responses between the two groups of patients

Treatment response	Complete response (CR)	Partial response (PR)	Stable disease (SD)	Progressive disease (PD)	Overall response rate (CR + PR)
Observation Group (<i>n</i> = 40)	2 (5.0)	16 (40.0)	14 (35.0)	8 (20.0)	18 (45.0)
Control Group (<i>n</i> = 40)	7 (17.5)	26 (65.0)	10 (25.0)	3 (7.5)	33 (82.5)
χ^2 -value					12.170
<i>P</i> -value					0.001

3.4. Comparison of short-term prognosis between the two groups

After a 3-month follow-up, the recurrence and metastasis rate in the observation group was significantly higher than that in the control group, with a statistically significant difference ($\chi^2 = 8.658$, $P = 0.003$). See **Table 5**.

Table 5. Comparison of recurrence and metastasis sites between the two groups of patients

Recurrence/Metastasis Type	Lung Metastasis	Local Recurrence	Overall Recurrence Rate
Observation Group (<i>n</i> = 40)	8 (20.0)	4 (10.0)	12 (30.0)
Control Group (<i>n</i> = 40)	4 (10.0)	0 (0.0)	4 (10.0)
χ^2 -value			8.658
<i>P</i> -value			0.003

4. Discussion

Osteosarcoma is one of the leading causes of cancer-related deaths among adolescents. Although its treatment approach has evolved from simple surgical intervention to a comprehensive model of “neoadjuvant chemotherapy + surgery + adjuvant chemotherapy,” there are still significant individual differences in prognosis ^[5]. In clinical practice, even patients with the same Enneking stage and similar clinical characteristics may exhibit vastly different responses to chemotherapy and risks of recurrence and metastasis. The core reason for this phenomenon lies in the heterogeneity of tumor molecular phenotypes. The STAT3 and P53 pathways,

as key regulators of tumor cell proliferation, apoptosis, invasion, and metastasis, play a central role in the development and progression of osteosarcoma when their functions are imbalanced^[6,7].

This study, stratified by gene signatures, found significant differences between the observation group and the control group in terms of tumor size, Enneking stage, and LDH levels, with the observation group having a notably higher proportion of stage III-IV tumors compared to the control group. Enneking stages III-IV indicate a high risk of tumor invasion and metastasis and a poor prognosis, while elevated LDH levels reflect a high degree of tumor malignancy and strong metastatic potential, consistent with previous research. There were no significant differences in basic characteristics such as age, gender, and tumor location between the two groups, suggesting that the risk stratification based on gene signatures is not influenced by these variables and is applicable to diverse populations. It can provide a unified molecular assessment standard for osteosarcoma patients across different age groups and tumor locations, compensating for the individualized assessment limitations of traditional staging systems and offering significant discriminatory value for patients with similar clinical characteristics but different molecular phenotypes.

Functional imbalance in the STAT3/P53 pathway is a key molecular mechanism underlying the development and progression of osteosarcoma, and the core genes screened in this study are all involved in regulating this pathway. In the observation group, the expression of pro-oncogenes (BCL2, MDM2, MYC) was significantly increased, while the expression of tumor suppressor genes (BAX, FAS, P21) was significantly decreased: BCL2 inhibits mitochondrial apoptosis, MDM2 degrades P53 leading to the loss of its tumor suppressor function, and MYC promotes cell proliferation and metabolic reprogramming; low expression of BAX weakens apoptotic signals, reduced FAS levels aid tumor immune evasion, and decreased P21 triggers cell cycle disorders. These gene expression differences collectively contribute to the malignant phenotype of “active proliferation, apoptosis inhibition, and immune evasion” observed in the observation group, whereas the control group maintains a balance between tumor suppressor and pro-oncogene expression, exhibiting relatively mild biological behavior^[8].

The chemotherapy effectiveness rate in the observation group was significantly lower than that in the control group, with a 3-month recurrence and metastasis rate reaching 30.0%. This suggests that high-risk patients respond poorly to standard chemotherapy and are prone to early progression. This is highly correlated with gene expression patterns: high expression of MDM2 leads to the loss of P53 function, reducing chemotherapy sensitivity; imbalanced expression of BCL2 and BAX enhances anti-apoptotic capacity; high expression of MYC activates DNA damage repair pathways, reducing chemotherapy-induced cell death, collectively contributing to chemotherapy resistance. In clinical practice, high-risk patients can be identified in advance through gene signatures, and treatment plans can be adjusted accordingly: for those with high MDM2 expression, inhibitors such as Nutlin-3 can be combined to restore P53 function; for those with high BCL2 expression, Venetoclax can be added to reverse the anti-apoptotic phenotype^[8,9]. Additionally, the increased recurrence and metastasis rate in the observation group may be related to the activation of the STAT3 pathway promoting epithelial-mesenchymal transition (EMT). Therefore, high-risk patients should undergo chest CT and local MRI examinations every 1-2 months postoperatively to strengthen follow-up monitoring.

Compared with traditional clinical indicators, this gene signature offers significant advantages: it provides a more comprehensive assessment by capturing functional imbalances at the molecular level; it enables more precise stratification, distinguishing patients with similar clinical characteristics but significantly different prognoses; it offers more specific guidance, providing clear targets for targeted therapy; and it is

operationally convenient, being detectable and promotable through routine qRT-PCR. It can promote the transition of osteosarcoma treatment from “standardization” to “personalization”. Patients with high-risk stage I-II osteosarcoma can receive intensified neoadjuvant chemotherapy, while those with low-risk stage III-IV osteosarcoma can avoid the toxic and side effects of excessive chemotherapy. Moreover, it can be used for dynamic monitoring of treatment efficacy, with core gene expression re-examined after two cycles of chemotherapy to adjust treatment strategies.

5. Conclusion

In summary, the gene signature based on the STAT3/P53 pathway can effectively distinguish between high- and low-risk patients with osteosarcoma, with significant differences observed between the two groups in terms of clinical characteristics, gene expression, and short-term prognosis. This gene signature provides a reliable basis for prognosis prediction and treatment plan formulation.

Disclosure statement

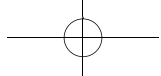
The authors declare no conflict of interest.

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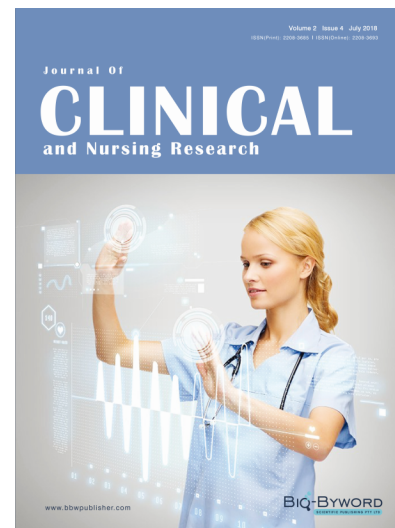
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